Structure-Based Design of a Parallel Synthetic Array Directed Toward the Discovery of Irreversible Inhibitors of Human Rhinovirus 3C Protease

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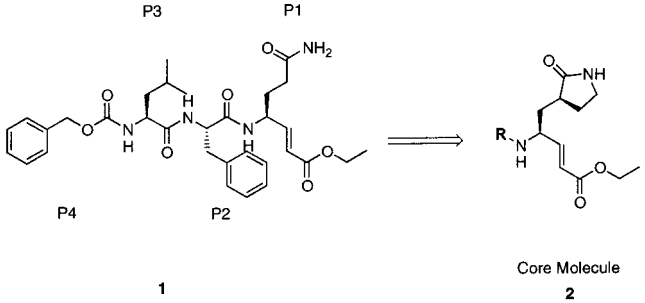
Utilizing the tools of parallel synthesis and structure-based design, a new class of Michael acceptor-containing, irreversible inhibitors of human rhinovirus 3C protease (HRV 3CP) was discovered. These inhibitors are shown to inhibit HRV-14 3CP with rates of inactivation ranging from 886 to 31 400 M^{-1} sec^{-1}. These inhibitors exhibit antiviral activity when tested against HRV-14 infected H1-HeLa cells, with EC_{50} values ranging from 1.94 to 0.15 μM. No cytotoxicity was observed at the limits of the assay concentration. A crystal structure of one of the more potent inhibitors covalently bound to HRV-2 3CP is detailed. These compounds were also tested against HRV serotypes other than type 14 and were found to have highly variable activities.

Introduction

The human rhinoviruses (HRVs) are members of the picornavirus family and are the single most causative agent of the common cold.[1] Because over 100 serotypes of the virus exist, immunization would be an impractical approach to prevention of infection. Rhinoviruses contain a positive-sense strand of RNA that is translated to a large polyprotein in infected cells. This polyprotein is cleaved by viral proteases to yield mature viral enzymes and structural proteins. The 3C protease (3CP) does the majority of the proteolytic processing. Inhibition of this viral protease by a small molecule agent should stop viral replication and thus control the extent of infection.

Much work has been previously described regarding the design and development of substrate-derived tripeptidyl, Michael acceptor-containing HRV 3CP inhibitors,[2] such as compound **1**.[2b] This molecule is comprised of a substrate-derived tripeptide binding determinant that provides affinity and selectivity for the target protease and a Michael acceptor moiety that forms a covalent adduct with the active site cysteine (Cys-147) of the enzyme. It has been our goal to develop low molecular weight, nonpeptidic HRV 3CP inhibitors. Other nonpeptidic inhibitors such as isatins[3] and homophthalimides[4] have been described in the literature. Typically, these molecules have suffered from problems such as cellular toxicity and modest antiviral activity.

Efforts have been made toward finding a replacement for the Michael acceptor-ethyl ester of **1** that maintains antiviral potency, increases metabolic stability, and retains its negligible cytotoxicity. Tripeptidyl aldehydes[5] and ketones[6] have been reported, but these achieve only modest levels of antiviral activity. Many analogues of the unsaturated ester, such as unsaturated ketones, amides, and cyclic analogues have been tested, but unacceptable losses in antiviral activity were encountered.[7] Stability tests such as incubation in the presence of dog and human blood plasma esterases have shown that this unsaturated ester has a degree of stability that may lead to resistance toward metabolic degradation.[2e] The Michael acceptor-ethyl ester compound **1** also did not react when incubated with high concentrations of dithiothreitol (5 mM), suggesting that the inhibitor does not react readily with nonenzymatic thiols.[2e]



1 **2**

Figure 1. Selection of a core of parallel synthesis.

The benefits of converting the P1 glutamine side chain into a lactam have been discussed in a previous paper.[2e] Cyclization of the P1 glutamine side chain into a lactam generally increases enzyme activity and antiviral activity, presumably by favorably altering the change in entropy of the formation of the inhibitor−protein complex. This modification also improves the physical properties (less peptidelike) of the resulting inhibitors. With these factors in mind, we sought to develop a small molecular version of **1**, using the ethyl ester Michael acceptor P1 lactam **2** as a core for high-throughput synthesis of a large number of potential inhibitor molecules. Specifically, we sought to replace the P2−P4 portion of **1** with a smaller, nonpeptidic substituent.

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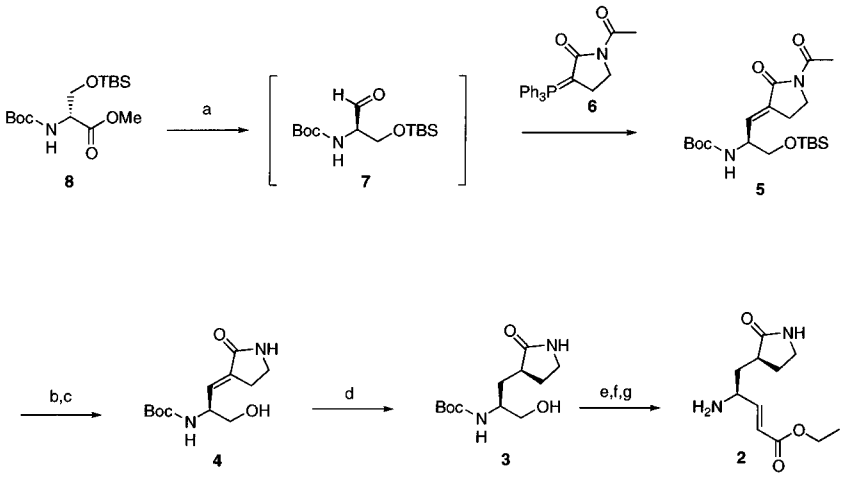
Scheme 1. Synthesis of **2**[a]



[a] Reagents: (a) DIBAH; MeOH; **6**, 60%. (b) TBAF. (c) K_2CO_3, MeOH, 73% from **5**. (d) *S*-BINAP-RuCl, H_2 (1200 psi) 70%. (e) IBX; $Ph_3P{=}CH{-}CO_2Et$. (f) HCl. (g)K_2CO_3, 60% from **3**.

Structure-Based Design of a Parallel Synthetic Array

It was envisioned that a large number of compounds could be produced in 96 well plate format by a solution phase coupling reaction between the selected carboxylic acids and the terminal nitrogen of the P1-Michael acceptor compound **2,** mediated by a resin-bound coupling reagent. Because a large number of carboxylic acids are commercially available, we sought to condense this list to a more manageable number by selecting reagents based on information from the cocrystal structure of the covalently bound complex between **1** and HRV-2 3CP.[8] Our goal was not to attempt the difficult task of predicting the most active candidates but rather to eliminate carboxylic acids that had little chance of leading to a potent inhibitor, for reasons such as large steric clashes with the target protein.

A virtual library of two-dimensional structures was derived by coupling a list of carboxylic acids obtained from the Available Chemicals Directory (ACD)[9] to the P1-Michael acceptor core **2** using proprietary software. The resulting set of two-dimensional structures was converted to three-dimensional structures using CORINA version 1.7.[10] These structures were then energy-minimized in the Batchmin module of Macromodel,[11] using the AMBER* force field. The minimized structures were docked into the target protein using an in-house automated docking program.[12] The P1-Michael acceptor core of each docked structure was fixed in the position observed in the cocrystal structure of **1** (active site Cys-147 covalently bound to the β-carbon of the former Michael acceptor at this point), and the remaining portion of the molecule was docked into the S2−S3−S4 sites of the protein. The docking procedure was reiterated eight times, a process that resulted in some molecules having several docked conformations. The resulting set of docked structures was evaluated by criteria such as number of hydrogen-bonding interactions, hydrophobic interactions, and steric interactions. For compounds with several docked conformations, only the lowest energy docked structure was retained. Structures that led to high energy interactions in the docking process were removed from consideration for inclusion in the parallel synthetic array.

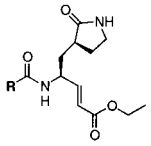
Chemistry

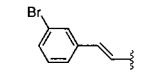
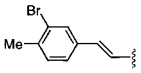
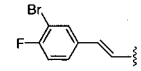
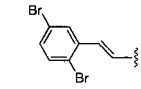
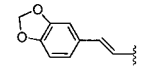
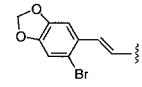
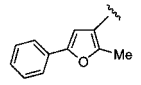
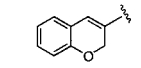
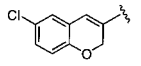
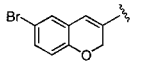
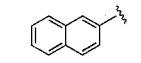
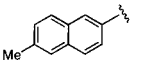
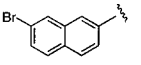
The synthesis of compounds such as **2** was described in a previous paper;[2e] however, we sought to make this preparation more amenable to scale-up by setting the side chain chirality through the use of an asymmetric hydrogenation. This change results in a shorter overall route and eliminates the need for a stoichiometric amount of chiral auxiliary that was used in the previous route.

The synthetic route is shown in Scheme 1. Compound **8**, derived from D-serine, is reduced with diisobutylaluminum hydride (DIBAH) to the corresponding aldehyde **7** and then treated with Wittig reagent **6** to give compound **5**. This sequence was analogous to the one described by Hanessian for the synthesis of Kainic acid derivatives.[13] The lactam Wittig reagent **6** was prepared using a modified procedure of Ikuta.[14] Compound **5** was deprotected with potassium carbonate and methanol to remove the acetate, followed by desilylation with TBAF to produce **4**. Several attempts were made at utilizing a directed hydrogenation of **4** to set the side chain chirality.[15] These reactions gave low yields and low selectivities. The use of S-2,2′-bis(diphenylphophino)-1,1′-binaphthyl (BINAP)·RuCl[16] as a chiral catalyst in the hydrogenation reaction produced **3** in good yields with excellent diastereomeric purity. This reaction could be easily carried out in 10 g scale using a Paar bomb hydrogenator.[17] The alcohol **3** was converted to the desired product **2** by a one pot IBX[18] oxidation−Wittig sequence,[19] followed by HCl-mediated deprotection of the Boc-amine. The stereochemistry of **2** was confirmed by correlation to material prepared by the literature route[2e] and ultimately by examination of the crystal structure of an inhibitor−protein complex (within this paper).

A mixture of **2** and a selected carboxylic acid was coupled using polystyrene-bound carbodiimide.[25] No attempts were made to purify the products. The product purity was examined by liquid chromatography-mass spectrometry (LC-MS) for a selection of representative wells (the diagonals) and was typically found to be greater than 80%. Synthesis of selected hits in larger scale was carried out utilizing O-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium (HATU) as a cou-

Table 1. Active Compounds



Cmpd No.	R	k_{obs}/[I] HRV-14 ($M^{-1}s^{-1}$)	EC_{50} HRV-14 (uM)	CC_{50} (uM)
9		2,560	1.40	>10
10		4,080	1.29	>10
11		2,857	0.63	>100
12		6,850	ND*	ND*
13		1,958	1.81	>10
14		14,400	0.49	>100
15		4,745	1.94	>100
16		886	1.19	>100
17		19,700	0.16	>100
18		25,000	0.18	>100
19		1,090	0.15	>100
20		5,000	0.88	>100
21		31,400	0.18	>50
1		25,000	0.54	>100

* ND = not determined.

pling reagent to react **2** with the desired carboxylic acid. These resynthesized compounds were all purified by preparative reverse phase high-performance liquid chromatography (HPLC).

Results

Table 1 shows a selection of the most active 3CP inhibitors in this series. The library hits were picked out of a high-throughput screen based on their rates of inactivation of HRV-14 3CP.[2f,2g,3,5] All of the compounds **9**−**21**, with additional information from an X-ray cocrystal structure of **17**, are predicted to occupy S2 of HRV 3CP, analogous to the Phe side chain of **1**. All of the compounds **9**−**21** contain planar, hydrophobic, aromatic moieties, reflective of the hydrophobic nature of S2. No highly active compound was found that appeared to have a substituent that extended to S3 or S4. Compounds **16**, **18**, and **21** were not found in the parallel array but were synthesized as follow-ups to compounds **17** and **19**, to test the effects of halogenation. The halogenation pattern of the P2 substituent is shown to have a large influence on activity. Comparison between **16**, **17**, and **18** and between **19** and **21** shows that a properly placed halogen can increase the rate of enzyme inactivation by as much as 30-fold against HRV-14 3CP.

Table 2. Multiple HRV Serotypes

compd no.	HRV serotype	k_{obs}/[I] ($M^{-1}\,s^{-1}$)	EC_{50} (μM)
17	14	19 700	0.16
	16	2200	ND
	2	385	0.65
	1A	ND	5.10
	10	ND	0.46
18	14	25 000	0.18
	2	372	ND
	1A	ND	5.10
	10	ND	0.55
19	14	1090	0.15
	2	110	1.40
1	14	25 000	0.54
	16	6529	2.3
	89	3439	6.3
	2	2000	1.6
	10	ND	0.6

The antiviral activities of compounds **9**−**21** loosely follow the order of their rates of inactivation. Compound **19** appears to be an anomaly, having much better antiviral activity than would be predicted by its rate of enzyme inactivation.[20] All of the compounds in Table 1 were nontoxic (CC_{50}) to the cell line used in the antiviral assay, at the limits of the testing concentrations. Compounds **17**, **18**, and **21** show rates of inactivation comparable to the peptide lead **1** but have an increased degree of antiviral activity. This may reflect the improved physical properties, leading to improved cellular permeability, of the smaller molecules as compared to the larger peptidic compound **1**.

Compounds **17**−**19** were tested against several other HRV serotypes with highly variable results (Table 2). The activity against HRV-14, the serotype used in the high-throughput assay, was consistently the highest. The activity against other serotypes fell off dramatically. The enzyme inhibitory activity of the tripeptide lead **1** varied approximately 10-fold over the serotypes tested. Examination of the cocrystal structure of the HRV-2 3CP−**17** covalent complex leads to a plausible explanation of the differences in activities across different serotypes, as discussed below. Despite their reduced enzyme activity across other serotypes, **17**−**19** still maintained better antiviral activity in comparable serotypes than the larger, more peptidic **1**.

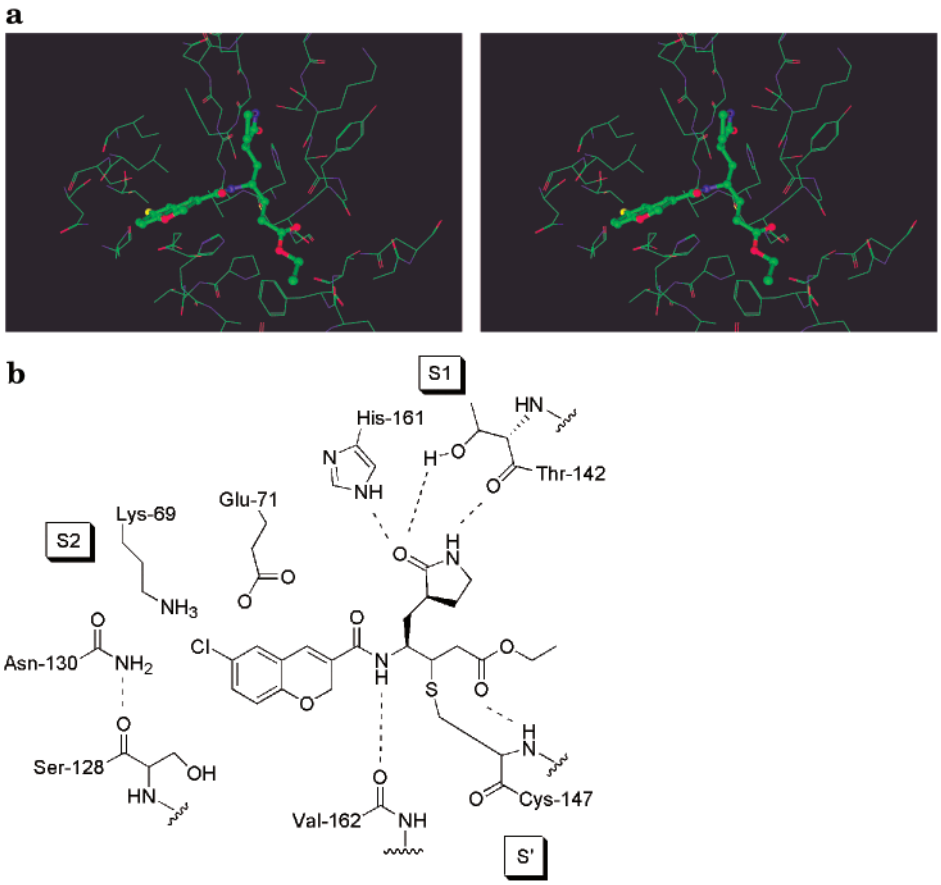


Figure 2. (a) Cocrystal structure of complex between **17** and HRV-2 3CP. (b) Map of complex between **17** and HRV-2 3CP.

X-ray Analysis of a Cocrystal Structure

A crystal structure was obtained for the covalent complex between **17** and HRV-2 3CP (Figure 2). Inhibitor−protein complexes of serotypes other than type 2 have not produced crystals of high enough quality to be used for X-ray crystallography. As expected, the Michael acceptor and the lactam groups of **17** are oriented in a fashion similar to the corresponding cocrystal structure of **1**.[8] Cys-147 is covalently linked to the inhibitor's unsaturated ester β-carbon. The lactam lies in the S1 specificity pocket where it makes hydrogen bonds with His-161 and Thr-142 residues. The bulky bicyclic ring substituent is coplanar with the inhibitor's carboxamide group and binds deep in the S2 pocket.

Although the extended substrate binding site for HRV 3CP is highly conserved among various rhinoviral serotypes, the differences that do exist involve residues that form the back portion of the S2 specificity pocket.[8] It is likely that these differences account for much of the variability in the enzyme inhibitory activity observed for **17** against the proteases from different HRV serotypes. The two nonconserved residues in the S2 pocket of HRV-2 3CP are Lys-69 and Asn-130.

The Cl of bound **17** is buried underneath the side chain carboxylate of Glu-71, where it forces the side chain of Lys-69 to adopt a conformation not seen in other HRV-2 3CP cocrystal structures with inhibitors having less bulky S2 substituents. There is also a close approach (3.55 Å) of the 7-position of the chromene ring to the side chain amide nitrogen of Asn-130, which is also expected to be unfavorable for the binding of **17** to HRV-2 3CP. This Asn-130 side chain hydrogen bonds with the backbone carbonyl oxygen of Ser-128 and is probably important for stabilizing the β-hairpin turn that forms one wall of the S2 pocket.

Unfavorable steric interactions between the P2 substituent of **17** and the residues at the back of the S2 pocket also force the inhibitor's amide NH away from the backbone carbonyl oxygen of Val-162, disrupting a potential hydrogen bond. In the current structure, this distance has increased to 3.7 Å as compared to 3.0 Å for the corresponding distance in the complex between **1** and HRV-2 3CP.[8]

The two nonconserved residues in the S2 pocket of HRV-2 3CP are Lys-69 and Asn-130. These residues are replaced by less bulky amino acids (Asn and Thr, respectively) in HRV-14 3CP. The increased inhibitory activity for **17** against HRV-14 3CP is most likely a result of the fact that the larger S2 pocket can accommodate the bulky P2 substituent without unfavorable steric interactions, while allowing the formation of the hydrogen bond between the inhibitor's amide and Val-162. This hydrogen bond is probably important for proper alignment of the Michael acceptor to facilitate the Michael reaction with the active site cysteine.

HRV-16 3CP has an S2 pocket containing intermediate size amino acids. Residues 69 and 130 are Lys and Thr, respectively. Consistent with these arguments, the potency of **17** against HRV 3CP serotypes increases in the order type 2 > type 16 > type 14.

Conclusions

The use of parallel synthesis guided with information from target structural data led to the discovery of a new

class of HRV-3CP inhibitors. We have used this strategy to focus high diversity on a small portion of the protein target (S2), while conserving a known binding element of the inhibitor (the P1-Michael acceptor moiety). Using available structural information also served to reduce the number of potential synthons to a manageable number. We have shown that this technique is a powerful tool to optimize the biological activity of a lead compound.

We have shown that for HRV-14 serotype, it is possible to obtain inhibitors with potent antiviral activity without having to build the molecules into S3 and S4. For type 14 serotype, protein−inhibitor interactions at S2 can have a large beneficial impact on inhibitor activity; however, because of the problem of high serotype variability at S2, broad spectrum activity may be a problem. Despite reduced enzyme inhibition against serotypes other than type 14, these new small molecule inhibitors still maintain slightly better antiviral activity than the larger, peptidic **1**.

Experimental Section

General descriptions of experimental procedures, reagent purifications, and instrumentation, along with conditions and uncertainties for enzyme and antiviral assays, are provided elsewhere.[2f,2g,3,5] ^{1}H nuclear magnetic resonance (NMR) coupling constants are given in Hz. Analytical HPLC analysis was performed on a Hewlett-Packard Series 1100, running a 20−100% acetonitrile−water gradient elution over 10 min. A Zorbax SB-C18 4.6 mm × 15 cm column was used. Preparative HPLC was performed using a Gilson 215 liquid handling robot, along with the Gilson 306 pump, and a Gilson 119 detector using 215 and 254 nm wavelengths. A Phenomenex LUNA C8-(2) 250 mm × 21 mm column was used, with an acetonitrile−water gradient elution. The method used for analysis of proteolytic processing has been described previously.[20] Abbreviations: DMSO, dimethyl sulfoxide; ADA, N-(2-acetamido)iminodiacetic acid; DTT, dithiothreitol; THF, tetrahydrofuran; TFA, trifluoroacetic acid.

Protein Crystallography. HRV-2 3CP was incubated with a 4-fold molar excess of **17** in the presence of 2% (vol/vol) DMSO for 24 h at 4 °C. The complex was concentrated to 10 mg/mL and then passed through a 0.22 μm cellulose−acetate filter. Crystals were grown at 13 °C, using a hanging drop vapor diffusion method in which equal volumes (3 uL) of the protein−ligand complex and reservoir solution were mixed on plastic coverslips and sealed over individual wells filled with 1 mL of reservoir solution containing 2 M sodium acetate, 0.1 M ADA, pH 6.5, and 10 mM DTT.

A single crystal measuring 0.3 mm × 0.2 mm × 0.1 mm (space group $P2_12_12$; a = 62.6, b = 77.9, c = 34.1 Å) was prepared for low-temperature X-ray data collection by transfer to an artificial mother liquor solution consisting of 300 μL of the reservoir solution mixed with 100 μL of glycerol and then flash-freezing in liquid nitrogen. X-ray diffraction data were collected at −180 °C on a MAR Research 345 mm imaging plate and processed with DENZO and SCALEPACK.[21] Diffraction data were 95.4% complete to a resolution of 1.73 Å with R(sym) = 3.1%. Protein atomic coordinates from an isomorphous cocrystal structure of HRV-2 3CP[7] were used to initiate rigid body refinement in X-PLOR[22] followed by simulated annealing and conjugate gradient minimization protocols. Placement of the inhibitor, addition of ordered solvent, and further refinement proceeded as described previously.[5a] The final R factor was 20.9% (16 017 reflections with $F > 2\sigma(F)$). The root mean square deviations from ideal bond lengths and angles were 0.014 Å and 2.6 degrees, respectively. The final model consisted of all atoms for residues 1−180 (excluding side chains of residues 12, 45, and 167), the inhibitor, and 175 ordered waters.

1-Acetyl-3-(triphenylphosphanylidine)pyrrolidin-2-one (6). 2,4-Dibromobutyride[14] (46.1 g, 188.2 mmol) in THF (1 L) was cooled to 0 °C and treated with a solution of lithium bis(trimethylsilylamide) (40.9 g, 244.6 mmol) in THF (200 mL). The solution was held at 0 °C for 2.5 h and then poured into brine (800 mL), extracted with ethyl acetate (2 L), and then dried ($MgSO_4$). Evaporation yielded 25.5 g of 3-bromo-pyrrolidin-2-one as a brown oil. This material was treated with Ac_2O (76 mL) and refluxed for 5 h. Evaporation followed by purification (silica gel filtration, EtOAc elutant) yielded 28 g of 1-acetyl-3-bromo-pyrrolidin-2-one as a dark oil. THF (272 mL) and triphenylphosphine (42.8 g, 163.3 mmol) were added, and the resulting solution was refluxed for 8 h. After it was cooled to room temperature, a precipitate of 1-acetyl-3-(triphenylphosphanyl)pyrrolidin-2-one bromide formed and was collected by filtration (27.1 g). Concentration of the mother liquor, followed by cooling to 0 °C, yielded an additional 6.6 g. The combined material in CH_2Cl_2 (1 L) was washed with 1 N NaOH (100 mL) and then brine (2 × 100 mL). Evaporation of the organic layer yielded 26.9 g (37% overall) of a tan oil. ^{1}H NMR ($CDCl_3$): δ 7.76−7.32 (15H, m), 3.90−3.85 (2H, m), 2.50 (3H, s), 2.56−2.30 (2H, m).

2*R*-*tert*-Butoxycarbonyl-3-(*tert*-butyldimethylsilanoxy)propionic Acid Methyl Ester (8). Boc-D-serine methyl ester (20.0 g, 91.2 mmol) in DMF (300 mL) was treated with imidazole (18.6 g, 273.7 mmol) and then TBSCl (13.0 g, 86.7 mmol). The solution was held at room temperature for 8 h and then was washed with saturated aqueous ammonium chloride (300 mL) and then extracted with ethyl acetate (800 mL). The organic layer was washed with brine (300 mL) and then dried ($MgSO_4$) to yield 30.2 g (100%) of a colorless oil. ^{1}H NMR ($CDCl_3$): δ 5.32 (1H, d, J = 8.3), 4.33 (1H, dt, J = 8.8, 2.7), 4.02 (1H, dd, J = 10.1, 2.6), 3.80 (1H, dd, J = 9.8, 3.1), 3.72 (3H, s), 1.43 (9H, s), 0.85 (9H, s), 0.00 (6H, s).

[1*S*-(1-Acetyl-2-oxopyrrolidin-3-ylidenemethyl)-2-(*tert*-butyldimethylsilanyloxy)ethyl]carbamic Acid *tert*-Butyl Ester (5). Compound **8** (12.7 g, 38.0 mmol) in toluene (190 mL) was cooled to −78 °C and treated with a solution of diisobutylaluminum hydride (15.6 mL, 87.4 mmol) in toluene (175 mL). Internal temperature was kept below −70 °C. The solution was held at −78 °C for an additional 90 min and then methanol (7.7 mL, 190 mmol) was added. 1-Acetyl-3-(triphenylphosphanylidine)pyrrolidin-2-one (**6**, 11.1 g, 28.6 mmol) in CH_2Cl_2 (50 mL) was added at −78 °C, and the resulting solution was allowed to warm to room temperature and held for 30 min. A solution of sodium potassium tartrate (150 g) in water (600 mL) was added and stirred vigorously for 30 min. The mixture was extracted with ethyl acetate (4 × 250 mL), dried ($MgSO_4$), and evaporated. Purification by silica gel chromatography yielded 7.04 g (60%) of a colorless oil. ^{1}H NMR ($CDCl_3$): δ 6.59 (1H, dt, J = 8.7, 2.9), 4.98 (1H, d, J = 6.8), 4.37−4.25 (1H, m), 3.77 (2H, t, J = 7.3), 3.70−3.58 (2H, m), 2.90−2.80 (1H, m), 2.75−2.60 (1H, m), 5.53 (3H, s), 1.41 (9H, s), 0.87 (9H, s), 0.04 (6H, s).

[2-Hydroxy-1*S*-(2-oxopyrrolidin-3-ylidenemethyl)ethyl]carbamic Acid *tert*-Butyl Ester (4). Compound **5** (9.18 g, 22.2 mmol) in THF (150 mL) was treated with tetrabutylammonium fluoride (22.2 mL of a 1 M solution in THF, 22.2 mmol) at 0 °C and held for 1 h. A solution of saturated aqueous ammonium chloride was added and stirred for 10 min and then, the solution was extracted with ethyl acetate (2 × 200 mL). The organic layer was dried ($MgSO_4$) and then evaporated. Purification by silica gel chromatography yielded 4.82 g (73%) of a colorless oil. This material was taken up in methanol (160 mL), treated with potassium carbonate (223 mg, 1.62 mmol), and held for 1 h at room temperature. The mixture was then treated with solid citric acid (311 mg, 1.62 mmol), and ethyl acetate (800 mL) was added. The solution was filtered through silica gel. Evaporation yielded 4.30 g (73% overall) of a colorless oil. ^{1}H NMR ($CDCl_3$): δ 7.03 (1H, br s), 6.35 (1H, dt, J = 8.6, 2.6), 5.37 (1H, d, J = 6.5), 4.40−4.20 (1H, m), 3.66 (br s, 3H), 3.40 (2H, t, J = 6.7), 3.10−2.80 (1H, m), 2.80−2.70 (1H, m), 1.41 (9H, s).

[2-Hydroxy-1*S*-(2-oxopyrrolidin-3*S*-ylmethyl)ethyl]carbamic Acid *tert*-Butyl Ester (3). Compound **4** (9.56 g, 37.3 mmol) in methanol (230 mL) was treated with (*S*)-BINAP−

$RuCl^{23}$ (69 mg, 74 μmol) and then put under a hydrogen atmosphere (1200 psi) at room temperature for 72 h. The solution was evaporated and then taken up in EtOAc (200 mL) and allowed to precipitate. The precipitate was filtered and dried to give 6.6 g (69%) of a white powder. This material was determined by ^{1}H NMR to consist of a single diastereomer. ^{1}H NMR ($CDCl_3$): δ 6.66 (1H, br s), 5.51 (1H, d, J = 8.2), 3.72−3.57 (3H, m), 3.34−3.31 (2H, m), 2.52−2.33 (2H, m), 2.20−1.86 (1H, m), 1.86−1.70 (1H, m), 1.62−1.50 (1H, m), 1.40 (9H, s).

4*S*-Amino-5-(2-oxopyrrolidin-3*S*-yl)pent-2-enoic Acid Ethyl Ester (2). Compound **3** (2.02 g, 7.83 mmol) in DMSO (78 mL) was treated with IBX[18] (3.30 g, 11.7 mmol) and held at room temperature for 1.5 h. Carbethoxymethylene triphenylphosphorane (5.45 g, 15.7 mmol) was added, and the solution was held at room temperature for 1 h. Brine (500 mL) was added, and the solution was extracted with ethyl acetate (4 × 150 mL) and then dried ($MgSO_4$). Evaporation yielded 8.50 g of a brown oil. Purification by silica gel chromatography yielded 1.54 g (60%) of a colorless oil. ^{1}H NMR ($CDCl_3$): δ 6.85 (1H, dd, J = 15.6, 5.2), 5.97 (1 H, dd, J = 15.6, 1.6), 4.5−4.3 (1H, m), 4.19 (2H, q, J = 7.1), 3.40−3.25 (2H, m), 2.50−2.35 (1H, m), 2.35−2.24 (1H, m), 2.11−1.55 (3H, m), 1.43 (9H, s), 1.28 (3H, t, J = 7.1). MS (FAB) 349 (MNa^+) 327 (MH^+). 4*S*-*tert*-Butoxycarbonylamino-5-(2-oxopyrrolidin-3*S*-yl)pent-2-enoic acid ethyl ester (2.13 g, 6.53 mmol) was treated with EtOH saturated with HCl gas (100 mL), held at room temperature for 1 h, and then evaporated. The residue was taken up in $CHCl_3$ (50 mL), then washed with 10% K_2CO_3−H_2O (100 mL), followed by brine (50 mL). The organic layer was dried over K_2CO_3 and then evaporated to give 1.50 g (100%) of the deprotected amine. ^{1}H NMR ($CDCl_3$): δ 6.89 (1H, dd, J = 15.7, 6.7), 5.96 (1H, d, J = 15.7), 4.18 (2H, q, J = 7.1), 3.74−3.63 (1H, m), 3.38−3.29 (2H, m), 2.65−2.53 (1H, m), 2.40−2.28 (1H, m), 2.23−1.92 (1H, m), 1.85−1.70 (1H, m), 1.60−1.48 (1H, m), 1.26 (3H, t, J = 7.1). Anal. ($C_{11}H_{18}N_2O_3$) C, H, N.

4*S*-[(Naphthalene-2-carbonyl)amino]-5-(2-oxopyrrolidin-3*S*-yl)pent-2-enoic Acid Ethyl Ester (19). Representative Experimental. Compound **2** (30 mg, 0.13 mmol) in DMF (1 mL) was treated with diisopropylethylamine (0.07 mL, 0.40 mmol), 2-naphthoic acid (22 mg, 0.13 mmol), and HATU (49 mg, 0.13 mmol) and held at room temperature for 1 h. The solution was washed with brine (5 mL) and extracted with EtOAc (10 mL). Evaporation yielded 34 mg of crude product. Purification by preparative reverse phase chromatography (CH_3CN−H_2O gradient) yielded 20 mg (41%) of product. ^{1}H NMR ($CDCl_3$): δ 8.48 (1H, s), 8.01−7.85 (4H, m), 7.58−7.50 (2H, m), 6.98 (1H, dd, J = 15.6, 5.3), 6.04 (1H, d, J = 15.8), 4.85−4.78 (1H, m), 4.17 (2H, q, J = 7.0), 3.39−3.34 (2H, m), 2.64−2.47 (2H, m), 2.17−2.06 (1H, m), 1.97−1.82 (3H, m), 1.34 (3H, t, J = 7.0). MS (FAB): 381 (MH^+), 403 (MNa^+). Anal. ($C_{22}H_{24}N_2O_4$·0.5H_2O) C, H, N.

4*S*-{3-(3-Bromophenyl)acryloylamino]-5-(2-oxopyrrolidin-3*S*-yl)pent-2-enoic Acid Ethyl Ester (9). Compound **9** was prepared according to the representative experimental, using 3-bromocinnamic acid. ^{1}H NMR ($CDCl_3$): δ 7.67 (1H, s), 7.53 (1H, d, J = 15.6), 7.50−7.38 (2H, m), 7.22 (1H, t, J = 7.8), 6.89 (1H, dd, J = 15.6, 5.3), 6.47 (1H, d, J = 15.7), 5.98 (1H, d, J = 15.6), 4.74−4.63 (1H, m), 4.17 (2H, q, J = 7.1), 3.42−3.35 (2H, m), 2.60−2.40 (2H, m), 2.10−1.70 (3H, m), 1.26 (3H, t, J = 7.1). MS (FAB): 435 (MH^+), 457 (MNa^+). Anal. ($C_{20}H_{23}BrN_2O_4$·0.3H_2O) C, H, N.

4*S*-[3-(3-Bromo-4-methyl-phenyl)-acryloylamino]-5-(2-oxopyrrolidin-3*S*-yl)pent-2-enoic Acid Ethyl Ester (10). Compound **10** was prepared according to the representative experimental, using 3-bromo-4-methylcinnamic acid. ^{1}H NMR ($CDCl_3$): δ 7.81 (1H, d, J = 7.0), 7.65 (1H, s), 7.52 (1H, d, J = 15.6), 7.35−7.20 (2H, m), 6.89 (1H, dd, J = 15.6, 5.3), 6.46 (1H, d, J = 15.7), 6.15 (1H, s), 5.95 (1H, d, J = 15.6), 4.78−4.65 (1H, m), 4.17 (2H, q, J = 7.1), 3.40−3.30 (2H, m), 2.60−2.35 (5H, m), 2.10−1.70 (3H, m), 1.27 (3H, t, J = 7.1). MS (FAB): 449.1090 (MH^+, calcd 449.1076), 471 (MNa^+).

4*S*-[3-(3-Bromo-4-fluorophenyl)acryloylamino]-5-(2-oxopyrrolidin-3*S*-yl)pent-2-enoic Acid Ethyl Ester (11). Compound **11** was prepared according to the representative experimental, using 3-bromo-4-fluorocinnamic acid. ^{1}H NMR ($CDCl_3$): δ 8.02 (1H, d, J = 6.6), 7.71 (1H, d, J = 8.6), 7.52 (1H, d, J = 15.6), 7.42−7.39 (1H, m), 7.11 (1H, t, J = 8.3), 6.88 (1H, dd, J = 15.6, 5.4), 6.41 (1H, d, J = 15.6), 5.97 (1H, d, J = 15.7), 5.94 (1H, s), 4.75−4.61 (1H, m), 4.17 (2H, q, J = 7.1), 3.41−3.37 (2H, m), 2.59−2.45 (2H, m), 2.17−1.82 (3H, m), 1.27 (3H, t, J = 7.2). MS (FAB): 453.0812 (MH^+, calcd. 453.0825), 475 (MNa^+).

4*S*-[3-(2,5-Dibromophenyl)acryloylamino]-5-(2-oxopyrrolidin-3*S*-yl)pent-2-enoic Acid Ethyl Ester (12). Compound **12** was prepared according to the representative experimental, using 2,5-dibromocinnamic acid. ^{1}H NMR ($CDCl_3$): δ 8.15 (1H, d, J = 6.4), 7.92 (1H, d, J = 15.6), 7.71 (1H, s), 7.47 (1H, d, J = 8.6), 7.32 (1H, d, J = 8.6), 6.93 (1H, dd, J = 15.6, 5.4), 6.43 (1H, d, J = 15.6), 6.03 (1H, d, J = 15.6), 5.62 (1H, s), 4.68−4.63 (1H, m), 4.22 (2H, q, J = 7.1), 3.42−3.37 (2H, m), 2.54−2.44 (1H, m), 2.05−1.58 (4H, m), 1.30 (3H, t, J = 7.1). MS (FAB): 515.0021 (MH^+, calcd. 515.0005).

4*S*-(3-Benzo[1,3]dioxol-5-yl-acryloylamino)-5-(2-oxopyrrolidin-3*S*-yl)pent-2-enoic Acid Ethyl Ester (13). Compound **13** was prepared according to the representative experimental, using 3,4-methylenedioxycinnamic acid. ^{1}H NMR ($CDCl_3$): δ 7.54 (1H, d, J = 6.3), 7.51 (1H, d, J = 15.1), 7.01 (1H, s), 6.97 (1H, d, J = 8.1), 6.89 (1H, dd, J = 15.7, 5.3), 6.78 (1H, d, J = 7.9) 6.35 (1H, d, J = 15.6), 6.01−5.93 (4H, m), 4.74−4.72 (1H, m), 4.20 (2H, q, J = 7.1), 3.37−3.34 (2H, m), 2.53−2.42 (2H, m), 2.04−1.70 (3H, m), 1.26 (3H, t, J = 7.1). MS (FAB): 423.1545 (MNa^+, calcd. 423.1532), 423 (MNa^+).

4*S*-[3′-(6′-Bromo-benzo[1,3]dioxol-5-yl)acryloylamino]-5-(2-oxopyrrolidin-3*S*-yl)pent-2-enoic Acid Ethyl Ester (14). Compound **14** was prepared according to the representative experimental, using 2-bromo-3,4-methylenedioxycinnamic acid. ^{1}H NMR ($CDCl_3$): δ 7.91 (1H, d, J = 15.5), 7.78 (1H, d, J = 6.8), 7.05 (1H, s), 7.03 (1H, s), 6.89 (1H, dd, J = 15.6, 5.4), 6.29 (1H, d, J = 15.5,), 6.03 (2H, s), 6.01, (1H, s), 5.98 (1H, d, J = 14.5), 4.72−4.67 (1H, m), 4.16 (2H, q, J = 7.1), 3.49−3.36 (2H, m), 2.56−2.43 (2H, m), 2.17−1.82 (3H, m), 1.28 (3H, t, J = 7.1). MS (FAB): 479.0807 (MH^+, calcd. 479.0818), 501 (MNa^+). Anal. ($C_{21}H_{23}BrN_2O_6$·0.6H_2O) C, H, N.

4*S*-[(2-Methyl-5-phenylfuran-3-carbonyl)amino]-5-(2-oxopyrrolidin-3*S*-yl)pent-2-enoic Acid Ethyl Ester (15). Compound **15** was prepared according to the representative experimental, using 2-methyl-5-phenyl-furan-3-carboxylic acid. ^{1}H NMR ($CDCl_3$): δ 8.16 (1H, d, J = 5.8), 7.69−7.62 (2H, m), 7.42−7.34 (2H, m), 7.29−7.22 (1H, m), 6.93 (1H, s), 6.92 (1H, dd, J = 15.6, 5.3), 6.01 (1H, d, J = 5.6), 5.68 (1H, s), 4.74−4.63 (1H, m), 4.18 (2H, q, J = 7.1), 3.42−3.34 (2H, m), 2.68 (3H, s), 2.62−2.42 (2H, m), 2.10−1.77 (3H, m), 1.27 (3H, t, J = 7.1). MS (FAB): 411.1929 (MH^+, calcd 411.1920), 433 (MNa^+).

4*S*-[(2*H*-Chromene-3-carbonyl)amino]-5-(2-oxopyrrolidin-3*S*-yl)pent-2-enoic Acid Ethyl Ester (16). Compound **16** was prepared according to the representative experimental, using 2*H*-chromene-3-carboxylic acid. ^{1}H NMR ($CDCl_3$): δ 8.46 (1H, d, J = 5.4), 7.23−7.12 (3H, m), 6.94−6.81 (3H, m), 5.97 (1H, dd, J = 15.6, 1.4), 5.62 (1H, s), 5.03 (1H, d, J = 1.2), 4.64−4.53 (1H, m), 4.17 (2H, q, J = 7.2), 3.42−3.38 (2H, m), 2.58−2.40 (2H, m), 2.03−1.75 (3H, m), 1.27 (3H, t, J = 7.2). MS (FAB): 385.1774 (MH^+, calcd. 385.1763).

4*S*-[(6-Chloro-2*H*-chromene-3-carbonyl)amino]-5-(2-oxopyrrolidin-3*S*-yl)pent-2-enoic Acid Ethyl Ester (17). Compound **17** was prepared according to the representative experimental, using 6-chloro-2*H*-chromene-3-carboxylic acid. ^{1}H NMR ($CDCl_3$): δ 8.61 (1H, d, J = 5.1), 7.18−7.09 (3H, m), 6.86 (1H, dd, J = 15.6, 5.6), 6.76 (1H, d, J = 8.2), 5.96 (1H, d, J = 15.6), 5.88 (1H, s), 5.03 (2H, s), 4.60−4.50 (1H, m), 4.18 (2H, q, J = 7.2), 3.43−3.36 (2H, m), 2.59−2.40 (2H, m), 2.04−1.76 (3H, m), 1.27 (3H, t, J = 7.1). MS (FAB): 419.1361 (MH^+, calcd 419.1374), 441 (MNa^+). Anal. ($C_{21}H_{23}ClN_2O_5$·0.7H_2O) C, H, N.

4*S*-[(6-Bromo-2*H*-chromene-3-carbonyl)amino]-5-(2-oxopyrrolidin-3*S*-yl)pent-2-enoic Acid Ethyl Ester (18).

Compound **18** was prepared according to the representative experimental, using 6-bromo-2*H*-chromene-3-carboxylic acid. ^{1}H NMR ($CDCl_3$): δ 8.72 (1H, d, J = 5.2), 7.28−7.23 (2H, m), 7.16 (1H, s), 6.86 (1H, dd, J = 15.6, 5.6), 6.72 (1H, d, J = 9.1), 5.96 (1H, dd, J = 5.6, 1.4), 5.02 (d, 2H, J = 1.2), 4.60−4.49 (1H, m), 4.17 (2H, q, J = 7.1), 3.43−3.36 (2H, m), 2.59−2.40 (2H, m), 2.04−1.76 (3H, m), 1.27 (3H, t, J = 7.1). MS (FAB): 463.0883 (MH^+, calcd 463.0869), 485 (MNa^+). Anal. ($C_{21}H_{23}BrN_2O_5 \cdot 0.6H_2O$) C, H, N.

4*S*-[(6-Methyl-naphthalene-2-carbonyl)amino]-5-(2-oxopyrrolidin-3*S*-yl)pent-2-enoic Acid Ethyl Ester (20). Compound **20** was prepared according to the representative experimental, using 6-methyl-2-naphthoic acid. ^{1}H NMR ($CDCl_3$): δ 8.42 (1H, s), 7.95 (1H, dd, J = 8.6, 1.7), 7.84 (1H, d, J = 8.4), 7.79 (1H, d, J = 8.6), 7.63 (1H, s), 7.36 (1H, dd, J = 8.6, 1.4), 6.97 (1H, dd, J = 15.6, 5.3), 6.01 (1H, d, J = 15.6), 5.99 (1H, s), 4.90−4.78 (1H, m), 4.17 (2H, q, J = 7.1), 3.43−3.30 (2H, m), 2.70−2.60 (2H, m), 2.52 (3H, s), 2.20−2.05 (1H, m), 2.00−1.80 (2H, m), 1.26 (3H, t, J = 1.7). MS (FAB): 395.1964 (MH^+, calcd. 395.1971), 417 (MNa^+).

4*S*-[(7-Bromonaphthalene-2-carbonyl)amino]-5-(2-oxopyrrolidin-3*S*-yl)pent-2-enoic Acid Ethyl Ester (21). Compound **21** was prepared according to the representative experimental, using 7-bromo-2-naphthoic acid.[24] ^{1}H NMR ($CDCl_3$): δ 8.82 (1H, d, J = 5.7), 8.39 (1H, s), 8.09 (1H, d, J = 1.6), 8.02 (1H, dd, J = 8.6, 1.6), 7.85 (1H, d, J = 8.6), 7.73 (1H, d, J = 8.7), 7.61 (1H, dd, J = 8.7, 1.9), 6.96 (1H, dd, J = 15.6, 5.4), 6.06 (1H, s), 6.03 (1H, d, J = 15.6), 4.85−4.70 (1H, m), 4.17 (2H, q, J = 7.1), 3.45−3.30 (2H, m), 2.70−2.40 (2H, m), 2.20−1.80 (3H, m), 1.27 (3H, t, J = 7.1). MS (FAB): 459.0906 (MH^+, calcd. 459.0919), 481 (MNa^+). Anal. ($C_{22}H_{23}BrN_2O_4$) C, H, N.

Parallel Synthesis

PS-carbodiimide[25] (15.9 umol, 1.59 mmol/g, approximately 10 mg) was loaded into each well of a filter bottom 96 well plate (Charybdis Calypso NXT-75 reaction block system[26]) using a resin-loading device (Millipore).[27] Compound **2** (5 μmol, 0.5 mL of a 10 mM solution in DMF) was subsequently loaded into each well using a multichannel pipet. A solution of the selected carboxylic acid (7.5 μmol, 0.1 mL of a 75 mM solution in DMF) was added to each well using an automated liquid handler (Charybdis Iliad PS2).[26] The plate was agitated overnight at room temperature, then filtered into a second solid-bottom 96 well plate, and then evaporated using a Genevac Atlas HT-12[28] evaporator.

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