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Selective and Potent Morpholinone Inhibitors of the MDM2–p53 Protein–Protein Interaction

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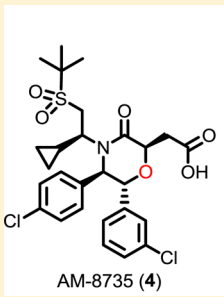
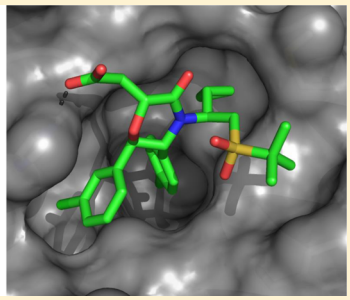
Supporting Information

ABSTRACT: We previously reported the discovery of AMG 232, a highly potent and selective piperidinone inhibitor of the MDM2–p53 interaction. Our continued search for potent and diverse analogues led to the discovery of novel morpholinone MDM2 inhibitors. This change to a morpholinone core has a significant impact on both potency and metabolic stability compared to the piperidinone series. Within this morpholinone series, AM-8735 emerged as an inhibitor with remarkable biochemical potency (HTRF IC_{50} = 0.4 nM) and cellular potency (SJSA-1 EdU IC_{50} = 25 nM), as well as pharmacokinetic properties. Compound **4** also shows excellent antitumor activity in the SJSA-1 osteosarcoma xenograft model with an ED_{50} of 41 mg/kg. Lead optimization toward the discovery of this inhibitor as well as key differences between the morpholinone and the piperidinone series will be described herein.





INTRODUCTION

Recognized as the "guardian of the genome" the transcription factor p53 is the cell's main tumor suppressor and one of the most frequently inactivated genes in human cancers.[1,2] Over 30 years of research has shown that upon cellular stress, p53 response triggers the expression of multiple genes that control cell cycle arrest, apoptosis, senescence, and DNA repair.[3] In about 50% of cancer cells p53 inactivation takes place through mutations within the p53 gene (*TP53*) or by translational modifications of its gene product.[4] In those tumors that retain wild-type p53 function, loss of activity occurs by other means, such as direct inhibition by its natural inhibitors.[5] Discovered in 1987, the oncogene MDM2 has been identified as p53's main inhibitor and is found to be amplified in many tumor tissues.[6−8] Research has shown that overexpression of MDM2 blocks p53-mediated cell cycle arrest and apoptosis.[9] As a result, disruption of MDM2 binding to p53 has emerged as an attractive strategy for the reactivation of the p53 pathway.[10] Despite the inherent challenges associated with this task, the relatively small hydrophobic interface between MDM2 and p53 suggested the possibility of designing a small molecule inhibitor that could bind to MDM2 in the p53 binding region.[5,11−14] Several small molecule inhibitors of the MDM2–p53 interaction are now being tested in the clinic.[15−18]

We recently described the structure-based design of a novel class of potent and selective piperidinone inhibitors of the MDM2–p53 interaction exemplified by AM-8553 (**1**, Figure 1) and AMG 232 (**2**, Figure 1).[19−22] While **1** is a potent inhibitor (SJSA-1 EdU IC_{50} = 72 nM),[23] continued investigation of the *N*-alkyl substituent in **1** resulted in a substantial improvement in the biochemical and cellular potency of our inhibitors.[21]

These efforts led to the discovery of AMG 232 (**2**), an exquisitely potent and selective piperidinone inhibitor.[21] Compound **2** demonstrated significant inhibition in both biochemical (HTRF) and cellular (SJSA-1, EdU) assays with an IC_{50} of 0.6 and 9.2 nM, respectively.[23] Furthermore, **2** has an outstanding pharmacokinetic profile and shows robust in vivo antitumor activity inhibiting tumor growth in an SJSA-1 osteosarcoma xenograft model with an ED_{50} of 9.2 mg/kg.[21]

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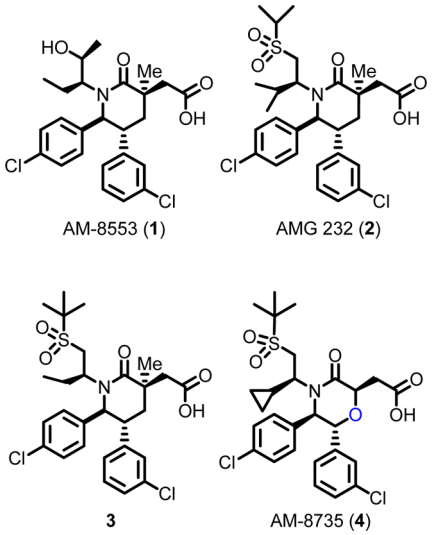


Figure 1. Potent morpholinone and piperidinone inhibitors of the MDM2−p53 interaction.

The exceptional potency of **2** can be understood by the cocrystal structure of analogue **3** (Figure 2).[19] Compound **3** occupies three critical binding sites on MDM2, namely, the Leu26$_{(p53)}$, Trp23$_{(p53)}$, and Phe19$_{(p53)}$ pockets. The carboxylate of **3** presumably engages in a salt bridge with the imidazole moiety on His96. The C3-Me points directly toward solvent, supporting our hypothesis that the improvement in potency observed with this substitution in the piperidinone series is due to the stabilization of the gauche conformation rather than from an additional contact with the MDM2 protein.[24] Additionally, the sulfone moiety plays an important role in directing the small lipophilic ethyl group on **3** toward the Phe19$_{(p53)}$ binding pocket while placing the *tert*-butyl moiety in close contact with the protein, in a shelf region located above Gly58. Direct comparison of the single X-ray crystal structure of **2** with the structure of analogue **3** bound to MDM2 shows a nearly perfect overlap between the two, suggesting that **2** binds to MDM2 in a low energy conformation that contributes to its high potency.[21]

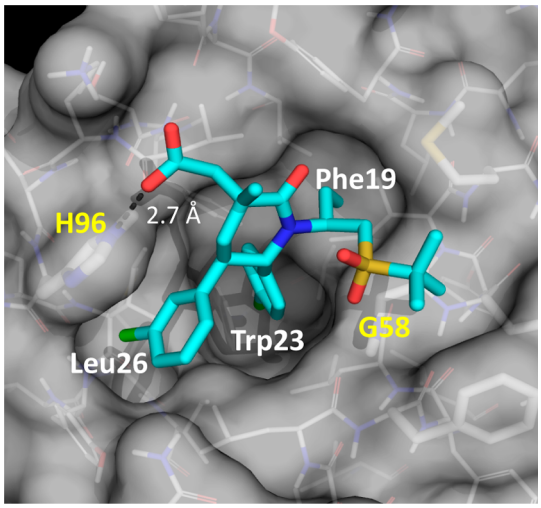


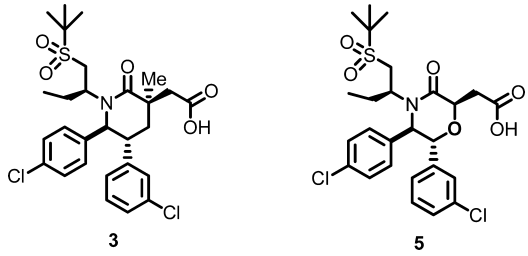
Figure 2. Cocrystal structure of **3** bound to human MDM2 (17−111) at 1.7 Å resolution. White labels indicate positions normally occupied by key p53 residues. MDM2 residues His96 and Gly58 are labeled in yellow. The coordinates of **3** with MDM2 have been deposited in the PDB with accession code 4OAS.

Initial efforts on morpholinone compounds indicated that this scaffold could be a template for the design of very potent MDM2−p53 inhibitors.[25] In this article, we report how our continued quest to find molecules with diverse structures that maintain the favorable efficacy of **2** led to the discovery of novel morpholinone-derived inhibitors of the MDM2−p53 interaction. Among these, AM-8735 (**4**, Figure 1), a potent and selective MDM2 inhibitor (SJSA-1 EdU IC_{50} = 25 nM), shows remarkable pharmacokinetic properties and in vivo efficacy in a SJSA-1 osteosarcoma xenograft model (ED_{50} = 41 mg/kg). In addition, we will discuss the significant differences in metabolic stability observed between the morpholinone inhibitors and their piperidinone counterparts.

RESULTS AND DISCUSSION

We commenced our research in this area by synthesizing morpholinone compounds using knowledge gained from the piperidinone series. From this effort, two findings became evident that are best exemplified by comparing morpholinone **5** and the aforementioned piperidinone **3** (Table 1): whereas morpholinone inhibitors are 5- to 10-fold less potent than their piperidinone counterparts, most morpholinones are more stable in hepatocytes, a finding that is consistent across preclinical species and human.[26] Given that the observed metabolism of most analogues is driven by glucuronidation of the parent acid (<10% turnover in human microsomes), we hoped that the morpholinone series could provide a pharmacokinetic advantage over the piperidinones that would compensate for the observed loss in potency. Besides this core change, there is another key difference between inhibitors **3** and **5**. Piperidinone inhibitor **3** requires the C3-methyl substituent to enforce a conformational equilibrium in favor of the gauche binding conformation (Figure 3; C3-monosubstituted, $\Delta E_{anti\text{-}gauche}$ = −0.5 kcal/mol, 3:1 anti/gauche; C3-disubstituted,

Table 1. Comparison between Piperidinone 3 and Morpholinone 5



	3	5
Biochemical Potency		
K_D (SPR, nM)	0.036	0.392
Potency in SJSA-1 Cells		
p21 IC_{50} (nM)[a,c]	18.4 ± 0.6	240 ± 90
EdU IC_{50} (nM)[b,c]	3.2 ± 1.2	37 ± 19
Hepatocyte Intrinsic Clearance (mL/min per 10^6 cells)		
human	15.0	1.9
rat	6.7	1.8
mouse	18.0	12.0
dog	68.0	15.5

[a]Assay conducted in the absence of human serum. [b]Assay conducted in the presence of 10% human serum. [c]Mean and standard deviation of at least two runs.

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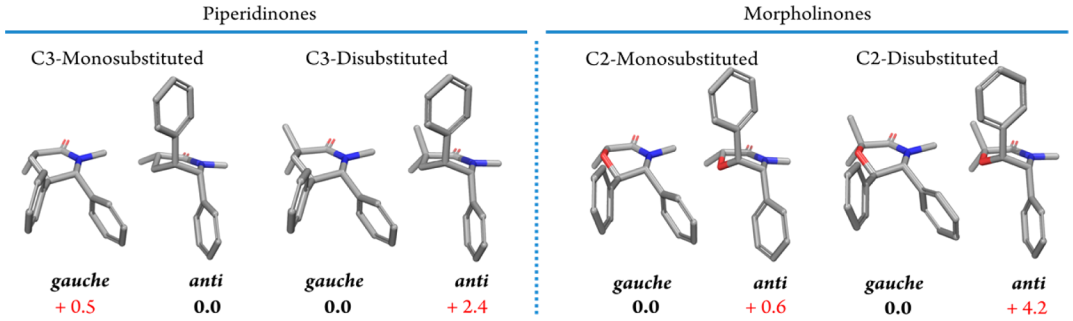
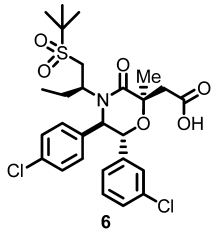


Figure 3. B3LYP/6-31G* structures and relative energies (kcal/mol) for model mono- and disubstituted piperidinone and morpholinone inhibitors of the MDM2−p53 interaction.

$\Delta E_{anti\text{-}gauche}$ = 2.8 kcal/mol, 1:46 anti/gauche), resulting in a 2- to 3-fold improvement in potency.[24] Conversely, analogous quantum mechanical calculations on the morpholinone core predict that the MDM2-binding gauche conformer is preferred, even in the absence of a stabilizing methyl substituent (Figure 3). These calculations are in agreement with the observed in vitro data showing that the C2-Me-substituted morpholinone **6** (Figure 4) exhibits similar potency (HTRF ± HS IC_{50} = 0.6/2.3 nM, EdU IC_{50} = 32 nM) to its *des*-Me counterpart **5** (HTRF ± HS IC_{50} = 0.5/2.0 nM, EdU IC_{50} = 37 nM).[23]



6

SJSA-1 EdU IC_{50} = 32 ± 7 nM

Figure 4. C2-Me-substituted morpholinone **6**.

With these data in hand, we began our research efforts by exploring modifications around the *N*-alkyl substituent. Thus, a series of sulfonamides, reverse sulfonamides, and sulfones were synthesized (Table 2). From these, incorporation of *N*-arylsulfonamide substituents provided some of our most potent morpholinone inhibitors (Table 2, entries 2−7). Introducing a fluorine at the ortho position of the arene moiety resulted in increased potency and decreased PXR activity (Table 2, entry 3). The para-fluoro substitution also resulted in decreased PXR activation (Table 2, entry 4 vs entry 2). With the potent ortho-phenyl substituent in place, the size of the *S*-alkyl substituent proved to be inconsequential to potency but did affect stability in human hepatocytes (Table 2, entries 6 and 7 vs entry 3). In the case of the sulfone substituent, increasing the size of the group from ethyl to *tert*-butyl resulted in a significant improvement in both biochemical and cellular potency (Table 2, entries 8−10). Nonetheless, further increasing the size of this small lipophilic group did not lead to more potent analogues and in most cases was also detrimental to the hepatocyte stability (Table 2, entries 11−13). Finally, substituents such as sulfonamide **19** and oxetanesulfone **20** (Table 2, entries 14−15) provided inhibitors with reduced cellular potency when compared to **5**.

Representative sulfonamides and sulfones in Table 2 were evaluated for their potential to cause drug−drug interactions by inhibition of the CYP enzymes. Whereas a majority of sulfones show minimal liabilities in these assays, many sulfonamides demonstrate considerable time dependent inhibition of CYP3A4 (e.g., **9**, 70%; **13**, 74%; **14**, 0.4%; **15**, 3%; **5**, 23%).[27]

With the potent *tert*-butyl sulfone in place we studied the interaction of the small alkyl group with the $Phe19_{(p53)}$ pocket in the MDM2 protein. As shown in Table 3, increasing the group size from methyl to ethyl resulted in a 3-fold improvement in cellular potency (entries 1 and 2). However, attempts to further improve potency by increasing the size of this substituent to isopropyl or *tert*-butyl were not successful (Table 3, entries 3−5). Gratifyingly, the cyclopropyl functionality provided a good balance for this position, yielding the most potent analogue with an IC_{50} of 25 nM in the SJSA-1 EdU assay (Table 3, entry 6) and a decrease in PXR activity. Importantly, all analogues studied within this series maintained favorable stability in human hepatocytes. The majority of inhibitors within this group exhibit low clearance in rats. On the basis of its good potency and stability in human hepatocytes, **4** was selected for further evaluation.

First, we evaluated the selectivity of **4** by examining its ability to inhibit the proliferation of HCT116 $p53^{wt}$ and $p53^{-/-}$ tumor cells in vitro (Figure 5).[28] Compound **4** displayed substantial growth inhibition of wild-type p53 cells (BrdU, 10% HS, IC_{50} = 63 nM) and no growth inhibition of p53-deficient cells (IC_{50} > 25 μM). Similarly, **4** exhibited a dose-dependent increase of p21 mRNA, a direct transcriptional readout of p53 activity, in HCT116 $p53^{wt}$ cells (p21, 10% HS, IC_{50} = 160 nM). No induction of p21 was observed in HTC116 $p53^{-/-}$ tumor cells treated with **4** at concentrations up to 10 μM. Compound **4** also demonstrated significant time and concentration dependent p21 mRNA induction in vivo in a pharmacodynamic assay in SJSA-1 osteosarcoma tumors (Figure 6).[19,21] A maximum p21 induction of 8-fold was observed 2 h after dosing on day 4 at 25 mg/kg. These data indicate in vivo p53-mediated activity displayed by **4** and provided dose-selection guidance for a xenograft efficacy study.

Thus, we tested the ability of **4** to inhibit tumor growth in a mouse xenograft model bearing the same SJSA-1 osteosarcoma tumor used in the pharmacodynamic assay (Figure 7).[29] In this study, SJSA-1 tumor cells were implanted subcutaneously and grown in mice for 9 days to an average volume of 200 mm^3. After this period, **4** was administered by oral gavage (as a solution in 15% HPβCD, 1% Pluronic F68, pH 8) at 5, 25, 50, and 100 mg/kg q.d. for a period of 2 weeks, and tumor volume was quantified. This compound caused robust dose-dependent tumor growth inhibition with the highest dose of 100 mg/kg resulting in tumor stasis. The calculated ED_{50} was 41 mg/kg (95% confidence interval of 34.11−50.25).

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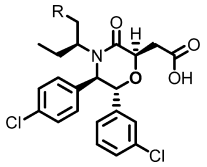
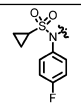
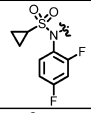
Table 2. Optimization of the *N*-Alkyl Group: Sulfonamides, Reverse Sulfonamides, and Sulfones



Entry	Compd	R	HTRF[a] Serum Free IC_{50} (nM)[f]	HTRF[b] 15% HS IC_{50} (nM)[f]	SJSA-1 EdU[c] IC_{50} (nM)[f]	hPXR (%)[d,f]	hHep CL (μL/min per 10^6 cells)[e]
1	**7**		1.1 ± 0.5	11 ± 2	500 ± 100	7 ± 3	---
2	**8**		0.8 ± 0.4	3.7 ±1.5	54 ± 11	93 ± 7	3.3
3	**9**		0.3 ± 0.1	2.3 ± 0.8	35 ± 4	7 ± 1	< 0.1
4	**10**		0.6 ± 0.2	6.0 ± 2.0	103 ± 22	19 ± 4	7
5	**11**		0.5 ± 0.1	2 ± 1	37 ± 1	8 ± 3	4.8
6	**12**		0.3	2.4	33 ± 1	31 ± 3	15
7	**13**		0.4 ± 0.1	4 ± 1	58 ± 9	71 ± 11	4.7
8	**14**		3 ± 1	26 ± 2	800 ± 200	4 ± 1	---
9	**15**		1.3 ± 0.6	10.1 ± 0.1	150 ± 12	5 ± 1	2.1
10	**5**		0.5 ± 0.3	2.0 ± 0.1	37 ± 19	50 ± 21	1.9
11	**16**		1.0 ± 0.4	18 ± 5	198 ± 12	19 ± 5	8.6
12	**17**		5 ± 3	20 ± 5	421 ± 46	4.6 ± 0.3	12
13	**18**		0.9 ± 0.2	17 ± 3	315 ± 66	114 ± 11	10
14	**19**		0.9 ± 0.2	10 ± 2	195 ± 61	4.0 ± 0.4	19
15	**20**		5	23	641	1.4 ± 0.6	6.6

[a]IC_{50} in biochemical assay using serum free buffer. [b]IC_{50} in biochemical assay using buffer containing 15% human serum. [c]Cellular potency in SJSA-1 in the presence of 10% human serum. [d]Human PXR activation, % of control, rafampin, at 2 μM. [e]Human hepatocyte stability. [f]Mean and standard deviation of at least two runs.

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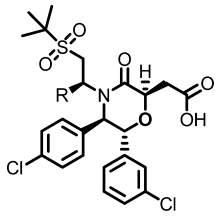
Table 3. Modification to the R Group that Occupies the MDM2 Phe19$_{p53}$ Pocket



Entry	Compd	R	HTRF[a,f] Serum Free IC_{50} (nM)[f]	HTRF[b] 15% HS IC_{50} (nM)[f]	SJSA-1 EdU[c] IC_{50} (nM)[f]	hPXR (%)[d,f]	hHep CL (μL/min per 10^6 cells)[e]	Rat PK[g] CL (L h^{-1} kg^{-1})/$t_{1/2}$ (h)
1	**21**	Me	1.1 ± 0.1	6.9 ± 2.1	129 ± 54	22 ± 11	0.7	
2	**5**	Et	0.5 ± 0.3	2.0 ± 0.1	37 ± 19	50 ± 21	1.9	0.23/4.1
3	**22**	CH_2CF_3	0.4 ± 0.2	2.1 ± 0.6	33 ± 8	12 ± 2	4.7	0.43/4.0
4	**23**	*i*-Pr	2.0	2.5	67 ± 14	42 ± 5	1.7	0.11/6.0
5	**24**	*t*-Bu	0.5 ± 0.1	5.1 ± 1.6	120 ± 16	49 ± 9	2.1	
6	**4**	*c*-Pr	0.4 ± 0.1	1.8 ± 0.9	25 ± 9	19.2	1.4	0.35/5.7

[a]IC_{50} in biochemical assay using serum free buffer. [b]IC_{50} in biochemical assay using buffer containing 15% human serum. [c]Cellular potency in SJSA-1 in the presence of 10% human serum. [d]Human PXR activation, % of control, rafampin, at 2 μM. [e]Human hepatocyte stability. [f]Mean and standard deviation of at least two runs. [g]Rat PK, iv, 0.5 mg/kg.

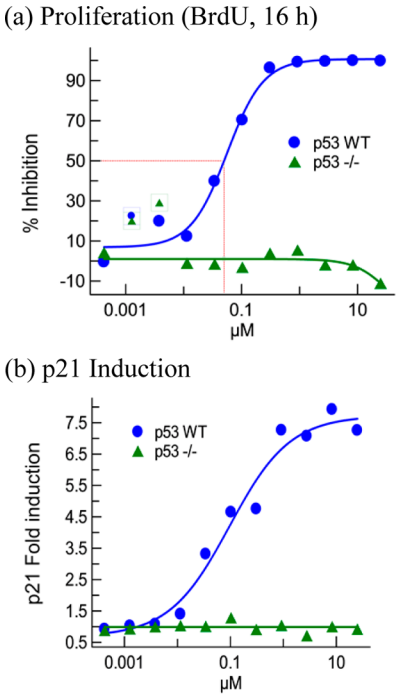


Figure 5. Cell activity of **4** is p53 dependent. (a) In HCT116 p53^{wt} and p53$^{-/-}$ cells, the percentage of BrdU positive cells was measured 16 h after compound treatment by flow cytometry. DMSO control was designated as 0% inhibition. (b) In HCT116 p53^{wt} and p53 −/− cells, total RNA was extracted 7 h after compound treatment and p21 mRNA was measured by quantitative RT-PCR.

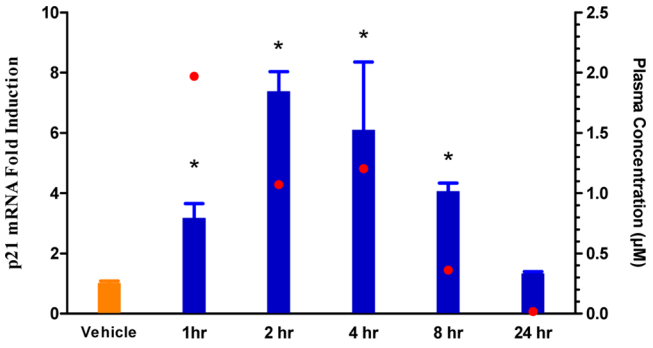


Figure 6. PD study results of **4** in SJSA-1 tumor xenograft: (∗) $p < 0.05$. Female athymic nude mice (n = 5/group) were implanted subcutaneously with 5 × 10^6 SJSA-1 cells. When tumors reached ∼175 mm^3, 25 mg/kg **4** or vehicle was administered orally once daily (q.d.) for 4 days. Mice were sacrificed on day 4 at 1, 2, 4, 8, and 24 h postdose. Tumors were immediately removed and snap-frozen. p21 mRNA levels were measured by quantitative RT-PCR. Tumors treated with vehicle served as a negative control and indicated the baseline p21 mRNA level. Data are represented as mean p21 fold induction over vehicle (blue bars) + SEM of data from five mice. Concentrations in plasma were analyzed by LC/MS/MS and plotted as red dots against the right y-axis.

Potency and hepatocyte stability comparisons between piperidinone **25** and morpholinone **4** are shown in Table 4. The most significant advantage morpholinone **4** has over piperidinone **25** is its improved stability in hepatocytes across all tested species. This observation supports what was observed with compounds **3** and **5** (Table 1).

In order to better understand their metabolic pathways, analogues **4** and **25** were incubated separately in hepatocytes (10^6 cells/mL) from five different species and their degradation products evaluated by LC−MS/MS (Figure 8). In the case of **25**, the chromatograms show that both the oxidative metabolite **M1** and acylglucuronide **M2** are generated across species, including human hepatocytes (Figure 8b).[30] Formation of the acylglucuronide of **25** in dog and human hepatocytes was qualitatively more significant than that in hepatocytes of other species.

In contrast, the oxidative metabolite of **4** was the major metabolite in mouse and rat hepatocytes. Although both the oxidative metabolite and acylglucuronide of **4** were detected in other species, they were barely detected in dog and human hepatocytes (Figure 8a). It appeared that this seemingly simple core substitution is enough to change the formation rates of metabolites. Moreover it impacted their intrinsic clearances across species, especially in human hepatocytes.

In an X-ray cocrystal with human MDM2 (Figure 9), compound **4** binds to the MDM2 protein in a similar fashion to several piperidinone inhibitors (Figure 2).[31] The C6-*m*-Cl-phenyl, C5-*p*-Cl-phenyl, and cyclopropyl moieties occupy the Leu26$_{(p53)}$, Trp23$_{(p53)}$, and Phe19$_{(p53)}$ pockets, respectively. The

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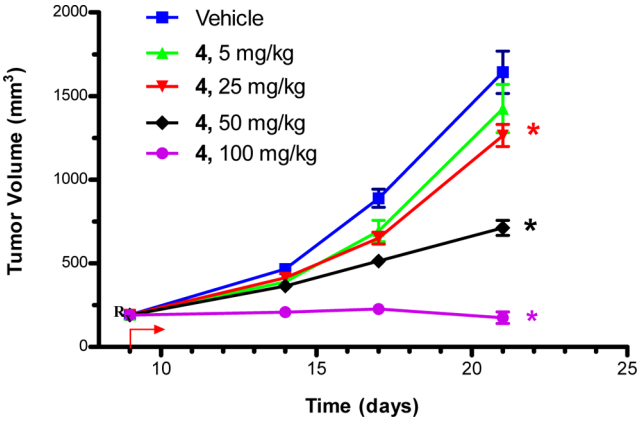
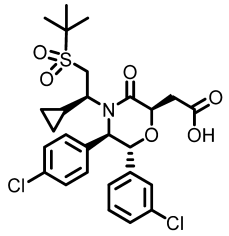


Figure 7. SJSA-1 cells (5×10^6) were implanted subcutaneously into female athymic nude mice. Treatment with vehicle or **4** at 5, 25, 50, or 100 mg/kg q.d. by oral gavage began on day 9 when tumors had reached ~200 mm^3 (n = 10/group). Tumor sizes and body weights were measured twice per week. Data are represented as mean tumor volumes, and the error bars represent SEM of data from 10 mice. No significant (>5%) body weight loss was observed in any of the groups: (*) $p < 0.05$.

Table 4. Comparison between Morpholinone 4 and Piperidinone 25



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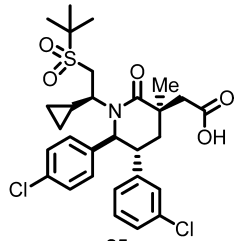

25

Biochemical Potency		
IC_{50} (HTRF, nM)[a,b]	0.4 ± 0.1	0.10 ± 0.01
IC_{50} (HTRF, 15% HS, nM)[b]	1.8 ± 0.9	1.1 ± 0.6
Rat PK (iv, 0.5 mg/kg)		
CL (L h^{-1} kg^{-1})	0.35	1.1
$t_{1/2}$ (L/kg)	5.7	3.8
Hepatocyte Stability (μL/min per 10^6 cells)		
human	1	16
rat	3	24
mouse	6	25
dog	13	24

[a]IC_{50} in biochemical assay using serum free buffer. [b]Mean and standard deviation of at least two runs.

carboxylate appears to engage in a salt bridge with the imidazole moiety on His96, a residue that also engages in a face-to-face, π-stacking interaction with the C6-arene. The *tert*-butyl moiety on the sulfone projects onto the glycine shelf region maximizing the hydrophobic contact with the MDM2 protein.

CHEMISTRY

Synthesis of amino alcohol **32** commenced with commercially available amino acid **27** (Scheme 1). Reduction of **27** with lithium aluminum hydride followed by Boc protection gave carbamate **29**. Dess–Martin periodinane-mediated oxidation of the alcohol produced aldehyde **30**. Addition of 3-chlorophenylmagnesium bromide at room temperature to **30** yielded *trans*-amino alcohol **31** which could be subsequently hydrolyzed to amino alcohol **32** hydrochloride.

Alternatively, amino alcohol **32** was also synthesized through a Henry reaction between nitroalkane **34** and 3-chlorobenzaldehyde followed by in situ protection of the secondary carbinol to its corresponding silyl ether **35** (Scheme 2). Reduction of the nitro group in **35** followed by chiral resolution of **32** provided the desired amino alcohol **36** in 97% ee.

With the desired amino alcohol at hand, the secondary alcohol was converted to the TBS ether **37** (Scheme 3). This transformation can be accomplished in good yields starting from either the free-base amino alcohol **36** or its hydrochloride salt **32**. Ytterbium-catalyzed addition of the free amine on the least hindered side of a chosen epoxide generated intermediates **38a–e**. This transformation proved to be tolerant to a wide variety of monosubstituted epoxides. Moreover, starting from the corresponding enantiomerically enriched epoxide allowed for diastereoselective formation of **38** with retention of configuration at the carbinol center. Aziridines **39a–d** could be formed under standard Mitsunobu conditions in good yields. However, for **38e**, the *tert*-butyl moiety proved to be too hindered for these conditions, and thus, mesylate formation followed by an intramolecular displacement of the mesylate yielded aziridine **39e** albeit in low yields.[32]

Indium-catalyzed aziridine opening with the appropriate thiol occurred smoothly.[33] Alkylation of the free alcohol with bromoacetic acid produced acids **41** that could then undergo an intramolecular amide coupling reaction to form morpholinones **42**. Interestingly, attempts to produce this amide bond in an intermolecular fashion with either acid chlorides or carboxylic acids failed with a variety of reagents. Thus, proceeding in an intramolecular fashion was pivotal for the success of this synthetic route. Finally, allylation at C2 followed by oxidation of both the thioether and the terminal alkene to their corresponding sulfones and carboxylic acids, respectively, afforded the desired inhibitors as a nearly 1.5:1 mixture of diastereoisomers in favor of the desired C2-*R*-isomer.[25]

Since reaction of **37** with 1-cyclopropyloxirane gave a complex mixture of products under the ytterbium-catalyzed reaction conditions depicted in Scheme 3, an alternative synthetic route was devised to access the cyclopropyl-substituted analogues (Scheme 4). First, formation of the morpholinone core was achieved by addition of chloroacetyl chloride to **36** under basic conditions.[25] From **43**, N-alkylation proceeded smoothly to **44**, yielding a 45:55 mixture of isomers favoring **44b**.

However, after isolation of the desired isomer **44a**, **44b** could be easily re-equilibrated to a 45:55 mixture of **44a**/**44b** that could subsequently be separated to give more of the desired isomer. Compound **44a** could then be reduced to the primary alcohol with superhydride. Alcohol **45** could easily be transformed to a thioether under Mitsunobu conditions and then to inhibitor **4** following a similar alkylation/oxidation sequence as that depicted previously in Scheme 2.[34]

Given the versatility of the Mitsunobu reaction, intermediate **49** was synthesized to quickly access a variety of sulfone- and sulfonamide-substituted inhibitors (Scheme 5). To this end, enantiomerically pure brosylate **47** was synthesized from *R*-2-ethoxyoxirane and 3,4-dimethoxybenzyl alcohol. N-Alkylation of **43** with **47** formed isomerically pure **48**. Subsequently, DDQ effected the formation of the desired primary carbinol **49** that served as a common intermediate toward the synthesis of both sulfonamide- and sulfone-containing analogues.

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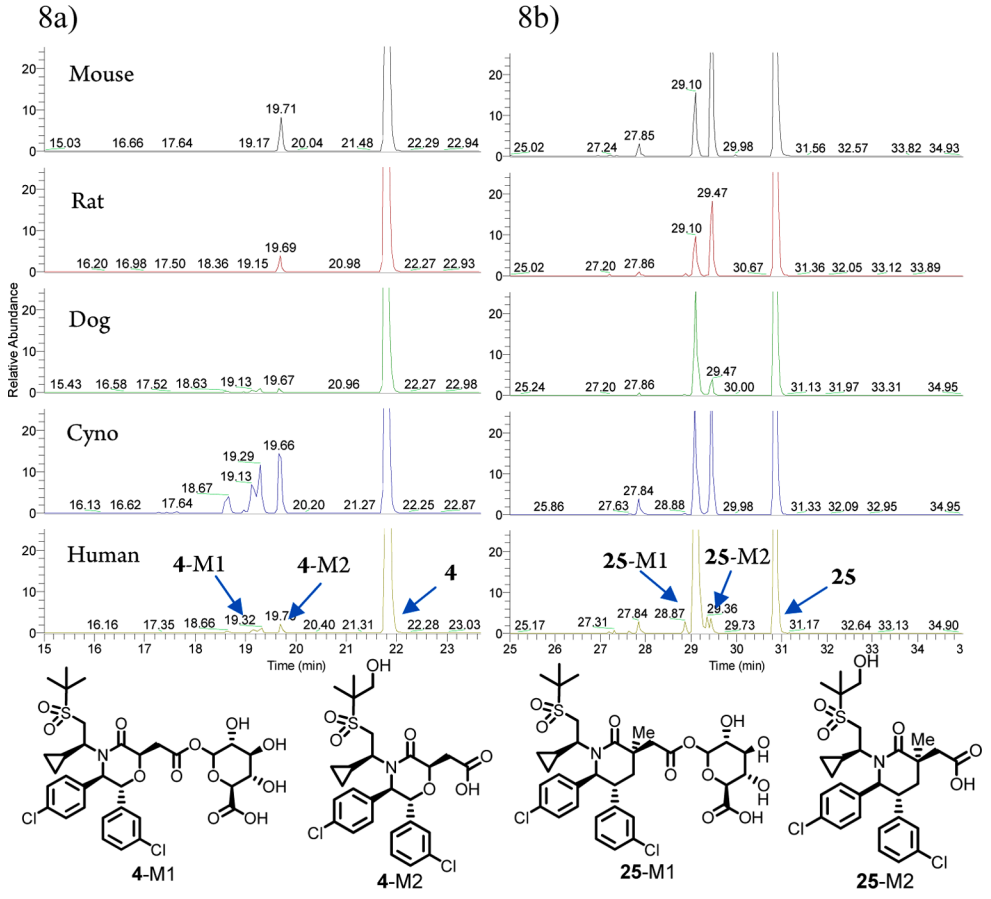


Figure 8. Metabolite profiles of **4** (a) and **25** (b) from hepatocyte incubation at 10 μM.

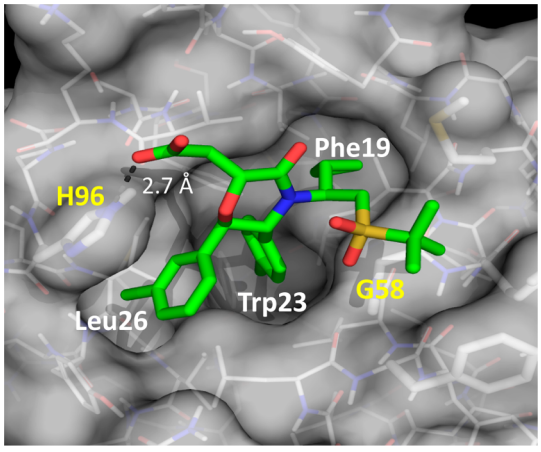
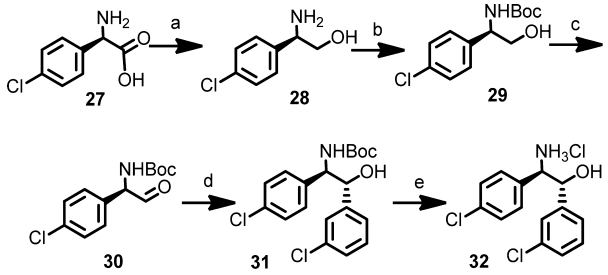


Figure 9. Cocrystal structure of **4** bound to human MDM2 (17−111). White labels indicate positions normally occupied by key p53 residues. MDM2 residues His96 and Gly58 are labeled in yellow. The coordinates of **4** with MDM2 have been deposited in the PDB with accession code 4OBA.

Cyclopropylsulfonamide inhibitor **7** could be prepared from intermediate **48** by alkylation at C2 followed by deprotection of the 3,4-dimethoxybenzyl ether to yield **52** (Scheme 6). Dess−Martin periodinane oxidation of **52** produced aldehyde **53** which, after a quick workup, was converted to methylamine **54** through a reductive amination. Addition of cyclopropanesulfonyl chloride to **54** rendered sulfonamide **55** that could be easily converted to inhibitor **7** utilizing our standard oxidation conditions.

Finally, tetrasubstituted inhibitor **6** was synthesized from **42b** by two subsequent alkylation reactions followed by our standard oxidative conditions (Scheme 7).

Scheme 1[a]



[a]Reagents and conditions: (a) $LiAlH_4$, THF, reflux, 3 h, quant; (b) Boc_2O, TEA, DCM, 12 h, 73%; (c) DMP, DCM, rt, 2 h, 95%; (d) 3-chlorophenylmagnesium bromide, THF, rt, 12 h, 47%; (e) HCl/dioxane, 2 h, quant.

CONCLUSION

Novel, potent, and selective morpholinone inhibitors of the MDM2−p53 interaction were described. The morpholinone inhibitors are less efficacious but have improved hepatocyte stability relative to piperidinone inhibitors. Among these, compound **4** shows excellent biochemical potency (HTRF IC_{50} = 0.4 nM, Table 4), MDM2 selectivity over MDMX (MDMX HTRF IC_{50} > 100 μM), cellular potency (SJSA-1 EdU

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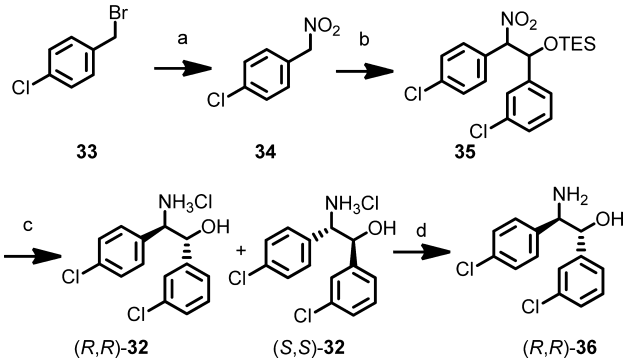
Scheme 2[a]



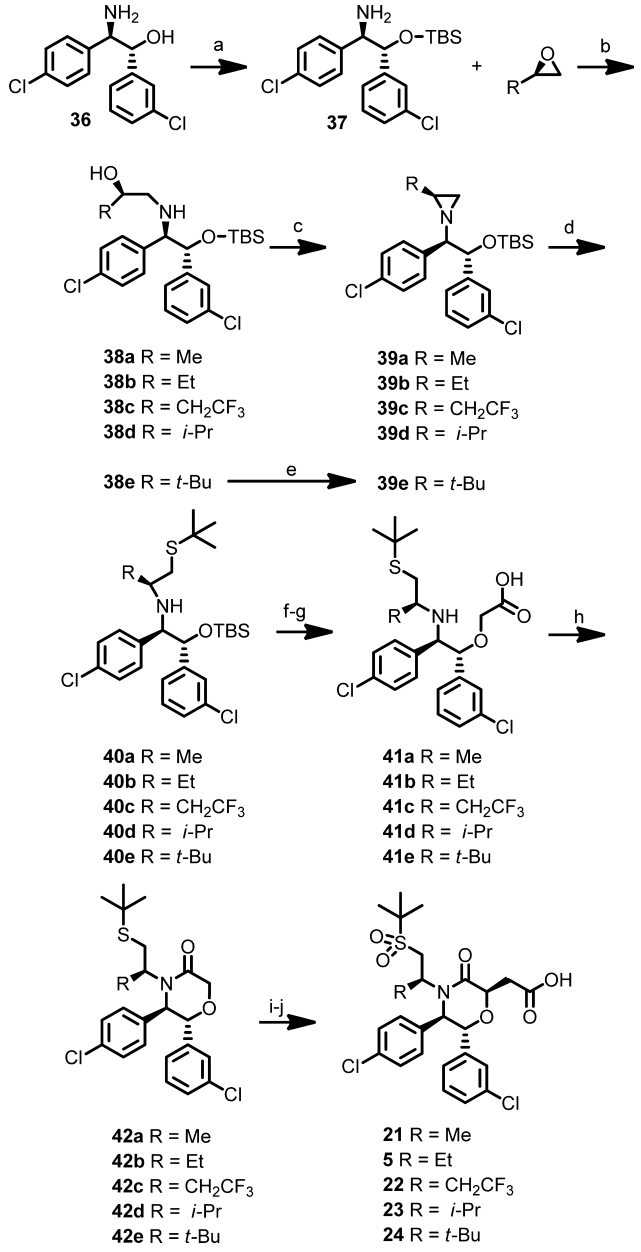
[a]Reagents and conditions: (a) $AgNO_2$, Et_2O, 0 °C, 62%; (b) 3-chlorobenzaldehyde, pyridine, alumina, TESCl, 55 °C, 12 h; (c) Zn/HCl, 40 °C, 2 h, 20% over two steps; (d) (+)-di-*p*-toluoyl-D-tartaric acid, EtOH/water, 38% (97% ee).

IC_{50} = 25 nM), as well as good pharmacokinetic properties (Table 4).[35] Importantly, compound **4** shows a marked improvement in hepatocyte stability compared to piperidinone inhibitor **25**. Oxidation is the main metabolic pathway for the morpholinone series and the morpholinone inhibitors have significantly less formation of the acylglucuronide metabolite than the piperidinones across preclinical species. This molecule also demonstrates robust antitumor activity in the SJSA-1 osteosarcoma xenograft study with a calculated ED_{50} of 41 mg/kg.

EXPERIMENTAL SECTION

General Chemistry. Reactions, unless otherwise stated, were conducted under an inert gas atmosphere (nitrogen or argon) at the temperature indicated. Commercial reagents and anhydrous solvents were used without further purification. Analytical thin layer chromatography (TLC) was performed on Analtech silica gel with organic binder 250 μm TLC plates. Removal of solvents was conducted by using a rotary evaporator, and residual solvent was removed from nonvolatile compounds using a vacuum manifold maintained at approximately 1 Torr. All yields reported are isolated yields. Preparative reversed-phase high pressure liquid chromatography (RP-HPLC) was performed using an Agilent 1100 series HPLC and Phenomenex Gemini C18 column (5 μm, 100 mm × 30 mm i.d.), eluting with a binary solvent system A and B using a gradient elusion [A, H_2O with 0.1% trifluoroacetic acid (TFA); B, CH_3CN with 0.1% TFA] with UV detection at 220 nm. All final compounds were purified to ≥95% purity as determined by a Agilent 1100 series HPLC with UV detection at 220 nm using the following method: Zorbax SB-C8 column (3.5 μm, 150 mm × 4.6 mm i.d.); mobile phase, A = H_2O with 0.1% TFA, B = CH_3CN with 0.1% TFA; gradient 5–95% B (0–15 min); flow rate, 1.5 mL/min. Low-resolution mass spectrometry (MS) data were determined on an Agilent 1100 series LCMS with UV detection at 254 nm and a low resolution electrospray mode (ESI). High-resolution mass spectra were obtained on an Agilent 6510 Q-TOF MS instrument with an Agilent 1200 LC on the front end. ^1H NMR spectra were obtained on a Bruker Avance III 500 (500 MHz) or Bruker Avance II 400 (400 MHz) spectrometer. Chemical shifts (δ) are reported in parts per million (ppm) relative to residual undeuterated solvent as an internal reference. The following abbreviations were used to explain the multiplicities: s = single, d = doublet, t = triplet, q = quartet, dd = doublet of doublets, dt = doublet of triplets, m = multiplet, br = broad. Optical rotations ($[\alpha]_D$) were measured on a JASCO P-1020 polarimeter. Specific rotations are given as deg/dm, and the concentrations are reported as g/100 mL of the specific solvent and were recorded at the temperature indicated. Absolute stereochemistry of inhibitors **1**–**5** was unambiguously determined through crystallographic analysis; for the rest of the inhibitors stereochemistry at C2 was assigned as *R* in agreement with that observed from inhibitors **1**–**5**.

Scheme 3[a]



[a]Reagents and conditions: (a) TBSCl, imidazole, DCM 25 °C, 90%; (b) $Yb(OTf)_3$, CH_3CN, 6 h, 130 °C, 79–93%; (c) DEAD, PPh_3, THF, 40 °C, 12 h, 69–82% (d) *t*-BuSH, $InCl_3$, DCM, 25 °C, 12 h, 88–98%; (e) MsCl, TEA, DCM, −78 °C to rt, 12 h, 31%; (f) TBAF, THF, 15 min, 25 °C, 73–95%; (g) bromoacetic acid, NaH, THF, 25 °C, 12 h, 100% conversion; (h) HATU, DMF, DIPEA, 25 °C, 1 h, 61–90% (last two steps); (i) LiHMDS, allyl bromide, −78 °C, 1 h, 85%, 2:1 mixture of isomers; (j) $NaIO_4$, $RuCl_3$, $CH_3CN/CCl_4/H_2O$, 32–75% combined yield for both isomers.

(*R*)-2-Amino-2-(4-chlorophenyl)ethanol (28). A solution of 1 M $LiAlH_4$ in THF was diluted with 460 mL of anhydrous THF and was heated to 75 °C under a nitrogen atmosphere. (*R*)-2-Amino-2-(4-chlorophenyl)acetic acid (**27**) (25.0 g, 135 mmol; Asta Tech, Inc., Bristol, PA) was added in several portions. The reaction mixture was heated to reflux for about 3 h, cooled to room temperature, and quenched by adding water (8.0 mL), aqueous 15% NaOH (8.0 mL),

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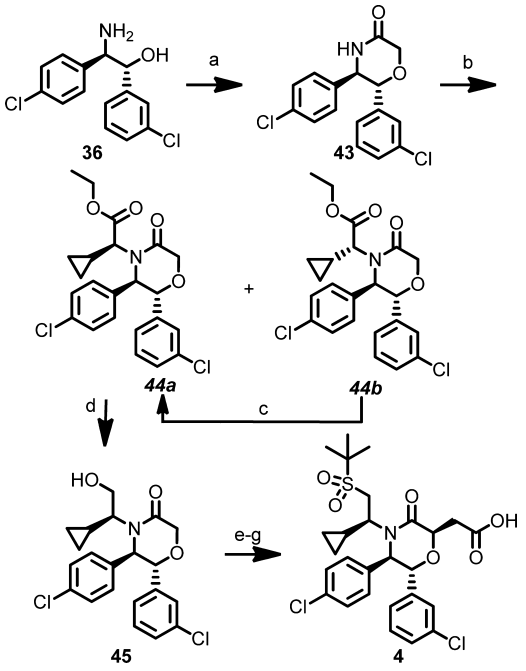
Scheme 4[a]



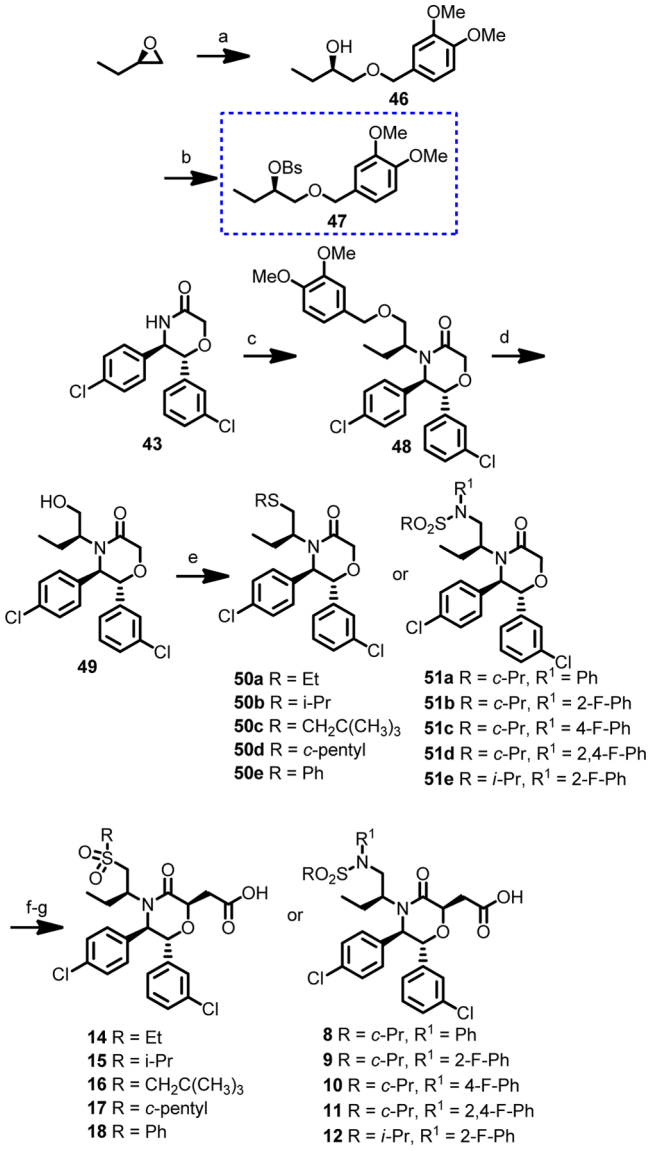
[a]Reagents and conditions: (a) chloroacetyl chloride, TEA, 0 °C, THF, 2 h; then NaH, rt, 12 h, 86%; (b) ethyl 2-bromo-2-cyclopropylacetate, NaH, DMF, rt, 12 h, 82%; (c) NaOEt/EtOH, rt, 4 h, 57%; (d) $LiEt_3BH$, THF, rt, 30 min, 90%; (e) *t*-BuSH, cyanomethyltributylphosphorane, toluene, 110 °C, 12 h, 43%. (f) LiHMDS, allyl bromide, −78 °C, 1 h, 85%, 2:1 mixture of isomers; (g) $NaIO_4$, $RuCl_3$, $CH_3CN/CCl_4/H_2O$, 32−75% combined yield for both isomers.

and water (25.6 mL), successively. The mixture was stirred vigorously for 30 min. The solids were filtered off, rinsed with THF and the combined organics were concentrated in vacuo to provide **28** as a yellow solid (>98% yield, 23 g). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31−7.35 (m, 2H), 7.29 (m, 2 H), 4.05 (br s, 1H), 3.69−3.78 (m, 1H), 3.53 (dd, J = 10.56, 8.02 Hz, 1H), 2.48 (br s, 1H), 1.59 (br s, 2 H). MS (ESI) m/z = 172.0 $[M + H]^+$.

(*R*)-*tert*-Butyl 1-(4-Chlorophenyl)-2-hydroxyethylcarbamate (29). A solution of di-*tert*-butyl dicarbonate (36.6 g, 168 mmol) in DCM (50 mL) was added dropwise to a solution of **28** (23 g, 135 mmol) in DCM (200 mL) at 0 °C. After the addition was completed, the ice bath was removed and the reaction mixture was stirred at room temperature for 1.5 h. The reaction mixture was concentrated in vacuo and the crude material was triturated with 10% DCM/hexane (850 mL) to provide **29** as a white powder (73% yield, 27 g). ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ 7.33−7.39 (m, 2 H), 7.27−7.32 (m, 2 H), 7.24 (d, J = 8.02 Hz, 1 H), 4.75−4.84 (m, 1 H), 4.43−4.56 (m, 1 H), 3.41−3.52 (m, 2 H), 1.36 (s, 9 H). MS (CI+) m/z = 565.0 $[2M + Na]^+$.

(*R*)-*tert*-Butyl 1-(4-Chlorophenyl)-2-oxoethylcarbamate (30). 1,1,1-Tris(acetoxy)-1,1-dihydro-1,2-benziodoxol-3-(1*H*)-one ("Dess−Martin periodinane") (17.4 g, 41.1 mmol) was added in several portions to a stirred suspension of **29** (5.5 g, 20.5 mmol) in wet DCM (40 mL of DCM/40 μL of water) at room temperature. The mixture was stirred for 2 h, diluted with diethyl ether (40 mL), and quenched by adding a solution of $Na_2S_2O_3$ (10 equiv) in saturated aqueous $NaHCO_3$ solution (80 mL) at room temperature. The mixture was stirred vigorously for 10 min, and the layers were separated. The aqueous layer was extracted with diethyl ether (40 mL). The organics were pooled, washed with saturated aqueous $NaHCO_3$ solution and brine, dried over $MgSO_4$, filtered, and the filtrate was concentrated. The crude material was used in the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.46 (br s, 1H), 7.31−7.37 (m, 2H), 7.17−7.24 (m, 2H), 5.58−5.92 (m, 1H), 5.25 (d, J = 3.72 Hz, 1H), 5.14−5.34 (m, 1H), 1.27−1.45 (m, 9H).

Scheme 5[a]



[a]Reagents and conditions: (a) 3,4-dimethoxybenzyl alcohol, NaH, DMF, 60 °C, 2 h, 44%; (b) 4-bromobenzenesulfonyl chloride, DMAP, DCM, rt, 78%; (c) **47**, NaO^tBu, DMF, 85 °C, 12 h, 66%; (d) DDQ, CH_2Cl_2/H_2O, 0 °C, 2 h, 87%; (e) cyanomethyltributylphosphorane, toluene, 110 °C, 12 h, 10−85%; (f) LiHMDS, allyl bromide, −78 °C, 1 h, 85%, 2:1 mixture of isomers; (g) $NaIO_4$, $RuCl_3$, $CH_3CN/CCl_4/H_2O$, 32−75% combined yield for both isomers.

***tert*-Butyl (1*R*,2*R*)-2-(3-Chlorophenyl)-1-(4-chlorophenyl)-2-hydroxyethylcarbamate (31).** A 500 mL three-neck flask was charged with a magnetic stir bar, magnesium (2.163 g, 89 mmol), a crystal of iodine, and anhydrous diethyl ether (100 mL). The flask was equipped with an addition funnel and a reflux condensor. The addition funnel was charged with a solution of 1-bromo-3-chlorobenzene (89 mmol) in anhydrous ether. An amount of 15 mL of this solution was added dropwise into the flask under vigorous stirring. Then the reaction mixture was heated to reflux for about 5 min until the color of the iodine was no longer visible. The heating source was removed, and the remainder of the 1-bromo-3-chlorobenzene solution was added dropwise at a rate to maintain gentle reflux. After the addition was completed, the reaction mixture was maintained at reflux temperature for another 30 min. The reaction mixture turned light brown during the heating. The oil bath was removed, and without cooling, a solution of **30** (4.80 g, 17.80 mmol) in anhydrous diethyl ethyl ether (66 mL)

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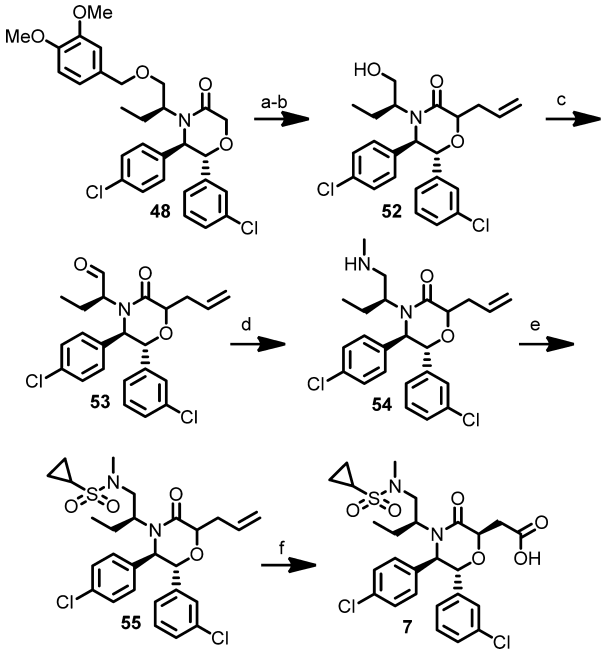
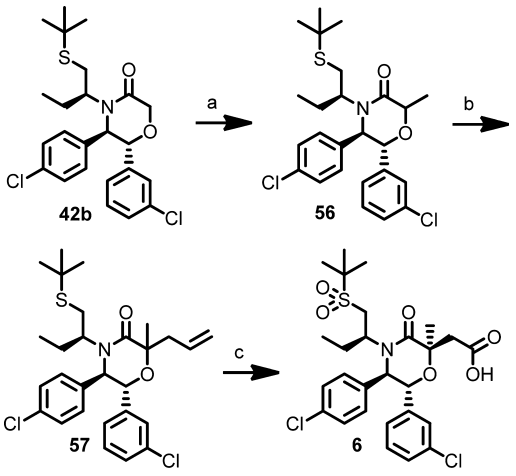
Scheme 6[a]



[a]Reagents and conditions: (a) LHMDS, allyl bromide, −78 °C, 1 h, 85%, 2:1 mixture of isomers; (b) DDQ, CH_2Cl_2/H_2O, 0 °C, 2 h, 87%; (c) DMP, DCM, 1 h, rt, quant; (d) $MeNH_2$, $Na(OEt)_3BH$, DCE, 12 h, 83%; (e) cyclopropanesulfonyl chloride, TEA, DCM, rt, 24 h, 38% (three steps); (f) $NaIO_4$, $RuCl_3$, $CH_3CN/CCl_4/H_2O$, 65%, combined yield for both isomers.

Scheme 7[a]



[a]Reagents and conditions: (a) LiHMDS, MeI, −78 °C, 1 h, 85%, ∼1.5:1 mixture of isomers; (b) LiHMDS, allyl bromide, −30 °C, 12 h, 45%, ∼1.5:1 mixture of isomers; (c) $NaIO_4$, $RuCl_3$, $CH_3CN/CCl_4/H_2O$, 49%, combined yield for both isomers.

was added over a period of 30 min. The mixture was stirred for an additional 2 h at room temperature. The reaction mixture was poured into 200 mL of a cold saturated aqueous NH_4Cl solution. The two layers were separated, and the aqueous layer was extracted with ethyl acetate (2 × 100 mL). The combined organic layers were washed with water and then brine, dried over Na_2SO_4, filtered, and the filtrate was concentrated. The residue was washed with 100 mL of hexanes, filtered, and dried to provide the title compound as a mixture of two diastereomers (anti/syn = 9:1 by NMR). The crude material was used in the next step without further purification (49% combined yield, 3.2 g). ^1H NMR (500 MHz, $CDCl_3$) δ 7.16−7.32 (8H, m), 5.38 (1H, d, J = 4.4 Hz), 4.85 (1H, d, J = 5.0 Hz), 1.32 (9H, s). MS (ESI) m/z = 404.0 $[M + Na]^+$.

(1*R*,2*R*)-2-Amino-1-(3-chlorophenyl)-2-(4-chlorophenyl)-ethanol Hydrochloride (32). Compound **31** (3.0 g, 7.49 mmol) was treated with 20 mL of 4 N HCl in dioxane at room temperature for 2 h. The solvent was evaporated. The residue was triturated with diethyl ether, filtered, washed with diethyl ether, and dried under vacuum to give **32** (>98% yield, 2.4 g). ^1H NMR (400 MHz, CD_3OD) δ 7.36 (2H, d, J = 12 Hz), 7.27 (5H, m), 7.07 (1H, d, J = 8 Hz), 4.90 (1H, d, J = 8 Hz), 4.36 (1H, d, J = 12 Hz). MS (ESI) m/z = 282.0 $[M + H]^+$.

1-Chloro-4-(nitromethyl)benzene (34). A suspension of $AgNO_2$ (392 g) in diethyl ether (1.6 L) was cooled to 0 °C, and a solution of 4-chlorobenzyl bromide (395 g, 1.92 mol) in diethyl ether (1.6 L) was added dropwise over 1 h (temperature maintained below 3 °C during addition). The reaction mixture was stirred for 16 h at 0 °C in the dark. Then the mixture was filtered and the solids were washed with diethyl ether (3×). The combined filtrates were concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (eluent, 0−10% EtOAc in heptane; gradient elution) to give **34** (62% yield, 205 g). ^1H NMR (300 MHz, $CDCl_3$) δ 7.41 (s, 4H), 5.42 (s, 2H). MS (ESI) m/z = 172.0 $[M + H]^+$.

(±)-2-Amino-1-(3-chlorophenyl)-2-(4-chlorophenyl)ethanol ((±)-32). To a flask containing 3-chlorobenzaldehyde (135 mL, 168 g, 1.19 mol) were added **34** (205 g, 1.19 mol), alumina (135 g), pyridine (96 mL, 1.19 mol), and chlorotriethylsilane (200 mL, 180 g, 1.19 mol). The flask was covered in aluminum foil and spun for 16 h in the dark at room temperature on a rotary evaporator. The resulting thick paste was then filtered and washed with isopropanol. The filtrate was divided into two equal portions. The procedure for each portion is as follows: To each solution was added 1 M HCl (7 L, 7 mol), and then Zn powder (800 g, 12.3 mol) was added in several portions. The reaction mixture was stirred until the observed exothermic reaction (to 35 °C) was complete (90 min). Then the mixture was cooled to 0 °C and basified with 30% NaOH to approximately pH 10. The suspension was filtered through a pad of Celite and washed with DCM. The filtrate was transferred to a separatory funnel, and the layers were separated. The aqueous layer was extracted with DCM. The combined organic layers were dried over Na_2SO_4, filtered, and the filtrate was concentrated under reduced pressure. The residue was dissolved in MTBE (1.5 L) and cooled to 0 °C. Then 4 N HCl in dioxane (375 mL, 1.5 mol) was added dropwise. The solid was collected by filtration. The solid was purified by crystallization from dioxane/ethanol to give a racemic mixture of **32** (20% overall yield, 76 g). MS (ESI) m/z = 281.9 $[M + H]^+$.

(*R*,*R*)-2-Amino-1-(3-chlorophenyl)-2-(4-chlorophenyl)-ethanol ((*R*,*R*-)36). *trans*-**32** (900 g, 2.8 mols) (>99.5% purity) was basified with 1 M NaOH (8 L) and extracted with EtOAc (6 L x 2). The EtOAc layers were combined, washed with water and brine, dried and concentrated, affording 800 g of free amine. The free amine was taken in EtOH (32 L) and (+)-di-*p*-toluoyl-D-tartaric acid (1095 g) was added. The mixture was heated to reflux and water was added until the solution became clear (2.4 L). The solution was allowed to cool overnight. The solid was filtered, washed with EtOH, and dried, affording 1.2 kg of wet salt (74% ee). The salt was taken in EtOH (24 L) and water (1.8 L), heated to reflux, and cooled overnight. The solid was filtered, washed with EtOH, and dried, affording 900 g of wet salt (92% ee). The salt was taken in EtOH (18 L) and water (1.2 L), heated to reflux, and cooled overnight. The solid was filtered, washed with EtOH, and dried, affording 600 g of wet salt (>97% ee). The salt was basified with 2 M NaOH (8 L) and extracted with EtOAc (4 L x 2). The EtOAc layers were combined, dried over Na_2SO_4, and concentrated in vacuo. **36** (>95% purity, > 97%ee) was obtained as a white solid (38% yield, 302 g). ^1H NMR (300 MHz, CD_3OD) δ 7.11−7.21 (m, 7H), 6.96−6.98 (m, 1H), 4.67 (d, J = 7.8 Hz, 1H), 3.92 (d, J = 7.8 Hz, 1H). MS (ESI) m/z = 282.0 $[M + H]^+$. Enantiomeric excess determined via chiral HPLC (OD-H, 250 × 4.6 mm, hexane:IPA = 90:10, 215, and 230 nm, 20 min method) 11.3 min (major, 99.8%) and 15.9 min (minor, 0.2%). Optical rotation for major isomer: $[\alpha]_D^{23.5}$ = +92.7° (c = 0.385, in MeOH).

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(1*R*,2*R*)-2-((*tert*-Butyldimethylsilyl)oxy)-2-(3-chlorophenyl)-1-(4-chlorophenyl)ethanamine (37). To a solution of (1*R*,2*R*)-2-amino-1-(3-chlorophenyl)-2-(4-chlorophenyl)ethanol (**36**) (1.0 g, 3.54 mmol) and imidazole (0.483 g, 7.09 mmol) in DCM (3.54 mL) was added *tert*-butyldimethylchlorosilane (0.588 g, 3.90 mmol) at room temperature. The mixture was stirred overnight at room temperature. After this period the reaction mixture was diluted with water (10 mL) and extracted with DCM (3 × 20 mL). The organic extract was dried over $MgSO_4$. The solution was filtered and concentrated under vacuum to give the crude material as a light-yellow oil. The crude material was adsorbed onto a plug of silica gel and purified by chromatography through a Redi-Sep prepacked silica gel column (120 g), eluting with isocratic 70/25/5 DCM/acetone/MeOH with 0.1% of triethylamine to provide **37** (90% yield, 1.26 g) as a light-yellow oil. ^{1}H NMR (400 MHz, CD_3OD) δ 7.12−7.30 (m, 7H), 7.07 (dt, J = 1.76, 4.11 Hz, 1H), 4.71 (d, J = 5.87 Hz, 1H), 3.97 (d, J = 5.67 Hz, 1H), 0.86−0.90 (m, 9H), −0.13 to −0.06 (m, 3H), −0.31 to −0.21 (m, 3H). MS (ESI) m/z = 396.2 $[M + H]^+$.

(*R*)-1-(((1*R*,2*R*)-2-((*tert*-Butyldimethylsilyl)oxy)-2-(3-chlorophenyl)-1-(4-chlorophenyl)ethyl)amino)butan-2-ol (38b). A solution of (1*R*,2*R*)-2-((*tert*-butyldimethylsilyl)oxy)-2-(3-chlorophenyl)-1-(4-chlorophenyl)ethanamine (**37**) (1.22 g, 3.08 mmol), (*R*)-(+)-1,2-epoxybutane (0.288 mL, 4.00 mmol), and ytterbium(III) trifluoromethanesulfonate (0.345 mL, 0.462 mmol) in acetonitrile (10 mL) was heated at 130 °C for 3 h in a microwave. This was diluted with water and extracted with ethyl acetate (3×). The combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated to provide **38b** (1.39 g, 96% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.19 (d, J = 8.61 Hz, 4H), 7.14−7.22 (m, 1H), 7.06−7.13 (m, 2H), 7.01 (d, J = 8.41 Hz, 2H), 6.80 (d, J = 7.63 Hz, 1H), 4.55 (d, J = 7.24 Hz, 1H), 3.73 (d, J = 7.24 Hz, 1H), 3.42−3.54 (m, 1H), 2.56 (d, J = 11.93 Hz, 1H), 1.31−1.49 (m, 3H), 0.91 (s, 9H), −0.04 (s, 3H), −0.23 (s, 3H). MS (ESI) m/z = 468.1 $[M + H]^+$.

(*S*)-1-((1*R*,2*R*)-2-((*tert*-Butyldimethylsilyl)oxy)-2-(3-chlorophenyl)-1-(4-chlorophenyl)ethyl)-2-ethylaziridine (39b). To a solution of triphenylphosphine (2.55 g, 9.72 mmol) and **38b** (4.14 g, 8.84 mmol) dissolved in THF (15 mL) was added diethyl azodicarboxylate, 40 wt % solution in toluene (3.83 mL, 9.72 mmol). The mixture was stirred at 40 °C overnight. The crude mixture was concentrated, adsorbed onto a plug of silica gel, and purified by chromatography through a Redi-Sep prepacked silica gel column (120 g), eluting with isocratic 5−10% EtOAc in hexanes to provide **39b** (69% yield, 3.0 g) as a light-yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.29−7.38 (m, 3H), 7.12 (d, J = 7.83 Hz, 2H), 7.03 (d, J = 16.38 Hz, 1H), 6.96 (d, J = 8.31 Hz, 1H), 6.73−6.78 (m, 1H), 4.84 (d, J = 6.36 Hz, 1H), 3.72−3.87 (m, 1H), 3.49−3.66 (m, 1H), 2.50 (d, J = 6.36 Hz, 1H), 1.79 (d, J = 3.42 Hz, 1H), 1.64 (d, J = 6.36 Hz, 1H), 1.26−1.30 (m, 2H), 0.85−0.90 (m, 9H), 0.68 (t, J = 7.34 Hz, 3H), 0.10 (s, 3H), −0.18 (s, 3H). MS (ESI) m/z = 450.2 $[M + H]^+$.

(*S*)-*N*-((1*R*,2*R*)-2-((*tert*-Butyldimethylsilyl)oxy)-2-(3-chlorophenyl)-1-(4-chlorophenyl)ethyl)-1-(*tert*-butylthio)butan-2-amine (40b). To a solution of (*S*)-1-((1*R*,2*R*)-2-((*tert*-butyldimethylsilyl)oxy)-2-(3-chlorophenyl)-1-(4-chlorophenyl)ethyl)-2-ethylaziridine (**39b**) (170 mg, 0.377 mmol) in DCM (1.9 mL) at room temperature were added 2-methyl-2-propanethiol (85 μL, 0.755 mmol) and indium(III) chloride (0.835 mg, 3.77 μmol). The light yellow slurry mixture was stirred at room temperature overnight. The mixture was concentrated to give quantitatively **40b** (>98% yield, 206 mg). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.04 (br s, 7H), 6.57−6.81 (m, 1H), 4.34−4.57 (m, 1H), 3.69−3.98 (m, 1H), 2.46−2.68 (m, 2H), 2.21−2.44 (m, 2H), 1.45 (bs, 2H), 1.32 (br s, 9H), 0.94 (s, 9H), 0.89 (t, J = 7.35 Hz, 3H), 0.05 (br s, 3H), −0.21 (s, 3H). MS (ESI) m/z = 540.3 $[M + H]^+$.

(5*R*,6*R*)-4-((*S*)-1-(*tert*-Butylthio)butan-2-yl)-6-(3-chlorophenyl)-5-(4-chlorophenyl)morpholin-3-one (42b). *Step A.* To a 250 mL round-bottomed flask were added (*S*)-*N*-((1*R*,2*R*)-2-((*tert*-butyldimethylsilyl)oxy)-2-(3-chlorophenyl)-1-(4-chlorophenyl)ethyl)-1-(*tert*-butylthio)butan-2-amine **40b** (3.07 g, 5.68 mmol) and tetrabutylammonium fluoride, 1.0 M in tetrahydrofuran (14.19 mL, 14.19 mmol) in THF (10 mL), at room temperature. The mixture was stirred for 1 h at this temperature. The reaction was quenched with water (10 mL). The mixture was extracted with diethyl ether (3 × 10 mL), and the organic extracts were dried over $MgSO_4$. The solution was filtered and concentrated under vacuum to give the crude material as a light-yellow oil.

The crude material was adsorbed onto a plug of silica gel and purified by chromatography through a Redi-Sep prepacked silica gel column (40 g), eluting with three-step isocratic 8−20% acetone in hexanes to provide (1*R*,2*R*)-2-(((*S*)-1-(*tert*-butylthio)butan-2-yl)-amino)-1-(3-chlorophenyl)-2-(4-chlorophenyl)ethanol (73.1% yield, 1.8 g). MS (ESI) m/z = 426.2 $[M + H]^+$.

Step B. To a 100 mL round-bottomed flask were added (1*R*,2*R*)-2-(((*S*)-1-(*tert*-butylthio)butan-2-yl)amino)-1-(3-chlorophenyl)-2-(4-chlorophenyl)ethanol (1.74 g, 4.08 mmol) and bromoacetic acid (0.680 g, 4.90 mmol) in THF (13.60 mL). To this solution sodium hydride, 60% in oil (0.408 g, 10.20 mmol), was added in portions over a period of 10 min. The mixture was allowed to stir at room temperature overnight. After this period the crude mixture was diluted in water (10 mL) and extracted with diethyl ether (3 × 20 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated. The residue was used in the next step without further purification. MS (ESI) m/z = 484.2 $[M + H]^+$.

Step C. To a solution of 2-((1*R*,2*R*)-2-(((*S*)-1-(*tert*-butylthio)butan-2-yl)amino)-1-(3-chlorophenyl)-2-(4-chlorophenyl)ethoxy)acetic acid (1.98 g, 4.09 mmol) and *N*,*N*-diethylpropan-2-amine (1.273 mL, 8.17 mmol) in DMF (13.62 mL) was added HATU (1.865 g, 4.90 mmol). The mixture was stirred overnight at room temperature. After this period, the crude mixture was quenched with water and extracted with diethyl ether (3 × 30 mL). The organic extracts were dried over $MgSO_4$, filtered, and concentrated. The crude material was adsorbed onto a plug of silica gel and purified by chromatography through a Redi-Sep prepacked silica gel column (40 g), eluting with three-step isocratic 5−15% acetone in hexanes to provide **42b** (70% yield over the last two steps, 1.34 g) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30 (d, J = 8.41 Hz, 2H), 7.23−7.27 (m, 1H), 7.12−7.20 (m, 2H), 7.08 (d, J = 8.41 Hz, 2H), 6.83 (d, J = 6.65 Hz, 1H), 4.82 (d, J = 8.80 Hz, 1H), 4.56 (d, J = 9.00 Hz, 1H), 4.43 (q, J = 16.43 Hz, 2H), 3.27 (dd, J = 9.98, 12.52 Hz, 1H), 2.87−3.03 (m, 1H), 2.58 (dd, J = 4.50, 12.52 Hz, 1H), 1.94−2.22 (m, 1H), 1.55−1.61 (m, 1H), 1.31−1.37 (m, 9H), 0.64 (t, J = 7.53 Hz, 3H). MS (ESI) m/z = 466.2 $[M + H]^+$.

2-((2*R*,5*R*,6*R*)-4-((*S*)-1-(*tert*-Butylsulfonyl)butan-2-yl)-6-(3-chlorophenyl)-5-(4-chlorophenyl)-3-oxomorpholin-2-yl)acetic Acid (5). Compound **5** was prepared from **42b** as an amorphous solid through a procedure similar to that described elsewhere.[25] ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.18−7.27 (m, 5H), 7.11−7.17 (m, 1H), 7.07 (t, J = 7.82 Hz, 1H), 6.97 (d, J = 7.63 Hz, 1H), 5.06 (d, J = 6.46 Hz, 1H), 4.86 (d, J = 6.65 Hz, 1H), 4.63 (t, J = 5.97 Hz, 1H), 3.85 (dd, J = 9.00, 13.69 Hz, 1H), 3.23−3.39 (m, 1H), 3.00 (s, 2H), 2.87 (d, J = 13.69 Hz, 1H), 1.97−2.17 (m, 1H), 1.45−1.64 (m, 1H), 1.31−1.40 (m, 9H), 0.45 (t, J = 7.53 Hz, 3H). MS (ESI) m/z = 556.0 $[M + H]^+$. HRMS (ESI) m/z found 556.1334 $[M + H]^+$, calcd for $C_{26}H_{32}Cl_2NO_6S$ 556.1322.

2-((2*R*,5*R*,6*R*)-4-((*S*)-1-(*tert*-Butylsulfonyl)propan-2-yl)-6-(3-chlorophenyl)-5-(4-chlorophenyl)-3-oxomorpholin-2-yl)acetic Acid (21). Compound **21** was prepared as a single isomer from **37** (11% overall yield for the last eight steps) through similar procedures to those described for the synthesis of **5** in Scheme 2. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31 (d, J = 8.41 Hz, 2H), 7.20−7.22 (m, 1H), 7.04−7.16 (m, 4H), 6.81 (d, J = 7.82 Hz, 1H), 5.02 (d, J = 9.59 Hz, 1H), 4.73 (dd, J = 7.04, 4.69 Hz, 1H), 4.64 (d, J = 9.78 Hz, 1H), 4.15 (dd, J = 13.40, 9.68 Hz, 1H), 3.57−3.71 (m, 1H), 3.26 (dd, J = 16.24, 7.24 Hz, 1H), 2.97 (dd, J = 16.24, 4.70 Hz, 1H), 2.85 (dd, J = 13.40, 3.42 Hz, 1H), 1.44 (br s, 9 H), 1.37 (d, J = 6.85 Hz, 3H). MS (ESI) m/z = 542 $[M + H]^+$. HRMS (ESI) m/z found 542.1177 $[M + H]^+$, calcd for $C_{25}H_{30}Cl_2NO_6S$ 542.1165.

2-((2*R*,5*R*,6*R*)-4-((*S*)-1-(*tert*-Butylsulfonyl)-4,4,4-trifluorobutan-2-yl)-6-(3-chlorophenyl)-5-(4-chlorophenyl)-3-oxomorpholin-2-yl)acetic Acid (22). Compound **22** was prepared as a single isomer from **37** (2% overall yield for the last eight steps)

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through procedures similar to those described for the synthesis of **5** in Scheme 2. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31−7.36 (m, 2H), 7.25−7.30 (m, 2H), 7.20−7.24 (m, 2H), 7.13 (t, J = 8.12 Hz, 1H), 6.88−7.04 (m, 1H), 5.18 (d, J = 7.83 Hz, 1H), 5.04 (d, J = 7.83 Hz, 1H), 4.82 (dd, J = 6.85, 4.11 Hz, 1H), 4.05−4.19 (m, 1H), 3.81−3.94 (m, 1H), 3.22 (dd, J = 16.92, 6.94 Hz, 2H), 2.92−3.14 (m, 2H), 2.26−2.42 (m, 1H), 1.36−1.51 (m, 9H). MS (ESI) m/z = 610 $[M + H]^+$. HRMS (ESI) m/z found 610.1050 $[M + H]^+$, calcd for $C_{26}H_{29}Cl_2NO_6S$ 610.1039.

2-((2*R*,5*R*,6*R*)-4-((*S*)-1-(*tert*-Butylsulfonyl)-3-methylbutan-2-yl)-6-(3-chlorophenyl)-5-(4-chlorophenyl)-3-oxomorpholin-2-yl)acetic Acid (23). Compound **23** was prepared as a single isomer from **37** (3% overall yield for the last eight steps) through procedures similar to those described for the synthesis of **5** in Scheme 2. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.49 (d, J = 8.22 Hz, 2H), 7.33−7.42 (m, 3H), 7.22 (d, J = 0.98 Hz, 3H), 5.28−5.39 (m, 1H), 5.16 (s, 1H), 4.66 (t, J = 6.16 Hz, 1H), 3.77 (dd, J = 7.43, 14.09 Hz, 1H), 3.30 (t, J = 7.53 Hz, 1H), 3.09−3.22 (m, J = 3.23, 6.16 Hz, 3H), 2.33−2.49 (m, 1H), 1.49 (s, 9H), 0.78 (d, J = 6.65 Hz, 3H), 0.53 (d, J = 6.85 Hz, 3H). MS (ESI) m/z = 570.0 $[M + H]^+$. HRMS (ESI) m/z found 570.1490 $[M + H]^+$, calcd for $C_{27}H_{34}Cl_2NO_6S$ 570.1478.

2-((2*R*,5*R*,6*R*)-4-((*S*)-1-(*tert*-Butylsulfonyl)-3,3-dimethylbutan-2-yl)-6-(3-chlorophenyl)-5-(4-chlorophenyl)-3-oxomorpholin-2-yl)acetic Acid (24). Compound **24** was prepared as a single isomer from **37** (2% overall yield for the last eight steps) through procedures similar to those described for the synthesis of **5** in Scheme 2. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33 (d, J = 8.41 Hz, 2H), 7.27−7.30 (m, 3H), 7.08−7.23 (m, 3H), 5.34 (d, J = 7.24 Hz, 1H), 5.20 (d, J = 7.43 Hz, 1H), 4.76 (t, J = 6.26 Hz, 1H), 3.95 (dd, J = 9.59, 13.69 Hz, 1H), 3.60 (d, J = 2.35 Hz, 1H), 3.51 (s, 1H), 3.12 (d, J = 6.26 Hz, 1H), 2.99 (dd, J = 2.35, 13.69 Hz, 1H), 1.48 (s, 9H), 0.78 (s, 9H). MS (ESI) m/z = 584.0 $[M + H]^+$. HRMS (ESI) m/z found 584.1642 $[M + H]^+$, calcd for $C_{28}H_{36}Cl_2NO_6S$ 584.1635.

(5*R*,6*R*)-6-(3-Chlorophenyl)-5-(4-chlorophenyl)morpholin-3-one (43). To a solution of (1*R*,2*R*)-2-amino-1-(3-chlorophenyl)-2-(4-chlorophenyl)ethanol (**36**) (0.47 g, 1.7 mmol) and triethylamine (0.349 mL, 2.5 mmol) in THF at 0 °C was added chloroacetyl chloride (0.16 mL, 2.0 mmol). The reaction mixture was stirred at 0 °C for 1 h. After this time, saturated aqueous NH_4Cl solution and ethyl acetate were added. The layers were separated and the combined organic layers were washed with water (3 × 10 mL), dried over $MgSO_4$, filtered and the filtrate was concentrated in vacuum to give 2-chloro-*N*-((1*R*,2*R*)-2-(3-chlorophenyl)-1-(4-chlorophenyl)-2-hydroxyethyl)-acetamide as a light yellow oil which was taken to the next step without further purification. MS (ESI) m/z 380.0 $[M + Na]^+$.

The product above was dissolved in THF (15 mL) and treated with several portions of sodium hydride (60% dispersion in mineral oil, 0.167 g, 4.16 mmol) over a period of 5 min. The mixture was stirred at room temperature for 5 h. After this time, saturated aqueous NH_4Cl solution and ethyl acetate were added. The organic extracts were separated and the combined organic layers were washed with water (3 × 10 mL), dried over $MgSO_4$, filtered and the filtrate was concentrated in vacuo to give the crude material as a yellow oil. The crude was absorbed onto a plug of silica gel and purified by silica gel column chromatography, eluting with a gradient of 0−30% acetone in hexanes to provide **43** (86% yield, 0.49 g). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47−7.54 (m, 1H), 7.46−7.54 (m, 1H), 7.34 −7.42 (m, 2H), 7.29−7.32 (m, 2H), 7.26−7.29 (m, 1H), 7.13−7.25 (m, 1H), 6.98 (d, J = 8.41 Hz, 1H), 4.57−4.65 (m, 1H), 4.45 (d, J = 9.98 Hz, 1H), 4.00 (d, J = 5.67 Hz, 2H). MS (ESI) m/z = 322.2 $[M]^+$.

Ethyl 2-((2*R*,3*R*)-2-(3-Chlorophenyl)-3-(4-chlorophenyl)-5-oxomorpholino)-2-cyclopropylacetate (44). To a solution of (5*R*,6*R*)-6-(3-chlorophenyl)-5-(4-chlorophenyl)morpholin-3-one (**43**) (4.3 g, 13.35 mmol) in DMF (26.7 mL) was added sodium hydride (1.068 g, 26.7 mmol) at 0 °C, and the mixture was stirred at this temperature for 30 min. To the mixture was added racemic ethyl 2-bromo-2-cyclopropylacetate (3.71 mL, 26.7 mmol) in DMF (40 mL) dropwise and was stirred at room temperature for 2 h. The reaction was quenched with saturated aqueous NH_4Cl (10 mL) and diluted with diethyl ether (10 mL). The solution was washed with 10% citric acid (10 mL), 5% $NaHCO_3$ (10 mL), water (10 mL), brine (10 mL). The combined organic extracts were dried with $MgSO_4$. The solvent was evaporated under reduced pressure, and the residue (a 45:55 **44a**/**44b** mixture of epimers) was purified by flash chromatography on silica gel (120 g), eluting with 5% ethyl acetate in DCM to give first **44a** (40% yield, 2.4 g) and then **44b** (48% yield, 2.9 g).

Characterization of (*S*)-Ethyl 2-((2*R*,3*R*)-2-(3-Chlorophenyl)-3-(4-chlorophenyl)-5-oxomorpholino)-2-cyclopropylacetate (44a). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.28−7.31 (m, 2H), 7.21−7.27 (m, 2H), 7.12−7.20 (m, 3H), 6.90 (d, J = 7.83 Hz, 1H), 4.88 (d, J = 7.34 Hz, 1H), 4.73 (d, J = 7.34 Hz, 1H), 4.35−4.54 (m, 2H), 4.13 (q, J = 7.09 Hz, 1H), 3.95−4.09 (m, 1H), 3.53 (d, J = 10.51 Hz, 1H), 1.30−1.42 (m, 1H), 1.23 (t, J = 7.09 Hz, 3H), 0.65−0.77 (m, 1H), 0.56 (dd, J = 4.89, 7.83 Hz, 1H), 0.33 (dd, J = 5.26, 9.90 Hz, 1H), −0.05 (dd, J = 5.87, 9.54 Hz, 1H). MS (ESI) m/z = 448.0 $[M + H]^+$.

Characterization of (*R*)-Ethyl 2-((2*R*,3*R*)-2-(3-Chlorophenyl)-3-(4-chlorophenyl)-5-oxomorpholino)-2-cyclopropylacetate (44b). ^{1}H NMR (500 MHz, $CDCl_3$) d 7.28 (s, 1H), 7.24−7.27 (m, 2H), 7.21 (t, J = 1.83 Hz, 1H), 7.09−7.18 (m, 3H), 6.83 (d, J = 7.83 Hz, 1H), 4.83 (d, J = 8.07 Hz, 1H), 4.65 (d, J = 8.07 Hz, 1H), 4.46 (d, J = 3.42 Hz, 2H), 4.09−4.28 (m, 2H), 2.85 (d, J = 10.03 Hz, 1H), 1.36−1.48 (m, 1H), 1.28−1.32 (m, 3H), 0.68 (d, J = 8.56 Hz, 1H), 0.53 (d, J = 7.82 Hz, 1H), 0.10−0.20 (m, 2H). MS (ESI) m/z = 448.0 $[M + H]^+$.

(*S*)-Ethyl 2-((2*R*,3*R*)-2-(3-Chlorophenyl)-3-(4-chlorophenyl)-5-oxomorpholino)-2-cyclopropylacetate (44a) from (*R*)-Ethyl 2-((2*R*,3*R*)-2-(3-Chlorophenyl)-3-(4-chlorophenyl)-5-oxomorpholino)-2-cyclopropylacetate (44b). Sodium ethoxide (0.144 mL, 0.386 mmol) was added to a solution of (*R*)-ethyl 2-((2*R*,3*R*)-2-(3-chlorophenyl)-3-(4-chlorophenyl)-5-oxomorpholino)-2-cyclopropylacetate (0.346 g, 0.772 mmol) in anhydrous ethanol (1.543 mL) at room temperature. After 4 h, the reaction mixture was neutralized with 3 M HCl (0.13 mL). The mixture was partitioned between water and EtOAc, and the layers were separated. The aqueous layer was extracted with EtOAc twice, and the organics were pooled and washed with brine. The combined organic phase was dried over Na_2SO_4, filtered, and concentrated in vacuo to provide a crude that was subsequently purified by flash chromatography on silica gel, eluting with 5% ethyl acetate in DCM to provide **44b** (57% yield, 0.2 g) (see experimental above for characterization of **44b**).

(5*R*,6*R*)-6-(3-Chlorophenyl)-5-(4-chlorophenyl)-4-((*S*)-1-cyclopropyl-2-hydroxyethyl)morpholin-3-one (45). To a solution of **44a** (3.6 g, 8.29 mmol) in THF (8.29 mL) was added superhydride (lithium triethylborohydride, 1.0 M solution in THF) (17.4 mL, 17.4 mmol) at 0 °C. After 15 min, LCMS showed complete conversion to the desired product. MeOH (3 mL) was added dropwise over a period of 1 min. Then potassium peroxymonosulfate (Oxone) (15.29 g, 24.87 mmol) in water (60 mL) was added dropwise over a period of 10 min. After 1 h, saturated aqueous $NaHSO_3$ (9 mL) was added at room temperature. Extraction was with diethyl ether (2 × 60 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated under a vacuum. The crude material was adsorbed onto a plug of silica gel and purified by flash chromatography through a silica gel column (330 g), eluting with isocratic 20% acetone in hexanes to provide **45** as an off-white solid (90% yield, 3.0 g). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.04−7.25 (m, 7H), 6.79 (d, J = 7.63 Hz, 1H), 4.87 (d, J = 7.43 Hz, 1H), 4.52 (d, J = 7.43 Hz, 1H), 4.26−4.42 (m, 2H), 3.50−3.59 (m, 1H), 3.13−3.36 (m, 2H), 2.88 (br s, 1H), 0.79 (ddd, J = 3.03, 4.94, 7.87 Hz, 1H), 0.40−0.57 (m, 2H), 0.11−0.23 (m, 1H), −0.10 to 0.06 (m, 1H). MS (ESI) m/z = 405.4 $[M + H]^+$.

2-((2*R*,5*R*,6*R*)-4-((*S*)-2-(*tert*-Butylsulfonyl)-1-cyclopropylethyl)-6-(3-chlorophenyl)-5-(4-chlorophenyl)-3-oxomorpholin-2-yl)acetic Acid (4). Compound **4** was prepared as a single isomer from **45** (24% yield, last three steps) through a procedure similar to that described for the synthesis of **5** (for Mitsunobu reaction conditions see synthesis of **50a** below and ref 34). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.28 (s, 5H), 7.06−7.17 (m, 3H), 5.10 (d, J = 4.70 Hz, 1H), 4.86 (d, J = 4.70 Hz, 1H), 4.50 (t, J = 6.16 Hz, 1H), 3.74−3.98 (m, 1H), 2.90−3.16 (m, J = 5.67 Hz, 2H), 2.01−2.08 (m, 1H), 1.35 (s, 9H), 1.09−1.28 (m, 1H), 0.22−0.43 (m, 2H), −0.13 to 0.07 (m, 1H), −0.69 to

−0.66 (bs, 1H). MS (ESI) m/z = 568.1 $[M + H]^+$. HRMS (ESI) m/z found 568.1331 $[M + H]^+$, calcd for $C_{27}H_{32}Cl_2NO_6S$ 568.1322.

(*R*)-1-((3,4-Dimethoxybenzyl)oxy)butan-2-ol (46). To a suspension of sodium hydride (60% dispersion in mineral oil, 8.91 g, 223 mmol) in DMF (300 mL) at 60 °C was added dropwise a solution of 3,4-dimethoxybenzyl alcohol (29.4 mL, 202 mmol) in DMF (100 mL) over a period of 30 min. The mixture was stirred at 60 °C for 0.5 h until H_2 evolution ceased. The mixture was cooled to 45 °C, and then (*R*)-2-ethyloxirane (17.61 mL, 202 mmol) was added dropwise. The mixture was allowed to stir at 45 °C overnight. The reaction mixture was diluted with saturated aqueous $NaHCO_3$ solution (300 mL) and extracted with diethyl ether (3 × 300 mL). The organic extract was washed with water (3 × 300 mL) and dried over $MgSO_4$. The solution was filtered and concentrated under reduced pressure. The residue was adsorbed onto a plug of silica gel and purified by chromatography through a silica gel column (330 g, RediSep Rf, Teledyne Isco, Lincoln, NE), eluting with a gradient of 10−40% acetone in hexanes to provide **46** as an off-white oil (44% yield, 21.4 g). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.81−6.93 (m, 3H), 4.51 (s, 2H), 3.91 (d, J = 1.00 Hz, 6H), 3.69−3.82 (m, 1H), 3.52 (dd, J = 3.03, 9.49 Hz, 1H), 3.33 (dd, J = 8.02, 9.39 Hz, 1H), 1.44−1.58 (m, 2H), 0.98 (t, J = 7.53 Hz, 3H). MS (ESI) m/z = 263.2 $[M + Na]^+$.

(*R*)-1-((3,4-Dimethoxybenzyl)oxy)butan-2-yl 4-Bromobenzenesulfonate (47). To DMAP (14.54 g, 119 mmol) and (*R*)-1-((3,4-dimethoxybenzyl)oxy)butan-2-ol (**46**) (13.0 g, 54.1 mmol) in CH_2Cl_2 (180 mL) was added 4-bromobenzene-1-sulfonyl chloride (20.74 g, 81 mmol). The mixture was stirred at room temperature overnight. The crude material was partitioned between ethyl acetate (50 mL) and saturated aqueous sodium bicarbonate (100 mL). The organics were sequestered, and the aqueous portion was extracted further with ethyl acetate (50 mL). The organics were combined and washed with 0.1 M HCl (2 × 50 mL). The organics were then washed with brine (50 mL), dried over $MgSO_4$, filtered, and the filtrate was concentrated under vacuum. The residue was adsorbed onto a plug of silica gel and purified by chromatography through a silica gel column (330 g, RediSep Rf, Teledyne Isco, Lincoln, NE), eluting with a three-step gradient of 10−30% acetone in hexanes which afforded **47** (78% yield, 19 g). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75 (d, J = 8.56 Hz, 2H), 7.59 (d, J = 8.31 Hz, 2H), 6.66−6.89 (m, 3H), 4.67 (quin, J = 5.62 Hz, 1H), 4.25−4.45 (m, 2H), 3.90 (s, 3H), 3.89 (s, 3H), 3.42−3.58 (m, 2H), 1.64−1.82 (m, 2H), 0.88 (t, J = 7.46 Hz, 3H). MS (ESI) m/z = 481.0 $[M + Na]^+$.

(5*R*,6*R*)-6-(3-Chlorophenyl)-5-(4-chlorophenyl)-4-((*S*)-1-((3,4-dimethoxybenzyl)oxy)butan-2-yl)morpholin-3-one (48). A solution of **43** (260 mg, 0.807 mmol), (*R*)-1-((3,4-dimethoxybenzyl)-oxy)butan-2-yl 4-bromobenzenesulfonate (**47**) (556 mg, 1.210 mmol), and sodium *tert*-butoxide (116 mg, 1.210 mmol) in 1,4-dioxane (2 mL) was stirred at 85 °C overnight. After this period the reaction mixture was poured into saturated aqueous $NaHCO_3$ solution (10 mL) and brine (10 mL) and extracted with 2.5% MeOH in CH_2Cl_2 (3 × 30 mL). The combined organics were dried over sodium sulfate, filtered, and the filtrate was concentrated under vacuum. Silica gel chromatography (gradient elution with 10−40% acetone in hexanes) afforded **48** (66% yield, 289 mg). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29 (s, 4H), 7.21−7.25 (m, 1H), 7.18 (t, J = 1.66 Hz, 1H), 7.11 (t, J = 7.92 Hz, 1H), 7.06 (d, J = 8.41 Hz, 1H), 6.78−6.92 (m, 3H), 4.77 (d, J = 7.83 Hz, 1H), 4.58 (d, J = 7.83 Hz, 1H), 4.45 (s, 2H), 4.37−4.40 (m, 2H), 3.96 (t, J = 9.49 Hz, 1H), 3.91 (s, 3H), 3.89 (s, 3H), 1.80−1.95 (m, J = 7.14, 14.77 Hz, 1H), 1.61 (bs, 2H), 1.64−1.72 (m, 1H), 0.63 (t, J = 7.53 Hz, 3H). MS (ESI) m/z = 566.2 $[M + Na]^+$.

(5*R*,6*R*)-6-(3-Chlorophenyl)-5-(4-chlorophenyl)-4-((*S*)-1-hydroxybutan-2-yl)morpholin-3-one (49). To a 0 °C solution of **48** (140 mg, 0.257 mmol) in CH_2Cl_2 (2.4 mL) and water (129 μL) was added DDQ (70.0 mg, 0.309 mmol). The mixture was stirred at 0 °C. After 2 h the reaction contents were poured into saturated aqueous $NaHCO_3$ solution (50 mL) and CH_2Cl_2 (30 mL). The layers were separated, and the aqueous layer was extracted further with CH_2Cl_2 (2 × 20 mL). The combined organics were dried over sodium sulfate, filtered, and the filtrate was concentrated under vacuum. Silica gel chromatography (gradient elution 10−40% acetone in hexanes) afforded **49** (87% yield, 89 mg). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.22−7.26 (m, 2H), 7.17−7.21 (m, 1H), 7.01−7.13 (m, 4H), 6.75 (d, J = 7.83 Hz, 1H), 4.54 (d, J = 4.70 Hz, 2H), 4.35 (d, J = 2.74 Hz, 2H), 3.53−3.62 (m, 2H), 3.41 (q, J = 7.04 Hz, 1H), 3.26−3.37 (m, 1H), 1.76−1.92 (m, 1H), 1.41 (ddd, J = 6.06, 7.53, 13.79 Hz, 1H), 1.14 (t, J = 6.94 Hz, 1H), 0.62−0.70 (m, 3H). MS (ESI) m/z = 394.2 $[M + H]^+$.

(5*R*,6*R*)-6-(3-Chlorophenyl)-5-(4-chlorophenyl)-4-((*S*)-1-(ethylthio)butan-2-yl)morpholin-3-one (50a). To a solution of **49** (300 mg, 0.761 mmol) and ethanethiol (0.225 mL, 3.04 mmol) in 0.2 mL of toluene was added cyanomethylenetributylphosphorane (0.735 mL, 3.04 mmol). The resulting solution was stirred at 110 °C overnight. After this period, the mixture was concentrated, and the crude material absorbed onto a plug of silica gel and purified by chromatography on silica gel, eluting with 20% acetone in hexanes, provided **50a** as a light yellow oil (85% yield, 283 mg). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.21−7.34 (m, 3H), 7.11−7.18 (m, 2H), 7.07 (d, J = 8.41 Hz, 2H), 6.79 (d, J = 7.83 Hz, 1H), 4.77 (d, J = 9.00 Hz, 1H), 4.56 (d, J = 9.00 Hz, 1H), 4.44 (q, J = 16.43 Hz, 2H), 3.06−3.15 (m, 2H), 2.44−2.62 (m, 3H), 2.04 (td, J = 7.36, 14.43 Hz, 1H), 1.56−1.68 (m, 1H), 1.23−1.31 (m, 3H), 0.66 (t, J = 7.43 Hz, 3H). MS (ESI) m/z = 438.2 $[M + H]^+$

2-((2*R*,5*R*,6*R*)-6-(3-Chlorophenyl)-5-(4-chlorophenyl)-3-oxo-4-((*S*)-1-(*N*-phenylcyclopropanesulfonamido)butan-2-yl)-morpholin-2-yl)acetic Acid (8). Compound **8** was prepared as a single isomer from **49** (6% combined yield for the last three steps) through procedures similar to those described for the synthesis of **5**. The isomers at C2 were separated by reverse phase preparatory HPLC (Gemini Prep C_{18} 5 μm column; Phenomenex, Torrance, CA; gradient elution of 25−75% MeCN in water, both solvents containing 0.1% TFA, 30 min method) to provide **8** as the first eluting isomer. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43−7.54 (m, 4H), 7.33−7.43 (m, 2H), 7.31 (d, J = 1.37 Hz, 1H), 7.16−7.27 (m, 2H), 6.95−7.14 (m, 3H), 4.79 (d, J = 12.52 Hz, 2H), 4.44−4.62 (m, 1H), 4.33 (s, 1H), 3.72−3.92 (m, 1H), 2.91−3.16 (m, 3H), 2.30−2.47 (m, 1H), 1.83−2.07 (m, 1H), 1.45−1.67 (m, 1H), 1.06 (d, J = 4.70 Hz, 2H), 0.93 (d, J = 6.46 Hz, 2H), 0.51 (t, J = 7.53 Hz, 3H). MS (ESI) m/z = 631.1 $[M + H]^+$. HRMS (ESI) m/z found 631.1434 $[M + H]^+$, calcd for $C_{31}H_{33}Cl_2N_2O_6S$ 631.1431.

2-((2*R*,5*R*,6*R*)-6-(3-Chlorophenyl)-5-(4-chlorophenyl)-4-((*S*)-1-(*N*-(2-fluorophenyl)cyclopropanesulfonamido)butan-2-yl)-3-oxomorpholin-2-yl)acetic Acid (9). Compound **9** was prepared as a single isomer from **49** (16% combined yield for the last three steps) through procedures similar to those described for the synthesis of **5**. The isomers at C2 were separated by reverse phase preparatory HPLC (Gemini Prep C_{18} 5 μm column; Phenomenex, Torrance, CA; gradient elution of 25−75% MeCN in water, both solvents containing 0.1% TFA, 30 min method) to provide **9** as the first eluting isomer. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52 (dt, J = 1.56, 7.82 Hz, 1H), 7.35−7.43 (m, 1H), 7.29−7.34 (m, 2H), 7.26−7.28 (m, 1H), 7.25 (s, 1H), 7.14−7.24 (m, 5H), 7.07 (d, J = 7.43 Hz, 1H), 4.90−4.98 (m, 1H), 4.82−4.89 (m, 1H), 4.44 (t, J = 6.36 Hz, 1H), 4.34 (dd, J = 8.90, 14.77 Hz, 1H), 3.81 (dd, J = 4.60, 14.77 Hz, 1H), 3.15 (br s, 1H), 3.04 (d, J = 6.46 Hz, 2H), 2.41−2.55 (m, 1H), 1.83−2.00 (m, J = 7.04, 8.22 Hz, 1H), 1.47−1.65 (m, J = 2.25, 7.14 Hz, 1H), 0.87−1.12 (m, 4H), 0.49 (t, J = 7.53 Hz, 3H). MS (ESI) m/z = 649.0 $[M + H]^+$. HRMS (ESI) m/z found 649.1349 $[M + H]^+$, calcd for $C_{31}H_{32}Cl_2FN_2O_6S$ 649.1337.

2-((2*R*,5*R*,6*R*)-6-(3-Chlorophenyl)-5-(4-chlorophenyl)-4-((*S*)-1-(*N*-(4-fluorophenyl)cyclopropanesulfonamido)butan-2-yl)-3-oxomorpholin-2-yl)acetic Acid (10). Compound **10** was prepared as a single isomer from **49** (6% combined yield for the last three steps) through procedures similar to those described for the synthesis of **5**. The isomers at C2 were separated by reverse phase preparatory HPLC (Gemini Prep C_{18} 5 μm column; Phenomenex, Torrance, CA; gradient elution of 25−75% MeCN in water, both solvents containing 0.1% TFA, 30 min method) to provide **10** as the first eluting isomer. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39−7.46 (m, 2H), 7.32 (d, J = 8.61 Hz, 2H), 7.19−7.28 (m, 3H), 7.10−7.18 (m, 4H), 7.06 (d, J = 7.43 Hz, 1H), 4.84 (td, J = 7.43, 9.19 Hz, 2H), 4.33−4.48 (m, J = 8.80 Hz, 2H), 3.80 (dd, J = 4.79, 14.38 Hz, 2H), 2.95−3.14 (m, 2H), 2.31−2.42 (m, 1H), 1.84−2.00 (m, 1H), 1.51−1.65 (m, 1H), 1.00−1.10 (m, 2H),

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0.89−1.00 (m, 2H), 0.50 (t, J = 7.53 Hz, 3H). MS (ESI) m/z = 649.0 $[M + H]^+$. HRMS (ESI) m/z found 649.1336 $[M + H]^+$, calcd for $C_{31}H_{32}Cl_2FN_2O_6S$ 649.1337.

2-((2*R*,5*R*,6*R*)-6-(3-Chlorophenyl)-5-(4-chlorophenyl)-4-((*S*)-1-(*N*-(2,4-difluorophenyl)cyclopropanesulfonamido)butan-2-yl)-3-oxomorpholin-2-yl)acetic Acid (11). Compound **11** (4%) was prepared as a single isomer through procedures similar to those described for the synthesis of **5**. The isomers at C2 were separated by reverse phase preparatory HPLC (Gemini Prep C_{18} 5 μm column; Phenomenex, Torrance, CA; gradient elution of 25−75% MeCN in water, both solvents containing 0.1% TFA, 30 min method) to provide **11** as the first eluting isomer. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.38−7.54 (m, 1H), 7.13−7.37 (m, 7H), 7.03−7.13 (m, 1H), 6.90−7.03 (m, 2H), 4.96 (d, J = 7.09 Hz, 1H), 4.87 (d, J = 7.34 Hz, 1H), 4.48 (t, J = 6.48 Hz, 1H), 4.20−4.34 (m, 1H), 3.69−3.82 (m, 1H), 3.20 (br s, 1H), 2.97−3.15 (m, 2H), 2.42−2.53 (m, 1H), 1.85−1.94 (m, 1H), 1.48−1.64 (m, 1H), 0.87−1.09 (m, 4H), 0.49 (t, J = 7.46 Hz, 3H). MS (ESI) m/z = 667 $[M + H]^+$.

2-((2*R*,5*R*,6*R*)-6-(3-Chlorophenyl)-5-(4-chlorophenyl)-4-((*S*)-1-(*N*-(2-fluorophenyl)propan-2-ylsulfonamido)butan-2-yl)-3-oxomorpholin-2-yl)acetic Acid (12). Compound **12** was prepared as a single isomer from **49** (28% combined yield for the last three steps) through procedures similar to those described for the synthesis of **5**. The isomers at C2 were separated by reverse phase preparatory HPLC (Gemini Prep C_{18} 5 μm column; Phenomenex, Torrance, CA; gradient elution of 25−75% MeCN in water, both solvents containing 0.1% TFA, 30 min method) to provide **12** as the first eluting isomer. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.67 (dt, J = 1.47, 7.97 Hz, 1H), 7.34−7.43 (m, J = 1.47, 4.79 Hz, 1H), 7.31 (d, J = 8.41 Hz, 2H), 7.26−7.28 (m, 1H), 7.13−7.25 (m, 6H), 7.04 (d, J = 7.63 Hz, 1H), 4.89−4.98 (m, 1H), 4.77−4.87 (m, 1H), 4.37 (t, J = 6.16 Hz, 2H), 3.78 (dd, J = 4.50, 14.87 Hz, 1H), 2.92−3.28 (m, 4H), 1.90 (quint, J = 7.53, 15.06 Hz, 1H), 1.47−1.63 (m, 1H), 1.39 (d, J = 6.85 Hz, 6H), 0.45 (t, J = 7.53 Hz, 3H). MS (ESI) m/z = 651.2 $[M + H]^+$.

2-((2*R*,5*R*,6*R*)-6-(3-Chlorophenyl)-5-(4-chlorophenyl)-4-((*S*)-1-(*N*-(2-fluorophenyl)methylsulfonamido)butan-2-yl)-3-oxomorpholin-2-yl)acetic Acid (13). Compound **13** was prepared as a single isomer from **52** (20% combined yield for the last two steps) through procedures similar to those described for the synthesis of **5**. The isomers at C2 were separated by reverse phase preparatory HPLC (Gemini Prep C_{18} 5 μm column; Phenomenex, Torrance, CA; gradient elution of 25−75% MeCN in water, both solvents containing 0.1% TFA, 30 min method) to provide **13** as the first eluting isomer. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46−7.52 (m, J = 1.57 Hz, 1H), 7.35−7.44 (m, 1H), 7.32 (d, J = 8.41 Hz, 3H), 7.14−7.26 (m, 6H), 7.05 (d, J = 7.63 Hz, 1H), 4.89−4.98 (m, 1H), 4.87 (s, 1H), 4.41 (d, J = 12.72 Hz, 1H), 4.29 (dd, J = 8.71, 14.77 Hz, 1H), 3.73 (dd, J = 4.70, 14.87 Hz, 1H), 3.03 (dd, J = 3.23, 6.36 Hz, 3H), 2.93 (br s, 3H), 1.80−1.98 (m, 1H), 1.49−1.64 (m, 1H), 0.48 (t, J = 7.53 Hz, 3H). MS (ESI) m/z = 623.1 $[M + H]^+$.

2-((2*R*,5*R*,6*R*)-6-(3-Chlorophenyl)-5-(4-chlorophenyl)-4-((*S*)-1-(ethylsulfonyl)butan-2-yl)-3-oxomorpholin-2-yl)acetic Acid (14). Compound **14** was prepared as a single isomer from **49** (14% combined yield for the last three steps) through procedures similar to those described for the synthesis of **5**. The isomers at C2 were separated by reverse phase preparatory HPLC (Gemini Prep C_{18} 5 μm column; Phenomenex, Torrance, CA; gradient elution of 25−75% MeCN in water, both solvents containing 0.1% TFA, 30 min method) to provide **14** as the first eluting isomer. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33−7.38 (m, 2H), 7.25 (td, J = 2.15, 3.91 Hz, 4H), 7.14−7.21 (m, 1H), 7.02 (d, J = 7.63 Hz, 1H), 5.09 (d, J = 7.04 Hz, 1H), 4.95 (d, J = 7.04 Hz, 1H), 4.79 (t, J = 5.87 Hz, 1H), 4.05 (br s, 1H), 3.44 (br s, 1H), 3.01−3.16 (m, 4H), 2.97 (d, J = 12.91 Hz, 1H), 2.14 (ddd, J = 7.34, 9.44, 14.13 Hz, 1H), 1.60 (ddd, J = 4.21, 7.53, 13.89 Hz, 1H), 1.38−1.50 (m, 3H), 0.57 (t, J = 7.43 Hz, 3H). MS (ESI) m/z = 528.0 $[M + H]^+$. HRMS (ESI) m/z found 528.1021 $[M + H]^+$, calcd for $C_{24}H_{28}Cl_2NO_6S$ 528.1009.

2-((2*R*,5*R*,6*R*)-6-(3-Chlorophenyl)-5-(4-chlorophenyl)-4-((*S*)-1-(isopropylsulfonyl)butan-2-yl)-3-oxomorpholin-2-yl)acetic Acid (15). Compound **15** was prepared as a single isomer from **49** (3% combined yield for the last three steps) through procedures similar to those described for the synthesis of **5**. The isomers at C2 were separated by reverse phase preparatory HPLC (Gemini Prep C_{18} 5 μm column; Phenomenex, Torrance, CA; gradient elution of 25−75% MeCN in water, both solvents containing 0.1% TFA, 30 min method) to provide **15** as the first eluting isomer. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32−7.39 (m, 3H), 7.30 (s, 2H), 7.21−7.27 (m, 2H), 7.17 (s, 1H), 6.99−7.07 (m, 1H), 5.07−5.17 (m, 1H), 4.95 (s, 1H), 4.74 (s, 1H), 3.11 (d, J = 6.06 Hz, 3H), 2.86−2.98 (m, 1H), 2.05−2.21 (m, 1H), 1.55−1.69 (m, 1H), 1.43 (dd, J = 4.30, 6.85 Hz, 6H), 0.56 (t, J = 7.53 Hz, 3H). MS (ESI) m/z = 542.0 $[M + H]^+$. HRMS (ESI) m/z found 542.1177 $[M + H]^+$, calcd for $C_{25}H_{30}Cl_2NO_6S$ 542.1165.

2-((2*R*,5*R*,6*R*)-6-(3-Chlorophenyl)-5-(4-chlorophenyl)-4-((*S*)-1-(neopentylsulfonyl)butan-2-yl)-3-oxomorpholin-2-yl)acetic Acid (16). Compound **16** was prepared as a single isomer from **49** (4% combined yield for the last three steps) through procedures similar to those described for the synthesis of **5**. The isomers at C2 were separated by reverse phase preparatory HPLC (Gemini Prep C_{18} 5 μm column; Phenomenex, Torrance, CA; gradient elution of 25−75% MeCN in water, both solvents containing 0.1% TFA, 30 min method) to provide **16** as the first eluting isomer. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.23−7.28 (m, 2H), 7.21 (s, 3H), 7.14−7.17 (m, 1H), 7.05−7.12 (m, 1H), 6.95 (d, J = 7.63 Hz, 1H), 5.00 (d, J = 6.46 Hz, 1H), 4.85 (d, J = 6.65 Hz, 1H), 4.60 (t, J = 6.06 Hz, 1H), 3.82−3.97 (m, 1H), 3.01 (d, J = 6.46 Hz, 2H), 2.77−2.95 (m, 3H), 1.95−2.10 (m, 1H), 1.41−1.56 (m, 1H), 1.16 (s, 10H), 0.38−0.52 (m, 3H). MS (ESI) m/z = 570.2 $[M + H]^+$.

2-((2*R*,5*R*,6*R*)-6-(3-Chlorophenyl)-5-(4-chlorophenyl)-4-((*S*)-1-(cyclopentylsulfonyl)butan-2-yl)-3-oxomorpholin-2-yl)acetic Acid (17). Compound **17** was prepared as a single isomer from **49** (12% combined yield for the last three steps) through similar procedures to those described for the synthesis of **5**. The isomers at C2 were separated by reverse phase preparatory HPLC (Gemini Prep C_{18} 5 μm column; Phenomenex, Torrance, CA; gradient elution of 25−75% MeCN in water, both solvents containing 0.1% TFA, 30 min method) to provide **17** as the first eluting isomer. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.22 (ddd, J = 6.46, 8.61, 12.52 Hz, 5H), 7.12−7.17 (m, J = 1.17, 1.96 Hz, 1H), 7.08 (t, J = 7.82 Hz, 1H), 6.95 (d, J = 7.63 Hz, 1H), 5.02 (d, J = 6.65 Hz, 1H), 4.85 (d, J = 6.65 Hz, 1H), 4.62 (t, J = 6.06 Hz, 1H), 3.89 (dd, J = 9.00, 13.50 Hz, 1H), 3.19−3.39 (m, 2H), 2.98−3.04 (m, 2H), 2.84 (dd, J = 2.64, 13.99 Hz, 1H), 1.87−2.12 (m, 5H), 1.75 (br s, 2H), 1.60 (d, J = 4.11 Hz, 3H), 0.46 (t, J = 7.53 Hz, 3H). MS (ESI) m/z = 568.1 $[M + H]^+$. HRMS (ESI) m/z found 568.1332 $[M + H]^+$, calcd for $C_{27}H_{33}Cl_2NO_6S$ 568.1322.

2-((2*R*,5*R*,6*R*)-6-(3-Chlorophenyl)-5-(4-chlorophenyl)-3-oxo-4-((*S*)-1-(phenylsulfonyl)butan-2-yl)morpholin-2-yl)acetic Acid (18). Compound **18** was prepared as a single isomer from **49** (37% combined yield for the last three steps) through procedures similar to those described for the synthesis of **5**. The isomers at C2 were separated by reverse phase preparatory HPLC (Gemini Prep C_{18} 5 μm column; Phenomenex, Torrance, CA; gradient elution of 25−75% MeCN in water, both solvents containing 0.1% TFA, 30 min method) to provide **18** as the first eluting isomer. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.96 (d, J = 7.24 Hz, 2H), 7.56−7.77 (m, 3H), 7.14−7.46 (m, 7H), 7.02 (d, J = 7.63 Hz, 1H), 4.88−5.12 (m, 2H), 4.63 (dd, J = 4.89, 6.85 Hz, 1H), 4.21 (dd, J = 9.39, 14.28 Hz, 1H), 3.42 (br s, 1H), 2.98−3.21 (m, 3H), 2.09 (ddd, J = 7.43, 9.44, 14.23 Hz, 1H), 1.57 (ddd, J = 4.11, 7.58, 13.94 Hz, 1H), 0.48 (t, J = 7.43 Hz, 3H). MS (ESI) m/z = 576.0 $[M + H]^+$. HRMS (ESI) m/z found 576.1020 $[M + H]^+$, calcd for $C_{28}H_{28}Cl_2NO_6S$ 576.1009.

2-((2*R*,5*R*,6*R*)-6-(3-Chlorophenyl)-5-(4-chlorophenyl)-4-((*S*)-1-(*N*,*N*-dimethylsulfamoyl)butan-2-yl)-3-oxomorpholin-2-yl)-acetic Acid (19). Compound **8** was prepared as a single isomer from **42** (>1% combined yield from **42**) through procedures similar to those described for the synthesis of **5**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31−7.36 (m, 2H), 7.20−7.26 (m, 4H), 7.12−7.19 (m, 1H), 7.01 (d, J = 7.6 Hz, 1H), 5.03 (d, J = 7.0 Hz, 1H), 4.93 (d, J = 7.0 Hz, 1H), 4.77 (t, J = 6.0 Hz, 1H), 3.87 (dd, J = 13.7, 9.4 Hz, 1H), 3.29 (br s, 1H), 3.11 (d, J = 6.1 Hz, 2H), 2.89 (s, 3H), 2.89 (s, 3H), 2.82−2.87 (m, 1H), 2.05−2.20 (m, 1H), 1.63 (ddd, J = 13.9, 7.7, 4.2 Hz, 1H), 0.56 (t, J = 7.5 Hz, 3H). MS (ESI) m/z = 543.2 $[M + H]^+$.

2-((2*R*,5*R*,6*R*)-6-(3-Chlorophenyl)-5-(4-chlorophenyl)-4-((*S*)-1-(oxetan-3-ylsulfonyl)butan-2-yl)-3-oxomorpholin-2-yl)acetic Acid (20). Compound **20** was prepared as a single isomer from **39b** (10% combined yield for the six three steps) through procedures similar to those described for the synthesis of **5** in Scheme 3. The isomers at C2 were separated by reverse phase preparatory HPLC (Gemini Prep C_{18} 5 μm column; Phenomenex, Torrance, CA; gradient elution of 25−75% MeCN in water, both solvents containing 0.1% TFA, 30 min method) to provide **20** as the first eluting isomer. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35 (s, 2H), 7.30 (s, 2H), 7.23−7.26 (m, 2H), 7.19 (t, J = 7.82 Hz, 1H), 7.04 (d, J = 7.63 Hz, 1H), 4.86−5.13 (m, 5H), 4.74 (dd, J = 5.67, 6.65 Hz, 1H), 4.33−4.45 (m, 1H), 3.91−4.05 (m, 1H), 3.39−3.54 (m, 1H), 3.04−3.19 (m, 2H), 2.86 (d, J = 16.43 Hz, 1H), 2.04−2.17 (m, 1H), 1.50−1.70 (m, 1H), 0.58 (t, J = 7.43 Hz, 3H). MS (ESI) m/z = 556.0 $[M + H]^+$. HRMS (ESI) m/z found 556.0966 $[M + H]^+$, calcd for $C_{25}H_{28}Cl_2NO_7S$ 556.0958.

(2*S*)-2-((5*R*,6*R*)-2-Allyl-6-(3-chlorophenyl)-5-(4-chlorophenyl)-3-oxomorpholino)butanal (53). To a solution of a 2:1.3 mixture of (2*R*,5*R*,6*R*)-2-allyl-6-(3-chlorophenyl)-5-(4-chlorophenyl)-4-((*S*)-1-hydroxybutan-2-yl)morpholin-3-one and (2*S*,5*R*,6*R*)-2-allyl-6-(3-chlorophenyl)-5-(4-chlorophenyl)-4-((*S*)-1-hydroxybutan-2-yl)-morpholin-3-one (**52**) (163 mg, 0.375 mmol, synthesized from **48** through procedures similar to those described in Scheme 5) in DCM (1.8 mL) was added Dess−Martin periodinane (175 mg, 0.413 mmol) and water (7.44 μL, 0.413 mmol). After 30 min, saturated aqueous sodium thiosulfate was added. The mixture was extracted with DCM. The combined organic layers were washed with saturated aqueous $NaHCO_3$, dried over Na_2SO_4, and concentrated to provide **53** (>98% yield, 159 mg). ^{1}H NMR (400 MHz, $CDCl_3$) (1.5:1 mixture of isomers at C2) δ 9.54 (s, 1H), 9.42 (s, 1.5H), 8.27 (d, J = 12 Hz, 1H), 8.01 (d, J = 12 Hz, 1H), 7.94 (t, J = 6.3 Hz, 1H), 7.36 (t, J = 6 Hz, 1H), 7.34−7.22 (m, 10H), 7.16−7.05 (m, 7.5H), 6.71 (d, J = 8 Hz, 1.5H), 5.99−5.93 (m, 2.5H), 5.31−5.16 (m, 6H), 4.96 (d, J = 8 Hz, 1H), 4.75 (d, J = 12 HZ, 1.5H), 4.71 (d, J = 8 Hz, 1H), 4.70−4.68 (M, 1H), 4.57 (d, J = 12 Hz, 1.5H), 4.37 (m, 1H), 3.80 (t, J = 8 Hz, 1.5H), 3.59 (m, J = 8 Hz, 1H), 2.84−2.80 (m, 5H), 2.23−2.15 (m, 2.5H), 0.84−0.79 (m, 7.5H). MS (ESI) m/z = 432.2 $[M + H]^+$.

(5*R*,6*R*)-2-Allyl-6-(3-chlorophenyl)-5-(4-chlorophenyl)-4-((*S*)-1-(methylamino)butan-2-yl)morpholin-3-one (54). To a solution of (2*S*)-2-((5*R*,6*R*)-2-allyl-6-(3-chlorophenyl)-5-(4-chlorophenyl)-3-oxomorpholino)butanal (**53**) (188 mg, 0.435 mmol) in DCE (4 mL) were added methanamine (1.305 mL, 2.61 mmol) 2.0 M solution in THF, sodium triacetoxyborohydride (276 mg, 1.305 mmol), and 3 drops of HOAc. After being stirred overnight, the reaction mixture was quenched with water and saturated aqueous $NaHCO_3$. The reaction mixture was extracted with ethyl acetate. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated to provide (5*R*,6*R*)-2-allyl-6-(3-chlorophenyl)-5-(4-chlorophenyl)-4-((*S*)-1-(methylamino)butan-2-yl)morpholin-3-one (**54**) (83% yield, 162 mg), used without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) (1.5:1 mixture of isomers at C2) δ 7.34−7.22 (m, 8H), 7.13−7.09 (m, 4H), 6.71 (d, J = 12 Hz, 1H), 6.05−5.87 (m, 1.5H), 5.31−5.15 (m, 4H), 4.90−4.88 (m, 1H), 4.61 (d, J = 8 Hz, 1.5H), 4.50 (t, J = 8 Hz, 1H), 3.74 (s, 1H), 2.83−2.79 (m, 3H), 2.42 (m, J = 12 Hz, 6H), 1.89−1.75 (m, 2H), 1.66−1.52 (m, 3H), 0.71−0.64 (m, 7.5H). MS (ESI) m/z = 447.1 $[M + H]^+$.

***N*-((2*S*)-2-((5*R*,6*R*)-2-Allyl-6-(3-chlorophenyl)-5-(4-chlorophenyl)-3-oxomorpholino)butyl)-*N*-methylcyclopropanesulfonamide (55).** To a solution of (2*R*,5*R*,6*R*)-2-allyl-6-(3-chlorophenyl)-5-(4-chlorophenyl)-4-((*S*)-1-(methylamino)butan-2-yl)morpholin-3-one and (2*S*,5*R*,6*R*)-2-allyl-6-(3-chlorophenyl)-5-(4-chlorophenyl)-4-((*S*)-1-(methylamino)butan-2-yl)morpholin-3-one (**54**) (162 mg, 0.362 mmol) and pyridine (0.070 mL, 0.869 mmol) in DCM (4 mL) was added cyclopropanesulfonyl chloride (0.044 mL, 0.435 mmol). After 90 min, DMAP (5.0 mg, 0.041 mmol) was added. After 1 h, cyclopropanesulfonyl chloride (0.044 mL, 0.435 mmol) and pyridine (0.070 mL, 0.869 mmol) were added. After being stirred for 3 days, the mixture was quenched with water and extracted with DCM (2×), and saturated aqueous NH_4Cl was added. The combined organic layers were dried over Na_2SO_4, and concentrated. The crude residue was purified by flash chromatography on silica gel (12 g column, gradient elution with 5−70% ethyl acetate in hexanes) to provide **55** (38% yield, 75 mg). ^{1}H NMR (400 MHz, $CDCl_3$) (1.5:1 mixture of isomers at C2) δ 7.33−7.19 (m, 5.5H), 7.10−7.02 (m, 5.5H), 6.82 (d, J = 8 Hz, 1H), 5.98−5.93 (m, 2H), 5.24−5.11 (m, 4H), 4.72 (m, J = 12H, 1H), 4.61 (m, J = 12 Hz, 2H), 4.40−4.36 (m, 2H), 2.97 (s, 4.5H), 2.93 (s, 3H), 2.40−2.22 (m, 2H), 1.96−1.94 (m, 2H), 1.69−1.61 (m, 1.5H), 1.27−1.10 (m, 3.5H), 0.99−0.96 (m, 3.5H), 0.67−0.57 (m, 5H). MS (ESI) m/z = 551.0 $[M + H]^+$.

2-((2*R*,5*R*,6*R*)-6-(3-Chlorophenyl)-5-(4-chlorophenyl)-4-((*S*)-1-(*N*-methylcyclopropanesulfonamido)butan-2-yl)-3-oxomorpholin-2-yl)acetic Acid (7). Compound **7** was prepared as a single isomer from **55** (39% yield) through a procedure similar to that described for the synthesis of **5**. The isomers at C2 were separated by reverse phase preparatory HPLC (Gemini Prep C_{18} 5 μm column; Phenomenex, Torrance, CA; gradient elution of 25−75% MeCN in water, where both solvents contain 0.1% TFA, 30 min method) to provide **7** as the faster eluting isomer. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.28−7.38 (m, 4H), 7.14−7.27 (m, 4H), 4.82−4.98 (m, 2H), 4.71 (t, J = 6.16 Hz, 1H), 3.11 (dd, J = 6.26, 9.00 Hz, 3H), 2.88 (s, 3H), 2.33 (s, 1H), 1.77−1.96 (m, 1H), 1.53−1.73 (m, 1H), 1.08−1.29 (m, 2H), 0.85−1.07 (m, 4H), 0.57 (t, J = 7.53 Hz, 3H). MS (ESI) m/z = 569.2 $[M + H]^+$. HRMS (ESI) m/z found 569.1279 $[M + H]^+$, calcd for $C_{26}H_{31}Cl_2NO_6S$ 569.1274.

2-((2*R*,5*R*,6*R*)-4-((*S*)-1-(*tert*-Butylsulfonyl)butan-2-yl)-6-(3-chlorophenyl)-5-(4-chlorophenyl)-2-methyl-3-oxomorpholin-2-yl)acetic Acid (6). Compound **6** was prepared from **42b** (10% combined yield for the last three steps) as a single isomer through procedures similar to those described for the synthesis of **5**. The isomers at C2 were separated by reverse phase preparatory HPLC (Gemini Prep C_{18} 5 μm column; Phenomenex, Torrance, CA; gradient elution of 25−75% MeCN in water, where both solvents contain 0.1% TFA, 30 min method) to provide **6** as the faster eluting isomer. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.27−7.34 (m, 2H), 7.08 (d, J = 16.43 Hz, 5H), 6.81 (d, J = 7.83 Hz, 1H), 5.07 (q, J = 9.59 Hz, 2H), 4.01 (dd, J = 10.56, 13.30 Hz, 1H), 3.32 (dd, J = 7.24, 9.39 Hz, 2H), 3.18 (d, J = 15.85 Hz, 1H), 3.01 (d, J = 15.65 Hz, 1H), 2.80 (d, J = 12.32 Hz, 1H), 2.12−2.33 (m, 1H), 1.69−1.78 (m, 3H), 1.43 (s, 9H), 0.53 (t, J = 7.43 Hz, 3H). MS (ESI) m/z = 570.2 $[M + H]^+$. HRMS (ESI) m/z found 570.1479 $[M + H]^+$, calcd for $C_{27}H_{34}Cl_2NO_6S$ 570.1478.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

(i) In vitro biological assays, (ii) in vivo protocols, and (iii) determination of cocrystal structures of **4** bound to MDM2. This material is available free of charge via the Internet at http://pubs.acs.org.

Accession Codes

The coordinates of **4** with MDM2 have been deposited in the PDB with accession code 4OBA.

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Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS USED

AcCN, acetonitrile; Boc, *tert*-butoxycarbonyl; BrdU, 5-bromo-2-deoxyuridine; CL, clearance; CYP3A4, cytochrome P450 3A4; DCM, dichloromethane; DMBCl, 2,4-dimethoxybenzyl chloride; DMF, *N*,*N*-diemethylformamide; DMP, Dess−Martin periodinane; DMSO, dimethylsulfoxide; dr, diastereoselectivity ratio; EdU, 5-ethynyl-2′-deoxyuridine; EtOAc, ethyl acetate; FACS, fluorescence-activated cell sorting; hPXR, human

pregnane X receptor; HTRF, homogeneous time-resolved fluorescence; LiHMDS, lithium bis(trimethylsilyl)amide; MDM2, murine double minute 2; MsCl, methanesulfonyl chloride; NaHMDS, sodium bis(trimethylsilyl)amide; NMO, *N*-methylmorpholinine *N*-oxide; q.d., once a day dosing; qRT-PCR, quantitative reverse transcription polymerase chain reaction; SEM, standard error of mean; SPR, surface plasmon resonance; TBAF, tetrabutylammonium fluoride; TDI, time dependent inhibition; TEA, triethylamine; TES, triethylsilyl; TESCl, triethylsilyl chloride; TFA, trifluoroacetic acid; THF, tetrahydrofuran

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(23) Experimental details of the in vitro biological assays can be found in the Supporting Information.

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(27) Time-dependent inhibition, % remaining of CYP3A4 over positive control, rifampin, after 30 min at 10 μM.

(28) Experimental details of the BrdU proliferation and HCT116 p21 TaqMan assays can be found in the Supporting Information.

(29) Experimental details of in vivo studies can be found in the Supporting Information.

(30) Metabolites structures determined by analogy with previous results; see ref 21.

(31) The atomic coordinates have been deposited in the Protein Data Bank under accession code 40BA.

(32) Intermediate **39e** was synthesized through a procedure similar to that published in the following: Bae, J. H.; Shin, S.-H.; Park, C. S.; Lee, W. K. Preparation of cysteinol derivatives by highly regioselective ring opening of non-activated chiral aziridines by thiols. *Tetrahedron* **1999**, *55*, 10041−10046.

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