Sulfonamidopyrrolidinone Factor Xa Inhibitors: Potency and Selectivity Enhancements via P-1 and P-4 Optimization

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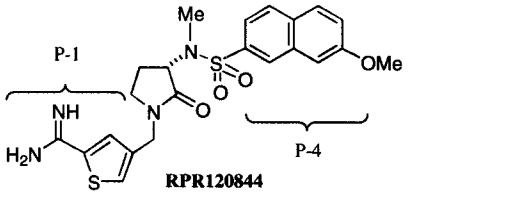
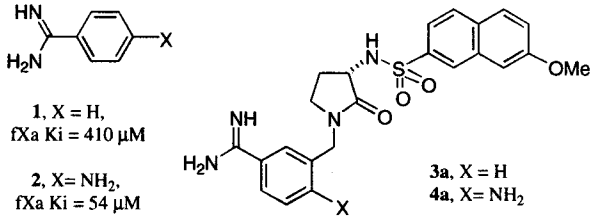
Sulfonamidopyrrolidinones were previously disclosed as a selective class of factor Xa (fXa) inhibitors, culminating in the identification of RPR120844 as a potent member with efficacy in vivo. Recognizing the usefulness of the central pyrrolidinone template for the presentation of ligands to the S-1 and S-4 subsites of fXa, studies to optimize the P-1 and P-4 groups were initiated. Sulfonamidopyrrolidinones containing 4-hydroxy- and 4-aminobenzamidines were discovered to be effective inhibitors of fXa. X-ray crystallographic experiments in trypsin and molecular modeling studies suggest that our inhibitors bind by insertion of the 4-hydroxybenzamidine moiety into the S-1 subsite of the fXa active site. Of the P-4 groups examined, the pyridylthienyl sulfonamides were found to confer excellent potency and selectivity especially in combination with 4-hydroxybenzamidine. Compound **20b** (RPR130737) was shown to be a potent fXa inhibitor ($K_i = 2$ nM) with selectivity against structurally related serine proteinases (>1000 times). Preliminary biological evaluation demonstrates the effectiveness of this inhibitor in common assays of thrombosis in vitro (e.g. activated partial thromboplastin time) and in vivo (e.g. rat $FeCl_2$-induced carotid artery thrombosis model).

Introduction

Vascular injury stimulates a series of proteolytic cleavage reactions culminating in the sequential activation of coagulation factor X and prothrombin.[1] Factor X is secreted by the liver as a zymogen which is hydrolytically activated to factor Xa (fXa) by the factor VIIa/tisssue factor complex (extrinsic pathway) or by the factor IXa/factor VIIIa complex (intrinsic pathway). The trypsin-like serine proteinase fXa combines with factor Va and calcium on membrane surfaces to form the prothrombinase complex. fXa is responsible for the catalytic activity of prothrombinase, i.e. the conversion of prothrombin (factor II) to thrombin (factor IIa), the last enzyme in the coagulation cascade. Upon assembly, one unit of prothrombinase can generate over 100 molecules of thrombin,[2] which in turn is responsible for fibrin formation, platelet activation, and other physiological events.[3] Although direct thrombin inhibitors have been pursued[4] as therapies for thrombosis, the inhibition of fXa in the prothrombinase complex has emerged as an alternative antithrombotic approach.[5] Indeed, the unique position of fXa at the convergence of the intrinsic and extrinsic pathways and its role as the singular enzyme for thrombin activation make fXa an attractive intervention point for the development of antithrombotic therapy.[6]





A common approach to the design of proteinase inhibitors is the modification of peptidyl substrates;[7] inhibitors which result are often burdened with the drawbacks associated with peptides in general.[8] Previously we disclosed novel non-peptidyl inhibitors[9] which used the X-ray structure of the fXa active site[10] and existing SAR as the departure points for design. This resulted in the discovery of a 3-sulfonamidopyrrolidinone as a useful template for the presentation of ligands to the S-1 and S-4 subsites of fXa. Further optimization led to RPR120844, a reversible inhibitor of fXa (K_i = 6.9 nM) which displays moderate selectivity against related serine proteinases and efficacy in vivo. Having established optimal template characteristics (ring size, chirality, and linkage type) in the previous work,[9] we anticipated that potency and selectivity enhancements

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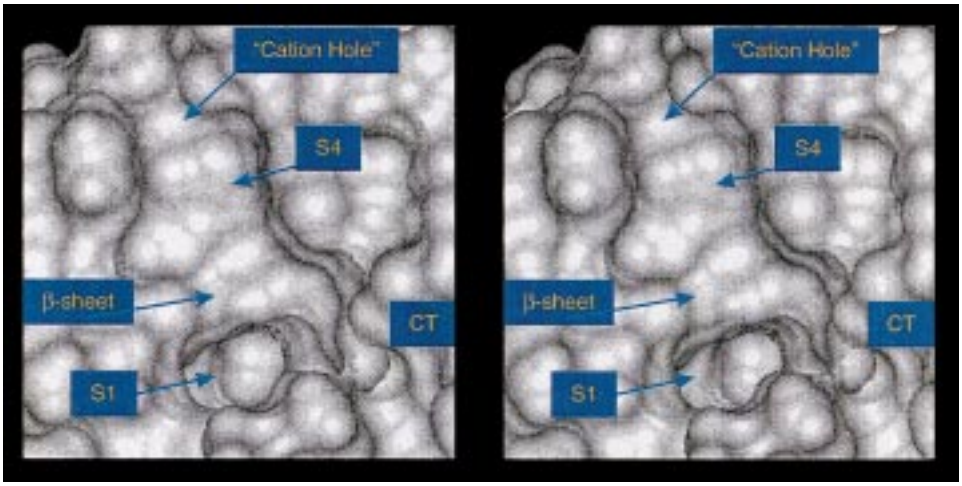
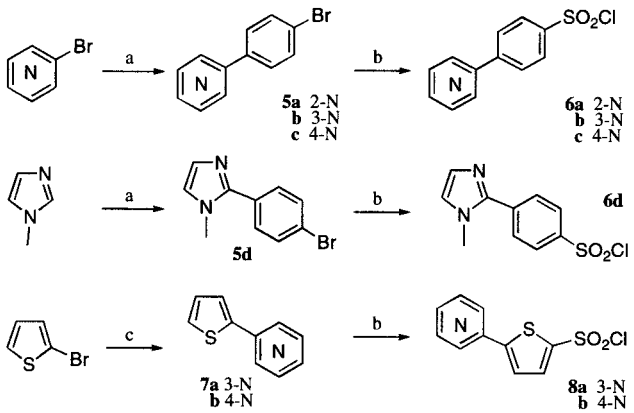


Figure 1. Connolly surface of the fXa active site showing features which are potentially important for binding: S-1, S-4 (with "cation hole"), β-sheet, and the catalytic triad region (CT). This stereoview was generated using fXa coordinates[10] available in the Brookhaven database: 1HCG.

Scheme 1. General Synthesis of Sulfonyl Chlorides[a]



[a] (a) i. *t*-BuLi or *n*-BuLi, THF, −78 °C, 10 min, ii. $ZnCl_2$, −78 °C to rt, iii. 1-bromo-4-iodobenzene, $Pd(Ph_3)_4$; (b) i. *t*-BuLi or *n*-BuLi, THF, −78 °C, ii. SO_2 gas, −78 °C to rt, iii. SO_2Cl_2, hexane; (c) i. Mg, Et_2O, reflux, ii. 3-bromopyridine or 4-bromopyridine, $NiCl_2$dppp, Et_2O, rt.

could be realized by further manipulation of the P-1 and P-4 ligands.

In this respect we were intrigued by the publications of Sturzebecher and Walsmann in the field of trypsin-like serine proteinases.[11] In these papers the effect of heteroatom substitution on the activity of benzamidine "needles"[12] was described. Modest increases in activity against several trypsin-like serine proteinases, including fXa, trypsin, and thrombin (fIIa), were reported for 4-aminobenzamidine (**2**) versus benzamidine (**1**) (vide infra). We envisioned that similar modifications could be made for the P-1 groups of the pyrrolidinone inhibitors. Our modeling efforts indicated that substituents *para* to the P-1 benzamidine of a sulfonamidopyrrolidinone would be placed in an environment rich in H-bond donors and acceptors. Therefore, we set about to synthesize fXa inhibitors which incorporated *p*-aminobenzamidines.

In addition to potency enhancement, we were interested in modifications which could significantly alter the physicochemical properties of our inhibitors. Modifications were incorporated into the aromatic P-4 group because of the unique features of the aryl binding pocket of fXa (S-4). X-ray structures of fXa/inhibitor complexes were unavailable at the time this work was initiated; however, a truncated fXa structure[10] had been published (Figure 1). The S-4 subsite is an open-ended box with the floor and walls defined by two electron-rich aromatic side chains (Trp215, Tyr99) and a phenylalanine residue (Phe174). The terminus of the box is lined with H-bond acceptors, and an acidic side chain is located at the periphery (Glu97). This juxtaposition of functionality, termed the "cation hole", is not found in other trypsin-like serine proteinases. It has been invoked to explain the potency of inhibitors with positively charged and/or basic moieties at P-4.[13] Of the substitution patterns explored in our earlier work, biaryls were found to be effective P-4 ligands.[9] We incorporated weakly basic sp^2-hybridized nitrogens into the distal ring of the biaryl moiety in hopes of modifying physicochemical properties while increasing anti-fXa potency.

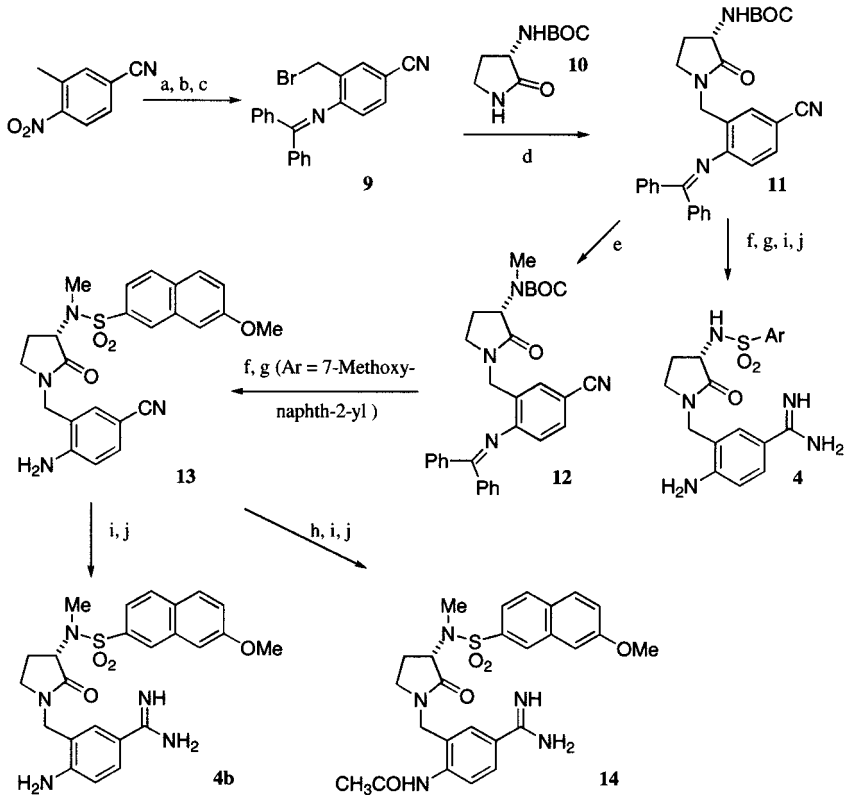
Herein we report our findings on the use of *para*-substituted benzamidines as effective P-1 ligands in the pyrrolidinone series. In addition, the selectivity enhancements obtained by P-4 manipulation are described. The synergies realized by the combination of these ligands into hybrid molecules, as demonstrated by in vitro potencies, and the in vivo effectiveness of a potent inhibitor **20b** (RPR130737) will be discussed.

Chemistry

The pyrrolidinone benzamidine inhibitors **3** were prepared using the methods of the companion paper;[9] however, a number of novel P-4 groups are described herein. The requisite sulfonyl chlorides were prepared by lithiation of the biaryl intermediates **5** and **7**, then treatment with sulfur dioxide, and conversion of the resulting sulfinic acid to the corresponding sulfonyl chloride (Scheme 1). Palladium(0)-mediated cross-coupling reactions were used to generate the biaryl intermediates **5**. Cross-coupling reaction of 2-thienylmagnesium bromide with bromopyridines utilizing $NiCl_2$(dppp) catalyst[14] gave better yields of the pyridylthiophene intermediates **7** than Suzuki coupling.[15]

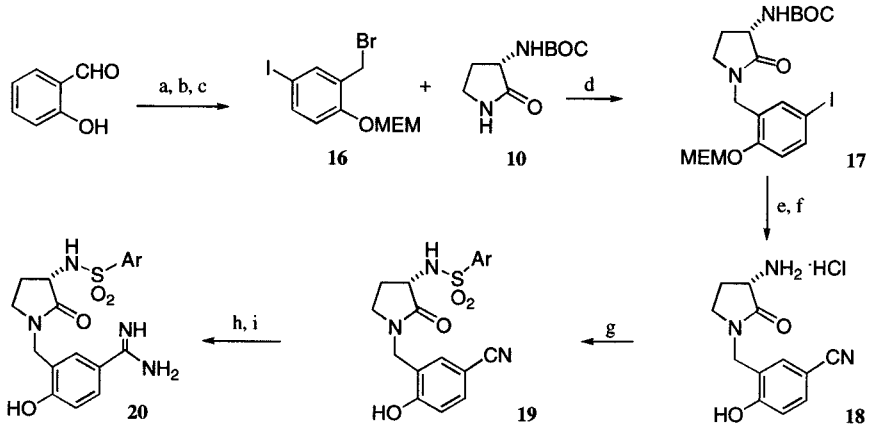
The aminobenzamidine inhibitors were prepared as shown in Scheme 2. (2-Oxopyrrolidin-3(*S*)-yl)carbamic acid *tert*-butyl ester (**10**)[9] was alkylated with the appropriate benzyl bromide (obtained in three steps start-

Scheme 2. Synthesis of Aminobenzamidine Derivatives[a]



[a] (a) $SnCl_2$, EtOH; (b) benzophenone, pTsOH, toluene; (c) NBS, $(C_6H_5CO)_2O_2$, CCl_4; (d) NaH, THF/DMF, 0 °C; (e) NaH, MeI, DMF, 0 °C; (f) HCl gas, EtOAc, 0 °C; (g) $ArSO_2Cl$, Et_3N, CH_3CN; (h) acetyl chloride, DMAP, Et_3N, 1,2-DCE; (i) HCl gas, EtOH, rt; (j) NH_3 gas, MeOH, reflux.

Scheme 3. Synthesis of Hydroxybenzamidine Derivatives[a]



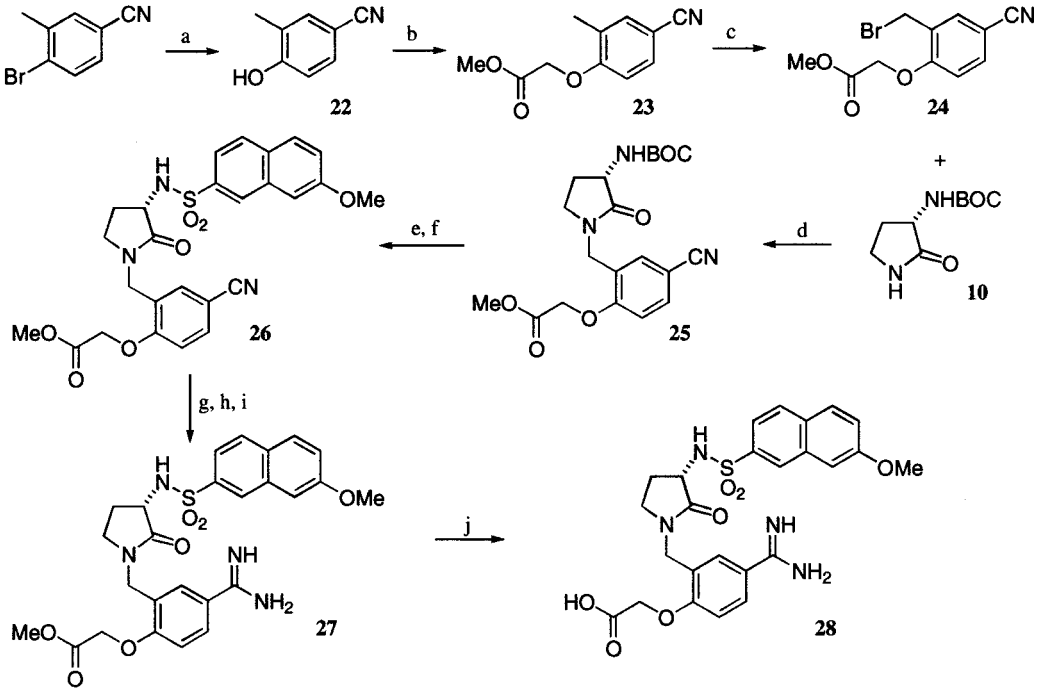
[a] (a) ICl, CH_2Cl_2; (b) i. NaH, MEMCl, DMPU, THF, ii. $NaBH_4$; (c) NBS, Ph_3P, THF; (d) NaH, THF, DMPU; (e) $Zn(CN)_2$, $Pd(PPh_3)_4$, DMF; (f) HCl gas, EtOAc, 0 °C; (g) $ArSO_2Cl$, pyridine, rt; (h) HCl gas, EtOH; (i) NH_3 gas, MeOH, reflux.

ing from 3-methyl-4-nitrobenzonitrile in the case of **9**); subsequent BOC deprotection and sulfonylation yielded the penultimate intermediate. Depending on the reaction time and temperature, deprotection of the BOC group using HCl gas led to partial or complete removal of the benzhydrylidene protecting group. Nevertheless, the sulfonylation step proceeded smoothly on either intermediate to give the desired sulfonamides (see Experimental Section). Standard conditions[9] (i.e. Pinner reaction and ammonolysis) were used to convert the nitrile to the target amidine **4**. The *meta*-isomer **15** was prepared similarly using 3-methyl-5-nitrobenzonitrile as the starting material. For the acetylated derivative **14** an alkylation step (**11** to **12**) was inserted prior to BOC removal and sulfonylation. Nitrile **13** was acetylated and converted to the amidine as usual.

The hydroxybenzamidine inhibitor **20** was prepared as described in Scheme 3 by alkylation of **10** with the appropriate bromide (**16** in the case of **20**). Cyanation of the iodo intermediate **17** followed by deprotection gave the 3-aminopyrrolidinone **18**. Sulfonylation in pyridine provided the intermediate nitrile **19**; standard conditions were used to convert the nitrile to the target compound **20**. Inhibitor **21** was prepared similarly using *p*-hydroxybenzaldehyde as the starting material.

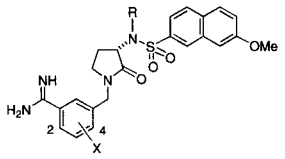
Compound **27** was prepared by alkylation of **10** with benzyl bromide **24**, deprotection, sulfonylation, and then conversion to the amidine using mild conditions[16]

Scheme 4. Synthesis of Acylating Inhibitor **27** and Acid **28**[a]



[a] (a) *t*-BuLi, O_2, THF, −78 °C; (b) methyl bromoacetate, K_2CO_3, *n*-Bu_4NI, DMF, 80 °C; (c) NBS, $(C_6H_5CO)_2O_2$, CCl_4; (d) NaH, THF/DMF, 0 °C; (e) HCl(g), EtOAc; (f) 7-methoxynaphthalene-2-sulfonyl chloride, Et_3N, CH_3CN, rt; (g) H_2S, Et_3N, pyridine; (h) MeI, acetone, reflux; (i) NH_4OAc, MeOH, reflux; (j) 10 N NaOH, EtOH, rt.

Table 1. Dependence of fXa Inhibition on Benzamidine and Sulfonamide Substitution Patterns



Cmpd.	X	R	Ki (nM) fXa	fIIa	Tryp.
3a[a]	H	H	47	1,430	853
4a	4-NH_2	H	6	278	299
3b[a]	H	Me	22	1,051	704
4b	4-NH_2	Me	4	201	189
14	4-NHCOMe	Me	919	>4000	>2900
15	3-NH_2	H	1,200	>4000	>2900
20a	4-OH	H	3	206	305
21	2-OH	H	983	>4000	>2900
27	4-OCH_2CO_2Me	H	8	263	12
28	4-OCH_2CO_2H	H	1,300	>4000	>2900

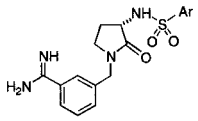
[a]Synthesis described in preceding paper.[9]

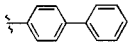
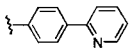
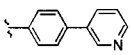
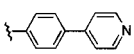
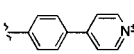
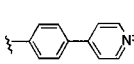
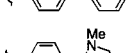
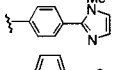
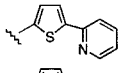
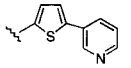
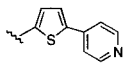
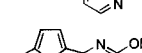
(Scheme 4). The functionalized benzyl bromide **24** was prepared in three steps from 4-bromo-3-methylbenzonitrile. Treatment of 4-bromo-3-methylbenzonitrile with *t*-BuLi followed by oxygen gas gave the hydroxybenzonitrile **22**. Alkylation with methyl bromoacetate followed by benzylic bromination yielded the desired benzyl bromide intermediate **24**. The acid derivative **28** was obtained by base hydrolysis of the ester **27**.

Results and Discussion

Our initial efforts at P-1 optimization were fruitful and led to the identification of inhibitor **4a** as a

Table 2. SAR of Biaryl P-4 Ligands



Cmpd.	Ar	Ki (nM) fXa	fIIa	Tryp.
3c[a]		160	>4,000	>2,900
3d		150	~4,000	>2,900
3e		210	~1960	>2,900
3f		130	>4,000	>2,900
3g		$>10^3$	>4,000	>2,900
3h		330	>4,000	>2,900
3i		2,600	>4,000	>2,900
3j		140	>4,000	2,100
3k		41	>4,000	>2,900
3l		55	~3,000	>2,900
3m		150	>4,000	>2,900
3n		49	>4,000	1,900

[a]Synthesis described in preceding paper.[9]

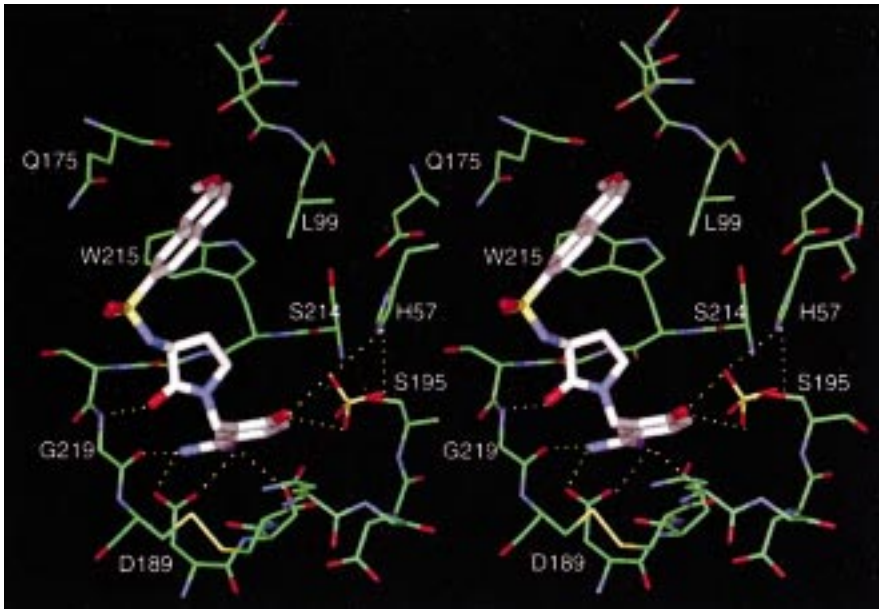


Figure 2. Stereoview of the trypsin/**20a** crystal structure.

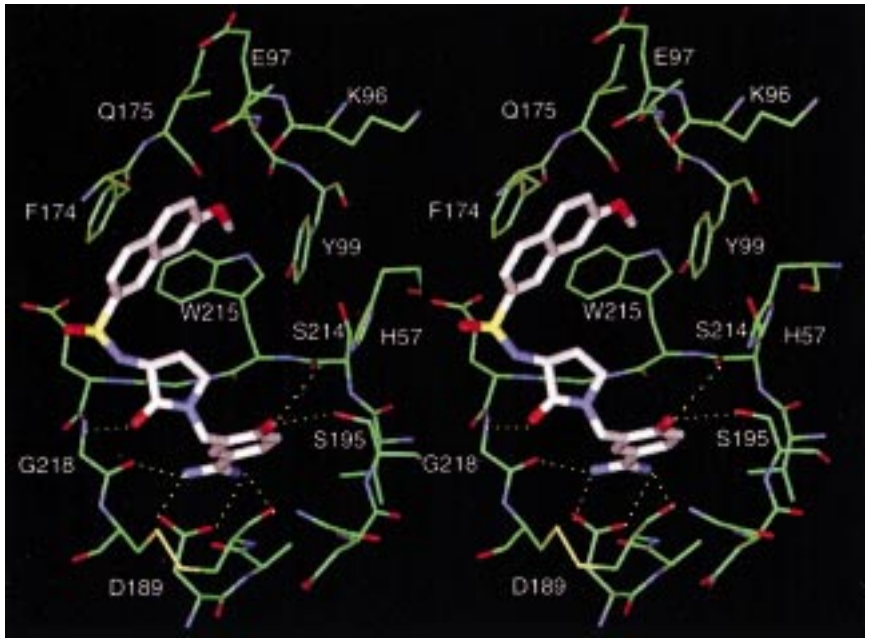


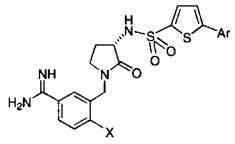
Figure 3. Stereoview of the binding model of **20a** in the fXa active site.

significant step forward (Table 1). This compound was 8-fold more potent against fXa than its parent **3a**,[9] and in this case, the activity against other serine proteinases (thrombin, 5-fold; bovine trypsin, 3-fold) was also enhanced. Our earlier work[9] had demonstrated that N-methylation of the sulfonamide was tolerated and in some instances led to modest enhancements in potency (**3a** vs **3b**); this result was paralleled by the aminobenzamidine pair **4a** and **4b**.

The requirements of the P-1 ligand were explored in more detail. Work on simple benzamidines[11] had suggested that 4-hydroxy substitution could also lead to potency enhancements, albeit less than 4-amino substitution. Thus, we were gratified to discover that the corresponding compound **20a** was more potent than the parent **3a** (16-fold). Once again, activity against other serine proteinases also increased (thrombin, 7-fold; bovine trypsin, 3-fold) although selectivity favored fXa.

The activities of **15** and **21** demonstrate the strict positional requirements for heteroatom substitution. Examination of the S-1 pocket suggests that substitution in the 3-position of the benzamidine ring results in steric clashes upon binding; correspondingly, compound **15** was weakly active. Substitution at the 2-position would appear to be sterically tolerated; nonetheless compound **21** was much less active than its 4-positional isomer. How can the large potency increases seen with the 4-heterobenzamidines and the 4-hydroxy compounds, in particular, be rationalized? Benzamidines substituted in the *para*-position generally exist in solution as protonated cations;[17] similarly, unsubstituted benzamidines are protonated at physiological pH

Table 3. Heterobenzamidine Hybrids



Cmpd.	Ar	X	Ki (nM) fXa	fIIa	Tryp.
29		F	130	~4,000	>2,900
4c		NH_2	6.0	2,600	1,500
20b		OH	2.0	2,800	~ 2,900
4d		NH_2	7.0	2,228	1700
20c		OH	3.0	2,219	~2,900

Table 4. In Vitro Comparisons of Selected fXa Inhibitors

Cmpd.	Ratio: Ki enz/Ki fXa fIIa	Tryp.	APC	Plasmin	tPA	ClogP	2 X APTT (μM),
3a	42	25	35	137	>255	2.4	NA
RPR120844	156	77	342	631	>1240	2.7	1.5
20a	69	102	657	784	356	2.0	NA
20b (RPR130737)	1379	1448	>9246	1465	3347	1.1	0.32

(pK_a = 11.4). Thus, invoking the increased basicity of amino- and hydroxybenzamidines to explain the potency increases observed is not a compelling argument.

The possibility of obtaining fXa/**20a** crystal structures to help explain our SAR was considered. Unfortunately, unlike the related serine proteinases, trypsin and thrombin, X-ray structures of fXa with small molecule inhibitors are rare. Bode et al. have obtained X-ray structures of the bisamidine inhibitor DX-9065a in both fXa[13] and trypsin.[18] Similar inhibitor binding modes were observed for both complexes, reflecting the large degree of homology between the two enzymes; these results were the impetus for our own protein structure work. A crystal structure of **20a** (trypsin K_i = 206 nM) with trypsin was solved to 1.9 Å resolution (Figure 2). The salient features of this structure include a twin−twin interaction between the P-1 amidine and the S-1 carboxylate (Asp189) which is part of an extensive H-bonding network (Ser190 and Gly219). An H-bond from the pyrrolidinone carbonyl to Gly219 is observed, and the methoxynaphthyl group is in close contact with the S-4 subsite. The role of the hydroxyl is difficult to define since it hydrogen bonds to an artifactual sulfate ion; this anion also forms H-bonds to the catalytic triad residues Ser195 and His57.

All the important interactions of **20a** with the trypsin active site are duplicated in the fXa/**20a** binding model (Figure 3), and the conformations of the inhibitor in the two active sites are very similar. The protonated benzamidine engages in a hydrogen bond network to Asp189, Ser190, and Gly218 of the S-1 subsite. An H-bond from the pyrrolidinone carbonyl to Gly218 is observed and the methoxynaphthyl inserts deep into the S-4 subsite. The 4-hydroxybenzamidine cation is expected to be extensively delocalized with participation of the substituent; consequently the hydroxyl is an excellent H-bond donor or may ionize readily. Several H-bond acceptors (and donors) are found in this region of fXa; our binding model indicates that the Ser195 or His57 side chains and the Ser214 carbonyl are likely candidates for direct or water-mediated interaction. This additional interaction could account for the potency enhancements seen in fXa, and similar arguments can be made for the 4-aminobenzamidines. In contrast, the hydroxyl group of the less active isomer **21** cannot access this region of the enzyme.

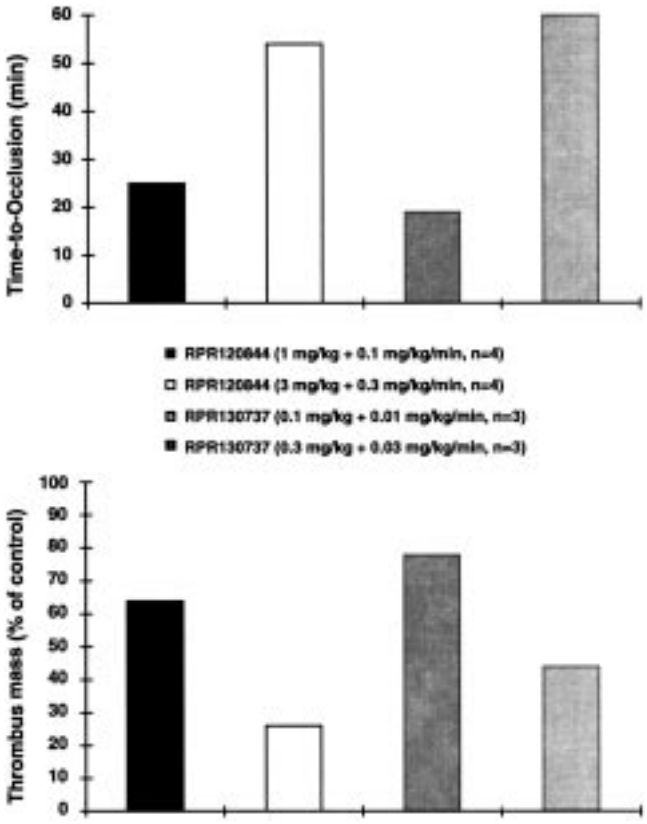


Figure 4. Comparison of RPR120844 and RPR130737 in the rat $FeCl_2$-induced carotid artery thrombosis model. Dose is bolus plus 60 min of infusion. Vehicle time-to-occlusion: 18 min.

A recent publication from Berlex Biosciences describes a very similar *para*-substituted benzamidine effect on an unrelated series of fXa inhibitors, namely 1,6-diphenoxypyridines.[19] The potency enhancements for 4-amino substitution were comparable to our own observations on pyrrolidinones. However, the 4-hydroxy analogue was 67-fold more active than the unsubstituted parent of the Berlex series. This contrasts with the more modest enhancements (up to 20-fold) observed for the pyrrolidinones. It is by no means certain that the rather small potency increases from *para*-substitution first described by Sturzebecher and Walsmann for trypsin-like serine proteinases in general[11] would apply equally to all classes of fXa inhibitors, and it appears that the magnitude and relative specificity of this effect is class-dependent.

It occurred to us that the 4-hydroxy and/or 4-amino functions could serve as handles to append moieties with potential to interact covalently with the catalytic triad (i.e. Ser195). Thus, compounds **14** and **27** were prepared

as potential acylating probes, and in fact, the latter compound was shown to be an effective inhibitor of fXa (K_i = 8 nM). We propose that the ester is functioning as an alternate substrate inhibitor of fXa, ultimately hydrolyzed by water to yield acid **28**, a relatively weak inhibitor. In support of this mechanism we were able to demonstrate, by HPLC analysis, that incubation of ester **27** with fXa yielded acid **28** and that the inhibition of fXa by **27** was time-dependent. Compound **27** also showed significantly enhanced trypsin activity. Because of the chemical lability and lack of selectivity of **27**, we decided to focus our efforts on nonacylating inhibitors. Nonetheless, this was a useful result since it provided further support for the pyrrolidinone binding model in fXa.

Efforts to optimize the P-4 group are summarized in Table 2. Initial attempts to incorporate nitrogen into the distal ring yielded compounds **3d**−**3f**, equipotent to the simple biphenyl **3c**. It was observed, as was typical for the biaryl P-4s, that selectivities against trypsin and thrombin were 20-fold or better. Attempts to incorporate discrete positive charges in P-4, entries **3g** and **3i**, did not increase potency. It is possible that the very nearly linear presentation of these biphenyl systems does not allow for productive interaction of the basic and/or charged systems with the S-4 "cation hole".

In contrast, similar substitutions with thiophene as the proximal aromatic ring yielded two compounds (**3k** and **3l**) with 3-fold enhancements in fXa activity with respect to the simple biphenyl. For this pair, relative selectivity against fIIa and trypsin was high; the isoxazole **3n** was also potent and selective. In the two X-ray structures of fXa with small molecule inhibitors,[13,20] a bound water molecule at the terminus of the S-4 pocket is observed. A weak interaction with this water may be invoked to rationalize the modest enhancements in activity observed for compounds **3k**, **3l**, and **3n**. In the less active isomer **3j**, the nitrogen lone pair is directed away from the bound water.

To further exploit these observations, the pyridin-3-yl and isoxazolylthienyl P-4s were grafted onto the hydroxy- and aminobenzamidine-containing templates. The hydroxybenzamidine hybrids in Table 3 (**20b** and **20c**) were found to be potent inhibitors of fXa (K_i = 2 and 3 nM, respectively), comparable to the best of the naphthalene-containing compounds (**20a**) with the additional benefit of improved selectivity against trypsin and thrombin (3 orders of magnitude). The aminobenzamidines **4c** and **4d** were somewhat less effective than the hydroxy counterparts and less selective. Note that, in contrast, the fluoro analogue **29** (K_i = 130 nM) was significantly less active than even the unsubstituted parent **3k** (K_i = 41 nM). Although the amino function conferred good activity, it appears that hydroxybenzamidine is generally a superior ligand for S-1 in this series. Compound **20b**, in particular (designated RPR130737), had an attractive profile and was chosen for further study as an intravenous fXa inhibitor.

Table 4 compares in vitro data for RPR130737 with earlier pyrrolidinone prototypes. Optimal selectivity is obtained by the combination of the pyridylthiophene P-4 and the hydroxybenzamidine. Most significantly, the compound is selective against anticoagulant serine proteinases such as aPC and plasmin. In addition, it has low activity against the fibrinolytic serine proteinase tissue plasminogen activator (t-PA). Kinetic analysis showed that RPR130737 was a reversible, competitive inhibitor of fXa.[21] This compound inhibited coagulation of human plasma in vitro: i.e. RPR130737 doubled activated partial thromboplastin time at a concentration of 0.32 μM. This result indicates its effectiveness against fXa in the prothrombinase complex which is the physiologically relevant form of the proteinase. In this respect, note also the improvement in activity versus RPR120844; similar results were seen in rat and dog plasma (data not shown).

The more polar nature of the pyridyl-containing inhibitors is reflected in the calculated log *P* (Table 4). The Clog *P* for RPR130737 (1.1) is significantly less than for the naphthalene-containing inhibitors (Clog $P \geq 2$).

The fXa inhibitor RPR130737 was evaluated in vivo in a rat model of ferrous chloride-induced arterial thrombosis; this data is presented in Figure 4. A dose-dependent reduction in thrombus mass and a concomitant increase in the time to occlusion were observed. Efficacy is comparable to that of RPR120844 at about one-tenth the dose. Qualitatively similar results were obtained in a canine model of electrolytic injury-induced thrombosis in the jugular vein and carotid artery and will be published separately. Clearly, the potency enhancements measured in vitro are reflected in the animal models of thrombosis. Inhibitors containing positively charged amidino or guanidino functions are often compromised by their nonspecific effects on cardiovascular function.[22] Blood pressure and heart rate were monitored during the course of our in vivo experiments with RPR130737; hemodynamic parameters were unchanged.

Conclusion

The SAR of the pyrrolidinone class of fXa inhibitors has been expanded to yield **20b** (RPR130737) as a potent member (K_i = 2 nM) with high selectivity (>1000 times) against other serine proteinases. RPR130737 is a reversible inhibitor of fXa and inhibits coagulation of human plasma at submicromolar levels. In vivo experiments have shown that this compound is an effective antithrombotic agent in animal models of thrombosis.

X-ray studies in trypsin support our model for the inhibition of fXa by pyrrolidinone inhibitors with a hydroxybenzamidine moiety in P-1. It suggests that the twin−twin interaction between the amidine and Asp189 is augmented by hydrogen bonding between the *p*-hydroxyl and the proximal residues of the enzyme. The excellent selectivity achieved for RPR130737 is, in part, due to the P-4 pyridylthiophene, which is complementary to the unique S-4 subsite of fXa.

Experimental Section

Enzyme Assays. Human fXa, thrombin, and activated protein C (aPC) were obtained from Enzyme Research Laboratories, Inc. (South Bend, IN). Bovine trypsin was obtained from Sigma Chemical Co. (St. Louis, MO). Plasmin was purchased from Pharmacia Hepar (Franklin, OH). Tissue plasminogen activator (t-PA) was obtained from Genentech (San Fransisco, CA). The chromogenic substrates used were Spectrozyme FXa (American Diagonostica Inc., Greenwich, CT) for fXa; Pefachrome TH (Centerchem, Inc., Stamford, CT) for thrombin; S-2765 (Diapharma Group Inc., Franklin, OH) for trypsin; Pefachrome-tPA (Centerchem Inc.) for t-PA. The

chromogenic substrate S-2366 (Diapharma Group Inc.) was used for both plasmin and aPC assays.

fXa, thrombin, and plasmin were assayed in a buffer containing 0.05 M Tris, 0.15 M NaCl, and 0.1% PEG-8000 at pH 7.5.[23] Trypsin and aPC were assayed in the aforementioned buffer with addition of 0.02 M $CaCl_2$. Tissue plasminogen activator was assayed in the first buffer, but PEG-8000 was replaced with 0.1% (w/v) BSA (bovine serum albumin; Sigma).

The final substrate concentrations in the reactions were 200, 50, 250, and 500 μM for Spectrozyme FXa, Pefachrome TH, S-2765, and Pefachrome-tPA, respectively. The final concentrations of S-2366 for the plasmin and aPC assays were 300 and 500 μM, respectively. All enzyme assays were carried out at room temperature in 96-well microtiter plates with a final enzyme concentration of 1 nM. Compound dilutions were added to the wells containing buffer and enzyme and preincubated for 30 min. The enzyme reactions were initiated by the addition of substrate, and the color developed from the release of *p*-nitroanilide from each chromogenic substrate was monitored continuously for 5 min at 405 nm on a Thermomax microtiter plate reader (Molecular Devices, Sunnyvale, CA). Under the experimental conditions, less than 10% of the substrate was consumed in all assays. The initial velocities measured were used to determine the amount of inhibitor required to diminish 50% of the control velocity; this concentration was defined as the IC_{50} of the inhibitor. Assuming the kinetic mechanisms were all competitive, the apparent K_i values were then calculated according to the Cheng−Prusoff equation: $K_i = IC_{50}/1 + [S]/K_m$.[24]

fXa-Induced Cleavage of Inhibitor 27. Compound **27** and fXa were combined in buffer at a final concentration of 62.5 and 125 nM, respectively. The ratio of **27** and fXa was high (500:1) to enable HPLC analysis of the reaction mixture. The mixture was incubated at 37 °C, and aliquots were removed for analysis and anti-fXa activity estimation.

At the early time points (5 and 30 min) fXa was completely inhibited, and very little conversion had taken place. After 1-h incubation with fXa, HPLC analysis revealed that 48% of compound **27** (K_i = 8 nM) was converted to acid **28** (K_i = 1.3 μM). This corresponded to an anti-fXa activity of 98% for the mixture. After 3-h incubation, the ester **27** was completely converted to acid **28**; the anti-fXa activity of the mixture was 14%.

Coagulation Assay. Activated partial thromboplastin time (APTT) was measured with a MLA Electra 800 automatic coagulation timer (Orthodiagnostics, NJ). Citrated human (George King Biomedical Inc: Overland Park, KS), dog (mongrel; Covance Research Product, Alice, TX), and rat (Sprague−Dawley; Charles River) plasma were used in the assays. One hundred microliters of freshly thawed plasma was mixed with 100 μL of compound dilutions followed by automatic addition of 100 μL of actin-activated cephaloplastin reagent (Dade, Miami, FL) and 100 μL of 0.035 M calcium chloride to start the clot formation. Anticoagulant activity of a compound was reported as the concentration required for doubling the plasma clotting time.

Rat $FeCl_2$-Induced Carotid Artery Thrombosis Model.[25] Experiments were performed on male Sprague−Dawley rats anesthetized with inactin (100 mg/kg, ip). The trachea was intubated via a tracheostomy with polyethylene tubing (PE 205) to ensure airway patency. Catheters (PE50) were inserted into the right jugular vein for drug administration and the right femoral artery for collection of blood samples and recording of blood pressure. The right carotid artery was isolated, and a piece of parafilm "M" (American Can Co., Greenwich, CT) was placed under the vessel to separate it from the surrounding tissue. An electromagnetic flow probe (0.95 mm lumen) was placed on the carotid artery and attached to a model 1401 flow meter (Skalar, Delft, The Netherlands). Following baseline flow measurements of 10 min, compounds were administered as an intravenous bolus plus a constant infusion for 75 min. After 15 min of drug infusion, vascular damage was produced by the local application of a piece of filter paper (2 × 5 mm) saturated with a 50% solution of $FeCl_2$ placed on top of the vessel downstream from the flow probe as described in the methods of Kurz et al., with minor modifications.[25] The filter paper was removed after 10 min. The carotid artery was removed 60 min after the application of the filter paper whether or not occlusion had occurred. Time-to-occlusion was defined as the time from the application of $FeCl_2$ until blood flow decreased to zero. If the vessel did not occlude by 60 min, the time to occlusion was assigned a 60-min value for data analysis. The thrombus was removed, and the wet weight was determined immediately on a Sartorius BP160P balance (Sartorius AG, Gottingen, Germany). Blood pressure and heart rate were continuously monitored on a Gould TA6000 recorder (Gould Instruments, Valley View, OH) and reported at control, after 15 min of infusion, and at 15, 30, 45, and 60 min after $FeCl_2$ application.

Molecular Modeling. The inhibitor **20a** was flexibly docked into the fXa active site using an automated protocol developed in-house.[26] In this method, lower-energy conformations of the ligand are generated on the fly using a modifed version of a rule-based method implemented in Chem-X (January 1997 release, Chemical Design Ltd., Chipping Norton, Oxfordshire OX7 5SR, U.K.). Using ChemDBS-3D with in-house customization, an attempt is made to fit each conformation in turn onto a predefined, minimal pharmacophore model using a steric shell of the active site as an added constraint. Matching conformations are then passed to the program DISCOVER (MSI, 9685 Scranton Rd, San Diego, CA 92121-3752) for optimization in a partially relaxed active site model using the CFF97 force field (April 1997 release, developed by the Potential Energy Functions Consortium, MSI, 9685 Scranton Rd, San Diego, CA 92121-3752).[27] The resulting docked complexes are scored on the basis of total force field energy.

Clog *P*'s were calculated using ACD Lab software (Advanced Chemistry Development, 133 Richmond St W, Suite 605, Toronto, Ontario, Canada, M5A 2L3).

Trypsin/20a Crystal Structure Determination. Crystallization and inhibitor soaking: Bovine pancreatic trypsin (Sigma T8003) was solubilized in 1 mM $CaCl_2$, 60 mM benzamidine, 50 mM Mes-NaOH (pH 6.0) buffer to a final concentration of 30 mg/mL. The hanging drops crystallization method was used. Single crystals were obtained with ammonium sulfate (1.4−1.8 M), pH 6.0, in the "open" form ($P2_12_12_1$, a = 59.9 Å, b = 64.2 Å, c = 69.9 Å) for which the binding site is free of any contact with crystallographic symmetry-related molecules. The crystals were soaked in 50 μL of 3.3 M ammonium sulfate, 1 mM **20a**, 6% DMF, 1 mM $CaCl_2$, 50 mM Mes-NaOH (pH 6.0) for 5 h.

Data collection and processing: For data collection a crystal was placed in the previous soaking solution + 20% glycerol for 5 min and then flash-cooled in a gas stream of N_2 at 90 K. Data collection was performed on a FR591 rotating anode generator (Nonius, The Netherlands) equipped with a DIP2020K image plate detector (Mac Science, Japan). Data images were processed using DENZO and SCALEPACK,[28] and structure refinement was performed using X-PLOR.[29] The trypsin structure used as a starting point is the uncomplexed 1.55 Å form (2ptn Protein Data Bank entry).[30]

Chemistry. All starting materials, reagents, and solvents were used as received from commercial sources except for *N*-bromosuccinimide which was recrystallized from water and dried under vacuum. In general, reactions were performed under an inert atmosphere such as nitrogen or argon. Workup indicates drying over sodium or magnesium sulfate, filtering, and concentrating in vacuo. Flash column chromatography of intermediates was performed using 230−400 mesh silica gel 60 (E. Merck). Proton NMRs were recorded on a Bruker ARX 300 MHz or ACF 300 spectrometer, and mass spectra were obtained from a Varian VG-70SE spectrometer. Elemental analyses were performed by Quantitative Technologies of Whitehorse, NJ.

Final products were generally purified by preparative reverse-phase HPLC. Purification was performed on a Rainin SD-1 Dynamax system with a 2-in. C-18 reverse-phase Dynamax 60 Å column using a gradient of 10−40% acetonitrile/

0.1% TFA water to 80−100% acetonitrile/0.1% TFA water over a 30−50-min period and a flow rate of 40−50 mL/min. Fractions containing the desired material were concentrated and lyophilized to obtain the final products as white solids.

Preparation of 4-Pyridin-2-ylbenzenesulfonyl Chloride (6a). Representative Procedure for the Synthesis of Biaryl Sulfonyl Chlorides. Part A: A solution of 2-bromopyridine (1.0 g, 6.3 mmol) in THF (25 mL) was treated with *tert*-butyllithium (7.5 mL, 1.7 M solution in pentane) at −78 °C for 10 min followed by zinc chloride in ether (6.6 mL, 6.6 mmol). The reaction was warmed to room temperature over a 2-h period and then treated with palladium tetrakis(triphenylphosphine) (35.4 mg, 0.031 mmol) and 1-bromo-4-iodobenzene (1.78 g, 6.29 mmol) in THF (10 mL). The reaction mixture was stirred for 72 h, then concentrated in vacuo, and diluted with 10% NH_4OH solution and EtOAc. The organic layer was washed with saturated NaCl and worked up. The crude residue was purified by flash chromatography to give 4-(pyridin-2-yl)bromobenzene (**5a**) (0.75 g, 51%): ^{1}H NMR ($CDCl_3$) δ 8.71 (d, 1H), 7.88 (d, 2H), 7.66−7.79 (m, 2H), 7.56 (d, 2H), 7.22 (d, 1H); MS (EI) *m*/*z* 233, 235 (M^+).

4-(Pyridin-3-yl)bromobenzene (5b). 3-Bromopyridine (6 g, 38 mmol) was treated as described above with *n*-butyllithium (28.5 mL, 1.6 M solution in THF) and 1-bromo-4-iodobenzene (8.96 g, 31.7 mmol). The crude product was purified by chromatography (30% EtOAc/hexanes) to obtain **5b** (3.5 g, 47%): MS (EI) *m*/*z* 233, 235 (M^+).

4-(Pyridin-4-yl)bromobenzene (5c). 4-Bromopyridine hydrochloride was free based with 1 N NaOH solution (0.95 equiv) and extracted into methylene chloride. The organic solution was concentrated at 0 °C and used immediately without further purification. A portion of the solid obtained (3.0 g, 19 mmol) was treated as described above with *n*-butyllithium (14.25 mL, 1.6 M solution in THF) and 1-bromo-4-iodobenzene (5.39 g, 19 mmol). The crude product was purified by chromatography (30% EtOAc/hexanes to 60% EtOAc/hexanes) to obtain 4-(pyridin-4-yl)bromobenzene (**5c**) (2.59 g, 58%): MS (EI) *m*/*z* 233, 235 (M^+).

(1-Methyl-1*H*-imidazol-2-yl)benzene (5d). (1-Methyl-1*H*-imidazol-2-yl)benzene was prepared using the method of Bell *et al.*[31] with 1-bromo-4-iodobenzene and 1-methylimidazole as starting materials: ^{1}H NMR ($CDCl_3$) δ 7.58 (d, 2H), 7.5 (d, 2H), 7.11 (d, 1H), 6.98 (d, 1H), 3.75 (s, 3H); MS (EI) *m*/*z* 236, 238 (M^+).

Part B: *tert*-Butyllithium (3.72 mL, 1.7 M solution in pentane) was added dropwise to a solution of 4-(pyridin-2-yl)-bromobenzene (0.75 g, 3.16 mmol) in THF/ether (20 mL, 1:1 v/v) at −78 °C. In a separate flask, SO_2 gas was condensed (10 mL) at −78 °C and then diluted with ether (20 mL). The lithium anion was added dropwise via cannula to the solution of sulfur dioxide, and the reaction mixture was allowed to warm to room temperature overnight. The suspension was concentrated in vacuo, and the resulting solid washed with ether. The collected solid was diluted with anhydrous hexane (20 mL) and treated with SO_2Cl_2 (3.32 mL, 3.32 mmol, 1 M solution in CH_2Cl_2) at 0 °C. The suspension was allowed to warm to room temperature over 4 h and then concentrated in vacuo. The crude solid product was washed with hexane and triturated with warm benzene followed by methylene chloride to give 4-pyridin-2-ylbenzenesulfonyl chloride (**6a**) (0.5 g, 63%): ^{1}H NMR (CD_3OD) δ 8.72 (d, 1H), 8.34 (AB, 2H), 8.19 (AB, 2H), 7.85−8.05 (m, 2H), 7.47 (t, 1H); MS (EI) *m*/*z* 253, 255 (M^+).

4-Pyridin-3-ylbenzenesulfonyl Chloride (6b). The title compound was prepared from 4-(pyridin-3-yl)bromobenzene (1.75 g, 7.5 mmol) as described in part B except that 2 equiv of *tert*-butyllithium were used to generate the starting anion. The crude solid product was purified by washing with copious amounts of hexane followed by 100 mL of hot anhydrous CH_2Cl_2 and was used without further purification (1.98 g, 100%): ^{1}H NMR (DMSO-d_6) δ 9.23 (bs, 1H), 8.80 (m, 2H), 8.04 (m, 1H), 7.65−7.83 (AB, 4H); MS (EI) *m*/*z* 253, 255 (M^+).

4-Pyridin-4-ylbenzenesulfonyl Chloride (6c). The title compound was prepared from 4-(pyridin-4-yl)bromobenzene as described in part B except that 2 equiv of *tert*-butyllithium were used to generate the starting anion. The crude solid product was purified by washing with copious amounts of hexane and ether and was used without further purification: MS (EI) *m*/*z* 253, 255 (M^+).

(1-Methyl-1*H*-imidazol-2-yl)benzene-4-sulfonyl Chloride (6d). (1-Methyl-1*H*-imidazol-2-yl)benzene was treated as described in part B but using 1.02 equiv of *n*-butyllithium: MS (EI) *m*/*z* 256, 258 (M^+).

5-Pyridin-3-ylthiophene-2-sulfonyl Chloride (8a). 3-Thiophene-2-ylpyridine[14a] was treated as described in part B but using 1.02 equiv of *n*-butyllithium. The crude solid product was diluted with EtOAc and water. The organic layer was washed with saturated $NaHCO_3$ and saturated NaCl and then worked up. The crude solid was suspended in ether and the insoluble black solid filtered off. The ethereal solution was concentrated in vacuo to give a light yellow solid which was used without further purification: ^{1}H NMR ($CDCl_3$) δ 8.95 (bs, 1H), 8.70 (bs, 1H), 7.95 (d, 1H), 7.90 (d, 1H), 7.45 (bs, 1H), 7.44 (d, 1H); MS (EI) *m*/*z* 259, 261 (M^+).

5-Pyridin-4-ylthiophene-2-sulfonyl Chloride (8b). 4-Thiophene-2-ylpyridine[14b] was treated as described in part B but using 1.02 equiv of *n*-butyllithium. The crude solid product was diluted with EtOAc and water. The organic layer was washed with saturated $NaHCO_3$ and saturated NaCl and then worked up to give the product as a yellow solid which was used without further purification: ^{1}H NMR ($CDCl_3$) δ 8.75 (d, 2H), 8.60 (d, 1H), 7.90 (d, 1H), 7.51 (d, 2H); MS (EI) *m*/*z* 259, 261 (M^+).

Preparation of 4-Amino-3-[3-(*S*)-(7-methoxynaphthalen-2-ylsulfonylamino)-2-oxopyrrolidin-1-ylmethyl]benzamidine Trifluoroacetate (4a). Representative Procedure for the Synthesis of Compounds 4c and 4d. Part A: Tin(II) chloride (13.9 g, 61.7 mmol) was added to a solution of 3-methyl-4-nitrobenzonitrile (2.0 g, 12.3 mmol) in EtOH (160 mL). The resulting mixture was refluxed for 2 h and then cooled to ambient temperatures. The reaction mixture was poured into ice water (100 mL), and the pH of the solution was adjusted to >7 with saturated $NaHCO_3$. The solution was diluted with EtOAc, and the resulting mixture was filtered through Celite. The organic layer was separated and the aqueous layer further extracted with EtOAc. The combined organic layers were worked up to yield 4-amino-3-methylbenzonitrile (1.57 g, 96%) as a white solid: ^{1}H NMR ($CDCl_3$) δ 7.30−7.35 (m, 2H), 6.63 (d, 1H), 4.10 (bs, 2H), 2.15 (m, 3H); MS (EI) *m*/*z* 132 (M^+).

Part B: Benzophenone (1.74 g, 9.53 mmol) and *p*-toluenesulfonic acid (0.43 g, 2.1 mmol) were added to a solution of 4-amino-3-methylbenzonitrile (1.2 g, 9.08 mmol) in toluene (75 mL). The reaction vessel was fitted with a Dean−Stark trap and heated to reflux. After 24 h, the solution was cooled to ambient temperature and concentrated in vacuo. The residue was chromatographed on silica gel eluting with a gradient of 3% EtOAc/hexanes to 10% EtOAc/hexanes to give 4-(benzhydrylidenylamino)-3-methylbenzonitrile (2.43 g, 90%) as an oil: ^{1}H NMR ($CDCl_3$) δ 7.80 (m, 2H), 7.23−7.48 (m, 7H), 7.15 (d, 1H), 7.05 (bs, 2H), 6.50 (d, 1H), 2.20 (s, 3H); MS (EI) *m*/*z* 296 (M^+).

Part C: *N*-Bromosuccinimide (0.84 g, 4.7 mmol) and benzoyl peroxide (0.22 g, 0.64 mmol) were added to a solution of 4-(benzhydrylidenylamino)-3-methylbenzonitrile (1.36 g, 4.27 mmol) in CCl_4 (40 mL). The solution was refluxed for 16 h and then cooled to ambient temperature. The mixture was diluted with CH_2Cl_2 and washed with 1 N NaOH and saturated NaCl. The organic layer was worked up, and the residue was chromatographed on silica gel eluting with a gradient of 5% EtOAc/hexanes to 10% EtOAc/hexanes to give 4-(benzhydrylidenylamino)-3-(bromomethyl)benzonitrile (**9**) (0.91 g, 57%) as an oil: ^{1}H NMR ($CDCl_3$) δ 7.70−7.80 (m, 2H), 7.60 (d, 1H), 7.25−7.53 (m, 8H), 7.15 (dd, 1H), 6.35 (d, 1H), 4.55 (s, 2H); MS (EI) *m*/*z* 374, 376 (M^+).

Part D: Sodium hydride (0.028 g, 0.70 mmol, 60% oil dispersion) was added to a solution of (2-oxopyrrolidin-3-(*S*)-yl)carbamic acid *tert*-butyl ester[9] (**10**) (0.134 g, 0.67 mmol) in

THF/DMF (10 mL, 9:1 v/v) at 0 °C. After 5 min, 4-(benzhydrylidenylamino)-3-(bromomethyl)benzonitrile (**9**) (0.252 g, 0.67 mmol) in THF (1 mL) was added dropwise via cannula to the reaction mixture. The ice bath was removed and the resulting solution stirred for an additional 4 h during which time the solution turned cloudy. The reaction was quenched with saturated NH_4Cl solution and diluted with EtOAc. The organic layer was separated and the aqueous layer washed further with EtOAc. The combined organic layers were worked up, and the crude residue was chromatographed on silica gel eluting with a gradient of 20% EtOAc/hexanes to 30% EtOAc/hexanes to give {1-[2-(benzhydrylidenylamino)-5-cyanobenzyl]-2-oxopyrrolidin-3-yl}carbamic acid *tert*-butyl ester (**11**) (0.284 g, 86%) as a foamy solid: ^{1}H NMR ($CDCl_3$) δ 7.60−7.80 (bs, 2H), 7.40 (s, 1H), 7.25−7.40 (bs, 6H), 7.22 (d, 1H), 7.00−7.15 (bs, 2H), 6.48 (d, 1H), 5.00 (d, 1H, 4.45 (AB, 2H), 4.15 (m, 1H), 3.30 (m, 2H), 2.61 (m, 1H), 1.90 (m, 1H), 1.45 (s, 9H); MS (FAB) *m*/*z* 495 $(M + H)^+$.

Part E: Hydrogen chloride gas was bubbled through a solution of {1-[2-(benzhydrylidenylamino)-5-cyanobenzyl]-2-oxopyrrolidin-3-yl}carbamic acid *tert*-butyl ester (**11**) (0.70 g, 1.42 mmol) in EtOAc (75 mL) at 0 °C for 5 min. After 1 h, the solution was concentrated in vacuo. The resulting residue was dissolved in CH_3CN (50 mL); then triethylamine (0.79 mL, 5.68 mmol) and 7-methoxynaphthalene-2-sulfonyl chloride (0.38 g, 1.49 mmol) were added. After 5 h, the reaction mixture was concentrated in vacuo, diluted with EtOAc, and washed with saturated $NaHCO_3$ and saturated NaCl. The organic layer was worked up, and the crude material was purified by chromatography eluting with 5% CH_3OH/CH_2Cl_2 to give 7-methoxynaphthalene-2-sulfonic acid [1-(2-amino-5-cyanobenzyl)-2-oxopyrrolidin-3-(*S*)-yl]amide (0.60 g, 94%) as a yellow solid: ^{1}H NMR ($CDCl_3$) δ 8.30 (s, 1H), 7.90 (d, 1H), 7.80 (d, 1H), 7.70 (d, 1H), 7.30−7.41 (m, 4H), 6.55 (d, 1H), 5.25 (d, 1H), 4.90 (s, 2H), 4.30 (AB, 2H), 3.95 (s, 3H), 3.75 (m, 1H), 3.20 (m, 2H), 2.55 (m, 1H), 2.00 (m, 1H); MS (FAB) *m*/*z* 451 $(M + H)^+$.

5-Pyridin-3-ylthiophene-2-sulfonic Acid [1-(2-Amino-5-cyanobenzyl)-2-oxopyrrolidin-3-(*S*)-yl]amide. ^{1}H NMR ($CDCl_3$) δ 8.85 (d, 1H), 8.61 (dd, 1H), 7.82 (m, 1H), 7.68 (d, 1H), 7.26−7.38 (m, 4H), 6.60 (d, 1H), 5.35 (d, 1H), 4.89 (s, 2H), 4.30 (AB, 2H), 3.97 (m, 1H), 3.30 (m, 2H), 2.65 (m, 1H), 2.09 (m, 1H); MS (FAB) *m*/*z* 454 $(M + H)^+$.

5-Isoxazol-3-ylthiophene-2-sulfonic Acid [1-(2-Amino-5-cyanobenzyl)-2-oxopyrrolidin-3-(*S*)-yl]amide. ^{1}H NMR ($CDCl_3$) δ 8.35 (d, 1H), 7.81 (d, 1H), 7.69 (d, 1H), 7.50 (d, 2H), 7.39 (dd, 1H), 6.65 (d, 1H), 6.59 (d, 1H), 4.32 (AB, 2H), 4.02 (m, 1H), 3.30 (m, 2H), 2.60 (m, 1H), 2.08 (m, 1H).

Part F: Hydrogen chloride gas was bubbled through a solution of 7-methoxynaphthalene-2-sulfonic acid [1-(2-amino-5-cyanobenzyl)-2-oxopyrrolidin-3-yl]amide (0.60 g, 1.33 mmol) in EtOH (60 mL) at 0 °C for 10 min. The reaction flask was stoppered and allowed to warm to ambient temperature overnight. The reaction mixture was concentrated in vacuo, and the crude imidate was taken up in MeOH (50 mL) and cooled to 0 °C. Ammonia gas was bubbled through the solution for 10 min; then the reaction flask was fitted with a reflux condenser and balloon. The reaction mixture was heated at 60 °C for 4.5 h and then concentrated in vacuo to yield the crude product. Purification by reverse-phase HPLC and lyophilization of the aqueous fractions containing the desired product yielded 4-amino-3-[3-(*S*)-(7-methoxynaphthalen-2-ylsulfonylamino)-2-oxopyrrolidin-1-ylmethyl]benzamidine trifluoroacetate (**4a**) (0.58 g, 75%) as a white solid: ^{1}H NMR (DMSO-d_6) δ 8.80 (bs, 2H), 8.45 (bs, 2H), 8.35 (s, 1H), 8.10 (d, 1H), 8.00 (d, 1H), 7.90 (d, 1H), 7.70 (dd, 1H), 7.50 (m, 2H), 7.40 (d, 1H), 7.35 (dd, 1H), 6.70 (d, 1H), 6.20 (bs, 2H), 4.15 (AB, 2H), 4.10 (m, 1H), 3.90 (s, 3H), 3.12 (m, 2H), 1.98 (m, 1H), 1.55 (m, 1H); MS (FAB) *m*/*z* 468 $(M + H)^+$. Anal. ($C_{23}H_{25}N_5O_4S \cdot 1.5TFA \cdot 0.5H_2O$) C, H, N.

4-Amino-3-[2-oxo-3-(*S*)-(5-pyridin-3-ylthiophene-2-ylsulfonylamino)pyrrolidin-1-ylmethyl]benzamidine Trifluoroacetate (4c). ^{1}H NMR (DMSO-d_6) δ 8.95 (d, 1H), 8.75 (s, 2H), 8.58 (d, 1H), 8.50 (d, 1H), 8.48 (s, 2H), 8.10 (d, 1H), 7.70 (s, 2H), 7.50 (m, 2H), 7.40 (d, 1H), 6.71 (d, 1H), 6.20 (bs, 2H), 4.11−4.25 (m, 3H), 3.15 (m, 2H), 2.18 (m, 1H), 1.62 (m, 1H); MS (FAB) *m*/*z* 471 $(M + H)^+$; HRMS (ESI) calcd for $C_{21}H_{22}N_6O_3S_2$ 471.1273, found 471.1268 $(M + H)^+$; HPLC analysis (C18, 15-min linear gradient; elution 10−80% CH_3CN/0.1% TFA H_2O; flow rate 1.0 mL/min) indicated that the product was 99A%.

4-Amino-3-[3-(*S*)-(5-isoxazol-3-ylthiophene-2-ylsulfonylamino)-2-oxopyrrolidin-1-ylmethyl]benzamidine Trifluoroacetate (4d). ^{1}H NMR (DMSO-d_6) δ 8.80 (bs, 2H), 8.75 (d, 1H), 8.70 (d, 1H), 8.52 (bs, 2H), 7.75 (s, 2H), 7.52 (dd, 1H), 7.45 (d, 1H), 7.10 (d, 1H), 6.71 (d, 1H), 6.20 (bs, 1H), 4.28 (m, 1H), 4.21 (AB, 2H), 3.20 (m, 2H), 2.20 (m, 1H), 1.70 (m, 1H); MS (ESI) *m*/*z* 461 $(M + H)^+$. Anal. ($C_{19}H_{20}N_6O_4S \cdot TFA \cdot 1.07H_2O$) C, H, N.

Preparation of 4-Amino-3-[3-(*S*)-(7-methoxynaphthalen-2-ylsulfonylmethylamino)-2-oxopyrrolidin-1-ylmethyl]benzamidine Trifluoroacetate (4b). Part A: Sodium hydride (0.35 g, 8.7 mmol, 60% mineral oil dispersion) was added to a solution of {1-[2-(benzhydrylidenylamino)-5-cyanobenzyl]-2-oxopyrrolidin-3-yl}carbamic acid *tert*-butyl ester (11) (3.94 g, 7.98 mmol) in DMF (8 mL) at 0 °C. After 20 min, methyl iodide (0.99 mL, 15.9 mmol) was added. The reaction mixture was stirred for 2 h, then treated with saturated NH_4Cl, and diluted with EtOAc. The organic layer was separated and the aqueous layer washed with additional EtOAc. The combined organic layers were washed with saturated NaCl and worked up. The crude material was purified by column chromatography eluting with a gradient of 30% EtOAc/hexanes to 50% EtOAc/hexanes to give {1-[2-(benzhydrylidenylamino)-5-cyanobenzyl]-2-oxopyrrolidin-3-yl}-*N*-methylcarbamic acid *tert*-butyl ester (12) (3.72 g, 92%) as a yellow solid: ^{1}H NMR ($CDCl_3$) δ 7.70 (bs, 2H), 7.45 (m, 8H), 7.10 (bs, 2H), 6.45 (dd, 1H), 4.70 (m, 1H), 4.49 (AB, 2H), 3.30 (m, 2H), 2.83 (s, 3H), 2.35 (m, 1H), 2.10 (m, 1H), 1.50 (s, 9H); MS (FAB) *m*/*z* 509 $(M + H)^+$.

Part B: {1-[2-(Benzhydrylidenylamino)-5-cyanobenzyl]-2-oxopyrrolidin-3-yl}-*N*-methylcarbamic acid *tert*-butyl ester (**12**) was treated as described in example **4a**, part E, to give 7-methoxynaphthalene-2-sulfonic acid [1-(2-amino-5-cyanobenzyl)-2-oxopyrrolidin-3-yl]methylamide (**13**) as a foamy yellow solid: ^{1}H NMR ($CDCl_3$) δ 8.38 (d, 1H), 7.90 (d, 1H), 7.78 (d, 1H), 7.72 (dd, 1H), 7.32 (dd, 1H), 7.30 (dd, 1H), 7.28 (d, 1H), 7.23 (d, 1H), 6.55 (d, 1H), 4.98 (s, 2H), 4.95 (m, 1H), 4.25 (AB, 2H), 3.98 (s, 3H), 3.20 (m, 2H), 2.70 (s, 3H), 1.95 (m, 1H); MS (FAB) *m*/*z* 465 $(M + H)^+$.

Part C: 7-Methoxynaphthalene-2-sulfonic acid [1-(2-amino-5-cyanobenzyl)-2-oxopyrrolidin-3-yl]methylamide (**13**) was treated as described in example **4a**, part F, to give 4-amino-3-[3-(*S*)-(7-methoxynaphthalen-2-ylsulfonylmethylamino)-2-oxopyrrolidin-1-ylmethyl]benzamidine trifluoroacetate (**4b**) as a white solid: ^{1}H NMR (DMSO-d_6) δ 8.90 (bs, 2H), 8.75 (bs, 2H), 8.40 (s, 1H), 8.050 (d, 1H), 7.95 (d, 1H), 7.70 (dd, 1H), 7.60 (d, 1H), 7.55 (dd, 1H), 7.48 (d, 1H), 7.39 (dd, 1H), 6.70 (d, 1H), 6.00 (bs, 1H), 4.98 (m, 1H), 4.20 (AB, 2H), 3.90 (s, 3H), 3.15 (m, 2H), 2.67 (s, 3H), 2.05 (m, 1H), 1.70 (m, 1H); MS (FAB) *m*/*z* 482 $(M + H)^+$. Anal. ($C_{23}H_{25}N_5O_4S \cdot 2.0TFA$) C, H, N.

Preparation of *N*-(4-Carbamimidoyl-2-{3-[(7-methoxynaphthalen-2-ylsulfonyl)methylamino]-2-oxopyrrolidin-1-(*S*)-ylmethyl}phenyl)acetamide Trifluroacetate (14). Part A: Triethylamine (0.25 mL, 1.81 mmol), *N*,*N*-(dimethylamino)pyridine (0.01 g, 0.061 mmol) and acetyl chloride (0.43 mL, 6.05 mmol), were added to a solution of 7-methoxynaphthalene-2-sulfonic acid [1-(2-amino-5-cyanobenzyl)-2-oxopyrrolidin-3-yl]methylamide (**13**) (0.28 g, 0.61 mmol) in 1,2-dichloroethane (20 mL). The solution was heated to 60 °C for 16 h, then cooled to ambient temperature, and diluted with EtOAc. The organic layer was washed with saturated $NaHCO_3$ and saturated NaCl and then worked up. The crude residue was purified by column chromatography eluting with 20% EtOAc/CH_2Cl_2 to give *N*-(4-cyano-2-{3-[(7-methoxynaphthalen-2-ylsulfonyl)methylamino]-2-oxopyrrolidin-1-ylmethyl}-phenyl)acetamide (0.232 g, 76%) as a white solid: ^{1}H NMR ($CDCl_3$) δ 9.50 (s, 1H), 8.50 (d, 1H), 8.30 (s, 1H), 7.89 (d, 1H), 7.80 (d, 1H), 7.76 (dd, 1H), 7.60 (d, 1H), 7.40 (d, 1H), 7.20 (m,

2H), 4.90 (m, 1H), 4.30 (AB, 2H), 3.90 (s, 3H), 3.30 (m, 2H), 2.75 (s, 3H), 2.35 (m, 1H), 2.05 (m, 1H), 1.90 (s, 3H); MS (FAB) *m*/*z* 507 $(M + H)^+$.

Part B: *N*-(4-Cyano-2-{3-[(7-methoxynaphthalen-2-ylsulfonyl)methylamino]-2-oxopyrrolidin-1-ylmethyl}phenyl)-acetamide was treated as described in example **4a**, part F, to yield the title compound **14**: ^{1}H NMR (DMSO-d_6) δ 9.70 (s, 1H), 9.23 (bs, 2H), 9.00 (bs, 1H), 8.40 (s, 1H), 8.00 (d, 1H), 7.98 (d, 1H), 7.70 (m, 2H), 7.60 (m, 2H), 7.35 (dd, 1H), 4.97 (m, 1H), 4.40 (AB, 2H), 3.90 (s, 3H), 3.20 (m, 2H), 2.68 (s, 3H), 2.10 (m, 1H), 2.00 (s, 3H), 1.80 (m, 1H); MS (FAB) *m*/*z* 524 $(M + H)^+$. Anal. ($C_{26}H_{29}N_5O_5S$·TFA·1.5H_2O) C, H, N.

Preparation of 3-Amino-5-[3-(*S*)-(7-methoxynaphthalen-2-ylsulfonylamino)-2-oxopyrrolidin-1-ylmethyl]benzamidine Bistrifluoroacetate (15). Part A: 3-Methyl-5-nitrobenzonitrile[32] was treated in a similar manner as example **4a**, part A, to give 3-amino-5-methylbenzonitrile: ^{1}H NMR ($CDCl_3$) δ 6.83 (s, 1H), 6.70 (s, 1H), 6.68 (s, 1H), 3.70 (bs, 2H), 2.30 (s, 3H); MS (EI) *m*/*z* 132 (M^+).

Part B: 3-Amino-5-methylbenzonitrile was treated as described in example **4a**, part B, to give 3-(benzhydrylidenylamino)-5-methylbenzonitrile: ^{1}H NMR ($CDCl_3$) δ 7.73 (d, 2H), 7.45 (m, 2H), 7.30 (m, 4H), 7.05 (dd, 2H), 7.00 (s, 1H), 6.78 (s, 2H), 6.71 (s, 1H), 2.20 (s, 3H); MS (EI) *m*/*z* 296 $(M)^+$.

Part C: 3-(Benzhydrylidenylamino)-5-methylbenzonitrile was treated in the same manner as described in example **4a**, part C, to yield 3-(benzhydrylidenylamino)-5-(bromomethyl)-benzonitrile: ^{1}H NMR ($CDCl_3$) δ 7.75 (d, 2H), 7.50 (m, 1H), 7.40 (m, 2H), 7.30 (m, 4H), 7.05 (m, 2H), 6.95 (s, 1H), 6.89 (s, 1H), 4.30 (s, 2H); MS (EI) *m*/*z* 374 $(M)^+$.

Part D: 3-(Benzhydrylidenylamino)-5-(bromomethyl)benzonitrile was treated in the same manner as described in example **4a**, part D, to give {1-[3-(benzhydrylidenylamino)-5-cyanobenzyl]-2-oxopyrrolidin-3-yl}carbamic acid *tert*-butyl ester: ^{1}H NMR ($CDCl_3$) δ 7.75 (d, 2H), 7.50 (m, 1H), 7.40 (m, 2H), 7.30 (m, 4H), 7.10 (m, 1H), 6.95 (s, 1H), 6.65 (s, 1H), 5.10 (bs, 1H), 4.30 (AB, 2H), 4.05 (m, 1H), 3.85 (m, 2H), 2.55 (m, 1H), 1.75 (m, 1H), 1.40 (s, 9H); MS (EI) *m*/*z* 495 $(M)^+$.

Part E: {1-[3-(Benzhydrylidenylamino)-5-cyanobenzyl]-2-oxopyrrolidin-3-yl}carbamic acid *tert*-butyl ester was treated in the same manner as described in example **4a**, part E, to give 7-methoxynaphthalene-2-sulfonic acid [1-(3-benzhydrylidenylamino-5-cyanobenzyl)-2-oxopyrrolidin-3-yl]amide: ^{1}H NMR ($CDCl_3$) δ 8.35 (s, 1H), 7.90 (d, 1H), 7.80 (d, 1H), 7.75 (dd, 1H), 7.70 (d, 2H), 7.50 (m, 1H), 7.40 (m, 2H), 7.25 (m, 5H), 7.00 (m, 4H), 6.55 (s, 1H), 5.25 (s, 1H), 4.25 (AB, 2H), 3.95 (s, 3H), 3.65 (m, 1H), 2.80 (m, 2H), 2.45 (m, 1H), 1.95 (m, 1H); MS (FAB) *m*/*z* 615 $(M + H)^+$.

Part F: 7-Methoxynaphthalene-2-sulfonic acid [1-(3-benzhydrylidenylamino-5-cyanobenzyl)-2-oxopyrrolidin-3-yl]-amide was treated in the same manner as described in example **4a**, part F, to give 3-amino-5-[3-(*S*)-(7-methoxynaphthalen-2-ylsulfonylamino)-2-oxopyrrolidin-1-ylmethyl]benzamidine bistrifluoroacetate (**15**): ^{1}H NMR (DMSO-d_6) δ 9.15 (s, 1H), 9.00 (bs, 2H), 8.35 (s, 1H), 8.20 (d, 1H), 8.05 (d, 1H), 7.95 (d, 1H), 7.70 (dd, 1H), 7.60 (d, 1H), 7.20 (dd, 1H), 6.70 (s, 1H), 6.65 (s, 1H), 6.60 (s, 1H), 5.80 (bs, 2H), 4.20 (AB, 2H), 4.10 (m, 1H), 3.90 (s, 3H), 3.00 (m, 2H), 2.00 (m, 1H), 1.50 (m, 1H); MS (FAB) *m*/*z* 468 $(M + H)^+$. Anal. ($C_{26}H_{29}N_5O_5S$·2.5TFA·1.25H_2O) C, H, N.

Preparation of 4-Hydroxy-3-[2-oxo-3-(*S*)-(5-pyridin-3-ylthiophene-2-ylsulfonylamino)pyrrolidin-1-ylmethyl]-benzamidine Trifluoroacetate (20b). Representative Procedure for the Synthesis of Compounds 20a and 20c. Part A: Iodine monochloride (250 mL, 1 M solution in CH_2Cl_2) was added to a solution of salicylaldehyde (30.6 g, 251 mmol) in CH_2Cl_2 (150 mL). The reaction mixture was stirred for 14 h; then saturated sodium sulfite (500 mL) was added until the color disappeared. The mixture was diluted with CH_2Cl_2, and the layers were separated. The organic layer was washed with water and saturated NaCl and then worked up. The crude material was recrystallized from cyclohexane to give 2-hydroxy-5-iodobenzaldehyde (31.3 g, 50%) as white solid: ^{1}H NMR ($CDCl_3$) δ 10.91 (s, 1H), 9.82 (s, 1H), 7.84 (d, 1H), 7.75 (dd, 1H), 6.75 (d, 1H); MS (EI) *m*/*z* 248 $(M)^+$.

Part B: Sodium hydride (1.2 g, 52 mmol, 60% mineral oil dispersion) was added to a solution of 2-hydroxy-5-iodobenzaldehyde (7.0 g, 28 mmol) in THF (25 mL) at 0 °C. After 5 min, 2-methoxyethoxymethoxy chloride (3.4 mL, 30 mmol) and 1,3-dimethyl-3,4,5,6-tetrahydro-2(1*H*)-pyrimidinone (4 mL) were added to the reaction mixture. The solution was allowed to warm to ambient temperature. After 45 min, the solution was cooled to −15 °C and sodium borohydride (6 mL, 2 M solution in THF) was added dropwise. The solution was stirred for 10 min; then 2 M HCl (24 mL) was added. The resulting solution was diluted with ether and washed with water and saturated NaCl. The organic layer was worked up and the crude material purified by column chromatography eluting with 40% EtOAc/hexanes to give 5-iodo-2-(2-methoxyethoxymethoxy)benzyl alcohol (7.6 g, 80%) as a white solid: ^{1}H NMR ($CDCl_3$) δ 7.64 (d, 1H), 7.52 (dd, 1H), 6.90 (d, 1H), 5.30 (s, 2H), 4.62 (d, 2H), 3.82 (m, 2H), 3.54 (m, 2H), 3.35 (s, 3H), 2.53 (t, 1H); MS (EI) *m*/*z* 338 $(M)^+$.

Part C: Triphenylphosphine (30.6 g, 117 mmol) and *N*-bromosuccinimide (20.8 g, 117 mmol) were added to a solution of 5-iodo-2-(2-methoxyethoxymethoxy)benzyl alcohol (36.2 g, 107 mmol) in THF (290 mL) at 5 °C. The solution was stirred for 10 min then allowed to warm to ambient temperature. After 20 min, the solution was concentrated in vacuo and the crude material purified by column chromatography eluting with EtOAc:CH_2Cl_2:hexane (3:1:6) to give 5-iodo-2-(2-methoxyethoxymethoxy)benzyl bromide (**16**) (29.6 g, 69%) as an oil: ^{1}H NMR ($CDCl_3$) δ 7.60 (d, 1H), 7.50 (dd, 1H), 6.91 (s, 1H), 5.30 (s, 2H), 4.43 (dd, 1h), 3.84 (m, 2H), 3.53 (m, 2H), 3.35 (s, 3H); MS (EI) *m*/*z* 400 $(M)^+$.

Part D: 3-(*S*)-(*tert*-Butoxycarbonylamino)-1-(5-iodo-2-(2-methoxyethoxymethoxy)benzyl)pyrrolidin-2-one (**17**) was prepared as described in example **4a**, part D, substituting 5-iodo-2-(2-methoxyethoxymethoxy)benzyl bromide (**16**) for 4-(benzhydrylidenylamino)-3-(bromomethyl)benzonitrile. The product was purified by column chromatography eluting with EtOAc:CH_2Cl_2:hexane (3:1:1): ^{1}H NMR ($CDCl_3$) δ 7.53 (dd, 1H), 7.45 (d, 1H), 6.93 (d, 1H),. 5.26 (s, 2H), 5.20 (bs, 1H), 4.50 (d, 1H), 4.45 (d, 1H), 4.19 (m, 1H), 3.80 (m, 2H), 3.53 (m, 2H), 3.37 (s, 3H), 3.22 (m, 2H), 2.62 (m, 1H), 1.84 (m, 1H), 1.46 (s, 9H); MS (ESI) *m*/*z* 521 $(M + H)^+$.

Part E: Zinc cyanide (16.6 g, 141 mmol) and tetrakis-(triphenylphosphine)palladium(0) (3.93 g, 3.40 mmol) were added to a solution of 3-(*S*)-(*tert*-butoxycarbonylamino)-1-(5-iodo-2-(2-methoxyethoxymethoxy)benzyl)pyrrolidin-2-one (**17**) (25.3 g, 48.6 mmol) in DMF (150 mL). The solution was heated to 80 °C for 4 h, then cooled to room temperature, and diluted with EtOAc. The resulting solution was washed with 1 N NH_4OH, water, and saturated NaCl. The organic layer was worked up and the crude product purified by column chromatography eluting with EtOAc:CH_2Cl_2:hexane (3:1:1) to give 3-(*S*)-(*tert*-butoxycarbonylamino)-1-(5-cyano-2-(2-methoxyethoxymethoxy-)benzyl)pyrrolidin-2-one (18.8 g, 92%) as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 7.54 (dd, 1H), 7.45 (d, 1H), 7.20 (d, 1H), 5.35 (s, 2H), 5.20 (bs, 1H), 4.50 (d, 1H),1.45 (s, 9H), 4.45 (d, 1H), 4.15 (m, 1H), 3.77 (m, 2H), 3.51 (m, 2H), 3.35 (s, 3H), 3.24 (m, 2H), 2.60 (m, 1H), 1.90 (m, 1H); MS (EI) *m*/*z* 420 $(M)^+$.

Part F: Hydrogen chloride gas was bubbled through EtOAc (340 mL) at 0 °C for 10 min; then a solution of 3-(*S*)-(*tert*-butoxycarbonylamino)-1-(5-cyano-2-(2-methoxyethoxymethoxy-)benzyl)pyrrolidin-2-one (18.8 g, 44.9 mmol) in EtOAc (160 mL) was added dropwise via cannula. The mixture was stirred for 30 min during which time a precipitate formed. The reaction mixture was diluted with diethyl ether and then quickly filtered to yield 3-(*S*)-[(3-amino-2-oxopyrrolidin-1-yl)methyl]-4-hydroxybenzonitrile hydrochloride (**18**) (10.6 g, 88%) as a white hygroscopic solid: ^{1}H NMR (DMSO-d_6) δ 8.50 (bs, 3H), 7.50 (dd, 1H), 7.40 (d, 1H), 7.00 (d, 1H), 4.26 (d, 2H), 4.00 (m, 1H), 3.25 (m, 2H), 2.30 (m, 1H), 1.90 (m, 1H); MS (EI) *m*/*z* 231 $(M)^+$.

Part G: 5-Pyridin-3-ylthiophene-2-sulfonyl chloride (3.38 g, 13.0 mmol) and 3-(*S*)-[(3-amino-2-oxopyrrolidin-1-yl)methyl]-

4-hydroxybenzonitrile hydrochloride (**18**) (3.47 g, 13.0 mmol) were stirred in pyridine (70 mL) at ambient temperature overnight. The reaction mixture was concentrated in vacuo and then purified by chromatography eluting with 10% MeOH/CH_2Cl_2 to afford 5-pyridin-3-ylthiophene-2-sulfonic acid {1-[5-cyano-2-hydroxybenzyl]-2-oxopyrrolidin-3-(*S*)-yl}amide (3.6 g, 61%) as a foamy white solid: ^{1}H NMR ($CDCl_3$) δ 8.82 (bs, 1H), 8.61 (bs, 1H), 7.85 (d, 1H), 7.65 (d, 1H), 7.63 (d, 1H), 7.55 (s, 1H), 7.53 (dd, 1H), 7.41 (d, 1H), 7.31 (m, 1H), 7.28 (d, 1H), 6.91 (d, 1H), 4.41 (s, 2H), 4.12 (m, 1H), 3.35 (m, 2H), 2.55 (m, 1H), 2.09 (m, 1H); MS (FAB) *m*/*z* 455 $(M + H)^+$.

7-Methoxynaphthalene-2-sulfonic Acid {1-[5-Cyano-2-hydroxybenzyl]-2-oxopyrrolidin-3-(*S*)-yl}amide. MS (FAB) *m*/*z* 452 $(M + H)^+$.

5-Isoxazol-3-ylthiophene-2-sulfonic Acid [1-(5-Cyano-2-hydroxybenzyl)-2-oxopyrrolidin-3-(*S*)-yl]amide. ^{1}H NMR ($CDCl_3$) δ 9.37 (bs, 1H), 8.35 (d, 2H), 7.50 (m, 2H), 7.41 (d, 1H), 7.00 (d, 1H), 6.57 (d, 1H), 5.48 (bs, 1H), 4.35 (AB, 2H), 4.10 (m, 1H), 3.50 (m, 2H), 2.70 (m, 1H), 2.20 (m, 1H).

Part H: 5-Pyridin-3-ylthiophene-2-sulfonic acid {1-[5-cyano-2-hydroxybenzyl]-2-oxopyrrolidin-3-(*S*)-yl}amide was treated in the same manner as described in example **4a**, part F, to afford 4-hydroxy-3-[2-oxo-3-(*S*)-(5-pyridin-3-ylthiophene-2-ylsulfonylamino)pyrrolidin-1-ylmethyl]benzamidine trifluoroacetate (**20b**) as an amorphous white solid: ^{1}H NMR (DMSO-d_6) δ 10.91 (s, 1H), 9.06 (s, 2H), 8.98 (s, 1H), 8.67 (s, 2H), 8.61 (d, 1H), 8.55 (d, 1H), 8.16 (d, 1H), 7.71 (m, 2H), 7.60 (d, 1H), 7.51 (m, 1H), 7.42 (s, 1H), 7.00 (d, 1H), 4.35 (AB, 2H), 4.21 (m, 1H), 3.21 (m, 2H), 2.20 (m, 1H), 1.71 (m, 1H); MS (FAB) *m*/*z* 472 $(M + H)^+$. Anal. ($C_{21}H_{21}N_5O_4S\cdot 2TFA\cdot 1.4H_2O$) C, H, N.

4-Hydroxy-3-[3-(*S*)-(7-methoxynaphthalen-2-ylsulfonylamino)-2-oxopyrrolidin-1-ylmethyl]benzamidine (20a). 7-Methoxynaphthalene-2-sulfonic acid {1-[5-cyano-2-hydroxybenzyl]-2-oxopyrrolidin-3-(*S*)-yl}amide was treated in the same manner as described in example **4a**, part F, except the crude material was purified by chromatography eluting with 1% Et_3N/15% MeOH in CH_2Cl_2 to afford the title product as an amorphous solid: ^{1}H NMR (DMSO-d_6) δ 8.36 (bs, 1H), 8.0 (d, 1H), 7.93 (d, 1H), 7.72 (m, 1H), 7.53 (bs, 1H), 7.40 (m, 1H), 7.32 (m, 1H), 7.19 (bs, 1H), 6.22 (d, 1H), 4.20 (d, 1H), 4.12 (d, 1H), 4.10 (t, 1H), 3.90 (s, 3H), 3.12 (m, 2H), 1.94 (m, 1H), 1.55 (m, 1H); MS (FAB) *m*/*z* 469 $(M + H)^+$. Anal. ($C_{23}H_{24}N_4O_5S\cdot 0.75H_2O$) C, H, N.

4-Hydroxy-3-[3-(*S*)-(5-isoxazol-3-ylthiophene-2-ylsulfonylamino)-2-oxopyrrolidin-1-ylmethyl]benzamidine Trifluoroacetate (20c). ^{1}H NMR (DMSO-d_6) δ 9.04 (bs, 2H), 8.87 (bs, 2H), 8.73 (d, 1H), 8.67 (d, 1H), 7.74 (m, 2H), 7.61 (dd, 1H), 7.42 (d, 1H), 7.09 (d, 1H), 6.97 (d, 1H), 4.33 (AB, 2H), 4.23 (m, 1H), 3.21 (m, 2H), 2.21 (m, 1H), 1.75 (m, 1H); MS (ESI) *m*/*z* 462 $(M + H)^+$. Anal. ($C_{19}H_{19}N_5O_5S_2\cdot TFA\cdot 0.875H_2O$) C, H, N.

Preparation of 2-Hydroxy-5-[3-(*S*)-(7-methoxynaphthalen-2-ylsulfonylamino)-2-oxopyrrolidin-1-ylmethyl]benzamidine (21). Part A: *p*-Hydroxybenzaldehyde was treated in the same manner as described in example **20b**, part A, to give 4-hydroxy-3-iodobenzaldehyde: ^{1}H NMR ($CDCl_3$) δ 9.82 (s, 1H), 8.24 (s, 1H), 7.80 (d, 1H), 7.12 (d, 1H), 5.95 (s, 1H); MS (EI) *m*/*z* 248 $(M)^+$.

Part B: 4-Hydroxy-3-iodobenzaldehyde was treated in the same manner as described in example **20b**, part B, to give 3-iodo-4-(2-methoxyethoxymethoxy)benzyl alcohol: ^{1}H NMR ($CDCl_3$) δ 7.78 (s, 1H),7.25 (dd, 1H), 7.10 (d, 1H), 5.31 (s, 2H), 4.59 (d, 2H), 3.88 (t, 2H), 3.55 (t, 2H), 3.36 (s, 3H); MS (EI) *m*/*z* 338 $(M)^+$.

Part C: 3-Iodo-4-(2-methoxyethoxymethoxy)benzyl alcohol was treated in the same manner as described in example **20b**, part C, to give 3-iodo-4-(2-methoxyethoxymethoxy)benzyl bromide: ^{1}H NMR ($CDCl_3$) δ 7.80 (s, 1H), 7.30 (d, 1H), 7.05 (d, 1H), 5.32 (s, 2H), 4.40 (s, 2H), 3.85 (t, 2H), 3.55 (t, 2H), 3.37 (s, 3H); MS (EI) *m*/*z* 400 $(M)^+$.

Part D: 3-Iodo-4-(2-methoxyethoxymethoxy)benzyl bromide was treated in the same manner as described in example **20b**, part D, to give 3-(*S*)-(*tert*-butoxycarbonylamino)-1-(3-iodo-4-(2-methoxyethoxymethoxy)benzyl)pyrrolidin-2-one: ^{1}H NMR ($CDCl_3$) δ 7.62 (s, 1H), 7.14 (dd, 1H), 7.05 (d, 1H), 5.31 (s, 2H), 5.13 (s, 1H), 4.35 (AB, 2H), 4.18 (m, 1H), 3.85 (t, 2H), 3.55 (t, 2H), 3.35 (s, 3H), 3.18 (m, 2H), 2.60 (m, 1H), 1.85 (m, 1H), 1.45 (s, 9H); MS (EI) *m*/*z* 520 $(M)^+$.

Part E: 3-(*S*)-(*tert*-Butoxycarbonylamino)-1-(3-iodo-4-(2-methoxyethoxymethoxy)benzyl)pyrrolidin-2-one was treated in the same manner as described in example **20b**, part E, to give 3-(*S*)-(*tert*-butoxycarbonylamino)-1-(3-cyano-4-(2-methoxyethoxymethoxy)benzyl)pyrrolidin-2-one: ^{1}H NMR ($CDCl_3$) δ 7.42 (d, 1H), 7.40 (dd, 1 H), 7.23 (s, 1H), 5.38 (s, 2H), 5.13 (, 1H), 4.40 (AB, 2H), 4.17 (m, 1 H), 3.88 (t, 2H), 3.55 (t, 2H), 3.38 (s, 3H), 3.20 (m, 2H), 2.60 (m, 1H), 1.91 (m, 1H) 1.45 (s, 9H); MS (FAB) *m*/*z* 420 $(M + H)^+$.

Part F: 3-(*S*)-(*tert*-Butoxycarbonylamino)-1-(3-cyano-4-(2-methoxy)ethoxymethoxy)benzyl)pyrrolidin-2-one was treated in the same manner as described in example **20b**, part F, to give 5-[(3-(*S*)-amino-2-oxopyrrolidin-1-yl)methyl]-2-hydroxybenzonitrile hydrochloride: ^{1}H NMR (CD_3OD) δ 7.45 (d, 1H), 7.40 (dd, 1H), 4.40 (AB, 2H), 4.10 (m, 1 H), 3.35 (m, 2H), 2.55 (m, 1H), 1.95 (m, 1H).

Part G: 5-[(3-(*S*)-Amino-2-oxopyrrolidin-1-yl)methyl]-2-hydroxybenzonitrile hydrochloride was treated in the same manner as described in example **20b**, part G, to give 7-methoxynaphthalene-2-sulfonic acid [1-(3-cyano-4-hydroxybenzyl)-2-oxopyrrolidin-3-(*S*)-yl]amide: ^{1}H NMR (CD_3OD) δ 8.40 (s, 1H), 7.93 (d, 1H), 7.85 (d, 1H), 7.73 (dd, 1H), 7.40 (dd, 2H), 7.30 (m, 2H), 6.90 (d, 1H), 4.30 (AB, 2H), 4.10 (m, 1H), 3.91 (s, 3H), 3.10 (m, 2H), 2.15 (m, 1H), 1.65 (m, 1H); MS (FAB) *m*/*z* 452 $(M + H)^+$.

Part H: 7-Methoxynaphthalene-2-sulfonic acid [1-(3-cyano-4-hydroxybenzyl)-2-oxopyrrolidin-3-(*S*)-yl]amide was treated in the same manner as described in example **20b**, part H, to give 2-hydroxy-5-[3-(7-methoxynaphthalen-1-ylsulfonylamino)-2-oxopyrrolidin-1-yl]benzamidine, trifluoroacetic acid (**21**): ^{1}H NMR (CD_3OD) δ 8.4 (s, 1H), 7.95 (d, 1H), 7.88 (d, 1H), 7.75 (dd, 1H), 7.45 (d, 1H), 7.40 (m, 1H), 7.30 (dd, 1H), 6.98 (d, 1H), 4.35 (d, 2H), 4.15 (t, 1H), 3.95 (s, 3H), 3.20 (m, 2H), 2.20 (m, 1H), 1.70 (m, 1H); MS (FAB) *m*/*z* 469 $(M + H)^+$; HRMS (ESI) calcd for $C_{23}H_{24}N_4O_5S$ 469.1545, found 469.1531 $(M + H)^+$; HPLC analysis (C18, 20-min linear gradient; elution 10−100% CH_3CN/0.1% TFA H_2O; flow rate 1.0 mL/min) indicated that the product was 99A%.

Preparation of {4-(Aminoiminomethyl)-2-[3-(*S*)-(7-methoxynaphthalen-2-ylsulfonylamino)-2-oxopyrrolidin-1-ylmethyl]phenoxy}acetic Acid Methyl Ester Trifluoroacetate (27). Part A: *tert*-Butyllithium (31.1 mL, 52.9 mmol, 1.7 M solution in pentane) was added dropwise to a solution of 4-bromo-3-methylbenzonitrile (5.18 g, 26.4 mmol) in THF (165 mL) at −78 °C. After 5 min, $CuBr\cdot SMe_2$ (11.42 g, 55.5 mmol) was added. The resulting solution was stirred for 10 min; then O_2 was slowly and **carefully** bubbled through the reaction mixture for 30 min. After this time, the solution was allowed to warm to ambient temperature and the solution was stirred for 16 h. The reaction mixture was then poured into cold H_2O (75 mL) and diluted with EtOAc. The organic layer was washed with saturated NH_4SO_4 (3 × 75 mL) and then extracted with 10 N NaOH (2 × 30 mL). The basic aqueous layers were acidified to pH 6 with 6 N HCl and then extracted with EtOAc. The combined organic layers were worked up to yield 4-hydroxy-3-methylbenzonitrile (**22**) (1.27 g, 36%) as a white solid which was used in the subsequent step without further purification: ^{1}H NMR ($CDCl_3$) δ 9.00 (s, 1H), 7.45 (s, 1H), 7.40 (d, 1H), 6.80 (d, 1H), 2.26 (s, 3H); MS (EI) *m*/*z* 133 $(M)^+$.

Part B: Methyl bromoacetate (0.56 mL, 5.92 mmol) was added to a solution of 4-hydroxy-3-methylbenzonitrile (**22**) (0.70 g, 5.26 mmol), K_2CO_3 (1.61 g, 11.6 mmol), and tetrabutylammonium iodide (0.57 g, 1.53 mmol) in DMF (30 mL). The resulting mixture was heated to 80 °C for 16 h and then cooled to ambient temperature. The reaction mixture was diluted with EtOAc and washed with H_2O and saturated NaCl. The organic layer was worked up and the crude material purified by column chromatography eluting with a gradient of 10% EtOAc/hexanes to 50% EtOAc/hexanes to afford (4-cyano-2-

methylphenoxy)acetic acid methyl ester (**23**) (0.82 g, 76%): ^{1}H NMR ($CDCl_3$) δ 7.40−7.48 (m, 2H), 6.70 (d, 1H), 4.68 (s, 2H), 3.80 (s, 3H), 2.25 (s, 3H); MS (EI) *m*/*z* 205 $(M)^+$.

Part C: (4-Cyano-2-methylphenoxy)acetic acid methyl ester (**23**) was treated in the same manner as described in example **4a**, part C, to give (2-bromomethyl-4-cyanophenoxy)acetic acid methyl ester (**24**) as a white solid: ^{1}H NMR ($CDCl_3$) δ 7.65 (d, 1H), 7.55 (dd, 1H), 6.80 (d, 1H), 4.80 (s, 2H), 4.55 (s, 2H), 3.80 (s, 3H); MS (EI) *m*/*z* 283, 285 $(M)^+$.

Part D: (2-Bromomethyl-4-cyanophenoxy)acetic acid methyl ester (**24**) was treated in the same manner as described in example **4a**, part D, to give [2-(3-*tert*-butoxycarbonylamino-2-oxopyrrolidin-1-ylmethyl)-4-cyanophenoxy]acetic acid methyl ester (**25**) as a white solid: ^{1}H NMR ($CDCl_3$) δ 7.50−7.58 (m, 2H), 6.78 (d, 1H), 5.10 (bs, 1H), 4.70 (s, 2H), 4.55 (AB, 2H), 4.15 (m, 1H), 3.80 (s, 3H), 3.20 (m, 2H), 2.60 (s, 2H), 1.90 (m, 1H), 1.58 (s, 9H).

Part E: [2-(3-*tert*-Butoxycarbonylamino-2-oxopyrrolidin-1-ylmethyl)-4-cyanophenoxy]acetic acid methyl ester (**25**) was treated in the same manner as described in example **4a**, part E, to give {4-cyano-2-[3-(7-methoxynaphthalen-2-ylsulfonylamino)-2-oxopyrrolidin-1-ylmethyl]phenoxy}acetic acid methyl ester (**26**) as a foamy white solid: ^{1}H NMR ($CDCl_3$) δ 8.35 (s, 1H), 7.90 (d, 1H), 7.75 (dd, 1H), 7.55 (dd, 1H), 7.42 (d, 1H), 7.30 (dd, 1H), 7.20 (m, 1H), 6.70 (d, 1H), 5.40 (d, 1H), 4.65 (s, 2H), 3.95 (s, 3H), 3.70 (m, 1H), 3.20 (m, 2H), 2.50 (m, 1H), 2.05 (m, 1H); MS (FAB) *m*/*z* 524 $(M + H)^+$.

Part F: Hydrogen sulfide gas was bubbled for 5 min through a solution of {4-cyano-2-[3-(7-methoxynaphthalen-2-ylsulfonylamino)-2-oxopyrrolidin-1-ylmethyl]phenoxy}acetic acid methyl ester (**26**) (0.12 g, 0.23 mmol) in pyridine/TEA (5.4 mL, 10:1 v/v) at room temperature. After stirring the pale green solution for a period of 18 h, the reaction mixture was concentrated in vacuo. The residue was diluted with EtOAc and washed with 0.5 N HCl and saturated NaCl. The organic layer was worked up to give the crude thioamide. Methyl iodide (0.56 mL, 9.00 mmol) was added to a solution of the thioamide in acetone (9 mL) and then heated to reflux for 2 h. The reaction mixture was cooled to room temperature and concentrated in vacuo to provide the crude thioimidate hydroiodide. The thioimidate hydroiodide was dissolved in MeOH (6 mL), and NH_4OAc (0.058 g, 0.75 mmol) was added. The resulting mixture was heated at reflux for 4 h, allowed to cool to room temperature, and stirred overnight. The reaction mixture was concentrated in vacuo and the crude product purified by reverse-phase HPLC. Lyophilization of the aqueous fractions containing the desired product yielded {4-(aminoiminomethyl)-2-[3-(*S*)-(7-methoxynaphthalen-2-ylsulfonylamino)-2-oxopyrrolidin-1-ylmethyl]phenoxy}acetic acid methyl ester trifluoroacetate (**27**) (0.030 g, 21%) as a white solid: ^{1}H NMR (DMSO-d_6) δ 9.00 (bs, 4H), 8.30 (s, 1H), 7.97 (d, 1H), 7.90 (d, 1H), 7.65 (m, 2H), 7.50 (s, 1H), 7.37 (s, 1H), 7.25 (dd, 1H), 7.10 (d, 1H), 4.95 (AB, 2H), 4.30 (AB, 2H), 4.05 (m, 1H), 3.80 (s, 3H), 3.60 (s, 3H), 3.15 (m, 2H), 1.95 (m, 1H), 1.55 (m, 1H); MS (FAB) *m*/*z* 541 $(M + H)^+$. Anal. ($C_{26}H_{28}N_4O_7S$·TFA·3.40H_2O) C, H, N.

Preparation of {4-(Aminoiminomethyl)-2-[3-(*S*)-(7-methoxynaphthalen-2-ylsulfonylamino)-2-oxopyrrolidin-1-ylmethyl]phenoxy}acetic Acid Trifluoroacetate (28). 10 N NaOH (0.05 mL) was added to a solution of {4-(aminoiminomethyl)-2-[3-(*S*)-(7-methoxynaphthalen-2-ylsulfonylamino)-2-oxopyrrolidin-1-ylmethyl]phenoxy}acetic acid methyl ester trifluoroacetate (**27**) (0.1 g, 0.18 mmol) in EtOH (2 mL). The reaction mixture was stirred for 5 h and then concentrated in vacuo. The residue was dissolved in H_2O (2 mL), and the pH was adjusted to 3 using 1 N HCl. The solid which formed was collected by filtration and purified by reverse-phase HPLC. The appropriate fractions were lyophilized to afford the title compound **28** (0.05 g, 44%) as a white solid: ^{1}H NMR (DMSO-d_6) δ 9.10 (bs, 2H), 8.70 (bs, 2H), 8.35 (s, 1H), 8.15 (d, 1H), 8.00 (d, 1H), 7.90 (d, 1H), 7.70 (m, 2H), 7.50 (s, 1H), 7.45 (s, 1H), 7.30 (m, 1H), 7.10 (m, 1H), 4.85 (s, 1H), 4.30 (AB, 2H), 4.05 (m, 1H), 3.80 (s, 3H), 3.10 (m, 2H), 1.95 (m, 1H), 1.55 (m, 1H); MS (FAB) *m*/*z* 527 $(M + H)^+$. Anal. ($C_{25}H_{26}N_4O_7S$·2.0TFA) C, H, N.

Preparation of Biphenyl-4-sulfonic Acid {1-[3-(Aminoiminomethyl)benzyl]-2-oxopyrrolidin-3-(*S*)-yl}amide Trifluoroacetate (3c). Representative Procedure for the Synthesis of Compounds 3d−3f, 3i−3l, 3n. Part A: Biphenyl-4-sulfonyl chloride (0.28 g, 1.09 mmol) was added to a solution of triethylamine (0.42 mL, 3.0 mml) and 3-(3-(*S*)-amino-2-oxopyrrolidin-1-ylmethyl)benzonitrile hydrochloride[9] (0.25 g, 1.0 mmol) in CH_3CN (10 mL). After 4 h, the reaction mixture was concentrated in vacuo, diluted with EtOAc, and then washed with saturated $NaHCO_3$ and saturated NaCl. The organic layer was worked up and purified by chromatography eluting with 50% EtOAc/CH_2Cl_2 to give biphenyl-4-sulfonic acid [1-(3-cyanobenzyl)-2-oxopyrrolidin-3-(*S*)-yl]amide (0.30 g, 70%): ^{1}H NMR ($CDCl_3$) δ 8.14 (s, 1H), 7.95 (d, 1H), 7.82 (d, 1H), 7.64 (m, 5H), 7.47 (m, 6H), 5.42 (bs, 1H), 4.42 (AB, 2H), 3.82 (m, 1H), 3.22 (m, 1H), 2.62 (m, 1H), 2.13 (m, 1H).

4-Pyridin-2-ylbenzenesulfonic Acid [1-(3-Cyanobenzyl)-2-oxopyrrolidin-3-(*S*)-yl]amide. ^{1}H NMR (CD_3OD) δ 8.73 (bs, 2H), 8.16 (d, 2H), 8.02 (d, 1H), 7.80 (m, 2H), 7.57 (d, 1H), 7.46 (m, 3H), 7.32 (m, 1H), 7.26 (s, 2H), 5.42 (bs, 2H), 4.55 (s, 1H), 4.23 (t, 1H), 3.20 (m, 2H), 2.33 (m, 1H), 1.87 (m, 1H); MS (FAB) *m*/*z* 433 $(M + H)^+$.

4-Pyridin-3-ylbenzenesulfonic Acid [1-(3-Cyanobenzyl)-2-oxopyrrolidin-3-(*S*)-yl]amide. ^{1}H NMR (CD_3OD) δ 8.85 (bs, 2H), 8.57 (bs, 2H), 8.16 (d, 1H), 7.94 (AB, 4H), 7.46−7.65 (m, 5H), 4.44 (AB, 2H), 4.23 (t, 1H), 3.20 (m, 2H), 2.33 (m, 1H), 1.87 (m, 1H); MS (FAB) *m*/*z* 433 $(M + H)^+$.

4-Pyridin-4-ylbenzenesulfonic Acid [1-(3-Cyanobenzyl)-2-oxopyrrolidin-3-(*S*)-yl]amide. ^{1}H NMR ($CDCl_3$) δ 8.78 (m, 2H), 8.11 (d, 2H), 7.66 (m, 2H), 7.47−7.58 (m, 5H), 5.48 (s, 1H), 4.50 (AB, 2H), 3.88 (t, 1H), 3.29 (dd, 2H), 2.58 (m, 1H), 2.17 (m, 1H); MS (FAB) *m*/*z* 433 $(M + H)^+$.

(1-Methyl-1*H*-imidazol-2-yl)benzene-4-sulfonic Acid [1-(3-Cyanobenzyl)-2-oxopyrrolidin-3-(*S*)-yl]amide. ^{1}H NMR ($CDCl_3$) δ 7.60 (m, 3H), 7.45 (m, 5H), 7.15 (s, 1H), 6.98 (s, 1H), 4.48 (AB, 2H), 3.95 (s, 3H), 3.75 (m, 1H), 3.20 (m, 2H), 2.60 (m, 1H), 2.00 (m, 1H); MS (FAB) *m*/*z* 436 $(M + H)^+$.

5-Pyrid-2-ylthiophene-2-sulfonic Acid [1-(3-Cyanobenzyl)-2-oxopyrrolidin-3-(*S*)-yl]amide. ^{1}H NMR ($CDCl_3$) δ 8.62 (m, 1H), 7.78 (m, 1H), 7.69 (m, 1H), 7.58 (m, 2H), 7.50 (d, 1H), 7.46 (m, 2H), 7.20 (m, 2H), 5.43 (bs, 1H), 4.42 (AB, 2H), 3.98 (m, 1H), 3.26 (m, 2H), 2.68 (m, 1H), 2.15 (m, 1H); MS (FAB) *m*/*z* 439 $(M + H)^+$.

5-Pyridin-3-ylthiophene-2-sulfonic Acid [1-(3-Cyanobenzyl)-2-oxopyrrolidin-3-(*S*)-yl]amide. ^{1}H NMR ($CDCl_3$) δ 8.90 (d, 1H), 8.63 (d, 1H), 7.85 (dd, 1H), 7.68 (d, 1H), 7.61 (m, 1H), 7.41−7.45 (m, 3H), 7.34 (dd, 1H), 7.31 (d, 1H), 5.45 (s, 1H), 4.50 (s, 2H), 3.95 (m, 1H), 3.25 (m, 2H), 2.68 (m, 1H), 2.15 (m, 1H); MS (FAB) *m*/*z* 439 $(M + H)^+$.

5-Pyridin-4-ylthiophene-2-sulfonic Acid [1-(3-Cyanobenzyl)-2-oxopyrrolidin-3-(*S*)-yl]amide. ^{1}H NMR ($CDCl_3$) δ 8.70 (d, 2H), 7.68 (d, 1H), 7.60 (m, 1H), 7.40−7.50 (m, 6H), 5.65 (bs, 1H), 4.48 (AB, 2H), 3.98 (m, 1H), 3.28 (m, 2H), 2.68 (m, 1H), 2.17 (m, 1H); MS (FAB) *m*/*z* 439 $(M + H)^+$.

5-Isoxazol-3-ylthiophene-2-sulfonic Acid [1-(3-Cyanobenzyl)-2-oxopyrrolidin-3-(*S*)-yl]amide. ^{1}H NMR ($CDCl_3$) δ 8.36 (d, 1H), 7.70 (d, 1H), 7.61 (m, 1H), 7.49 (m, 4H), 6.57 (d, 1H), 5.55 (bs, 1H), 4.51 (s, 2H), 3.97 (m, 1H), 3.29 (m, 2H), 2.69 (m, 1H), 2.17 (m, 1H).

Part B: Biphenyl-4-sulfonic acid [1-(3-cyanobenzyl)-2-oxopyrrolidin-3-(*S*)-yl]amide was treated in the same manner as described in example **4a**, part F, to give biphenyl-4-sulfonic acid {1-[3-(aminoiminomethyl)benzyl]-2-oxopyrrolidin-3-(*S*)-yl}amide trifluoroacetate **3c** as a white solid: ^{1}H NMR (DMSO-d_6) δ 9.31 (bs, 2H), 9.14 (bs, 2H), 8.22 (d, 1H), 7.91 (m, 6H), 7.60 (m, 8H), 4.45 (AB, 2H), 4.16 (m, 1H), 3.12 (m, 1H), 2.07 (m, 1H), 1.65 (m, 1H); MS (FAB) *m*/*z* 449 $(M + H)^+$. Anal. ($C_{24}H_{24}N_4O_3S$·TFA·0.25H_2O) C, H, N.

4-Pyridin-2-ylbenzenesulfonic Acid {1-[3-(Aminoiminomethyl)benzyl]-2-oxopyrrolidin-3-(*S*)-yl}amide Bistrifluoroacetate (3d). ^{1}H NMR (CD_3OD) δ 8.72 (d, 2H), 8.15

(d, 2H), 7.97−8.06 (m, 4H), 7.55−7.83 (m, 4H), 7.50 (m, 1H), 4.54 (AB, 2H), 4.21 (t, 1H), 3.28 (m, 2H), 2.30 (m, 1H), 1.80 (m, 1H); MS (FAB) *m/z* 450 (M + H)$^+$. Anal. ($C_{23}H_{23}N_5O_3S$·2TFA·H_2O) C, H, N.

4-Pyridin-3-ylbenzene Sulfonic Acid {1-[3-(Aminoiminomethyl)benzyl]-2-oxopyrrolidin-3-(*S*)-yl}amide Bistrifluoroacetate (3e). ^{1}H NMR (DMSO-d_6) δ 9.27 (bs, 2H), 9.05 (bs, 2H), 8.23 (m, 2H), 7.93 (m, 4H), 7.62 (m, 2H), 7.51 (m, 3H), 4.40 (m, 2H), 4.15 (m, 1H), 3.10 (m, 2H), 2.05 (m, 1H), 1.60 (m, 1H); MS (FAB) *m/z* 450 (M + H)$^+$; HRMS (ESI) calcd for $C_{23}H_{23}N_5O_3S$ 450.1600, found 450.1611 (M + H)$^+$; HPLC analysis (C18, 15-min linear gradient; elution 10−80% CH_3-CN/0.1% TFA H_2O; flow rate 1.0 mL/min) indicated that the product was >97A%.

4-Pyridin-4-ylbenzenesulfonic Acid {1-[3-(Aminoiminomethyl)benzyl]-2-oxopyrrolidin-3-(*S*)-yl}amide Bistrifluoroacetate (3f). ^{1}H NMR (DMSO-d_6) δ 9.28 bs, 2H), 9.13 (bs, 2H), 8.80(bs, 1H), 8.33 (m, 1H), 7.97 (m, 5H), 7.62 (m, 1H), 7.51 (m, 3H), 4.40 (m, 2H), 4.15 (m, 1H), 3.10 (m, 2H), 2.05 (m, 1H), 1.60 (m, 1H); MS (FAB) *m/z* 450 (M + H)$^+$. Anal. ($C_{23}H_{23}N_5O_3S$·2TFA) C, H, N.

(1-Methyl-1H-imidazol-2-yl)benzene-4-sulfonic Acid {1-[3-(Aminoiminomethyl)benzyl]-2-oxopyrrolidin-3-(*S*)-yl}amide Trifluoroacetate (3i). ^{1}H NMR (DMSO-d_6) δ 9.30 (bs, 2H), 8.89 (bs, 2H), 8.70 (d, 1H), 7.90 (m, 1H), 7.69 (m, 4H), 7.55 (m, 5H), 4.45 (s, 2H), 4.10 (m, 1H), 3.90 (s, 3H), 3.20 (m, 2H), 2.20 (m, 1H), 1.80 (m, 1H). MS (FAB) *m/z* 453 (M + H)$^+$. Anal. ($C_{22}H_{24}N_6O_3S$·2TFA·0.8H_2O) C, H, N.

5-Pyrid-2-ylthiophene-2-sulfonic Acid {1-[3-(Aminoiminomethyl)benzyl]-2-oxopyrrolidin-3-(*S*)-yl}amide trifluoroacetate (3j). ^{1}H NMR (DMSO-d_6) δ 9.32 (bs, 2H), 9.13 (bs, 2H), 8.56 (d, 1H), 8.49 (d, 1H), 8.04 (d, 1H), 7.89 (m, 3H), 7.58 (m, 4H), 7.38 (m, 1H), 4.46 (AB, 2H), 4.23 (m, 1H), 3.16 (m, 2H), 2.16 (m, 1H), 1.70 (m, 1H); MS (FAB) *m/z* 456 (M + H)$^+$. Anal. ($C_{21}H_{21}N_5O_3S_2$·2TFA) C, H, N.

3-[2-Oxo-3-(*S*)-(5-pyridin-3-ylthiophene-2-ylsulfonylamino)pyrrolidin-1-ylmethyl]benzamidine Trifluoroacetate (3k). ^{1}H NMR (DMSO-d_6) δ 9.30 (s, 2H), 9.05 (s, 2H), 8.95 (s, 1H), 8.63 (d, 1H), 8.55 (d, 1H), 8.16 (d, 1H), 7.65−7.71 (m, 3H), 7.48−7.60 (m, 4H), 4.48 (AB, 2H), 4.28 (m, 1H), 3.15 (m, 2H), 2.20 (m, 1H), 1.71 (m, 1H); MS (FAB) *m/z* 456 (M + H)$^+$. Anal. ($C_{21}H_{21}N_5O_3S_2$·2TFA·0.8H_2O) C, H, N.

3-[2-Oxo-3-(*S*)-(5-pyridin-4-ylthiophene-2-ylsulfonylamino)pyrrolidin-1-ylmethyl]benzamidine Trifluoroacetate (3l). ^{1}H NMR (DMSO-d_6) δ 9.30 (s, 2H), 9.05 (s, 2H), 8.69 (d, 2H), 8.67 (d, 1H), 7.89 (d, 1H), 7.85 (d, 2H), 7.77 (d, 1H), 7.68 (m, 2H), 7.51−7.60 (m, 3H), 4.42 (AB, 2H), 4.26 (m, 1H), 3.18 (m, 2H), 2.21 (m, 1H), 1.70 (m, 1H); MS (FAB) *m/z* 456 (M + H)$^+$; HRMS (FAB) calcd for $C_{21}H_{21}N_5O_3S_2$ 456.1164, found 456.1205 (M + H)$^+$; HPLC analysis (C18, 15-min linear gradient; elution 10−80% CH_3CN/0.1% TFA H_2O; flow rate 1.0 mL/min) indicated that the product was 99A%.

3-[3-(*S*)-(5-Isoxazol-3-ylthiophene-2-ylsulfonylamino)-2-oxopyrrolidin-1-ylmethyl]benzamidine Trifluoroacetate (3n). ^{1}H NMR (DMSO-d_6) δ 9.31 (bs, 2H), 9.22 (bs, 2H), 8.74 (s, 1H), 8.70 (d, 1H), 7.75 (m, 3H), 7.57 (m, 3H), 7.10 (d, 1H), 4.44 (AB, 2H), 4.26 (m, 1H), 3.15 (m, 2H), 2.20 (m, 1H), 1.71 (m, 1H); MS (FAB) *m/z* 446 (M + H)$^+$. Anal. ($C_{19}H_{19}N_5O_4S_2$·1.5TFA) C, H, N.

Preparation of *N*-Methylpyrid-4-ylbenzene-4-sulfonic Acid {1-[3-(Aminoiminomethyl)benzyl]-2-oxopyrrolidin-3(S)-yl}amide Trifluoroacetate (3g). 4-Pyridin-4-ylbenzenesulfonic acid [1-(3-cyanobenzyl)-2-oxopyrrolidin-3-(*S*)-yl]-amide was treated as described in example **27**, part F: ^{1}H NMR (CD_3OD) δ 8.42,8.98 (AB, 4H), 8.16 (s, 4H) 7.56−7.73 (m, 3H), 7.59 (s, 1H), 4.50 (AB, 2H), 4.43 (s, 3H), 4.27 (t, 1H), 3.26 (m, 2H), 2.33 (m, 1H), 1.80 (m, 1H); MS (FAB) *m/z* 464 (M + H)$^+$; HRMS (ESI) calcd for $C_{24}H_{26}N_5O_3S$ 464.1756, found 464.1751 (M)$^+$. HPLC analysis (C18, 15-min linear gradient; elution 10−80% CH_3CN/0.1% TFA H_2O; flow rate 1.0 mL/min) indicated that the product was >97A%.

Preparation of 4-(1-Oxidopyridin-4-yl)benzene-4-sulfonic Acid {1-[3-(Aminoiminomethyl)benzyl]-2-oxopyrrolidin-3-(*S*)-yl}amide Trifluoroacetate (3h). Part A: 3-Chloroperoxybenzoic acid (0.017 g, 0.966 mmol) was added to a solution of 4-pyridin-4-ylbenzenesulfonic acid [1-(3-cyanobenzyl)-2-oxopyrrolidin-3-(*S*)-yl]amide (0.038 g, 0.088 mmol) in $CHCl_3$ (2 mL) at room temperature. The mixture was stirred for 18 h and then diluted with CH_2Cl_2. The organic layer was washed with saturated $NaHCO_3$ and saturated NaCl and then worked up to yield 4-(1-oxidopyridin-4-yl)benzenesulfonic acid [1-(3-cyanobenzyl)-2-oxopyrrolidin-3-(*S*)-yl]amide (0.039 g, 100%) which was used in the subsequent step without further purification: ^{1}H NMR ($CDCl_3$) δ 8.34 (d, 2H), 8.09 (d, 2H), 7.8 (d, 2H), 7.69 (m, 2H), 7.64 (m, 1H), 7.51 (d, 1H), 7.48 (s, 1H), 4.48 (ABq, 2H), 4.55 (s, 1H), 4.02 (t, 1H), 3.26 (m, 2H), 2.56 (m, 1H), 2.04 (m, 1H); MS (ESI) *m/z* 449 (M + H)$^+$.

Part B: 4-(1-Oxidopyridin-4-yl)benzenesulfonic acid [1-(3-cyanobenzyl)-2-oxopyrrolidin-3-(*S*)-yl]amide was treated as described in example **4a**, part F, to give the title compound **3h**: ^{1}H NMR (DMSO-d_6) δ 9.28 (bs, 2H), 8.98 (bs, 2H), 8.30 (m, 3H), 7.75−8.02 (m, 6H), 7.44−7.78 (m, 4H), 4.35 (m, 2H), 4.12 (m, 1H), 3.10 (m, 2H), 2.05 (m, 1H), 1.64 (m, 1H); HRMS (ESI) calcd for $C_{23}H_{23}N_5O_4S$ 466.1549, found 466.1526 (M + H)$^+$; HPLC analysis (C18, 15-min linear gradient; elution 10−80% CH_3CN/0.1% TFA H_2O; flow rate 1.0 mL/min) indicated that the product was >90A%.

Preparation of 3-{3-(*S*)-[5-(2-Methoxypyrimidin-4-yl)thiophene-2-ylsulfonylamino]-2-oxopyrrolidin-1-ylmethyl}benzamidine Trifluoroacetate (3m). Part A: [5-(2-Methylsulfanylpyrimidin-4-yl)thiophene-2-sulfonic acid [1-(3-cyanobenzyl)-2-oxopyrrolidin-3-(*S*)-yl]amide was prepared in a similar manner as described in example **3c**, Part A. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.55 (d, 1H), 7.70 (d, 1H), 7.65 (d, 1H), 7.60 (m, 1H), 7.50 (s, 1H), 7.46 (m, 2H), 7.23 (d, 1H), 5.84 (s, 1H), 4.48 (s, 2H), 3.97 (m, 1H), 3.28 (m, 2H), 2.66 (m, 1H), 2.60 (s, 3H), 2.15 (m, 1H).

Part B: 3-Chloroperoxybenzoic acid (75%, 1.05 g, 4.58 mmol) in $CHCl_3$ (50 mL) was added dropwise to a solution of [5-(2-methylsulfanylpyrimidin-4-yl)thiophene-2-sulfonic acid [1-(3-cyanobenzyl)-2-oxopyrrolidin-3-(*S*)-yl]amide (1.48 g, 3.05 mmol) in $CHCl_3$ (30 mL) at 0 °C. The resulting mixture was allowed to warm to room temperature and stirred for 16 h. The reaction mixture was diluted with CH_2Cl_2 and washed successively with saturated Na_2SO_3, 10% Na_2CO_3, and water. The organic layer was worked up to give a mixture of [5-(2-methylsulfinylpyrimidin-4-yl)thiophene-2-sulfonic acid [1-(3-cyanobenzyl)-2-oxopyrrolidin-3-(*S*)-yl]amide and [5-(2-methylsulfonylpyrimidin-4-yl)thiophene-2-sulfonic acid [1-(3-cyanobenzyl)-2-oxopyrrolidin-3-(*S*)-yl]amide (1.46 g, 2.82 mmol) as a solid which was used in the subsequent step without further purification: MS (FAB) *m/z* 502, 518 (M + H)$^+$.

Part C: A mixture of [5-(2-methylsulfinylpyrimidin-4-yl)-thiophene-2-sulfonic acid [1-(3-cyanobenzyl)-2-oxopyrrolidin-3-(*S*)-yl]amide and [5-(2-methylsulfonylpyrimidin-4-yl)thiophene-2-sulfonic acid [1-(3-cyanobenzyl)-2-oxopyrrolidin-3-(*S*)-yl]amide (0.19 g, 0.37 mmol) was dissolved in MeOH (10 mL) and CH_2-Cl_2 (1 mL). Ammonia gas was bubbled through the solution for 5 min; then the reaction mixture was heated to reflux for 4 h. After this time, the solution was concentrated in vacuo to give [5-(2-methoxypyrimidin-4-yl)thiophene-2-sulfonic acid [1-(3-cyanobenzyl)-2-oxopyrrolidin-3-(*S*)-yl]amide (0.17 g, 97%) as a solid: ^{1}H NMR (DMSO-d_6) δ 8.70 (d, 1H), 8.09 (d, 1H), 7.75 (m, 3H), 7.66 (s, 1H), 7.55 (m, 3H), 4.42 (AB, 2H), 4.27 (m, 1H), 3.95 (s, 3H), 3.15 (m, 2H), 2.17 (m, 1H), 1.68 (m, 1H).

Part D: [5-(2-Methoxypyrimidin-4-yl)thiophene-2-sulfonic acid [1-(3-cyanobenzyl)-2-oxopyrrolidin-3-(*S*)-yl]amide was treated in the same manner as described in example **4a**, part F, to give 3-{3-(*S*)-[5-(2-methoxypyrimidin-4-yl)thiophene-2-ylsulfonylamino]-2-oxopyrrolidin-1-ylmethyl}benzamidine trifluoroacetate (**3m**): ^{1}H NMR (DMSO-d_6) δ 9.29 (bs, 2H), 9.11 (bs, 2H), 8.70 (d, 1H), 8.62 (d, 1H), 8.09 (d, 1H), 7.75 (m, 2H), 7.68 (m, 1H), 7.57 (m, 3H), 4.45 (AB, 2H), 4.25 (m, 1H), 3.95 (s, 3H), 3.16 (m, 2H), 2.18 (m, 1H), 1.70 (m, 1H); MS (FAB) *m/z* 487 (M + H)$^+$. Anal. ($C_{21}H_{22}N_6O_4S_2$·1.5TFA·0.975H_2O) C, H, N.

Preparation of 4-Fluoro-3-[3-(*S*)-(5-pyridin-3-ylthiophene-2-ylsulfonylamino)-2-oxopyrrolidin-1-ylmethyl-

]benzamide Trifluoroacetate (29). Part A: Sodium borohydride (5 mL, 2.0 M solution in triglyme, 10.0 mmol) was added to a solution of 5-bromo-2-fluorobenzaldehyde (6.10 g, 30.0 mmol) in THF (30 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 25 min and then quenched by the addition of 1 N HCl. The mixture was diluted with EtOAc, and the layers were separated. The organic layer was washed with water and saturated NaCl and then worked up. The crude product was purified by column chromatography eluting with 15% EtOAc/hexanes to afford 5-bromo-2-fluorobenzyl alcohol (6.00 g, 98%): ^{1}H NMR ($CDCl_3$) δ 7.55 (dd, 1H), 7.36 (m, 1H), 6.89 (t, 1H), 4.71 (d, 2H), 2.11 (bs, 1H); MS (EI) *m*/*z* 204, 206 $(M)^+$.

Part B: Triphenylphosphine (4.10 g, 15.6 mmol) and *N*-bromosuccinimide (2.67 g, 15.0 mmol) were added to a solution of 5-bromo-2-fluorobenzyl alcohol (3.10 g, 15.1 mmol) in THF (30 mL) at 10 °C. The ice bath was removed and the resulting solution stirred for 20 min at room temperature. The reaction mixture was concentrated in vacuo and the crude material purified by column chromatography eluting with 5% EtOAc/hexanes to give 5-bromo-2-fluorobenzyl bromide (3.90 g, 93%): ^{1}H NMR ($CDCl_3$) δ 7.50 (dd, 1H), 7.37 (m, 1H), 6.92 (t, 1H), 4.42 (s, 2H); MS (EI) *m*/*z* 266, 268, 270 $(M)^+$.

Part C: 3-(*S*)-(*tert*-Butoxycarbonylamino)-1-(5-bromo-2-fluorobenzyl)pyrrolidin-2-one was prepared as described in example **4a**, part D, substituting 5-bromo-2-fluorobenzyl bromide for 4-(benzhydrylidenylamino)-3-(bromomethyl)benzonitrile. The product was purified by column chromatography eluting with EtOAc:CH_2Cl_2:hexane (3:1:1): ^{1}H NMR ($CDCl_3$) δ 7.40 (m, 2H), 6.96 (t, 1H), 5.20 (bs, 1H), 4.50 (s, 2H), 4.18 (m, 1H), 3.28 (m, 2H), 2.64 (m, 1H), 1.90 (m, 1H), 1.45 (s, 9H); MS (EI) *m*/*z* 287, 389 $(M)^+$.

Part D: 3-(*S*)-(*tert*-Butoxycarbonylamino)-1-(5-cyano-2-fluorobenzyl)pyrrolidin-2-one was prepared as described in example **20b**, part E, substituting 3-(*S*)-(*tert*-butoxycarbonylamino)-1-(5-bromo-2-fluorobenzyl)pyrrolidin-2-one for 3-(*S*)-(*tert*-butoxycarbonylamino)-1-(5-iodo-2-(2-methoxyethoxymethoxy)benzyl)pyrrolidin-2-one (**17**). The crude material was purified by column chromatography eluting with 3:1:1 EtOAc:hexane:CH_2Cl_2: ^{1}H NMR ($CDCl_3$) δ 7.70 (bd, 1H), 7.61 (m, 1H), 7.19 (t, 1H), 5.18 (bs, 1H), 4.63 (d, 1H), 4.50 (d, 1H), 4.18 (m, 1H), 3.32 (m, 2H), 2.64 (m, 1H), 2.00 (m, 1H), 1.47 (s, 9H); MS (EI) *m*/*z* 334 $(M)^+$.

Part E: 3-(*S*)-[(3-Amino-2-oxopyrrolidin-1-yl)methyl]-4-fluorobenzonitrile hydrochloride was prepared as described in example **20b**, part F, substituting 3-(*S*)-(*tert*-butoxycarbonylamino)-1-(5-cyano-2-fluorobenzyl)pyrrolidin-2-one for 3-(*S*)-(*tert*-butoxycarbonylamino)-1-(5-cyano-2-(2-methoxyethoxymethoxy)benzyl)pyrrolidin-2-one: ^{1}H NMR (DMSO-d_6) δ 8.73 (bs, 3H), 7.92 (m, 1H), 7.86 (d, 1H), 7.50 (t, 1H), 4.54 (s, 2H), 4.10 (m, 1H), 3.38 (m, 2H), 2.42 (m, 1H), 2.07 (m, 1H); MS (EI) *m*/*z* 233 $(M)^+$.

Part F: 5-Pyridin-3-ylthiophene-2-sulfonic acid [1-(5-cyano-2-fluorobenzyl)-2-oxopyrrolidin-3-(*S*)-yl]amide was prepared as described in example **20b**, part G, using 3-(*S*)-[(3-amino-2-oxopyrrolidin-1-yl)methyl]-4-fluorobenzonitrile hydrochloride and 5-pyridin-3-ylthiophene-2-sulfonyl chloride. The crude product was triturated with Et_2O to give the product as a white solid: ^{1}H NMR ($CDCl_3$ + CD_3OD) δ 8.87 (s, 1H), 8.55 (d, 1H), 8.08 (d, 1H), 7.73 (m, 3H), 7.53 (m, 1H), 7.48 (d, 1H), 7.28 (t, 1H), 4.54 (AB, 2H), 4.20 (m, 1H), 3.34 (m, 2H), 2.49 (m, 1H), 1.98 (m, 1H); MS (FAB) *m*/*z* 457 $(M + H)^+$.

Part G: 5-Pyridin-3-ylthiophene-2-sulfonic acid [1-(5-cyano-2-fluorobenzyl)-2-oxopyrrolidin-3-(*S*)-yl]amide was treated in the same manner as described in example **4a**, part F, to give 4-fluoro-3-[3-(*S*)-(5-pyridin-3-ylthiophene-2-ylsulfonylamino)-2-oxopyrrolidin-1-ylmethyl]benzamide trifluoroacetate (**29**): ^{1}H NMR (CD_3OD) δ 8.77 (s, 1H), 8.44 (d, 1H), 8.03 (d, 1H), 7.67 (m, 2H), 7.58 (d, 1H), 7.45 (d, 1H), 7.41 (m, 1H), 7.28 (t, 1H), 5.10 (s, 1H), 4.47 (s, 2H), 4.13 (m, 1H), 3.23 (m, 2H), 2.28 (m, 1H), 1.81 (m, 1H); MS (FAB) *m*/*z* 473 $(M + H)^+$; HRMS (ESI) calcd for $C_{21}H_{20}N_5O_3S_2F$ 474.1070, found 474.1069 $(M + H)^+$; HPLC analysis (C18, 20-min linear gradient; elution 10−100% CH_3CN/0.1% TFA H_2O; flow rate 1.0 mL/min) indicated that the product was 99A%.

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