Design and Synthesis of Potent Nonpeptidic Farnesyltransferase Inhibitors Based on a Terphenyl Scaffold

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By modification of key carboxylate, hydrophobic, and zinc-binding groups projected from a sterically restricted terphenyl scaffold, a series of simple and nonpeptide mimetics of the Cys-Val-Ile-Met tetrapeptide substrate of protein farnesyltransferase (FTase) have been designed and synthesized. A crystal structure of 4-nitro-2-phenyl-3′-methoxycarbonylbiphenyl shows that the triphenyl fragment provides a large hydrophobic surface that potentially mimics the hydrophobic side chains of the three terminal residues in the tetrapeptide. 2-Phenyl-3-(*N*-(1-(4-cyanobenzyl)-1H-imidazol-5-yl)methyl)amino-3′carboxylbiphenyl, in which the free thiol group was replaced with a 1-(4-cyanobenzyl)imidazole group, shows submicromolar inhibition activity against FTase in vitro and inhibits H-Ras processing in whole cells.

Introduction

The four Ras proteins (H, N, K_A and K_B) are a family of 21 kDa GTPases encoded by three *ras* genes.[1] In normal cells, Ras cycles between its GTP (active)- and its GDP (inactive)-bound states and plays a crucial function by transducing intracellular signals from growth factor receptor tyrosine kinases to several signal transduction pathways, such as the PI3K/Akt and the Raf/MEK/Erk cascades.[2] Single amino acid substitutions at positions 12, 13, or 61 render Ras GTPase deficient and therefore lock it in its GTP-bound state resulting in uncontrolled cell growth. These mutations are found in over 30% of human cancers, particularly in 50% of colon and 95% of pancreatic cancers.[3] To fulfill its signaling function, Ras must associate with the plasma membrane. This translocation is facilitated in Ras, and many other small GTPases, by the attachment of prenyl and, in some cases, palmitoyl groups onto the surface of the protein.

Protein prenylation plays a crucial role in intracellular signal transduction. In this process, farnesyl (C_{15}) or geranylgeranyl (C_{20}) groups are covalently attached to a key cysteine residue, near the protein carboxyl terminus, through a thiol alkylation reaction that is catalyzed by the isoprenyltransferase enzymes.[4] Three classes of the enzymes catalyzing the prenylation of proteins have been identified in mammalian cells: protein farnesyltransferase (FTase), type I protein geranylgeranyltransferase (GGTase-I), and type II protein geranylgeranyltransferase (GGTase-II).[5−7] FTase catalyzes farnesylation of proteins (such as Ras, Lamin B, etc.) with a carboxyl terminal sequence CAAX, where C is cysteine, A is an aliphatic amino acid, and X is methionine, serine, alanine, glutamine, or cysteine. GGTase-I catalyzes geranylgeranylation of proteins (such as Rho, Rap, and Rac)[8] with similar sequences at the carboxyl terminus, except that the X residue is leucine, isoleucine, or phenylalanine. GGTase-II catalyzes the geranylgeranylation of Rab proteins, with particular specificity for CXC, CCXX, or XXCC C-terminal sequences.[9] Because the function of Ras is dependent on the increase in hydrophobicity that results from farnesylation, inhibition of FTase has become a target for blocking oncogenic Ras function and thus drug design in cancer chemotherapy.[10,11]

FTase is a heterodimeric zinc metalloenzyme composed of a 48 kDa α-subunit and a 46 kDa *β*-subunit. A crystal structure of FTase with a tetrapeptide mimetic Ac-Cys-Val-Ile-Met(Se)-OH and a farnesylpyrophosphate (FPP) analogue bound into the active site shows that the tetrapeptide adopts an extended conformation in which the cysteine thiol group coordinates to the catalytic zinc ion.[12] The terminal carboxylate of the peptide binds to Gln 167α through a hydrogen bond, and another hydrogen bond is formed between the C-terminal amide oxygen and the Arg 202*β*.

Over the past few years, remarkable progress has been made in the development of synthetic inhibitors for FTase. We and others have previously reported peptidomimetics and pseudopeptide derivatives of the CAAX tetrapeptide sequence.[9,13−18] These molecules showed high inhibitory potency for FTase; however, in some cases, they retain peptide characteristics such as a methionine or metabolically unstable cysteine residues. In this paper, we report the design and synthesis of a new series of CAAX mimetics as FTase inhibitors based on a fully nonpeptidic terphenyl scaffold.

Design

Tetrapeptide Cys-Val-Ile-Met (**1**, Figure 1), the C-terminal tetrapeptide sequence of K-Ras-4B, inhibits FTase in vitro (IC_{50} = 340 nM) but is not active in whole cells. In an attempt to improve its poor membrane permeability and metabolic instability, we had previ-

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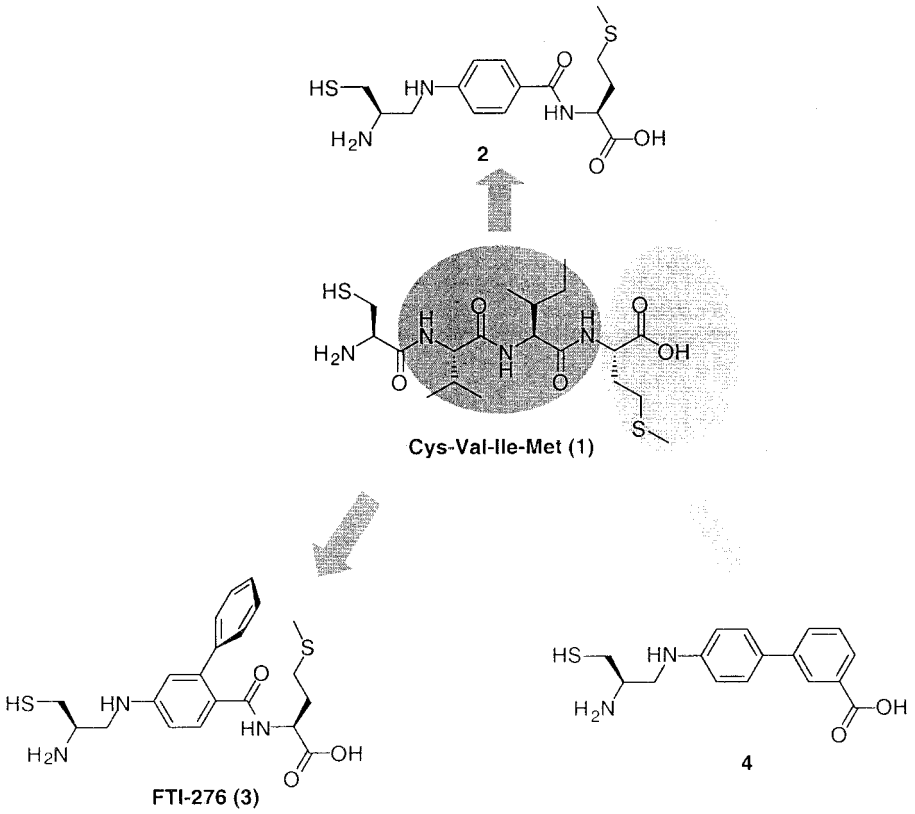


Figure 1. Structures of previously reported inhibitors.

ously synthesized a series of peptidomimetics wherein the hydrophobic Val-Ile dipeptide core was replaced by a 4-aminobenzoic acid spacer.[18] This provided a rigid and hydrophobic link between the cysteine and the methionine residues. For example, **2** inhibited FTase with an IC_{50} of 150 nM,[18] confirming that the central residues in the tetrapeptide sequence could be replaced with a structurally defined nonamino acid peptidomimetic.

To reduce the peptidic character of **2**, the Val-Ile-Met tripeptide of **1** was replaced with a more conformationally restricted 4-amino-3′-carboxybiphenyl group, resulting in **4**. Moreover, further modifications lead to the introduction of an additional phenyl group onto the aromatic spacer of **2**. The *ortho*-substituted benzene of **3** represented a better mimic of the hydrophobic side chains of Val-Ile within tetrapeptide **1**. The resulting compound, FTI-276 (**3**), showed extremely high inhibition activity (IC_{50} = 0.61 nM), improving by 10^3 relative to **1**.[16]

The success of scaffolds **3** and **4** suggested that combining their structural features (hydrophobic substitution on the phenyl ring and a C-terminal benzoate) could lead to improved and entirely nonpeptide FTase inhibitors. These features would mimic important properties of CAAX, as the crystal structure of ternary complex of FTase clearly demonstrates that the substrate (Ac-Cys-Val-Ile-Met(Se)-OH) in the active site is recognized by (1) hydrogen bonding of the methionine C-terminal carboxylate with Arg 202β, (2) hydrophobic interactions of the isobutyl and isopropyl groups of the Val-Ile dipeptide with a hydrophobic pocket formed by aromatic residues, and (3) chelation of the thiol group of cysteine to the active site zinc ion.[12] Thus, we further modified our inhibitor design to optimize the functionality of (a) the carboxylate group, (b) the hydrophobic group, and (c) the zinc-binding group based on the biphenyl scaffold, as shown in Figure 2.

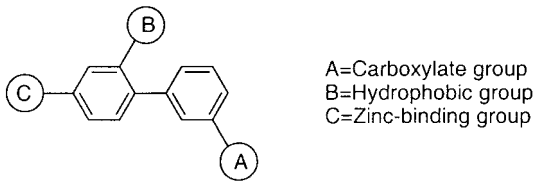
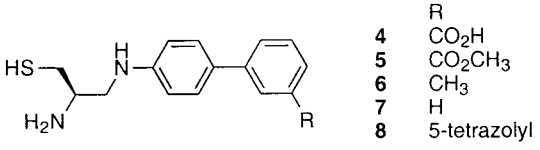


Figure 2. Modified functional groups of nonpeptidic inhibitors.

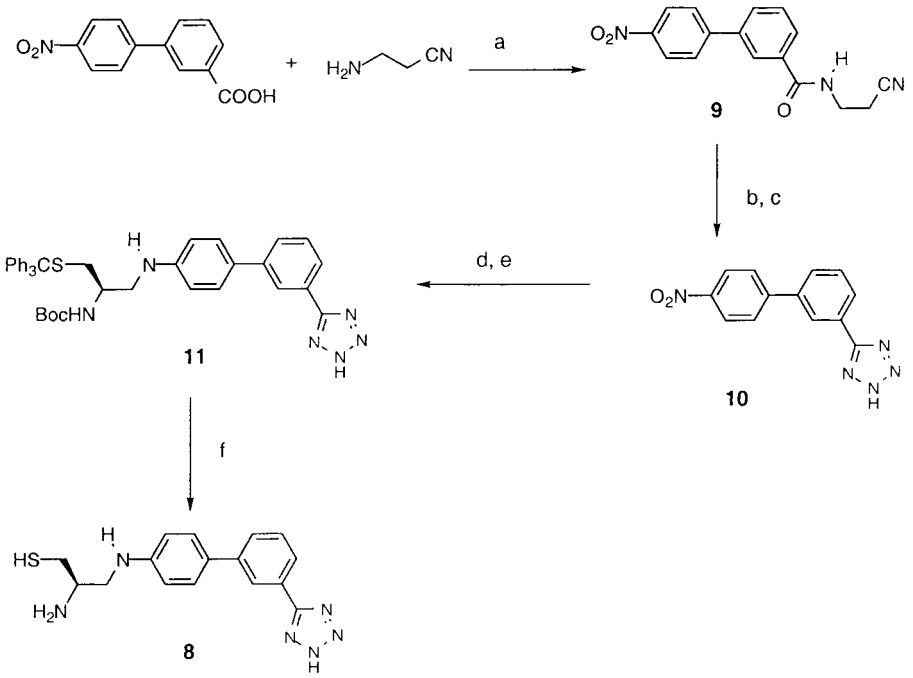
Results and Discussion

(A) Modification of the Carboxyl Group. Previous work had shown that the location of the carboxyl group in the 3-position of the biphenyl moiety was important for FTase inhibition activity.[17] We further investigated the substituent effects on biological activity by varying the size and polarity of the groups in this position. One interesting modification was to replace the carboxylic acid with a tetrazole group, which has been successfully used as a carboxylate isostere in many enzyme inhibitor studies.[19] The pK_a value of a tetrazole (5−6) is very similar to that of a carboxylic acid[20] and should allow it to interact favorably with carboxylate-binding residues in the active site of FTase.



The synthesis of the tetrazole-containing compound **8** is shown in Scheme 1. The tetrazole group was formed

Scheme 1. Synthesis of Tetrazole-Containing Compound **8**[a]

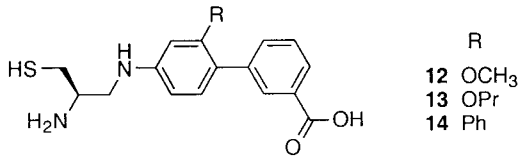


[a] Reagents: (a) EDCI, HOBt, 80%; (b) Ph_3P, DEAD, $TMSN_3$, 48 h; (c) 1N NaOH, 24 h, 53%; (d) H_2, Pd/C; (e) N-Boc-S-trityl-cysteinal, $NaBCNH_3$, 30%; (f) TFA, Et_3SiH, 53%.

through Mitsunobu reaction of amide **9** with trimethylsilyl azide.[20] Protection of the tetrazole in **10** with a triphenylmethyl group failed because conditions required for reduction of the nitro group lead to cleavage of the protecting group. Therefore, the tetrazole was left unprotected in the reductive amination and deprotection steps with the final product purified by reverse phase preparative high-performance liquid chromatography (HPLC).

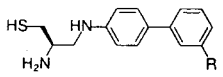
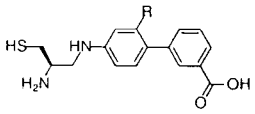
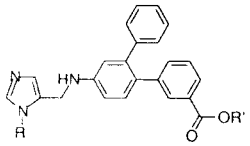
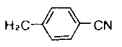
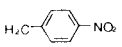
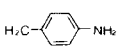
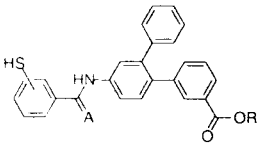
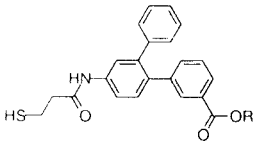
The ability of these synthetic compounds to inhibit FTase and GGTase-I in vitro was investigated by using partially purified FTase and GGTase-I from human Burkitt lymphoma (Daudi) cells.[21] Enzyme preparations were incubated with [^{3}H]FPP and recombinant H-Ras-CVLS (FTase) or [^{3}H]GGPP and H-Ras-CVLL (GGTase-I) in the presence of different concentrations of inhibitors. After the preparations were incubated for 30 min at 37 °C, the reaction was stopped and filtered on glass fiber filters to separate free [^{3}H]FPP from [^{3}H]F-Ras. The IC_{50} values of **4**−**8** for the inhibition of FTase are reported in Table 1. As a comparison, the tetrapeptide Cys-Val-Ile-Met (**1**) inhibited FTase with an IC_{50} of 340 nM. The high potency of **4** bearing a carboxyl group at the 3-position relative to **1** shows that an appropriately functionalized biphenyl group can act as an effective mimic of the extended backbone conformation of the last three amino acids of the Ras protein. Methylation of the carboxylic acid, as in **5**, or replacement of the carboxyl group with a hydrogen or methyl group resulted in lower activity.[17] However, replacement of carboxylic acid by tetrazole as in **8** increased potency by ~5-fold as compared to **4**. Like the carboxylic acid group, tetrazole bears a negative charge under neutral aqueous conditions, confirming the importance of an ionic interaction between the inhibitor and the enzyme active site. The difference in charge distribution between tetrazole and carboxylate presumably accounts for their different inhibition potencies. Furthermore, none of these derivatives inhibited potently GGTase-I, suggesting that the biphenyl group is a better mimic of AAM than of AAL. The results also suggest that methionine replacement in these inhibitors is better tolerated by FTase than is leucine replacement by GGTase-I.

(B) Modification of the Hydrophobic Group. The large increase in potency that came from a phenyl substituent in FTI-276 (**3**) on the unsubstituted spacer in **2** prompted us to modify the biphenyl scaffold of **4** by incorporating a hydrophobic group. Methoxy, propoxy, and phenyl groups were introduced at the 2-position of the biphenyl scaffold in **4**, as in **12**−**14**.



The synthetic procedure for **13** is shown in Scheme 2. Deprotection of the methoxy group under acidic conditions generated a substituted phenol (**15**) that was alkylated to give **16**. Reduction of the nitro group followed by reductive amination provided **17**, which was deprotected to give the final product **13**. To synthesize the terphenyl scaffold in **14**, we applied recently developed Suzuki chloride coupling reactions[22,23] for chemoselective arylation of commercially available 3-bromo-4-chloro-nitrobenzene (Scheme 3). The coupling reaction of 3-bromo-4-chloronitrobenzene with one equivalent of phenylboronic acid, $Pd(PPh_3)_4$ as the catalyst, and K_2CO_3 as the base in 20% EtOH/toluene afforded chloride **18**[24] in 94% yield. Recently, Buchwald et al. have reported electron rich and sterically bulky phosphine ligands as effective additives to activate the Pd^0 center.[22] By using a $Pd(OAc)_2$/2-(dicyclohexylphosphino)biphenyl[22] catalyst system, successive modified Suzuki chloride couplings of **18** to *m*-methoxycarbonylphenylboronic

Table 1. IC_{50} Values for Enzyme Inhibition of CAAX Mimetic FTase Inhibitors

Structures		Inhibitors	FTase IC_{50} (nM)[a]	GGTase-I IC_{50} (nM)[a]
		1 (Cys-Val-Ile-Met)	340[b]	nd
	R			
	CO_2H	**4** (FTI-265)	150 ± 30	90,000 ± 17,000
	CO_2CH_3	**5** (FTI-259)	930	100,000
	CH_3	**6** (FTI-263)	770	>100,000
	H	**7** (FTI-268)	1200	>100,000
	tetrazole	**8** (FTI-2102)	30 ± 3.5	4400 ± 1030
	R			
	OCH_3	**12** (FTI-281)	37 ± 15	35,000 ± 21,000
	OPr	**13** (FTI-288)	41 ± 35	2400 ± 1300
	Ph	**14** (FTI-289)	20 ± 15	640 ± 250
	R R'			
	H H	**22** (FTI-2195)	2300	5000
	H Me	**23** (FTI-2296)	>10,000	>10,000
	 H	**24** (FTI-2312)	430 ± 120	4400 ± 3400
	Me	**25** (FTI-2297)	380 ± 140	6900 ± 560
	 H	**26** (FTI-2325)	450 ± 71	3900 ± 890
	Me	**27** (FTI-2307)	310 ± 26	5900 ± 890
	 H	**28** (FTI-2347)	2900	7000
	Me	**29** (FTI-2310)	5600	>10,000
	SH R A			
	p- H HH	**30** (FTI-2342)	>10,000	>10,000
	p- Me HH	**31** (FTI-2316)	>10,000	>10,000
	m- H HH	**32** (FTI-2343)	>10,000	>10,000
	m- Me HH	**33** (FTI-2314)	>10,000	>10,000
	m- H O	**34** (FTI-2313)	>10,000	>10,000
	m- Me O	**35** (FTI-2302)	>10,000	>10,000
	o- Me HH	**36** (FTI-2315)	>10,000	>10,000
	R			
	H	**37** (FTI-2317)	3800	>10,000
	Me	**38** (FTI-2306)	>10,000	>10,000

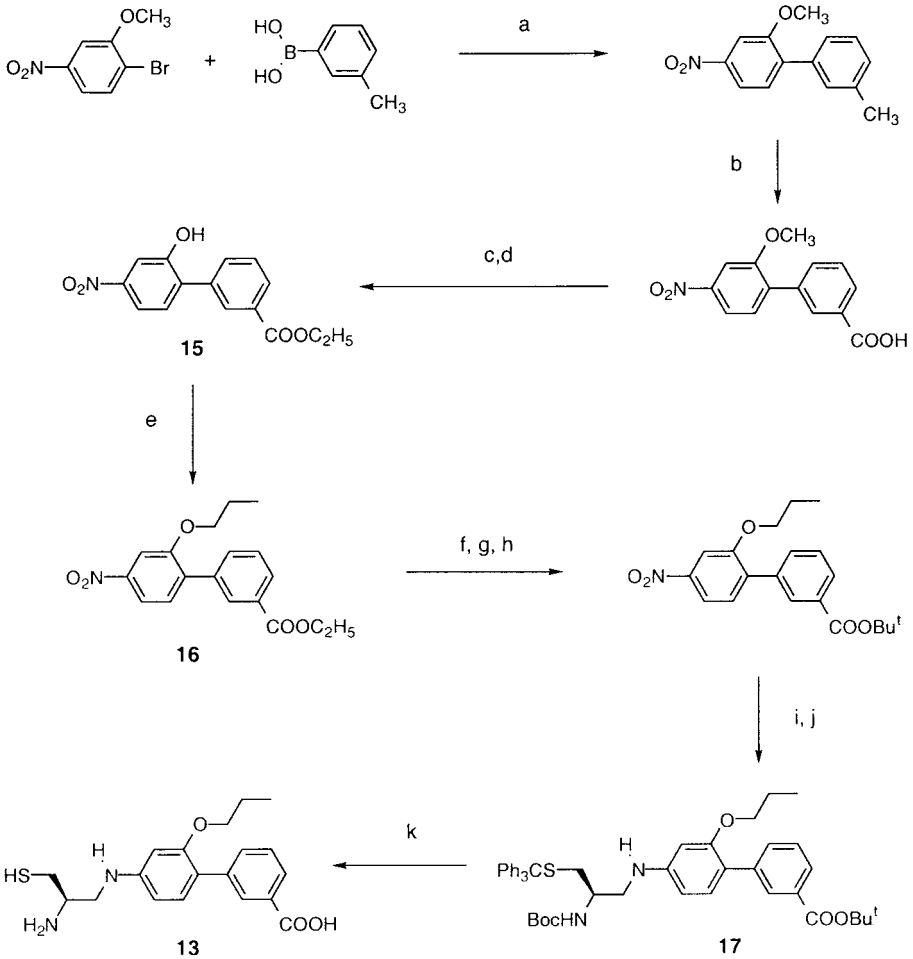
[a] Where s.d. is given $n \geq 3$, otherwise $n = 1$. [b] Ref 18.

acid[25] with K_3PO_4 as the base under reflux in toluene gave terphenyl derivative **19** in 86% yield.[24] The methyl ester in **19** was hydrolyzed, and the nitro group was reduced with stannous chloride to afford amino carboxylic acid **21**. Reductive amination with protected cysteinal followed by deprotection gave inhibitor **14**.

We have solved the X-ray crystal structure of 4-nitro-2-phenyl-3′-methoxycarbonylbiphenyl (**19**), an intermediate in the synthesis of **14**. The structure gives an insight into the relative orientation of the three phenyl rings and shows dihedral angles of 53° for the biphenyl unit and 45° for the phenyl substituent. Figure 3 shows a superimposition of the crystal structures of **19** and the tripeptide fragment (Val-Ile-Met(Se)-OH) from the FTase complex with the AcCys-Val-Ile-Met(Se)-OH tetrapeptide.[12] When the nitro-N and the ester-C in **19** are aligned with the Val-N and the Met carboxylate-C on the tripeptide, the phenyl substituent occupies a similar position to the isobutyl group of the Ile residue. Overall, the triphenyl fragment provides a large hydrophobic area covering the isopropyl, isobutyl, and part of methylthioethyl groups in the tripeptide.

The FTase inhibition activities of **12**−**14** are shown in Table 1. Substitution of hydrogen by alkyloxy or aryl groups at the 2-position of the biphenyl ring in **4** gave improved inhibition potency in each case by 3- to 8-fold. This established that the key hydrophobic interaction between the active site and the groups at the 2-position of the inhibitors, preserved on going from the 4-aminobenzoic acid spacer (in **3**) to the 4-amino-3′-carboxy-

Scheme 2. Synthesis of **13**[a]



[a] Reagents: (a) $Pd(OAc)_2$, acetone/H_2O reflux, 83%; (b) $KMnO_4$, pyridine/H_2O, reflux, 75%; (c) 48% aqueous HBr, reflux; (d) EtOH, $SOCl_2$, reflux, 2 steps 88%; (e) *n*-C_3H_7I, acetone, K_2CO_3, reflux, 77%; (f) aqueous NaOH/MeOH; (g) $(COCl)_2$; (h) Bu^tOK, 3 steps 30%; (i)H_2, Pd/C; (j) N-Boc-S-trityl-cysteinal, $NaBCNH_3$, 60%; (k) TFA, Et_3SiH.

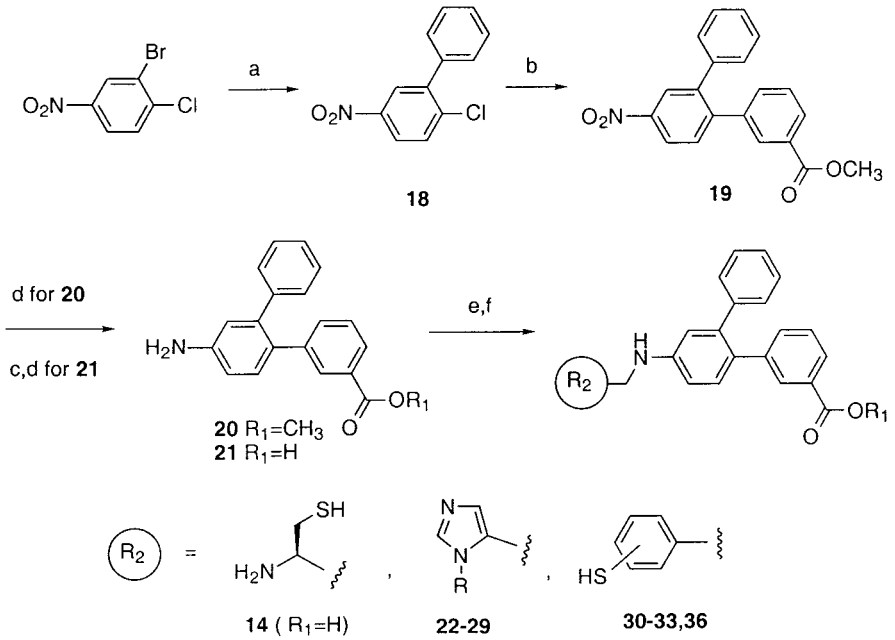
biphenyl scaffold in **12**−**14**, is important for biological activity. Moreover, introducing a phenyl group (as in **14**) improved the FTase inhibition activity of **4** by 8-fold. As shown in Figure 3, the phenyl substituent is presumably located at the same position as the isobutyl side chain of the Ile residue in Cys-Val-Ile-Met and binds in the large hydrophobic pocket formed by aromatic residues in the FTase active site.[12] Here again, with the exception of **14**, the derivatives were highly selective for FTase over GGTase-I.

(C) Modification of the Zinc-Binding Group. The free thiol functionality in **14**, while important for binding to the Zn^{2+} ion, is oxidatively unstable and complicates further biological evaluations. To overcome this problem, we and others have previously developed nonthiol-containing FTase inhibitors in which the cysteine residue was replaced by an imidazole.[13,26−29] In this study, a similar strategy would be to replace the cysteine residue of **14** with alternative Zn^{2+} ion coordinating groups such as imidazole, arylthiol, and alkylthiol groups. The structures of the noncysteine inhibitors **22**−**38** are shown in Figure 4. It is noteworthy that these are nonpeptide, achiral derivatives.

The representative synthetic approach used for the preparation of the compounds is shown in Schemes 3 and 4. Protection of 4(5)-imidazocarboxyaldehyde with a trityl group, followed by alkylation with *p*-cyano- or *p*-nitrobenzyl bromide with simultaneous deprotection gave the corresponding aldehydes **40a**[13] and **40b**, respectively (Scheme 4). Coupling of **40** with terphenyl scaffold **20** or **21** under reductive amination conditions gave imidazole-containing derivatives **24**−**27**, respectively (Scheme 3). Compounds **28** and **29** were derived from further reduction of the nitro group in **26** and **27**. Protection of the free thiol in *p*-, *m*-, or *o*-mercaptobenzoic acid with ^{t}butoxycarbonyl (Boc) and reduction of the benzoic acids to the alcohols **42a**−**c** via mixed anhydride formation, followed by Swern oxidation, gave the corresponding aldehydes **43a**−**c**, respectively (Scheme 4). As with the imidazole derivatives, reductive amination of these aldehydes with **20** or **21** followed by deprotection under acidic conditions gave the corresponding mercaptoaryl-containing derivatives **30**−**33** and **36** (Scheme 3).

The results of an in vitro assay for inhibition of FTase by **22**−**38** are shown in Table 1. In most cases, replacement of the cysteine residue of **14** by an imidazole group as in **22**−**29** leads to a retention of FTase inhibition activity with IC_{50} values at submicromolar levels. Moreover, introducing a benzyl substituent onto the nitrogen of the imidazole improved the activity significantly. For example, **24**, which contains a *p*-cyanobenzyl group, showed 5-fold higher inhibition potency against FTase than nonsubstituted **22**. These observations

Scheme 3. Representative Synthesis of FTase Inhibitors Based on a Terphenyl Scaffold[a]



[a] Reagents: (a) $Pd(PPh_3)_4$, phenylboronic acid, K_2CO_3, reflux, overnight, 94%; (b) $Pd(OAc)_2$, *m*-methoxycarbonylphenylboronic acid, 2-(dicyclohexylphosphino)biphenyl, K_3PO_4, toluene, 85 °C, 1 h, 86%; (c) LiOH, THF/H_2O, reflux, 95%; (d) $SnCl_2$, AcOEt, reflux; (e) R_2CHO, AcOH, $NaBH_4$, room temperature; (f) TFA/CH_2Cl_2.

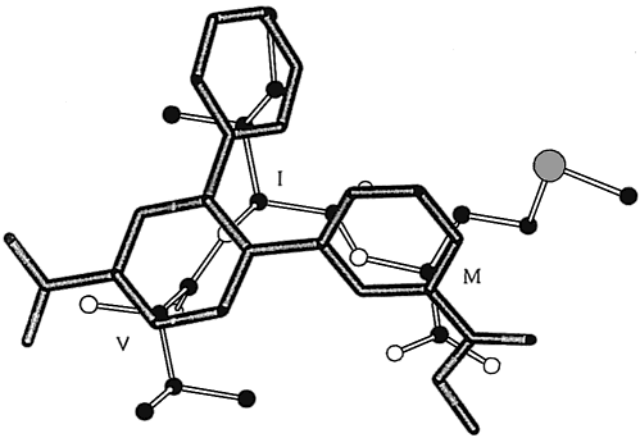


Figure 3. The crystal structures of tripeptide Val-Ile-Met (shown below as ball and stick) and that of **19** (shown above as bold lines): most hydrogens are excluded for clarity.

suggest that the imidazole acts as a chelator to Zn^{2+}, and its aromatic substituent binds to a hydrophobic pocket in FTase. Furthermore, the nitro and cyano groups were better tolerated than the amino group at this position. Replacement of the cysteine of **14** by a mercaptoarylamido or mercaptoarylmethylamino group (**30**−**36**), to rigidify the link between the thiol and the terphenyl groups, was unsuccessful, presumably due to the molecules being constrained in a conformation less optimal for binding to Zn^{2+}. In contrast, **37**, in which the cysteine was replaced by a mercaptoalkylamido group, retained moderate FTase inhibition activity (IC_{50} = 3.8 μM). The flexible thiol group may be able to adopt a more appropriate conformation for binding to the zinc ion. However, **14** was 200-fold more potent than **37** suggesting that in addition to the thiol group, the alkylamino group from the reduced cysteine fragment may play an important role in binding to the FTase active site.

Inhibition activity of the achiral compounds in whole cells was also investigated. The whole cell inhibition of farnesylation and geranylgeranylation was determined based on the level of inhibition of H-Ras and Rap1A processing, respectively. Oncogenic H-Ras-transformed NIH 3T3 cells were treated with various concentrations of inhibitors and incubated for 48 h. The cell lysates were separated on 12.5% sodium dodecyl sulfate polyacrylamide gel electrophoresis. The separated proteins were transferred to nitrocellulose and immunoblotted using an anti-Ras antibody (Y13-238) or an anti-Rap1 antibody (SC-65). Antibody reactions were visualized using HRP-conjugated anti-rat or anti-rabbit IgG and an enhanced chemiluminescence detection system. As a reference, FTI-2153[26] and GGTI-298[30] were used for H-Ras and Rap1A processing inhibition, respectively. The results of the Western blot are shown in Figure 5. The imidazole derivatives containing *p*-substituted benzyl groups, **24** (FTI-2312; lanes 12 and 13), **25** (FTI-2297; lanes 15−19), **26** (FTI-2325; lanes 27 and 28), and **27** (FTI-2307; lanes 8 and 9), inhibited H-Ras processing at 1−10 μM concentrations, whereas unsubstituted imidazole derivative **23** (FTI-2296) did not show inhibition activity (lanes 4 and 5). Mercaptoarylmethylamino derivatives **30** (FTI-2342; lane 29) and **31** (FTI-2316; lanes 23 and 24) or mercaptoalkylamido derivatives **37** (FTI-2317; lanes 25 and 26) and **38** (FTI-2306; lanes 6 and 7) did not show whole cell activity. The methyl ester forms (**25** and **27**, lanes 15−19 and 8 and 9, IC_{50} values ~2 and 4 μM) showed higher inhibition activity against H-Ras processing than the corresponding free carboxylate derivatives (**24** and **26**, lanes 12 and 13 and 27 and 28, IC_{50} values ~10 and 50 μM) suggesting that an increase of the hydrophobicity of the inhibitors could lead to higher cell permeability.

In summary, we have developed a potent series of peptidomimetics that inhibit FTase with submicromolar IC_{50} values. The inhibitors are based on a terphenyl

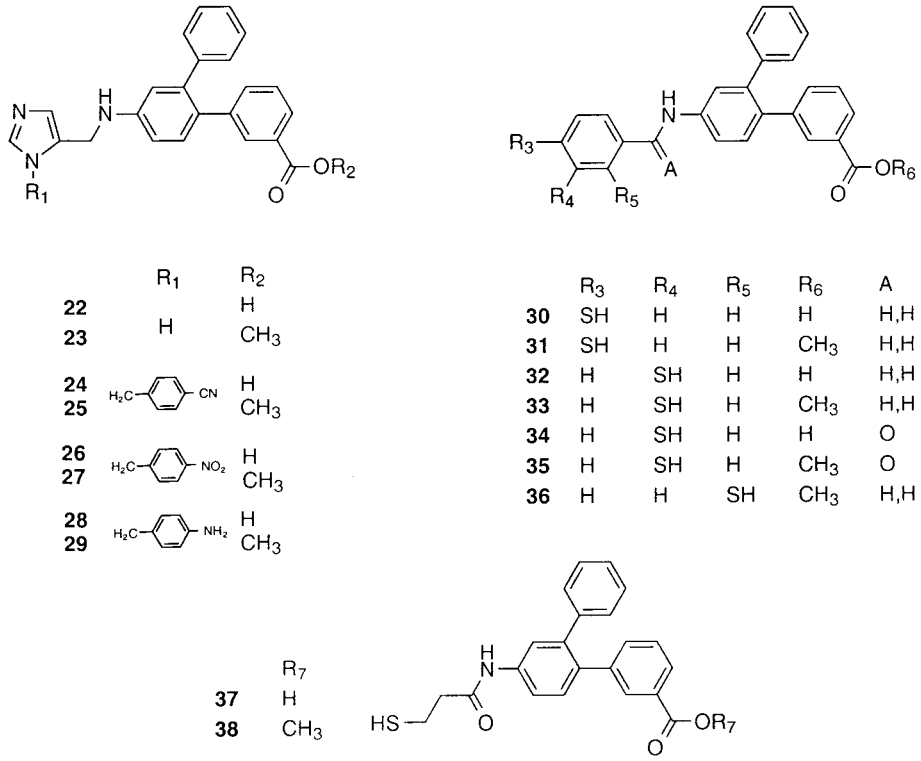
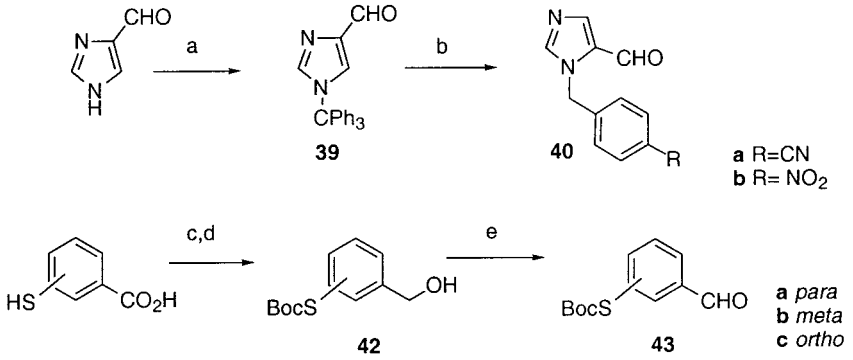


Figure 4. Nonpeptide and achiral inhibitors based on a terphenyl scaffold.

Scheme 4[a]



[a] Reagents: (a) Ph_3CCl, Et_3N, DMF, 99%; (b) *p*-substituted benzylbromide/CH_3CN, 60 °C, overnight, 70%; (c) Boc_2O, Et_3N, DMAP, THF; (d) EtOCOCl, $NaBH_4$; (e) $(COCl)_2$, DMSO, Et_3N.

scaffold that functions as a nonpeptidic mimetic of the key CAAX tetrapeptide substrate of FTase. By incorporating imidazole and benzoate groups as replacements for Cys and Met residues, we have generated simple and achiral inhibitors (e.g., **25**) that show whole cell inhibition activity against H-Ras processing.

Experimental Section

Melting points were determined with an Electrothermal capillary melting point apparatus and are uncorrected. ^1H and ^{13}C NMR spectra were recorded on a Bruker AM-500, 400, or QE-plus 300 spectrometer. Chemical shifts were reported in δ (ppm) relative to tetramethylsilane. All coupling constants were described in Hz. Elemental analyses were performed by Atlantic Microlab, Inc., GA. Flash column chromatography was performed on silica gel (40−63 μm) under a pressure of about 4 psi. Solvents were obtained from commercial suppliers and purified as follows: tetrahydrofuran and ether were distilled from sodium benzophenone ketyl; methylene chloride was distilled over calcium hydride. All synthesized final compounds were checked for purity by analytical HPLC, which was performed using a Rainin HP controller and a Rainin UV-C detector with a Rainin 250 mm × 4.6 mm, 5 μm Microsorb C-18 column. High-resolution mass spectra (HRMS) and low-resolution mass spectra (LRMS) were performed on a Varian MAT-CH-5 (HRMS) or VG 7070 (LRMS) mass spectrometer. Compounds **14**, **34**, and **37** were prepared by previously reported procedures.[24]

4-Nitro-3′-[(2-cyanoethyl)aminocarbonyl]biphenyl (9). 4-Nitro-3′-carboxybiphenyl[17] (1.70 g, 7.0 mmol) was coupled with 3-aminopropionitrile (0.566 mL, 7.70 mmol) by using EDCI (1.48 g, 1.10 equiv) and HOBt (1.04 g, 1.10 equiv) to give **9** (1.64 g, 80%). mp 142−143 °C. ^1H NMR ($CDCl_3$): δ 8.32 (d, J = 8.8 Hz, 2H), 8.07 (s, 1H), 7.74−7.81 (m, 4H), 7.59 (t, J = 7.8 Hz, 1H), 6.67 (br t, 1H, amide), 3.76 (q, J = 6.1 Hz, 2H), 2.80 (t, J = 6.1 Hz, 2H). ^{13}C NMR ($CDCl_3$): δ 167.4, 147.4, 146.3, 139.5, 134.5, 130.8, 129.5, 127.9, 126.9, 126.4, 124.2, 118.2, 36.3, 18.5.

4-*N*-[2-(*R*)-Amino-3-mercaptopropyl]amino-3′-(2H-tetrazol-5-yl)biphenyl Trifluoroacetate (8). Compound **9** (1.47 g, 5.0 mmol) was dissolved in 50 mL of tetrahydrofuran (THF). To this solution were added triphenylphosphine (2.64 g, 10 mmol) and DEAD (1.58 mL, 10.0 mmol) at 0 °C. After the solution was stirred for 20 min, trimethylsilyl azide (1.34 mL, 10.0 mmol) was added. The mixture was stirred at room

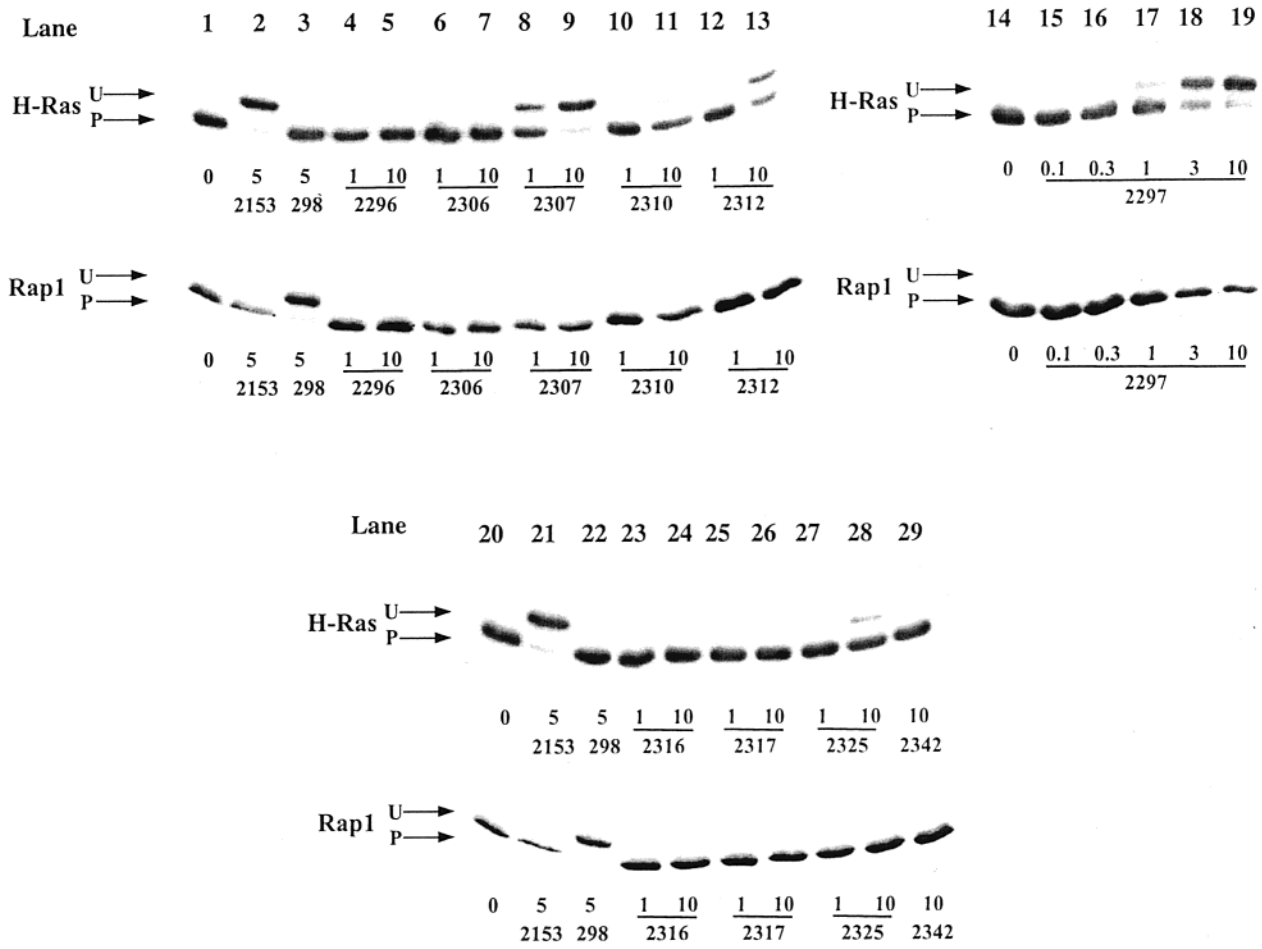


Figure 5. Effect of imidazole-containing terphenyl derivatives on processing of H-Ras and Rap1A in NIH 3T3 cells. Western blot analysis demonstrates the inhibition of farnesylated H-Ras or geranylgeranylated Rap 1A as seen by the band shift from processed (P) to unprocessed (U) protein. Lanes 1, 14 and 20: vehicle control; lanes 2, and 21: 5 μM FTI-2153;[26] lanes 3 and 22: 5 μM GGTI-298; lanes 4 and 5: 1 and 10 μM FTI-2296 (**23**); lanes 6 and 7: 1 and 10 μM FTI-2306 (**38**); lanes 8 and 9: 1 and 10 μM FTI-2307 (**27**); lanes 10 and 11: 1 and 10 μM FTI-2310 (**29**); lanes 12 and 13: 1 and 10 μM FTI-2312 (**24**); lanes 15−19: 0.1, 0.3, 1, 3, and 10 μM FTI-2297 (**25**); lanes 23 and 24: 1 and 10 μM FTI-2316 (**31**); lanes 25 and 26: 1 and 10 μM FTI-2317 (**37**); lanes 27 and 28: 1 and 10 μM FTI-2325 (**26**); lane 29: 10 μM FTI-2342 (**30**).

temperature for 48 h. After the solvents were evaporated, the residue was dissolved in 10 mL of THF and 10 mL of 1 N sodium hydroxide was added. The mixture was stirred at room temperature for 24 h, and then solvents were evaporated. The residue was extracted into 1 N NaOH solution, and the aqueous phase was acidified with 1 N HCl. The precipitate was collected and dried to give **10** (702 mg, 53%).

Compound **10** (530 mg, 2.0 mmol) was dissolved in a mixture of THF and methanol, and the solution was hydrogenated at 40 psi with Pd−C (5% equiv). Hydrogenation was stopped after 30 min, and the hydrogenated product was treated with N-Boc-S-trityl-L-cysteinal (1.0 equiv). $NaBCNH_3$ (252 mg, 2.0 eq) was added, and the mixture was stirred overnight. After the solvents were evaporated, the residue was extracted with methylene chloride and 0.5 N HCl. Solvents were evaporated, and the residue was purified by flash column chromatography (CH_2Cl_2:methanol = 10:1) to give the reductive amination product **11** (398 mg, 30%). mp 89−90 °C (decomp). ^{1}H NMR ($CDCl_3$): δ 7.97 (s, 1H), 7.90 (d, J = 7.7 Hz, 1H), 7.55 (d, J = 7.9 Hz, 1H), 7.35−7.42 (m, 9H), 7.14−7.31 (m, 9H), 6.47 (d, J = 8.6 Hz, 2H), 4.80 (d, J = 8.4 Hz, 1H, Boc amide), 3.92 (br, 1H, Cys α H), 3.08 (m, 2H, CH_2N), 2.40−2.48 (m, 2H, CH_2S), 1.45 (s, 9H, Boc).

The compound prepared above (70 mg) was deprotected with trifluoroacetic acid (TFA), and the crude product (70% purity according to analytical HPLC) was purified by preparative HPLC to give **8** as a hydroscopic TFA salt (28 mg, 53%). mp 90 °C (decomp). ^{1}H NMR (CD_3OD): δ 8.23 (s, 1H), 7.87 (d, J = 7.7 Hz, 1H), 7.73 (d, J = 7.7 Hz, 1H), 7.57 (m, 3H), 6.83 (d, J = 8.6 Hz, 2H), 3.50−3.57 (m, 2H, Cys α H, CH_2N), 3.42 (m, 1H, CH_2N), 2.96 (dd, J = 4.9, 14.8 Hz, 1H, CH_2S), 2.83 (dd, J = 5.6, 14.8 Hz, 1H, CH_2S). ^{13}C NMR (CD_3OD): δ 149.3, 143.7, 130.9, 130.8, 130.4, 130.0, 128.9, 125.6, 125.5, 114.5, 53.9, 45.3, 25.3 (expect 11 aromatic C, observed 10). Anal. Calcd for $C_{16}H_{18}N_6S\cdot1.3CF_3COOH\cdot H_2O$: C, 45.34; H, 4.32; N, 17.06. Found: C, 45.47; H, 4.17; N, 17.07.

4-Nitro-2-hydroxy-3′-ethoxycarbonylbiphenyl (15). The coupling of 1-bromo-2-methoxy-4-nitrobenzene with 3-methylphenylboronic acid gave 2-methoxy-4-nitro-3′-methylbiphenyl, which was then oxidized to give 2-methoxy-4-nitro-3′-carboxybiphenyl (75%). This compound (1 g, 3.6 mmol) was suspended in 25 mL of 48% aqueous HBr and 20 mL of acetic acid. The mixture was refluxed for 5 h, and then, 50 mL of water was added. The pale yellow solid was filtered to give the demethylated product (88%). mp 236−237 °C. This intermediate (730 mg, 2.81 mmol) was mixed with ethanol (25 mL) and thionyl chloride (0.4 mL, 2 equiv). The mixture was refluxed for 2 h and then cooled in an ice bath. The precipitate was filtered off (0.32 g), and the filtrate was first evaporated and then washed with $NaHCO_3$ to give another fraction of product (0.48 g, combined yield 98%). mp 201−202 °C. ^{1}H NMR (acetone-d_6): δ 9.55 (s, 1H, phenol), 8.30 (s, 1H), 8.06 (d, J = 7.7 Hz, 1H), 7.84−7.93 (m, 3H), 7.60−7.65 (m, 2H), 4.41 (q, J = 7.1 Hz, 2H), 1.38 (t, J = 7.1 Hz, 3H). HRMS (EI): calcd for $C_{15}H_{13}NO_5$, 287.0794; observed, 287.0796.

4-Nitro-2-propanoxy-3′-ethoxycarbonylbiphenyl (16). Compound **15** (750 mg, 2.61 mmol) was dissolved in 20 mL of acetone, and 1-iodopropane (0.38 mL, 2.50 equiv) and potas-

sium carbonate (1.08 g, 3 equiv) were added. The mixture was refluxed for 5 h and then filtered. The filtrate was evaporated and then recrystallized from 95% ethanol to give white crystals (657 mg, 77%). mp 90.5−91.0 °C. ^{1}H NMR ($CDCl_3$): δ 8.27 (s, 1H), 8.09 (d, J = 7.8 Hz, 1H), 7.92 (d, J = 8.3 Hz, 1H), 7.82 (s, 1H), 7.75 (d, J = 7.8 Hz, 1H), 7.48−7.54 (m, 2H), 4.41 (q, J = 7.1 Hz, 2H), 4.07 (t, J = 6.3 Hz, 2H), 1.86 (m, 2H), 1.43 (t, J = 7.1 Hz, 3H), 1.01 (t, J = 7.3 Hz, 3H). HRMS: calcd for $C_{18}H_{19}NO_5$, 329.1263; observed, 329.1261.

2-Propanoxy-4-*N*-[2-(*R*)-*N*-*tert*-butoxycarbonylamino-3-(triphenylmethyl)thiopropyl]amino-3′-*tert*-butoxycarbonylbiphenyl (17). Compound **16** was hydrolyzed with NaOH−MeOH to give 2-propanoxy-4-nitro-3′-carboxybiphenyl (100%, mp 198−199 °C). This intermediate (513 mg, 1.70 mmol) was converted to an acid chloride with oxalyl chloride (0.22 mL, 1.5 equiv) and then reacted with KOBu^t (382 mg, 2.0 equiv) to give 2-propanoxy-4-nitro-3′-*tert*-butoxycarbonylbiphenyl (182 mg) as a pale yellow waxy product after column chromatography purification (30%). ^{1}H NMR ($CDCl_3$): δ 8.18 (s, 1H), 8.17 (d, J = 7.7 Hz, 1H), 7.92 (d, J = 8.3 Hz, 1H), 7.81 (s, 1H), 7.71 (d, J = 7.5 Hz, 1H), 7.50 (t, 2H), 4.05 (t, J = 6.3 Hz, 2H), 1.83 (m, 2H), 1.60 (s, 9H), 1.01 (t, J = 7.3 Hz, 3H).

The above intermediate (124 mg, 0.34 mmol) was hydrogenated and then treated with N-Boc-S-trityl-L-cysteinal (1.0 equiv) in the presence of $NaBCNH_3$ (34 mg, 1.5 equiv). After flash column chromatography (hexane:AcOEt = 4:1), **17** was obtained (160 mg, 60%). mp 68−70 °C (decomp). ^{1}H NMR ($CDCl_3$): δ 8.13 (s, 1H), 7.86 (d, J = 7.8 Hz, 1H), 7.65 (d, J = 7.8 Hz, 1H), 7.40−7.44 (m, 7H), 7.20−7.38 (m, 9H), 7.14 (d, J = 8.2 Hz, 1H), 6.20 (m, 2H), 4.58 (br d, J = 7.2 Hz, 1H, Boc amide), 3.88 (m, 4H, Cys α H, NH, and OCH_2), 3.13 (m, 2H, CH_2N), 2.49 (m, 2H, CH_2S), 1.76 (m, 2H, propyl), 1.58 (s, 9H, Bu^t), 1.43 (s, 9H, Boc), 0.97 (t, J = 7.3 Hz, 3H, propyl). ^{13}C NMR ($CDCl_3$): δ 166.1, 157.0, 155.5, 148.8, 144.4, 138.9, 133.3, 131.4, 131.3, 130.3, 129.5, 128.0, 127.6, 126.9, 126.7, 119.1, 104.8, 97.1, 80.6, 79.6, 69.7, 67,0, 49.4, 47.2, 34.3, 28.3, 28.2, 22.6, 10.8. Anal. Calcd for $C_{47}H_{54}O_5N_2S$: C, 74.40; H, 7.12; N, 3.69; S, 4.22. Found: C, 74.59; H, 7.52; N, 3.54; S, 4.04.

2-Propanoxy-4-*N*-[2-(*R*)-amino-3-mercaptopropyl]amino-3′-carboxybiphenyl Hydrochloride (13). Compound **17** (100 mg, 0.13 mmol) was deprotected with TFA in the presence of triethylsilane, and the TFA salt was converted to the hydrochloride salt to give **13** (45 mg, 79%). mp 170−172 °C (decomp). ^{1}H NMR (CD_3OD): δ 8.18 (s, 1H), 7.89 (d, J = 7.8 Hz, 1H), 7.68 (d, J = 7.8 Hz, 1H), 7.44 (t, J = 7.8 Hz, 1H), 7.20 (d, J = 8.1 Hz, 1H), 6.56 (s, 1H), 6.53 (d, J = 8.1 Hz, 1H), 3.94 (t, J = 6.3 Hz, 2H, OCH_2), 3.60 (m, 2H, Cys α H and CH_2N), 3.47 (m, 1H, CH_2N), 2.99 (dd, J = 4.8, 14.6 Hz, 1H, CH_2S), 2.85 (dd, J = 5.5, 14.6 Hz, 1H, CH_2S), 1.74 (m, 2H, propyl), 0.98 (t, J = 7.3 Hz, 3H, propyl). LRMS (EI): calcd for $C_{19}H_{24}O_3N_2S$: 360 (M^+, 30), 284 (100).

Procedure for the Synthesis of Imidazole-Containing Inhibitors 22−29. Typical Example Given for Compound 26. 1-Triphenylmethyl-4-imidazolecarboxaldehyde (39). 4(5)-Imidazolecarboxaldehylde (3.0 g, 31 mmol) and Et_3N (6.24 g, 62 mmol) were stirred at room temperature in dimethylformamide (DMF) (20 mL) under nitrogen. Triphenylmethyl chloride (8.7 g, 31 mmol) in DMF (45 mL) was added dropwise, and the resulting solution was stirred at room temperature overnight. The mixture was poured into 300 mL of water, which resulted in a white precipitate. This solid was isolated by filtration and dissolved in AcOEt (approximately 600 mL). The solution was dried with sodium sulfate and the solvent then removed under vacuum to give the product as a white solid (10.45 g, 99%). mp 186 °C. ^{1}H NMR ($CDCl_3$): δ 9.86 (s, 1H, CHO), 7.59 (s, 1H, NCHN), 7.51 (s, 1H, NC*H*CCHO), 7.32−7.38 (m, 12H, Trt), 7.08−7.12 (m, 7H, Trt).

1-(4-Nitrobenzyl)-5-imidazolecarboxaldehyde (40b). Compound **39** (0.5 g, 1.5 mmol) and 4-nitrobenzyl bromide (0.33 g, 1.5 mmol) were stirred in acetonitrile (10 mL) at 60 °C under nitrogen overnight. The solvent was removed by evaporation, and the resulting paste was triturated with acetone (approximately 20 mL). The resulting solid was isolated by filtration and extracted with CH_2Cl_2 and saturated $NaHCO_3$. The organic layers were dried with Na_2SO_4 and then removed by evaporation to give the product as a yellow solid (233 mg, 70%). mp 120 °C. ^{1}H NMR ($CDCl_3$): δ 9.73 (s, 1H, CHO), 8.16 (d, J = 8 Hz, 2H, ortho to NO_2), 7.88 (s, 1H, NCHN), 7.84 (s, 1H, NC*H*CCHO), 7.31 (d, J = 8 Hz, 2H, meta to NO_2), 5.64 (s, 2H, CH_2Ar).

2-Phenyl-3-(*N*-(1-(4-nitrobenzyl)-1*H*-imidazol-5-yl)-methyl)amino-3′-carboxylbiphenyl (26) Compound **40b** (75 mg, 0.32 mmol) and **21**[24] (94 mg, 0.32 mmol) were added to MeOH (6 mL) containing 0.4 g of molecular sieves. The solution was stirred at room temperature under nitrogen for 1 h, after which acetic acid (262 mg, 4.4 mmol) was added. After 5 min, $NaCNBH_3$ (40 mg, 0.64 mmol) was added in portions. The mixture was stirred at room temperature under nitrogen overnight. The reaction mixture was then extracted from CH_2Cl_2 and saturated $NaHCO_3$. The aqueous layer was extracted with CH_2Cl_2, after which the combined organic layers were washed with brine. The organic layer was then dried with Na_2SO_4, and the solvent was removed by evaporation. The crude product was purified using flash chromotography (100:40:8 = $CHCl_3$:acetone:EtOH) and then preparative HPLC to give a yellow amorphous solid (19 mg, 12%). ^{1}H NMR (CD_3OD): δ 8.04 (d, 2H, J = 9 Hz, ortho to NO_2), 7.84 (s, 1H, NC*H*NAr), 7.69 (m, 2H, Aryl H), 7.19 (d, 2H, J = 9 Hz, meta to NO_2), 7.08 (m, 7H, Aryl H), 6.92 (m, 2H, Aryl H), 6.54 (dd, 1H, J = 8 and 2 Hz, Aryl H), 6.36 (d, 1H, J = 2 Hz, Aryl H), 5.44 (s, 2H, CH_2Ar), 4.19 (s, 2H, CH_2N). HRMS (FAB): calcd for $C_{30}H_{24}O_4N_4$, 505.1875; observed, 505.1874.

2-Phenyl-3-(*N*-(1-(4-nitrobenzyl)-1*H*-imidazol-5-yl)-methyl)amino-3′-methoxycarbonylbiphenyl (27). Compound **40b** was reacted with **20**[24] in a manner similar to that described for **26**. The crude product (yellow oil) was purified using flash chromotography ($CHCl_3$:acetone:EtOH = 100:40:8). After the product was dried under vacuum, a yellow amorphous solid was obtained (71%). ^{1}H NMR ($CDCl_3$): δ 8.15 (d, 2H, J = 12 Hz, ortho to NO_2), 7.89 (t, 1H, J = 1.5 Hz, Aryl H), 7.80 (dt, 1H, J = 1.5 and 8 Hz, Aryl H), 7.63 (s, 1H, NC*H*N), 7.16−7.22 (m, 7H, Aryl H), 7.12 (dt, 1H, J = 2 and 8 Hz, Aryl H), 7.04 (m, 2H, Aryl H), 6.61 (dd, 1H, J = 2 and 8 Hz, ortho to NH), 6.54 (d, 1H, 3 Hz, Aryl H), 5.35 (s, 2H, CH_2Ar), 4.18 (d, 2H, 4 Hz, CH_2NH), 3.87 (s, 3H, COOCH_3), 3.69 (broad, 1H, NH). ^{13}C NMR ($CDCl_3$): δ 167.14, 147.70, 146.73, 143.50, 141.73, 141.54, 141.18, 134.58, 132.11, 131.57, 130.63, 130.10, 129.93, 129.63, 128.03, 127.67, 127.26, 127.09, 126.81, 124.27, 115.08, 112.49, 52.08, 48.21, 38.32 (expected 22 aryl carbons, found 21). LRMS (M^+): 519.2. HRMS (FAB): calcd for $C_{31}H_{26}O_4N_4S$, 519.2032; observed, 519.2033.

2-Phenyl-3-(*N*-(1*H*-imidazol-5-yl)methyl)amino-3′-methoxycarbonylbiphenyl (23). 4(5)-Imidazolecarboxaldehyde (24 mg, 0.25 mmol) was reacted with **20** (50 mg, 0.17 mmol) in a manner similar to that described for **26.** The crude oil was purified by flash column chromatography ($CHCl_3$:acetone:EtOH = 100:40:8) to give the product as a colorless amorphous solid (62%). ^{1}H NMR ($CDCl_3$): δ 7.88 (m, 1H, Aryl H), 7.77−7.79 (m, 1H, Aryl H), 7.61 (s, 1H, imid-2H), 7.24−7.26 (m, 2H, Aryl H), 7.08−7.18 (m, 7H, Aryl H), 6.99 (s, 1H, imid-5H), 6.73 (br s, 1H, Aryl H), 6.70 (br s, 1H, Aryl H), 4.37 (s, 2H, CH_2N), 3.85 (s, 3H, CO_2CH_3). LRMS: (M^+, intensity 100) calcd for $C_{24}H_{21}N_3O_2$, 383. HRMS (FAB): calcd, 383.1711; observed, 383.1712.

2-Phenyl-3-(*N*-(1-(4-cyanobenzyl)-1*H*-imidazol-5-yl)-methyl)amino-3′-carboxylbiphenyl (24). Compound **21** was reacted with **40a**[13] in a manner similar to that described for **26** (67%). ^{1}H NMR (10% CD_3OD in $CDCl_3$): δ 7.92 (s, 1H, Aryl H), 7.82 (d, J = 6.4 Hz, 1H, Aryl H), 7.63 (br s, 1H, imid-2H), 7.59 (d, J = 8.0 Hz, 2H, Aryl H), 7.26 (d, J = 8.4 Hz, 1H, Aryl H), 7.06−7.20 (m, 10H, Aryl H and imid-4H), 6.60 (dd, J = 2.4 and 8.0 Hz, 1H, Aryl H), 6.54 (d, J = 2.4 Hz, 1H, Aryl H), 5.31 (s, 2H, CH_2N), 4.16 (s, 2H, CH_2NH). LRMS: (M^+, intensity 100) calcd for $C_{31}H_{24}N_4O_2$, 485. HRMS (FAB): calcd, 485.1980; observed, 485.1977.

2-Phenyl-3-(*N*-(1-(4-cyanobenzyl)-1*H*-imidazol-5-yl)-methyl)amino-3′-methoxycarbonylbiphenyl (25). Compound **20** was reacted with **40a** (33 mg, 0.15 mmol) in a

manner similar to that described for **26** (45%). ^{1}H NMR ($CDCl_3$): δ 7.89 (t, J = 1.5 Hz, 1H, Aryl H), 7.81 (dt, J = 2.0 and 7.0 Hz, 1H, Aryl H), 7.60 (s, 1H, imid-2H), 7.59 (d, J = 8.5 Hz, 2H, Aryl H), 7.06−7.22 (m, 11H, Aryl H and imid-4H), 6.61 (dd, J = 2.5 and 8.0 Hz, 1H, Aryl H), 6.55 (d, J = 2.5 Hz, 1H, Aryl H), 5.30 (s, 2H, CH_2N), 4.17 (d, J = 5.0 Hz, 2H, CH_2-NH), 3.83 (s, 3H, CO_2CH_3), 3.68 (br s, 1H, NH). LRMS: (M^+, intensity 100) calcd for $C_{32}H_{26}N_4O_2$, 499. HRMS (FAB): calcd, 499.2132; observed, 499.2134.

2-Phenyl-3-(*N*-(1-(4-aminobenzyl)-1*H*-imidazol-5-yl)-methyl)amino-3′-methoxycarbonylbiphenyl (28). Compound **26** (58 mg, 0.115 mmol) and $SnCl_2$ (135 mg, 0.60 mmol) were added to AcOEt (4 mL). A few drops of MeOH were added to cause **26** to dissolve. The solution was heated at reflux for 2 h, after which it was extracted with AcOEt and saturated $NaHCO_3$. The combined organic layers were dried over Na_2SO_4 and concentrated to yield a clear oil. The crude product was purified using flash chromotography (5:1 = $CHCl_3$:MeOH followed by 100:40:8 = $CHCl_3$:acetone:EtOH) yielding a white oily solid after it was dried under vacuum overnight (1.4 mg, 3%). ^{1}H NMR (CD_3OD): δ 7.92 (s, 1H, Aryl H), 7.82 (m, 1H, Aryl H), 7.55 (s, 1H, Aryl H), 7.27 (d, 2H, J = 8 Hz, Aryl H), 7.17 (m, 9H, Aryl H), 7.03 (s, 1H, Aryl H), 6.92 (d, 2H, 8 Hz, Aryl H), 6.62 (m, 4H, Aryl H), 5.08 (s, 2H, CH_2Ar), 4.20 (s, 2H, CH_2N). LRMS: (M^+, intensity 35) calcd, 475.2.

2-Phenyl-3-(*N*-(1-(4-aminobenzyl)-1*H*-imidazol-5-yl)-methyl)amino-3′-methoxycarbonylbiphenyl (29). Compound **27** (81 mg, 0.16 mmol) and $SnCl_2$ (205 mg, 0.91 mmol) were added to AcOEt (6 mL). The solution was heated under reflux for 2 h, and then, saturated $NaHCO_3$ (15 mL) was added. The mixture was extracted, and the aqueous layer was washed with AcOEt (10 mL, 2×). The combined organic portions were dried with sodium sulfate, and the solvent was removed by evaporation. A white amorphous solid was obtained (57 mg, 75%). ^{1}H NMR ($CDCl_3$): δ 7.90 (s, 1H, Aryl H), 7.80 (d, 1H, J = 7 Hz, Aryl H), 7.53 (s, 1H, NC*H*N), 7.09−7.21 (m, 8H, Aryl H), 7.06 (s, 1H, NC*H*CCH$_2$N), 6.89 (d, 2H, J = 10 Hz, meta to NH_2), 6.55−6.61 (m, 4H, Aryl H), 5.04 (s, 2H, CH_2Ar), 4.19 (s, 2H, CH_2NH), 3.86 (s, 3H, COOCH_3), 3.77 (s, 1H, NH). ^{13}C NMR ($CDCl_3$): δ 167.19, 147.07, 146.51, 141.79, 141.49, 138.70, 134.64, 131.48, 130.65, 129.85, 129.77, 129.45, 129.27, 128.49, 128.29, 127.94, 127.60, 126.92, 129.61, 125.38, 115.33, 112.35, 52.03, 48.71, 38.18. LRMS: (M^+, intensity 40) calcd, 489.2. HRMS (FAB): calcd, 489.2991; observed, 489.2993.

Synthetic Procedure for Arylthiol-Containing Inhibitors 30−36: Typical Example Given for Compound 31. 4-*S*-(*t*-Butoxycarbonyl)mercaptobenzoic Acid (41a). To a solution of 4-mercaptobenzoic acid (1.0 g, 6.5 mmol), Boc_2O (1.6 g, 7.1 mmol), and DMAP (8 mg, 0.06 mmol) in THF (10 mL) was added Et_3N (992 μL, 7.1 mmol), and the mixture was stirred at room temperature overnight. After the solvent evaporated, the residual oil was dissolved in AcOEt (100 mL) and the organic layer was washed with 10% citric acid, brine, and dried ($MgSO_4$). Evaporation of the solvent gave a crude pale yellow solid (1.7 g). The crude solid was recrystallized from an Et_2O/petroleum ether mixture to give the product as colorless crystals (1.4 g, 82%). mp 129−130 °C. ^{1}H NMR ($CDCl_3$): δ 8.12 (d, J = 8.4 Hz, 2H, Aryl H), 7.65 (d, J = 8.4 Hz, 2H, Aryl H), 1.53 (s, 9H, ^{t}Bu). ^{13}C NMR ($CDCl_3$): δ 134.78, 134.15, 130.66, 130.42, 86.38, 28.17.

4-*S*-(*t*-Butoxycarbonyl)mercaptobenzyl Alcohol (42a). To a solution of **41a** (600 mg, 2.36 mmol) and NEt_3 (362 μL, 2.60 mmol) in THF (10 mL) was added ethyl chloroformate (248 μL, 2.60 mmol) in THF (2 mL) dropwise at 0 °C. After the solution was stirred at 0 °C for 30 min, additional dry THF (15 mL) was added to the mixture and the precipitate was filtered off. To the filtrate was added $NaBH_4$ (197 mg, 5.20 mmol) at 0 °C, and the mixture was stirred at 0 °C for 1 h. The reaction was quenched by adding 0.5 N HCl (2 mL) at 0 °C, and the THF was evaporated. The product was extracted with AcOEt (100 mL) and 5% $NaHCO_3$, and the organic layer was washed with brine and dried ($MgSO_4$). The crude oil was purified by flash column chromatography (hexane:AcOEt = 2:1) to give the product as a colorless oil (516 mg, 91%). ^{1}H NMR ($CDCl_3$): δ 1.49 (s, 9H, ^{t}Bu), 3.60 (br s, 1H, OH), 4.53 (s, 2H, CH_2), 7.28 (d, J = 7.6 Hz, 2H, Aryl H), 7.43 (d, J = 7.6 Hz, 2H, Aryl H). ^{13}C NMR ($CDCl_3$): δ 28.12, 64.08, 85.51, 127.07, 127.28, 142.60, 128.06.

4-*S*-(*t*-Butoxycarbonyl)mercaptobenzaldehyde (43a). To a solution of oxalyl chloride (55 μL, 0.63 mmol) in dry CH_2Cl_2 (0.5 mL) was added freshly distilled DMSO (96 μL, 1.35 mmol) in dry CH_2Cl_2 (0.5 mL) by syringe at −70 °C under a N_2 stream. The solution was stirred for 20 min at −70 °C, and then **42a** (126 mg, 1.35 mmol) in CH_2Cl_2 (0.5 mL) was slowly added by syringe at −70 °C. After the solution was stirred for 1 h at −70 °C, freshly distilled Et_3N (362 μL, 2.60 mmol) was added, and the bath was removed to allow the temperature to rise to room temperature. To the solution was added Et_2O (50 mL) and saturated $NaHCO_3$, and the organic layer was washed with H_2O and brine and dried ($MgSO_4$). Evaporation of the solvent gave the product as a colorless oil (111 mg, 90%). The aldehyde was used for the next step reaction immediately without further purification. ^{1}H NMR ($CDCl_3$): δ 10.02 (s, 1H, Aryl H), 7.88 (d, J = 6.4 Hz, 2H, Aryl H), 7.69 (d, J = 6.4 Hz, 2H, Aryl H), 1.52 (s, 9H, ^{t}Bu). ^{13}C NMR ($CDCl_3$): δ 191.43, 166.18, 136.43, 136.18, 134.37, 127.82, 86.47, 27.91.

2-Phenyl-3-(4-mercapto)benzylamino-3′-methoxycarbonylbiphenyl (31). The aldehyde **43a** (60 mg, 0.25 mmol) and **20** (51 mg, 0.17 mmol) were dissolved in CH_2Cl_2 (1 mL), and AcOH (20 mg) was added to the mixture. The solution was stirred for 1 h at room temperature, and then, $NaBH_4$ (9 mg, 0.25 mmol) was added. After the solution was stirred for an additional 2 h at room temperature, the solvent was evaporated. The residue was dissolved in CH_2Cl_2 (50 mL), and the organic layer was washed with saturated $NaHCO_3$ and brine and dried ($MgSO_4$). The crude product was purified by flash column chromatography (hexane:AcOEt = 4:1) to give 2-phenyl-3-(4-*t*-butoxycarbonylmercapto)benzylamino-3′-methoxycarbonylbiphenyl as a white fine powder (69 mg, 78%). mp 170−171 °C. ^{1}H NMR ($CDCl_3$): δ 7.89 (s, 1H, Aryl H), 7.78−7.81 (m, 1H, Aryl H), 7.52 (d, J = 8.1 Hz, 2H, Aryl H), 7.43 (d, J = 8.1 Hz, 2H, Aryl H), 7.08−7.27 (m, 8H, Aryl H), 6.66−6.69 (m, 2H, Aryl H), 4.42 (s, 2H, CH_2), 4.25 (br s, 1H, NH), 3.86 (s, 3H, OCH_3), 1.51 (s, 9H, ^{t}Bu). ^{13}C NMR ($CDCl_3$): δ 167.91, 167.19, 147.43, 141.88, 141.50, 140.69, 135.22, 134.64, 131.58, 130.66, 129.84, 129.79, 129.13, 128.33, 128.10, 127.90, 127.53, 127.38, 126.85, 126.57, 114.88, 111.92, 85.65, 51.99, 47.83, 28.18. LRMS: (M^+, intensity 80) calcd for $C_{27}H_{23}NO_2S$, 525. HRMS (FAB): calcd, 525.1975; observed, 525.1974.

To a solution of 2-phenyl-3-(4-*t*-butoxycarbonylmercapto)-benzylamino-3′-methoxycarbonylbiphenyl (55 mg, 0.11 mmol) in CH_2Cl_2 (1 mL) was added TFA (1 mL), and the solution was stirred at room temperature for 30 min. Evaporation of the solvent gave **31** as a yellow amorphous solid (45 mg, 100%). ^{1}H NMR ($CDCl_3$): δ 7.87−7.90 (m, 2H, Aryl H), 7.45 (d, J = 8.8 Hz, 1H, Aryl H), 7.34−7.36 (m, 2H, Aryl H), 7.11−7.23 (m, 9H, Aryl H), 6.95−6.97 (m, 2H, Aryl H), 4.43 (s, 2H, CH_2), 3.88 (s, 3H, CO_2CH_3), 3.47 (br s, 1H, SH). ^{13}C NMR ($CDCl_3$): δ 167.08, 142.79, 141.00, 139.95, 138.86, 134.39, 134.35, 134.00, 132.23, 131.18, 130.58, 130.16, 129.53, 129.28, 128.52, 128.37, 128.15, 127.65, 126.50, 125.04, 121.95, 56.36, 52.36. LRMS: (M^+, intensity 90) calcd for $C_{27}H_{23}NO_2S$, 424. HRMS (FAB): calcd, 424.1369; observed, 424.1371.

2-Phenyl-3-(4-mercapto)benzylamino-3′-carboxylbiphenyl (30). Compound **43a** and amine **21** were reacted in a manner similar to that described for **31** to give S-Boc-protected form **30** as a white solid (23%). mp 138 °C. ^{1}H NMR (CD_3OD): δ 7.93−7.75 (m, 2H, ArH), 7.56 (s, 1H, ArH), 7.38 (m, 4H, ArH), 7.13−6.98 (m, 7H, ArH), 6.60 (m, 2H, ArH), 4.35 (s, 2H, $ArCH_2$), 1.41 (s, 9H, $(CH_3)_3C$). HRMS (FAB): calcd for $C_{31}H_{29}NO_4S$, 511.1817; observed, 511.1816.

The above compound was deprotected in a manner similar to that described for **31** to afford **30** as a yellow amorphous solid (87%). ^{1}H NMR ($CDCl_3$): δ 7.86 (m, 3H, ArH), 7.20−7.04 (m, 12H, ArH and NH or SH), 6.64−6.62 (m, 2H, ArH), 4.95−4.90 (br s, 1H, SH or NH), 4.29 (s, 2H, ArH). ^{13}C NMR ($CDCl_3$): δ 170.08, 145.84, 140.62, 140.28, 140.00, 134.05,

130.21, 129.86, 128.39, 128.25, 127.81, 127.55, 127.01, 126.97, 126.80, 126.76, 126.60, 126.36, 126.14, 125.30, 116.01, 113.80, 110.91, 64.50. HRMS (FAB): calcd for $C_{26}H_{21}NO_2S$, 411.1293; observed, 411.1291.

2-Phenyl-[4-(3-mercaptobenzyl)amino]-3′-carboxylbiphenyl (32). 3-(*S*-*t*-Butoxycarbonyl)mercaptobenzoic acid (41b). This compound was prepared by the same method as described for **41a**. Recrystallization from a Et_2O/hexanes mixture afforded a colorless powder (97%). mp 127−128 °C. ^{1}H NMR ($CDCl_3$): δ 8.27 (s, 1H, Aryl H), 8.13 (d, J = 7.5 Hz, 1H, Aryl H), 7.77 (d, J = 7.5 Hz, 1H, Aryl H), 7.51 (t, J = 8.1 Hz, 1H, Aryl H), 1.52 (s, 9H, Boc). ^{13}C NMR ($CDCl_3$): δ 168.60, 167.47, 139.45, 136.23, 131.19, 130.82, 129.32, 129.16, 86.18, 28.16.

3-*S*-(*t*-Butoxycarbonyl)mercaptobenzyl Alcohol (42b). This compound was prepared from **41b** by the same method as for **42a** (100%). ^{1}H NMR ($CDCl_3$): δ 7.33−7.48 (m, 4H, Aryl H), 4.61 (s, 2H, CH_2), 3.30 (br s, 1H, OH), 1.49 (s, 9H, ^{t}Bu). ^{13}C NMR ($CDCl_3$): δ 168.07, 142.04, 133.81, 133.16, 129.07, 128.50, 127.91, 85.87, 64.42, 27.93.

3-*S*-(*t*-Butoxycarbonyl)mercaptobenzaldehyde (43b). This compound was prepared from **42b** by the same method as for **43a** (60%). This aldehyde was used for the next step reaction immediately without further purification. ^{1}H NMR ($CDCl_3$): δ 10.02 (s, 1H, CHO), 8.03 (s, 1H, Aryl H), 7.90 (d, J = 8.0 Hz, 1H, Aryl H), 7.78 (d, J = 8.0 Hz, 1H, Aryl H), 7.57 (t, J = 7.6 Hz, 1H, Aryl H), 1.52 (s, 9H, ^{t}Bu).

Compound **43b** and amine **21** (0.12 g, 0.42 mmol) were reacted in a manner similar to **31** to afford the S-Boc-protected form of **32** as a white solid (10 mg, 4%). mp 150 °C. TLC: R_f, 0.41 (silica gel, $CHCl_2$:MeOH 8:1). ^{1}H NMR ($CDCl_3$:CD_3OD = 1:1): δ 7.65 (m, 2H, ArH), 7.45−7.28 (s, 4H, ArH), 7.07−6.95 (m, 8H, ArH), 6.60−6.55 (m, 2H, ArH), 4.31 (s, 2H, $ArCH_2$), 1.35 (s, 9H, $(CH_3)_3C$). HRMS (FAB): calcd for $C_{31}H_{29}NO_4S$, 511.1817; observed, 511.1816.

The above 2-phenyl-4-[3-*S*-(*t*-butoxylcarbonyl)mercaptobenzyl]amino-3′-carboxylbiphenyl was deprotected to afford **32** as a yellow amorphous solid (7 mg, 88%). ^{1}H NMR ($CDCl_3$:CD_3OD = 1:1): δ 7.66−7.64 (m, 2H, ArH), 7.24−6.95 (m, 13H, ArH), 6.63 (m, 1H, ArH), 4.27 (s, 2H, ArH). HRMS (FAB): calcd for $C_{26}H_{21}NO_2S$, 411.1293; observed, 411.1291.

2-Phenyl-3-(3-mercapto)benzylamino-3′-methoxycarbonylbiphenyl (33). The fully protected form of **33** was prepared from the reaction of **43b** with **20** by the same procedure as for **31**. The crude yellow oil was purified by flash column chromatography (hexane:AcOEt = 10:1) to give 2-phenyl-3-(3-*t*-butoxycarbonylmercapto)benzylamino-3′-methoxycarbonylbiphenyl as colorless oil (50%). ^{1}H NMR ($CDCl_3$): δ 7.90 (s, 1H, Aryl H), 7.80 (dd, J = 1.8 and 6.6 Hz, 1H, Aryl H), 7.56 (s, 1H, Aryl H), 7.38−7.47 (m, 3H, Aryl H), 7.25−7.28 (m, 1H, Aryl H), 7.08−7.20 (m, 7H, Aryl H), 6.67−6.69 (m, 2H, Aryl H), 4.41 (s, 2H, CH_2N), 4.26 (br s, 1H, NH), 3.86 (s, 3H, CO_2-Me), 1.51 (s, 9H, ^{t}Bu). LRMS: (M^+, intensity 95) calcd for $C_{32}H_{31}NO_4S$, 525. HRMS (FAB): calcd, 525.1975; observed, 525.1974.

Deprotection of the above compound by the same manner as for **31** gave **33** as a yellow amorphous solid (100%). ^{1}H NMR ($CDCl_3$): δ 7.88−7.91 (m, 2H, Aryl H), 7.46 (d, J = 8.0 Hz, 1H, Aryl H), 7.34−7.38 (m, 2H, Aryl H), 7.09−7.24 (m, 10H, Aryl H), 6.98−7.00 (m, 2H, Aryl H), 4.42 (s, 2H, CH_2N), 3.89 (s, 3H, CO_2CH_3), 3.44 (s, 1H, SH). LRMS: (M^+, intensity 100) calcd for $C_{27}H_{23}NO_2S$, 425. HRMS (FAB): calcd, 425.1252; observed, 425.1450.

2-Phenyl-4-(3-mercaptobenzoyl)amino-3′-methoxycarbonylbiphenyl (35). The S-protected **35** was prepared by the reaction of 3-(*S*-*tert*-butoxycarbonyl)mercaptobenzoic acid and **20** in a similar procedure to **34**[24] (23%). ^{1}H NMR ($CDCl_3$): δ 8.03 (s, 1H, Aryl H), 7.95 (s, 2H, Aryl H), 7.93 (s, 1H, Aryl H), 7.87 (dt, J = 8.0 and 2.5 Hz, 1H, Aryl H), 7.78 (dd, J = 8.5 and 2.5 Hz, 1H, Aryl H), 7.71 (d, J = 8.0 Hz, 1H, Aryl H), 7.68 (d, J = 2.5 Hz, 1H, Aryl H), 7.54 (t, J = 8.0 Hz, 1H, Aryl H), 7.46 (d, J = 8.0 Hz, 1H, Aryl H), 7.19−7.23 (m, 4H, Aryl H), 7.14−7.15 (m, 2H, Aryl H), 3.89 (s, 3H, CO_2CH_3), 1.53 (s, 9H, Boc).

Deprotection of the above compound by the same procedure as for **34** gave **35** as a yellow amorphous solid (93%). ^{1}H NMR ($CDCl_3$): δ 7.96 (s, 1H, Aryl H), 7.93 (s, 1H, Aryl H), 7.88 (dt, J = 2.5 and 7.5 Hz, 1H, Aryl H), 7.83 (s, 1H, Aryl H), 7.78 (dd, J = 2.5 and 8.5 Hz, 1H, Aryl H), 7.65 (d, J = 8.0 Hz, 2H, Aryl H), 7.47 (d, J = 7.5 Hz, 1H, Aryl H), 7.39 (t, J = 7.0 Hz, 1H, Aryl H), 7.21−7.23 (m, 4H, Aryl H), 7.14−7.17 (m, 2H, Aryl H), 3.89 (s, 3H, CO_2CO_3). LRMS: (M^+, intensity 40) calcd for $C_{27}H_{21}NO_3S$, 440. HRMS (FAB): calcd, 440.1319; observed, 440.1320.

2-Phenyl-3-(2-mercapto)benzylamino-3′-methoxycarbonylbiphenyl (36). 2-(*S*-*t*-Butoxycarbonyl)mercaptobenzoic acid (41c). Protection of thiosalicylic acid with Boc_2O was carried out by the same procedure as for **41a** to give a pale yellow solid (83%). This protected acid was used for the next step reaction without further purification. mp 121−123 °C. ^{1}H NMR (10% CD_3OD in $CDCl_3$): δ 7.97 (dd, J = 1.6 and 7.6 Hz, 1H, Aryl H), 7.69 (dd, J = 1.2 and 9.2 Hz, 1H, Aryl H), 7.51 (dd, J = 1.2 and 6.4 Hz, 1H, Aryl H), 7.43 (td, J = 1.2 and 6.4 Hz, 1H, Aryl H), 1.50 (s, 9H, ^{t}Bu). ^{13}C NMR ($CDCl_3$): δ 168.64, 167.32, 136.09, 131.51, 130.72, 129.39, 128.68, 128.64, 85.55, 27.71.

2-*S*-(*t*-Butoxycarbonyl)mercaptobenzyl Alcohol (42c). This compound was prepared from **41c** by the same method as for **42a**, and the crude product was purified by flash column chromatography (hexane:AcOEt = 10:1 to 4:1) to give the product as a colorless oil (65%). ^{1}H NMR ($CDCl_3$): δ 7.55 (dd, J = 1.2 and 7.6 Hz, 1H, Aryl H), 7.50 (dd, J = 1.2 and 7.6 Hz, 1H, Aryl H), 7.42 (td, J = 1.6 and 7.6 Hz, 1H, Aryl H), 7.28 (td, J = 1.6 and 7.6 Hz, 1H, Aryl H), 4.72 (d, J = 4.4 Hz, 2H, CH_2), 2.90 (s, 1H, OH), 1.48 (s, 9H, ^{t}Bu). ^{13}C NMR ($CDCl_3$): δ 168.15, 144.44, 136.56, 130.55, 128.16, 126.39, 86.01, 63.33, 28.07.

2-*S*-(*t*-Butoxycarbonyl)mercaptobenzaldehyde (43c). This compound was prepared from **42c** by the same method as **43a** (79%). ^{1}H NMR ($CDCl_3$): δ 10.43 (s, 1H, CHO), 8.00−8.03 (m, 1H, Aryl H), 7.54−7.63 (m, 3H, Aryl H), 1.50 (s, 9H, ^{t}Bu). This compound was used for the next step reaction without further purification.

The fully protected compound was prepared from **43c** and **20** by the same method as for **31**. The crude compound was purified by column chromatography (hexane:AcOEt = 10:1) to give 2-phenyl-3-(2-*t*-butoxycarbonylmercapto)benzylamino-3′-methoxycarbonylbiphenyl as a colorless oil (64%). ^{1}H NMR ($CDCl_3$): δ 7.79 (dt, J = 1.5 and 1.5 Hz, 1H, Aryl H), 7.55−7.60 (m, 2H, Aryl H), 7.43 (td, J = 1.5 and 8.0 Hz, 1H, Aryl H), 7.33 (td, J = 1.5 and 8.0 Hz, 1H, Aryl H), 7.25 (d, J = 8.0 Hz, 1H, Aryl H), 7.10−7.18 (m, 7H, Aryl H), 6.68−6.69 (m, 2H, Aryl H), 4.52 (s, 2H, NCH_2), 4.30 (br s, 1H, NH), 3.86 (s, 3H, CO_2CH_3), 1.48 (s, 9H, ^{t}Bu). ^{13}C NMR ($CDCl_3$): δ 167.68, 167.19, 147.54, 142.65, 141.97, 141.56, 137.18, 134.66, 131.54, 130.65, 130.58, 129.81, 129.20, 128.82, 128.13, 127.87, 127.53, 127.33, 126.78, 126.51, 114.82, 111.81, 85.93, 51.98, 46.85, 28.13.

Compound **36** was obtained from the deprotection of the above compound in the same manner as for **31** (100%). ^{1}H NMR ($CDCl_3$): δ 7.88−7.90 (m, 2H, Aryl H), 7.39−7.49 (m, 3H, Aryl H), 7.15−7.27 (m, 10H, Aryl H), 6.99−7.02 (m, 2H, Aryl H), 4.72 (s, 2H, CH_2), 3.88 (s, 4H, CO_2CH_3 and SH). ^{13}C NMR ($CDCl_3$): δ 167.12, 142.65, 140.67, 140.08, 139.04, 134.85, 134.40, 133.73, 132.31, 132.10, 130.98, 130.75, 130.59, 130.31, 130.15, 129.57, 128.46, 128.34, 128.14, 127.92, 127.61, 124.47, 121.44, 54.96, 52.34. LRMS: (M^+, intensity 100) calcd for $C_{27}H_{23}NO_2S$, 424. HRMS (FAB): calcd, 424.1369; observed, 424.1371.

2-Phenyl-4-(3-mercaptoethylcarbonyl)amino-3′-methoxycarbonylbiphenyl (38). The S-protected compound was prepared form S-^{t}butoxycarbonyl-β-mercaptopropionic acid and **20** by a manner similar to **37**[24] (61%). ^{1}H NMR ($CDCl_3$): δ 7.91 (s, 1H, Aryl H), 7.85 (dt, J = 2.1 and 6.0 Hz, 1H, Aryl H), 7.73 (s, 1H, Aryl H), 7.65 (dd, J = 7.8 and 2.1 Hz, 1H, Aryl H), 7.58 (d, J = 2.1 Hz, 1H, Aryl H), 7.37 (d, J = 8.7 Hz, 1H, Aryl

H), 7.08−7.22 (m, 7H, Aryl H), 3.87 (s, 3H, CO_2CH_3), 3.16 (t, J = 6.3 Hz, 2H, CH_2S), 2.78 (t, J = 6.3 Hz, 2H, CH_2CO), 1.49 (s, 9H, ^{t}Bu).

The Boc-protected compound was treated with TFA to give **38** as a yellow oil (12 mg, 75%). ^{1}H NMR ($CDCl_3$): δ 7.90 (s, 1H, Aryl H), 7.85 (d, J = 8.0 Hz, 1H, Aryl H), 7.82 (s, 1H, Aryl H), 7.39 (d, J = 8.0 Hz, 1H, Aryl H), 7.16−7.24 (m, 5H, Aryl H), 7.07−7.09 (m, 2H, Aryl H), 3.89 (s, 3H, CO_2CH_3), 2.90−2.94 (m, 2H, SCH_2), 2.76 (t, J = 7.0 Hz, 2H, CH_2CO), 1.70 (t, J = 7.5 Hz, 1H, SH). LRMS: (M^+, intensity 100) calcd for $C_{23}H_{21}NO_3$, 392. HRMS (FAB): calcd, 392.1321; observed, 392.1320.

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Supporting Information Available: Crystallographic details for **19** (Figure 3) including tables of atomic coordinates, thermal parameters, bond angles, and bond lengths (17 pages). This material is available free of charge via the Internet at http://pubs.acs.org.

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