Synthesis and Crystal Structures of Substituted Benzenes and Benzoquinones as Tissue Factor VIIa Inhibitors

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Several multistep syntheses of substituted benzenes are reported. The benzene analogues were designed such that their substitution pattern would occupy and interact with the S_1, S_2, and S_3 pockets of the tissue Factor VIIa enzyme. A variety of chemical transformations including nucleophilic additions, reductive aminations, Stille couplings, and polymer-assisted solution-phase (PASP) techniques were used to prepare key intermediates and final products. The initial analogues identified some weakly active compounds which ultimately led to a 340 nM (IC_{50}) tissue Factor VIIa inhibitor with selectivity over other related enzymes. The structure−activity relationship of these inhibitors and the synthetic progression from the discovery of the lead compound to the development of potent analogues will be discussed. The X-ray crystal structures of fluorobenzene **50c** and benzoquinone **54** inhibitors complexed with the TF/VIIa enzyme will also be described.

Introduction

Acute coronary syndromes (ACS) consisting of unstable angina, myocardial infarction, and sudden death are the most frequent cause of mortality in the United States and Western countries.[1] ACS is associated with acute thrombus formation, often as the result of plaque rupture. Thrombosis occurs in transient ischemic attack, stroke, peripheral occlusive arterial disease, deep vein thrombosis, pulmonary embolism, abrupt closure following angioplasty, and the disseminated intravascular coagulation associated with sepsis and certain cancers. Effective and safe antithrombotics are needed to combat these diseases. Most research has focused on thrombin and Factor Xa inhibitors as potentially valuable therapeutic agents for these diseases.[2] More recently, small molecule inhibitors of tissue Factor (TF) VIIa have been the point of much research effort because of their potential to inhibit the coagulation cascade while lessening the risk of bleeding side effects.[3] The extrinsic coagulation cascade is triggered by the binding of Factor VIIa to cell surface TF. This cascade is critical in normal hemostasis, but is also involved in the pathogenesis of various thrombotic diseases. Under normal conditions, TF expressed in the subendothelium of healthy blood vessels is not exposed to blood. However, in a disease state or during injury, TF comes in contact with Factor VIIa and the ensuing TF/VIIa complex activates Factors X and IX to Xa and IXa, respectively. The complex of Factor Xa and Factor Va on a membrane surface converts prothrombin to thrombin, leading to fibrin formation, deposition, and subsequent thrombus formation.[4]

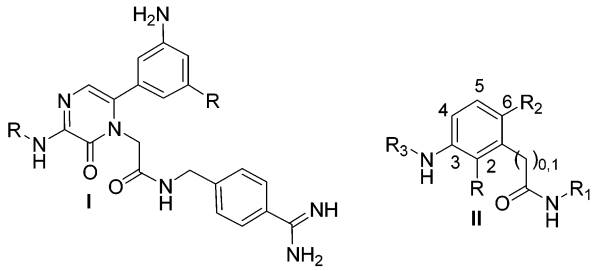

Figure 1. Pyrazinone and benzene core structures.

We previously reported the preparation of pyrazinone analogues as tissue Factor VIIa inhibitors.[5] These pyrazinone compounds are active-site inhibitors for TF/VIIa exhibiting potency at the single-digit nanomolar level with excellent selectivity over thrombin (IIa) and Factor Xa. In an effort to increase the potency and influence the pharmocokinetic properties, other core ring systems were evaluated. Depicted in Figure 1 is the lead pyrazinone structure **I**. Focusing on the central ring, one of the exercises was to replace the nitrogens with carbons on the pyrazinone ring, resulting in benzene **II** and benzoquinone as central ring systems.[6] On the basis of the X-ray crystal structures of TF/VIIa,[5,7] several key interactions were crucial for both potency on TF/VIIa and selectivity over thrombin and Factor Xa. It was found in the pyrazinone series that the benzamidine binds, as expected, mediated by an ion-pair between the basic amidine moiety and the carboxylate of Asp 189. The substituted *m*-aminophenyl ring occupies the S_2 pocket, and the substituted amino group at the 3-position of the pyrazinone occupies and interacts with the S_3 pocket. In addition, the ketone at the 2-position on the pyrazinone ring forms a hydrogen bond with the peptide backbone of Gly 216. The hypothesis was that the benzene ring could orient the substituents in the correct spatial arrangement to probe the S_1, S_2,

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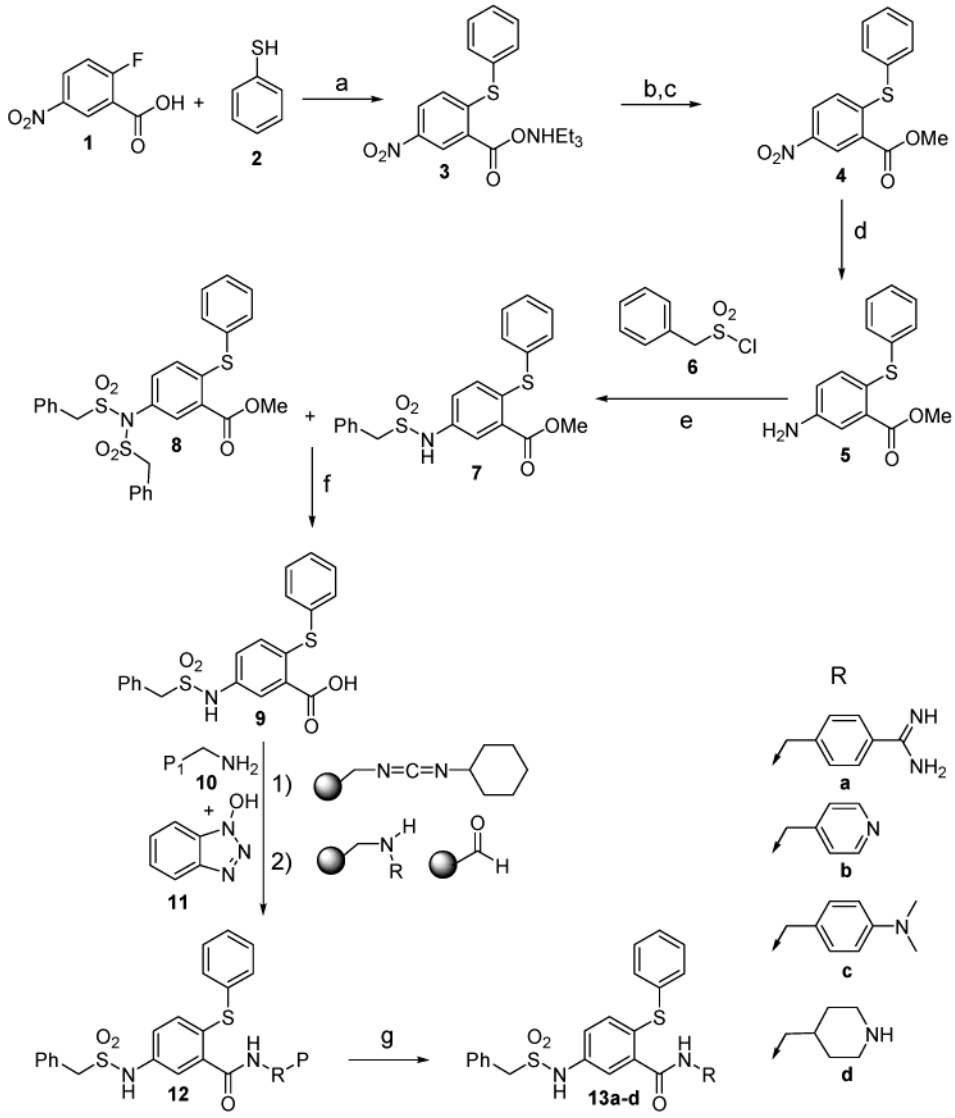
Scheme 1. Synthesis of Substituted Benzamides[a]



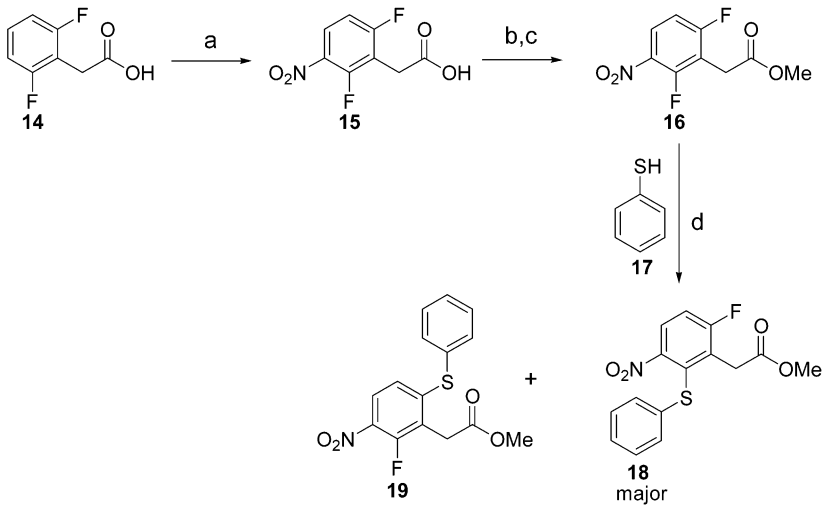
[a] Reagents: (a) TEA, THF, 65 °C; (b) $(ClCO)_2$, DCM; (c) MeOH, pyridine; (d) Fe, $MeCO_2H$, 80 °C; (e) **6**, DIEA, DCM; (f) NaOH, MeOH, 65 °C; (g) 4 N HCl/dioxane.

and S_3 pockets. In addition, the benzene ring could be substituted at the 2-position with various hydrogen bond accepting groups to engage Gly 216. Reported herein is the discovery, synthesis, X-ray crystal structures, and biological activity of a series of novel substituted benzene analogues as tissue Factor VIIa inhibitors.

Results and Discussion

Early in the program, the first set of compounds was designed in an attempt to occupy and interact with the S_1, S_2, and S_3 pockets of the enzyme. With little knowledge of what was required for the key interactions, the first set of analogues was prepared as shown in Scheme 1. These compounds were designed with a benzylsulfonamide at the 3-position of the benzene ring to probe the S_3 pocket. This moiety was chosen as a starting point based on the structure−activity relationship (SAR) from the thrombin literature and the similarity of the S_3 pocket of TF/VIIa with thrombin. Considering the larger size of the S_2 pocket of TF/VIIa relative to thrombin and Factor Xa, a phenyl ring was selected as a potential group to probe the S_2 pocket of TF/VIIa with the hope of providing selectivity. To allow for these types of substitutions, commercially available 2-fluoro-5-nitrobenzoic acid **1** was used as the starting material. Nucleophilic displacement of **1** with thiophenol **2** afforded the triethylamine salt of **3**. The salt **3** was converted to the acid chloride using oxalyl chloride, and quenching with methanol formed the methyl ester **4**. An iron reduction of the nitro compound **4** in acetic acid afforded the aniline **5**. The aniline **5** was reacted with α-toluenesulfonyl chloride **6** to afford a mixture of the mono-sulfonamide **7**, bis-sulfonamide **8**, and starting material **5**. Excess α-toluenesulfonyl chloride **6** was added to consume the remaining starting material affording both the mono- and bis-sulfonamide **7** and **8**, respectively. Hydrolysis of the methyl ester of the mono-sulfonamide **7** with sodium hydroxide afforded the free carboxylic acid **9**. Hydrolysis of the bis-sulfonamide **8** resulted in both hydrolysis of the methyl ester and hydrolysis of one of the sulfonamides, affording the same

Scheme 2. Thiophenol Displacement[a]



[a] Reagents: (a) Fuming HNO_3, −10 °C; (b) $(ClCO)_2$, DCM; (c) MeOH, pyridine; (d) TEA, THF, reflux.

desired acid **9**. The intermediate compound **9** possessed the unsubstituted thiophenyl ring to occupy the S_2 pocket and the benzylsulfonamide to occupy the S_3 region. It was desirable at this stage of the synthesis to rapidly prepare a variety of amides to occupy the S_1 pocket. The carboxylic acid **9** was coupled with different amines **10**, each containing a basic moiety in an attempt to engage the Asp 189. The amide coupling was accomplished in a parallel format using polymer-assisted solution-phase (PASP) technology[8] by treating the acid with polymer-bound carbodiimide, hydroxybenzotriazole, and *N*-methylmorpholine as base followed by addition of the amine **10** to afford the products **12**. Upon completion of the reaction, the polyamine and aldehyde sequestering resins were added to each reaction vial to remove any remaining benzoic acid/ester, hydroxybenzotriazole, and primary amine. Simple filtration and rinsing with dichloromethane yielded a filtrate, whereupon evaporation of the solvents afforded the desired products **12a**−**d**. The BOC protecting group of compounds **12a** and **12d** was removed/deprotected using 4 N hydrochloric acid in dioxane followed by evaporation to afford the desired products **13a** and **13d**, respectively. The target compounds **13a**−**d** were screened for potency on tissue Factor VIIa, and for other enzymes affecting coagulation to determine specificity including Factor Xa and thrombin (Table 1). This set of compounds **13a**−**d** was inactive against the various enzymes with the exception of **13a** showing slight activity on thrombin.

The next set of analogues was designed with an acetate linker between the benzene ring and the amide. This would permit more flexibility and allow the basic group on the amide to engage with the Asp 189, which is thought to be the anchor point and essential for biological activity. In addition to the acetate linker, a fluorine in the 2-position of the benzene ring was designed to hydrogen bond with Gly 216, allowing for another point of interaction. Thus, a second synthesis using 2,6-difluorophenylacetic acid **14** as the starting material was considered as shown in Scheme 2. The nitration of 2,6-difluorophenylacetic acid **14** using fuming nitric acid afforded a single regioisomer **15** distinguished by proton and fluorine NMR. The phenylacetic acid **15** was converted to the methyl ester **16** by forming the acid chloride with oxalyl chloride and reacting the resulting acid chloride with methanol. Nucleophilic displacement of the difluoro compound **16** with thiophenol **17** resulted in two isomers, compounds **18** and **19**, with the major (undesired) product **18** resulting from displacement of the fluorine at the 2-position.

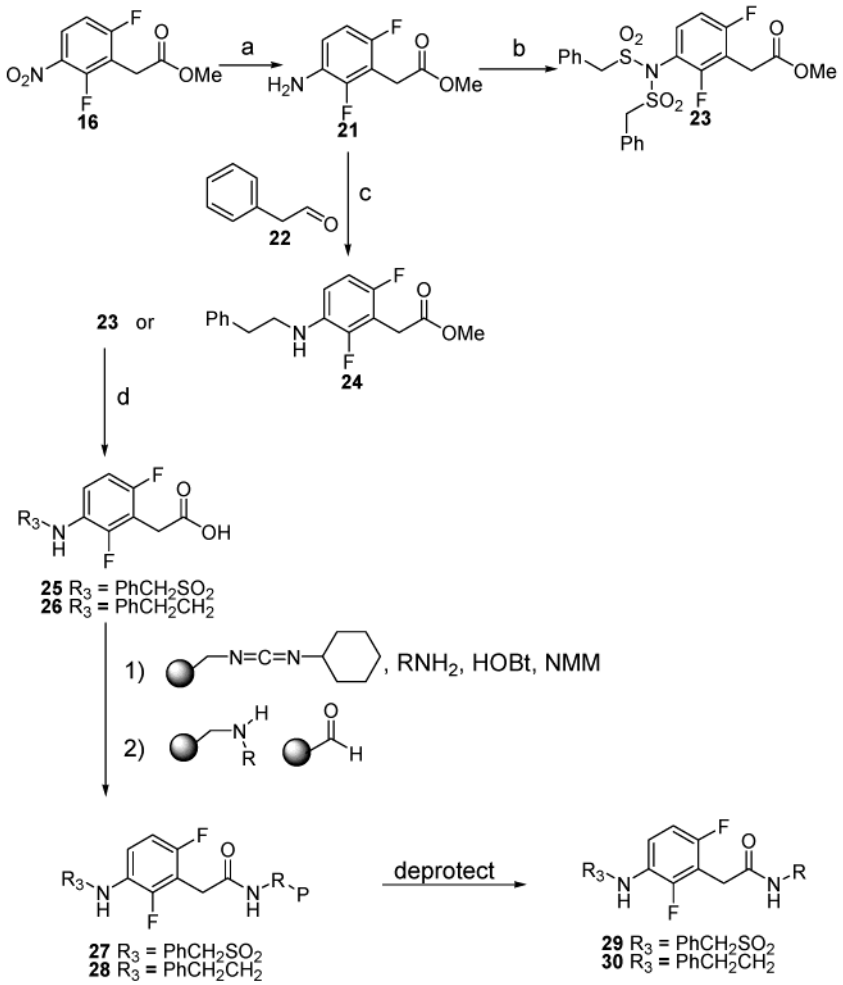
Table 1. IC_{50} Values

	IC_{50} (μM)		
compd	VIIa	Xa	thrombin
13a	>30[a]	>30	22% @30
13b	>30	>30	>30
13c	>30	>30	>30
13d	>30	>30	>30
29a	>30	>30	>30
29b	>30	>30	26.9
29c	>30	>30	>30
29d	>30	>30	>30
29e	>30	>30	>30
29f	>30	>30	>30
29g	>30	>30	>30
30b	16.4	>30	1.56
50a	3.98	>30	7.0
50b	0.50	>30	10
50c	0.34	>30	0.95
50d	25	>30	9.5
50e	14.7	>30	7.9
51	2.7	>30	6.8
53	2.5	>30	>30
54	2.8	>30	>30

[a] No inhibition was observed at a concentration of 30 μM.

Since nucleophlic displacement of the difluoro compound **16** led to the undesired regioisomer **18**, it was decided to maintain the 6-fluoro and address the ability to substitute at the 6-position at a later time. Continuing with the synthesis in Scheme 3, reduction of the nitro compound **16** using iron in acetic acid afforded the aniline **21**. Derivatization of the aniline nitrogen was achieved using two different electrophiles. The first involved reacting aniline **21** with an excess of α-toluenesulfonyl chloride **6** to afford exclusively the bis-sulfonamide **23**. An excess of **6** was used to avoid forming a mixture of both mono- and bis-sulfonamide. Hydrolysis of the bis-sulfonamide **23** resulted in both hydrolysis of the methyl ester and hydrolysis of one of the sulfonamides, affording the desired acid **25**. The carboxylic acid **25** was coupled with a variety of amines,

Scheme 3. Synthesis of 2,6-Difluorobenenzene Analogues[a]

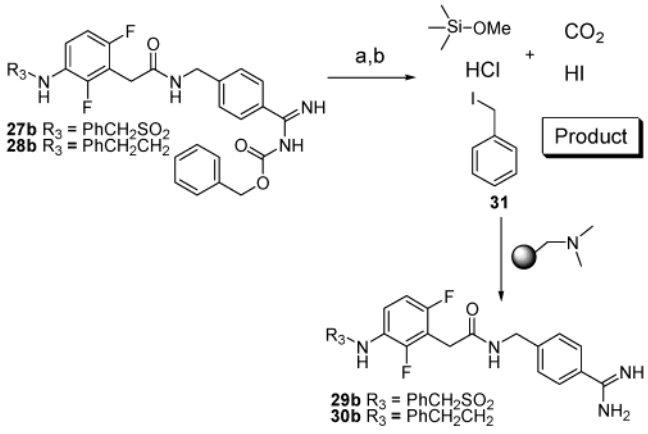


[a] Reagents: (a) Fe, $MeCO_2H$, 80 °C; (b) **6**, DIEA, DCM; (c) $NaHB(OAc)_3$, DCM, THF; (d) NaOH, MeOH, 65 °C.

each containing a basic moiety to engage Asp 189. The amide coupling was run in a parallel format using PASP technology as previously mentioned to afford the desired amide products **27**/**29**. Another compound was designed with phenethylamine as the substituent at the 3-position of the benzene ring. Reductive amination of aniline **21** with phenylacetaldehyde **22** using sodium triacetoxyborohydride afforded the desired phenethylamine product **24**. Reaction of **24** with sodium hydroxide at 65 °C in methanol resulted in the desired carboxylic acid **26**. On the basis of the biological data generated with the products **29**, the carboxylic acid **26** was coupled with only one of the amines, benzyl amino[4-(aminomethyl)-phenyl]methylcarbamate, to afford the Cbz protected amide **28b**.

Several of the amine inputs for the amide coupling were protected with either a BOC or Cbz protecting group. The BOC protecting group was easily removed by incubating the compound with 4 N hydrochloric acid in dioxane followed by evaporation to afford the desired products **29e** and **29f** (Scheme 5). A deprotection protocol for the Cbz protecting group was designed allowing for the reaction to be run in a parallel format using PASP technology. The Cbz protecting group was removed using an in-situ trimethylsilyl idodide (TMSI) deprotection protocol.[9] The procedure (Scheme 4) generates TMSI by stirring the compound in acetonitrile with an excess of sodium iodide and adding trimethylsilyl

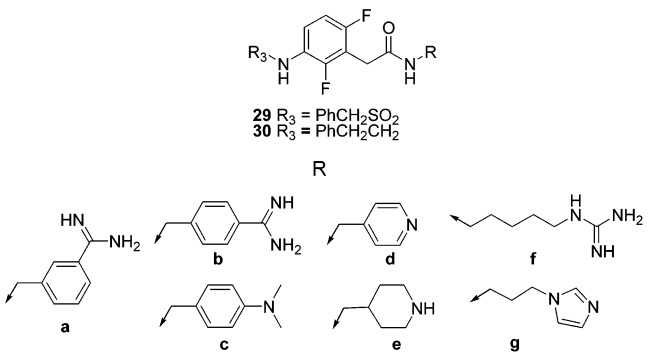
Scheme 4. Cbz Deprotection Protocol[a]



[a] Reagents: (a) TMSCl, NaI, MeCN, 55 °C; (b) MeOH.

chloride followed by heating. Upon completion of the reaction, methanol is added to quench the reaction and react with the remaining TMSI, affording volatile byproducts. Polyvinyl pyridine or a resin containing a tertiary amine group is then added to sequester the benzyl iodide byproduct **31** and remove the salts. It was found that if a polymer-bound primary or secondary amine is used as a sequestering resin with products that contain an amidine group, the resin reacts with the amidine group resulting in sequestration of the product.

Scheme 5. 2,6-Difluorobenzene Products



The in-situ trimethylsilyl iodide deprotection protocol afforded the desired products **29a**,**b** and **30b** as shown in Scheme 5.

With the exception of **29b**, which exhibited potency on thrombin, the sulfonamide compounds **29a**−**g** were inactive. Of all of the amides tested thus far, only the analogues with the *p*-benzamidine moiety have exhibited low levels of activity. As a result, that amine input was used for the amide coupling with the 3-phenethylamine acid **26**, followed by deprotection to afford the desired product **30b** (Scheme 5). Compound **30b** exhibited activity against TF/VIIa and thrombin (Table 1). This was the first benzene analogue of the series that demonstrated activity ($IC_{50} = 16.4\ \mu M$) against TF/VIIa.

With the TF/VIIa activity exhibited from the 6-fluoro analogue **30b**, a synthesis was required that would allow for a phenyl ring substitution at the 6-position of the benzene ring in an attempt to improve the binding affinity by interacting with the S_2 pocket. The reaction of 2,6-difluorobenzeneacetate **16** with thiophenol **17** afforded the wrong regioisomer **18** as the major product (Scheme 2). However, when 2,6-difluoroacetate **16** was reacted with pivalic acid **31** using potassium carbonate as the base in dimethyl sulfoxide at 80 °C (Scheme 6),[10] the product mixture contained a 1:2 mixture of the 6-hydroxy **32** to 2-hydroxy **33** compounds as determined by NMR. It is believed that the steric crowding of the pivalic acid **31** influenced the regioselectivity. Both regioisomers **32** and **33** can be utilized as starting materials. The 6-hydroxybenzene **32** allows for substitution at the 6-position while maintaining the fluorine at the 2-position. The 2-hydroxybenzene **33** permits substitution at the 6-position and derivatization at the 2-position.

Both the 2-hydroxybenzene **33** and its 2-methoxy derivative have the potential to hydrogen bond to Gly 216. The 2-methoxy starting material was prepared as shown in Scheme 6. The 2-hydroxybenzene **33** was reacted with methyl iodide to afford the methyl ether **34**. Nucleophilic displacement of the 6-fluoro with sodium hydoxide and concomitant hydrolysis of the ester afforded the acetic acid **35**. The acid **35** was converted back to the methyl ester **36** by forming the acid chloride and reacting with methanol. The methyl ester **36** was desired due to the ease of purification over the acid in the subsequent reactions.

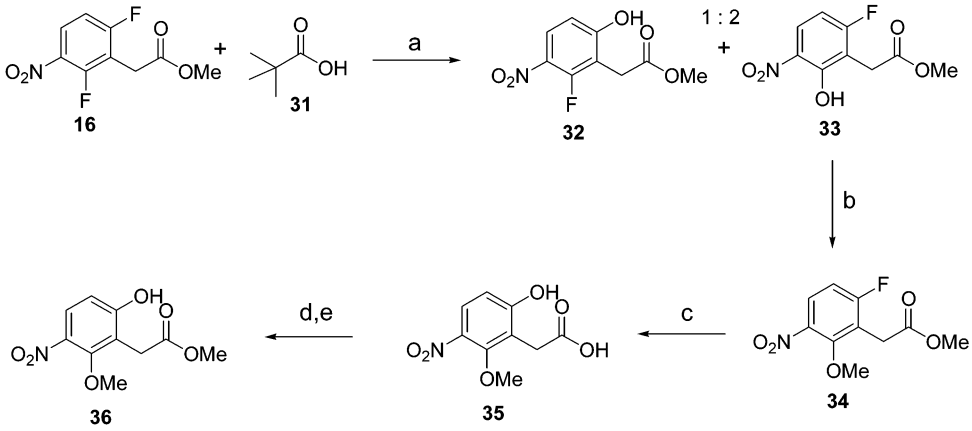
The intermediates **32** and **36** allow for substitution at the 6-position with (substituted) phenyl rings via Stille couplings. They also allow for varying the amines at the 3-position via reductive amination. The synthesis of such analogues is shown in Scheme 7. The hydroxy compounds **32** and **36** were reacted with triflic anhydride to afford the triflates **40** and **41**, respectively. The triflates **40** and **41** underwent Stille couplings with *tri-n*-butylphenylstannanes **39a** and **39b** using tetrakis(triphenylphosphine)palladium(0) and lithium chloride in dioxane at 65 °C to afford the 6-phenyl derivatives **42a**−**c**.[11] Iron reduction of the nitro group afforded the desired amines **43a**−**c**. The Stille reaction involving the 2-fluoro derivative **40** and stannane **39b** afforded the 3-aminobenzene **43b** as the major product, as reduction of the nitro group occurred during the Stille coupling. Reductive amination of the amino compounds **43a**−**c** with benzaldehyde **44**, phenylacetaldehyde **45**, or acetone **46** using sodium triacetoxyborohydride afforded the substituted amines **47a**−**e**. Hydrolysis of the methyl ester using sodium hydroxide afforded the carboxylic acids **48a**−**e**. The acids **48a**−**e** were coupled with benzyl amino[4-(aminomethyl)phenyl]methylcarbamate to afford the Cbz protected products **49a**−**e**. Hydrogenation deprotection of the Cbz group was used to afford the desired products **50a**,**c**−**e**. However, hydrogenation of compound **49b** resulted in debenzylation of the amine. As a result, hydrogen bromide in acetic acid was used to afford the desired product **50b**.

In an effort to obtain an analogue with a hydroxy in the 2-position for hydrogen bonding with the Gly 216, the methyl ether **49d** was reacted using the in-situ trimethylsilyl idodide protocol (Scheme 8). Upon purification, the desired hydroxy product **51** was isolated as the minor component with the lactone **52** as the major product. Under the reaction conditions, intramolecular cyclization occurs with the hydroxy attacking the amide carbonyl, followed by loss of the benzamidine to afford the lactone product **52**.

A benzoquinone ring system was prepared as shown in Scheme 9. The benzoquinone maintains the ketone in the same position as the pyrazinone **I** for hydrogen bonding with Gly 216. The precursor **49e** was prepared as shown in Scheme 7. Compound **49e** was designed with the isopropylamine at the 3-position and the unsubstituted phenyl ring at the 6-position. The unsubstituted phenyl ring was chosen over the aminophenyl ring at the 6-position to avoid quinone formation of that phenyl ring. The synthesis was carried out as shown in Scheme 9. The methoxybenzene **49e** was treated with boron tribromide to afford the phenol **53**. Under these conditions no sign of lactone formation was observed. Reacting the phenol **53** with Fremy's salt[12] afforded the benzoquinone **54**. The benzoquinone analogues proved to be unstable and, as a result, further synthetic efforts with benzoquinone as the core ring were not pursued.

All of the compounds were screened against several serine protease enzymes involved in the coagulation cascade, including TF/VIIa, thrombin (IIa), and Factor Xa. Each enzyme assay consisted of the specific enzyme and the chromogenic substrate for that enzyme. Reported are the inhibition data as IC_{50} values for TF/VIIa and thrombin (Table 1). The structure−activity relationships of the benzene series can be divided into four parts: The amide which occupies the S_1 pocket and interacts with Asp 189, the 3-amino substituent that occupies the S_3 pocket, the substituent at the 6-position

Scheme 6. Displacement Using Pivalic Acid[a]



[a] Reagents: (a) K_2CO_3, DMSO, 80 °C; (b) MeI, K_2CO_3, THF, 65 °C; (c) NaOH, THF, 90 °C; (d) $(ClCO)_2$, DCM; (e) MeOH, pyridine.

that occupies the S_2 pocket, and the group at the 2-position that hydrogen bonds with the peptide backbone. In general, these compounds were selective against Factor Xa with no activity observed at concentrations of 30 μM. Factor Xa has a tyrosine at position 60 and the S_2 pocket of Factor Xa is occluded by the side chain of Tyr 99. Thus, selectivity against Factor Xa is probably due to a collision of the P_2 phenyl ring with the Tyr 99 of Factor Xa.

The first set of compounds **29** and **30** identified that *p*-benzamidine as the amide was required for any type of biological activity and that the *p*-benzamidine needed to be connected by the acetate linker (Table 1).

Having identified compound **30b** as showing promising activity, further enhancements were made to probe the S_2 pocket, leading to compound **50a**. Compound **50a** maintained the substituents of **30b** (2-fluoro-3-phenethylamine), but added an unsubstituted phenyl ring at the 6-position. This resulted in an increase in potency on TF/VIIa (IC_{50} = 3.98 μM) with selectivity against Factor Xa and 2-fold selectivity against thrombin. The S_2 pocket of TF/VIIa is larger than that of thrombin and Factor Xa and contains a key residue, Asp 60, which is not present in thrombin and Factor Xa. In an attempt to engage the Asp 60, an amine was placed on the 6-phenyl ring with compounds **50b** and **50c**. An increase in potency was observed with **50c**, which is the most potent compound of the series (IC_{50} = 340 nM) on TF/VIIa. In addition to the potency, the amine substituent enhanced the selectivity profile of both **50b** and **50c**. There was no activity on Factor Xa at 30 μM and a 20-fold selectivity factor over thrombin with **50b**.

An X-ray crystal structure of compound **50c** bound to TF/VIIa is shown in Figure 2. The bound conformation of fluorobenzene inhibitor **50c** in the active site of VIIa resembles that of pyrazinone inhibitors that have been reported previously.[5] The benzamidine moiety engages the carboxylate of Asp 189 in the S_1 site, similar to the interactions observed in the crystal structures of other serine proteases. The peptide nitrogen of the acetate linker forms a hydrogen bond (3.2 Å) with the carbonyl oxygen of Ser 214. The anilino nitrogen attached to the central fluorobenzene core donates a hydrogen bond (3.4 Å) to the main chain oxygen of Gly 216. As anticipated, the fluorine atom in the central ring accepts a hydrogen bond (3.4 Å) from the amide nitrogen of Gly 216. Some of these hydrogen bonds are longer

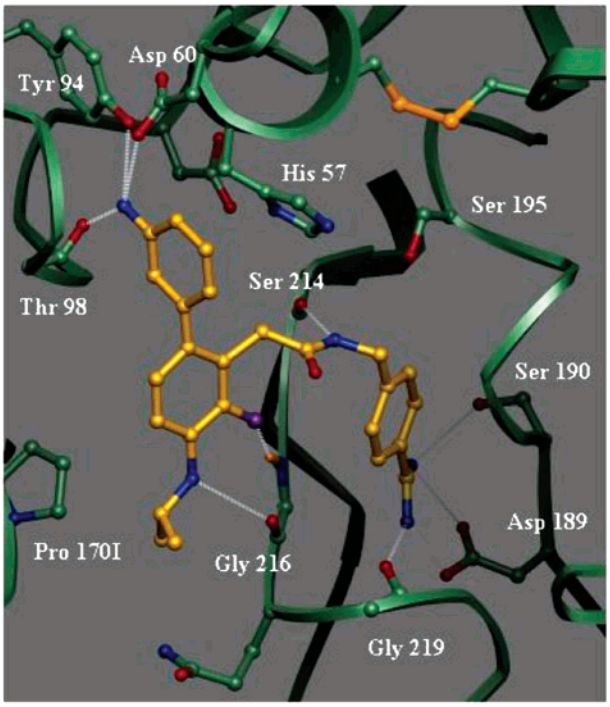
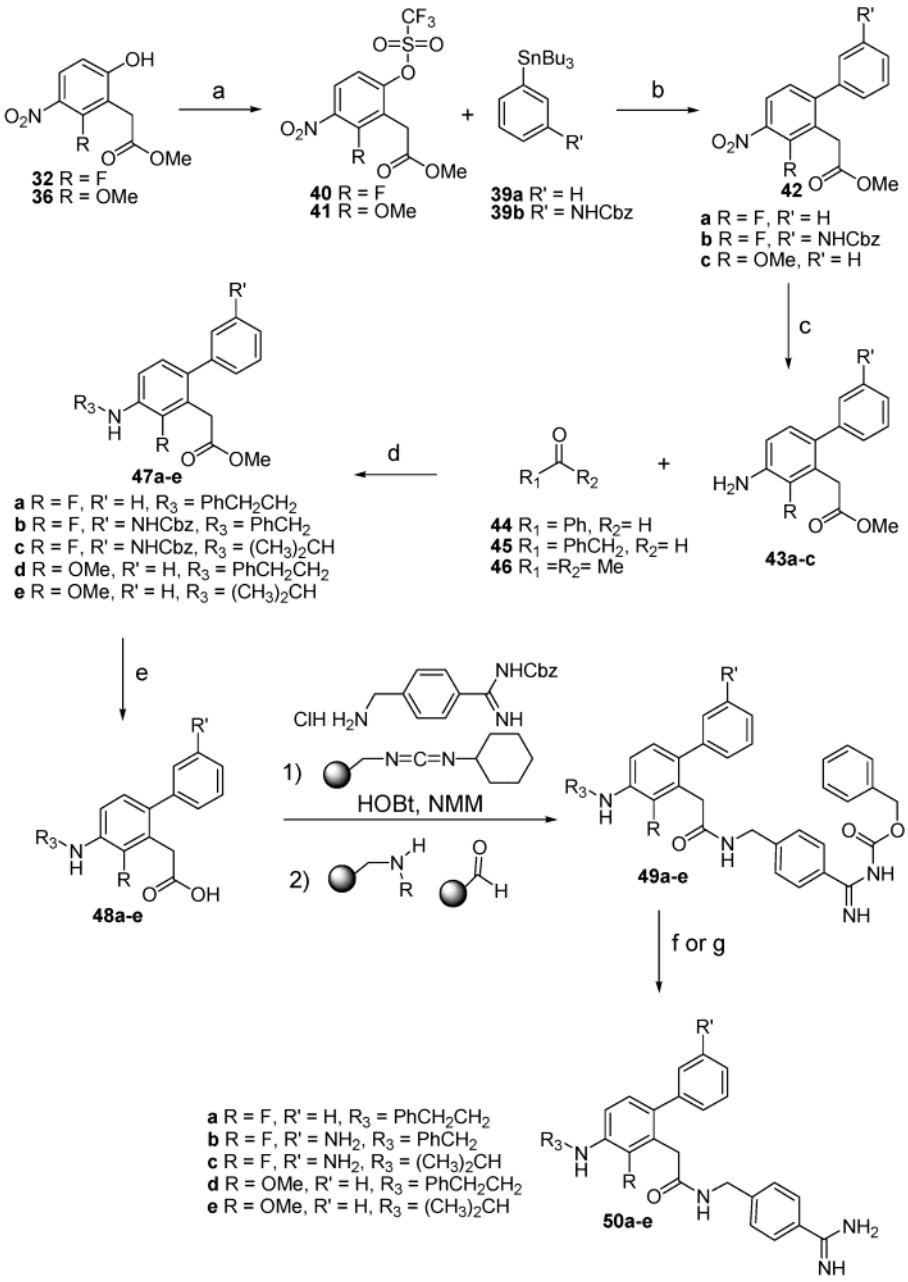


Figure 2. Crystal structure of compound **50c** (fluorobenzene) bound in the active site of TF/VIIa complex. Crystals of TF/VIIa complex were obtained by slight modification of the procedure described by Banner et al. (ref 7a). The structure has been refined to an R_{free} of 29.1% at 2.4 Å resolution ($R_{crystal}$: 23.7%). Some of the key side chains of Factor VIIa are displayed (C: green, N: dark blue, O: red, S: yellow, and H: orange). The inhibitor is represented with carbon, nitrogen, oxygen, and fluorine atoms displayed in gold, blue, red, and purple, respectively. The hydrogen bonds formed by the inhibitor are shown in dotted white lines.

and perhaps indicate nonoptimized interactions of the inhibitor in the active site. This might explain the relatively weaker binding affinity of this class of inhibitors compared to that of pyrazinone inhibitors. The amino group attached to the P_2 phenyl ring forms tight interactions with Asp 60, Tyr 94, and the carbonyl oxygen of Thr 98. Potential collision of the phenyl group of the inhibitor at P_2 with the side chain of Tyr 99 in Factor Xa is probably why it does not inhibit Factor Xa.

The S_3 pocket was accommodating to all 3-amino substituents (phenethylamine, benzylamine, and isopropylamine). In the 2-fluoro series, the 3-isopropyl-

Scheme 7. Synthesis of Substituted Benzene TF/VIIa Inhibitors[a]



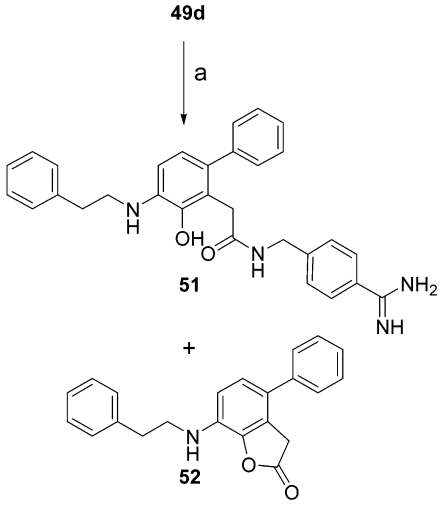
[a] Reagents: (a) $(CF_3SO_2)_2O$, TEA, DCM, −10 °C; (b) $(Ph_3P)_4Pd$, LiCl, dioxane, 65 °C; (c) Fe, $MeCO_2H$, 80 °C; (d) $NaHB(OAc)_3$, DCM, THF; (e) NaOH, MeOH, 65 °C; (f) H_2, Pd/C, MeOH; (g) HBr, $MeCO_2H$.

amine (**50c**) was ~2-fold more potent than the 3-benzylamine (**50b**). In the 2-methoxy series, the 3-isopropylamine (**50e**) was ~2-fold more potent than the 3-phenethylamine (**50d**). Thus, the small aliphatic isopropyl group was the most potent 3-amino substituent of the series.

The ketone at the 4-position on the pyrazinone ring hydrogen bonds with Gly 216 of the peptide backbone. In an attempt to maintain this key interaction with Gly 216, the benzene ring was substituted with various hydrogen bond accepting groups at the 4-position including fluorine, hydroxy, and methoxy. The fluoro and hydroxy analogues had roughly the same potency on TF/VIIa, whereas the methoxy was ~10-fold less active. Included in this set was a benzoquinone **54** which maintained the ketone from the pyrazinone core. This compound exhibited modest potency on TF/VIIa with selectivity over both thrombin and Factor Xa.

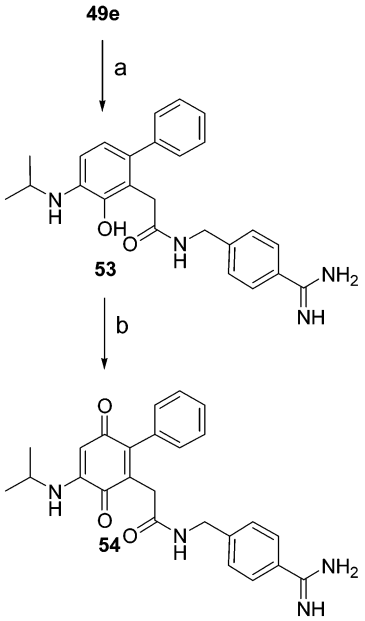
The crystal structure of the benzoquinone compound **54** bound to TF/VIIa is shown in Figure 3. The binding orientation of the benzoquinone inhibitor **54** is similar to that of the fluorobenzene **50c**. The ion-pair that is formed by the amidine moiety of the inhibitor with the carboxylate of Asp 189 functions as the main anchor for the inhibitor in the enzyme active site. In addition to this, the amidine group also forms hydrogen bonds to the main chain carbonyl of Gly 219 and the hydroxyl group of the side chain of Ser 190. Three other hydrogen bonds are formed by the inhibitor with the peptide backbone of residues Ser 214 -Gly 216 of VIIa. The amide nitrogen of the acetate linker interacts with the main chain oxygen of Ser 214 (3.2 Å) while the second-

Scheme 8. Synthesis of 2-Hydroxy Analogue[a]



[a] Reagents: (a) TMSCl, NaI, MeCN, 55 °C.

Scheme 9. Benzoquinone Synthesis[a]



[a] Reagents: (a) 1 M BBr_3, DCM, −10 °C; (b) $ON(SO_3K)_2$, THF, H_2O.

ary nitrogen attached to the quinone scaffold donates a hydrogen bond (3.0 Å) to the carbonyl oxygen of Gly 216. One of the quinone oxygen atoms accepts a hydrogen bond (3.3 Å) from the peptide nitrogen of Gly 216 as anticipated in our design. The other quinone oxygen forms van der Waals interactions (3.5 Å) with the carbonyl oxygen of Gly 97.

In summary, we have prepared a novel series of tissue Factor VIIa inhibitors. The initial set of analogues identified some weakly active compounds. Optimization of the initial lead was accomplished by novel synthesis of benzene analogues to occupy and interact with the S_1, S_2, and S_3 pockets of the TF/VIIa enzyme. The substituted benzene analogues were successfully prepared via multistep synthesis using various chemical transformations and regioselective additions with a benzene nucleus to afford novel benzene derivatives. In addition, a novel substituted benzoquinone analogue was prepared. The X-ray crystal structures of fluorobenzene **50c** and benzoquinone **54** inhibitors bound to TF/VIIa were obtained. The crystal structure of the fluorobenzene **50c** analogue clearly shows that the fluorine acts as the hydrogen bond acceptor and engages with Gly 216. Similarly, one of the ketones of the benzoquinone **54** registers with the peptide backbone of Gly 216 via a hydrogen bond. These compounds exhibit modest potency for TF/VIIa and some of them display selectivity over Factor Xa and thrombin.

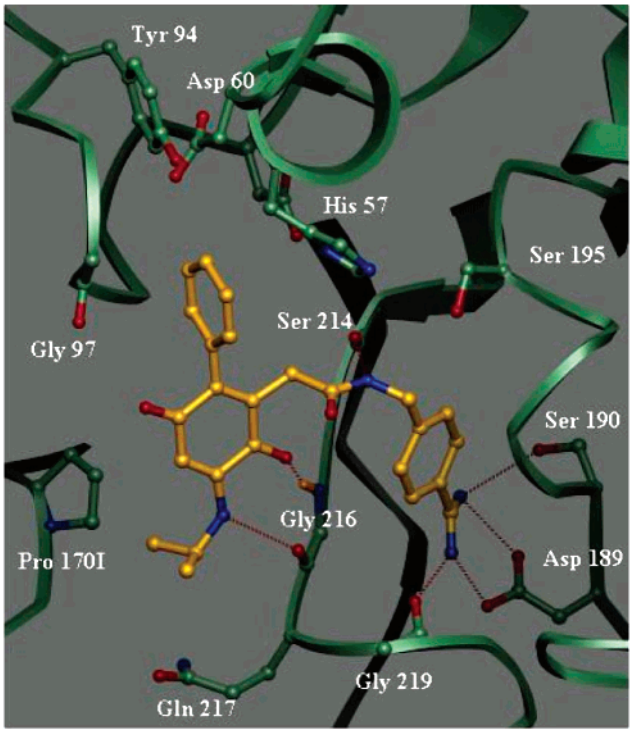


Figure 3. Crystal structure of compound **54** (benzoquinone) bound in the active site of TF/VIIa. The structure was refined to an R_{free} of 28.0% at 2.2 Å resolution ($R_{crystal}$: 22.5%). The atoms are colored as in Figure 2. Some of the key side chains of Factor VIIa are displayed. The hydrogen bonds are shown as dotted lines (magenta). One of the quinone oxygen atoms accepts a hydrogen bond from the amide nitrogen of Gly 216 as observed in the structures of pyrazinone inhibitors.

Experimental Section

General. Solvents and chemicals were reagent grade or better and were obtained from commercial sources. All polymeric reagents and sequestering resins were obtained from commercial sources. ^1H and ^{13}C NMR spectra were recorded using a 300 or 400 MHz NMR spectrometer. Sample purities were determined by HPLC analysis equipped with a mass spec detector using a C18 3.5 um 30 × 2.1 mm column, eluting with a gradient system of 5/95 to 95/5 acetonitrile/H_2O with a buffer consisting of 0.1% TFA over 4.5 min at 1 mL/min and detected by UV at 254 and 210 nm using a diode array detector. Column chromatography was performed on a preparative liquid chromatography instrument using silica gel columns and on a HPLC system using a 15 μm 100A, C18 column (25 mm I.D. × 100 mm length). Reported yields are not optimized with emphasis on purity of products rather than quantity.

5-Nitro-2-(phenylthio)benzoic Acid (3). Triethylamine (8.3 mL, 0.060 mol) was added to a solution of 2-fluoro-5-nitrobenzoic acid **1** (5.0 g, 0.027 mol) and thiophenol **2** (2.8 mL, 0.027 mol) in tetrahydrofuran. After stirring at reflux for 20 h, a saturated solution of ammonium chloride was added until solution became neutral. The solution was extracted with dichloromethane, and the organic layer was washed with

water, brine, dried over magnesium sulfate, and filtered. The solvent was removed by evaporation to afford 8.85 g (87%) of a yellow solid of product **3**; ^{1}H NMR ppm: 1.40 (t, 9H), 3.21 (q, 6H), 6.75 (d, 1H, J = 8.7 Hz), 7.47 (m, 3H), 7.57 (m, 2H), 7.85 (d, 1H, J = 8.7 Hz), 8.85 (d, 1H, J = 2.4 Hz); HRMS calcd for $C_{13}H_9O_4N_1S_1$ ($M^+ + NH_4$) 293.0596, found 293.0602.

General Procedure A. Preparation of Carboxylate Esters. Oxalyl chloride (5.0 mmol) was added to a solution of the carboxylic acid (1.0 mmol) in dichloromethane followed by a drop of dimethylformamide. After being stirred at room temperature for one to several hours, the solvent was removed by evaporation to afford the acid chloride. The acid chloride was redissolved into dichloromethane and an excess of methanol (50 mL) was added followed by addition of pyridine (1.4 mmol). The solution stirred at room temperature for one to several hours. The solution was washed with water, brine, dried over magnesium sulfate, and filtered. The solvent was removed by evaporation to afford the crude product. When necessary, the product was purified by column chromatography.

Methyl 5-Nitro-2-(phenylthio)benzoate (4). General procedure A. Using oxalyl chloride (7.7 mL, 0.088 mol), acid **3** (6.68 g, 0.017 mol), and pyridine (2.0 mL, 0.024 mol) to afford the crude product. The product was purified by column chromatography (30% ethyl acetate−hexane) to afford 4.21 g (82%) of a yellow solid of product **4**; ^{1}H NMR ppm: 4.05 (s, 3H), 6.89 (d, 1H, J = 9.0 Hz), 7.61 (m, 5H), 8.05 (dd, 1H, J = 8.7, 2.4 Hz), 8.88 (d, 1H, J = 2.4 Hz); HRMS calcd for $C_{14}H_{11}O_4N_1S_1$ (M^+ + H) 290.0487, found 290.0491.

General Procedure B. Iron Reduction of the Nitro Group. The substituted nitrobenzene (1.0 mmol) was stirred in glacial acetic acid (8 mL). Powdered iron (5.0 mmol) was added, and the solution was heated to 80 °C with vigorous stirring. The solution was stirred at 80 °C for 15−60 min at which point the iron had turned gray. The reaction mixture was filtered through Celite, and the solid was washed with diethyl ether. The resultant organic layer was washed with water, stirred with saturated sodium bicarbonate until basic, and washed with water again. The solution was dried over magnesium sulfate and filtered, and the solvent was removed by evaporation to afford the product. When necessary, the product was purified by column chromatography.

Methyl 5-Amino-2-(phenylthio)benzoate (5). General procedure B. Using the nitro compound **4** (3.71 g, 0.012 mmol) and powdered iron (3.58 g, 0.064 mmol) to afford 2.48 g (75%) of a yellow oil of product **5**; ^{1}H NMR ppm: 3.90 (s, 3H), 6.89 (dd, 1H, J = 8.4, 2.7 Hz), 6.96 (d, 1H, J = 8.4 Hz), 7.22 (d, 1H, J = 2.7 Hz), 7.32 (m, 5H); HPLC purity (retention time): >99% (2.81 min); HRMS calcd for $C_{14}H_{13}O_2N_1S_1$ (M^+ + H) 260.0745, found 260.0718.

Methyl 5-[(Benzylsulfonyl)amino]-2-(phenylthio)benzoate (7). Diisopropylethylamine (2.5 mL, 1.42 mmol) was added to a solution of the aniline **5** (2.46 g, 9.48 mmol) and α-toluenesulfonyl chloride **6** (3.17 g, 16.6 mmol) in dichloromethane, and the resulting solution stirred at room temperature for 3 h. The solution was washed with 2 N hydrochloric acid and brine, dried over magnesium sulfate, and filtered. The solvent was removed by evaporation to afford a mixture of two products. Fraction two of column chromatography (30% ethyl acetate−hexane) afforded 1.16 g (30%) of a white solid of product **7**; ^{1}H NMR ppm: 3.98 (s, 3H), 4.32 (s, 2H), 6.66 (s, 1H), 6.80 (d, 1H), 7.28 (dd, 1H), 7.29−7.73 (m, 11H); HPLC purity (retention time): 97.2% (4.20 min); HRMS calcd for $C_{21}H_{19}O_4N_1S_2$ ($M^+ + NH_4$) 431.1099, found 431.1069.

Methyl 5-[Bis(benzylsulfonyl)amino]-2-(phenylthio)-benzoate (8). Following the same procedure described for **7**, fraction one of column chromatography (30% ethyl acetate−hexane) afforded 2.64 g (49%) of a white solid of product **8**; ^{1}H NMR ppm: 3.94 (s, 3H), 4.85 (s, 4H), 6.22 (dd, 1H, J = 9.0, 2.4 Hz), 6.44 (d, 1H, J = 9.0 Hz), 7.07 (d, 1H, J = 2.4 Hz), 7.40 (m, 7H), 7.50 (m, 9H); HRMS calcd for $C_{28}H_{25}O_6N_1S_3$ ($M^+ + NH_4$) 585.1188, found 585.1141.

General Procedure C. Hydrolysis of the Methyl Ester. Aqueous sodium hydroxide (10%) (4.5 mmol) was added to a solution of the methyl ester (1.0 mmol) in methanol and the resulting solution stirred at 65 °C for one to several hours. The solution was acidified with 2 N hydrochloric acid and extracted with diethyl ether. The solution was washed with brine, dried over magnesium sulfate, and filtered. The solvent was removed by evaporation to afford the product. When necessary, the product was purified by column chromatography.

5-[(Benzylsulfonyl)amino]-2-(phenylthio)benzoic Acid (9). General procedure C. Using sodium hydroxide (3.0 mL, 7.5 mmol) and methyl ester **7** (0.70 g, 1.7 mmol) to afford 0.66 g (98%) of a white solid of product **9**; ^{1}H NMR ppm: 3.86 (s, 2H), 6.31 (dd, 1H), 6.83 (m, 6H), 6.97 (m, 3H), 7.06 (m, 2H), 7.52 (d, 1H), 9.35 (s, 1H); HPLC purity (retention time): 93.9% (3.69 min); HRMS calcd for $C_{20}H_{17}O_4N_1S_2$ ($M^+ + NH_4$) 417.0943, found 417.0933.

General Procedure D. Amide Coupling. Under conditions of parallel reaction synthesis, 1-hydroxybenzotriazole (0.10 mmol) was added to a solution or slurry of the acid (0.10 mmol) and PS-carbodiimide (1.00 mmol/g) (0.20 mmol) in a mixture of 3 mL of dichloromethane and 0.5 mL of dimethylformamide. The suspension was agitated for 15 min. The amine (0.10 mmol) was added, along with *N*-methylmorpholine (0.12 mmol) when the amine is a hydrochloride salt. The suspension was agitated for one to several hours. Upon completion of the reaction, the polyamine resin (2.69 mmol/g) (1.0 mmol) and polymer-bound aldehyde (2.3 mmol/g) (1.0 mmol) was added, and the suspension was agitated for one to several hours. The solution was filtered and the polymer was rinsed with dimethylformamide and dichloromethane until no more UV activity was seen in the dichloromethane washing. The combined filtrate and washings were evaporated to afford the product. When necessary, the product was purified by column chromatography.

***tert*-Butyl [4-({[5-[(Benzylsulfonyl)amino]-2-(phenylthio)benzoyl]amino}methyl)phenyl](imino)methylcarbamate (12a).** General procedure D afforded 89.9 mg (71%) of a clear oil of product **12a**; ^{1}H NMR ppm: 1.56 (s, 9H), 4.31 (s, 2H), 4.52 (d, 2H), 7.12−7.68 (m, 19H); HPLC purity (retention time): 66.4% (3.41 min).

5-[(Benzylsulfonyl)amino]-2-(phenylthio)-*N*-(pyridin-4-ylmethyl)benzamide (12b). General procedure D afforded 30.5 mg (62%) of a white solid of product **12b**; ^{1}H NMR ppm: 4.35 (s, 2H), 4.55 (d, 2H), 7.13 (d, 2H), 7.26 (m, 13H), 7.66 (s, 2H), 8.39 (d, 2H); HPLC purity (retention time): >99% (3.00 min); HRMS calcd for $C_{26}H_{23}O_3N_3S_2$ (M^+ + H) 490.1259, found 490.1222.

5-[(Benzylsulfonyl)amino]-*N*-[4-(dimethylamino)benzyl]-2-(phenylthio)benzamide (12c). General procedure D afforded 30.0 mg (70%) of a white solid of product **12c**; ^{1}H NMR ppm: 2.95 (s, 6H), 4.35 (s, 2H), 4.44 (d, 2H), 6.70 (d, 2H), 7.23 (m, 16H), 7.62 (s, 1H); HPLC purity (retention time): >99% (3.15 min); HRMS calcd for $C_{29}H_{29}O_3N_3S_2$ (M^+ + H) 532.1729, found 532.1681.

***tert*-Butyl 4-({[5-[(Benzylsulfonyl)amino]-2-(phenylthio)-benzoyl]amino}methyl)piperidine-1-carboxylate (12d).** General procedure D afforded 58.4 mg (98%) of a clear oil of product **12d**; ^{1}H NMR ppm: 1.21 (m, 2H), 1.45 (s, 9H), 1.62 (m, 3H), 2.63 (m, 2H), 3.25 (m, 2H), 4.03 (m, 2H), 4.35 (s, 2H), 7.27 (m, 13H), 7.66 (s, 1H); HPLC purity (retention time): >99% (4.41 min).

General Procedure E. BOC Deprotection. Under conditions of parallel reaction synthesis, hydrochloric acid in dioxane (4 N) (3 mL) was added to the BOC protected compound, and the solution was agitated at room temperature for one to several hours. Evaporation of the solvents afforded the product.

***N*-{4-[Amino(imino)methyl]benzyl}-5-[(benzylsulfonyl)-amino]-2-(phenylthio)benzamide (13a).** General procedure E afforded 89.9 mg (71%) of a clear oil of product **13a**; ^{1}H NMR ppm: 4.85 (s, 2H), 4.90 (d, 2H), 7.52−8.22 (m, 18H), 9.31 (m, 1H), 9.82 (bs, 1H), 10.22 (s, 1H), 10.49 (s, 1H); HPLC purity (retention time): >83.7% (3.15 min); HRMS calcd for $C_{28}H_{26}O_3N_4S_2$ (M^+ + H) 531.1525, found 531.1583.

5-[(Benzylsulfonyl)amino]-2-(phenylthio)-*N*-(piperidin-4-ylmethyl)benzamide (13d). General procedure E afforded 35.3 mg (71%) of a clear oil of product **13d**; ^{1}H NMR ppm: 1.31 (m, 2H), 2.17 (m, 2H), 3.14 (m, 3H), 3.59 (m, 2H), 3.79 (m, 2H), 4.73 (s, 2H), 7.52 (m, 12H), 7.71 (s, 1H), 8.62 (bs, 1H), 9.60 (m, 1H), 10.34 (s, 1H); HPLC purity (retention time): >99% (2.97 min); HRMS calcd for $C_{26}H_{29}O_3N_3S_2$ (M^+ + H) 496.1729, found 496.1710.

(2,6-Difluoro-3-nitrophenyl)acetic Acid (15). 2,6-Difluorophelacetic acid **14** (5.0 g, 0.029 mol) was added in small portions over a period of 20 min to fuming nitric acid chilled to −10 °C (methanol/ice). The addition was closely monitored to keep the temperature below 5 °C. Upon complete addition, the solution stirred at −10 °C for 15 min. The solution was poured over ice/water and extracted with diethyl ether. The organic layer was washed with brine, dried over magnesium sulfate, and filtered. The solvent was removed by evaporation to afford 5.75 g (91%) of a white solid of product **15**; ^{1}H NMR ppm: 3.89 (s, 2H), 7.11 (m, 1H), 8.16 (m, 1H); ^{19}F NMR ppm: −101.66 (m, 1F), −115.43 (m, 1F); HRMS calcd for $C_8H_5O_4N_1F_2$ (M^+) 217.0186, found 217.0166.

Methyl (2,6-Difluoro-3-nitrophenyl)acetate (16). General procedure A. Using oxalyl chloride (12.6 mL, 0.144 mol), acid **15** (5.75 g, 0.026 mol), and pyridine (3.5 mL, 0.043 mol) to afford the crude product. The product was purified by column chromatography (30% ethyl acetate−hexane) to afford 5.47 g (91%) of an orange oil of product **16**; ^{1}H NMR ppm: 3.78 (s, 3H), 3.84 (s, 2H), 7.09 (m, 1H), 8.12 (m, 1H); ^{19}F NMR ppm: −101.76 (m, 1F), −115.58 (m, 1F); HPLC purity (retention time): >99% (2.88 min); HRMS calcd for $C_9H_7O_4N_1F_2$ (M^+ + NH_4) 249.0660, found 249.0687.

Methyl [6-Fluoro-3-nitro-2-(phenylthio)phenyl]acetate (18). Triethylamine (3.8 mL, 0.0270 mol) was added to a solution of methyl 2,6-difluoro-3-nitrophenyl acetate **16** (5.4 g, 0.023 mol) and thiophenol **17** (2.4 mL, 0.023 mol) in tetrahydrofuran. After stirring at reflux for 20 h, a 2 N hydrochloric acid solution was added until solution became acidic. The solution was extracted with diethyl ether, and the organic layer was washed with water and brine, dried over magnesium sulfate, and filtered. The solvent was removed by evaporation to afford a mixture of two products. Fraction one of column chromatography (10% ethyl acetate−hexane) afforded 4.41 g (60%) of a yellow oil of product **18**; ^{1}H NMR ppm: 3.57 (s, 3H), 3.98 (d, 2H), 7.29 (m, 6H), 7.76 (m, 1H); ^{19}F NMR ppm: −104.28 (m, 1F); ^{13}C NMR ppm (deuteriochloroform): 33.3, 52.5, 117.1, 124.9, 127.5, 129.1, 129.3, 129.5, 129.6, 130.4, 134.4, 161.1, 164.5, 169.4; HPLC purity (retention time): 83% (4.02 min); HRMS calcd for $C_{15}H_{12}O_4N_1S_1F_1$ (M^+ + NH_4) 339.0809, found 339.0797.

Methyl [2-Fluoro-3-nitro-6-(phenylthio)phenyl]acetate (19). Following the same procedure described for **18**, fraction two of column chromatography (30% ethyl acetate−hexane) afforded 1.2 g (16%) of a yellow oil of product **19**; ^{1}H NMR ppm: 3.78 (s, 3H), 3.99 (d, 2H), 6.80 (dd, 1H), 7.49 (m, 5H), 7.83 (m, 1H); ^{19}F NMR ppm: −119.24 (d, 1F); ^{13}C NMR ppm (deuteriochloroform): 32.0, 52.8, 123.2, 125.0, 130.0, 130.4, 134.8, 149.6, 152.5, 156.0, 169.5; HPLC purity (retention time): 83% (4.02 min); HRMS calcd for $C_{15}H_{12}O_4N_1S_1F_1$ (M^+ + NH_4) 339.0809, found 339.0793.

Methyl (3-Amino-2,6-difluorophenyl)acetate (21). General procedure B. Using the nitro compound **16** (6.87 g, 0.029 mmol) and powdered iron (8.29 g, 0.148 mmol) to afford the crude product. The product was purified by column chromatography (30% ethyl acetate−hexane) to afford 4.07 g (68%) of a clear oil of product **21**; ^{1}H NMR ppm: 3.62 (bs, 2H), 3.71 (s, 2H), 3.74 (s, 3H), 6.70 (m, 2H); ^{19}F NMR ppm: −115.58 (m, 1F), −129.12 (m, 1F); HPLC purity (retention time): >99% (1.57 min); LRMS for $C_9H_9O_2N_1F_2$ (ES, *m*/*z*) 202 (M + H).

Methyl {3-[Bis(benzylsulfonyl)amino]-2,6-difluorophenyl}acetate (23). Diisopropylethylamine (5.1 mL, 0.029 mol) was added to a solution of the aniline **21** (4.0 g, 0.019 mmol) and α-toluenesulfonyl chloride **6** (8.34 g, 0.042 mol) in dichloromethane, and the resulting solution stirred at room temperature for 20 h. The solution was washed with 2 N hydrochloric acid and brine, dried over magnesium sulfate, and filtered. The solvent was removed by evaporation to afford the crude product. The product was purified by column chromatography (30% ethyl acetate−hexane) to afford 6.2 g (61%) of a tan solid of product **23**; ^{1}H NMR ppm: 3.73 (s, 2H), 3.75 (s, 3H), 4.63 (d, 2H), 5.10 (d, 2H), 5.91 (m, 1H), 6.47 (m, 1H), 7.45 (m, 10H); ^{19}F NMR ppm: −109.09 (m, 1F), −115.57 (m, 1F); HPLC purity (retention time): 85.3% (4.45 min); HRMS calcd for $C_{23}H_{21}O_6N_1S_2F_2$ (M^+ + NH_4) 527.1122, found 527.1122.

General Procedure F. Reductive Amination. Sodium triacetoxyborohydride (4.0 mmol) was added to a solution of the aniline (1.0 mmol), aldehyde or ketone (1.0 mmol), and a drop of acetic acid in a tetrahydrofuran−dichloromethane (1:1) solution. After being stirred at room temperature for one to several hours, the solution was diluted with diethyl ether and water. The organic layer was washed with brine, dried over magnesium sulfate, and filtered. The solvent was removed by evaporation to afford the product. When necessary, the product was purified by column chromatography.

Methyl {2,6-Difluoro-3-[(2-phenylethyl)amino]phenyl}acetate (24). General procedure F. Using sodium triacetoxyborohydride (7.2 g, 0.033 mol), aniline **21** (1.72 g, 0.0085 mol), and phenylacetaldehyde **22** (2.0 mL, 0.015 mol) to afford a mixture of two products. Fraction two of column chromatography (10% ethyl acetate−hexane) afforded 1.06 g (41%) of a clear oil of product **24**; ^{1}H NMR ppm: 2.97 (t, 2H), 3.42 (t, 2H), 3.73 (s, 2H), 3.75 (s, 3H), 6.62 (m, 1H), 6.82 (m, 1H), 7.29 (m, 5H); ^{19}F NMR ppm: −131.28 (m, 1F), −137.00 (m, 1F); HPLC purity (retention time): 96.1% (3.93 min); HRMS calcd for $C_{17}H_{17}O_2N_1F_2$ (M^+ + H) 306.1306, found 306.1309.

{3-[(Benzylsulfonyl)amino]-2,6-difluorophenyl}acetic Acid (25). General procedure C. Using sodium hydroxide (10%) (10.3 mL, 0.025 mol) and methyl ester **23** (3.29 g, 0.0064 mol) to afford 2.1 g (95%) of a white solid of product **25**; ^{1}H NMR ppm: 3.87 (s, 2H), 4.60 (s, 2H), 7.11 (m, 1H), 7.51 (m, 6H), 9.77 (s, 1H); ^{19}F NMR ppm: −119.10 (m, 1F), −123.54 (m, 1F); HPLC purity (retention time): >99% (2.86 min); HRMS calcd for $C_{15}H_{13}O_4N_1S_1F_2$ (M^+ + NH_4) 359.0877, found 359.0867.

{2,6-Difluoro-3-[(2-phenylethyl)amino]phenyl}acetic Acid (26). General procedure C. Using sodium hydroxide (10%) (5.5 mL, 13.7 mmol) and methyl ester **24** (1.06 g, 3.47 mmol) to afford 0.93 g (92%) of a white solid of product **26**; ^{1}H NMR ppm: 2.64 (t, 2H), 3.02 (t, 2H), 3.19 (s, 2H), 6.46 (m, 1H), 6.75 (m, 6H), 7.10 (bd, 1H), 9.30 (bs, 1H); ^{19}F NMR ppm: −119.37 (m, 1F), −128.26 (m, 1F); HPLC purity (retention time): >99% (3.26 min); HRMS calcd for $C_{16}H_{15}O_2N_1F_2$ (M^+ + H) 292.1149, found 292.1150.

Benzyl (1*Z*)-Amino(3-{[({3-[(benzylsulfonyl)amino]-2,6-difluorophenyl}acetyl)amino]methyl}phenyl)-methylidenecarbamate (27a). General procedure D afforded 37.7 mg (62%) of a clear oil of product **27a**; ^{1}H NMR ppm: 3.62 (m, 2H), 4.29 (s, 2H), 4.42 (d, 2H), 5.12 (s, 2H), 6.80 (m, 2H), 7.38 (m, 14H), 7.71 (m, 2H); ^{19}F NMR ppm: −117.61 (m, 1F), −125.19 (m, 1F); HPLC purity (retention time): 69.6% (3.25 min); LRMS for $C_{31}H_{28}O_5N_4S_1F_2$ (ES, *m*/*z*) 607 (M + H).

Benzyl (4-{[({3-[(Benzylsulfonyl)amino]-2,6-difluorophenyl}acetyl)amino]methyl}phenyl)(imino)methylcarbamate (27b). General procedure D afforded 49.9 mg (82%) of a clear oil of product **27b**; ^{1}H NMR ppm: 3.58 (m, 2H), 4.27 (s, 2H), 4.32 (d, 2H), 5.18 (s, 2H), 6.74 (m, 2H), 7.14 (d, 2H), 7.37 (m, 12H), 7.68 (d, 2H); ^{19}F NMR ppm: −117.58 (m, 1F), −124.63 (m, 1F); HPLC purity (retention time): 81.7% (3.14 min); LRMS for $C_{31}H_{28}O_5N_4S_1F_2$ (ES, *m*/*z*) 607 (M + H).

***tert*-Butyl 4-{[({3-[(Benzylsulfonyl)amino]-2,6-difluorophenyl}acetyl)amino]methyl}piperidine-1-carboxylate (27e).** General procedure D afforded 67.2 mg (100%) of a clear oil of product **27e**; ^{1}H NMR ppm: 1.14 (m, 2H), 1.47 (s, 9H), 1.65 (m, 3H), 2.69 (m, 2H), 3.18 (m, 2H), 3.62 (s, 2H), 4.10 (m, 2H), 4.38 (s, 2H), 6.01 (m, 1H), 6.90 (m, 2H), 7.35 (m, 6H); ^{19}F NMR ppm: −117.94 (m, 1F), −126.43 (m, 1F); HPLC purity (retention time): 56.6% (3.57 min); LRMS for $C_{26}H_{33}O_5N_3S_1F_2$ (ES, *m*/*z*) 560 (M + Na).

Bis-*tert*-butyl (1*E*)-Amino({5-[({3-[(benzylsulfonyl)-amino]-2,6-difluorophenyl}acetyl)amino]pentyl}amino)-dimethylidenecarbamate (27f). General procedure D afforded 67.1 mg (100%) of a clear oil of product **27f**; ^{1}H NMR ppm: 1.57 (m, 18H), 1.58 (m, 6H), 3.26 (m, 2H), 3.40 (m, 2H), 3.60 (s, 2H), 4.35 (s, 2H), 6.10 (m, 1H), 6.86 (m, 1H), 7.37 (m, 8H), 8.31 (m, 1H); ^{19}F NMR ppm: −117.86 (m, 1F), −125.96 (m, 1F); HPLC purity (retention time): 39.2% (3.48 min); LRMS for $C_{31}H_{43}O_7N_5S_1F_2$ (ES, *m*/*z*) 668 (M + H).

Benzyl (4-{[({2,6-Difluoro-3-[(2-phenylethyl)amino]-phenyl}acetyl)amino]methyl}phenyl)(imino)methyl-carbamate (28b). General procedure D. Using 1-hydroxybenzotriazole **11** (43.1 mg, 0.31 mmol), acid **26** (0.93 g, 3.19 mmol), PS-carbodiimide (1.00 mmol/g) (9.57 g, 9.57 mmol), amine (0.96 g, 3.16 mmol), and *N*-methylmorpholine (2.0 mL, 18.1 mmol) afforded the crude product. The product was purified by column chromatography (70% ethyl acetate−hexane) to afford 1.14 g (64%) of a white solid of product **28b**; ^{1}H NMR ppm: 2.92 (t, 2H), 3.37 (m, 2H), 3.64 (s, 2H), 3.86 (bs, 1H), 4.39 (d, 2H), 5.23 (s, 2H), 6.41 (m, 1H), 6.57 (m, 1H), 6.79 (m, 1H), 7.34 (m, 14H), 7.72 (d, 2H), 9.43 (bs, 1H); ^{19}F NMR ppm: −131.01 (m, 1F), −136.68 (m, 1F); HPLC purity (retention time): 83.6% (3.38 min); HRMS calcd for $C_{32}H_{30}O_3N_4F_2$ (M^+ + H) 557.2364, found 557.2326.

General Procedure G. In Situ TMSI Deprotection Protocol. The benzyloxycarbonyl (Cbz) compound (0.010 mmol), sodium iodide (60.0 mg, 0.040 mmol), and trimethylsilyl chloride (50.7 uL, 0.040 mmol) were stirred in acetonitrile at 55 °C for one to several hours. Methanol (100 uL) was added and the solution stirred at room temperature for 1−4 h. The PS-dimethylbenzylamine resin (3.58 mequiv/g) (0.60 g, 2.1 mmol) was added and the solution stirred at room temperature for 1−6 h. The reaction mixture was filtered through Celite, and the solid was rinsed with acetonitrile. The combined filtrate and washings were evaporated to afford the product. When necessary, the product was purified by column chromatography.

***N*-{3-[Amino(imino)methyl]benzyl}-2-{3-[(benzylsulfonyl)amino]-2,6-difluorophenyl}acetamide (29a).** General Procedure G afforded 89.9 mg (71%) of a white solid of product **29a**; ^{1}H NMR ppm: 3.00 (s, 2H), 3.62 (s, 2H), 3.76 (d, 2H), 6.12 (m, 1H), 6.58−7.32 (m, 13H), 7.88 (m, 1H); ^{19}F NMR ppm: −120.82 (m, 1F), −124.52 (m, 1F); HPLC purity (retention time): 66.4% (2.60 min); HRMS calcd for $C_{23}H_{22}O_3N_4S_1F_2$ (M^+ + H) 473.1459, found 473.1449.

***N*-{4-[Amino(imino)methyl]benzyl}-2-{3-[(benzylsulfonyl)amino]-2,6-difluorophenyl}acetamide (29b).** General Procedure G afforded 89.9 mg (71%) of a white solid of product **29b**; ^{1}H NMR ppm: 3.21 (s, 2H), 3.94 (s, 2H), 4.06 (m, 2H), 5.94 (bs, 1H), 6.42 (m, 1H), 6.91 (m, 8H), 7.12 (d, 2H), 7.49 (d, 2H), 8.18 (m, 1H); ^{19}F NMR ppm: −119.65 (m, 1F), −124.41 (m, 1F); HPLC purity (retention time): 44.1% (2.57 min); HRMS calcd for $C_{23}H_{22}O_3N_4S_1F_2$ (M^+ + H) 473.1459, found 473.1447.

2-{3-[(Benzylsulfonyl)amino]-2,6-difluorophenyl}-*N*-[4-(dimethylamino)benzyl]acetamide (29c). General procedure D afforded 55.5 mg (100%) of a white solid of product **29c**; ^{1}H NMR ppm: 2.50 (s, 6H), 3.26 (s, 2H), 3.89 (s, 2H), 3.93 (d, 2H), 6.27 (bd, 1H), 6.43 (m, 1H), 6.72 (m, 2H), 6.93 (m, 6H), 7.44 (m, 1H), 7.58 (s, 1H), 8.84 (s, 1H); ^{19}F NMR ppm: −118.49 (m, 1F), −124.01 (m, 1F); HPLC purity (retention time): >99% (2.57 min); HRMS calcd for $C_{24}H_{25}O_3N_3S_1F_2$ (M^+ + H) 474.1663, found 474.1647.

2-{3-[(Benzylsulfonyl)amino]-2,6-difluorophenyl}-*N*-(pyridin-4-ylmethyl)acetamide (29d). General procedure D afforded 51.2 mg (100%) of a white solid of product **29d**; ^{1}H NMR ppm: 3.21 (s, 2H), 3.82 (s, 2H), 3.93 (d, 2H), 6.37 (m, 1H), 6.78 (m, 8H), 7.29 (s, 1H), 7.46 (s, 1H), 8.91 (s, 1H); ^{19}F NMR ppm: −118.95 (m, 1F), −123.95 (m, 1F); HPLC purity (retention time): 91% (2.34 min); HRMS calcd for $C_{21}H_{19}O_3N_3S_1F_2$ (M^+ + H) 432.1193, found 432.1164.

2-{3-[(Benzylsulfonyl)amino]-2,6-difluorophenyl}-*N*-(piperidin-4-ylmethyl)acetamide (29e). General procedure E afforded 40.6 mg (93%) of a white solid of product **29e**; ^{1}H NMR ppm: 1.04 (m, 2H), 1.32 (m, 3H), 2.35 (m, 3H), 2.86 (m, 2H), 3.06 (s, 2H), 3.78 (s, 2H), 6.26 (m, 1H), 6.74 (m, 6H), 7.57 (bs, 1H), 8.73 (m, 1H), 8.99 (m, 1H); ^{19}F NMR ppm: −118.93 (m, 1F), −123.81 (m, 1F); HPLC purity (retention time): 88.1% (2.36 min); HRMS calcd for $C_{21}H_{25}O_3N_3S_1F_2$ (M^+ + H) 438.1663, found 438.1642.

***N*-(5-{[Amino(imino)methyl]amino}pentyl)-2-{3-[(benzylsulfonyl)amino]-2,6-difluorophenyl}acetamide (29f).** General procedure E afforded 41.2 mg (88%) of a white solid of product **29f**; ^{1}H NMR ppm: 0.83 (m, 6H), 2.71 (m, 2H), 3.05 (s, 2H), 3.16 (m, 2H), 3.68 (s, 2H), 6.25 (m, 1H), 6.74 (m, 8H); HPLC purity (retention time): >99% (2.66 min); HRMS calcd for $C_{21}H_{27}O_3N_5S_1F_2$ (M^+ + H) 468.1881, found 468.1842.

2-{3-[(Benzylsulfonyl)amino]-2,6-difluorophenyl}-*N*-[3-(1*H*-imidazol-1-yl)propyl]acetamide (29g). General procedure D afforded 55.5 mg (100%) of a clear oil of product **29g**; ^{1}H NMR ppm: 1.97 (m, 2H), 3.24 (m, 2H), 3.58 (s, 2H), 3.94 (m, 2H), 4.34 (s, 2H), 6.61 (m, 1H), 7.71 (m, 2H), 6.89 (m, 3H), 7.32 (m, 7H), 8.02 (s, 1H); ^{19}F NMR ppm: −118.56 (m, 1F), −125.29 (m, 1F); HPLC purity (retention time): 51.0% (2.28 min); HRMS calcd for $C_{21}H_{22}O_3N_4S_1F_2$ (M^+ + H) 449.1459, found 449.1455.

***N*-{4-[Amino(imino)methyl]benzyl}-2-{2,6-difluoro-3-[(2-phenylethyl)amino]phenyl}acetamide (30b).** General Procedure G afforded 0.58 g (82%) of a yellow oil of product **30b**; ^{1}H NMR ppm: 3.16 (t, 2H), 3.72 (m, 2H), 3.86 (s, 2H), 4.66 (s, 2H), 7.32 (m, 7H), 7.68 (m, 3H), 7.82 (d, 2H), 8.78 (bs, 1H), 9.27 (bs, 1H); ^{19}F NMR ppm: −112.97 (m, 1F), −125.49 (m, 1F); HPLC purity (retention time): 63.3% (2.77 min); HRMS calcd for $C_{24}H_{24}O_1N_4F_2$ (M^+ + H) 423.1996, found 423.1953.

Methyl (2-Fluoro-6-hydroxy-3-nitrophenyl)acetate (32). The nitro compound **16** (18.3 g, 0.079 mol) was added to a solution of trimethylacetic acid **31** (24.3 g, 0.23 mol) and potassium carbonate (54.5 g, 0.39 mol) in dimethyl sulfoxide and the resulting solution stirred at 80 °C for 15 min. The reaction was cooled to room temperature and diluted with water and diethyl ether. The resulting solution was acidified with 2 N hydrochloric acid and extracted with diethyl ether. The solution was washed with brine, dried over magnesium sulfate, and filtered. The solvent was removed by evaporation to afford a mixture of two products. Fraction two of column chromatography (40% ethyl acetate−hexane) afforded 4.97 g (27%) of a yellow solid of product **32**; ^{1}H NMR ppm: 3.71 (s, 3H), 3.80 (d, 2H, J_{HF} = 1.7 Hz), 6.95 (dd, 1H, J_{HH} = 9.2 Hz, J_{HF} = 1.1 Hz), 8.05 (m, 1H); ^{19}F NMR ppm: −120.02 (d, 1F, J_{HF} = 8.7 Hz); HPLC purity (retention time): >99% (2.25 min); HRMS calcd for C9H8O5N1F1 (M^+ + NH_4) 247.0730, found 247.0700.

Methyl (6-Fluoro-2-hydroxy-3-nitrophenyl)acetate (33). Following the same procedure described for **32**, fraction one of column chromatography (40% ethyl acetate−hexane) afforded 12.1 g (67%) of a yellow solid of product **33**; ^{1}H NMR ppm: 3.71 (s, 3H), 3.81 (s, 2H), 6.80 (m, 1H), 8.18 (m, 1H); ^{19}F NMR ppm: −99.71 (m, 1F); HPLC purity (retention time): >99% (2.63 min); HRMS calcd for $C_9H_8O_5N_1F_1$ (M^+ + NH_4) 247.0730, found 247.0700.

Methyl (6-Fluoro-2-methoxy-3-nitrophenyl)acetate (34). Iodomethane (20.0 mL, 0.32 mol) was added to a solution of the phenol **33** (5.0 g, 0.022 mol) and potassium carbonate (9.0 g, 0.065 mol) in tetrahydrofuran. After stirring at 65 °C for 18 h, the solution was diluted with water and extracted with diethyl ether. The organic layer was washed with brine, dried over magnesium sulfate, and filtered. The solvent was removed by evaporation to afford the crude product. The product was purified by column chromatography (15% ethyl acetate−hexane) to afford 4.18 g (79%) of a yellow oil of product **34**; ^{1}H NMR ppm: 3.76 (s, 3H), 3.70 (d, 2H, J_{HF} = 1.5 Hz), 3.93 (s, 3H), 7.00 (m, 1H), 7.94 (m, 1H); ^{19}F NMR ppm: −103.51 (m, 1F); HPLC purity (retention time): >99% (2.60 min); HRMS calcd for $C_{10}H_{10}O_5N_1F_1$ (M^+ + NH_4) 261.0887, found 261.0905.

Methyl (6-hydroxy-2-methoxy-3-nitrophenyl)acetate (36). Aqueous sodium hydroxide (10%) (25 mL, 0.062 mol) was added to a solution of the fluoro compound **34** (4.0 g, 0.016

mol) in tetrahydrofuran and the resulting solution stirred at 90 °C for 14 h. The solution was acidified with 4 N hydrochloric acid and extracted with diethyl ether. The solution was washed with brine, dried over magnesium sulfate, and filtered. The solvent was removed by evaporation to afford the crude product **35** in which fluorine NMR revealed no signal. The product was dissolved into dichloromethane and oxalyl chloride (7.0 mL, 0.080 mol) was added to the solution followed by a drop of dimethylformamide. After being stirred at room temperature for 1.5 h, the solvent was removed by evaporation to afford the acid chloride. The acid chloride was redissolved into dichloromethane, and an excess of methanol (50 mL) was added followed by addition of pyridine (2.6 mL, 0.032 mol). The solution stirred at room temperature for 5 h. The solution was washed with water and brine, dried over magnesium sulfate, and filtered. The solvent was removed by evaporation to afford a mixture of two products. Fraction two of column chromatography (25% ethyl acetate−hexane) afforded 1.33 g (34%) of a white solid of product **36**; ^{1}H NMR ppm: 3.84 (s, 3H), 3.85 (s, 2H), 3.94 (s, 3H), 6.78 (d, 1H, J = 8.9 Hz), 7.90 (d, 1H, J = 8.9 Hz), 8.19 (s, 1H); HPLC purity (retention time): >99% (2.14 min); HRMS calcd for $C_{11}H_{13}O_6N_1$ ($M^+ + NH_4$) 259.0930, found 259.0921.

General Procedure H. Triflate Formation. Triethylamine (1.2 mmol) was added to a solution of the phenol (1.0 mmol) and triflic anhydride (1.1 mmol) in dichloromethane at −10 °C. After being stirred at room temperature for 1−18 h, the solution was acidified with hydrochloric acid 2 N and extracted with dichloromethane. The organic layer was washed with brine, dried over magnesium sulfate, and filtered. The solvent was removed by evaporation to afford the product. When necessary, the product was purified by column chromatography.

Methyl (2-Fluoro-3-nitro-6-{[(trifluoromethyl)sulfonyl]-oxy}phenyl)acetate (40). General procedure H. Using triethylamine (729 uL, 5.2 mmol), phenol **32** (1.0 g, 4.36 mmol), and triflic anhydride (807 uL, 4.7 mmol) to afford the crude product. The product was purified by column chromatography (20% ethyl acetate−hexane) to afford 1.0 g (68%) of a clear oil of product **40**; ^{1}H NMR ppm: 3.79 (s, 3H), 3.90 (d, 2H, J_{HF} = 2.0 Hz), 7.38 (dd, 1H,, J_{HH} = 7.6 Hz, J_{HF} = 1.8 Hz), 8.18 (m, 1H); ^{19}F NMR ppm: −73.61 (s, 3F), −113.78 (m, 1F); HPLC purity (retention time): 94.6% (3.69 min); LRMS for $C_9H_8O_5N_1F_1$ (ES, m/z) 3.62 (M + H).

Methyl (2-Methoxy-3-nitro-6-{[(trifluoromethyl)sulfonyl]oxy}phenyl)acetate (41). General procedure H. Using triethylamine (910 uL, 6.5 mmol), phenol **36** (1.3 g, 5.5 mmol), and triflic anhydride (1.0 mL, 5.9 mmol) to afford the crude product. The product was purified by column chromatography (15% ethyl acetate−hexane) to afford 1.7 g (83%) of a clear oil of product **41**; ^{1}H NMR ppm: 3.78 (s, 3H), 3.87 (d, 2H), 3.96 (s, 3H), 7.27 (d, 1H, J = 9.2 Hz), 7.95 (d, 1H, J = 9.2 Hz); ^{19}F NMR ppm: −73.89 (s, 3F); HPLC purity (retention time): >99% (3.62 min); HRMS calcd for $C_{11}H_{10}O_8N_1F_3$ ($M^+ + NH_4$) 391.0423, found 391.0396.

General Procedure I. Stille Coupling. The triflate compound (1.0 mmol) was added to a solution of stannane (1.1 mmol), lithium chloride (3.0 mmol), and tetrakis(triphenylphosphine)palladium(0) (0.02 mmol) in anhydrous dioxane (0.2 mM). The solution was heated to 85 °C for one to several hours. The solution was allowed to cool to room temperature followed by addition of diethyl ether and water. The organic layer was washed with brine, dried over magnesium sulfate, and filtered. The solvent was removed by evaporation to afford the product. When necessary, the product was purified by column chromatography.

Methyl (3-Fluoro-4-nitro-1,1′-biphenyl-2-yl)acetate (42a). General procedure I. Using triflate **40** (1.0 g, 2.76 mmol), tri-*n*-butylphenylstannane **39a** (1.0 mL, 3.0 mmol), lithium chloride (351 mg, 8.28 mmol), and tetrakis(triphenylphosphine)palladium(0) (63.9 mg, 0.055 mmol) to afford the crude product. The product was purified by column chromatography (20% ethyl acetate−hexane) to afford 0.62 g (78%) of a yellow oil of product **42a**; ^{1}H NMR ppm: 3.73 (s, 5H), 7.28 (m, 3H), 7.49 (m, 3H), 8.07 (m, 1H); ^{19}F NMR ppm: −118.75 (m, 1F); HPLC purity (retention time): >99% (3.81 min); HRMS calcd for $C_{15}H_{12}O_4N_1F_1$ ($M^+ + NH_4$) 307.1094, found 307.1093.

Methyl (3′-{[(Benzyloxy)carbonyl]amino}-3-fluoro-4-nitro-1,1′-biphenyl-2-yl)acetate (42b). General procedure I. Using triflate **40** (6.5 g, 0.018 mol), the stannane **39b** (11.2 mL, 0.022 mol), lithium chloride (2.2 g, 0.051 mol), and tetrakis(triphenylphosphine)palladium(0) (410 mg, 0.35 mmol) to afford a mixture of products. Fraction two of column chromatography (30% ethyl acetate−hexane) afforded 3.8 g (48%) of an orange oil of product **42b**; ^{1}H NMR ppm: 3.71 (m, 5H), 5.21 (s, 2H), 6.86 (bs, 1H), 6.99 (m, 1H), 7.23 (d, 1H), 7.37 (m, 8H), 8.03 (t, 1H); ^{19}F NMR ppm: −118.69 (m, 1F); HPLC purity (retention time): >99% (4.09 min); HRMS calcd for $C_{23}H_{19}O_6N_2F_1$ ($M^+ + NH_4$) 456.1571, found 456.1564.

Methyl (3-Methoxy-4-nitro-1,1′-biphenyl-2-yl)acetate (42c). General procedure I. Using triflate **41** (5.53 g, 14.8 mmol), *tri-n*-butylphenylstannane **39a** (5.8 mL, 17.7 mmol), lithium chloride (1.9 g, 44.8 mmol), and tetrakis(triphenylphosphine)palladium(0) (34.0 mg, 0.29 mmol) to afford the crude product. The product was purified by column chromatography (15% ethyl acetate−hexane) to afford 3.32 g (74%) of a white solid of product **42c**; ^{1}H NMR ppm: 3.69 (s, 5H), 3.94 (s, 3H), 7.17 (d, 1H, J = 8.3 Hz), 7.29 (m, 2H), 7.45 (m, 3H), 7.89 (d, 1H, J = 8.3 Hz); HPLC purity (retention time): >99% (3.60 min); HRMS calcd for $C_{16}H_{15}O_5N_1$ ($M^+ + NH_4$) 319.1294, found 319.1312.

Methyl (4-Amino-3-fluoro-1,1′-biphenyl-2-yl)acetate (43a). General procedure B. Using the nitro compound **42a** (0.60 g, 2.0 mmol) and powdered iron (0.60 g, 10.7 mmol) to afford 0.47 g (91%) of a clear oil of product **43a**; ^{1}H NMR ppm: 3.64 (d, 2H, J_{HF} = 2.3 Hz), 3.71 (s, 3H), 6.83 (m, 1H), 7.27 (dd, 1H), 7.37 (m, 5H); ^{19}F NMR ppm: −137.27 (d, 1F, J_{HF} = 8.7 Hz); HPLC purity (retention time): 93.2% (2.76 min); HRMS calcd for $C_{15}H_{14}O_2N_1F_1$ ($M^+ + NH_4$) 277.1352, found 277.1337.

Methyl (4-Amino-3′-{[(benzyloxy)carbonyl]amino}-3-fluoro-1,1′-biphenyl-2-yl)acetate (43b). General procedure I. Using the triflate **40** (1.31 g, 3.6 mmol), stannane **39b** (2.17 mL, 4.2 mmol), lithium chloride (440 mg, 10.0 mmol), and tetrakis(triphenylphosphine)palladium(0) (80.8 mg, 0.070 mmol) in 18 mL of anhydrous dioxane. The solution was heated to 85 °C for 21 h. The organic layer was washed with brine, dried over magnesium sulfate, and filtered. The solvent was removed by evaporation to afford the crude product. The product was purified by column chromatography (40% ethyl acetate−hexane) to afford 0.56 g (38%) of a yellow oil of product **43b**; ^{1}H NMR ppm: 3.64 (d, 2H), 3.70 (s, 3H), 5.23 (s, 2H), 6.90 (m, 4H), 7.40 (m, 8H); ^{19}F NMR ppm: −136.68 (d, 1F); HPLC purity (retention time): >99% (3.50 min); HRMS calcd for $C_{23}H_{21}O_4N_2F_1$ ($M^+ + NH_4$) 426.1829, found 426.1846.

Methyl (4-Amino-3-methoxy-1,1′-biphenyl-2-yl)acetate (43c). General procedure B. Using the nitro compound **42c** (1.0 g, 3.3 mmol) and powdered iron (0.92 g, 16.4 mmol) to afford the product **43c**. The product was not purified and carried on to the next step. HPLC purity (retention time): >99% (2.48 min); HRMS calcd for $C_{16}H_{17}O_3N_1$ ($M^+ + H$) 272.1278, found 272.1325.

Methyl {3-Fluoro-4-[(2-phenylethyl)amino]-1,1′-biphenyl-2-yl}acetate (47a). General procedure F. Using sodium triacetoxyborohydride (1.7 g, 8.0 mmol), aniline **43a** (0.051 g, 2.0 mmol), and phenylacetaldehyde **45** (281 uL, 0.015 mol) to afford 0.58 g (80%) of a yellow oil of product **47a**; ^{1}H NMR ppm: 3.02 (t, 2H), 3.51 (m, 2H), 3.64 (d, 2H, J_{HF} = 2.9 Hz), 3.70 (s, 3H), 6.80 (m, 1H), 7.02 (dd, 1H), 7.31 (m, 10H); ^{19}F NMR ppm: −138.50 (d, 1F); HPLC purity (retention time): 83.2% (4.69 min); HRMS calcd for $C_{23}H_{22}O_2N_1F_1$ ($M^+ + H$) 364.1713, found 364.1703.

Methyl (4-(Benzylamino)-3′-{[(benzyloxy)carbonyl]-amino}-3-fluoro-1,1′-biphenyl-2-yl)acetate (47b). General procedure F. Using sodium triacetoxyborohydride (1.12 g, 5.28 mmol), aniline **43b** (0.54 g, 1.32 mmol), and benzaldehyde **44** (147.8 uL, 1.45 mmol) to afford the crude product. The product

was purified by column chromatography (20% ethyl acetate−hexane) to afford 0.44 g (67%) of a yellow oil of product **47b**; ^{1}H NMR ppm: 3.65 (d, 2H), 3.71 (s, 3H), 4.44 (s, 2H), 5.23 (s, 2H), 6.72 (m, 2H), 6.97 (m, 2H), 7.36 (m, 8H); ^{19}F NMR ppm: −138.06 (d, 1F); HPLC purity (retention time): >99% (4.58 min); HRMS calcd for $C_{30}H_{27}O_4N_2F_1$ ($M^+ + H$) 499.2033, found 499.2018.

Methyl [3′-{[(Benzyloxy)carbonyl]amino}-3-fluoro-4-(isopropylamino)-1,1′-biphenyl-2-yl]acetate (47c). General procedure F. Using sodium triacetoxyborohydride (5.6 g, 26.4 mmol), aniline **43b** (2.69 g, 6.6 mmol), and acetone **46** (8.0 mL, excess) to afford the crude product. The product was purified by column chromatography (30% ethyl acetate−hexane) to afford 1.34 g (45%) of a white solid of product **47c**; ^{1}H NMR ppm: 1.30 (d, 6H), 3.62 (d, 2H), 3.68 (s, 3H), 4.13 (m, 1H), 5.21 (s, 2H), 6.70 (m, 2H), 6.99 (m, 2H), 7.36 (m, 9H); HPLC purity (retention time): >99% (3.84 min); HRMS calcd for $C_{26}H_{27}O_4N_2F_1$ ($M^+ + H$) 451.2033, found 451.2023.

Methyl {3-methoxy-4-[(2-phenylethyl)amino]-1,1′-biphenyl-2-yl}acetate (47d). General procedure F. Using sodium triacetoxyborohydride (2.8 g, 13.2 mmol), aniline **43c** (0.89 g, 3.3 mmol), and phenylacetaldehyde **45** (540 uL, 39.2 mmol) to afford the crude product. The product was purified by column chromatography (25% ethyl acetate−hexane) to afford 0.69 g (56%) of a yellow oil of product **47d**; ^{1}H NMR ppm: 3.04 (t, 2H), 3.50 (t, 2H), 3.69 (s, 3H), 3.67 (s, 2H), 3.69 (s, 3H), 6.75 (d, 1H, J = 8.3 Hz), 7.02 (d, 1H, J = 8.3 Hz), 7.35 (m, 10H); HPLC purity (retention time): >99% (4.37 min); HRMS calcd for $C_{24}H_{25}O_3N_1$ ($M^+ + H$) 376.1913, found 376.1872.

Methyl [4-(isopropylamino)-3-methoxy-1,1′-biphenyl-2-yl]acetate (47e). General procedure F. Using sodium triacetoxyborohydride (9.3 g, 43.0 mmol), aniline **43c** (2.99 g, 11.0 mmol), and acetone **46** (1.0 mL, 13.6 mmol) to afford the crude product. The product was purified by column chromatography (15% ethyl acetate−hexane) to afford 3.55 g (100%) of a yellow oil of product **47e**; ^{1}H NMR ppm: 1.24 (d, 6H), 3.59 (s, 3H), 3.62 (m, 2H), 3.72 (s, 3H), 4.09 (m, 1H), 6.62 (d, 1H, J = 8.3 Hz), 6.92 (d, 1H, J = 8.3 Hz), 7.28 (m, 5H); HPLC purity (retention time): 97.5% (2.57 min); HRMS calcd for $C_{19}H_{23}O_3N_1$ ($M^+ + H$) 314.1756, found 314.1758.

{3-Fluoro-4-[(2-phenylethyl)amino]-1,1′-biphenyl-2-yl}acetic Acid (48a). General procedure C. Using sodium hydroxide (10%) (2.5 mL, 6.2 mmol) and methyl ester **47a** (0.58 g, 1.6 mmol) to afford 0.47 g (84%) of an orange glass of product **48a**; ^{1}H NMR ppm: 3.02 (t, 2H), 3.51 (m, 2H), 3.68 (d, 2H, J_{HF} = 2.6 Hz), 6.84 (m, 1H), 7.04 (dd, 1H), 7.31 (m, 10H); ^{19}F NMR ppm: −137.47 (d, 1F); HPLC purity (retention time): >99% (4.08 min); HRMS calcd for $C_{22}H_{20}O_2N_1F_1$ ($M^+ + H$) 350.1556, found 350.1532.

(4-(Benzylamino)-3′-{[(benzyloxy)carbonyl]amino}-3-fluoro-1,1′-biphenyl-2-yl)acetic Acid (48b). General procedure C. Using sodium hydroxide (10%) (1.4 mL, 3.5 mmol) and methyl ester **47b** (0.44 g, 0.88 mmol) to afford 0.29 g (100%) of a white solid of two products. Partial exchange of benzyloxy with methoxy of the Cbz group occurred resulting in a mixture of two products. The mixture of products were not separated and carried on to the next.

[3′-{[(Benzyloxy)carbonyl]amino}-3-fluoro-4-(isopropylamino)-1,1′-biphenyl-2-yl]acetic Acid (48c). Aqueous sodium hydroxide (10%) (4.3 mL, 10.0 mmol) was added to a solution of the methyl ester **47c** (1.21 g, 2.6 mmol) in tetrahydrofuran and the resulting solution stirred at 60 °C for 48 h. Partial Cbz deprotection occurred so, benzyl chloroformate (382 uL, 2.6 mmol) was added to the solution and the resulting solution stirred at room temperature for 1 h. The solution was acidified with 2 N hydrochloric acid and extracted with diethyl ether. The solution was washed with brine, dried over magnesium sulfate, and filtered. The solvent was removed by evaporation to afford the crude product. The product was purified by column chromatography (50% ethyl acetate−hexane) to afford 0.24 g (21%) of a brown oil of product **47c**; ^{1}H NMR ppm: 1.28 (d, 6H), 3.65 (d, 2H), 4.12 (m, 1H), 5.19 (s, 2H), 6.69 (m, 1H), 6.97 (m, 2H), 7.36 (m, 9H); HPLC purity (retention time): >99% (2.84 min); LRMS for $C_{25}H_{25}O_4N_2F_1$ (ES, m/z) 437 (M + H).

{3-Methoxy-4-[(2-phenylethyl)amino]-1,1′-biphenyl-2-yl}acetic Acid (48d). General procedure C. Using sodium hydroxide (10%) (2.9 mL, 7.2 mmol) and methyl ester **47d** (0.69 g, 1.8 mmol) to afford 0.66 g (100%) of an orange foam of product **48d**; HPLC purity (retention time): 80% (3.83 min).

[4-(Isopropylamino)-3-methoxy-1,1′-biphenyl-2-yl]acetic Acid (48e). General procedure C. Using sodium hydroxide (10%) (7.6 mL, 19.0 mmol) and methyl ester **47e** (1.51 g, 4.81 mmol) to afford the crude product. The product was purified by column chromatography (40% ethyl acetate−hexane) to afford 0.44 g (31%) of a white solid of product **48e**; ^{1}H NMR ppm: 1.25 (d, 6H), 3.62 (m, 3H), 3.75 (s, 3H), 6.50 (d, 1H, J = 8.3 Hz), 6.94 (d, 1H, J = 8.3 Hz), 7.27 (m, 5H); HPLC purity (retention time): >99% (2.11 min); HRMS calcd for $C_{18}H_{21}O_3N_1$ ($M^+ + H$) 300.1600, found 300.1587.

Benzyl (4-{[({3-Fluoro-4-[(2-phenylethyl)amino]-1,1′-biphenyl-2-yl}acetyl)amino]methyl}phenyl)(imino)methylcarbamate (49a). General procedure D. Using 1-hydroxybenzotriazole **11** (180 mg, 1.3 mmol), acid **48a** (0.47 g, 1.34 mmol), PS-carbodiimide (1.00 mmol/g) (2.6 g, 2.6 mmol), amine (0.51 g, 1.6 mmol), and *N*-methylmorpholine (295 uL, 2.6 mmol) to afford the product. The product was purified by column chromatography (60% ethyl acetate−hexane) to afford 0.79 g (96%) of a white solid of product **49a**; ^{1}H NMR ppm: 2.92 (m, 2H), 3.19 (m, 2H), 3.77 (d, 2H), 4.67 (d, 2H), 5.40 (s, 2H), 5.60 (bm, 1H), 7.08 (m, 1H), 7.15 (dd, 1H), 7.54 (m, 16H), 8.28 (m, 3H), 8.59 (bt, 1H), 9.40 (bs, 1H), 9.65 (bs, 1H); ^{19}F NMR ppm: 138.15 (d, 1F); HPLC purity (retention time): >99% (4.00 min); HRMS calcd for $C_{22}H_{20}O_2N_1F_1$ ($M^+ + H$) 615.2771, found 615.2760.

Benzyl [4-({[(4-(Benzylamino)-3′-{[(benzyloxy)carbonyl]amino}-3-fluoro-1,1′-biphenyl-2-yl)acetyl]amino}methyl)phenyl](imino)methylcarbamate (49b). General procedure D. Using PS-carbodiimide (1.00 mmol/g) (1.4 g, 1.4 mmol), acid **48b** (0.29 g, 0.71 mmol), 1-hydroxybenzotriazole **11** (95.9 mg, 0.71 mmol), amine (0.27 g, 0.84 mmol), and *N*-methylmorpholine (624 uL, 5.6 mmol) to afford a the crude product. The product was purified by reverse-phase chromatography to afford 180 mg (34%) of an orange oil of product **49b**; ^{1}H NMR ppm: 3.68 (m, 4H), 4.34 (s, 2H), 5.08 (s, 2H), 5.26 (s, 2H), 6.68−7.60 (m, 28H); ^{19}F NMR ppm: −136.67 (bs, 1F); HPLC purity (retention time): 61% (4.15 min); HRMS calcd for $C_{45}H_{40}O_5N_5F_1$ ($M^+ + H$) 672.2622, found 672.2604.

Benzyl {4-[({[3′-{[(Benzyloxy)carbonyl]amino}-3-fluoro-4-(isopropylamino)-1,1′-biphenyl-2-yl]acetyl}amino)methyl]phenyl}(imino)methylcarbamate (49c). General procedure D. Using PS-carbodiimide (1.00 mmol/g) (0.59 g, 0.59 mmol), acid **48c** (0.13 g, 0.29 mmol), 1-hydroxybenzotriazole **11** (40.2 mg, 0.29 mmol), amine (0.11 g, 0.34 mmol), and *N*-methylmorpholine (163 uL, 1.4 mmol) to afford the crude product. The product was carried on to the next step. HPLC purity (retention time): 79% (2.77 min); HRMS calcd for $C_{41}H_{40}O_5N_5F_1$ ($M^+ + H$) 702.3092, found 702.3114.

Benzyl Imino(4-{[({3-methoxy-4-[(2-phenylethyl)amino]-1,1′-biphenyl-2-yl}acetyl)amino]methyl}phenyl)methylcarbamate (49d). General procedure D. Using PS-carbodiimide (1.00 mmol/g) (3.6 g, 3.6 mmol), acid **48d** (0.66 g, 1.83 mmol), 1-hydroxybenzotriazole **11** (247 mg, 1.8 mmol), amine (0.70 g, 2.2 mmol), and *N*-methylmorpholine (1.0 mL, 9.0 mmol) to afford the product. The product was purified by column chromatography (60% ethyl acetate−hexane) to afford 0.52 g (45%) of a white solid of product **49d**; ^{1}H NMR ppm: 3.17 (m, 2H), 3.65 (m, 2H), 3.77 (s, 2H), 3.86 (s, 3H), 4.64 (d, 2H), 5.27 (bt, 1H), 4.40 (s, 2H), 6.96 (d, 1H), 7.11 (d, 1H), 7.56 (m, 16H), 8.23 (m, 3H), 8.41 (bt, 1H), 9.40 (bs, 1H), 9.75 (bs, 1H); HPLC purity (retention time): >99% (3.80 min); HRMS calcd for $C_{39}H_{38}O_4N_4$ ($M^+ + H$) 627.2971, found 627.2918.

Benzyl Imino{4-[({[4-(isopropylamino)-3-methoxy-1,1′-biphenyl-2-yl]acetyl}amino)methyl]phenyl}methylcarbamate (49e). General procedure D. Using PS-carbodiimide (1.00 mmol/g) (2.8 g, 2.8 mmol), acid **48e** (0.42 g, 1.4 mmol), 1-hydroxybenzotriazole **11** (0.19 mg, 1.4 mmol), amine

(0.54 g, 1.6 mmol), and *N*-methylmorpholine (620 uL, 5.6 mmol) to afford the crude product. The product was purified by column chromatography (60% ethyl acetate−hexane) but stuck to the column and was washed off with 100% ethyl acetate to afford 0.73 g (92%) of a white solid of product **49e**; ^{1}H NMR ppm: 1.25 (d, 6H), 3.62 (m, 3H), 3.71 (s, 3H), 4.33 (d, 2H), 5.19 (s, 2H), 6.05 (bt, 1H), 6.63 (d, 2H, J = 8.3 Hz), 6.93 (d, 2H, J = 8.3 Hz), 7.29 (m, 13H), 7.77 (d, 2H); HPLC purity (retention time): >99% (2.55 min); HRMS calcd for $C_{34}H_{36}O_4N_4$ (M^+ + H) 565.2815, found 565.2839.

***N*-{4-[Amino(imino)methyl]benzyl}-2-{3-fluoro-4-[(2-phenylethyl)amino]-1,1′-biphenyl-2-yl}acetamide (50a).** A catalytic amount of palladium on carbon (10%) in dioxane was added to a methanol−4 N hydrochloric acid/dioxane (3:1) solution of the Cbz compound **49a** (200 mg, 0.32 mmol) and the mixture was stirred under a balloon of hydrogen at room temperature for 12 h. The mixture was filtered through Celite, and the solvent was evaporated to afford the product. The product was purified by reverse-phase chromatography to afford 142 mg (92%) of a white solid of product **50a**; ^{1}H NMR ppm: 2.97 (t, 2H), 3.48 (t, 2H), 3.61 (d, 2H), 4.64 (d, 2H), 6.80 (m, 1H), 6.97 (dd, 1H), 7.33 (m, 11H), 7.47 (d, 2H), 7.79 (d, 2H); ^{19}F NMR ppm: −77.60 (s, 6F), −139.78 (d, 1F); HPLC purity (retention time): >99% (3.22 min); HRMS calcd for $C_{30}H_{29}O_1N_4F_1$ (M^+ + H) 481.2398, found 481.2413.

2-[3′-Amino-4-(benzylamino)-3-fluoro-1,1′-biphenyl-2-yl]-*N*-{4-[amino(imino)methyl]benzyl}acetamide (50b). Hydrogen bromide in acetic acid (30 wt %) was added to the carbamate **49b** and the solution stirred at room temperature for 16 h. The solution was evaporated to afford a mixture of two products. Fraction one of reverse-phase chromatography afforded 32 mg (52%) of an orange solid of product **50b**; ^{1}H NMR ppm: 3.64 (s, 3H), 4.36 (d, 2H), 4.45 (s, 2H), 6.64 (m, 1H), 6.84 (dd, 1H), 7.37 (m, 13H), 7.67 (d, 2H), 7.90 (bs, 2H), 8.97 (bs, 2H); ^{19}F NMR ppm: −76.96 (s, 9F), −138.72 (d, 1F); HPLC purity (retention time): >99% (2.62 min); HRMS calcd for $C_{29}H_{28}O_1N_5F_1$ (M^+ + H) 482.2356, found 482.2332.

General Procedure J. Hydrogenation. A catalytic amount of palladium on carbon (5%) in methanol was added to a methanol solution of the Cbz compound and the mixture was stirred under a balloon of hydrogen at room temperature for one to several hours. The mixture was filtered through Celite, and the solvent was evaporated to afford the product. When necessary, the product was purified by column chromatography.

2-[3′-Amino-3-fluoro-4-(isopropylamino)-1,1′-biphenyl-2-yl]-*N*-{4-[amino(imino)methyl]benzyl}acetamide (50c). General procedure J afforded 122.3 mg (95%) of a white solid of product **50c**; ^{1}H NMR ppm: 1.68 (d, 6H), 4.04 (d, 2H), 4.15 (sept, 1H), 4.85 (d, 2H), 7.27 (m, 6H), 7.65 (t, 1H), 7.83 (d, 2H), 8.07 (bt, 1H), 8.24 (d, 2H), 9.20 (bs, 1H), 10.38 (bs, 1H); ^{19}F NMR ppm: −76.41 (s, 9F), −138.02 (d, 1F); HPLC purity (retention time): >99% (1.37 min); HRMS calcd for $C_{25}H_{29}O_1N_5F_1$ (M^+ + H) 434.2356, found 434.2360.

***N*-{4-[Amino(imino)methyl]benzyl}-2-{3-methoxy-4-[(2-phenylethyl)amino]-1,1′-biphenyl-2-yl}acetamide (50d).** General procedure J afforded 14.6 mg (81%) of a white solid of product **50d**; ^{1}H NMR ppm: 3.00 (t, 2H), 3.50 (t, 2H), 3.63 (s, 2H), 3.67 (s, 3H), 4.41 (s, 2H), 6.86 (m, 1H), 6.96 (m, 1H), 7.35 (m, 10H), 7.46 (d, 2H), 7.77 (d, 2H), 8.21 (bs, 1H); HPLC purity (retention time): >99% (3.29 min); HRMS calcd for $C_{31}H_{32}O_2N_4$ (M^+ + H) 493.2604, found 493.2626.

***N*-{4-[Amino(imino)methyl]benzyl}-2-[4-(isopropylamino)-3-methoxy-1,1′-biphenyl-2-yl]acetamide (50e).** General procedure J afforded 120 mg (79%) of a purple-white solid of product **50e**; ^{1}H NMR ppm: 1.41 (m, 6H), 3.65 (s, 2H), 3.82 (m, 1H), 3.95 (s, 3H), 4.39 (s, 2H), 6.98 (d, 1H), 7.19 (d, 1H), 7.42 (m, 7H), 7.78 (d, 2H); HPLC purity (retention time): >99% (1.50 min); HRMS calcd for $C_{26}H_{30}O_2N_4$ (M^+ + H) 431.2447, found 431.2447.

***N*-{4-[Amino(imino)methyl]benzyl}-2-{3-hydroxy-4-[(2-phenylethyl)amino]-1,1′-biphenyl-2-yl}acetamide (51).** General procedure G. Using compound **49d** (0.10 g, 0.16 mmol), sodium iodide (0.19 g, 1.2 mmol), and trimethylsilyl chloride (162 uL, 1.2 mmol) to afford a mixture of two products. Fraction one of reverse-phase chromatography afforded 4.5 mg (4%) of a white solid of product **51**; ^{1}H NMR ppm: 3.12 (t, 2H), 3.66 (m, 4H), 4.51 (s, 2H), 6.96 (d, 1H), 7.36 (m, 12H), 7.55 (d, 2H), 7.80 (d, 2H); HPLC purity (retention time): 92% (2.53 min); HRMS calcd for $C_{30}H_{30}O_2N_4$ (M^+ + H) 479.2447, found 479.2446.

4-Phenyl-7-[(2-phenylethyl)amino]-1-benzofuran-2(3*H*)-one (52). Following the same procedure described for **51**, fraction two of reverse-phase chromatography afforded 17.7 mg (32%) of a white solid of product **52**; ^{1}H NMR ppm: 3.06 (t, 2H), 3.63 (t, 2H), 3.88 (s, 2H), 7.09 (d, 1H), 7.39 (m, 11H); HPLC purity (retention time): >99% (4.33 min); HRMS calcd for $C_{22}H_{22}O_2N_2$ (M^+ + H) 330.1494, found 330.1492.

***N*-{4-[Amino(imino)methyl]benzyl}-2-[3-hydroxy-4-(isopropylamino)-1,1′-biphenyl-2-yl]acetamide (53).** Boron tribromide 1 M (2.7 mL, 2.7 mmol) was added to a solution of the methyl ether **49e** (0.50 g, 0.88 mmol) in dichloromethane at −10 °C. The solution stirred at −10 °C for 1.5 h. The solution was quenched with water and evaporated to a minimal amount of solvent. The crude product mixture was purified directly by reverse-phase chromatography to afford a mixture of two products. Fraction one of reverse-phase chromatography afforded 0.22 mg (60%) of a purple-white solid of product **53**; ^{1}H NMR ppm: 1.41 (d, 6H), 3.68 (s, 2H), 3.89 (m, 1H), 4.28 (s, 2H), 6.97 (m, 1H), 7.25−7.92 (m, 11H); HPLC purity (retention time): >99% (1.42 min); HRMS calcd for $C_{25}H_{28}O_2N_4$ (M^+ + H) 417.2291, found 417.2295.

***N*-{4-[Amino(imino)methyl]benzyl}-2-[5-(isopropylamino)-3,6-dioxo-2-phenylcyclohexa-1,4-dien-1-yl]acetamide (54).** A solution of potassium nitrosodisulfonate (Fremy's salt) (0.15 g, 0.56 mol) in 0.5 mL of water was added to a solution of the phenol **53** (76.9 mg, 0.18 mmol) in 4 mL of a mixture of tetrahydrofuran and water (1:1). The solution stirred at room temperature for 2 h (turning deep red-brown). The solution was diluted with a saturated solution of sodium bicarbonate and extracted with diethyl ether. The organic layer was washed with brine, dried over magnesium sulfate, and filtered. The solvent was removed by evaporation to afford the crude product. The product was purified by reverse-phase chromatography to afford 4.2 mg (5%) of a red-brown solid of product **54**; ^{1}H NMR ppm: 1.25 (d, 6H), 3.69 (s, 2H), 3.71 (m, 1H), 4.38 (s, 2H), 7.28 (m, 3H), 7.38 (m, 5H), 7.75 (d, 2H), 7.89 (bs, 1H), 7.99 (bs, 1H); HPLC purity (retention time): >99% (1.52 min); HRMS calcd for $C_{25}H_{26}O_3N_4$ (M^+ + H) 431.2083, found 431.2102.

Assays for Biological Activity. Recombinant soluble TF, consisting of amino acids 1−219 of the mature protein sequence was expressed in E. coli and purified using a Mono Q Sepharose FPLC. Recombinant human VIIa was purchased from American Diagnostica, Greenwich CT and chromogenic substrate *N*-methylsulfonyl-D-phe-gly arg-*p*-nitroaniline was prepared by American Peptide Company, Inc., Sunnyvale, CA. Factor Xa was obtained from Enzyme Research Laboratories, South Bend, IN, thrombin from Calbiochem, La Jolla, CA, and trypsin and L-BAPNA from Sigma, St. Louis, MO. The chromogenic substrates S-2765 and S-2238 were purchased from Chromogenix, Sweden.

TF/VIIa Assay. 100 nM Recombinant soluble tissue factor and 2 nM recombinant human Factor VIIa were added to a 96-well assay plate containing 0.4 mM of the substrate, *N*-methylsulfonyl-D-phe-gly-arg-*p*-nitroaniline and either inhibitor or buffer (5 mM $CaCl_2$, 50 mM Tris-HCl, pH 8.0, 100 mM NaCl, 0.1% BSA). The reaction, in a final volume of 100 μL, was measured immediately at 405 nm to determine background absorbance. The plate was incubated at room temperature for 60 min, at which time the rate of hydrolysis of the substrate was measured by monitoring the reaction at 405 nm for the release of *p*-nitroaniline. All compounds were assayed in duplicate at seven concentrations. Percent inhibition at each concentration was calculated from OD_{405nm} value from the experimental and control sample. IC_{50} values were calculated from a four-parameter logistic regression equation.

For each compound the individual IC_{50} values were within 10% of each other. The reported IC_{50} represents an average of the duplicates.

Factor Xa Assay. 0.3 nM Human Factor Xa and 0.15 mM *N*-α-benzyloxycarbonyl-D-arginyl-L-glycyl-L-arginine-*p*-nitroaniline dihydrochloride (S-2765) were added to a 96-well assay plate containing either inhibitor or buffer (50 mM Tris-HCl, pH 8.0, 100 mM NaCl, 0.1% BSA). The reaction, in a final volume of 100 μL, was measured immediately at 405 nm to determine background absorbance. The plate was incubated at room temperature for 60 min, at which time the rate of hydrolysis of the substrate was measured by monitoring the reaction at 405 nm for the release of *p*-nitroaniline. All compounds were assayed in duplicate at seven concentrations. Percent inhibition at each concentration was calculated from OD_{405nm} value from the experimental and control sample. IC_{50} values were calculated from a four-parameter logistic regression equation. For each compound the individual IC_{50} values were within 10% of each other. The reported IC_{50} represents an average of the duplicates.

Thrombin Assay. Human thrombin (0.28 nM) and H-D-phenylalanyl-L-pipecolyl-L-arginine-*p*-nitroaniline dihydrochloride (0.06 mM) were added to a 96-well assay plate containing either inhibitor or buffer (50 mM Tris-HCl, pH 8.0, 100 mM NaCl, 0.1% BSA). The reaction, in a final volume of 100 μL, was measured immediately at 405 nm to determine background absorbance. The plate was incubated at room temperature for 60 min, at which time the rate of hydrolysis of the substrate was measured by monitoring the reaction at 405 nm for the release of *p*-nitroaniline. All compounds were assayed in duplicate at seven concentrations. Percent inhibition at each concentration was calculated from OD_{405nm} value from the experimental and control sample. IC_{50} values were calculated from a four-parameter logistic regression equation. For each compound the individual IC_{50} values were within 10% of each other. The reported IC_{50} represents an average of the duplicates.

Crystal Structure. Tissue factor was expressed in *E. coli* and was refolded from inclusion bodies.[13] Factor VII was expressed in mammalian cells (BHK cells) and purified using a Ca^{2+}-dependent monoclonal antibody directed against the GLA domain of factor VII. It was then eluted with a buffer that contains 20 mM EDTA, followed by further purification using a Mono Q column. Factor VII was subsequently activated to factor VIIa using recombinant factor Xa. The protein was dialyzed into a 9% NaCl solution at pH 6.0 and stored at −80 °C.

Prior to crystallization experiments, Factor VIIa was buffer-exchanged to a storage solution of 50 mM Tris pH 7.5, 50 mM NaCl, and 2 mM $CaCl_2$ and concentrated to 6 mg/mL. Crystals of the TF−VIIa complex were obtained by sitting drop vapor diffusion experiments using a reservoir solution of 16−24% PEG 4K, 50−150 mM $MgCl_2$, and 50 mM citrate pH 7.5.

The crystals are orthorhombic, space group $P2_12_12_1$ with lattice lengths $a = 69.71$ Å, $b = 81.02$ Å and $c = 125.90$ Å for the fluorobenzene complex and $a = 69.8$ Å, $b = 81.25$ Å, and $c = 126.12$ Å for the benzoquinone complex. Complete diffraction data were measured from crystals of the two complexes at the 17ID IMCA beamline at the Advanced Photon Source, Argonne National Laboratories. The overall R_{symm} was 10.8% for the fluorobenzene (**50c**) complex to 2.4 Å resolution and 9.1% for the benzoquinone (**54**) complex to 2.22 Å resolution. The structure of the two complexes was solved by difference Fourier methods using the structure of the covalent complex of TF−VIIa with a peptidomimetic inhibitor.[7a]

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Supporting Information Available: Analytical and spectral characterization data for all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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