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Design and Synthesis of Potent Macrocyclic HIV-1 Protease Inhibitors Involving P1-P2 Ligands

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A series of potent macrocyclic HIV-1 protease inhibitors have been designed and synthesized. The compounds incorporated 16- to 19-membered macrocyclic rings between a Nelfinavir-like P2 ligand and a tyrosine side chain containing a hydroxyethylamine sulfonamide isostere. All cyclic inhibitors are more potent than their corresponding acyclic counterparts. Saturated derivatives showed slight reduction of potency compared to respective unsaturated derivatives. Compound **8a** containing a 16-membered ring as the P1-P2 ligand showed the most potent enzyme inhibitory and antiviral activity.

Introduction

HIV-1 protease inhibitors are a critical component of antiretroviral therapy (ART) for the treatment of patients with HIV infection and AIDS.[1,2] The use of ART has reduced both the mortality and morbidity rates among HIV-infected patients. However, the emergence of drug resistance has raised serious concerns about the prospects of long-term treatment options.[3,4] In our continuing studies to combat drug resistance, our structure-based design strategies targeting the protein backbone has led to the discovery of a variety of novel HIV-1 protease inhibitors (PIs), including FDA approved HIV protease inhibitor darunavir with broad-spectrum activity against multidrug-resistant HIV-1 variants.[5-8]

In another approach to developing inhibitors with broad-spectrum activity, we have been exploring the design of various macrocyclic HIV-1 protease inhibitors. Recently, we have reported the design of a series of potent PIs that incorporate flexible macrocycles involving P1'-P2'-ligands and P1-P2 ligands to effectively fill in the S1'-S2' and S1-S2 subsites of HIV-1 protease, respectively.[9-11] The conception of this macrocyclic design evolved from the observation that certain mutations lead to decreased van der Waals interactions and increased the size of the subsite hydrophobic pocket.[12,13] On the basis of this insight of enzyme flexibility in accommodating alternate packing, we designed flexible macrocycles between the P1'-side chain and a suitable P2'-ligand to fill in the S2' and S1'-subsites. As shown in Figure 1, this effort led to a series of potent macrocyclic inhibitors, as represented by inhibitor **2,** containing the P1-P2-ligands of darunavir.

In an alternate design approach, we have designed macrocyclic inhibitors as represented by inhibitor **3,** where the macrocycles involve the P1-P2 ligands, incorporating 2,3-dihydroxybenzoic acid derivatives as the P2-ligand, and aliphatic chains as the P1 ligand. Fairlie and co-workers also designed a number of different macrocyclic HIV-1 protease inhibitors, as represented in inhibitor **4**.[14] Based upon our previous results, we have now investigated macrocyclic inhibitors involving P1-P2 ligands which incorporate 3-hydroxy-2-alkylbenzoic acid derivatives as the P2-ligand and alkylated tyrosine side chains as the P1 ligand. In particular, as shown in Figure 2, inhibitors are designed by taking advantage of the large hydrophobic pocket in the HIV protease S1-S2 active site. These inhibitors incorporate P2 ligand found in the FDA approved drug nelfinavir **5** and the P1′-P2′ ligands found in TMC-126 (**6**).[15,16] Various macrocyclic inhibitors can be synthesized conveniently by ring-closing metathesis of the dienes **7** using Grubbs' catalyst.

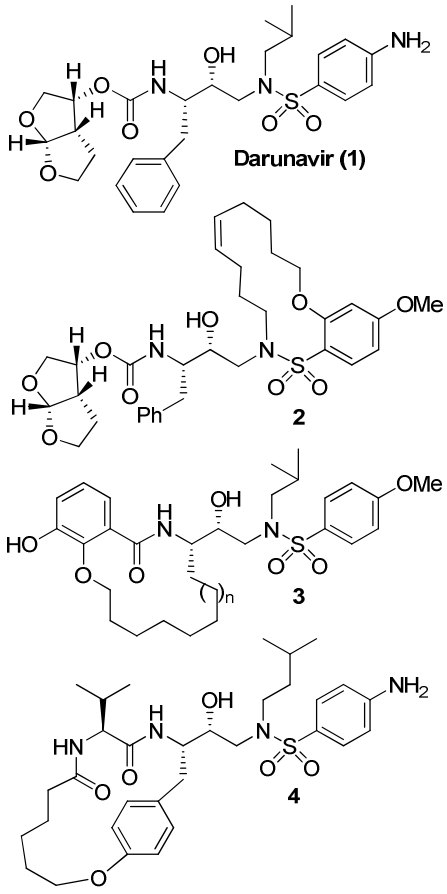


Figure 1. Structures of Darunavir and macrocyclic HIV-1 protease inhibitors **2-4**.

Results and discussion

In order to gain additional insight into the proposed inhibitors, a molecular model was obtained with one of the unsaturated macrocycles overlaid with nelfinavir (Figure 3).[17] As can be seen, the phenolic hydroxyl group of the macrocyclic inhibitor **8a** (16-membered macrocycle, n = 1) appears to be capable of forming hydrogen bonds with the Asp30 backbone NH as well as the side chain carbonyl residue in the S2 subsite. The benzamide carbonyl oxygen is positioned to form a hydrogen bond with the tight-bound water molecule that can interact with one of the sulfonamide oxygens. The 4-methoxy oxygen on the P2'-sulfonamide ligand can also form hydrogen bonding interactions with the Asp30′ backbone NH in the S2'-pocket. Furthermore, it appears that as the ring size increases, the P_2 and P_1 ligands would become distorted from their optimal position and bind less tightly in the S2 pockets. Based upon this model, it appears that inhibitors with 16-18 membered ring sizes could optimally fit in the S2 hydrophobic pocket.

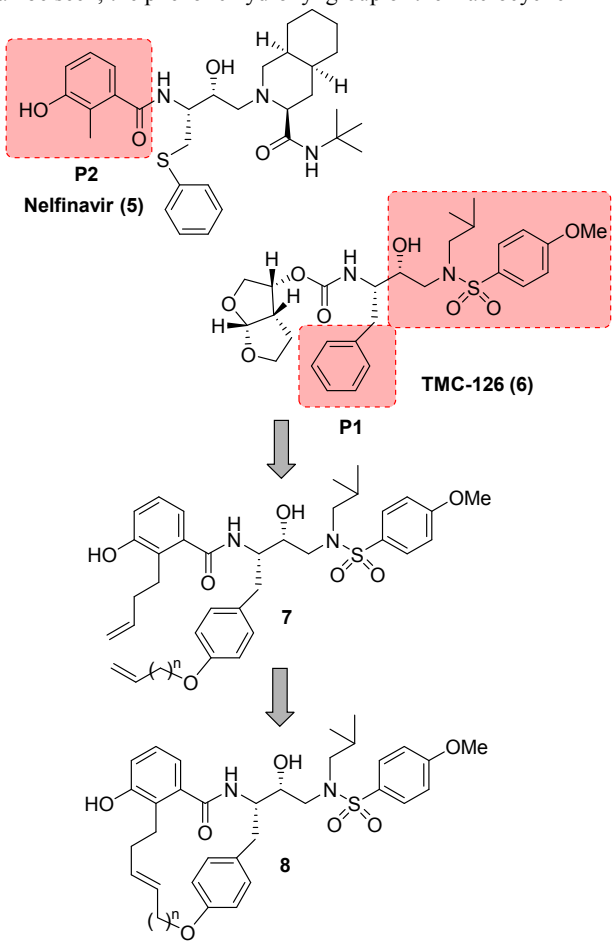


Figure 2. Design of Macrocyclic Inhibitor **8**.

Synthesis of the desired tyrosine-derived hydroxyethylamine sulfonamide isostere is shown in Scheme 1. The commercially available butadiene monoxide **9** was reacted with *p*-benzyloxyphenylmagnesium bromide in the presence of a catalytic amount of cuprous cyanide to provide the corresponding allylic alcohol.[18] Sharpless asymmetric epoxidation of the resulting alcohol using (-)-diethyl D-tartrate provided optically active epoxide **10** in very good yield.[19,20] Regioselective epoxide opening of **11** using $TMSN_3$ and Ti(O-*i*Pr)$_4$ as described by Sharpless and co-workers afforded the corresponding azido diol.[21] The resulting diol was converted to epoxide **11** by treatment with 2-acetoxyisobutyryl chloride in chloroform followed by reaction of the resulting chloroacetate with sodium methoxide.[22]

Preparation of the elaborated sulfonamide intermediate was accomplished by opening epoxide **11** with isobutylamine in 2-propanol at reflux.[23] The resulting amine was reacted with *p*-methoxyphenylsulfonyl chloride in the presence of aqueous $NaHCO_3$ to provide azidosulfonamide **12** in excellent yield. Catalytic hydrogenation of **12** over Pd/C in the presence of Boc_2O provided the Boc-protected amine as well as the free phenol in an efficient one-pot operation. The resulting phenol derivative was alkylated with appropriate alkenylbromide in acetone in the presence of K_2CO_3 to furnish the requisite olefins **13-16** in excellent yields.

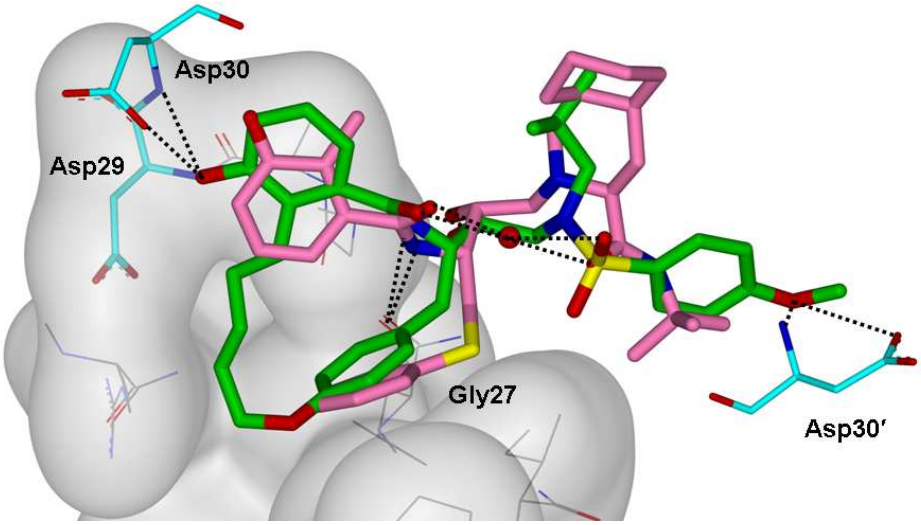
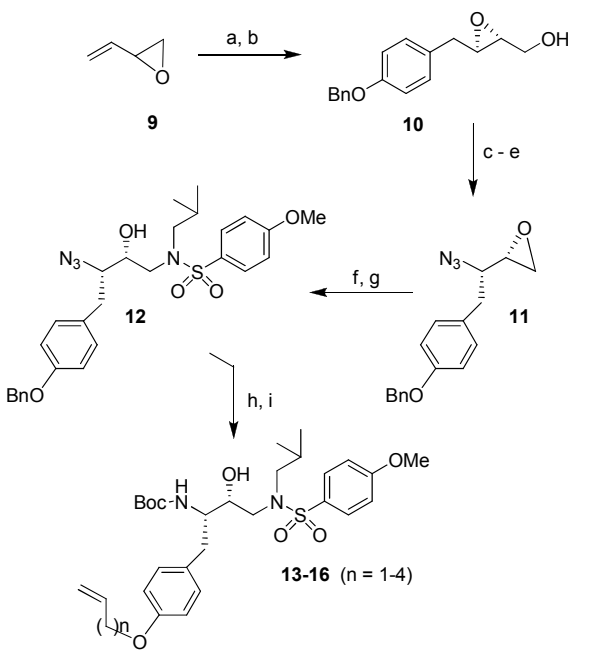


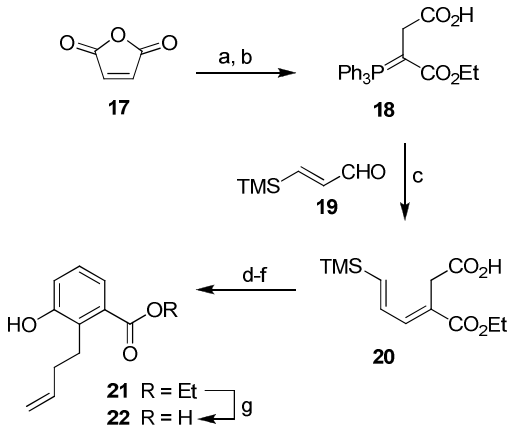
Figure 3. Model of inhibitor **8a** (green, n = 1) overlaid with Nelfinavir (magenta) in the HIV-1 protease active site.



Scheme 1. Reagents and conditions: (a) *p*-BnOPhMgBr, CuCN, THF, -78 °C; 39%; (b) D-DET, $Ti(O\text{-}iPr)_4$, 4 A MS, TBHP, CH_2Cl_2, 79%; (c) $Ti(O\text{-}iPr)_4$, $TMSN_3$, PhH, reflux, 55%; (d) $AcOMe_2COCl$, $CHCl_3$; (e) NaOMe, THF, 82% 2 steps; (f) *i*-$BuNH_2$, *i*-PrOH, reflux; (g) *p*-$MeOPhSO_2Cl$, aq. $NaHCO_3$, CH_2Cl_2, 97% 2 steps; (h) H_2, MeOH, Boc_2O, Pd/C, 75%; (i) acetone, K_2CO_3, Bromide, reflux, 94-96%.

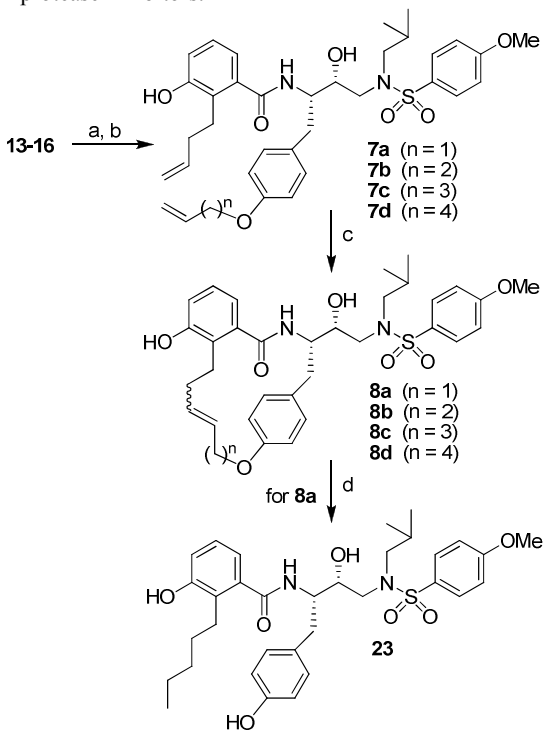
Synthesis of 3-hydroxy-2-alkenylbenzoic acid, the corresponding alkenyl metathesis substrate is shown in Scheme 2. Phosphorane **18** was prepared from maleic anhydride **17** using reported procedures.[24-26] Wittig reaction of phosphorane **18** with known[27,28] aldehyde **19** provided dienoic acid **20** in good yield (83%).[29] Alkylation of **20** with LDA and 1-bromo-3-butene in the presence of DMPU afforded the corresponding triene derivative. This was exposed to trifluoroacetic anhydride (TFAA) and triethylamine followed by $NaBH_4$ in ethanol which effected a 1,6-electrocyclization to afford benzoic acid ester **21**.[30] Saponification of ethyl ester **21** with KOH in methanol provided the desired acid **22**.

Synthesis of various acyclic and macrocyclic inhibitors is shown in Scheme 3. Treatment of Boc-derivatives **13-16** with trifluoroacetic acid in CH_2Cl_2 resulted in the deprotection of Boc group. The resulting amines were then coupled with acid **22** to provide acyclic dienes **7a-d** in good yields. Ring-closing metathesis of the dienes **7a-d** was carried out using Grubbs' first generation catalyst in CH_2Cl_2 at 23 °C for 4 h to provide macrocycles **8a-d** in excellent yields (78-89%).[31,32] The alkenes were formed as a mixture of *trans/cis* isomers. *Trans/cis* ratios of the unsaturated macrocycles were 1:1 for **8a** (16-membered ring); 3:1 for **8b** (17-membered ring); 5:1 for **8c** (18-membered ring); and essentially a single isomer for **8d** (19-membered ring). The *cis/trans* isomers could not be separated



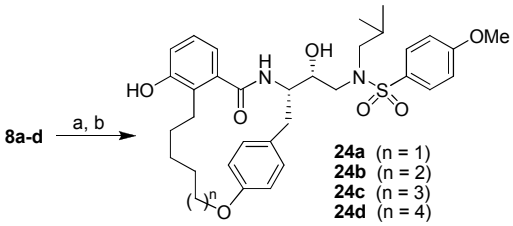
Scheme 2. Reagents and conditions: (a) PPh_3, acetone; (b) EtOH, 35% 2 steps; (c) $PhCH_3$, hydroquinone, 83%; (d) LDA, THF, DMPU, 4-bromo-1-butene; (e) TFAA, Et_3N; (f) $NaBH_4$, EtOH, 48% 2 steps; (g) KOH, MeOH, reflux, 89%.

by silica gel chromatography. To obtain the corresponding saturated macrocyclic inhibitors, we first attempted hydrogenation of allyl ether **8a** over 10% Pd-C in ethyl acetate for 12h. However, under these conditions, only ring opening compound **23** was isolated as the main byproduct. The use of PtO_2 as catalyst also resulted in allylic ring cleavage to afford the ring-opened byproduct. A similar effect has been reported by us in the context of the synthesis of cycloamide based HIV-1 protease inhibitors.[11]



Scheme 3. Reagents and conditions: (a) TFA, CH_2Cl_2; (b) acid **22**, EDCI, HOBt, Et_3N, DMF, 60-78%, 2 steps; (c) Grubbs' 1st Gen. Cat. (20 mol%), CH_2Cl_2, 23 °C, 78-89%; (d) H_2, Pd/C, EtOAc.

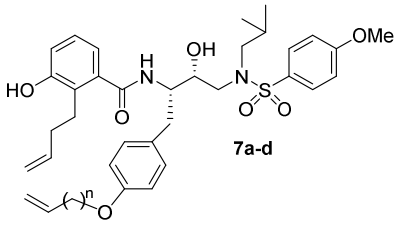
To prevent cleavage of the allylic ether, we carried out the hydrogenation according to the procedure reported by Sajiki and Hirota.[33] As shown in Scheme 4, hydrogenation of **8a** in the presence of 5% Pd/C in 1% NH_3 in methanol for 4 hours afforded the saturated macrocyclic inhibitor **24a** in 40% isolated yield. This condition also provided the ring opened compound **23** as the byproduct (27%). Catalytic hydrogenation of unsaturated macrocycles **8b-8d** however, proceeded smoothly over 10% Pd/C in ethyl acetate to provide saturated derivatives **24b-d** respectively in yields (88-94%).



Scheme 4. Reagents and conditions: (a) for n = 1, H_2, 5% Pd/C, 1% NH_3 in MeOH, 40%; (b) for n = 2-4, H_2, Pd/C, EtOAc, 88-94%.

The inhibitory potencies of acyclic and cyclic inhibitors were measured by the assay protocol of Toth and Marshall.[34] The results are shown in Tables 1 and 2. A number of selected compounds that showed potent enzyme inhibitory K_i

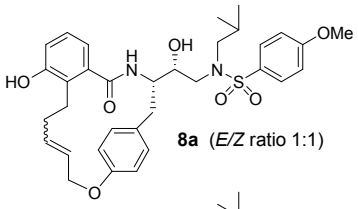
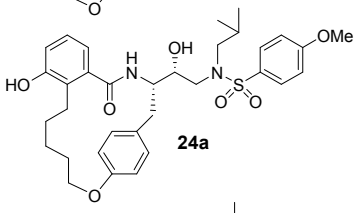
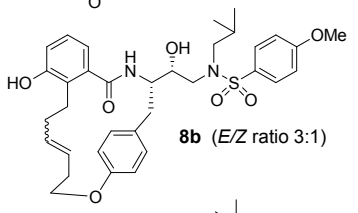
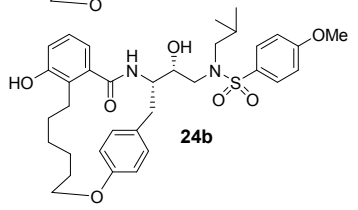
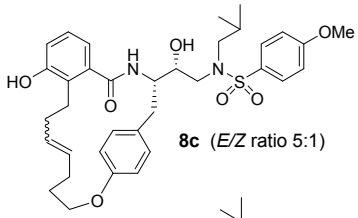
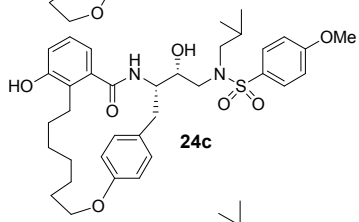
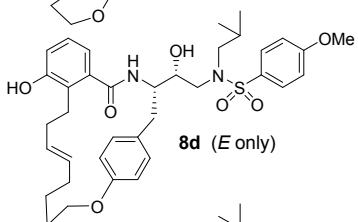
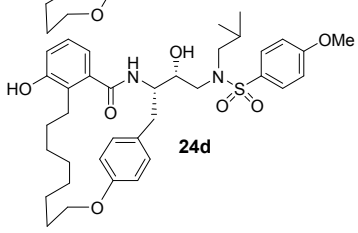
Table 1. Potency (K_i) of acyclic inhibitors



Compd	Ring size (after RCM)	n	K_i (nM)[a]
7a	16	1	5
7b	17	2	7
7c	18	3	45
7d	19	4	30
23	---	---	0.41

[a]Darunavir exhibited K_i = 16 pM.

Table 2. Enzymatic inhibitory and antiviral activity of macrocyclic inhibitors

Entry	Inhibitor	K_i (nM)	IC_{50}(μM)[a,b]
1.	 **8a** (*E/Z* ratio 1:1)	0.2	0.21
2.	 **24a**	0.25	nt
3.	 **8b** (*E/Z* ratio 3:1)	0.5	0.28
4.	 **24b**	2.3	0.31
5.	 **8c** (*E/Z* ratio 5:1)	10	0.42
6.	 **24c**	4	0.80
7.	 **8d** (*E* only)	4	nt
8.	 **24d**	20	nt

[a] Human T-lymphoid (MT-2) cells were exposed to 100 $TCID_{50}$ values of HIV-1_{LAI} and cultured in the presence of each PI, and IC_{50} values were determined using the MTT assay. Darunavir exhibited K_i = 16 pM, IC_{50} = 0.003 μM. [b]nt = not tested.

values were further evaluated in an antiviral assay. Antiviral activity was determined based upon previously published assay protocol.[35] As can be seen, acyclic inhibitors in Table 1 displayed enzyme inhibitory potency ranging from 5 nM to 45 nM. Acyclic ring opened product with P1-tyrosine side chain (compound **23**) showed enhanced potency (K_i = 0.41 nM). In general, inhibitors with longer chain showed reduction in potency. Interestingly, conversion of acyclic inhibitors to their corresponding cyclic derivatives after RCM resulted in significant improvement in enzyme inhibitory activity. As shown in Table 2, acyclic inhibitor **7a** (K_i = 5 nM and antiviral IC_{50} > 1μM) upon RCM provided 16-membered macrocycles **8a** (*E/Z*=1:1) which showed a K_i value of 0.2 nM. The mixture of isomers also displayed antiviral IC_{50} value of 0.21μM in MT-2 cells. Saturation of double bonds provided saturated inhibitor **24a** which showed comparable enzyme inhibitory activity. Acyclic inhibitor **7b** (K_i = 7 nM) following RCM afforded cyclic inhibitor **8b** (*E/Z* = 3:1) which also showed improvement of enzyme K_i over its acyclic derivatives. The 17-membered macrocycles showed comparable antiviral activity to 16-membered inhibitors. The corresponding saturated inhibitor **24b** displayed reduction in inhibitory potency (K_i = 2.3 nM). However, this compound maintained similar antiviral activity as its unsaturated mixtures.

Acyclic inhibitor **7c** upon cyclization provided cyclic inhibitor **8c** (*E/Z* = 5:1) which displayed K_i of 10 nM and antiviral activity of 420 nM. Interestingly, the corresponding saturated derivative **24c** showed improvement in enzyme inhibitory activity. The antiviral activity of inhibitor **24c** was reduced nearly a factor of 2 over its unsaturated derivative. Acyclic inhibitor **7d** (K_i = 30 nM) was subjected to RCM and provided cyclic derivative **8d** as a single *E*-isomer. This compound showed improvement in enzyme K_i over its acyclic derivative. Saturation of the double bond in **8d** provided saturated derivative **24d** which showed 5-fold reduction in inhibitory activity.

To gain insight into specific ligand-binding site interactions, an energy minimized model structure of inhibitor **24b** was created. The structure was modelled in the HIV-1 protease active site based upon our published X-ray crystal structure of inhibitor **1** and HIV-1 protease complex (Protein Data Bank entry 1S6G) as a template.[36] As can be seen in Figure 4, the 3-hydroxy group of the P2-ligand appeared to be within proximity to form hydrogen bond with Asp30 backbone NH as well as with the side chain carboxylic acid. The P2-carbonyl group appears to form effective hydrogen bonds with the tight-bound structural water molecule through one of the P2'-sulfonamide oxygens. The macrocyclic carbon chain is nicely accommodated in the S1-S2 hydrophobic pockets. Interestingly, saturation of the ring olefin in **8b** resulted in a more flexible carbon chain which may have resulted in unfavorable van der Waals interactions in the S2-subsite. This may explain the reduction of enzyme inhibitory activity for compound **24b**. It appears that the 16-membered saturated macrocycle in **24a** makes more favorable interactions in the S2-subsite of the protease active site than the corresponding 17-membered macrocycle in compound **24b**. The OMe-group on the P2'-sulfonamide of inhibitor **24b** appears to maintain hydrogen bonding interactions with Asp30' backbone NH as well as with the side chain carboxylic acid.

Conclusions

In summary, a novel series of macrocyclic HIV-1 protease inhibitors has been designed, synthesized and evaluated. The design of macrocycles is based upon the hypothesis that the cyclic flexible chain would effectively pack the hydrophobic pocket in the S1 to S2 subsites. We have synthesized acyclic derivatives involving P1-tyrosine and P2-3-hydroxy-2-alkenylbenzamide derivatives. Ring-closing metathesis using Grubbs' 1st generation catalyst efficiently provided 16-19 membered macrocyclic rings containing both *E/Z* olefins as the P1-P2 ligands in these inhibitors. Catalytic hydrogenation provided the corresponding saturated derivatives. In general, all cyclic inhibitors containing *E/Z* olefins showed improved enzyme inhibitory potency compared to their acyclic counterparts. The saturated derivatives are less potent than the

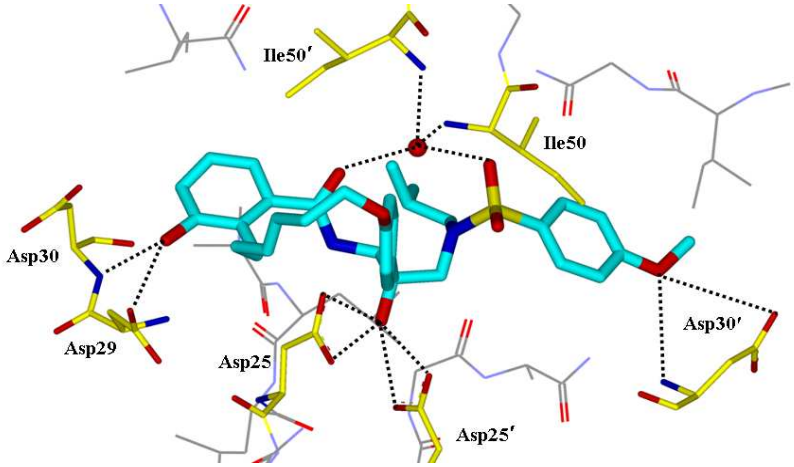


Figure 4. An energy-minimized model of 17-membered macrocyclic inhibitor **24b** in the HIV-1 protease active site. Putative hydrogen bonds are shown as dotted lines.

corresponding unsaturated derivatives. Compound **8a** showed the best enzyme inhibitory and antiviral activity in this series. A model of the *E*-isomer of **8b** was created to obtain ligand-binding site interactions. As it appears, the unsaturated macrocyclic ring is nicely accommodated in the S1-S2 hydrophobic pocket. Saturation of the ring olefin most likely resulted in unfavorable Van der Waals interaction in the S2-subsite. This may explain the reduction of enzyme inhibitory activity for the saturated compound **24b**. One of the important features of these macrocyclic inhibitors is that the inhibitors contain only two asymmetric centers and can be synthesized efficiently using RCM reaction. Further design and optimization of these inhibitors are in progress.

Experimental Section

All reactions were carried out under an inert atmosphere, either N_2 or Ar, using magnetic stirring and oven-dried glassware. All solvents were anhydrous and distilled prior to use. Dichloromethane and triethylamine were distilled from calcium hydride. Tetrahydrofuran, diethyl ether, and benzene were distilled from sodium/benzophenone. All other solvents were HPLC grade or better. Flash column chromatography was performed using EM Science 60-200 mesh silica gel. Thin-layer chromatography was performed using 60 F-254 E. Merck silica gel plates. ^{1}H- and ^{13}C-NMR were recorded using Bruker AV-500, Avance DRX-500, Varian Mercury-Vx-300, and Gemini-2300 spectrometers and use Me_4Si as an internal standard . Infrared spectra were recorded on a ATI Mattson Genesis Series FT-IR. Optical rotations were recorded on a Perkin-Elmer 341 polarimeter. A Thermo Finnigan LCQ Classic mass was used for MS analyses.

(1*S*,2*R*)-2-[1-Azido-2-(4-benzyloxyphenyl)-ethyl]-oxirane (10). To a suspension of magnesium turnings (101.6mg, 4.2 mmol) in 25 mL THF was added a solution of 4-Benzyloxybromobenzene (1.0 g, 3.8 mmol) in THF. The mixture was heated at 65 ^{o}C for 30 mins and the solution was cooled to room temperature. The Grignard solution was added dropwise to a solution of CuCN (26 mg, 0.30 mmol) and butadiene monoxide **9** (220 mg, 3.1 mmol) in 80 mL of anhydrous THF at -78 ^{o}C. The reaction was stirred for 30 min at -78 ^{o}C, after which it was quenched with 15 mL of saturated NH_4Cl followed by 15 mL of NH_4OH and 15 mL saturated NH_4Cl. The aqueous layer was extracted twice with ethyl acetate. The combined organic phases were dried over sodium sulfate and concentrated in vacuo. The residue was purified by flash chromatography on silica gel with 15-20% ethyl acetate/hexanes to give *trans*-4-(4-benzyloxy)phenyl-2-buten-1-ol (308 mg, 1.21 mmol, 39% yield). ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.44 (d, 2H, *J* = 7.0 Hz), 7.39 (dt, 2H, *J* = 2.0, 7.0 Hz), 7.33 (tt, 1H, *J* = 2.5, 7.5 Hz), 7.11 (td, 2H, *J* = 2.5, 9.0 Hz), 6.92 (td, 2H, *J* = 2.0, 8.5 Hz), 5.84 (dtt, 1H, *J* =1.5, 6.5, 15.0 Hz), 5.69 (dtt, 1H, j=1.5, 6.0, 15.5 Hz), 5.05 (s, 2H), 4.12 (dd, 2H, *J* =1.0, 55Hz), 3.34 (d, 2H, *J* =7.0 Hz), 1.48(s, 1H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 157.3, 137.2, 132.4, 132.0, 130.0, 129.6, 128.6, 128.0, 127.5, 114.9, 70.1, 63.6, 37.8

Molecular sieves (500 mg) were flame dried in a flask and then allowed to cool to room temperature. Dry dichloromethane (4 mL) and D-DET (11 mg, 0.05 mmol) were added and the suspension was cooled to -25 °C. To this, Ti(O*i*-Pr)$_4$ (20.7 μL, 0.07 mmol)) and TBHP (0.35 mL, 1.92 mmol) were added and the mixture was stirred at -25 °C for 30 minutes. A solution of (*E*)-4-(4-(benzyloxy)phenyl)but-2-en-1-ol (211 mg, 0.87 mmol) in dry DCM (1 mL) was added to the above mixture and it was kept in a freezer at about -25 °C for 18 h. To the reaction mixture H_2O (3 mL) was added and stirred at 0 °C for 30 minutes. A 10% aqueous NaOH was added and the mixture was warmed to room temperature for 1 h. The product was extracted with DCM (3x), dried with $NaSO_4$, filtered, and concentrated under reduced pressure. Purification by flash column chromatography (30-40% EtOAc/Hexanes) afforded epoxy alcohol **10** (177 mg, 79%). $[\alpha]_D^{23}$ +12.5° (c 0.14 $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.45 (d, 2H, *J* = 7.0 Hz), 7.41, (t, 2H *J* = 5.5 Hz), 7.34 (t, 1H, *J* = 7.5 Hz), 7.17 (td, 2H, *J* = 2.0, 8.5 Hz), 6.95 (td, 2H, *J* = 2.0, 8.5 Hz), 5.06 (s, 2H), 3.88 (dd, 1H, *J* = 2.5, 12.5 Hz), 3.60 (dd, 1H, *J* = 4.5, 12.5 Hz), 3.17 (dt, 1H, *J* = 2.5, 5.5 Hz), 2.98 (m, 1H), 2.86 (m, 2H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 157.7, 137.1, 130.1, 129.3, 128.6, 128.0, 127.5, 115.0, 70.1, 61.6, 58.4, 56.2, 37.0.

(1*S*,2*R*)-2-[1-Azido-2-(4-benzyloxyphenyl)-ethyl]-oxirane (11). Dry benzene (5 mL), Ti(O*i*-Pr)$_4$ (160 μL), and $TMSN_3$ (72 μL) were refluxed for 5 hours. A solution of epoxy alcohol **50** (0.123 g, 0.455 mmol) in dry benzene (3 mL) was added and the solution was refluxed for 30 minutes. After cooling to room temperature, 5% H_2SO_4 (2 mL) was added and the solution was stirred at room temperature for 1 hour. The layers were separated and the aqueous portion was extracted with ethyl acetate (3x). The organic solution was dried with Na_2SO_4, filtered, and concentrated under reduced pressure. Purification by flash column chromatography (40-50% EtOAc/Hexanes) gave (2*R*,3*S*)-3-Azido-4-(4-benzyloxyphenyl)-1,2-butanediol (78.9 mg, 55% yield). $[\alpha]_D^{23}$ +18.1° (c 0.19 $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.44 (d, 2H, *J* = 7.0 Hz), 7.39 (t, 2H, *J* = 7.5 Hz), 7.34 (t, 1H, *J* = 7.0 Hz), 7.19 (d, 2H, *J* = 8.5 Hz), 6.95 (td, 2H, *J* = 2.0, 8.5 Hz), 5.05 (s, 2H), 3.80-3.67 (m, 4H), 3.26 (bs, 1H), 2.99 (dd, 1H, *J* = 3.0, 14.0 Hz), 2.77-2.73 (m, 2H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 157.8, 137.0, 130.4, 129.5, 128.6, 128.0, 127.6, 115.1, 73.1, 70.1, 65.7, 63.3, 36.2; HRMS *m/z* $(M + Na)^+$ calc'd for $C_{17}H_{19}N_3O_3Na$ 336.1324, found 336.1317.

(2R,3S)-3-Azido-4-(4-benzyloxyphenyl)-1,2-butanediol (84.4 mg, 0.269 mmol) and chloroform (3 mL) were cooled to 0 °C. 1-Chlorocarbonyl-1-methylethyl acetate (58.5 μL, 0.404 mmol) was added and the solution was stirred at room temperature for 20 hours. A saturated solution of $NaHCO_{3(aq)}$ was added and the mixture stirred for 15 minutes. The crude chloroacetate was extracted with chloroform , dried with Na_2SO_4, filtered, and concentrated in vacuo. The residue was dissolved in dry THF (5 mL) and cooled to 0 °C. Solid NaOMe (24.7 mg) was added and the reaction was stirred at room temperature for 2 hours. The reaction was quenched with saturated $NH_4Cl_{(aq)}$ and extracted with ethyl acetate. Drying over Na_2SO_4, filtering, and concentrating under reduced pressure afforded a residue which was purified by flash column chromatography (12% EtOAc/Hexanes) to afford pure azido epoxide **11** (56.5 mg, 71% yield). $[\alpha]_D^{23}$ +11.3° (c 0.34 $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.44 (d, 2H, *J* = 7.5 Hz), 7.40 (t, 2H, *J* = 8.0 Hz), 7.34 (t, 1H, *J* = 7.0 Hz), 7.17 (d, 2H, *J* = 11.0 Hz), 6.95 (td, 2H, *J* = 2.0, 11.5 Hz), 5.06 (s, 2H), 3.56 (quintet, 1H, *J* = 4.0, 5.0 Hz), 3.07-3.05 (m, 1H), 2.94 (dd, 1H, *J* = 4.5, 14.0 Hz), 2.84-2.82 (m, 1H), 2.81-2.75 (m, 2H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 157.9, 137.0, 130.4, 128.9, 128.6, 128.0, 127.5, 115.0, 70.1, 63.8, 53.0, 45.2, 37.4.

(2*R*,3*S*)-N-[3-Azido-4-(4-benzyloxyphenyl)-2-hydroxy-butyl]-N-isobutyl-4-methoxy-benzenesulfonamide (12). To epoxide **11** (0.60 g, 2.03 mmol) and isopropanol (10 mL), was added isobutylamine (2 mL) and the solution was refluxed for 2 hours. The reaction was concentrated in vacuo and used as is in the next reaction. The residue was taken up in DCM (10 mL), saturated aqueous $NaHCO_3$ (2 mL) and 4-methoxybenzenesulfonyl chloride (0.50 g) were added and the reaction was stirred for 18 hours at room temperature. The organic layer was separated and the aqueous portion was extracted with DCM. The combined organics were dried with Na_2SO_4, filtered, and concentrated under reduced pressure. Flash column chromatography (15-25% EtOAc/Hexanes) afforded sulfonamide **12** (1.06 g, 97% yield over 2 steps). ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.75 (dd, 2H, *J* = 2.0, 9.0 Hz), 7.45-7.43 (m, 2H), 7.39 (t, 2H, *J* = 7.5 Hz), 7.34-7.33 (m, 1H), 7.19 (d, 2H, *J* = 9.0 Hz), 7.01 (dd, 2H, *J* = 2.0, 9.0 Hz), 6.94 (dd, 2H, *J* = 2.0, 8.5 Hz), 5.06 (s, 2H), 3.83 (s, 3H), 3.78-3.75 (m, 1H), 3.59-3.55 (m, 2H), 3.24 (dd, 1H, *J* = 9.5, 15.5 Hz), 3.09-3.02 (m, 3H), 2.80 (dd, 1H, *J* = 6.5, 13.5 Hz), 2.75 (dd, 1H, *J* = 9.0, 14.0 Hz), 1.71-1.69 (m, 1H), 0.94 (d, 3H, *J* = 6.5 Hz), 0.88 (d, 3H, *J* = 6.5 Hz); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 163.2, 157.8, 137.0, 130.4, 129.7, 129.5, 128.6, 128.0, 127.5, 115.1, 114.5, 71.8, 70.0, 66.7, 58.9, 55.7, 52.9, 36.0, 27.2, 20.2, 19.8; HRMS *m/z* (M + Na)$^+$ calc'd for $C_{28}H_{34}N_4O_5SNa$ 561.2148, found 561.2166.

(1*S*,2*R*)-{1-(4-Allyloxy-benzyl)-2-hydroxy-3-[isobutyl-(4-methoxy-benzenesulfonyl)-amino]-propyl}-carbamic acid tert-butyl ester (13). Azide **12** (55.1 mg, 0.10 mmol) was dissolved in methanol (5 mL) and Boc_2O (27 mg) and 10% Pd/C (10 mg) were added and the reaction stirred under a H_2 balloon for 18 hours. The mixture was filtered through Celite and concentrated under reduced pressure. Flash column chromatography (30% EtOAc/Hexanes) afforded (1*S*,2*R*)-{2-Hydroxy-1-(4-hydroxybenzyl)-3-[isobutyl-(4-methoxy-benzenesulfonyl)-amino]-propyl}-carbamic acid tert-butyl ester (40 mg, 75% yield). ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.68 (d, 2H, *J* = 8.5 Hz), 7.06 (m, 2H), 6.96 (d, 2H, *J* = 9.0 Hz), 6.73-6.71 (m, 2H), 6.35-6.25 (bs, 1H), 4.78 (d, 1H, *J* = 8.0 Hz), 4.01-3.99 (m, 1H), 3.84 (s, 3H), 3.81-3.79 (m, 1H), 3.71-3.69 (m, 1H), 3.10-2.77 (m, 6H), 1.86-1.81 (m, 1H), 0.89-0.85 (m, 6H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 163.0, 156.3, 154.7, 130.6, 129.8, 129.5, 129.3, 115.4, 114.4, 80.0, 72.8, 58.6, 55.6, 54.9, 53.6, 34.6, 28.3, 27.2, 20.1, 19.9.

Above phenol derivative (13 mg, 0.02 mmol) was dissolved in acetone (2 mL), K_2CO_3 (5.2 mg) and allyl bromide (22 μL) were added, and the reaction was refluxed for 16 hours, then concentrated under reduced pressure. Flash column chromatography (25% EtOAc/Hexanes) afforded allylic ether **13** (13.4 mg, 96% yield). ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.69 (dd, 2H, *J* = 2.0, 6.5 Hz), 7.15 (d, 2H, *J* = 8.5 Hz), 6.97 (dd, 2H, *J* = 2.0, 7.0 Hz), 6.85 (dd, 2H, *J* = 2.0, 6.5 Hz), 6.08-6.02 (m, 1H), 5.41 (dd, 1H, *J* = 1.5, 17.5 Hz), 5.28 (dd, 1H, *J* = 1.0, 10.5 Hz), 4.62-4.61 (m, 1H), 4.51 (dt, 2H, *J* = 1.5, 5.5 Hz), 3.86 (s, 3H), 1.35 (s, 9H), 0.90 (d, 3H, *J* = 6.5 Hz), 0.86 (d, 3H, *J* = 6.5 Hz); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 163.0, 157.3, 156.1, 133.4, 130.5, 129.9, 129.5, 117.6, 114.8 114.6, 114.3, 79.7, 72.7, 68.8, 58.7, 55.6, 54.7, 53.8, 34.5, 28.3, 27.2, 20.2, 19.9.

(1*S*,2*R*)-{1-(4-(3-butenyloxy)-benzyl)-2-hydroxy-3-[isobutyl-(4-methoxy-benzenesulfonyl)-amino]-propyl}-carbamic acid tert-butyl ester (14). (1*S*,2*R*)-{2-Hydroxy-1-(4-hydroxybenzyl)-3-[isobutyl-(4-methoxy-benzenesulfonyl)-amino]-propyl}-carbamic acid tert-butyl ester (36.4 mg, 0.07 mmol) was dissolved in acetone (5 mL). To this was added K_2CO_3 (24 mg) and 4-bromo-1-butene (7 μL) and the reaction was refluxed for 18 hours. After concentrating under reduced pressure, the product was purified by flash column chromatography (25% EtOAc/Hexanes) to afford **14** (37 mg, 96% yield). ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.69 (dd, 2H, *J* = 2.0, 9.0 Hz), 7.14 (d, 2H, *J* = 8.5 Hz), 6.96 (dd, 2H, *J* = 2.0, 9.0 Hz), 6.83 (d, 2H, *J* = 9.0 Hz), 5.94-5.86 (m, 1H), 5.16 (ddd, 1H, *J* = 1.5, 2.0, 17.0 Hz), 5.10 (ddd, 1H, *J* = 1.0, 1.5, 10.0 Hz), 4.64 (d, 1H, *J* = 8.0 Hz), 3.98 (t, 2H, *J* = 7.0 Hz), 3.94-3.92 (m, 1H), 3.86 (s, 3H), 3.78-3.75 (m, 1H), 3.71-3.68 (m, 1H), 3.10-2.77 (m, 6H), 2.55-2.51 (m, 2H), 1.84-1.79 (m, 1H), 1.35 (s, 9H), 0.89 (d, 3H, *J* = 6.5 Hz), 0.85 (d, 3H, *J* = 6.5 Hz); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 163.0, 157.6, 156.1, 134.5, 130.5, 130.0, 129.8, 129.5, 117.0, 114.6, 114.3, 79.6, 72.7, 67.2, 58.6, 55.6, 54.7, 53.8, 34.5, 29.3, 28.3, 27.2, 20.2, 19.9.

(1*S*,2*R*)-2-Hydroxy-3-[isobutyl-(4-methoxy-benzenesulfonyl)-amino]-1-(4-(4-pentenyloxy)-benzyl)-propyl]-carbamic acid tert-butyl ester (15). (1*S*,2*R*)-{2-Hydroxy-1-(4-hydroxybenzyl)-3-[isobutyl-(4-methoxy-benzenesulfonyl)-amino]-propyl}-carbamic acid tert-butyl ester (54.8 mg, 0.105 mmol), K_2CO_3 (43.5 mg), and 5-bromo-1-pentene (62 μL) were added to acetone (3 mL). The mixture was refluxed for 18 hours, then concentrated under reduced pressure. Flash column chromatography (30% EtOAc/Hexanes) afforded olefin **15** (59.6 mg, 94% yield). ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.69 (d, 2H, *J* = 8.0 Hz), 7.14 (d, 2H, *J* = 8.5 Hz), 6.96 (dd, 2H, *J* = 2.0, 7.0 Hz), 6.82 (d, 2H, *J* = 8.5 Hz), 5.89-5.80 (m, 1H), 5.05 (ddd, 1H, *J* = 1.5, 2.0, 17.0 Hz), 4.99 (dd, 1H, *J* = 1.5, 10.0 Hz), 4.65 (d, 1H, *J* = 8.0 Hz), 3.94 (t, 2H, *J* = 6.5 Hz), 3.86 (s, 3H), 3.77 (m, 1H), 3.70 (m, 1H), 3.07-3.03 (m, 2H), 2.95-2.86 (m, 3H), 2.81-2.77 (m, 1H), 2.23 (q, 2H, *J* = 7.0 Hz), 1.90-1.82 (m, 3H), 1.35 (s, 9H), 0.90-0.86 (m, 6H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 163.0, 157.7, 156.1, 137.9, 130.5, 130.0, 129.6, 129.5, 115.2, 114.5, 114.3, 79.6, 72.7, 67.2, 58.6, 55.6, 54.7, 53.7, 34.5, 30.1, 28.5, 28.3, 27.2, 20.2, 19.9; HRMS *m/z* (M + Na)$^+$ calc'd for $C_{31}H_{46}N_2O_7SNa$ 613.2923, found 613.2906.

(1*S*,2*R*)-{1-(4-(5-hexenyloxy)-benzyl)-2-hydroxy-3-[isobutyl-(4-methoxy-benzenesulfonyl)-amino]-propyl}-carbamic acid tert-butyl ester (16). (1*S*,2*R*)-{2-Hydroxy-1-(4-hydroxybenzyl)-3-[isobutyl-(4-methoxy-benzenesulfonyl)-amino]-propyl}-carbamic acid tert-butyl ester (51.5 mg, 0.0985 mmol), K_2CO_3 (40.9 mg), and 6-bromo-1-hexene (66 μL) were added to acetone (3 mL). The mixture was refluxed for 16 hours, then concentrated under reduced pressure. Flash column chromatography (25% EtOAc/Hexanes) afforded olefin **16** (58.5 mg, 96% yield). ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.69 (ddt, 2H, *J* = 3.0, 8.5 Hz), 7.14 (d, 2H, *J* = 8.5 Hz), 6.96 (ddt, 2H, *J* = 2.5, 9.0 Hz), 6.82 (d, 2H, *J* = 8.5 Hz), 5.86-5.78 (m, 1H), 5.04 (ddd, 1H, *J* = 1.5, 2.0, 17.0 Hz), 4.96 (ddd, 1H, *J* = 1.0, 2.0, 10.5 Hz), 4.65 (d, 1H, *J* = 8.5 Hz), 3.93 (t, 2H, *J* = 6.5 Hz), 3.86 (s, 3H), 3.77 (m, 1H), 3.70 (m, 1H), 3.07-3.02 (m, 2H), 2.93-2.86 (m, 3H), 2.81-2.78 (m, 1H), 2.12 (q, 2H, *J* = 7.0 Hz), 1.85-1.76 (m, 3H), 1.59-1.53 (m, 2H), 1.35 (s, 9H), 0.89 (d, 3H, *J* = 6.5 Hz), 0.86 (d, 3H, *J* = 6.5 Hz); ^{13}C-NMR (125 MHz, CDCl3) δ 163.0, 157.8, 156.1, 138.6, 130.5, 130.0, 129.6, 129.5, 114.8, 114.5, 114.3, 79.6, 72.7, 67.8, 58.6, 55.6, 54.7, 53.7, 34.5, 33.5, 28.8, 28.3, 27.2, 25.3, 20.2, 19.9; HRMS

m/z (M + Na)$^+$ calc'd for $C_{32}H_{48}N_2O_7S$ 627.3080, found 627.3087.

2-(Triphenylphosphoranylidene)-succinic acid 1-ethyl ester (18). To a solution of acetone (100 mL) and PPh_3 (28.8 g, 0.11 mol) was added a solution of maleic anhydride (**17**, 10.8 g, 0.11 mol) in acetone (100 mL). After stirring at room temperature for 1 hour, the suspension was cooled to 0 °C. The product was isolated by filtering through a scintered glass funnel and washing with cold acetone. The product was then dried under vacuum. To the resulting solid, EtOH (200 mL) was added and the solution was stirred for 2 days after which the mixture was concentrated under reduced pressure. The carboxylic acid was recrystallized from 1:1 EtOAc/Hexanes to afford **18** (15.8 g, 35% over 2 steps) as a tan solid. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.63-7.59 (m, 9H), 7.51-7.47 (m, 6H), 3.80 (q, 2H, *J* = 7.0 Hz), 2.83 (d, 2H, *J* = 14.5 Hz), 0.75 (t, 3H, *J* = 7.0 Hz); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 172.8, 172.7, 170.3, 133.8, 133.7, 133.7, 129.5, 129.4, 122.6, 121.8, 61.1, 39.6, 39.0, 35.2, 13.8.

3-Trimethylsilanyl-propenal (19). Magnesium turnings (24.35 g, 1.00 mol were added to dry THF (500 mL). Ethyl bromide (75 mL, 1.00 mmol was slowly added dropwise by addition funnel over 1.5 hours. The mixture was heated at 50 °C for 1 hour, then cooled to 0 °C. Propargyl alcohol (20.8 mL, 0.36 mol) in dry THF (21 mL) was then added over 1 hour, and the mixture was stirred at room temperature overnight. The mixture was cooled to 0 °C and TMSCl (127 mL, 1.91 mol) was slowly added, followed by refluxing for 2 hours. After cooling to room temperature, 1.4 M H_2SO_4 (400 mL) was slowly added and the reaction was stirred for 10 minutes. The reaction was extracted with diethyl ether, washed with water, brine, dried with Na_2SO_4, and concentrated under reduced pressure. Short-path distillation afforded 3-trimethylsilanyl-1-hydroxy-2-propyne (39.9 g, 87% yield). ^{1}H-NMR (500 MHz, $CDCl_3$) δ 4.27 (s, 2H), 1.61 (s, 1H), 0.18 (s, 9H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 130.8, 90.8, 51.7, -0.2.

Dry diethyl ether (10 mL) and Red-Al (6.2 mL, 21.8 mmol, 3.5 M in toluene) were cooled to 0 °C. 3-trimethylsilanyl-1-hydroxy-2-propyne (1.86 g, 14.5 mmol) and dry ether (15 mL) were added dropwise and the solution was stirred at room temperature for 2 hours. The reaction was cooled to 0 °C and 3.6 M H_2SO_4 (15 mL) was added. After extraction with diethyl ether (3x), drying with Na_2SO_4, filtering, and concentrating under reduced pressure, the residue was purified by flash column chromatography (2-15% EtOAc/Hexanes) to give 3-trimethylsilanyl-1-hydroxy-2-propene (1.19 g, 63% yield).

To 3-trimethylsilanyl-1-hydroxy-2-propene (0.11 g, 0.84 mmol) and dry DCM (5 mL) was added MnO_2 (0.51 g, 5.87 mmol) and the reaction was stirred for 30 minutes at room temperature and 3 hours at reflux. After cooling to room temperature, 2 additional equivalents of MnO_2 were added and the mixture was stirred at room temperature for 16 hours. Silica gel was added, the mixture was filtered through a silica gel plug washing with DCM, and concentrated under reduced pressure to provide conjugated aldehyde **19** (102 mg, 95%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 9.49 (d, 1H *J* = 7.5 Hz), 7.19 (d, 1H, *J* = 18.9 Hz), 6.50 (dd, 1H, *J* = 7.5, 20.7 Hz), 0.17 (s, 9H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ 194.7, 158.7, 144.0, -2.0.

(3*E*,5*E*)-3-ethoxycarbonyl-6-trimethylsilyl-hexadienoic acid (20). To a solution of aldehyde **19** (1.2 g, 7.8 mmol) in toluene (50 mL) was added Wittig reagent **18** (3.8 g, 9.4 mmol) and hydroquinone (8.6 mg) and the reaction was stirred for 2 days at room temperature. After concentrating the reaction under reduced pressure, flash column chromatography (0-20% EtOAc/Hexanes) afforded diene **20** (1.98 g, 83% yield). FT-IR (film) 1711, 1628, 1414, 1373, 1285, 1249, 1206, 1076, 990, 858, 841 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.30 (d, 1H, *J* = 11.0 Hz), 6.72 (ddd, 1H, *J* = 1.0, 10.0, 18.0 Hz), 6.45 (d, 1H, *J* = 18.0 Hz), 4.23 (q, 2H, 7.0 Hz), 3.52 (s, 2H), 1.29 (dt, 3H, *J* = 1.0, 7.0 Hz), 0.12 (d, 9H, 1.0 Hz); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 176.8, 167.5, 147.2, 143.0, 137.3, 123.5, 61.2, 32.5, 14.2, -1.6.

Ethyl-2-(3-butenyl)-3-hydroxybenzoate (21). Diisopropylamine (2.41 mL, 17.19 mmol) and dry THF (50 mL) were cooled to 0 °C. To this, *n*-Buli (11.5 mL, 18.4 mmol, 1.6 M in hexane) was added and the solution was stirred for 30 minutes at 0 °C. After adding DMPU (3.86 mL), acid **20** (2.05 g) in dry THF (10 mL) was added and the reaction was stirred at 0 °C for 1 hour. After cooling the mixture to -78° C, 4-bromo-1-butene (0.89 mL, 8.8 mmol) was added and the reaction was stirred for 1 hour, followed by 2 hours at 0 °C. The reaction was quenched with 5% $HCl_{(aq)}$, extracted with diethyl ether, dried with Na_2SO_4, filtered, and concentrated under reduced pressure. Filtration through a 10g silica gel column eluting with 15% EtOAc/Hexanes (100mL) afforded the crude tris-olefin, which was used as is in the next step. Dry THF (10 mL) was added to the crude olefin (0.42 g, 1.35 mmol), followed by TFAA (0.38 mL) and TEA (0.57 mL). The reaction was stirred at room temperature for 2 hours. The reaction was quenched with 5% $HCl_{(aq)}$ and extracted with diethyl ether. After drying with Na_2SO_4, filtration, and concentrating under reduced pressure, the residue was taken up in absolute ethanol (10 mL). The reaction was cooled to 0 °C and $NaBH_4$ (0.11 g, 2.71 mmol) was added. The mixture was stirred at room temperature for 2 hours, after which it was quenched with 5% $HCl_{(aq)}$. The crude product was extracted with diethyl ether, dried with Na_2SO_4, filtered, and concentrated in vacuo. Flash column chromatography (10% EtOAc/Hexanes) afforded aromatic ester **21** (0.85 g, 48% yield, 2 steps). ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.40 (d, 1H, *J* = 1.0, 8.0 Hz), 7.11 (t, 1H, *J* = 8.0 Hz), 6.93 (dd, 1H, *J* = 1.0, 8.0 Hz), 5.98-5.90 (m, 1H), 5.31 (bs, 1H), 5.09 (ddd, 1H, *J* = 1.5, 3.5, 17.5 Hz), 4.99 (dd, 1H, *J* = 1.0, 10.0 Hz), 4.36 (q, 2H, *J* = 7.5 Hz), 3.01 (dd, 2H, *J* = 7.5, 9.5 Hz), 2.39-2.35 (m, 2H), 1.39 (t, 3H, *J* = 7.0 Hz); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 168.2, 154.2, 138.5, 132.2, 129.2, 126.6, 122.7, 118.7, 115.0, 61.1, 34.0, 26.5, 14.3.

2-(3-butenyl)-3-hydroxybenzoic acid (22). Ester **21** (23.7 mg, 0.11 mmol) was taken up in MeOH (3 mL) and 2N $KOH_{(aq)}$ (3 mL) was added. The reaction was refluxed for 36 hours. The MeOH was removed under reduced pressure and the aqueous mixture was acidified to pH = 2 with 6N $HCl_{(aq)}$. Crude acid was extracted with EtOAc, dried with Na_2SO_4, filtered, and concentrated under reduced pressure. Flash column chromatography (20-50% EtOAc/Hexanes) afforded pure acid **22** (18.5 mg, 89% yield). ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.61 (d, 1H, *J* = 8.0 Hz), 7.17 (t, 1H, *J* = 8.0 Hz), 7.01 (d, 1H, *J* = 8.0 Hz), 5.96 (m, 1H), 5.09 (dd, 1H, *J* = 1.5, 17.0 Hz), 5.01 (dd, 1H, *J* = 1.0, 10.0 Hz), 3.12 (t, 2H, *J* = 7.5

Hz), 2.39 (dd, 2H, *J* = 7.5, 15.5 Hz); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 173.1, 154.2, 138.5, 130.6, 130.2, 126.7, 124.0, 119.9, 115.2, 34.0, 26.4. HRMS *m/z* (M - H)$^+$ calc'd for $C_{11}H_{12}O_3$ 191.0708, found 191.0706.

(1*S*,2*R*)-N-{1-(4-Allyloxy-benzyl)-2-hydroxy-3-[isobutyl-(4-methoxy-benzenesulfonyl)-amino]-propyl}-2-(3-butenyl)-3-hydroxy-benzamide (7a). Boc-amine **13** (8.1 mg, 0.014 mmol) was dissolved in DCM (1 mL) and stirred with 30% TFA/DCM (2 mL) for 2 hours at room temperature. After concentration under reduced pressure, the residue was taken up in DMF (2 mL). To the solution HOBt (2.1 mg), EDCI (3 mg), acid **22** (3 mg), and TEA (3 μL) were added. The reaction was stirred for 18 hours, and then brine was added. The crude product was extracted with ethyl acetate, dried with Na_2SO_4, filtered, and concentrated. Flash column chromatography (30-40% EtOAc/Hexanes) afforded diene **7a** (6.4 mg, 70% yield over 2 steps). ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.68 (dd, 2H, *J* = 2.0, 7.0 Hz), 7.19 (d, 2H, *J* = 9.0 Hz), 6.98-6.95 (m, 2H), 6.92 (d, 1H, *J* = 8.0 Hz), 6.85 (d, 2H, *J* = 8.5 Hz), 6.75 (d, 1H, *J* = 8.0 Hz), 6.54 (d, 1H, *J* = 7.5 Hz), 6.28 (bs, 1H), 6.10-6.08 (m, 2H), 5.74-5.72 (m, 1H), 5.40 (dd, 1H, *J* = 1.5, 17.0 Hz), 5.28 (dd, 1H, *J* = 1.5, 10.5 Hz), 4.93 (dd, 1H, *J* = 1.5, 17.0 Hz), 4.88 (d, 1H, *J* = 10.0 Hz), 4.50 (d, 2H), 450-4.49 (m, 2H), 4.13-4.11 (m, 1H), 3.86 (s, 3H), 3.15-3.13 (m, 1H), 3.09-3.04 (m, 2H), 2.96-2.92 (m, 2H), 2.84-2.82 (m, 1H), 2.55-2.49 (m, 2H), 2.19-2.18 (m, 2H), 2.05-2.03 (m, 1H), 0.92 (d, 3H, *J* = 6.5 Hz), 0.87 (d, 3H, *J* = 6.5 Hz); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 170.6, 163.1, 157.4, 154.5, 138.4, 137.6, 133.3, 130.3, 129.8, 129.7, 129.4, 126.9, 126.4, 118.7, 117.7, 117.1, 114.9, 114.9, 114.4, 73.1, 68.8, 58.9, 55.7, 54.3, 53.6, 33.9, 27.4, 26.5, 20.2, 19.9.

(1*S*,2*R*)-2-(3-butenyl)-N-{1-(4-(3-butenyloxy)-benzyl)-2-hydroxy-3-[isobutyl-(4-methoxy-benzenesulfonyl)-amino]-propyl}-3-hydroxy-benzamide (7b). Boc-protected amine **13** (0.15 g) and 30% TFA/DCM (2 mL) were stirred at room temperature for 2 hours, then concentrated under vacuum. The residue was taken up in DMF (2 mL) and acid **22** (48.8 mg), EDCI (53.5 mg), HOBt (37.7 mg), and TEA (53 μL) were added. The solution was stirred at room temperature for 16 hours. Brine was added and the reaction was extracted with EtOAc, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Flash column chromatography (30-50% EtOAc/Hexanes) afforded diene **7b** (0.1295 g, 78% yield over 2 steps). ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.66 (d, 2H, *J* = 9.0 Hz), 7.17 (d, 2H, *J* = 8.5 Hz), 6.94 (d, 2H, *J* = 9.0 Hz), 6.88 (t, 1H, *J* = 7.5 Hz), 6.82 (d, 2H, *J* = 8.5 Hz), 6.75 (d, 1H, 7.5 Hz), 6.50 (d, 1H, *J* = 7.5 Hz), 6.19 (d, 1H, *J* = 8.5 Hz), 5.93-5.84 (m, 1H), 5.75-5.67 (m, 1H), 5.15 (ddd, 1H, *J* = 1.5, 2.0, 15.5 Hz), 5.09 (dd, 1H, *J* = 1.5 Hz, 10.5), 4.89 (dd, 1H, *J* = 1.5, 17.5 Hz), 4.83 (d, 1H, *J* = 10.0 Hz), 4.33-4.28 (m, 1H), 3.99-3.95 (m, 3H), 3.83 (s, 3H), 3.18-3.04 (m, 3H), 2.98-2.85 (m, 3H), 2.61-2.46 (m, 4H), 2.17 (q, 2H, *J* = 7.0, 7.5 Hz), 1.91-1.83 (m, 1H), 0.90 (d, 3H, *J* = 6.5 Hz), 0.86 (d, 3H, *J* = 6.5 Hz); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 170.9, 163.1, 162.9, 157.7, 155.0, 138.6, 137.4, 134.5, 130.3, 129.7, 129.7, 129.4, 126.8, 126.5, 118.2, 117.1, 114.8, 114.6, 114.4, 73.0, 67.2, 58.8, 55.7, 54.3, 53.6, 33.9, 33.7, 31.7, 27.3, 26.5, 20.2, 19.9; HRMS *m/z* (M + H)$^+$ calc'd for $C_{36}H_{47}N_2O_7S$ 651.3104, found 651.3098.

(1*S*,2*R*)-2-(3-butenyl)-3-hydroxy-N-[2-hydroxy-3-[isobutyl-(4-methoxy-benzenesulfonyl)-amino]-1-(4-(4-pentenyloxy)-benzyl)-propyl]-benzamide (7c). Boc-amine **14** (40.4 mg, 0.0668 mmol) and 30% TFA/DCM (4 mL) were stirred for 3 hours at room temperature, then concentrated in vacuo. The residue was taken up in DMF (2 mL) and acid **22** (12.8 mg), HOBt (9.0 mg), EDCI (12.8 mg), and TEA were stirred for 18 hours at room temperature. Brine was added and the product was extracted with ethyl acetate. After drying with Na_2SO_4, filtering, and concentrating under reduced pressure, the product was purified by flash column chromatography (30% EtOAc/Hexanes) to afford diene **7c** (26.6 mg, 60% over 2 steps). ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.69 (d, 2H, *J* = 8.0 Hz), 7.19 (d, 2H, *J* = 8.5 Hz), 6.98-6.95 (m, 3H), 6.83 (d, 2H. *J* = 8.5 Hz), 6.77 (dd, 1H, *J* = 1.0, 8.0 Hz), 6.57 (dd, 1H, *J* = 1.0, 7.5 Hz), 6.02 (d, 1H, 8.5 Hz), 5.89-5.80 (m, 1H), 5.78-5.72 (m, 1H), 5.65 (bs, 1H), 5.06 (ddd, 1H, *J* = 1.5, 2.0, 17.0 Hz), 5.00 (dd, 1H, *J* = 1.5, 10.0 Hz), 4.95 (dd, 1H, *J* = 1.5, 17.0 Hz), 4.90 (d, 1H), 4.33-4.29 (m, 1H), 4.24 (bs, 1H), 3.99-3.96 (m, 1H), 3.94 (t, 2H, *J* = 6.5 Hz), 3.87 (s, 3H), 3.15 (d, 1H, *J* = 8.5 Hz), 3.10-3.04 (m, 2H), 2.99-2.90 (m, 2H), 2.85-2.81 (m, 1H), 2.62-2.51 (m, 2H), 2.26-2.20 (m, 4H), 1.91-1.85 (m, 3H), 1.68-1.61 (m, 1H), 0.93 (d, 3H, *J* = 6.5 Hz), 0.88 (d, 3H, *J* = 6.5 Hz); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 170.5, 163.1, 157.9, 154.3, 138.4, 137.8, 137.7, 130.3, 129.8, 129.4, 127.0, 126.3, 118.9, 117.1, 115.2, 115.0, 114.7, 114.4, 73.0, 67.2, 59.0, 55.7, 54.3, 53.7, 34.0, 30.1, 28.5, 27.4, 26.5, 20.2, 19.9; HRMS *m/z* (M + Na)$^+$ calc'd for $C_{37}H_{48}N_2O_7SNa$ 687.3080, found 637.3086.

(1*S*,2*R*)-2-(3-butenyl)-N-{1-(4-(5-hexenyloxy)-benzyl)-2-hydroxy-3-[isobutyl-(4-methoxy-benzenesulfonyl)-amino]-propyl}-3-hydroxy-benzamide (7d). Boc-amine **16** (37.8 mg, 0.061 mmol) and 30% TFA/DCM (5 mL) were stirred at room temperature for 6 hours, then concentrated under reduced pressure. The residue was taken up in DMF (2 mL) and acid **22** (11.7 mg), EDCI (11.7 mg), HOBt (8.3 mg), and TEA (12.8 μL) were added and the reaction stirred at room temperature for 18 hours. Brine was added to the solution, and the product was extracted with ethyl acetate. After drying with Na_2SO_4, filtering, and concentrating under reduced pressure, the product was purified by flash column chromatography (25% EtOAc/Hexanes) to afford diene **7d** (25.7 mg, 62% yield for 2 steps). ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.69 (d, 2H, *J* = 9.0 Hz), 7.19 (d, 2H, *J* = 8.5 Hz), 6.99-6.95 (m, 3H), 6.83 (d, 2H, *J* = 8.5 Hz), 6.77 (dd, 1H, *J* = 1.0, 8.0 Hz), 5.77 (dd, 1H, *J* = 1.0, 7.5 Hz), 6.04 (d, 1H, *J* = 9.0 Hz), 5.87-5.71 (m, 3H), 5.05 (ddd, 1H, *J* = 1.5, 2.0, 17.0 Hz), 4.98-4.93 (m, 2H), 4.99 (d, 1H, *J* = 10.0 Hz), 4.33-4.29 (m, 1H), 4.26-4.25 (bs, 1H), 3.99-3.96 (m, 1H), 3.93 (t, 2H, *J* = 6.5 Hz), 3.86 (s, 3H), 3.19-3.13 (m, 1H), 3.09-3.03 (m, 2H), 3.00-2.90 (m, 2H), 2.84-2.80 (m, 1H), 2.63-2.49 (m, 2H), 2.22-2.18 (m, 2H), 2.12 (q, 2H, *J* = 7.0 Hz), 1.91-1.85 (m, 1H), 1.82-1.76 (m, 2H), 1.60-1.53 (m, 2H), 0.93 (d, 3H, *J* = 6.5 Hz), 0.88 (d, 3H, *J* = 6.5 Hz); ^{13}C-NMR (125 MHz, CDCl3) δ 170.5, 1631, 157.9, 154.3, 138.5, 138.4, 137.7, 130.3, 129.8, 129.4, 127.0, 126.4, 118.9, 117.1, 115.0, 114.9, 114.8, 114.7, 114.4, 73.0, 67.8, 59.0, 55.7, 54.3, 53.7, 34.0, 33.5, 29.7, 28.8, 27.4, 26.5, 25.4, 20.2, 19.9; HRMS *m/z* (M + Na)$^+$ calc'd for $C_{38}H_{50}N_2O_7SNa$ 701.3236, found 701.3217.

(2'*R*,3''*S*)-N-[2'-Hydroxy-2-(10-hydroxy-5-oxo-17-oxa-4-aza-tricyclo[16.2.2.06,11]docosa-1(21),6,8,10,14,18(22),19-heptaen-3''-yl)-ethyl]-N-isobutyl-4-methoxy-benzenesulfonamide (8a). Diene **7a** (12 mg, 0.02 mmol) was dissolved in dry DCM (5 mL). First generation Grubbs' catalyst (2 mg) was added and the reaction was stirred for 4 h.

The solution was concentrated and purified by flash column chromatography (35% EtOAc/hexanes) to afford olefin **8a** (9.6 mg, 83 % yield) as an inseparable 1:1 mixture of cis/trans isomers. Mixture of diastereomers. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.76-7.73 (m, 4H), 7.02-6.87 (m, 12 H) 6.76-6.69 (m, 4 H), 5.86 (dd, 2H, *J* = 9.5, 21.5 Hz), 5.56-5.54 (m, 2H), 5.41-5.36 (m, 2H), 5.30-5.25 (m, 2H), 4.55-4.51 (m, 2H), 4.44-4.35 (m, 4H), 3.97-3.95 (m, 2H), 2.91-2.85 (m, 2H), 2.69-2.57 (m, 4H), 1.94-1.90 (m, 2H), 0.98 (dd, 3H, *J* = 2.5, 6.5 Hz), 0.92 (d, 3H, *J* = 6.5 Hz); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 170.0, 169.7, 163.2, 157. 2, 155.8, 154.6, 139.8, 138.8, 137.5, 137.1, 133.0, 131. 8, 131.2, 130.4, 129.6, 129.5, 128.7, 127.1, 126.9, 126.8, 125.2, 124.8, 124.3, 122.8, 122.3, 122.2, 122.1, 119.2, 118.9, 117.2, 116.5, 114.5, 73.8, 72.1, 70.0, 59.2, 55.7, 54.2, 54.0, 53.8, 34.8, 30.3, 29.7, 29.3, 27.4, 27.4, 26.6, 25.1, 20.2, 19.9; HRMS *m/z* $(M-H)^+$ calc'd $C_{33}H_{39}N_2O_7S$ 607.2478, found 607.2479.

(2'*R*,3''*S*)-N-[2'-Hydroxy-2-(10-hydroxy-5-oxo-18-oxa-4-aza-tricyclo[17.2.2.06,11]tricosa-1(22),6,8,10,14,19(23),20-heptaen-3''-yl)-ethyl]-N-isobutyl-4-methoxy-benzene sulfonamide (8b). Diene **7b** (7.5 mg) was dissolved in dry DCM (3 mL) and Grubbs' first generation catalyst (0.9 mg) was added. The reaction was stirred for 4 hours at room temperature, and then concentrated. Flash column chromatography (30-50% EtOAc/Hexanes) gave olefin **8b** (5.6 mg, 78% yield) as an inseparable 3:1 (by ^{1}H-NMR analysis) mixture of isomers. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.73 (d, 2H, *J* = 9.0 Hz), 6.99-6.96 (m, 3H), 6.89-6.85 (m, 3H), 6.71-6.70 (m, 1H), 6.64 (d, 1H, *J* = 7.5 Hz), 6.59 (d, 1H, *J* = 8.5 Hz), 6.14-6.12 (m, 1H), 5.33-5.28 (m, 1H), 5.15-5.09 (m, 1H), 4.47-4.41 (m, 1H), 4.36-4.32 (m, 1H), 4.20-4.17 (m, 1H), 3.98-3.95 (m, 1H), 3.85 (s, 3H), 3.31 (m, 1H), 3.18-3.15 (m, 1H), 3.08-3.01 (m, 2H), 2.92-2.88 (m, 1H), 2.53-2.45 (m, 1H), 2.42-2.40 (m, 1H), 2.30-2.26 (m, 2H), 1.93-1.89 (m, 2H), 1.76-1.73 (m, 1H), 1.38-1.33 (m, 1H), 0.95 (s, 3H), 0.91 (s, 3H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 170.5, 163.2, 158.6, 154.5, 138.7, 133.1, 129.6, 129.5, 126.7, 126.3, 125.5, 118.1, 116.3, 114.5, 73.6, 66.6, 58.9, 55.7, 53.9, 53.8, 35.8, 34.5, 34.5, 32.2, 27.3, 20.2, 19.9; HRMS *m/z* $(M + Na)^+$ calc'd for $C_{34}H_{42}N_2O_7SNa$ 645.2610, found 645.2622.

(2'*R*,3''*S*)-N-[2'-Hydroxy-2-(10-hydroxy-5-oxo-19-oxa-4-aza-tricyclo[18.2.2.06,11]tetracosa-1(23),6,8,10,14,20(24),21-heptaen-3''-yl)-ethyl]-N-isobutyl-4-methoxy-benzenesulfonamide (8c). To a solution of diene **7c** (12.4 mg, 0.0187 mmol) and dry DCM (5 mL) was added Grubbs' first generation catalyst (1.2 mg) and the reaction was stirred for 4 hours, then concentrated under reduced pressure. Flash column chromatography (30-40% EtOAc/Hexanes) afforded olefin **8c** (10.5 mg, 85% yield) as a 5:1 (by ^{1}H-NMR analysis) mixture of inseparable isomers. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.76 (dd, 2H, *J* = 2.0, 7.0 Hz), 7.08 (d, 2H, *J* = 8.5 Hz), 7.02-6.99 (m, 3H), 6.81 (d, 2H, *J* = 8.5 Hz), 6.75-6.07 (m, 2H), 5.86 (d, 1H, *J* = 9.5 Hz), 5.32 (bs, 1H), 5.26-5.20 (m, 1H), 5.12-5.06 (m, 1H), 4.35-4.29 (m, 1H), 4.14-4.11 (m, 1H), 3.98-3.92 (m, 2H), 3.87 (s, 3H), 3.82-3.77 (bs, 1H), 3.30 (dd, 1H, *J* = 9.0, 15.0 Hz), 3.14 (dd, 2H, *J* = 2.5, 15.0 Hz), 3.07 (dd, 1H, *J* = 3.5, 13.0 Hz), 2.90 (dd, 1H, *J* = 6.5, 13.5 Hz), 2.52-2.47 (m, 1H), 2.36-2.28 (m, 1H), 2.19-2.11 (m, 2H), 2.07-2.00 (m, 1H), 1.97-1.91 (m, 1H), 1.75-1.55 (m, 3H), 1.41-1.35 (m, 1H), 0.99 (d, 3H, *J* = 6.5 Hz), 0.94 (d, 3H, *J* = 6.5 Hz); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 169.9, 163.2, 158.9, 154.3, 138.5, 134.0, 130.1, 129.6, 127.3, 126.9, 125.9, 118.4, 116.5, 115.4, 114.5, 73.6, 66.6, 59.2, 55.7, 54.7, 54.0, 35.1, 32.0, 29.7, 29.4, 28.3, 27.7, 27.4, 20.2, 19.9; HRMS *m/z* $(M + Na)^+$ calc'd for $C_{35}H_{44}N_2O_7SNa$ 659.2767, found 659.2766.

(2'*R*,3''*S*)-N-[2'-Hydroxy-2-(10-hydroxy-5-oxo-20-oxa-4-aza-tricyclo[19.2.2.06,11]pentacosa-1(24),6,8,10,14,21(25),22-heptaen-3''-yl)-ethyl]-N-isobutyl-4-methoxy-benzenesulfonamide (8d). To diene **7d** (12.8 mg, 0.019 mmol) and dry DCM (4 mL) was added first generation Grubbs' catalyst (1.2 mg). The solution was stirred for 4 hours at room temperature, then concentrated under reduced pressure. Flash column chromatography (25-40% EtOAc/Hexanes) afforded olefin **8d** (10.7 mg, 87% yield) as a single (by ^{1}H-NMR analysis) isomer. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.76 (dd, 2H, *J* = 2.0, 7.0 Hz), 7.10 (d, 2H, *J* = 8.5), 7.04-6.98 (m, 3H), 6.82-6.76 (m, 4H), 5.94 (d, 1H, *J* = 10.0 Hz), 5.30-5.22 (m, 3H), 4.36-4.33 (m, 1H), 4.15-4.06 (m, 2H), 3.98-3.95 (m, 1H), 3.87 (s, 3H), 3.82-3.80 (bs, 1H), 3.31 (dd, 1H, *J* = 9.0, 15.5 Hz), 3.20-3.04 (m, 3H), 2.89 (dd, 1H, *J* = 6.5, 13.5 Hz), 2.53 (t, 1H, *J* = 13.0 Hz), 2.38 (dt, 1H, *J* = 3.5, 11.5 Hz), 2.09-2.05 (m, 2H), 1.95-1.77 (m, 6H); ^{13}C-NMR (125 MHz, CDCl3) δ 169.9, 163.2, 156.9, 154.1, 138.3, 130.7, 130.0, 129.9, 129.6, 129.5, 126.9, 126.4, 118.7, 116.7, 115.2, 114.5, 73.7, 67.1, 59.2, 55.7, 54.3, 54.1, 34.9, 32.7, 30.9, 27.9, 27.4, 26.5, 24.0, 20.2, 19.9; HRMS *m/z* $(M + H)^+$ calc'd for $C_{34}H_{46}N_2O_7SNa$ 673.2923, found 673.2921.

Inhibitor 23 and Saturated inhibitor 24a. To a stirred solution of olefin **8a** (9 mg, 0.02 mmol) in 5 mL of 1% NH_3 in MeOH solution, 5% Pd/C (1 mg) was added, and the resulting suspension was stirred at 23 °C under a hydrogen filled balloon for 4 h. The mixture was filtered through Celite and concentrated. Silica gel chromatography (40% EtOAc/hexanes) afforded ring-opening acyclic product **23** (2.5 mg, 27% yield) and saturated macrocycle **24a**. (3.6 mg, 40% yield).

Compound 23: ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.70 (d, 2 H, *J* = 9 Hz), 7.14 (d, 2 H, *J* = 8.5 Hz), 6.99-6.96 (m, 3 H), 6.78-6.75 (m, 3 H), 6.56 (d, 1 H, *J* = 7.5 Hz), 5.99 (d, 1 H, *J* = 8.5 Hz), 5.21 (bs, 1 H), 5.09 (bs, 1 H), 4.32-4.29 (m, 1 H), 4.20 (m, 1 H), 3.97-3.96 (m, 1 H), 3.87 (s, 3 H), 3.15-3.02 (m, 3 H), 2.98-2.82 (m, 3 H), 2.56-2.47 (m, 2 H), 1.91-1.88 (m, 1 H), 1.49-1.43 (m, 2 H), 1.27-1.24 (m, 3 H), 0.93 (d, 3 H, *J* = 7.0 Hz), 0.89 (d, 3 H, *J* = 6.5 Hz), 0.85 (t, 3 H, *J* = 6.5 Hz). ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.5, 163.1, 154.5, 154.1, 137.6, 130.5, 129.8, 129.6, 129.4, 127.2, 126.8, 119.0, 117.0, 115.5, 114.4, 72.9, 58.9, 55.6, 54.3, 53.7, 34.0, 32.1, 29.9, 29.7, 27.4, 26.9, 22.5, 20.1, 19.9, 14.0. LRMS-ESI (*m/z*): 635.6 $(M + Na)^+$; HRMS *m/z* $(M + H)^+$ calc'd for $C_{33}H_{44}N_2O_7SH$ 613.2947, found 613.2955.

Saturated macrocycle **24a:** ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.76 (d, 2 H, *J* = 9.0 Hz), 7.23 (d, 1 H, *J* = 9.5 Hz), 7.045-7.015 (m, 2 H), 6.99 (d, 2 H, *J* = 9 Hz), 6.91 (d, 1 H, *J* = 8 Hz), 6.82 (d, 2 H, *J* = 9.5 Hz), 6.77 (d, 1 H, *J* = 8 Hz), 5.80 (d, 1 H, *J* = 9.5 Hz), 4.89 (s, 1 H), 4.30-4.26 (m, 2 H), 4.15-4.11 (m, 1 H), 3.95 (m, 1 H), 3.86 (s, 3 H), 3.72 (d, 1 H, *J* = 2 Hz), 3.28-3.22 (m, 2 H), 3.12-3.04 (m, 3 H), 2.86 (dd, 1 H, *J* = 6.5, 13.5 Hz), 2.59-2.53 (m, 2 H), 2.24 (td, 1 H, *J* = 4 .0, 12.8 Hz), 2.01-1.96 (m, 1 H), 1.94-1.90 (m, 1 H), 1.27-1.245 (m, 3H), 0.99 (d, 3H, *J* = 6.5 Hz), 0.93 (d, 3H, *J* = 7.0 Hz). ^{13}C NMR (125 MHz, $CDCl_3$): δ 169.4, 163.1, 159.5, 154.8, 137.0, 130.8, 130.5, 129.5, 129.1, 127.8, 126.6, 119.7, 119.2, 117.3, 117.1, 114.5,

73.8, 69.5, 59.2, 55.6, 54.1, 53.8, 34.8, 30.8, 27.9, 27.4, 26.9, 26.0, 20.2, 19.9. LRMS-ESI *m/z:* 633.5 (M + Na)$^+$; HRMS *m/z* (M + Na)$^+$ calc'd for $C_{33}H_{42}N_2O_7SNa$ 633.2610, found 633.2631.

Saturated inhibitor 24b. Olefin **8b** (3.5 mg), EtOAc (2 mL), and 10% Pd/C (0.5 mg) was stirred under a hydrogen balloon for 2 hours. The reaction was filtered through Celite and concentrated. Flash column chromatography (30% EtOAc/Hexanes) afforded saturated macrocycle **24b** (3.3 mg, 94% yield). ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.75 (td, 2H, *J* = 3.0, 9.0), 7.13-7.05 (m, 1H), 6.98 (td, 2H, *J* = 2.0, 9.0 Hz), 6.92 (t, 1H, *J* = 7.5 Hz), 6.88 (d, 2H, *J* = 6.0 Hz), 6.71 (d, 2H, *J* = 9.0 Hz), 6.10 (bs, 1H), 6.04 (d, 1H, *J* = 9.5 Hz), 4.40-4.37 (m, 1H), 4.33-4.29 (m, 1H), 4.22 (td, 1H, *J* = 4.0, 12.0 Hz), 4.00-3.96 (m, 1H), 3.85 (s, 3H), 3.30 (dd, 1H, *J* = 9.0, 15.0 Hz), 3.16-3.11 (m, 2H), 3.05 (dd, 1H, *J* = 8.5, 13.0 Hz), 2.89 (dd, 1H, *J* = 6.5, 13.5 Hz), 2.57-2.51 (m, 1H), 2.38 (dt, 1H, *J* = 4.0, 13.0 Hz), 1.94-1.90 (m, 1H), 1.80-1.72 (m, 2H), 1.49-1.40 (m, 2H), 1.30-1.22 (m, 2H), 1.20-1.12 (m, 3H), 0.97 (d, 3H, *J* = 6.5 Hz), 0.92 (d, 3H, *J* = 6.5 Hz); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 170.1, 163.2, 158.0, 154.6, 137.7, 130.3, 130.2, 129.6, 129.5, 127.7, 126.5, 118.6, 117.2, 116.8, 114.5, 73.7, 68.2, 59.1, 55.7, 54.1, 53.9, 34.6, 29.1, 28.1, 27.4, 26.8, 24.0, 20.2, 19.9; HRMS *m/z* (M + Na)$^+$ calc'd for $C_{34}H_{44}N_2O_7SNa$ 647.2767, found 647.2761.

Saturated inhibitor 24c. Olefin **8c** (4.0 mg, 0.0063 mmol) was dissolved in ethyl acetate (5 mL) and 10% Pd/C (1 mg) was added. The mixture was stirred under a hydrogen balloon for 4 hours and then filtered through Celite. After concentration under reduced pressure, the product was purified by flash column chromatography (30% EtOAc/Hexanes) to afford macrocycle **24c** (3.5 mg, 88% yield). ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.76 (ddd, 2H, *J* = 2.0, 3.0, 9.0 Hz), 7.09-7.04 (m, 3H), 6.99 (ddd, 2H, *J* = 2.0, 3.0, 9.0 Hz), 6.87 (dd, 1H, *J* = 1.0, 7.5 Hz), 6.82 (d, 2H, *J* = 8.5 Hz), 6.80 (dd, 1H, *J* = 1.0, 8.0 Hz), 5.90 (d, 1H, *J* = 9.0 Hz), 4.91 (bs, 1H), 4.37 (dt, 1H, *J* = 4.0, 12.0 Hz), 4.28-4.23 (m, 1H), 4.17-4.13 (m, 1H), 3.98-3.95 (m, 1H), 3.86 (s, 3H), 3.30 (dd, 1H, *J* = 9.0, 15.5 Hz), 3.17-3.13 (m, 2H), 3.06 (dd, 1H, *J* = 8.6, 13.0 Hz), 2.87 (dd, 1H, *J* = 6.5, 13.5 Hz), 2.71-2.65 (m, 1H), 2.60 (dd, 1H, *J* = 12.0, 14.5 Hz), 2.03-1.97 (m, 1H), 1.96-1.90 (m, 2H), 1.62-1.55 (m, 3H), 1.45-1.42 (m, 2H), 1.39-1.34 (m, 1H), 1.05-1.01 (m, 3H), 0.98 (d, 3H, *J* = 6.5 Hz), 0.92 (d, 3H, *J* = 6.5 Hz); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 170.1, 163.1, 156.9, 154.3, 137.3, 130.1, 129.7, 129.7, 129.5, 127.5, 126.7, 119.2, 117.1, 115.8, 114.5, 73.6, 68.0, 59.2, 55.7, 55.0, 54.0, 34.9, 29.7, 29.4, 28.9, 27.6, 27.4, 26.7, 26.1, 25.4, 20.2, 19.9; HRMS *m/z* (M + Na)$^+$ calc'd for $C_{35}H_{46}N_2O_7SNa$ 661.2923, found 661.2921.

Saturated inhibitor 24d. Olefin **8d** (4.5 mg, 0.0069 mmol) was dissolved in EtOAc (3 mL) and 10% Pd/C (0.5 mg) was added. The mixture was stirred under a H_2 balloon for 4 hours, then filtered through celite and concentrated under reduced pressure. Flash column chromatography (40% EtOAc/Hexanes) afforded saturated macrocycle **24d** (4.0 mg, 89% yield). ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.75 (ddd, 2H, *J* = 2.0, 2.5, 9.0 Hz), 7.12 (d, 2H, *J* = 8.5 Hz), 7.03 (t, 1H, *J* = 8.0 Hz), 7.00 (ddd, 2H, *J* = 3.0, 4.0, 9.0 Hz), 6.82 (d, 2H, *J* = 9.0 Hz), 6.80-6.76 (m, 2H), 5.94 (d, 1H, *J* = 9.5 Hz), 5.05 (bs, 1H), 4.40-4.34 (m, 1H), 4.18-4.12 (m, 2H), 3.99-3.95 (m, 1H), 3.87 (s, 3H), 3.31 (dd, 1H, *J* = 9.0, 15.0 Hz), 3.15-3.11(m, 2H), 3.07 (dd, 1H, *J* = 9.0, 13.5 Hz), 2.88 (dd, 1H, *J* = 6.5, 13.5 Hz), 2.59 (dd, 1H, *J* = 12.0, 14.5 Hz), 2.50 (dt, 1H, *J* = 3.5, 12.5 Hz), 1.96-1.90 (m, 1H), 1.90-1.82 (m, 1H), 1.75-1.71 (m, 1H), 1.65-1.54 (m, 4H), 1.50-1.44 (m, 1H), 1.36-1.33 (m, 2H), 1.10-1.03 (m, 4H), 0.99 (d, 3H, *J* = 6.5 Hz), 0.93 (d, 3H, *J* = 6.5 Hz); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 170.1, 163.2, 156.8, 154.1, 138.0, 129.9, 129.6, 129.6, 129.5, 127.1, 126.7, 119.0, 116.8, 115.1, 114.5, 73.7, 67.0, 59.2, 55.7, 54.2, 54.0, 34.7, 30.0, 29.7, 28.3, 27.4, 27.1, 27.0, 26.8, 26.2, 23.0, 20.2, 19.9; HRMS *m/z* (M + Na)$^+$ calc'd for $C_{36}H_{48}N_2O_7SNa$ 675.3080, found 675.3086.

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