Design, Synthesis, and Evaluation of Inhibitors for Severe Acute Respiratory Syndrome 3C-Like Protease Based on Phthalhydrazide Ketones or Heteroaromatic Esters

Jianmin Zhang,[†] Hanna I. Pettersson,[†] Carly Huitema,[‡] Chunying Niu,[§] Jiang Yin,[§] Michael N. G. James,[§,||] Lindsay D. Eltis,[‡] and John C. Vederas*[,†,⊥]

Departments of Chemistry and Biochemistry, University of Alberta, Edmonton, Alberta, Canada T6G 2G2, and Departments of Microbiology and Biochemistry, University of British Columbia, Vancouver, British Columbia, Canada V6T 1Z3

Received December 11, 2006

The 3C-like protease (3CL^{pro}), which controls the severe acute respiratory syndrome (SARS) coronavirus replication, has been identified as a potential target for drug design in the treatment of SARS. A series of tetrapeptide phthalhydrazide ketones, pyridinyl esters, and their analogs have been designed, synthesized, and evaluated as potential SARS 3CL^{pro} inhibitors. Some pyridinyl esters are identified as very potent inhibitors, with IC_{50} values in the nanomolar range (50−65 nM). Electrospray mass spectrometry indicates a mechanism involving acylation of the active site cysteine thiol for this class of inhibitors.

Introduction

Severe acute respiratory syndrome (SARS) is a life-threatening form of atypical pneumonia that first occurred in Guangdong province in China and rapidly spread through several countries in other parts of the world in 2003.[1,2] The fatality rate of SARS is rather high. In the first wave of the SARS outbreak, around 8500 people worldwide were affected and over 900 died. The re-emergence of SARS in south China was reported in December 2003 and again in the spring of 2004.[3]

The causative agent of SARS has been identified as a novel human coronavirus (SARS-CoV).[1,2,4] The SARS coronavirus, including the well-characterized human CoV 229E (HCoV) and porcine transmissible gastroenteritis virus (TGEV), are positive-strand RNA viruses.[5] Studies of these viruses have identified at least three major processes that are essential for successful viral replication in the host and are good targets for novel therapeutics: viral entry, transcription of the viral genome, and proteolytic processing. The replicase polyproteins pp1a and pp1b encoded by the SARS CoV[6−8] are extensively processed to yield the functional subunits for successful viral propagation. The proteolysis is mediated by the main protease 3CL^{pro} (also called M^{pro}), a cysteine protease and a papain-like cysteine protease. The active site of SARS-CoV 3CL protease has a catalytic dyad with the sulfur of Cys145 as a nucleophile and the imidazole ring of His41 as a general base.[4,9,10] The 3CL^{pro} cleaves pp1a/pp1b at 11 conserved interdomain sites, in which the P1 position has a well-conserved Gln residue and the P2 position has a hydrophobic amino acid residue.[8,11]

A number of small molecules have been reported that are potent inhibitors of 3CL^{pro}, such as the HIV protease inhibitor TL-3,[12] bifunctional aryl boronic acids,[13] thiophenylcarboxylate,[14] keto-glutamine analogues,[15a] anilides,[16a] and benzotriazole esters.[17] One such study has recently indicated that the covalent inhibitor aza-peptide epoxide has been visualized in the SARS 3CL^{pro} crystal structure.[18] However, for many of the inhibitors it is still not determined how they interact with the SARS 3CL^{pro}. Clearly, further studies will assist understanding of the inhibition mechanisms and the possible discovery of therapeutic agents.

In our previous studies,[15] keto-glutamine analogues (e.g., **1**, IC_{50} = 0.6 μM, Figure 1) were found to be effective SARS 3CL^{pro} inhibitors and, therefore, interesting lead compounds for the development of anti-SARS drugs. Related investigations on the 3C cysteine protease of the hepatitis A virus (HAV)[19] have shown that these inhibitors display rapid competitive inhibition, followed by slow irreversible inactivation of the enzyme. Structure−activity studies also indicated that both the γ-lactam and the phthalhydrazide moieties are important structural factors for good inhibition. Interestingly, **2a** (AG7088,[20] Figure 1) was recently reported to show no inhibition of SARS 3CL^{pro} at 100 μM concentration. However, replacement of the γ-lactam moiety by a phenyl group generated modified AG7088 analogues that have significantly improved inhibition (e.g., **2b**, IC_{50} = 39 μM, Figure 1).[16b] Based on this concept, we decided to examine a modified keto-glutamine analog in which the γ-lactam is replaced with a phenyl group.

Screening of compound libraries demonstrated that aromatic ester **3** (Figure 1, IC_{50} = 0.5 μM) is approximately equipotent to keto-glutamine **1** (IC_{50} = 0.6 μM) as a 3CL^{pro} inhibitor.[14,15a] Hence, it also seemed reasonable to prepare "mix-and-match" combinations based on the assumption that the carbonyl of **3** and the ketone of **1** could bind at the same enzyme site (e.g., at or near the Cys145 nucleophile). This assumption is supported by the observation that **3** inhibits HAV 3C^{pro} as strongly as it

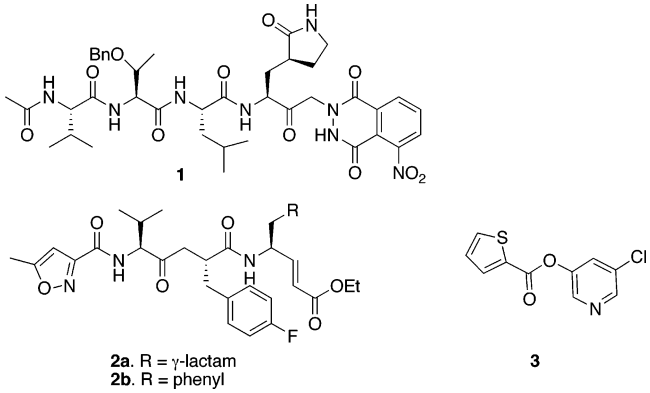
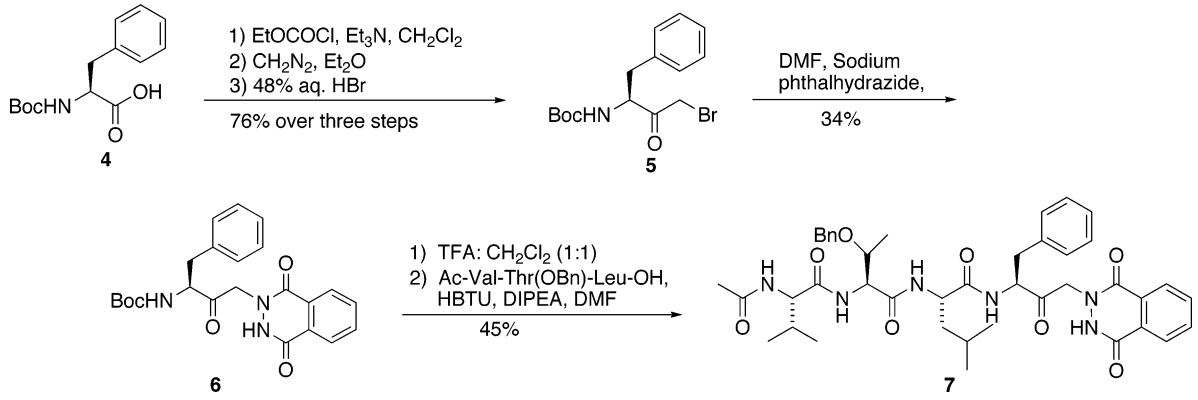


Figure 1. Structures of inhibitors **1**, **2a**, **2b**, and **3**.

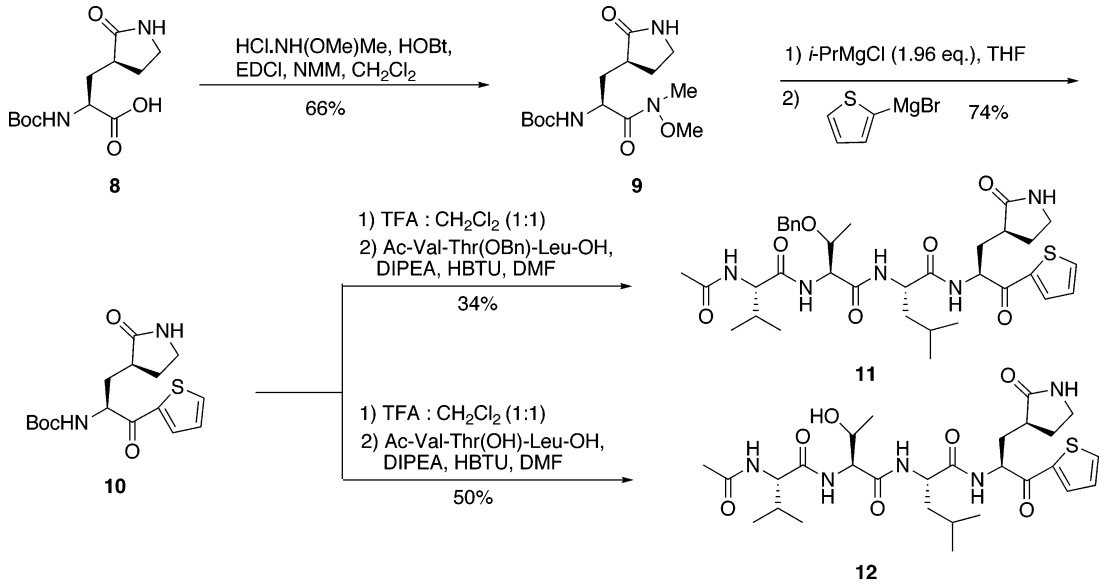
* To whom correspondence should be addressed. Tel.: +1-780-492-5475. Fax: +1-780-492-2134. E-mail: john.vederas@ualberta.ca.
[†] Department of Chemistry, University of Alberta.
[‡] Departments of Microbiology and Biochemistry, University of British Columbia.
[§] Department of Biochemistry, University of Alberta.
[||] Canada Research Chair in Protein Structure and Function.
[⊥] Canada Research Chair in Bioorganic and Medicinal Chemistry.

10.1021/jm061425k CCC: $37.00
Published on Web 03/24/2007

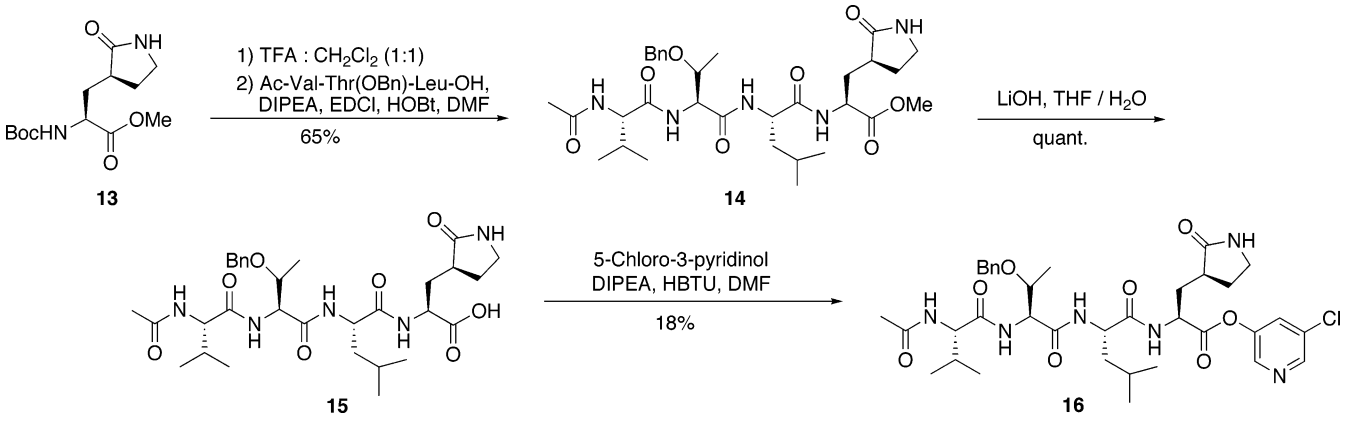
Scheme 1. Synthesis of Tetrapeptide **7** from *N*-Boc-L-phenylalanine **4**



Scheme 2. Synthesis of Tetrapeptide **11** and **12** from Cyclic Glutamic Acid **8**



Scheme 3. Synthesis of Tetrapeptide **16** from Cyclic Glutamic Acid Ester **13**



does SARS-CoV 3CL^{pro}. Although the two proteases have low sequence identity, they appear to accept similar substrate analogs in their active sites. Finally, in the present work we also examine a library of 103 structures based on a modification of the aromatic ester **3**.

Results and Discussion

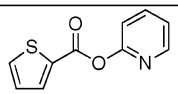
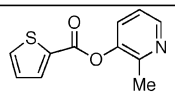
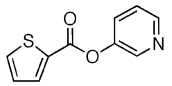
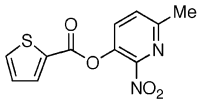
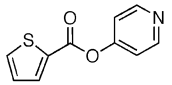
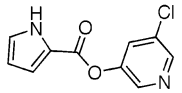
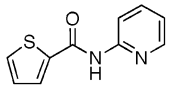
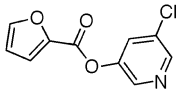
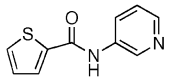
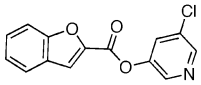
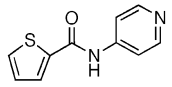
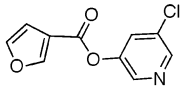
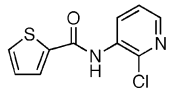
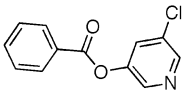
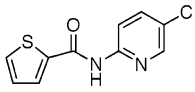
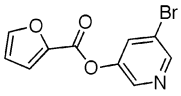
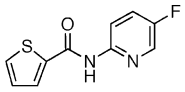
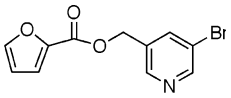
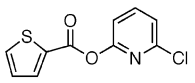
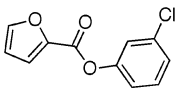
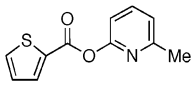
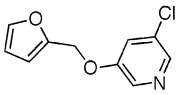
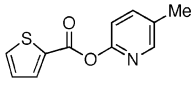
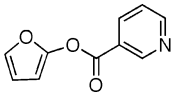
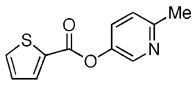
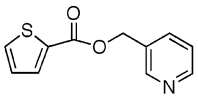
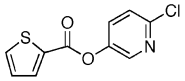
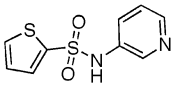
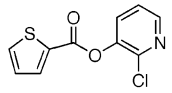
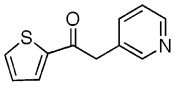
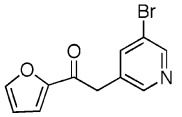
Peptide Analogues. The keto-glutamine analogue **7** with a phenyl substituent in the P1 position[21] was synthesized using an established method[15a−c] reported previously by our group (Scheme 1). Activation of the carboxylic acid of *N*-Boc-L-phenylalanine **4** with ethyl chloroformate, followed by diazomethane substitution, provided the diazo compound. Treating

Table 1. Evaluation of Compounds **7**, **11**, **12**, and **16** as SARS 3CL^{pro} Inhibitors

cmpd	% inhibition at 100 μM[a]
7	72
11	–
12	23
16	–

[a] Dash means <10% inhibition.

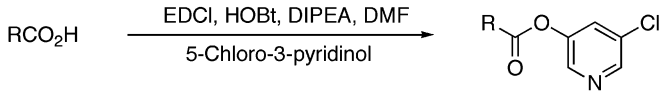
Table 2. Preliminary Evaluation of Selected Analogues as SARS 3CL^{pro} Inhibitors

cmpd	structure	% inhibition at 100 μM[a]	cmpd	structure	% inhibition at 100 μM[a]
17		13	**32**		-
18		91	**33**		15
19		-	**34**		65
20		-	**35**		99
21		13	**36**		94
22		-	**37**		89
23		-	**38**		92
24		-	**39**		99
25		-	**40**		90
26		-	**41**		40
27		38	**42**		11
28		-	**43**		80
29		-	**44**		19
30		13	**45**		-
31		20	**46**		-
			47		-

[a] Dash means <10% inhibition.

Scheme 4. Synthesis of a Library of 3-Chloropyridinyl Esters by Method A or B

Method A:



Method B:

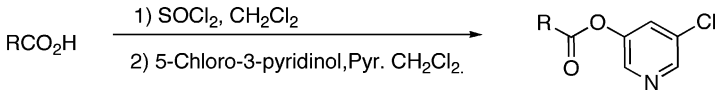


Table 3. Evaluation of Aldehydes as SARS 3CL^pro Inhibitors

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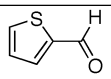
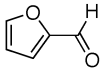
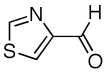
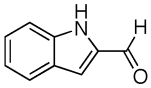
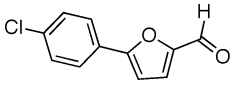
53		-
54		23
55		36
56		39
57		70

[a] Dash means <10% inhibition.

Table 4. Evaluation of Pyridinyl Esters as SARS 3CL^pro Inhibitors

cmpd	IC_{50} (nM)	$T_{1/2}$[a] (h)
35	60	12
36	170	28
39	50	119
48	65	42
49	95	32
50	270	125
51	340	53
52	63	41

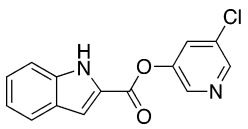
[a] Half-life for hydrolysis at pH 7.5 in phosphate buffer.

the diazo intermediate with 48% aqueous HBr gave compound **5** in 76% yield over three steps. Nucleophilic substitution of the bromide with sodium phthalhydrazide produced compound **6** in 34% yield. Removal of the Boc group with TFA, followed by coupling with the recognition tripeptide Ac-Val-Thr(OBn)-Leu-OH, afforded the desired tetrapeptide **7** in 45% yield.

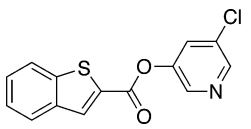
The target tetrapeptides **11** and **12** containing a thiophene moiety were prepared following the route shown in Scheme 2. The synthesis started from the advanced intermediate, cyclic glutamic acid **8**, prepared by a literature method.[15a,22] Conversion of carboxylic acid **8** to the Weinreb amide[23] **9** proceeded in 66% yield. Isopropyl magnesium bromide was added to deprotonate the two acidic protons of **9**; this was followed by nucleophilic attack by thiophene magnesium bromide to generate compound **10** in 74% yield. Removal of the Boc protecting group, followed by coupling with the tripeptide Ac-Val-Thr-(OBn)-Leu-OH, afforded the desired tetrapeptide **11** in 34% yield. However, removal of the benzyl group of compound **11** to yield compound **12** was not successful, either by palladium-catalyzed hydrogenation or with a Lewis acid. Hence, compound **12** was obtained in 50% yield by an alternative route: removal of the Boc group of compound **10**, followed by direct coupling with the tripeptide Ac-Val-Thr(OH)-Leu-OH. Surprisingly, our previous study[15] showed that removal of the threonine O-benzyl group of the corresponding keto-glutamine inhibitor **1** slightly *increased* the IC_{50} values with 3CL^pro but still gave low micromolar inhibition of 3CL^pro.

The tetrapeptide **16** with the pyridinyl moiety was prepared using a different synthetic strategy (Scheme 3). Removal of the Boc group of the cyclic glutamic acid ester **13**, followed by coupling with tripeptide Ac-Val-Thr(OBn)-Leu-OH, provided the tetrapeptide **14** in 65% yield. Hydrolysis of the ester group of **14** yielded compound **15** with a carboxylic acid in quantitative yield. Coupling of **15** with 5-chloro-3-pyridinol afforded the tetrapeptide **16** as a 1:1 mixture of diastereomers at the glutamine analogue α-carbon. As 5-chloro-3-pyridinol is a poor nucleophile, transient cyclization of the activated carboxyl group with the neighboring amide to an azlactone could compete with the coupling reaction, thereby leading to the epimerization.

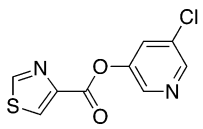
Compounds **7**, **11**, **12**, and **16** were tested against SARS 3CL^pro using a continuous fluorometric assay, as described previ-



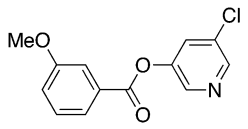
48, 99% inhibition at 1 μM



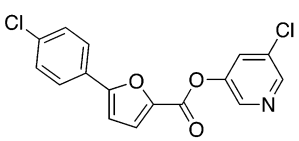
49, 96% inhibition at 1 μM



50, 98% inhibition at 1 μM



51, 97% inhibition at 1 μM



52, 98% inhibition at 1 μM

Figure 2. Structures of SARS 3CL^pro inhibitors **48**−**52**.

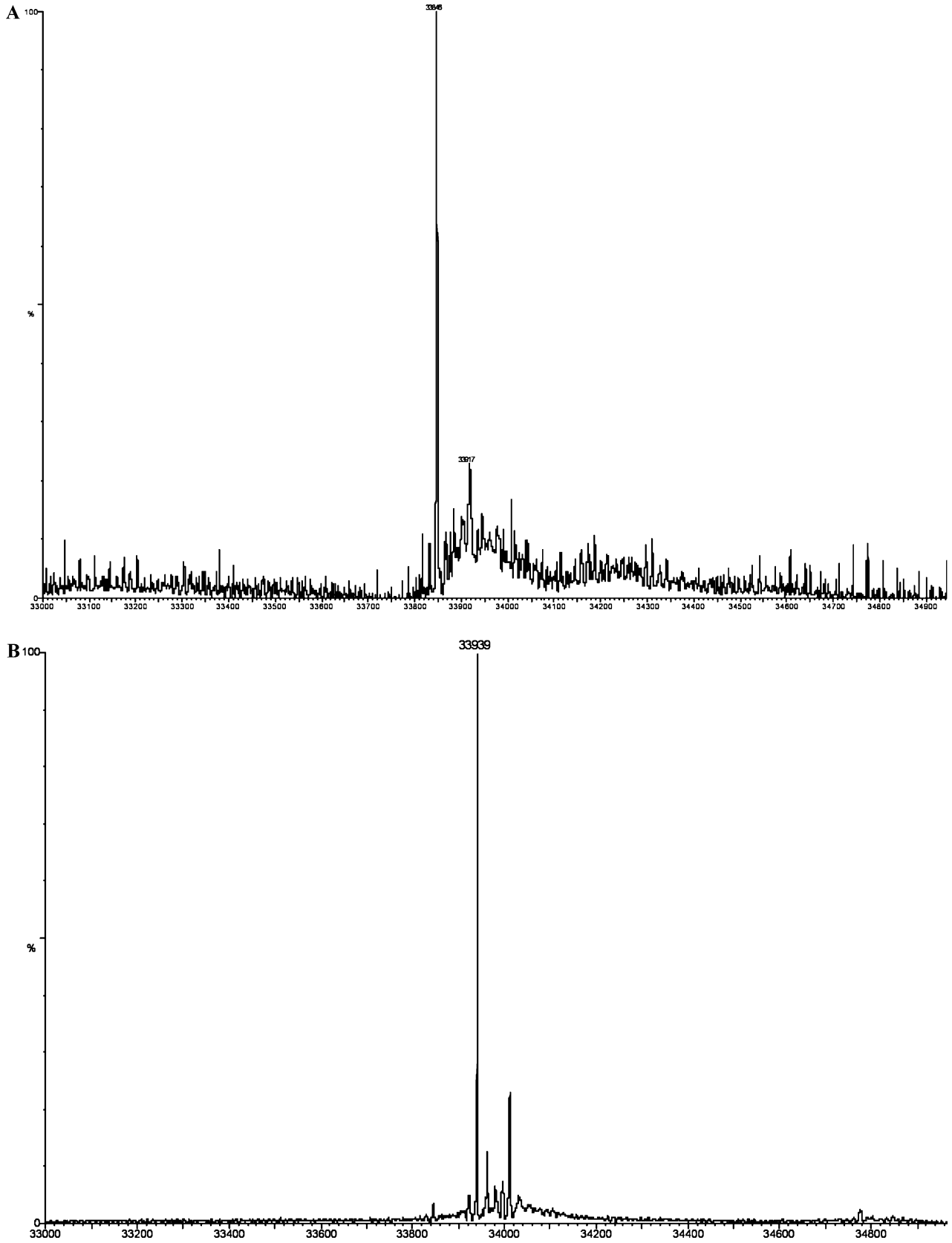


Figure 3. (A) Mass spectrum of wild type SARS 3CL^{pro} (M^+ = 33 846 Da). (B) Mass spectrum of the complex of 3CL^{pro} and inhibitor **39** (M^+ = 33 939 Da).

ously.[14,15a] In brief, the assay was performed at 22 °C in a solution containing 100 mM KH_2PO_4/K_2HPO_4 at pH 7.5, 2 mM EDTA, 10 μM fluorogenic substrate (Abz-SVTLQSG-Tyr(NO_2)R, 93% purity), 1.5 μM His-tagged protease (N-terminal, cleavable), and 1% DMSO without any preincubation. Increase in fluorescence (λ_{ex} 340 nm, λ_{em} 415 nm) was monitored using a

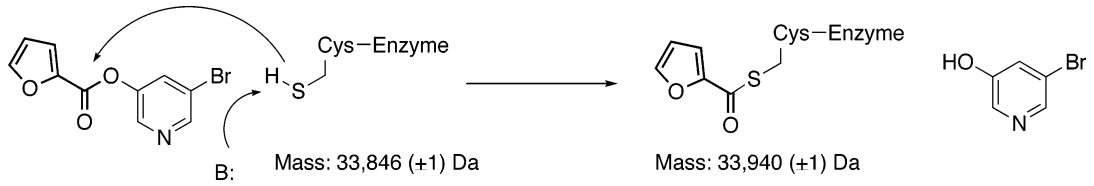


Figure 4. Proposed mechanism of inhibition of SARS 3CL^{pro} by pyridinyl ester inhibitors.

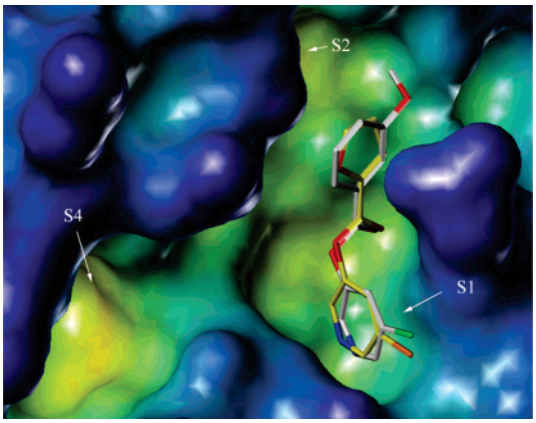


Figure 5. The modeling binding conformations of inhibitors **39** and **51** in the active site of SARS 3CL^{pro}. Inhibitors **39** (yellow) and **51** (white) are shown in the stick mode (oxygen atoms are red; nitrogen atoms are blue; chlorine is green; and bromine is orange). The color of the enzyme surface shows the cavity depth from the outside of the protein (blue) to the inside of the protein (yellow).

spectrofluorimeter. Initial rates were calculated using the first 3 min of the progress curves. The testing results are listed in Table 1.

As outlined in Table 1, in comparison to the keto-glutamine with the γ-lactam moiety (94% inhibition at 100 μM),[15a] the analog **7** with a phenyl substituent does not show improved inhibition in our case (72% inhibition at 100 μM). This is unexpected in view of the better inhibition of SARS 3CL^{pro} by **2b** compared to that of **2a**.[16b] All the other tetrapeptide analogs **11**, **12**, and **16** show only very weak inhibition against 3CL^{pro}, even though **16** would be expected to be a good substrate mimic. The results are consistent with our previous finding[15a] that both the γ-lactam and the phthalhydrazide moieties are important structural features for this class of tetrapeptide inhibitor.

Aromatic Esters and Related Analogues. The relatively accessible structure of inhibitor **3**, which has an IC_{50} of 0.5 μM with SARS 3CL^{pro},[14] encouraged us to make a focused library around this motif to examine structure−activity relationships. A series of 31 analogues were initially synthesized and tested against 3CL^{pro} using the fluorescent assay conditions described earlier. The results, which examine only the initial binding, are outlined in Table 2. As a result of self-quenching of fluorescence at high substrate concentrations, low concentrations of fluorogenic peptide and, consequently, low overall conversions (i.e., short times) were used. Compared to the ester analogues, the amides (e.g., **20**) show almost no inhibition at 100 μM concentration under these conditions. In addition, when the ester groups are at the *ortho* or *para* position, instead of the *meta* position, of the pyridinyl rings (e.g., **17**, **19**, **28**), poor or no inhibition is observed. Furthermore, if the chlorine substituent is at the 2 or 6 position (i.e., **30**, **31**) instead of the 3 position (e.g., **3**) of the pyridinyl ring, the inhibition decreases dramatically. However, the analogue with a hydrogen (i.e., **19**) instead of a chlorine substituent at the 3 position still displays reasonably good inhibition. From the analysis of compounds (**3**, **18**, **34**, **35**, **36**, **37**, **38**) with moderate to good inhibition, it appears that in addition to the pyridinyl ring, the other aromatic rings (furan or thiophene) are also key structural factors for potent inhibition. Compound **39** with a bromine substituent at the *meta* position of the pyridinyl ring showed very potent inhibition (98%) against 3CL^{pro} even at 1 μM concentration.

Compared to compounds **18** and **39**, the analogues **40** and **44** with one extra carbon inserted between the pyridinyl rings and the oxygen atom exhibited much weaker inhibition. In addition, the amide analog **21**, the ether **42**, the sulfonamide **45**, and the ketones **46** and **47** showed almost no activity against the enzyme. Compound **43** with a reversed ester linkage also showed decreased inhibition against 3CL^{pro} at 100 μM.

Based on the above screening results, a library of pyridinyl esters was prepared through automated parallel synthesis, by the coupling reactions (Method A or B, Scheme 4) between 5-chloro-3-pyridinol and 90 commercially available carboxylic acids, most of which are aromatic carboxylic acids (see Supporting Information for structures). Of the 90 targets, 18 compounds were relatively unstable to aqueous conditions and were not examined further, but 72 compounds were obtained and purified by automated HPLC-MS analysis.

This library of 3-chloropyridinyl esters was tested against 0.4 μM SARS 3CL^{pro} using the continuous fluorometric assay. Compounds **48** to **52** showed almost complete inhibition of 3CL^{pro} at 1 μM concentration (Figure 2).

Based on the testing results, it seemed that for this class of 3-chloropyridinyl esters, both noncovalent protein−inhibitor interactions as well as inherent chemical reactivity (i.e., propensity for enzyme acylation) may play important roles in the strong inhibition of 3CL^{pro}. It is well-known that compared to the esters aldehydes are chemically more reactive toward nucleophiles and are potentially good inhibitors for cysteine enzymes through formation of hemithioacetals.[24] Hence, several aldehydes having part of the structural motif of the most effective inhibitors were synthesized and tested (Table 3). However, none of these aldehydes showed very potent inhibition against 3CL^{pro}, which suggests that both the 3-chloropyridine unit and the other aromatic ring play important roles in strong binding to the enzyme (see Supporting Information for the structures of aromatic and nonaromatic carboxylic acids).

The activities of selected pyridinyl esters were further investigated using a non-His-tagged protease, which was more stable and had higher activity in the assay. Initial rates were calculated using the first 5 min of the progress curves. Under the assay conditions, all of the esters were reasonably stable to hydrolysis, as studied by ^{1}H NMR, and some were extremely potent inhibitors of 3CL^{pro}, as summarized in Table 4. For example, in an assay containing 100 nM enzyme, compound **39** had an IC_{50} of 50 nM. This corresponds to the lowest IC_{50} theoretically measurable in the assay and, to our knowledge, is one of the lowest IC_{50} values reported for the SARS 3CL^{pro}. Indeed, pyridinyl ester **39** was such an effective inhibitor of 3CL^{pro}, that it was not possible to measure k_{inact} and K_i, even in the presence of the fluorogenic substrate. Mixing the enzyme and the inhibitor in a 1:1 molar ratio completely inactivated

the enzyme within the dead-time of the assay (~6 s). However, the activity of 3CL^{pro} recovered with a $t_{1/2}$ of ~4 min. This behavior is consistent with inactivation of the enzyme through rapid acylation of the enzyme by the inhibitor, followed by its reactivation through slow deacylation.

The inhibition mechanism was also investigated by electrospray mass spectrometry. As shown in Figure 3A, the mass of the wild type enzyme is 33 846 Da, and the mass of the complex of enzyme and inhibitor **39** is 33 939 Da (Figure 3B, expected mass 33 940 = 33 845 + 95 Da, ±1 Da). This indicates covalent bond formation via acylation of the enzyme by the furoyl group (MW 95) of inhibitor **39** with departure of the 3-bromo-5-hydroxypyridine leaving group is the likely mechanism of inhibition. The electrospray mass spectra of the complexes of inhibitors **35**, **48**, and **51** with the enzyme (see Supporting Information for spectra) also confirmed an analogous acylation mechanism for these 3-chloropyridinyl esters (Figure 4).

Inhibitors **39** and **51** were modeled into the active site of SARS 3CL^{pro} (PDB code: 2A5K)[18] using the program Autodock 3.0.5[25] (Figure 5). The general trends of the predicted conformations follow the "Cys-S1" binding mode described for a group of compounds having a similar basic design. The halopyridine moieties fit comfortably in the S1 substrate binding site, where the majority of the interactions with the enzyme are contributed by van der Waals contacts between the pyridine function and the two "walls" of the S1 pocket comprised of residues Phe140, Leu141, Asn142, and residues Glu166 and His172, respectively. The halogen atom in either inhibitor does not interact significantly with the enzyme and points out toward the solvent. The pyridine nitrogen atom of inhibitor **39** forms a hydrogen bond with N$^{\epsilon 2}$ of His163, the P1 specificity-determining residue. The carbonyl oxygen of the central ester function is directed into the oxyanion hole and receives hydrogen bonds from O$^{\gamma}$ of Ser144 and the main chain N atoms of Gly143 and Cys145. The furan function in inhibitor **39** and anisole function in inhibitor **51** are located near the catalytic residue Cys145, forming mainly hydrophobic contacts. Because the S1 pocket is crucial to substrate recognition, the presence of the halopyridine function in the S1 subsite would effectively block the entry of peptidyl substrates.

Proteases are also known to hydrolyze ester substrates. The inhibition mechanism of the ester-based inhibitors described in this study likely involves covalent attachment of the inhibitors (at the carbonyl carbon) to the nucleophilic sulfur of Cys145. While the central ester bonds of these inhibitors provide the main interaction with the catalytic Cys145, the initial binding of the intact inhibitors into the active site of 3CL^{pro} (Figure 5) may critically depend on the derivatized pyridine moieties as well as the functional groups on the acid side of the ester bond. The docking results indicate that the halopyridine groups of these inhibitors have a strong propensity to bind inside the S1 pocket of the 3CL^{pro} substrate binding site. In addition, the derivatizations on either side of the central ester bond could affect the electrophilicity of the carbonyl function, which, in turn, may modify the reactivity of inhibitors to SARS 3CL^{pro}. The biochemical data presented in this study should be viewed as the consequence of combined influences of inhibitor binding affinity and chemical reactivity with the enzyme.

In conclusion, in this paper we have reported a series of peptidomimetics, pyridinyl esters, and their analogs, of which some halopyridinyl esters are very potent SARS 3CL^{pro} inhibitors (IC_{50} values as low as 50 to 65 nM). A covalent bond formation mechanism for the enzyme−inhibitor complexes has been proposed based on the electrospray mass spectrometry investigation. Structure−activity relationship studies indicated that the chemical structure plays a key role in the strong inhibition against 3CL^{pro}. Based on the structure−activity relationship, efforts to discover more effective reversible inhibitors and the study of the inhibition mechanism are ongoing in our lab.

Experimental Section

Materials and Reagents. SARS-CoV 3CL^{pro} was expressed and prepared according to the previously described procedure.[14] All of the reagents were of analytical grade and used without further purification.

(*R*)-*tert*-Butyl-4-bromo-3-oxo-1-phenylbutan-2-ylcarbamate (5). To a solution of *N*-Boc-L-phenylalanine **4** (1 g, 3.77 mmol) in THF (30 mL) at 0 °C was added triethylamine (0.58 mL, 4.15 mmol), followed by ethyl chloroformate (0.39 mL, 4.02 mmol). The resulting solution was stirred at 0 °C for half an hour. The formed salt was filtered out through gravity filtration quickly, the filtrate was transferred into an excess ethereal diazomethane solution (approximately 12 mmol), and the temperature was maintained at 0 °C. The reaction mixture was slowly warmed to rt and stirred for another hour. The solvent was removed under reduced pressure to obtain the crude diazo product (1.08 g, quant.), which was used in the following step without any purification. To the solution of the diazo-ketone compound in THF (30 mL) at 0 °C was added aq 48% HBr (0.67 mL, 4.00 mmol) dropwise over 15 min. The reaction mixture was stirred for additional 15 min, quenched with saturated aq $NaHCO_3$ (10 mL), and the solvent was removed in vacuo. The residue was diluted with H_2O and extracted with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$ and then concentrated in vacuo. Purification of the crude product by flash chromatography on silica gel (EtOAc) afforded **5** as a white solid (0.98 g, 76%). $[\alpha]^{25}_D = +6.1$ (*c* 0.18, $CHCl_3$); IR ($CHCl_3$, cast) 3364, 3029, 2985, 2936, 1733, 1679, 1515, 1456 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.12−7.33 (m, 5H), 5.05−5.12 (m, 1H), 4.64−4.74 (m, 1H), 3.95 (d, 1H, J = 13.7 Hz), 3.83 (d, 1H, J = 13.7 Hz), 3.04−3.13 (m, 1H), 2.94−3.04 (m, 1H), 1.40 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 200.8, 155.2, 135.8, 129.2, 128.9, 127.3, 80.5, 58.5, 37.8, 33.2, 28.3; HRMS (ES) calcd for $C_{15}H_{20}NO_3BrNa$, 364.0519; found, 364.0516.

(*S*)-*tert*-Butyl-4-(1,4-dioxo-3,4-dihydrophthalazin-2(1*H*)-yl)-3-oxo-1-phenylbutan-2-ylcarbamate (6). To a solution of phthalhydrazide (1 g, 6.15 mmol) in DMF (30 mL) was added NaH (0.17 g, 6.8 mmol), and the mixture was stirred at rt for 2 h after which it was filtered and washed with anhydrous Et_2O to yield sodium phthalhydrazide as a white solid (1.18 g, quant.). To a suspension of sodium phthalhydrazide (117 mg, 0.64 mmol) in DMF (4 mL) was added a solution of bromoketone **5** (200 mg, 0.58 mmol) in DMF (8 mL) dropwise over 1 h. After stirring for 6 h at rt, the solvent was removed in vacuo, and the residue was diluted with H_2O and extracted with EtOAc. The combined organic layers were dried over $MgSO_4$, and the solvent was removed in vacuo to obtain the crude product, which was purified by flash column chromatography on silica gel (50/50 EtOAc/hexanes) to obtain **6** (83 mg, 34%) as a white solid. $[\alpha]^{25}_D = -20.4$ (*c* 0.09, MeOH); IR (microscope) 3359, 3167, 3012, 2921, 1751, 1738, 1687, 1656, 1601, 1523, 1494 cm^{-1}; ^{1}H NMR (CD_3OD, 500 MHz) δ 8.28 (d, 1H, J = 7.6 Hz), 8.11 (d, 1H, J = 8.0 Hz), 7.91 (dd, 1H, J = 7.6, 7.3 Hz), 7.86 (dd, 1H, J = 8.0, 7.3 Hz), 7.15−7.28 (m, 5H), 5.12 (s, 2H), 4.56 (dd, 1H, J = 9.8, 5.0 Hz), 3.21 (dd, 1H, J = 14.0, 5.0 Hz), 2.83 (dd, 1H, J = 14.0, 9.8 Hz), 1.36 (s, 9H); ^{13}C NMR (CD_3OD, 125 MHz) δ 205.4, 161.7, 157.8, 151.4, 138.6, 135.0, 133.6, 130.5, 129.9, 129.5, 127.7, 127.4, 126.0, 125.0, 80.8, 70.2, 60.0, 37.5, 28.6; HRMS (ES) calcd for $C_{23}H_{25}N_3O_5Na$, 446.1686; found, 446.1683.

(*S*)-2-((2*S*,3*R*)-2-((*S*)-2-Acetamido-3-methylbutanamido)-3-(benzyloxy)butanamido)-*N*-((*S*)-4-(1,4-dioxo-3,4-dihydrophthalazin-2(1*H*)-yl)-3-oxo-1-phenylbutan-2-yl)-4-methylpentanamide (7). To a solution of **6** (16.5 mg, 0.045 mmol) was added TFA/DCM (2 mL, 1:1 ratio) at 0 °C. The resulting solution was stirred

for 1.5 h, after which the reaction mixture was concentrated in vacuo and the residue was triturated with Et_2O to yield the trifluoroacetate salt. To a solution of trifluoroacetate salt in DMF (4 mL) at rt was added Ac-Val-Thr(OBn)-Leu-OH (30.6 mg, 0.045 mmol), DIPEA (16 μL, 0.090 mmol), and HBTU (17.7 mg, 0.045 mmol). After 4 h of stirring, the solvent was removed in vacuo. The crude product was purified by HPLC (Waters C18 Bondpak 10 μm, 125 Å; 100 × 25 mm, 15 mL/min, 5 min elution of 20% acetonitrile, followed by a linear gradient elution over 25 min of 20 to 100% acetonitrile in 0.075% TFA/H_2O, t_R = 26 min) to afford **7** (13.6 mg, 45% yield). $[\alpha]^{25}_D = -49.4$ (*c* 0.05, DMSO); IR (microscope) 3064, 2958, 1740, 1655, 1625, 1535, 1493 cm^{-1}; ^{1}H NMR (CD_3OD, 500 MHz) δ 8.30 (ddd, 1H, *J* = 7.8, 1.4, 0.70 Hz), 8.10 (ddd, 1H, *J* = 7.8, 1.4, 0.64 Hz), 7.83 (dd, 1H, *J* = 7.8, 0.5 Hz), 7.63 (dd, 1H, *J* = 7.8, 0.5 Hz), 7.36−7.12 (m, 10H), 5.12 (d, 1H, *J* = 17.0 Hz), 5.04 (d, 1H, *J* = 17.0 Hz), 4.58 (d, 1H, *J* = 11.3 Hz), 4.42 (d, 1H, *J* = 11.3 Hz), 4.40−4.33 (m, 2H), 4.13 (dd, 1H, *J* = 6.4, 3.1 Hz), 4.07 (d, 1H, *J* = 6.4 Hz), 3.48 (dd, 1H, *J* = 14.0, 7.1 Hz), 3.26−3.18 (m, 1H), 2.94 (dd, 1H, *J* = 14.0, 9.7 Hz), 2.12 (dd, 1H, *J* = 13.4, 6.7 Hz), 1.95 (s, 3H), 1.57−1.46 (m, 2H), 1.40−1.28 (m, 1H), 1.22 (d, 3H, *J* = 6.4 Hz), 0.99 (d, 3H, *J* = 4.2 Hz), 0.98 (d, 3H, *J* = 4.2 Hz), 0.83 (d, 3H, *J* = 6.3 Hz), 0.79 (d, 3H, *J* = 6.3 Hz); HRMS (ES) calcd for $C_{42}H_{52}N_6O_8Na$, 791.3739; found, 791.3736.

(*S*)-*tert*-Butyl-1-(methoxy(methyl)amino)-1-oxo-3-((*S*)-2-oxopyrrolidin-3-yl)propan-2-ylcarbamate (9). To a solution of cyclic glutamic acid **8**[15] (200 mg, 0.73 mmol) in DCM (5 mL) at 0 °C was added Weinreb amine[23] (71 mg, 0.73 mmol), EDCI (141 mg, 0.73 mmol), HOBt (99 mg, 0.73 mmol), and NMM (0.16 mL, 1.46 mmol). The resulting solution was stirred overnight while allowing to warm slowly to rt. Then the reaction mixture was diluted with DCM (50 mL) and washed with water and brine. The combined organic layers were dried over $MgSO_4$ and then concentrated in vacuo. Purification of the crude product by flash chromatography on silica gel (90/10, EtOAc/MeOH) afforded **5** as a white foam (151 mg, 66%). $[\alpha]^{25}_D = -0.22$ (*c* 0.27, $CHCl_3$); IR ($CHCl_3$, cast) 3293, 2976, 1693 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.85 (br, 1H, NH), 5.45 (br, 1H, NH), 4.60 (t, 1H, *J* = 8.0 Hz), 3.77 (s, 3H), 3.33 (dd, 2H, *J* = 8.7, 4.0 Hz), 3.20 (s, 1H), 3.28−3.16 (m, 2H), 2.54−2.42 (m, 2H), 2.15−2.02 (m, 1H), 1.89−1.77 (m, 1H), 1.72−1.62 (m, 1H), 1.42 (s, 9H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 179.6, 172.6, 155.8, 79.6, 61.6, 49.3, 40.3, 38.0, 34.4, 32.1, 28.3, 28.0; HRMS (ES) calcd for $C_{14}H_{25}N_3O_5Na$, 338.1686; found, 338.1688.

(*S*)-*tert*-Butyl-1-oxo-3-((*S*)-2-oxopyrrolidin-3-yl)-1-(thiophen-2-yl)propan-2-ylcarbamate (10). To a solution of **9** (120 mg, 0.38 mmol) in THF (2 mL) at −15 °C was charged *i*-PrMgCl (0.37 mL, 2 M solution in THF, 0.74 mmol) dropwise to afford a clear solution. After stirring for 10 min, thiophen-2-yl-magnesium bromide (1.2 mL, 1.0 M solution in THF, 1.2 mmol) was added slowly, and the temperature was maintained lower than −5 °C. The cooling bath was removed, and the mixture was allowed to warm to rt over 30 min. After 4 h of stirring at rt, the reaction was complete, which was monitored by TLC. The reaction mixture was cooled on an ice bath, and 1.0 N HCl (2 mL) was added slowly, followed by an EtOAc extraction. The combined organic layer was dried over $MgSO_4$, and the solvent was removed in vacuo to obtain the crude mixture, which was purified by flash column chromatography on silica gel (EtOAc) to obtain product **10** (81 mg, 63%) as a white foam and recovered starting material **9** (18 mg, 15%). $[\alpha]^{25}_D = +33.7$ (*c* 0.25, $CHCl_3$); IR ($CHCl_3$, cast) 3283, 2977, 1663, 1515, 1440 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.84 (m, 1H), 7.66 (d, 1H, *J* = 4.8 Hz), 7.12 (dd, 1H, *J* = 4.8, 3.8 Hz), 6.29 (br, 1H, NH), 5.68 (br, 1H, NH), 5.10 (dd, 1H, *J* = 6.5, 0.6 Hz), 3.38−3.30 (m, 2H), 2.58−2.46 (m, 2H), 2.22−2.14 (m, 1H), 1.94−1.84 (m, 2H), 1.42 (s, 9H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 191.5, 179.5, 155.6, 141.2, 134.8, 133.2, 128.4, 79.9, 54.9, 40.3, 38.2, 35.3, 35.2, 28.3; HRMS (ES) calcd for $C_{16}H_{22}N_2O_4SNa$, 361.1193; found, 361.1190.

(*S*)-2-((2*S*,3*R*)-2-((*S*)-2-Acetamido-3-methylbutanamido)-3-(benzyloxy)butanamido)-4-methyl-*N*-((*S*)-1-oxo-3-((*S*)-2-oxopyrrolidin-3-yl)-1-(thiophen-2-yl)propan-2-yl)pentanamide (11). To a solution of **10** (33.8 mg, 0.1 mmol) was added TFA/CH_2Cl_2 (3 mL, 1:1 ratio) at 0 °C. The resulting solution was stirred for 1.5 h, after which the reaction mixture was concentrated in vacuo and the residue was triturated with Et_2O to yield the trifluoroacetate salt. To a solution of trifluoroacetate salt in DMF (5 mL) at rt was added Ac-Val-Thr(OBn)-Leu-OH (46.4 mg, 0.1 mmol), DIPEA (35 uL, 0.2 mmol), and HBTU (39.5 mg, 0.1 mmol). After 4 h of stirring, the solvent was removed in vacuo. The crude product was purified by HPLC (Waters C18 Bondpak 10 μm, 125 Å; 100 × 25 mm, 15 mL/min, 10 min elution of 10% acetonitrile followed by linear gradient elution over 45 min of 10 to 100% acetonitrile in 0.075% TFA/H_2O, t_R = 34 min) to afford **11** (23.4 mg, 34% yield). $[\alpha]^{25}_D = +2.8$ (*c* 0.07, MeOH); IR (microscope) 3280, 3070, 2960, 2873, 1635, 1517, 1438 cm^{-1}; ^{1}H NMR (CD_3OD, 500 MHz) δ 7.93 (dd, 1H, *J* = 3.8, 1.0 Hz), 7.83 (dd, 1H, *J* = 4.9, 1.0 Hz), 7.34−7.26 (m, 5H), 7.16 (dd, 1H, *J* = 4.9, 3.8 Hz), 5.14 (dd, 1H, *J* = 11.0, 4.0 Hz), 4.55 (d, 1H, *J* = 11.5 Hz), 4.46 (d, 1H, *J* = 11.5 Hz), 4.40 (d, 2H, *J* = 4.3 Hz), 4.23 (d, 1H, *J* = 7.2 Hz), 4.03 (dd, 1H, *J* = 6.3, 4.3 Hz), 3.28−3.16 (m, 2H), 2.32−2.18 (m, 3H), 2.14−2.04 (m, 1H), 2.05 (s, 3H), 1.86−1.72 (m, 2H), 1.64−1.52 (m, 3H), 1.19 (d, 3H, *J* = 6.3 Hz), 0.97 (d, 3H, *J* = 6.8 Hz), 0.93 (d, 3H, *J* = 6.8 Hz), 0.87 (d, 3H, *J* = 6.0 Hz), 0.85 (d, 3H, *J* = 6.0 Hz); ^{13}C NMR (CD_3OD, 125 MHz) δ 192.6, 182.1, 174.8, 174.0, 173.9, 171.9, 142.8, 139.8, 136.1, 134.5, 129.6, 129.5, 129.1, 128.8, 75.9, 72.4, 60.5, 59.4, 55.7, 53.2, 41.9, 41.6, 40.0, 33.7, 31.6, 29.3, 25.8, 23.6, 22.5, 21.4, 19.9, 18.6, 16.5; HRMS (ES) calcd for $C_{35}H_{49}N_5O_7SNa$, 706.3245; found, 706.3247.

(*S*)-2-((2*S*,3*R*)-2-((*S*)-2-Acetamido-3-methylbutanamido)-3-hydroxybutanamido)-4-methyl-*N*-((*S*)-1-oxo-3-((*S*)-2-oxopyrrolidin-3-yl)-1-(thiophen-2-yl)propan-2-yl)pentanamide (12). To a solution of **10** (9.5 mg, 0.028 mmol) was added TFA/CH_2Cl_2 (1 mL, 1:1 ratio) at 0 °C. The resulting solution was stirred for 1.5 h, after which the reaction mixture was concentrated in vacuo and the residue was triturated with Et_2O to yield the trifluoroacetate salt. To a solution of trifluoroacetate salt in DMF (1 mL) at rt was added Ac-Val-Thr(OBn)-Leu-OH (10.9 mg, 0.028 mmol), DIPEA (10 uL, 0.056 mmol), and HBTU (11.1 mg, 0.028 mmol). After 4 h of stirring, the solvent was removed in vacuo. The crude product was purified by HPLC (Waters C18 Bondpak 10 μm, 125 Å; 100 × 25 mm, 15 mL/min, 10 min elution of 10% acetonitrile followed by linear gradient elution over 45 min of 10 to 100% acetonitrile in 0.075% TFA/H_2O, t_R = 26 min) to afford **11** (8.3 mg, 48% yield). $[\alpha]^{25}_D = -53.1$ (*c* 0.11, MeOH); IR (microscope) 3277, 3083, 2962, 1632, 1537, 1438 cm^{-1}; ^{1}H NMR (CD_3OD, 400 MHz) δ 7.98 (dd, 1H, *J* = 3.9, 1.0 Hz), 7.88 (dd, 1H, *J* = 5.0, 1.0 Hz), 7.21 (dd, 1H, *J* = 5.0, 3.9 Hz), 5.33 (dd, 1H, *J* = 11.4, 4.0 Hz), 4.40 (dd, 1H, *J* = 8.5, 6.2 Hz), 4.36 (d, 1H, *J* = 4.4 Hz), 4.18 (d, 1H, *J* = 7.1 Hz), 4.14 (dd, 1H, *J* = 6.4, 4.4 Hz), 3.36−3.31 (m, 2H), 2.64−2.55 (m, 1H), 2.42−2.32 (m, 1H), 2.24 (ddd, 1H, *J* = 14.0, 11.4, 4.0 Hz), 2.11−2.01 (m, 1H), 2.00 (s, 3H), 1.92−1.74 (m, 2H), 1.66−1.54 (m, 3H), 1.16 (d, 3H, *J* = 6.4 Hz), 0.96 (d, 6H, *J* = 6.8 Hz), 0.91 (d, 3H, *J* = 6.2 Hz), 0.86 (d, 3H, *J* = 6.2 Hz); ^{13}C NMR (CD_3OD, 125 MHz) δ 192.1, 181.7, 174.5, 173.9, 173.6, 171.9, 142.7, 136.0, 134.4, 129.4, 68.2, 60.7, 59.5, 54.3, 53.1, 41.4, 41.3, 39.4, 34.0, 31.3, 28.8, 25.6, 23.2, 22.2, 21.7, 19.7, 19.5, 18.5; HRMS (ES) calcd for $C_{28}H_{43}N_5O_7SNa$, 616.2775; found, 616.2775.

(2*S*,5*S*,8*S*,11*S*)-8-((*R*)-1-(Benzyloxyl)ethyl)-5-isobutyl-11-isopropyl-4,7,10,13-tetraoxo-2-((*S*)-2-oxopyrrolidin-3-yl)methyl-3,6,9,12-tetraazatetradecan-1-oic Acid (15). To a solution of **14**[15] (45 mg, 0.071mmol) in THF/H_2O (10 mL, 1:1 ratio) at 0 °C was added LiOH (4.0 mg, 0.092 mmol). After 2 h of stirring, the solvent was removed in vacuo. Water (10 mL) and citric acid were added to adjust the pH of the solution to 3. The mixture was extracted with EtOAc and washed with water and brine. The combined organic layers were dried over $MgSO_4$ and then the solvent was removed in vacuo to afford the product **15** (42 mg, quant.) as a white foam. $[\alpha]^{25}_D = -22.9$ (*c* 0.30, MeOH); IR (microscope) 3276, 3089, 2961, 2873, 1633, 1545, 1438, 1404 cm^{-1}; ^{1}H NMR (CD_3OD, 400 MHz) δ 7.38−7.20 (m, 5H), 4.59 (d, 1H, *J* = 11.1 Hz), 4.47 (d, 1H, *J* = 11.1 Hz), 4.47 (d, 2H, *J* = 3.7 Hz), 4.18 (d, 1H, *J* = 6.8 Hz), 4.16−4.06 (m, 1H), 3.28−3.10 (m, 2H), 2.54−2.42

(m, 1H), 2.28−2.04 (m, 4H), 1.95 (s, 3H), 1.84−1.70 (m, 2H), 1.68−1.54 (m, 3H), 1.22 (d, 3H, J = 6.4 Hz), 0.97 (dd, 6H, J = 6.8, 1.9 Hz), 0.90−0.85 (m, 6H); ^{13}C NMR (CD_3OD, 125 MHz) δ 174.7, 174.5, 174.2, 173.9, 171.9, 171.8, 139.6, 129.4, 128.9, 128.7, 76.0, 72.5, 61.2, 58.9, 53.2, 52.0, 42.0, 41.4, 39.6, 34.3, 31.3, 28.7, 25.7, 23.5, 22.4, 22.0, 19.7, 18.7, 16.9; HRMS (ES) calcd for $C_{31}H_{47}N_5O_8Na$, 640.3317; found, 640.3320.

(5*S*,8*S*,11*S*)-5-Chloropyridin-3-yl-8-((*R*)-1-(benzyloxyl)ethyl)-5-isobutyl-11-isopropyl-4,7,10,13-tetraoxo-2-((*S*)-2-oxopyrrolidin-3-yl)methyl-3,6,9,12-tetraazatetradecan-1-oate (16). To a solution of **15** (26 mg, 0.043 mmol) in DMF (6 mL) at rt was added DIPEA (7.4 uL, 0.043 mmol) and HBTU (16.2 mg, 0.043 mmol) and 3-chloro-5-hydroxy-pyridine (5.5 mg, 0.043 mmol). After 4 h of stirring, the solvent was removed in vacuo. The crude product was purified by HPLC (Waters C18 Bondpak 10 μm, 125 Å; 100 × 25 mm, 15 mL/min, 10 min elution of 10% acetonitrile followed by a linear gradient elution over 45 min of 10 to 100% acetonitrile in 0.075% TFA/H_2O, t_R = 34 min) to afford **11** (5.6 mg, 18% yield). ^{1}H NMR (CD_3OD, 500 MHz) δ 8.50−8.43 (m, 1H), 8.39−8.34 (m, 1H), 7.78 (t, 0.5H, J = 2.3 Hz), 7.74 (t, 0.5H, J = 2.3 Hz), 7.32−7.22 (m, 5H), 4.57 (dd, 1H, J = 11.3, 7.5 Hz), 4.52−4.42 (m, 3H), 4.14 (d, 0.5H, J = 6.7 Hz), 4.14−4.10 (m, 1H), 4.09 (d, 0.5H, J = 6.7 Hz), 3.28−3.16 (m, 2H), 2.65−2.45 (m, 1H), 2.43−2.32 (m, 2H), 2.31−2.18 (m, 1H), 2.14−2.06 (m, 1H), 2.02−1.96 (m, 1H), 1.95 (s, 1.5H), 1.95 (s, 1.5H), 1.88−1.76 (m, 1H), 1.70−1.56 (m, 3H), 1.23 (d, 1.5H, J = 6.4 Hz), 1.22 (d, 1.5H, J = 6.4 Hz), 1.00−0.94 (m, 6H), 0.90−0.84 (m, 6H); HRMS (ES) calcd for $C_{36}H_{49}N_6O_8ClNa$, 751.3193; found, 751.3187.

General Procedure for the Preparation of Pyridinyl Esters. To a solution of carboxylic acid (2 mmol, 1.0 equiv) in DCM (5 mL) at rt was added thionyl chloride (0.4 mL, 1.3 equiv) and a catalytic amount of DMF (2 drops). After 20 h of stirring, the solvent was removed in vacuo to afford the acyl chloride product. A solution of the acyl chloride in DCM (5 mL) was added dropwise to a solution of pyridinyl alcohol or amine (1.0 equiv) and pyridine (0.18 mL, 1.1 equiv) in DCM (5 mL) at 0 °C. After 3 h of stirring, the solvent was removed in vacuo. The residue was diluted with H_2O and extracted with EtOAc. The combined organic layers were washed with brine, dried over $MgSO_4$, and then concentrated in vacuo. Purification of the crude product by flash chromatography on silica gel afforded the product as a solid in 43−90% yield.

Pyridin-2-yl Thiophene-2-carboxylate[26] **(17).** The title compound **17** was obtained following the standard procedure described above. Purification of the crude product by flash chromatography on silica gel (25/75 EtOAc/hexanes) afforded **17** as a white solid (270 mg, 66%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.45 (ddd, 1H, J = 4.9, 2.0, 0.8 Hz), 8.02 (dd, 1H, J = 3.8, 1.3 Hz), 7.84 (ddd, 1H, J = 8.1, 7.4, 2.0 Hz), 7.69 (dd, 1H, J = 4.9, 1.3 Hz), 7.27 (ddd, 1H, J = 7.4, 4.9, 0.9 Hz), 7.22 (dt, 1H, J = 8.1, 0.8 Hz), 7.18 (dd, 1H, J = 5.0, 3.8 Hz); HRMS [EI] calcd for $C_{10}H_7NO_2S$ (M^+), 205.0197; found, 205.0195.

Pyridin-3-yl Thiophene-2-carboxylate (18). The title compound **18** was obtained following the standard procedure described above. Purification of the crude product by flash chromatography on silica gel (50/50 EtOAc/hexanes) afforded **18** as a white solid (320 mg, 78%). IR ($CHCl_3$, cast) 3093, 1731, 1572, 1413 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.52 (m, 1H), 8.48 (dd, 1H, J = 4.6, 1.0 Hz), 7.96 (dd, 1H, J = 3.8, 1.3 Hz), 7.65 (dd, 1H, J = 5.0, 1.3 Hz), 7.57 (ddd, 1H, J = 8.3, 2.8, 1.0 Hz), 7.33 (ddd, 1H, J = 8.3, 4.6, 0.5 Hz), 7.15 (dd, 1H, J = 5.0, 3.8 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 160.0, 147.3, 147.1, 143.5, 135.2, 134.2, 131.9, 129.3, 128.2, 123.9; HRMS [EI] calcd for $C_{10}H_7NO_2S$ (M^+), 205.0197; found, 205.0197.

Pyridin-4-yl Thiophene-2-carboxylate (19). The title compound **19** was obtained following the standard procedure described above. Purification of the crude product by flash chromatography on silica gel (50/50 EtOAc/hexanes) afforded **19** as a white solid (300 mg, 73%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.67 (d, 2H, J = 6.1 Hz), 8.01 (dd, 1H, J = 3.8, 1.2 Hz), 7.72 (dd, 1H, J = 5.0, 1.3 Hz), 7.26 (dd, 2H, J = 4.6, 1.7 Hz), 7.21 (dd, 1H, J = 5.0, 3.8 Hz); HRMS [EI] calcd for $C_{10}H_7NO_2S$ (M^+), 205.0197; found, 205.0199.

***N*-(Pyridin-2-yl)thiophene-2-carboxamide**[27] **(20).** The title compound **20** was obtained following the standard procedure described above. Purification of the crude product by flash chromatography on silica gel (50/50 EtOAc/hexanes) afforded **20** (mixture of amide and enolate isomers) as a white solid (280 mg, 69%). ^{1}H NMR (CD_3OD, 300 MHz) δ 8.38 (ddd, 0.24H, J = 4.9, 1.9, 0.9 Hz), 8.33 (ddd, 0.76H, J = 4.9, 1.9, 0.9 Hz), 8.13 (dt, 0.76H, J = 8.4, 0.9 Hz), 7.92 (dd, 0.76H, J = 3.8, 1.2 Hz), 7.89 (ddd, 0.24H, J = 8.1, 7.5, 1.9 Hz), 7.80 (ddd, 0.76H, J = 8.1, 7.5, 1.9 Hz), 7.78 (ddd, 0.76H, J = 4.8, 1.2 Hz), 7.75 (dd, 0.76H, J = 5.0, 1.2 Hz), 7.53 (dd, 0.48H, J = 3.8, 1.2 Hz), 7.42 (dt, 0.24H, J = 8.1, 1.0 Hz), 7.34 (ddd, 0.24H, J = 7.5, 4.9, 1.0 Hz), 7.18 (dd, 0.76H, J = 5.0, 3.8 Hz), 7.14 (ddd, 0.76H, J = 7.4, 5.0, 1.0 Hz), 7.06 (dd, 0.48H, J = 5.0, 1.9 Hz); HRMS [EI] calcd for $C_{10}H_8NO_2S$ (M^+), 204.0357; found, 204.0357.

***N*-(Pyridin-3-yl)thiophene-2-carboxamide**[27] **(21).** The title compound **21** was obtained following the standard procedure described above. Purification of the crude product by flash chromatography on silica gel (EtOAc) afforded **21** as a slightly yellow solid (230 mg, 57%). IR ($CHCl_3$, cast) 3327, 3073, 2966, 1660, 1645, 1599, 1531, 1480 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 9.09 (br, 1H, NH), 8.64 (m, 1H), 8.26 (dd, 1H, J = 4.7, 1.3 Hz), 8.18 (ddd, 1H, J = 8.4, 2.5, 1.3 Hz), 7.72 (dd, 1H, J = 3.8, 1.1 Hz), 7.48 (dd, 1H, J = 5.0, 1.1 Hz), 7.21 (dd, 1H, J = 8.3, 4.7 Hz), 7.01 (dd, 1H, J = 5.0, 3.8 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 161.0, 145.1, 141.6, 138.7, 135.2, 131.5, 129.2, 128.2, 127.9, 123.9; HRMS [EI] calcd for $C_{10}H_8N_2OS$ (M^+), 206.0357; found, 206.03253.

***N*-(Pyridin-4-yl)thiophene-2-carboxamide**[27] **(22).** The title compound **22** was obtained following the standard procedure described above. Purification of the crude product by flash chromatography on silica gel (5/95 MeOH/EtOAc) afforded **22** as a slightly brown solid (190 mg, 47%). ^{1}H NMR (CD_3OD, 300 MHz) δ 8.41 (dd, 2H, J = 5.0, 1.6 Hz), 7.94 (dd, 1H, J = 3.8, 1.2 Hz), 7.79 (dd, 2H, J = 4.9, 1.6 Hz), 7.77 (dd, 1H, J = 5.0, 1.1 Hz), 7.19 (dd, 1H, J = 5.0, 3.8 Hz); HRMS [EI] calcd for $C_{10}H_8N_2OS$ (M^+), 204.0357; found, 204.0358.

***N*-(2-Chloropyridin-3-yl)thiophene-2-carboxamide**[28] **(23).** The title compound **23** was obtained following the standard procedure described above. Purification of the crude product by flash chromatography on silica gel (50/50 EtOAc/hexanes) afforded **23** as a white solid (400 mg, 84%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.85 (dd, 1H, J = 8.2, 1.7 Hz), 8.29 (br s, 1H), 8.15 (dd, 1H, J = 4.6, 1.5 Hz), 7.70 (dd, 1H, J = 3.8, 1.1 Hz), 7.63 (dd, 1H, J = 5.0, 1.1 Hz), 7.32 (dd, 1H, J = 8.2, 4.7 Hz), 7.19 (dd, 1H, J = 5.0, 3.8 Hz); HRMS [EI] calcd for $C_{10}H_7ClN_2OS$ (M^+), 237.9968; found, 237.9968.

***N*-(5-Chloropyridin-2-yl)thiophene-2-carboxamide**[29] **(24).** The title compound **24** was obtained following the standard procedure described above. Purification of the crude product by flash chromatography on silica gel (50/50 EtOAc/hexanes) afforded **24** as a white solid (430 mg, 90%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.41 (br s, 1H), 8.31 (d, 1H, J = 8.9 Hz), 8.26 (d, 1H, J = 2.5 Hz), 7.71 (dd, 1H, J = 8.9, 2.6 Hz), 7.67 (dd, 1H, J = 3.8, 1.1 Hz), 7.61 (dd, 1H, J = 5.0, 1.1 Hz), 7.16 (dd, 1H, J = 5.0, 3.8 Hz); HRMS [EI] calcd for $C_{10}H_7ClN_2OS$ (M^+), 237.9968; found, 237.9966.

***N*-(5-Fluoropyridin-2-yl)thiophene-2-carboxamide (25).** The title compound **25** was obtained following the standard procedure described above. Purification of the crude product by flash chromatography on silica gel (50/50 EtOAc/hexanes) afforded **25** as a yellow solid (350 mg, 79%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.45 (br s, 1H), 8.35 (dd, 1H, J = 9.1, 4.0 Hz), 8.18−8.12 (m, 1H), 7.67 (dd, 1H, J = 3.8, 1.2 Hz), 7.60 (dd, 1H, J = 5.0, 1.2 Hz), 7.49 (ddd, 1H, J = 9.2, 7.7, 3.0 Hz), 7.15 (dd, 1H, J = 5.0, 3.8 Hz); HRMS [EI] calcd for $C_{10}H_7FN_2OS$ (M^+), 222.0263; found, 222.0264.

6-Chloropyridin-2-yl Thiophene-2-carboxylate (26). The title compound **26** was obtained following the standard procedure described above. Purification of the crude product by flash chromatography on silica gel (50/50 EtOAc/hexanes) afforded **26** as a white solid (390 mg, 88%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ

8.02 (dd, 1H, J = 4.9, 1.3 Hz), 7.80 (t, 1H, J = 7.9 Hz), 7.71 (dd, 1H, J = 5.0, 1.3 Hz), 7.33 (d, 1H, J = 0.6 Hz), 7.19 (d, 1H, J = 7.7 Hz), 7.19 (dd, 1H, J = 4.2, 3.0 Hz); HRMS [EI] calcd for $C_{10}H_6ClNO_2S$ (M^+), 238.9808; found, 238.9812.

6-Methylpyridin-2-yl Thiophene-2-carboxylate (27). The title compound **27** was obtained following the standard procedure described above. Purification of the crude product by flash chromatography on silica gel (50/50 EtOAc/Hexanes) afforded **27** as a white solid (370 mg, 84%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.01 (dd, 1H, J = 3.8, 1.3 Hz), 7.71 (t, 1H, J = 7.7 Hz), 7.67 (dd, 1H, J = 4.9, 1.3 Hz), 7.17 (dd, 1H, J = 5.0, 3.8 Hz), 7.12 (ddd, 1H, J = 7.5, 0.8, 0.5 Hz), 7.01 (ddd, 1H, J = 8.0, 0.5, 0.5 Hz), 2.56 (s, 3H); HRMS [EI] calcd for $C_{11}H_9NO_2S$ (M^+), 219.0354; found, 219.0355.

5-Methylpyridin-2-yl Thiophene-2-carboxylate (28). The title compound **28** was obtained following the standard procedure described above. Purification of the crude product by flash chromatography on silica gel (50/50 EtOAc/hexanes) afforded **28** as a white solid (320 mg, 73%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.29 (dd, 1H, J = 5.1, 0.3 Hz), 8.00 (dd, 1H, J = 3.8, 1.3 Hz), 7.68 (dd, 1H, J = 5.0, 1.4 Hz), 7.18 (dd, 1H, J = 5.0, 3.8 Hz), 7.10−7.08 (m, 1H), 7.08−7.06 (m, 1H), 2.40 (s, 3H); HRMS [EI] calcd for $C_{11}H_9NO_2S$ (M^+), 219.0354; found, 219.0354.

6-Chloropyridin-3-yl Thiophene-2-carboxylate (29). The title compound **29** was obtained following the standard procedure described above. Purification of the crude product by flash chromatography on silica gel (50/50 EtOAc/hexanes) afforded **29** as a white solid (390 mg, 89%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.43 (d, 1H, J = 2.8 Hz), 8.00 (dd, 1H, J = 3.8, 1.2 Hz), 7.70 (dd, 1H, J = 5.0, 1.3 Hz), 7.49 (dd, 1H, J = 8.5, 2.8 Hz), 7.24−7.18 (m, 2H), 2.60 (s, 3H); HRMS [EI] calcd for $C_{11}H_9NO_2S$ (M^+), 219.0354; found, 219.0352.

6-Methylpyridin-3-yl Thiophene-2-carboxylate (30). The title compound **30** was obtained following the standard procedure described above. Purification of the crude product by flash chromatography on silica gel (50/50 EtOAc/hexanes) afforded **30** as a white solid (380 mg, 79%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.40 (d, 1H, J = 2.6 Hz), 8.03 (dd, 1H, J = 3.8, 1.2 Hz), 7.80 (dd, 1H, J = 8.6, 2.7 Hz), 7.71 (dd, 1H, J = 4.9, 1.2 Hz), 7.22 (d, 1H, J = 0.6 Hz), 7.19 (dd, 1H, J = 4.9, 3.9 Hz); HRMS [EI] calcd for $C_{10}H_6ClNO_2S$ (M^+), 238.9808; found, 238.9808.

6-Chloropyridin-3-yl Thiophene-2-carboxylate (31). The title compound **31** was obtained following the standard procedure described above. Purification of the crude product by flash chromatography on silica gel (50/50 EtOAc/hexanes) afforded **31** as a white solid (410 mg, 85%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.34 (dd, 1H, J = 4.7, 1.7 Hz), 8.06 (dd, 1H, J = 3.8, 1.2 Hz), 7.74 (dd, 1H, J = 5.0, 1.3 Hz), 7.69 (dd, 1H, J = 8.0, 1.7 Hz), 7.34 (dd, 1H, J = 8.0, 4.7 Hz), 7.22 (dd, 1H, J = 5.0, 3.8 Hz); HRMS [EI] calcd for $C_{10}H_6ClNO_2S$ (M^+), 238.9808; found, 238.9806.

2-Methylpyridin-3-yl Thiophene-2-carboxylate (32). The title compound **32** was obtained following the standard procedure described above. Purification of the crude product by flash chromatography on silica gel (EtOAc) afforded **32** as a white solid (380 mg, 87%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.48 (dd, 1H, J = 4.8, 1.5 Hz), 8.02 (dd, 1H, J = 3.8, 1.3 Hz), 7.71 (dd, 1H, J = 5.0, 1.3 Hz), 7.52 (dd, 1H, J = 8.1, 1.5 Hz), 7.25−7.19 (m, 2H), 2.50 (s, 3H); HRMS [EI] calcd for $C_{11}H_9NO_2S$ (M^+), 219.0354; found, 219.0354.

6-Methyl-2-nitropyridin-3-yl Thiophene-2-carboxylate (33). The title compound **33** was obtained following the standard procedure described above. Purification of the crude product by flash chromatography on silica gel (EtOAc) afforded **33** as a white solid (460 mg, 87%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.01 (dd, 1H, J = 3.8, 1.3 Hz), 7.78 (d, 1H, J = 8.3 Hz), 7.75 (dd, 1H, J = 5.0, 1.0 Hz), 7.53 (d, 1H, J = 8.3 Hz), 7.21 (dd, 1H, J = 5.0, 3.8 Hz), 2.68 (s, 3H); HRMS [EI] calcd for $C_{11}H_8N_2O_4S$ (M^+), 264.0205; found, 264.0206.

5-Chloropyridin-3-yl 1*H*-Pyrrole-2-carboxylate (34). The title compound **34** was obtained following the standard procedure described above. Purification of the crude product by flash chromatography on silica gel (25/75 EtOAc/hexanes) afforded **34** as a white solid (320 mg, 72%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.40−9.20 (br s, 1H), 8.49 (dd, 1H, J = 2.1, 0.4 Hz), 8.45 (dd, 1H, J = 2.3, 0.3 Hz), 7.66 (t, 1H, J = 2.3 Hz), 7.17 (ddd, 1H, J = 3.9, 2.5, 1.4 Hz), 7.11 (ddd, 1H, J = 2.8, 2.8, 1.4 Hz), 6.38 (ddd, 1H, J = 3.9, 2.8, 2.8 Hz); HRMS [EI] calcd for $C_{10}H_7N_2O_2Cl$ (M^+), 222.0196; found, 222.0198.

5-Chloro-pyridin-3-yl Furan-2-carboxylate (35). The title compound **35** was obtained following the standard procedure described above. Purification of the crude product by flash chromatography on silica gel (25/75 EtOAc/hexanes) afforded **35** as a white solid (360 mg, 81%). IR ($CHCl_3$, cast) 3131, 3077, 1749, 1564, 1464, 1438 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.60 (d, 1H, J = 2.0 Hz), 8.43 (d, 1H, J = 2.2 Hz), 7.67 (dd, 1H, J = 1.7, 0.8 Hz), 7.64 (t, 1H, J = 2.2 Hz), 7.40 (dd, 1H, J = 3.6, 0.8 Hz), 6.60 (dd, 1H, J = 3.6, 1.7 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 155.8, 147.9, 146.8, 146.1, 142.9, 141.3, 131.8, 129.5, 120.7, 112.5; HRMS [EI] calcd for $C_{10}H_6ClNO_3$ (M^+), 223.0036; found, 223.0035.

5-Chloropyridin-3-yl Benzofuran-2-carboxylate (36). The title compound **36** was obtained following the standard procedure described above. Purification of the crude product by flash chromatography on silica gel (25/75 EtOAc/hexanes) afforded **36** as a white solid (360 mg, 66%). IR ($CHCl_3$, cast) 3034, 1739, 1558, 1443 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.55−8.52 (m, 2H), 7.76 (d, 1H, J = 1.0 Hz), 7.73 (ddd, 1H, J = 8.0, 1.3, 0.7 Hz), 7.71 (t, 1H, J = 2.2 Hz), 7.62 (ddd, 1H, J = 8.5, 1.7, 1.0 Hz), 7.51 (ddd, 1H, J = 8.5, 7.2, 1.3 Hz), 7.35 (ddd, 1H, J = 8.0, 7.2, 1.0 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 156.8, 156.3, 146.8, 146.3, 143.5, 141.2, 131.9, 129.3, 128.7, 126.7, 124.3, 123.2, 116.6, 112.5; HRMS [EI] calcd for $C_{14}H_8ClNO_3$ (M^+), 273.0193; found, 273.0192.

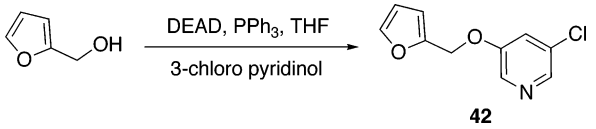
5-Chloropyridin-3-yl Furan-3-carboxylate (37). The title compound **37** was obtained following the standard procedure described above. Purification of the crude product by flash chromatography on silica gel (25/75 EtOAc/hexanes) afforded **37** as a white solid (400 mg, 90%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.50 (d, 1H, J = 2.1 Hz), 8.44 (d, 1H, J = 2.3 Hz), 8.23 (dd, 1H, J = 1.5, 0.8 Hz), 7.65 (t, 1H, J = 2.2 Hz), 7.53 (t, 1H, J = 1.7 Hz), 6.87 (dd, 1H, J = 1.9, 0.8 Hz); HRMS [EI] calcd for $C_{10}H_6ClNO_3$ (M^+), 223.0036; found, 223.0035.

5-Chloropyridin-3-yl Benzoate (38). The title compound **38** was obtained following the standard procedure described above. Purification of the crude product by flash chromatography on silica gel (25/75 EtOAc/hexanes) afforded **38** as a white solid (270 mg, 90%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.51 (d, 1H, J = 2.1 Hz), 8.48 (d, 1H, J = 2.3 Hz), 8.21 (dd, 1H, J = 1.3, 0.7 Hz), 8.18 (dd, 1H, J = 1.3, 0.6 Hz), 7.72−7.65 (m, 2H), 7.58−7.50 (m, 2H); HRMS [EI] calcd for $C_{12}H_8ClNO_2$ (M^+), 233.0244; found, 233.0242.

5-Bromo-pyridin-3-yl Furan-2-carboxylate (39). 3-Bromo pyridinol was prepared according to the literature procedure.[30] The title compound **39** was obtained following the standard procedure described above. Purification of the crude product by flash chromatography on silica gel (75/25 EtOAc/hexanes) afforded **39** as a white solid (330 mg, 62%). IR ($CHCl_3$, cast) 3131, 3071, 1745, 1559, 1436, 1421 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.62−8.58 (m, 1H), 8.62−8.58 (m, 1H), 7.79 (t, 1H, J = 2.0 Hz), 7.72 (dd, 1H, J = 1.7, 0.8 Hz), 7.44 (dd, 1H, J = 3.5, 0.8 Hz), 6.63 (dd, 1H, J = 3.5, 1.7 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 155.8, 148.2, 147.9, 146.9, 142.9, 141.6, 132.2, 120.7, 120.0, 112.5; HRMS [EI] calcd for $C_{10}H_6BrNO_3$ (M^+), 266.9531; found, 266.9535.

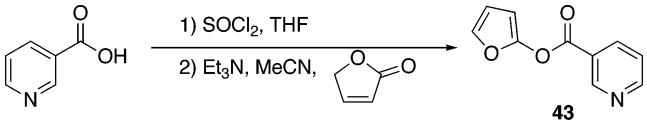
(5-Bromo-pyridin-3-yl)methyl Furan-2-carboxylate (40). 5-(Bromo-pyridin-3-yl)-methanol was prepared according to the literature procedure.[31] The title compound **40** was obtained following the standard procedure described above. Purification of the crude product by flash chromatography on silica gel (50/50 EtOAc/hexanes) afforded **40** as a white solid (310 mg, 58%). IR ($CHCl_3$, cast) 3139, 1725, 1580, 1473, 1424 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.73−8.60 (m, 2H), 7.98−7.96 (m, 1H), 7.59 (t, 1H, J = 0.8 Hz), 7.22 (d, 1H, J = 3.5 Hz), 6.52 (dd, 1H, J = 3.5, 1.7 Hz), 5.32 (s, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 158.1, 150.4, 147.2,

Scheme 5



42

Scheme 6



43

146.9, 143.9, 139.1, 133.2, 119.0, 118.9, 112.1, 63.0; HRMS [EI] calcd for $C_{11}H_8BrNO_3$ (M^+), 282.9759; found, 282.9763.

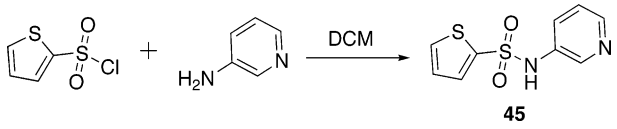
3-Chlorophenyl Furan-2-carboxylate (41). The title compound **41** was obtained following the standard procedure described above. Purification of the crude product by flash chromatography on silica gel (25/75 EtOAc/hexanes) afforded **41** as a white solid (370 mg, 84%). IR ($CHCl_3$, cast) 3141, 1743, 1593, 1574, 1468 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.65 (dd, 1H, J = 1.6, 0.7 Hz), 7.36 (dd, 1H, J = 3.5, 0.7 Hz), 7.36−7.32 (m, 1H), 7.24 (m, 1H, J = 1.6 Hz), 7.22 (dd, 1H, J = 1.9, 1.0 Hz), 7.15−7.12 (m, 1H), 6.57 (dd, 1H, J = 3.5, 1.6 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 156.4, 150.7, 147.4, 143.6, 134.8, 130.2, 126.4, 122.3, 120.0, 119.9, 112.3; HRMS [EI] calcd for $C_{11}H_7ClO_3$ (M^+), 222.0084; found, 220.0083.

3-Chloro-5-furan-(2-ylmethoxy)pyridine (42). To a solution of PPh_3 (1.24 g, 4.74 mmol) in THF (20 mL) at rt was added DEAD (0.75 mL, 4.74 mmol) dropwise. After 30 min of stirring at rt, furfuryl alcohol (0.31 mg, 3.16 mmol) and 3-chloro pyridinol (0.61 g, 4.74 mmol) were added to the reaction mixture (Scheme 5). The resulting solution was stirred overnight at rt and then the solvent was removed in vacuo. The residue was diluted with DCM, washed with water, 1 N HCl, satd $NaHCO_3$ solution, and brine, dried over $MgSO_4$, and then concentrated in vacuo. Purification of the crude product by flash chromatography on silica gel (gradient column, 25/75 EtOAc/hexanes to 50/50 EtOAc/hexanes) afforded **42** as a yellow liquid (150 mg, 23%), which solidified in the fridge. IR ($CHCl_3$, cast) 3048, 2932, 1575, 1449, 1422 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 8.22 (d, 1H, J = 2.6 Hz), 8.14 (d, 1H, J = 1.9 Hz), 7.41 (dd, 1H, J = 1.8, 0.8 Hz), 7.27 (t, 1H, J = 2.2 Hz), 6.43 (d, 1H, J = 3.3 Hz), 6.35 (dd, 1H, J = 2.2, 1.8 Hz), 5.00 (s, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 154.7, 148.8, 143.6, 141.2, 136.3, 132.0, 122.0, 110.9, 110.7, 62.9; HRMS [EI] calcd for $C_{10}H_8ClNO_2$ (M^+), 209.0244; found, 209.0244.

Furan-2-yl Nicotinate (43). The title compound **43** was prepared by a modified literature procedure.[32] To a solution of nicotinic acid (500 mg, 4 mmol) in THF (5 mL) at rt was added thionyl chloride (2 mL, 26 mmol; Scheme 6). After several hours of stirring, the solvent was removed in vacuo to afford the acyl chloride product. A solution of the acyl chloride in MeCN (5 mL) was added dropwise to a solution of 2(5*H*)-furanone (0.25 mL, 3.5 mmol) and triethylamine (1.6 mL, 12 mmol) in MeCN (5 mL) at 0 °C. The ice bath was replaced with an oil bath, and the reaction mixture was heated at 50 °C for 4 h. After cooling, the solvent was removed in vacuo. The residue was diluted with EtOAc and then washed with satd $NaHCO_3$ solution. The combined organic layers were washed with brine, dried over $MgSO_4$, and then concentrated in vacuo. Purification of the crude product by flash chromatography on silica gel (50/50 EtOAc/hexanes) afforded **43** as a slightly yellow oil (88 mg, 13%). IR ($CHCl_3$ cast) 3127, 1765, 1590, 1511, 1422 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 9.35 (dd, 1H, J = 2.1, 0.8 Hz), 8.85 (dd, 1H, J = 5.0, 1.7 Hz), 8.41 (ddd, 1H, J = 8.0, 2.1, 1.7 Hz), 7.46 (ddd, 1H, J = 8.0, 5.0, 0.9 Hz), 7.11 (dd, 1H, J = 2.1, 1.1 Hz), 6.41 (dd, 1H, J = 3.4, 2.1 Hz), 6.05 (dd, 1H, J = 3.4, 1.1 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 161.2, 154.3, 151.4, 150.7, 137.8, 135.7, 124.4, 123.6, 111.3, 92.9; HRMS [EI] calcd for $C_{10}H_7NO_3$ (M^+), 189.0426; found, 189.0424.

Pyridin-3-ylmethyl Thiophene-2-carboxylate (44). Pyridin-3-ylmethanol was prepared according to the literature procedure.[31]

Scheme 7



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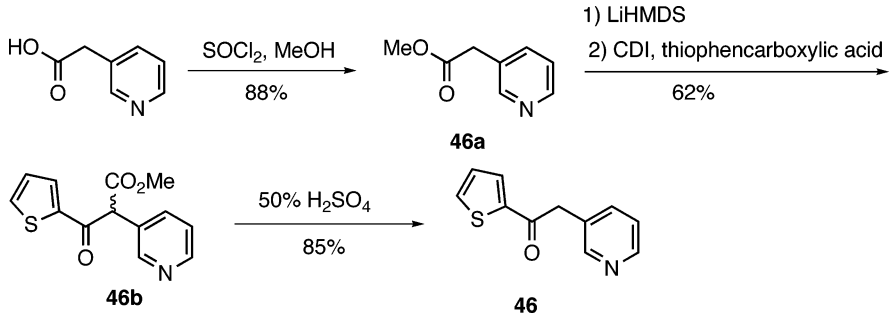
The title compound **44** was obtained following the standard procedure described above for the preparation of pyridinyl esters. Purification of the crude product by flash chromatography on silica gel (50/50 EtOAc/hexanes) afforded **44** as a white solid (190 mg, 43%). IR ($CHCl_3$, cast) 3019, 2926, 2852, 1711, 1525, 1417 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 8.64 (d, 1H, J = 1.8 Hz), 8.53 (dd, 1H, J = 5.0, 1.6 Hz), 7.94 (ddd, 1H, J = 7.9, 2.2, 1.7 Hz), 7.84 (dd, 1H, J = 3.8, 1.3 Hz), 7.77 (dd, 1H, J = 5.0, 1.3 Hz), 7.47 (ddd, 1H, J = 7.9, 5.0, 0.7 Hz), 7.16 (dd, 1H, J = 5.0, 3.8 Hz), 5.39 (s, 2H); HRMS [EI] calcd for $C_{11}H_9NO_2S$ (M^+), 219.0354; found, 219.0354.

***N*-(Pyridin-3-yl)thiophene-2-sulfonamide**[33] **(45).** Compound **45** was synthesized (Scheme 7) and then purified by automated HPLC-MS instrument to give a white solid. IR (microscope) 3128, 3091, 3067, 3006, 2937, 2620, 1584, 1518, 1475 cm^{-1}; ^1H NMR (CD_3OD, 500 MHz) δ 8.25 (d, 1H, J = 1.5 Hz), 8.24 (d, 1H, J = 1.3 Hz), 7.72 (dd, 1H, J = 4.9, 1.4 Hz), 7.67 (ddd, 1H, J = 8.3, 2.6, 1.5 Hz), 7.51 (dd, 1H, J = 3.8, 1.4 Hz), 7.35 (ddd, 1H, J = 8.3, 5.0, 0.6 Hz), 7.05 (dd, 1H, J = 5.0, 3.8 Hz); ^{13}C NMR (CD_3OD, 125 MHz) δ 146.1, 142.7, 141.1, 136.6, 134.1, 134.0, 130.8, 128.6, 125.7; HRMS [EI] calcd for $C_9H_8N_2O_2S_2$ (M^+), 240.0027; found, 240.0026.

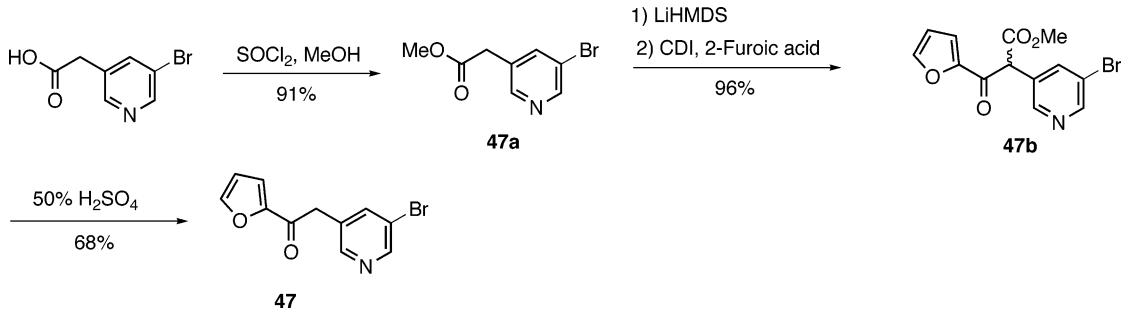
Methyl 3-oxo-2-(pyridin-3-yl)-3-(thiophen-2-yl)propanoate (46b). To a solution of 2-thiophenecarboxylic acid **(**0.79 g, 6.17 mmol) in THF (20 mL) was added CDI (1.11 g, 6.8 mmol; Scheme 8). The resulting solution was stirred at rt for 1 h. In a separate flask, to a solution of methyl 2-(pyridin-3-yl)acetate **46a**[34] (1.96 g, 13.0 mmol) in THF (45 mL) at −78 °C was added LiHMDS (14.2 mL, 1.0 M solution in THF, 14.2 mmol) dropwise. After stirring for 1.5 h at −78 °C, the thiophene carboxylic acid/CDI solution prepared above was added dropwise to this lithium enolate solution. The resulting mixture was stirred for another 2.5 h at −78 °C and then quenched with 1.0 M aqueous HCl (20 mL). The pH of the solution was adjusted to around 9 by satd $NaHCO_3$. The solution was extracted with EtOAc (3 × 50 mL). The combined organic layers were washed with brine (30 mL) and dried over $MgSO_4$. The solvent was removed under reduced pressure. Purification of the crude product by flash chromatography on silica gel (EtOAc) afforded **46b** as a yellow liquid with ∼10% impurities (1.0 g, 62%) and recovered starting material **46a** (0.71 g). IR ($CHCl_3$, cast) 3090, 2952, 2843, 1744, 1660, 1591, 1577, 1551, 1517, 1480, 1427, 1412 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 8.68−8.56 (m, 2H), 7.75 (dd, 1H, J = 3.8, 1.1 Hz), 7.71 (dd, 1H, J = 4.9, 1.1 Hz), 7.39 (dd, 2H, J = 4.6, 1.6 Hz), 7.13 (dd, 1H, J = 4.9, 3.8 Hz), 5.45 (s, 1H), 3.78 (s, 3H); HRMS [EI] calcd for $C_{13}H_{11}NO_3S$, 261.0460; found, 261.0453.

2-(Pyridin-3-yl)-1-(thiophen-2-yl)ethanone[35] **(46).** A solution of **46b** (1.0 g, 3.83 mmol) in 50% H_2SO_4 (15 mL) was refluxed at 100 °C overnight. NaOH (30 mL, 6.6 M) and satd $NaHCO_3$ (25 mL) was added to adjust the pH of the solution to 7. The solution was extracted with EtOAc (3 × 50 mL). The combined organic layers were washed with H_2O (20 mL) and brine (20 mL) and dried over $MgSO_4$. The solvent was removed under reduced pressure. Purification of the crude product by flash chromatography on silica gel (EtOAc) afforded **46** as a slightly yellow solid (0.66 g, 85%). IR ($CHCl_3$, cast) 3087, 3030, 2901, 1660, 1593, 1576, 1518, 1480, 1413 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 8.56−8.47 (m, 2H), 7.76 (dd, 1H, J = 3.8, 0.9 Hz), 7.62 (dd, 1H, J = 5.0, 0.9 Hz), 7.59 (d, 1H, J = 7.8 Hz), 7.21 (dd, 1H, J = 7.6, 5.0 Hz), 7.10 (dd, 1H, J = 4.8, 3.8 Hz), 4.19 (s, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 189.2, 150.5, 148.5, 143.4, 137.1, 134.5, 132.7, 129.9, 128.3, 123.5, 43.1; HRMS [EI] calcd for $C_{11}H_9NOS$, 203.0405; found, 203.0405.

Scheme 8



Scheme 9



Methyl 2-(5-Bromopyridin-3-yl)-3-(furan-2-yl)-3-oxopropanoate (47b). To a solution of **47a**[36] (0.91 g, 4.00 mmol) in THF (10 mL) at −78 °C was added LiHMDS (4.33 mL of 1.0 M solution in THF, 4.33 mmol, 2.3 equiv) dropwise over 25 min (Scheme 9). This solution was stirred for 1.5 h at −78 °C. To this solution was added dropwise over 25 min 2-furoic acid **(**0.21 g, 1.88 mmol) and CDI (0.34 g, 2.07 mmol) in anhydrous THF (10 mL), which had been previously stirred for 2 h at rt. The reaction mixture was stirred for 4 h at −78 °C. Then 1.0 M aqueous HCl (10 mL) was added to quench the reaction. The pH was adjusted to around 9 by adding satd aq $NaHCO_3$. The aqueous layer was extracted with EtOAc (3 × 25 mL). The organic fractions were combined and dried over anhydrous $MgSO_4$. The crude product was concentrated under reduced pressure and purified by column chromatography on silica gel (50/50 EtOAc/hexanes) to yield the product as a yellow liquid (0.59 g, 96%) and 0.45 g of recovered starting material. IR ($CHCl_3$, cast) 3134, 2954, 1746, 1676, 1567, 1464, 1426, 1393 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 8.61 (d, 2H, J = 2.0 Hz), 8.52 (d, 1H, J = 1.6 Hz), 8.05 (t, 1H, J = 2.0 Hz), 7.62 (d, 1H, J = 1.6 Hz), 7.23 (d, 1H, J = 3.7 Hz), 6.57 (dd, 1H, J = 3.7, 1.6 Hz), 5.49 (s, 1H), 3.76 (s, 3H); ^{13}CNMR ($CDCl_3$, 125 MHz) δ 180.2, 167.6, 151.0, 148.6, 147.5, 140.0, 120.7, 119.5, 130.0, 120.7, 119.5, 113.2, 56.4, 53.2; HRMS [EI] calcd for $C_{13}H_{10}BrNO_4$, 322.9793; found, 322.9793.

2-(5-Bromopyridin-3-yl)-1-(furan-2-yl)ethanone (47). A solution of **47b** (0.52 g, 1.59 mmol) in 50% H_2SO_4 (10 mL) was refluxed at 100 °C for 10 h. Then 6.25 M NaOH (20 mL) and satd $NaHCO_3$ (9 mL) was added to neutralize the solution. The aqueous layer was extracted with EtOAc (3 × 50 mL). The combined organic fractions were washed with brine (20 mL) and dried over anhydrous $MgSO_4$. The crude product was concentrated under reduced pressure and purified by column chromatography (EtOAc) to yield a brown solid (0.30 g, 68%), which appears to sublime under vacuum. IR ($CHCl_3$, cast) 3130, 3039, 2925, 1703, 1677, 1649, 1631, 1567, 1555, 1466, 1439, 1424, 1391, 1334 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 8.57 (s, 1H), 8.45 (s, 1H), 7.81 (s, 1H), 7.63 (s, 1H), 7.28 (d, 1H, J = 1.6 Hz), 6.57 (m, 1H), 4.10 (s, 2H); ^{13}CNMR ($CDCl_3$, 125 MHz) δ 184.7, 152.0, 149.5, 148.7, 147.0, 139.7, 131.3, 120.6, 118.1, 112.8, 41.5; HRMS [EI] calcd for $C_{11}H_8BrNO_2$, 264.9738; found, 264.9739.

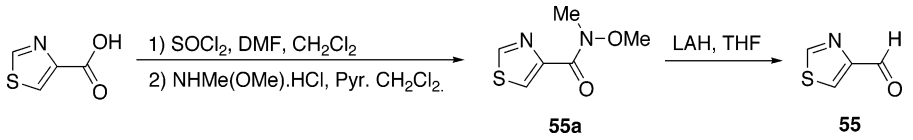
5-Chloro-pyridin-3-yl-1*H*-indole-2-carboxylate (48). The title compound **48** was obtained following the standard procedure described above for the preparation of pyridinyl esters. Purification of the crude product by flash chromatography on silica gel (25/75 EtOAc/hexanes) afforded **48** as a white solid (460 mg, 85%). IR ($CHCl_3$, cast) 3056, 1729, 1577, 1520, 1421 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 9.05 (br s, 1H), 8.50 (dd, 2H, J = 6.1, 1.8 Hz), 7.73 (dd, 1H, J = 8.2, 0.7 Hz), 7.70 (t, 1H, J = 2.2 Hz), 7.50−7.48 (m, 1H), 7.47−7.46 (m, 1H), 7.38 (ddd, 1H, J = 8.2, 7.0, 1.2 Hz), 7.20 (ddd, 1H, J = 8.1, 7.0, 1.0 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 159.3, 147.1, 146.1, 141.3, 137.6, 131.9, 129.5, 127.4, 126.6, 125.0, 123.0, 121.4, 112.0, 111.4; HRMS [EI] calcd for $C_{14}H_9ClN_2O_2$ (M^+), 272.0353; found, 272.0352.

5-Chloropyridin-3-yl-benzo-(b)-thiophene-2-carboxylate (49). The title compound **49** was obtained following the standard procedure described above for the preparation of pyridinyl esters. Purification of the crude product by flash chromatography on silica gel (25/75 EtOAc/hexanes) afforded **49** as a white solid **(**400 mg, 69%). IR ($CHCl_3$, cast) 3097, 1733, 1517, 1458, 1437 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 8.55−8.50 (m, 2H), 8.26 (d, 1H, J = 0.6 Hz), 7.97−7.91 (m, 2H), 7.71 (t, 1H, J = 2.2 Hz), 7.51 (ddd, 1H, J = 8.2, 7.1, 1.3 Hz), 7.45 (ddd, 1H, J = 8.1, 7.1, 1.1 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 160.3, 147.2, 146.2, 142.9, 141.3, 138.5, 132.9, 131.9, 131.1, 129.5, 127.8, 126.0, 125.4, 122.9; HRMS [EI] calcd for $C_{14}H_8ClNO_2S$ (M^+), 288.9964; found, 288.9956.

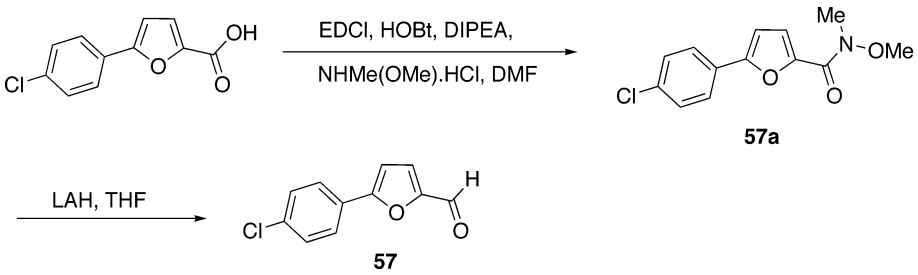
5-Chloropyridin-3-yl-thiazole-4-carboxylate (50). The title compound **50** was obtained following the standard procedure described above for the preparation of pyridinyl esters. Purification of the crude product by flash chromatography on silica gel (25/75 EtOAc/hexanes) afforded **50** as a white solid (330 mg, 69%). IR ($CHCl_3$, cast) 3121, 3087, 1745, 1573, 1491, 1419 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 8.93 (d, 1H, J = 2.1 Hz), 8.49 (d, 1H, J = 2.0 Hz), 8.47 (d, 1H, J = 2.4 Hz), 8.46 (d, 1H, J = 2.1 Hz), 7.68 (dd, 1H, J = 2.4, 2.1 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 158.6, 147.1, 146.3, 146.1, 141.3, 131.9, 129.9, 129.5; HRMS [EI] calcd for $C_9H_5ClN_2O_2S$ (M^+), 239.9760; found, 239.9765.

5-Chloropyridin-3-yl-3-methoxybenzoate (51). The title compound **51** was obtained following the standard procedure described above for the preparation of pyridinyl esters. Purification of the crude product by flash chromatography on silica gel (25/75 EtOAc/hexanes) afforded **51** as a white solid (460 mg, 87%). IR ($CHCl_3$, cast) 3072, 2836, 1744, 1600, 1586, 1488, 1420 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 8.49 (d, 1H, J = 2.1 Hz), 8.46 (d, 1H, J = 2.3 Hz), 7.77 (ddd, 1H, J = 7.8, 1.5, 1.0 Hz), 7.68−7.66 (m, 2H), 7.42 (ddd, 1H, J = 8.2, 7.8, 0.4 Hz), 7.20 (ddd, 1H, J = 8.3, 2.7, 1.0 Hz), 3.86 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 164.1, 159.8,

Scheme 10



Scheme 11



147.5, 146.0, 141.4, 131.8, 129.8, 129.6, 129.5, 122.7, 120.7, 114.7, 55.5; HRMS [EI] calcd for $C_{13}H_{10}ClNO_3$ (M^+), 263.0349; found, 263.0355.

5-Chloropyridin-3-yl-5-(4-chlorophenyl)furan-2-carboxylate (52). The title compound **52** was obtained following the standard procedure described above for the preparation of pyridinyl esters. Purification of the crude product by flash chromatography on silica gel (25/75 EtOAc/hexanes) afforded **52** as a white solid (500 mg, 75%). IR ($CHCl_3$, cast) 3135.7, 3077.4, 1736.4, 1602.4, 1602.4, 1574.6, 1564.6, 1521.3, 1473.1, 1438.1, 1420.8, 1412.2 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 8.48 (d, 1H, $J = 2.1$ Hz), 8.46 (d, 1H, $J = 2.3$ Hz), 7.73 (d, 2H, $J = 8.5$ Hz), 7.67 (t, 1H, $J = 2.3$ Hz), 7.49 (d, 1H, $J = 3.7$ Hz), 7.40 (d, 2H, $J = 8.5$ Hz), 6.81 (d, 1H, $J = 3.7$ Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 158.1, 155.7, 146.9, 146.1, 141.9, 141.3, 135.5, 131.8, 129.4, 129.3, 127.5, 126.3, 122.7, 107.7; HRMS [EI] calcd for $C_{16}H_9Cl_2NO_3$ (M^+), 332.9959; found, 332.9958.

***N*-Methoxy-*N*-methylthiazole-4-carboxamide (55a).** To a solution of thiazole-4-carboxylic acid (194 mg, 1.5 mmol) in DCM (20 mL) at rt was added thionyl chloride (0.4 mL, 5.2 mmol) and a catalytic amount of DMF (2 drops; Scheme 10). After 30 h of stirring, the solvent was removed in vacuo to afford the acyl chloride product. A solution of the acyl chloride in DCM (10 mL) was added dropwise to a solution of Weinreb amine (146 mg, 1.5 mmol) and pyridine (0.36 mL, 4.5 mmol) in DCM (10 mL) at 0 °C. After 3 h of stirring, the solvent was removed in vacuo. The residue was diluted with satd $NaHCO_3$ solution and extracted with EtOAc. The combined organic layers were washed with brine, dried over $MgSO_4$, and then concentrated in vacuo. Purification of the crude product by flash chromatography on silica gel (EtOAc) afforded **55a** as a slightly yellow oil (180 mg, 70%). IR ($CHCl_3$, cast) 3078, 2974, 2934, 1641, 1498, 1425 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 8.78 (d, 1H, $J = 2.0$ Hz), 8.05 (d, 1H, $J = 2.0$ Hz), 3.74 (s, 3H), 3.40 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 162.7, 149.6, 124.8, 124.6, 61.5, 61.4; HRMS [EI] calcd for $C_6H_8N_2O_2S$ (M^+), 172.0307; found, 172.0304.

Thiazole-4-carbaldehyde[37] **(55).** The title compound **55** was obtained following the standard procedure for the preparation of **56**.[38] To a solution of **55a** (100 mg, 0.58 mmol) in THF (5 mL) at −30 °C was added LAH (2.3 mL, 1 M solution in THF, 2.3 mmol) dropwise over 10 min (Scheme 10). After 4 h of stirring at −30 °C, the reaction was complete, which was monitored by TLC. The reaction mixture was cooled on an ice bath and water was added slowly, followed by DCM extraction. The combined organic layers were dried over $MgSO_4$, and the solvent was removed in vacuo to obtain the crude mixture, which was purified by flash column chromatography on silica gel (EtOAc) to obtain product **55** (30 mg, 46%) as a slightly yellow solid. IR ($CHCl_3$, cast) 2905, 1672, 1429 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 10.2 (s, 1H), 8.92 (d, 1H, $J = 2.0$ Hz), 8.26 (d, 1H, $J = 2.0$ Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 184.8, 155.7, 155.4, 126.6; HRMS (ES) calcd for C_4H_3NOS (M^+), 112.9935; found, 112.9935.

5-(4-Chlorophenyl)-*N*-methoxy-*N*-methylfuran-2-carboxamide (57a). To a solution of 5-(4-chlorophenyl)furan-2-carboxylic acid (334 mg, 2 mmol) in DMF (10 mL) at 0 °C was added Weinreb amine (147 mg, 2 mmol), EDCI (290 mg, 2 mmol), HOBt (204 mg, 2 mmol), and DIPEA (0.54 mL, 4 mmol; Scheme 11). The resulting solution was stirred overnight while allowing to warm slowly to rt. Then the reaction mixture was diluted with DCM (50 mL) and washed with water and brine. The combined organic layers were dried over $MgSO_4$ and then concentrated in vacuo. Purification of the crude product by flash chromatography on silica gel (50/50 EtOAc/hexanes) afforded **57a** as a white solid (280 mg, 53%). IR ($CHCl_3$, cast) 3109, 2971, 2935, 1640, 1583, 1519, 1477, 1414 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.66 (d, 2H, $J = 8.5$ Hz), 7.33 (d, 2H, $J = 8.5$ Hz), 7.17 (d, 1H, $J = 3.6$ Hz), 6.69 (d, 1H, $J = 3.6$ Hz), 3.80 (s, 3H), 3.38 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 159.0, 155.1, 145.2, 134.4, 129.1, 128.2, 126.0, 119.6, 107.1, 61.4, 33.3; HRMS [EI] calcd for $C_{13}H_{12}ClNO_3$ (M^+), 265.0506; found, 265.0502.

5-(4-Chlorophenyl)furan-2-carbaldehyde[39] **(57).** The title compound **57** was obtained following the standard procedure described above to the preparation of **55**. To a solution of **57a** (133 mg, 0.5 mmol) in THF (5 mL) at −30 °C was added LAH (1.5 mL, 1 M solution in THF, 1.5 mmol) dropwise over 10 min. After 4 h of stirring at −30 °C, the reaction was complete, which was monitored by TLC. The reaction mixture was cooled on an ice bath and water was added slowly, followed by DCM extraction. The combined organic layers were dried over $MgSO_4$, and the solvent was removed in vacuo to obtain the crude mixture, which was purified by flash column chromatography on silica gel (50/50 EtOAc/hexanes) to obtain product **57** (70 mg, 68%) as a slightly yellow solid. IR ($CHCl_3$, cast) 3111, 2855, 1685, 1662, 1478 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 9.61 (s, 1H), 7.71 (d, 2H, $J = 8.6$ Hz), 7.38 (d, 2H, $J = 8.6$ Hz), 7.28 (d, 1H, $J = 3.8$ Hz), 6.80 (d, 1H, $J = 3.8$ Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 177.2, 158.2, 152.2, 135.7, 129.3, 129.2, 127.5, 126.5, 108.0; HRMS [EI] calcd for $C_{11}H_7ClO_2$ (M^+), 206.0135; found, 206.0134.

Enzyme Assays. SARS-CoV $3CL^{pro}$ activity was measured by a quenched fluorescence resonance energy transfer assay with the peptide substrate (Abz-SVTLQSG-Tyr(NO_2)R, 93% purity). The rate of enzyme activity was determined by the increase in fluorescence (λ_{ex} 340 nm, λ_{em} 415 nm) upon continuous monitoring of reactions using a Shimadzu RF5301 spectrofluorimeter. The IC_{50} value of the individual inhibitor was measured at 22 °C in a reaction mixture (700 mL) containing 100 mM KH_2PO_4/K_2HPO_4 at pH 7.5, 2 mM EDTA, 10 μM fluorogenic substrate, 0.1 μM His-tagged protease, and 1% inhibitor solution without any preincubation. The initial 5 min of the reaction were used for calculation purposes. Initial stock solutions were prepared at 10 mM in DMSO, and serial dilutions were made in DMSO. The protease activity in the presence of the specified inhibitor was expressed as a percentage of that obtained from the respective control samples. For inhibitors displaying dose-dependent inhibition of the protease activity, IC_{50}

values were determined from plots of the relative protease activity versus the log of inhibitor concentration. IC_{50} values were not determined for compounds showing weak inhibition.

HPLC-MS Purification. The samples were purified on an 1100 HPLC coupling with an ES-MSD Agilent 1956B with positive ion detection: semi-prep column, Zorbax RX-C8, 9.4 × 250 mm, 5 μM with guard column; flow rate 3 mL/min, a linear gradient elution over 20 min of 35 to 100% acetonitrile in 0.05% formic acid/H_2O, then holding 2 min at 100% acetonitrile in 0.05% formic acid/H_2O, followed by return to 35% acetonitrile in 0.05% formic acid/H_2O over 0.5 min. The quality of some purified samples were confirmed by reinjection of purified samples to the analytical column: Zorbax RX-C18, 4.6 × 150 mm, 5 M; flow rate 0.7 mL/min, the same linear gradient elution as described above.

Mass Spectrometry of Enzyme−Inhibitor Complexes. The wild type enzyme (~0.2 mM) was mixed with 10 equiv of inhibitor at 22 °C without any preincubation. In addition, a control parallel experiment was preformed on the enzyme alone without any inhibitor. The samples were purified by C4 Ziptip (Millipore, MA) and eluted by 50% acetonitrile in 0.1% formic acid. Mass spectrometric analysis was performed on the Waters (Micromass) Q-TOF Premier using infusion at a flow rate of 0.5−1 mL/min.

Molecular Docking for SARS 3CL^{pro} Inhibitors. The crystal structure of SARS 3CL protease in complex with an aza-peptide epoxide (APE) (PDB code: 2A5K)[18] was selected to construct the predictive model after deleting the coordinates of the epoxide inhibitor from the pdb file. The three-dimensional (3D) coordinates of the 3CL enzyme (experimental) and those of the inhibitors (calculated) were processed in Sybyl 7.1.[40] The essential hydrogen atoms were added to the protein molecule and the Kollman united atom charges were applied; the 3D structures of inhibitors were constructed and energy-minimized using the Tripos force field in Sybyl 7.1; hydrogen atoms and Gasteiger−Marsili charges were added to the inhibitors.[41]

Autodock 3.0.5[25] was used to perform the automated molecular docking. The grid map with 60 × 60 × 60 points spaced at 0.375 Å was generated using the AUTOGRID program to evaluate the binding energies. The docked complexes of 3CL protease with inhibitors were evaluated according to the predicted binding energy and the geometric ideality of the docked inhibitors.

Acknowledgment. Financial support from the Natural Sciences and Engineering Research Council of Canada (NSERC), the Canada Research Chairs Program, the Alberta Heritage Foundation for Medical Research (AHFMR), and the Killam Foundation (Fellowship to J.Y.) is gratefully acknowledged. We thank Prof. Dennis Hall and Dr. Eric Pelletier for help with combinatorial synthesis and HPLC-MS purification and Dr. Randy Whittal and Ms. Jing Zheng for help with mass spectra. We also thank Reuben Mahaffy for assistance with enzyme inhibition studies.

Supporting Information Available: A library of 90 carboxylic acids, HPLC-MS data, ESI-MS of complexes of 3CL^{pro} with inhibitors **35**, **48**, and **51**, and spectra of new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Drosten, C.; Günther, S.; Preiser, W.; van der Werf, S.; Brodt, H.-R.; Becker, S.; Rabenau, H.; Panning, M.; Kolesnikova, L.; Fouchier, R. A. M.; Berger, A.; Burguière, A.-M.; Cinatl, J.; Eickmann, M.; Escriou, N.; Grywna, K.; Kramme, S.; Manuguerra, J.-C.; Müller, S.; Rickerts, V.; Stürmer, M.; Vieth, S.; Klenk, H.-D.; Osterhaus, A. D. M. E.; Schmitz, H.; Doerr, H. W. Identification of a novel coronavirus in patients with severe acute respiratory syndrome. *New Engl. J. Med.* **2003**, *348*, 1967−1976.

(2) Ksiazek, T. G.; Erdman, D.; Goldsmith, C. S.; Zaki, S. R.; Peret, T.; Emery, S.; Tong, S.; Urbani, C.; Comer, J. A.; Lim, W.; Rollin, P. E.; Dowell, S. F.; Ling, A.-E.; Humphrey, C. D.; Shieh, W.-J.; Guarner, J.; Paddock, C. D.; Roca, P.; Fields, B.; DeRisi, J.; Yang, J.-Y.; Cox, N.; Hughes, J. M.; LeDuc, J. W.; Bellini, W. J.; Anderson, L. J. A novel coronavirus associated with severe acute respiratory syndrome. *New Engl. J. Med*. **2003**, *348*, 1953−1966.

(3) (a) World Health Organization (15 August 2003). http://www.who.int/csr/sars/country/2003 08 15/en/. (b) World Health Organization (31 January 2004). http://www.who.int/csr/sars/country/2003 01 31/en/. (c) World Health Organization (30 April 2004). http://www.who.int/csr/sars/country/2003 01 31/en/.

(4) Anand, K.; Ziebuhr, J.; Wadhwani, P.; Mesturs, J. R.; Hilgenfeld, R. Coronavirus main proteinase (3CL^{pro}) structure: Basis for design of anti-SARS drugs. *Science* **2003**, *300*, 1763−1767.

(5) Myint, S. H. In *The Coronaviridae*; Siddell, S. G., Ed.; Plenum Press: New York, 1995; pp 389−401.

(6) Rota, P. A.; Oberste, M. S.; Nix, W. A.; Campagnoli, R.; Icenogle, J. P.; Peñaranda, S.; Bankamp, B.; Maher, K.; Chen, M.-H.; Tong, S.; Tamin, A.; Lowe, L.; Frace, M.; DeRisi, J. L.; Chen, Q.; Wang, D.; Erdman, D. D.; Peret, T. C. T.; Burns, C.; Ksiazek, T. G.; Rollin, P. E.; Sanchez, A.; Liffick, S.; Holloway, B.; Limor, J.; McCaustland, K.; Olsen-Rasmussen, M.; Fouchier, R.; Günther, S.; Osterhaus, A. D. H. E.; Drosten, C.; Pallansch, M. A.; Anderson, L. J.; Bellini, W. J. Characterization of a novel coronavirus associated with severe acute respiratory syndrome. *Science* **2003**, *300*, 1394−1399.

(7) Marra, M. A.; Jones, S. J. M.; Astell, C. R.; Holt, R. A.; Brooks-Wilson, A.; Butterfield, Y. S. N.; Khattra, J.; Asano, J. K.; Barber, S. A.; Chan, S. Y.; Cloutier, A.; Coughlin, S. M.; Freeman, D.; Girn, N.; Griffith, O. L.; Leach, S. R.; Mayo, M.; McDonald, H.; Montgomery, S. B.; Pandoh, P. K.; Petrescu, A. S.; Robertson, A. G.; Schein, J. E.; Siddiqui, A.; Smailus, D. E.; Stott, J. M.; Yang, G. S.; Plummer, F.; Andonov, A.; Artsob, H.; Bastien, N.; Bernard, K.; Booth, T. F.; Bowness, D.; Czub, M.; Drebot, M.; Fernando, L.; Flick, R.; Garbutt, M.; Gray, M.; Grolla, A.; Jones, S.; Feldmann, H.; Meyers, A.; Kabani, A.; Li, Y.; Normand, S.; Stroher, U.; Tipples, G. A.; Tyler, S.; Vogrig, R.; Ward, D.; Watson, B.; Brunham, R. C.; Krajden, M.; Petric, M.; Skowronski, D. M.; Upton, C.; Roper, R. L. The genome sequence of the SARS-associated coronavirus. *Science* **2003**, *300*, 1399−1404.

(8) Thiel, V.; Ivanov, K. A.; Putics, Á.; Hertzig, T.; Schelle, B.; Bayer, S.; Wei*β*brich, B.; Snijder, E. J.; Rabenau, H.; Doerr, H. W.; Ziebuhr, J. Mechanisms and enzymes involved in SARS coronavirus genome expression. *J. Gen. Virol.* **2003**, *84*, 2305−2315.

(9) Yang, H.; Yang, M.; Ding, Y.; Liu, Y.; Lou, Z.; Zhou, Z.; Sun, L.; Mo, L.; Ye, S.; Pang, H.; Gao, G.; Anand, K.; Bartlam, M.; Hilgenfeld, R.; Rao, Z. The crystal structures of SARS virus main protease M^{pro} and its complex with an inhibitor. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 13190−13195.

(10) Huang, C.; Wei, P.; Fan, K.; Liu, Y.; Lai, Y. 3C-like proteinase from SARS coronavirus catalyzes substrate hydrolysis by a general base mechanism. *Biochemistry* **2004**, *43*, 4568−4574.

(11) Hegyi, A.; Ziebuhr, J. Conservation of substrate specificities among coronavirus main proteases. *J. Gen. Virol.* **2002**, *83*, 595−599.

(12) Wu, C.-Y.; Jan, J.-T.; Ma, S.-H.; Kuo, C.-J.; Juan, H.-F.; Cheng, Y.-S. E.; Hsu, H.-H.; Huang, H.-C.; Wu, D.; Brik, A.; Liang, F.-S.; Liu, R.-S.; Fang, J.-M.; Chen, S.-T.; Liang, P.-H.; Wong, C.-H. Coronavirus main proteinase (3CL^{pro}) structure: Basis for design of anti-SARS drugs. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 13190−13195.

(13) Bacha, U.; Barrila, J.; Velazquez-Campoy, A.; Leavitt, S. A.; Freire, E. Identification of novel inhibitors of the SARS coronavirus main protease 3CL^{pro}. *Biochemistry* **2004**, *43*, 4906−4912.

(14) Blanchard, J. E.; Elowe, N. H.; Fortin, P. D.; Huitema, C.; Cechetto, J. D.; Eltis, L. D.; Brown, E. D. High-throughput screening identifies inhibitors of the SARS coronavirus main proteinase. *Chem. Biol*. **2004**, *11*, 1445−1453.

(15) (a) Jain, R. P.; Pettersson, H. I.; Zhang, J.; Aull, K. D.; Fortin, P. D.; Huitema, C.; Eltis, L. D.; Parrish, J. C.; James, M. N. G.; Wishart, D. S.; Vederas, J. C. Synthesis and evaluation of keto-glutamine analogues as potent inhibitors of severe acute respiratory syndrome 3CL^{pro}. *J. Med. Chem.* **2004**, *47*, 6113−6116. (b) Jain, R. P.; Vederas, J. C. Structural variations in keto-glutamines for improved inhibition against hepatitis A virus 3C proteinase. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3655−3658. (c) Ramtohul, Y. K.; James, M. N. G.; Vederas, J. C. Synthesis and evaluation of keto-glutamine analogues as inhibitors of hepatitis A virus 3C proteinase. *J. Org. Chem*. **2002**, *67*, 3169−3178. (d) Ramtohul, Y. K.; Martin, N. I.; Silkin, L.; James, M. N. G.; Vederas, J. C. Synthesis of pseudoxazolones and their inhibition of the 3C cysteine proteinases from hepatitis A virus and human rhinovirus-14. *J. Chem. Soc., Perkin Trans. 1* **2002**, 1351−1359. (e) Lall, M. S.; Ramtohul, Y. K.; James, M. N. G.; Vederas, J. C. Serine and threonine beta-lactones: A new class of hepatitis A virus 3C cysteine proteinase inhibitors. *J. Org. Chem.* **2002**, *67*,

1536−1547. (f) Ramtohul, Y. K.; Martin, N. I.; Silkin, L.; James, M. N. G.; Vederas, J. C. Pseudoxazolones, a new class of inhibitors for cysteine proteinases: Inhibition of hepatitis A virus and human rhinovirus 3C proteinases. *J. Chem. Soc., Chem. Commun.* **2001**, 2740−2741.

(16) (a) Shie, J.-J.; Fang, J.-M.; Kuo, C.-J.; Kuo, T.-H.; Liang, P.-H.; Huang, H.-J.; Yang, W.-B.; Lin, C.-H.; Chen, J.-L.; Wu, Y.-T.; Wong, C.-H. Discovery of potent anilide inhibitors against the severe acute respiratory syndrome 3CL protease. *J. Med. Chem.* **2004**, *48*, 4469−4473. (b) Shie, J. J.; Fang, J. M.; Kuo, T. H.; Kuo, C. J.; Liang, P. H.; Huang, H. J.; Wu, Y. T.; Jan, J. T.; Cheng, Y. S. E.; Wong, C. H. Inhibition of the severe acute respiratory syndrome 3CL protease by peptidomimetic α,β-unsaturated esters. *Bioorg. Med. Chem.* **2005**, *13*, 5240−5252.

(17) Wu, C. Y.; King, K.-Y.; Kuo, C.-J.; Fang, J.-M.; Wu, Y.-T.; Ho, M.-Y.; Liao, C.-L.; Shie, J.-J.; Liang, P.-H.; Wong, C.-H. Stable Benzotriazole esters as mechanism-based inactivators of the severe acute respiratory syndrome 3CL protease. *Chem. Biol*. **2006**, *13*, 261−268.

(18) Lee, T. W.; Cherney, M. M.; Huitema, C.; Liu, J.; James, K. E.; Powers, J. C.; Eltis, L. D.; James, M. N. Crystal structures of the main peptidase from the SARS coronavirus inhibited by a substrate-like aza-peptide epoxide. *J. Mol. Biol.* **2005**, *353* (5), 1137−1151.

(19) Yin, J.; Cherney, M. M.; Bergmann, E. M.; Zhang, J.; Huitema, C.; Pettersson, H. I.; Eltis, L. D.; Vederas, J. C.; James, M. N. G. An episulfide cation (thiiranium ring) trapped in the active site of HAV 3C proteinase inactivated by peptide-based ketone inhibitors. *J. Mol. Biol*. **2006**, *361*, 673−686.

(20) Matthews, D.; Dragovich, P. S.; Webber, S. E.; Fuhrman, S. A.; Patick, A. K.; Zalman, L. S.; Hendrickson, T. F.; Love, R. A.; Prins, T. J.; Marakovits, J. T.; Zhou, R.; Tikhe, J.; Ford, C. E.; Meador, J. W.; Ferre, R. A.; Brown, E. L.; Binford, S. L.; Brothers, M. A.; Delisle, D. M.; Worland, S. T. Structure-assisted design of mechanism-based irreversible inhibitors of human rhinovirus 3C protease with potent antiviral activity against multiple rhinovirus serotypes. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 11000−11007.

(21) Schechter, I.; Berger, A. On the size of the active site in proteases. I. Papain. *Biochem. Biophys. Res. Commun.* **1967**, *27*, 157−162.

(22) Tian, Q.; Nayyar, N. K.; Babu, S.; Chen, L.; Tao, J.; Lee, S.; Tibbetts, A.; Moran, T.; Liou, J.; Guo, M.; Kennedy, T. P. An efficient synthesis of a key intermediate for the preparation of the rhinovirus protease inhibitor AG7088 via asymmetric dianionic cyanomethylation of *N*-Boc-(+)-glutamic acid dimethyl ester. *Tetrahedron Lett.* **2001**, *42*, 6807−6809.

(23) Nahm, S.; Weinreb, S. M. *N*-Methoxy-*n*-methylamides as effective acylating agents. *Tetrahedron Lett.* **1981**, *22*, 3815−3818.

(24) Dufour, E.; Storer, A. C.; Menard, R. Peptide aldehydes and nitriles as transition state analog inhibitors of cysteine proteases. *Biochemistry* **1995**, *34*, 9136−9143.

(25) Morris, G. M.; Goodsell, D. S.; Halliday, R. S.; Huey, R.; Hart, W. E.; Belew, R. K.; Olson, A. J. Automated docking using Lamarckian genetic algorithm and empirical binding free energy function. *J. Comput. Chem.* **1998**, *19*, 1639−1662.

(26) Rayabarapu, D. K.; Majumdar, K. K.; Sambaiah, T.; Cheng, C.-H. Unusual 1,4-addition of 2-pyridyl carboxylates to benzynes: A convenient route to 1-(2-acylphenyl)-2-pyridones. *J. Org. Chem.* **2001**, *66*, 3646−3649.

(27) Kanaoka, Y.; San-nohe, K.; Hatanaka, Y.; Itoh, K.; Machida, M.; Terashima, M. Syntheses of thieno[2,3-*c*]-, pyrrolo[2,3-*c*]-, and indolo[2,3-*c*]diazanaphthalenes by photocyclization of acylaminopyridines. *Heterocycles* **1977**, *6*, 29−32.

(28) Couture, A.; Grandclaudon, P. 2-Aryl-oxazolo- and thiazolopyridines. Synthesis via cyclization of *N*-(2-chloro-3-pyridinyl)arylamides and thioamides. *Heterocycles* **1984**, *22*, 1383−1385.

(29) Kim, K.; Le, K. Two efficient *N*-acylation methods mediated by solid-supported reagents for weakly nucleophilic heterocyclic amines. *Synlett* **1999**, *12*, 1957−1959.

(30) Brenner, E.; Baldwin, R. M.; Tamagnan, G. Synthesis of a new precursor to the nicotinic receptor tracer 5-IA-85380 precursor using trimethylsilyl iodide as deblocking agent. *Tetrahedron Lett.* **2004**, *45*, 3607−3610.

(31) Gran, U. Synthesis of a new and versatile macrocyclic NADH model. *Tetrahedron* **2003**, *59*, 4303−4308.

(32) Montaña, A. M.; Grima, P. M. Asymmetry induction on the [4C-(4π)+3C(2π)] cycloaddition reaction of C2-functionalized furans: Influence of the chiral auxiliary nature. *Tetrahedron* **2002**, *58*, 4769−4786.

(33) Ballistreri, A.; Maccarone, E.; Musumarra, G. The acid dissociation of arenesulphonamides: σ_{Het} constants for thia- and oxa-substituents in five-membered *S*-linked heterocycles and effects of substituents in the *N*-linked aromatic ring. *J. Chem. Soc., Perkin Trans. 2* **1977**, *2*, 984−985.

(34) Horikawa, M.; Hashimoto, K.; Shirahama, H. Efficient syntheses of acromelic acids B and E, which are potent neuroexcitatory amino acids. *Tetrahedron Lett.* **1993**, *34*, 331−334.

(35) Miller, A. D.; Osuch, C.; Goldberg, N. N.; Levine, R. The synthesis of nitrogen-containing ketones versus the direct acylation of 3-picoline. *J. Am. Chem. Soc.* **1956**, *78*, 674−676.

(36) Thompson, W. J.; Gaudino, J. A general synthesis of 5-arylnicotinates. *J. Org. Chem.* **1984**, *49*, 5237−5243.

(37) Dondoni, A.; Fantin, G.; Fogagnolo, M.; Medici, A.; Pedrini, P. Synthesis of (trimethylsilyl)thiazoles and reactions with carbonyl compounds. Selectivity aspects and synthetic utility. *J. Org. Chem.* **1988**, *53*, 1748−1761.

(38) Kolhatkar, R. B.; Ghorai, S. K.; George, C.; Reith, M. E. A.; Dutta, A. K. Interaction of *cis*-(6-benzhydrylpiperidin-3-yl)benzylamine analogues with monoamine transporters: Structure−activity relationship study of structurally constrained 3,6-disubstituted piperidine analogues of (2,2-diphenylethyl)-[1-(4-fluorobenzyl)piperidin-4-ylmethyl]amine. *J. Med. Chem*. **2003**, *46*, 2205−2215.

(39) Du, X.; Guo, C.; Hansell, E.; Doyle, P. S.; Caffrey, C. R.; Holler, T. P.; McKerrow, J. H.; Cohen, F. E. Synthesis and structure−activity relationship study of potent trypanocidal thio semicarbazone inhibitors of the trypanosomal cysteine protease cruzain. *J. Med. Chem*. **2002**, *45*, 2695−2707.

(40) *SYBYL*, version 7.1; Tripos, Inc.: St. Louis, MO, 2005.

(41) Gasteiger, J.; Marsili, M. Iterative partial equalization of orbital electronegativity—a rapid access to atomic charges. *Tetrahedron* **1980**, *36*, 3219−3228.

JM061425K