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Development of a Tripeptide Mimetic Strategy for the Inhibition of Protein Farnesyltransferase

Mohit A. Kotharé,[a] Junko Ohkanda,[a] Jeffrey W. Lockman,[a] Yimin Qian,[a] Michelle A. Blaskovich,[b] Said M. Sebti[b,*] and Andrew D. Hamilton[a,*]

[a]*Department of Chemistry, Yale University, P. O. Box 208107, New Haven, CT 06520, USA*
[b]*Drug Discovery Program, H. Lee Moffitt Cancer Center and Research Institute, Department of Biochemistry and Molecular Biology, University of South Florida, Tampa, FL 33612, USA*

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Abstract—This paper describes the development of a novel terphenyl-based tripeptide mimetic of the CAAX carboxy terminal sequence of Ras. We employ a concise synthesis to form a series of differently functionalized terphenyl inhibitors of protein farnesyltransferase (PFTase), exemplified by **5**, **6** and **7**. The key reaction in the synthesis of the terphenyl methyl ester **13**, and therefore **6** and **7**, was the Pd-catalyzed chemoselective Suzuki cross-coupling of 3-bromo-4-chloronitrobenzene **16** with an appropriate boronic acid derivative utilizing a commercially available, electron rich phosphine ligand. We further show that one member of this series is a potent inhibitor of PFTase.

Introduction

The mammalian *ras* genes (H, K and N) encode a family of 21 kDa GTP binding proteins that are intimately involved in the regulation of mitogen-stimulated cell division.[1–3] The proteins cycle between their GTP (active) and GDP (inactive) bound states, to regulate the transduction of biological information from the plasma membrane to the nucleus. Since only the GTP–Ras complex can trigger mitogen-activated protein (MAP) kinase cascades (which eventually lead to cell division), the hydrolysis of GTP by Ras is vital to avoiding uncontrolled cell growth. Single amino acid mutations of Ras at positions 12, 13, or 61 lead to GTPase-deficient Ras, resulting in uncontrolled cell growth.[3] These mutations in the Ras proteins are common in human cancers and therefore blocking oncogenic Ras can provide a new avenue in cancer chemotherapy.[4]

Ras proteins must be membrane-bound to participate in the gene expression process through activation of the MAP kinase cascades.[5–7] Ras proteins are made in the cytosol, however, a series of post-translational modifications cause their translocation to the plasma membrane. The first modification of Ras is farnesylation of the cysteine residue of its carboxyl terminal sequence CAAX,[5] where the fourth residue is cysteine, the third and the second residues are aliphatic (valine or isoleucine) and X is any amino acid but usually methionine or serine. This modification which is required for Ras activity is catalyzed by the enzyme protein farnesyltransferase (PFTase).[8] The CAAX sequence is the minimum recognition element for PFTase. Subsequent post-translational modifications of the Ras protein include the proteolysis of the AAX residues and methylation of the resulting cysteine carboxylic acid. These steps are not obligatory for membrane association or malignant transforming activity.[6] Thus, inhibition of PFTase provides an attractive target for blocking Ras function and therefore as an anti-cancer therapy.

PFTase is a heterodimer composed of a 48 kDa α-subunit and a 46 kDa β-subunit, and also requires Zn^{2+} and Mg^{2+} ions for activity.[8] The PFTase active site is at the interface of the two subunits and forms a ternary complex with both farnesyl diphosphate (FPP) and the CAAX box of the Ras protein.[9] Crystallographic studies using α-hydroxyfarnesylphosphonic acid and a CVIM tetrapeptide as FPP and Ras analogues, respectively, suggest that both substrates bind to PFTase in extended conformations.[10] The tetrapeptide is bound within the active site through hydrophobic interactions in the region of the Ile-Met peptide bond and also utilizes hydrogen bonding to the Ile backbone carbonyl. Additionally, the cysteine sulfur is weakly bound to the active-site zinc.

Similar in function to PFTase are the two classes of protein geranylgeranyltransferases: PGGTase-I and PGGTase-II.[11] Both enzymes attach geranylgeranyl groups, 20-carbon

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* Corresponding authors. Tel.: +1-203-432-5570; fax: +1-203-432-3221; e-mail: andrew.hamilton@yale.edu; Said M. Sebti e-mail: sebti@moffitt.usf.edu; tel.: +813-979-6734; fax: +813-979-6748.

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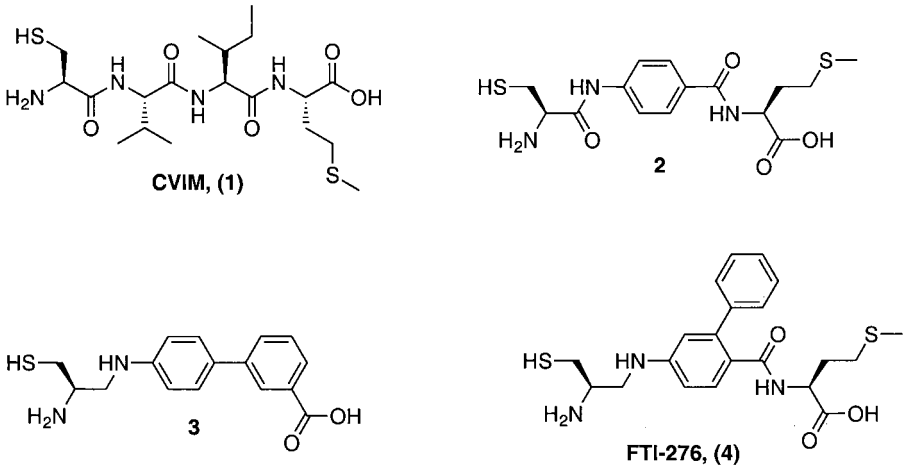


Figure 1. Previously reported PFTase inhibitors.

isoprenoid units, to the cysteine residues near their substrates' carboxy terminus. Like PFTase, PGGTase-I recognizes a CAAX tetrapeptide, but one with a terminal leucine instead of other amino acids. PGGTase-II recognizes proteins ending in Cys-Cys or Cys-X-Cys motifs. Since protein geranylgeranylation is more prevalent than farnesylation, it is imperative to design inhibitors that are selective for PFTase over PGGTase-I.

The CAAX tetrapeptides are potent inhibitors of PFTase.[12a] However, due to their peptide character, these inhibitors are metabolically unstable and are poorly taken up by the cells. In an endeavor to circumvent these problems, we[12b] and others[12c–e] have reported peptidomimetic inhibitors of PFTase. Herein, we report the design, synthesis and biological evaluation of novel, terphenyl-based tripeptide mimetics of CAAX, that are non-peptidic and cell permeable and are highly potent and selective inhibitors of PFTase.

Design of tripeptide mimics as inhibitors of PFTase

Tetrapeptide **CVIM** (Fig. 1) inhibited PFTase in vitro with an IC_{50} of 340 nM but is inactive at inhibiting Ras processing in whole cells. In an attempt to circumvent low cellular uptake and metabolic instability problems associated with peptide inhibitors, we had previously synthesized a series of peptidomimetics wherein the central hydrophobic dipeptide core (Val-Ile) was replaced by a 4-aminobenzoic acid spacer.[13] This provided a rigid and hydrophobic link between the cysteine and methionine residues. For example, analogue (**2**) inhibited PFTase potently (IC_{50}=150 nM), confirming that parts of the tetrapeptide sequence could be replaced with a structurally defined non-amino acid fragment.

In an endeavor to further reduce the peptidic character of **2**, the Val-Ile-Met residues of **CVIM** (**1**) were replaced with the more conformationally restricted 4-amino-3′-carboxybiphenyl moiety, resulting in analogue **3**. Since the *para*-substituted benzene of **2** was topographically a small mimic of the aliphatic Val-Ile moiety, further modifications were also investigated. It was envisioned that introduction of an additional phenyl ring onto the aromatic spacer of **2** would result in a more hydrophobic molecule, that could interact effectively with the hydrophobic binding pocket of PFTase. This resulted in the synthesis of **FTI-276 (4)**, an extremely potent inhibitor of PFTase (IC_{50}=0.61 nM).[14] The success of scaffolds **3** and **4** suggested that combining their essential features (phenyl substitution and a C-terminal benzoate) could lead to an improved non-peptide inhibitor of PFTase. Consequently, we designed a potential mimetic of the AAX tripeptide based on a terphenyl derivative, exemplified by **5** (Fig. 2).

The advantage of this design is that it combines a rigidly separated N- and C-terminus with a phenyl group that can mimic the hydrophobic side chains of the AAX tripeptide. To

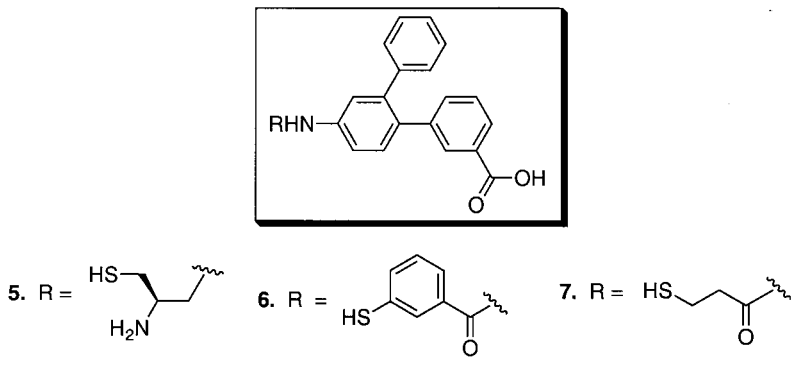


Figure 2. Designed PFTase inhibitors **5**–**7**.

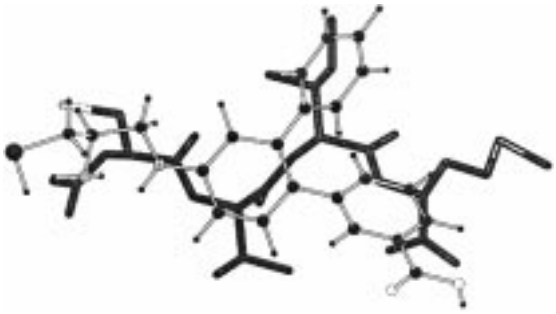


Figure 3. Overlaid structures of terphenyl inhibitor **5** and **Ac-CVIM**.

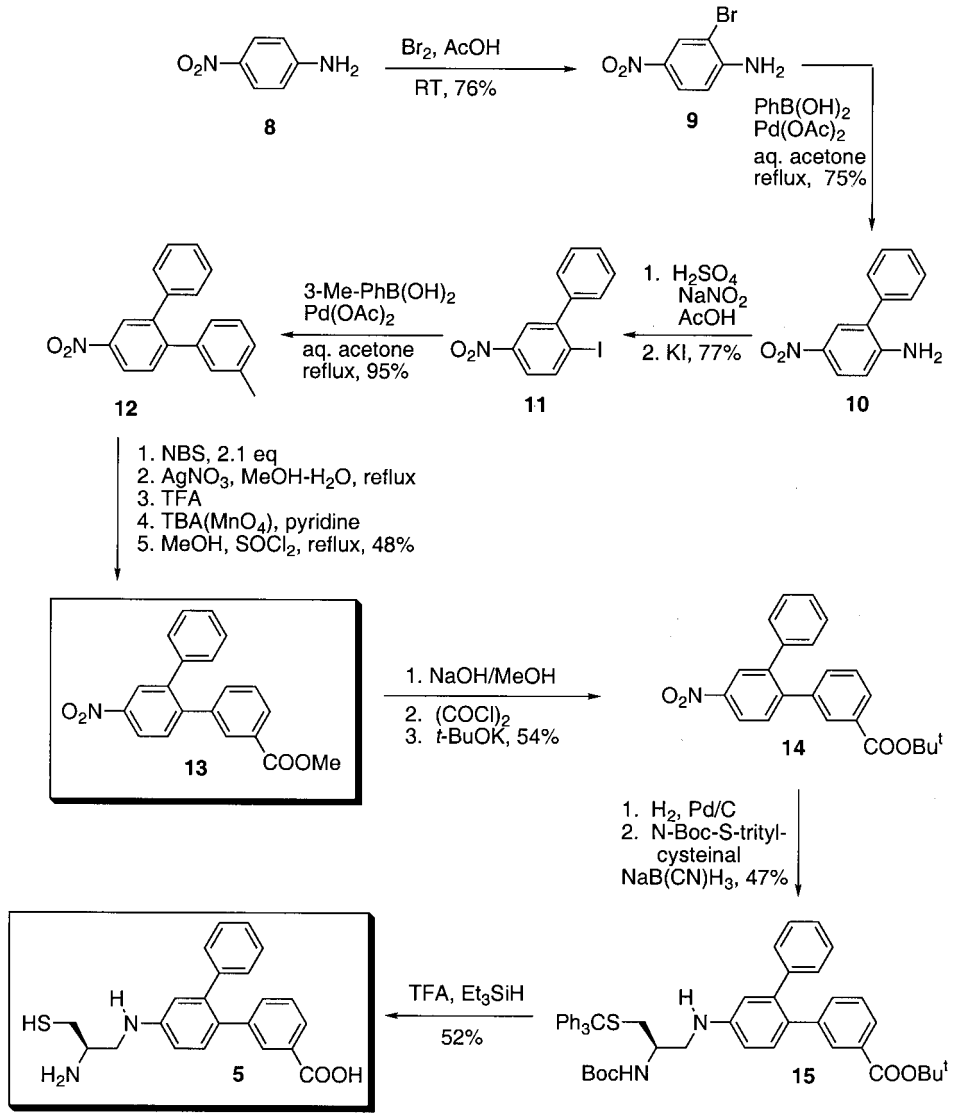
investigate the structural similarities of our terphenyl peptidomimetics with the Ras CAAX sequence, molecular modeling was employed (Fig. 3). Terphenyl compound **5** was modeled in the absence of solvent with the OPLS-AA force field, using the BOSS4.1 software package.[15] Although compound **5** is certain to exist in aqueous solution as its zwitterion, the neutral complex was modeled for computational simplicity.

Compound **5** superimposes well onto the extended conformation of **Ac-CVIM**[10] that is found in the X-ray crystal structure bound within the PFTase active site. The distance between the N-terminal cysteine sulfur and C-terminal carboxylate carbon on terphenyl inhibitor **5** (13.2 Å for **5**) is similar to that found in the substrate tetrapeptide (12.0 Å for **Ac-CVIM**). Superimposition of the N- and C-termini of the two molecules (Fig. 3) places the central phenyl ring of **5** near the substrate's valine residue and projects another phenyl ring in the position corresponding to the isoleucine side chain of the tetrapeptide. These results confirmed the potential mimicry of the AAX tripeptide by our terphenyl derivatives and encouraged us to seek an effective route to their synthesis.

Results and Discussion

Chemistry

Inhibitors exemplified by **3** and **4** were previously synthesized in this laboratory as peptidomimetics of the CAAX

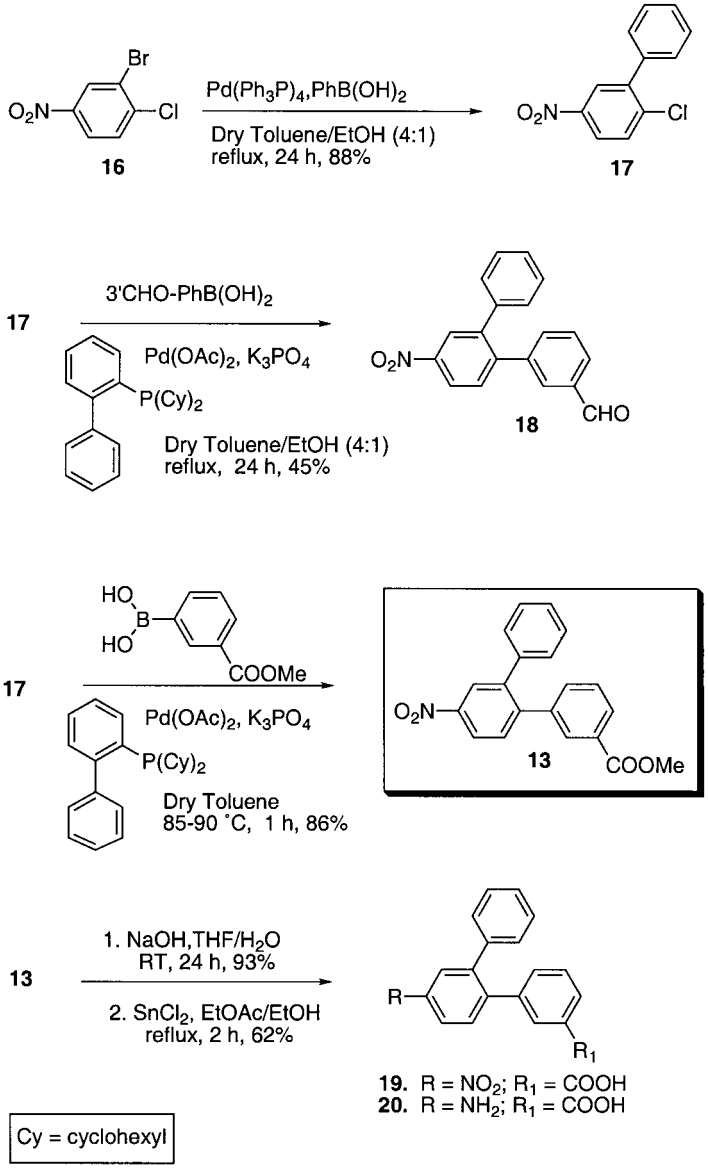


Scheme 1. Synthesis of analog **5**.

sequence (**1**) of protein farnesyltransferase (PFTase).[16–18] We were interested in pursuing the synthesis of compounds **5**–**7**, because of the above modeling studies and the novelty of these structures as PFTase inhibitors. In our initial strategy (Scheme 1), commercially available nitroaniline **8** was brominated and the resulting aryl bromide **9** was subjected to a Suzuki cross-coupling reaction with phenylboronic acid to afford biphenylaniline **10**, which was transformed under Sandmeyer conditions to aryl iodide **11**. Coupling of **11** with 3-methylphenylboronic acid under Suzuki cross-coupling conditions, afforded terphenyl **12**. The methyl moiety of **12** was oxidized to afford the corresponding terphenyl-carboxylic acid, which was then esterified to its terphenyl–methyl ester **13**. This ester was later hydrolyzed and re-esterified to the *t*-butyl ester **14**. Catalytic reduction of **14**, followed by reductive amination of the resultant amine with an appropriately protected cysteinal afforded **15**. Deprotection of **15** gave the desired terphenyl inhibitor **5**. While giving us access to the terphenyl peptidomimetics, this route was long and laborious. We therefore wanted to explore alternative routes to modified inhibitors such as **6** and **7**.

A facile entry into these terphenyl analogues would involve, the *chemoselective C–C bond formation* of a differentially functionalized aryl dihalide such as **16** with an appropriate arylboronic acid derivative (Scheme 2). Until the recent work of Buchwald et al.[19] and Fu et al.,[20] it was thought that aryl chlorides (particularly electron rich/neutral or sterically congested), were poor substrates for palladium-catalyzed Suzuki cross-coupling reactions. However, with the advent of electron rich ligands such as 2-(di-*t*-butylphosphino)biphenyl (**a**) and 2-(dicyclohexylphosphino)biphenyl (**b**) (Fig. 4), it is now possible to perform these reactions in an efficient manner.

We decided to apply this modification to the synthesis of the terphenyl mimetics, **6** and **7** (Schemes 2 and 3). Commercially available nitrobenzene **16**, was chosen as a retron for the synthesis of scaffold **13**. We envisioned a chemoselective



Scheme 2. Synthesis of the terphenyl scaffold **13**.

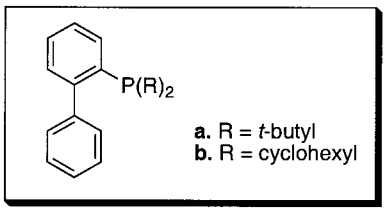


Figure 4. Electron rich phosphine ligands (Strem Chemicals).

Suzuki cross-coupling based on the differential reactivity of the bromine and chlorine substituents of **16**. Treatment of **16** with phenylboronic acid (1.0 equiv.) and tetrakis(triphenylphosphine)-palladium(0) afforded biphenyl chloride **17** in excellent yield. If excess boronic acid (1.3–1.5 equiv.), was present in the reaction mixture, substitution of the chloro resulted.

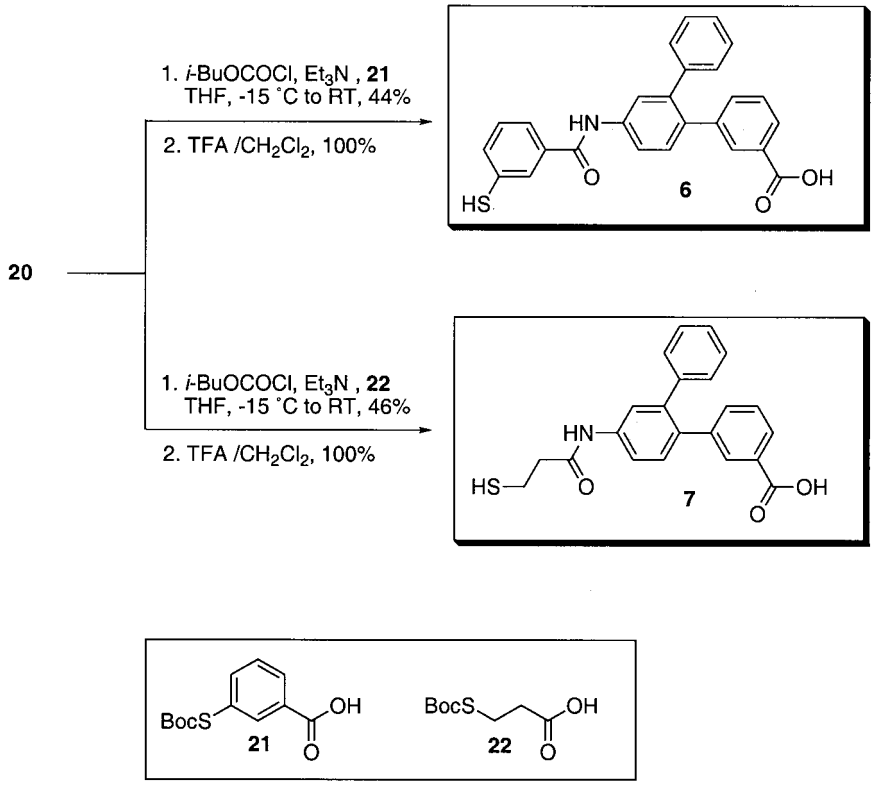
To test our synthetic strategy (chemoselective Suzuki), we attempted coupling of **17**, with commercially available 3-formylphenylboronic acid. Utilizing conditions outlined in Scheme 2, we obtained the formyl–terphenyl analog **18** in a moderate yield (45%), due presumably to the hydrolytic deboronation/instability of the formyl boronic acid under the reaction conditions.[21] To overcome this we synthesized terphenyl–methylester **13**, via 3-(methoxycarbonyl)phenylboronic acid[22] in a 86% yield (Table 1, entry 9).

In general, the choice of the palladium catalyst, base, substrate, and solvents affects the outcome of a Suzuki cross-coupling reaction.[19,20] As indicated in Table 1 (entry 9), the use of 10 mol% $Pd(OAc)_2$, 2-(dicyclohexylphosphino)biphenyl and K_3PO_4 was critical to the success of the chloride-coupling reaction. Ligands such as 2-(di-*t*-butylphosphino)biphenyl or *t*-tributylphosphine (which have

Table 1. Optimization of the chemoselective Suzuki cross-coupling of biphenyl chloride (**17**) with 3-(methoxycarbonyl)phenylboronic acid

Entry	mmol (**17**)	Catalyst (mol%)[a]	Ligand (4L/Pd)[b]	$ArB(OH)_2$ (equiv.)[c]	Base (equiv.)	Conditions[d]	Yield (%)
1	0.25	5	$P(t\text{-}Bu)_3$	1.3	Cs_2CO_3 (3)	60°C/24 h	19
2	0.25	5	**a**	1.3	KF (3)	RT to 60°C	<10[e]
3	0.25	5	**a**	1.3	Cs_2CO_3 (3)	60°C/30 h	none
4	0.10	5	**a**	1.3	K_3PO_4 (3)	60°C/overnight	trace
5	0.10	5	**b**	1.3	K_3PO_4 (2)	60°C/overnight	trace
6	0.10	10	**b**	1.3	K_3PO_4 (4)	70°C/1.5 h	>90[e]
7	1.0	10	**b**	1.3	K_3PO_4 (4)	70–75°C/1.5 h	69
8	1.5	10	**b**	1.3	K_3PO_4 (4)	85–90°C/3 h	55
9	0.7	10	**b**	1.5	K_3PO_4 (4)	85–90°C/1 h	86

[a] Catalyst: $Pd(OAc)_2$.
[b] **a**=2-(di-*t*-butylphosphino)biphenyl, **b**=2-(dicyclohexylphosphino)biphenyl. Refer, Fig. 4.
[c] $ArB(OH)_2$: 3-(methoxycarbonyl)phenylboronic acid.
[d] Solvent: toluene.
[e] Yield was estimated by ^1H NMR integration.



Scheme 3. Synthesis of analogues **6** and **7**.

been used in some aryl chloride Suzuki cross-couplings), either failed or gave the desired product in a poor yield. Finally, the use of the pinacol ester of 3-(methoxycarbonyl)-phenylboronic acid under the aforementioned reaction conditions did not afford the desired product. This underscores the importance of the reactivities of all the reagents, particularly due to the recalcitrant nature of the Ar–Cl bond to undergo oxidative addition.

Terphenyl ester **13** was then saponified to the corresponding acid **19** and was further reduced to the terphenyl amino acid **20**. Scaffold **20** was then utilized for synthesizing the PFTase inhibitors **6** and **7** (Scheme 3). Thus, mercaptobenzoic acid **21** and mercaptopropanoic acid **22** were each treated with isobutyl choloformate to give the corresponding mixed anhydrides, which upon treatment with terphenyl amino acid **20** afforded Boc-protected **6** and **7**. Removal of the protecting group under acidic conditions provided the target compounds, **6** and **7**. While this work was in progress, Fu et al.[23] also reported chemoselective Suzuki couplings of aryl dihalides with an appropriate boronic acid, to prepare substituted biaryls. However, to the best of our knowledge, our work is the first report of a chemoselective Suzuki cross-coupling involving a 1,2-dihalo arene, such as **16**, as a substrate. Furthermore, our method affords the desired peptidomimetic PFTase inhibitors in an efficient manner.

Biological results

Inhibitors **5–7** were evaluated against PFTase and PGGTase-I from human Burkitt lymphoma (Daudi) cells (Table 2).[24] The reaction mixture for the assay also contained [^{3}H]FPP and recombinant H-Ras-CVLS (PFTase), or [^{3}H]GGPP and H-Ras-CVLL (PGGTase-I) and different concentrations of inhibitors. The mixtures were incubated for 30 min at 37°C, filtered on glass fiber filters, and processed for scintillation counting as described previously.[24]

The IC_{50} values for inhibition of PFTase are collected in Table 2 and confirm the basis of our peptidomimetic strategy. The tetrapeptide CVIM (**1**) inhibited PFTase with an IC_{50} of 340 nM.[25] Compound **3**, bearing the comformationally restricted biphenyl scaffold (PFTase IC_{50} 114 nM), showed greater potency than **1**. This clearly confirms 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. Moreover, introducing an additional phenyl substituent onto the biphenyl system improved the PFTase inhibition activity by 8-fold as seen with **5**. The phenyl substituent presumably mimics the *iso*-butyl side chain of the Ile residue in CVIM (as in Fig. 3) and binds into the large and complementary hydrophobic pocket in PFTase. Replacement of the cysteine residue of **5** with an arylamido or an alkylamido thiol group (**6** and **7**) did not improve PFTase or PGGTase-I inhibition activity. These attempts to rigidify the spacer between the key thiol group and the terphenyl carboxylate were unsuccessful presumably due to the strict steric requirements of the PFTase active site. The flexible thioalkylamine **5** may be able to access an optimal conformation that permits binding to the zinc ion. In contrast, thioarylamide **6** and thioalkylamide **7** are less flexible and probably constrained in a less optimal conformation.

Table 2. Inhibition of PFTase and PGGTase-I by CAAX peptidomimetics

Compound	IC_{50} (nM) PFTase	IC_{50} (nM) PGGTase-I
CVIM, **(1)**[a]	340	–
3[b]	114	10 000
FTI-276, **(4)**[c]	0.61	50
5	15	640
6	>10 000	>10 000
7	3800	>10 000

[a] Ref. 25.
[b] Ref. 14.
[c] Ref. 17.

Conclusion

We have successfully demonstrated a chemoselective Suzuki cross-coupling of an 1,2-dihalo arene such as **16**, with an appropriate arylboronic acid derivative. We have also designed concise syntheses of PFTase inhibitors **5**, **6** and **7**. Incorporating a phenyl substituent into the inhibitors was clearly shown to be an advantage with the superior inhibition potency of **5** compared to **3**. However, further changes of the reduced cysteine N-terminus, as in **6** and **7**, gave a loss of activity.

Experimental

General

All solvents and reagents were purchased from Aldrich, WI and used directly without purification unless indicated otherwise. 3-Bromo-4-chloronitrobenzene was purchased from TCI, OR. 2-(Dicyclohexylphosphino)biphenyl was purchased from Strem Chemicals, MA. CH_2Cl_2 was distilled under nitrogen from calcium hydride. THF was distilled under argon from sodium benzophenone ketyl. All apparatus was oven-dried and cooled in a desiccator, prior to use. ^{1}H and ^{13}C NMR were procured on a Bruker-400 instrument, with $CDCl_3$ as the internal reference for ^{1}H (δ 7.26) and ^{13}C (δ 77.06). Mass spectral analyses were performed at University of Illinois at Urbana Champaign, IL. HRMS (FAB and EI) were procured on Micro-mass 70-4F and Micro-mass VSE instruments respectively. Reactions were monitored on silica gel TLC plates, (Aldrich) and visualized under a uv-lamp (254 nm) or staining with I_2, $KMnO_4$, Vanillin, Indophenol and/or Ninhydrin, where appropriate. Chromatographic separations were performed on silica gel columns (2.5×15 cm, Merck grade silica, 240–400 mesh, 60 Å) employing the gravity or flash technique. Fractions containing 20 mL of the eluent were collected and then combined, unless otherwise noted. Melting points were obtained on a Electrochem melting point apparatus and are uncorrected.

2-Phenyl-4-nitro-3′-methylbiphenyl (12). 4-Nitroaniline **8** (13.8 g, 0.10 mol) was suspended in 125 mL of acetic acid. To this solution was added a solution of bromine (15.92 g, 0.10 mol) in 75 mL of acetic acid through a dropping funnel over a period of 1 h. The mixture was stirred at room

temperature for 1 h and then warmed to 60°C. The resulting solution was poured into 300 mL of ice-water and the precipitate was filtered. The solid was dissolved in ether and washed with $NaHCO_3$. After evaporating solvents, the crude solid was recrystallized from methanol–water to give pale yellow crystals of **9** (16.5 g, 76%). mp 99–100°C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.38 (s, 1H, ArH), 8.04 (d, J=8.7 Hz, 1H, ArH), 6.74 (d, J=8.7 Hz, 1H, ArH), 4.83 (br s, 2H, NH_2).

2-Bromo-4-nitroaniline **9** (10.85 g, 50.0 mmol) was coupled with phenylboronic acid (6.4 g, 52.5 mmol) in the presence of $Pd(OAc)_2$ (575 mg, 5% equiv.) in aqueous acetone solution. The product was purified by flash column chromatography (ethyl acetate/hexane=2.5:1) to give 2-phenyl-4-nitroaniline **10** as pale yellow crystals (8.2 g, 77%). mp 124–125°C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.09 (d, J=8.5 Hz, 1H, ArH), 8.07 (s, 1H, ArH), 7.50 (d, J=8.1 Hz, 2H, ArH), 7.43 (m, 3H, ArH), 6.71 (d, J=8.5 Hz, 1H, ArH), 4.50 (br s, 2H, NH_2).

The diazonium salt of **10** was synthesized according to a known procedure.[26] Sodium nitrite (2.51 g, 36.4 mmol) was added in several portions to 19.4 mL of concentrated sulfuric acid in a water bath. To this solution was added 23 mL of glacial acetic acid dropwise at 10°C. The mixture was stirred for 20 min at this temperature and then 2-phenyl-4-nitroaniline (7.10 g, 33.0 mmol) was added in several portions within 30 min. The brown suspension was stirred at 10°C for 1 h (6 mL of water was added to obtain a clear solution) and at room temperature for 15 min. To this solution was added KI (8.71 g, 52.4 mmol) in 25 mL of 2N HCl. The brown mixture was stirred at room temperature for 20 min and then heated to 70°C. Sodium sulfite was added to the cold mixture to remove iodine. The solid was filtered and recrystallized from methanol to afford **11** as crystals (8.42 g, 77%). mp 112–114°C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.34 (d, J=8.6 Hz, 1H, ArH), 8.09 (s, 1H, ArH), 7.96 (d, J=8.5 Hz, 1H, ArH), 7.46–7.52 (m, 3H, ArH), 7.41–7.44 (m, 2H, ArH).

Coupling of 1-iodo-2-phenyl-4-nitrobenzene **11** (3.25 g, 10.0 mmol) with 3-methylphenylboronic acid (1.36 g, 10.0 mmol) in the presence of palladium acetate (112 mg, 5% equiv.) gave compound **12** as an oil (2.75 g, 95%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.30 (s, 1H, ArH), 8.25 (d, J=8.4 Hz, 1H, ArH), 7.58 (d, J=8.5 Hz, 1H, ArH), 7.26 (m, 3H, ArH), 7.09–7.16 (m, 4H, ArH), 6.99 (s, 1H, ArH), 6.88 (d, J=7.0 Hz, 1H, ArH), 2.27 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 400 MHz): δ 147.1, 146.9, 141.8, 139.3, 139.2, 137.8, 131.4, 130.1, 129.5, 128.4, 128.1, 127.9, 127.4, 126.7, 125.4, 122.1 (ArC), 21.3 ($ArCH_3$); LRMS (EI) 289.

2-Phenyl-4-nitro-3′-methoxycarbonylbiphenyl (13) and 2-phenyl-4-nitro-3′-*tert*-butoxycarbonylbiphenyl (14). (*Method A*: Scheme 1, Compound **13**). Compound **12** (2.25 g, 7.78 mmol) was dissolved in 45 mL of carbon tetrachloride and then NBS (2.91 g, 16.3 mmol, 2.10 equiv.) was added. After the addition of dibenzoyl peroxide (38 mg, 2% equiv.), the mixture was refluxed until all precipitate disappeared. ^{1}H NMR showed 25% of mono-brominated (δ 4.40, singlet) and 75% of di-brominated product (δ 6.44, singlet).The mixed brominated compounds (3.42 g) were dissolved in 50 mL of hot methanol and then $AgNO_3$ (3.63 g, 21.3 mmol) in 70 mL of methanol and 5 mL of water was added. The mixture was refluxed for 30 min and then filtered. The filtrate was washed and dried. ^{1}H NMR showed 30% of aldehyde, 48% of dimethyl acetal and 24% of methyl ether. This mixture was treated with 10 mL of TFA and 2 drops of water to convert the acetal to the aldehyde. After workup and evaporating solvents, the residue was treated with tetrabutylammonium permanganate (2.51 g, 6.95 mmol) in 20 mL of pyridine and was stirred for 10 h at room temperature. The mixture was poured into a solution of sodium sulfite (6 g) in 100 mL of 5N HCl and then extracted with ethyl acetate. After evaporating solvents, the residue was extracted with 1N NaOH and then acidified with 3N HCl. After ethyl acetate extraction and evaporating solvents, a solid (1.6 g) was obtained. This solid was mixed with 100 mL of methanol and 3.0 mL of thionyl chloride and refluxed for 5 h. After flash column chromatography, 2-phenyl-4-nitro-3′-methoxycarbonylbiphenyl **13** was obtained as an oil which solidified on standing (1.20 g, 46% for four steps).

The above methyl ester **13** (1.10 g, 3.3 mmol) was hydrolyzed with NaOH (1 equiv.) in methanol. The resulting carboxylic acid (1.05 g, 3.3 mmol) was treated with 0.43 mL of oxalyl chloride (4.93 mmol, 1.5 equiv.) and the acid chloride was reacted with $KOBu^t$ (0.55 g, 4.94 mmol) to give the *tert*-butyl ester (**14**). Compound **14** was purified by flash column chromatography (670 mg, 54.2%). mp 49–50°C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.31 (s, 1H, ArH), 8.27 (d, J=8.4 Hz, 1H, ArH), 7.91 (d, J=7.2 Hz, 1H, ArH), 7.86 (s, 1H, ArH), 7.62 (d, J=8.4 Hz, 1H, ArH), 7.21–7.29 (m, 5H, ArH), 7.12–7.15 (m, 2H, ArH), 1.56 (s, 9H, $C(CH_3)_3$); ^{13}C NMR ($CDCl_3$, 400 MHz) δ 165.1, 147.2, 145.9, 142.0, 139.2, 138.9, 133.5, 132.1, 131.4, 130.5, 129.6, 128.7, 128.4, 128.0, 127.6, 125.5, 122.3 (ArC), 81.2 $C(CH_3)_3$, $C(CH_3)_3$ 28.0; HRMS (EI, *m/z*) calcd for $C_{23}H_{21}NO_4$ 375.1470, obsd 375.1470.

(*Method B*: Scheme 2, Compound **13**). 3-(Methoxycarbonyl)-phenylboronic acid (0.81 g, 4.5 mmol),[22] palladium acetate (0.07 g, 0.30 mmol, 10 mol%), 2-(dicyclohexylphosphino)-biphenyl (0.42 g, 1.2 mmol, 4L/Pd) and K_3PO_4 (2.55 g, 12.0 mmol) were placed in a Schlenk flask under nitrogen. To this was added 5 mL of dry toluene and the mixture was stirred at room temperature for 5 min. 2-Chloro-5-nitrobiphenyl **17** (0.7 g, 3.0 mmol) was then added and the mixture was heated at 85–90°C for 1 h. At the end of this period, the mixture was filtered and the filtrate was washed with 10% citric acid (2×20 mL), followed by 1 M $NaHCO_3$ (2×20 mL) and brine (2×20 mL). The organic layers were combined and concentrated to afford a brown oil, which was chromatographed on a silica gel column, using a gradient of hexanes:EtOAc (100:0→90:10) to afford **13** as a yellow solid (0.84 g, 86%). mp 112°C; TLC: R_f: 0.31 (silica gel, hexanes:EtOAc 3:1); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.46–8.19 (m, 2H, ArH), 7.89 (t, 2H, ArH), 7.54 (d, 1H, ArH), 7.20–7.05 (m, 7H, ArH), 3.83 (s, 3H, $COOCH_3$); ^{13}C NMR ($CDCl_3$, 400 MHz): δ 166.37 ($COOCH_3$), 147.18, 145.61, 141.91, 139.47, 138.63, 133.87, 131.35, 131.23, 130.29, 129.45, 128.72, 128.22, 128.00, 127.55, 125.32, 122.15

(ArC), 52.06 (COO*CH*$_3$); HRMS (FAB, *m/z*) calcd for $C_{20}H_{16}NO_4$ $(M+H)^+$ 334.1080, found 334.1078.

2-Phenyl-4-*N*-[2(*R*)-*N*-*tert*-butoxycarbonylamino-3-(triphenylmethyl)thiopropyl]amino-3′-*tert*-butoxycarbonylbiphenyl (15). Compound **14** (660 mg, 1.76 mmol) was hydrogenated and then reacted with *N*-Boc-*S*-trityl-L-cysteinal (1.0 equiv.) in the presence of $NaCNBH_3$ to give the reductive amination product. After flash column chromatography (hexane/THF 4:1), compound **15** was isolated (650 mg, 47%). mp 85–86°C (dec.); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.76 (m, 2H, ArH), 7.41–7.44 (m, 6H, ArH), 7.17–7.30 (m, 13H, ArH), 7.09–7.14 (m, 4H, ArH), 6.61 (d, J=8.3 Hz, 1H, ArH), 6.55 (s, 1H, ArH), 4.57 (br, 1H, Boc amide), 3.87 (m, 2H, Cys α-H, NH), 3.14 (br t, 2H, CH_2N), 2.48 (m, 2H, CH_2S), 1.57 (s, 9H, Bu^t), 1.43 (s, 9H, Boc); Anal calcd for $C_{50}H_{52}O_4N_2S$: C 77.32, H 6.70, N 3.61; Found C 77.08, H 6.56, N 3.55.

2-Chloro-5-nitrobiphenyl (17). Phenylboronic acid (1.51 g, 10.05 mmol), tetrakis(triphenylphosphine)palladium(0) (0.35 g, 0.30 mmol, 3 mol%) and K_2CO_3 (2.76 g, 20.0 mmol) were placed in a Schlenk flask under nitrogen. To this was added 5 mL (4:1) dry toluene/EtOH and the mixture was stirred at room temperature for 5 min. 3-Bromo-4-chloronitrobenzene **16** (2.37 g, 10.0 mmol) was added and the mixture was refluxed for 24 h. At the end of this period, the mixture was filtered and the filtrate washed with 10% citric acid (2×20 mL), followed by 1 M $NaHCO_3$ (2×20 mL) and brine (2×20 mL). The organic layers were combined and concentrated to afford a brown oil, which was chromatographed on a silica gel column, using a gradient of hexanes:Et_2O (100:0→80:10) to afford **17** as a colourless oil, which solidified to a white residue on standing (1.56 g, 88%). mp 90°C; TLC: R_f: 0.75 (silica gel, hexanes:EtOAc 3:1); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.16–8.05 (m, 2H, ArH), 7.57–7.36 (m, 6H, ArH); ^{13}C NMR ($CDCl_3$, 400 MHz): δ 146.27, 141.63, 139.33, 136.92, 130.61, 128.97, 128.45, 128.23, 125.89, 122.91 (ArC); HRMS (EI, *m/z*): calcd for $C_{12}H_8NClO_2$ 233.0244 $(M)^+$, found 233.0243.

2-Phenyl-4-nitro-3′-formylbiphenyl (18). 3-Formylphenylboronic acid (0.02 g, 0.15 mmol), palladium acetate (0.002 g, 0.01 mmol, 10 mol%), 2-(dicyclohexylphosphino)biphenyl (0.01 g, 0.04 mmol, 4L/Pd) and K_3PO_4 (0.06 g, 0.30 mmol) were placed in a Schlenk flask under nitrogen. To this was added 5 mL (4:1) of dry toluene/EtOH and the mixture was stirred at room temperature for 5 min. This was followed by the addition of 2-chloro-5-nitrobiphenyl **17** (0.02 g, 0.10 mmol) and the mixture was refluxed for 24 h. At the end of this period, the mixture was filtered and the filtrate washed with 10% citric acid (2×20 mL), followed by 1 M $NaHCO_3$ (2×20 mL) and brine (2×20 mL). The organic layers were combined and concentrated to afford a brown oil, which was chromatographed on a silica gel column, using a gradient of hexanes:EtOAc (100:0→90:10) to afford **18** as a yellow oil (0.014 g, 45%). TLC: R_f: 0.40 (silica gel, hexanes:EtOAc 3:1); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 10.00 (s, 1H, ArCHO), 8.46 (s, 1H, ArH), 8.21 (d, 1H, ArH), 7.92 (d, 1H, ArH), 7.64–7.60 (m 4H, ArH), 7.52–7.44 (m 4H, ArH), 7.26 (s, 1H, ArH); HRMS (EI, *m/z*): calcd for $C_{19}H_{13}NO_3$ $(M)^+$ 303.0895, found 303.0898.

2-Phenyl-4-nitro-3′-carboxybiphenyl (19). 2-Phenyl-4-nitro-3′-methoxycarbonylbiphenyl **13** (0.44 g, 1.33 mmol) and NaOH (0.27 g, 6.0 mmol) were placed in a flask. To this was added 10 mL (1:5)H_2O/THF. The mixture was stirred at room temperature for 24 h. The mixture was washed with Et_2O (1×20 mL) to remove any starting material. The aqueous layer was acidified to pH~3 followed by extraction with EtOAc (3×30 mL). The organic layers were combined and concentrated to afford **19** as an off-white solid (0.39 g, 93%). mp 245°C (dec.); TLC: R_f: 0.22 (silica gel, $CHCl_3$:Me_2CO:EtOH 100:40:8); ^{1}H NMR ($CDCl_3$ and 1 drop of CD_3OD, 400 MHz): δ 8.28–8.31 (m, 2H, Ar), 7.97 (m, 2H, ArH), 7.63 (m, 1H, ArH), 7.15 (m, 7H, ArH); ^{13}C NMR ($CDCl_3$ and one drop of CD_3OD, 400 MHz): δ 167.98 (C═O), 146.99, 145.77, 141.86, 138.56, 133.73, 131.24, 131.19, 130.57, 130.39, 129.94, 129.31, 128.81, 128.05, 127.83, 122.01, 124.12 (ArC); HRMS (FAB, *m/z*) calcd for $C_{19}H_{14}NO_4$ $(M+H)^+$ 320.0924, found 320.0922.

2-Phenyl-4-amino-3′-carboxybiphenyl (20). 2-Phenyl-4-nitro-3′-carboxybiphenyl **19** (0.39 g, 1.23 mmol), $SnCl_2{\cdot}2H_2O$ (1.40 g, 6.15 mmol) were placed in a flask. To this was added 20 mL (1:1) EtOAc/EtOH. The mixture was stirred at reflux for 2 h and then cooled to room temperature. The mixture was then slowly poured into an ice-cold solution of saturated $NaHCO_3$ (100 mL). The pH of the solution was adjusted to pH~7, followed by extraction with EtOAc (3×30 mL). The organic layers were combined and concentrated to afford **20** as an off-white solid (0.22 g, 62%). mp 175°C; TLC: R_f: 0.18 (silica gel, $CHCl_3$:Me_2CO:EtOH 100:40:8); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.31–7.79 (m, 1H, ArH), 7.20–7.04 (m, 10H, ArH), 6.72–6.70 (m, 1H, ArH); ^{13}C NMR (10% CD_3OD in $CDCl_3$, 400 MHz): δ 169.64 (C═O), 147.01, 142.68, 135.20, 131.98, 131.95, 131.46, 130.73, 130.53 130.25, 128.33, 128.10, 127.60 126.98, 118.02, 115.33 (ArC); HRMS (FAB, *m/z*) calcd for $C_{19}H_{16}NO_2$ $(M+H)^+$ 290.1182, found 290.1180.

2-Phenyl-4-*N*-[2(*R*)-amino-3-mercaptopropyl]amino-3′-carboxybiphenyl trifluoroacetate (5). Compound **15** (440 mg) was deprotected by TFA in the presence of triethylsilane. The crude product (90% purity) was purified by preparative HPLC to give compound **5** (145 mg, 52%). mp 89–90°C (dec); $[\alpha]_D^{25}$=+12.1 (*c*=0.3, MeOH); ^{1}H NMR (CD_3OD, 400 MHz): δ 7.77 (m, 2H, ArH), 7.28 (d, J=8.3 Hz, 1H, ArH), 7.18–7.24 (m, 5H, ArH), 7.08–7.11 (m, 2H, ArH), 6.93 (d, J=8.3 Hz, 1H, ArH), 6.87 (s, 1H, ArH), 3.56–3.65 (m, 2H, Cys α H, CH_2N), 3.44–3.53 (m, 1H, CH_2N), 3.00 (dd, J=4.7, 14.6 Hz, 1H, CH_2SH), 2.88 (dd, J=5.5, 14.6 Hz, 1H, CH_2SH); ^{13}C NMR (CD_3OD, 400 MHz); δ 169.9 (C═O), 148.9, 143.5, 143.1, 143.0, 135.6, 132.5, 132.0, 131.5, 130.9, 130.7, 129.0, 128.8, 128.0, 127.7, 116.1, 113.2 (ArC), 53.9, 45.3, 25.3; Anal calcd for $C_{22}H_{22}O_2N_2S{\cdot}CF_3COOH{\cdot}1.2H_2O$: C 56.07, H 4.94, N 5.45; Found C 55.89, H 4.68, N 5.37.

2-Phenyl-[4-(3-mercaptobenzoyl)amino]-3′-carboxylbiphenyl (6). To a solution of **21** (24 mg, 0.10 mmol) and Et_3N (15 μL, 0.10 mmol) in THF (0.5 mL) was added isobutyl chloroformate (13.5 μL, 0.10 mmol) in THF (0.5 mL) by syringe at −20°C. After stirring for 15 min at −15°C, a solution of **20** (13 mg, 0.04 mmol) in THF (0.25 mL) was added by syringe at −15°C, and the mixture

was stirred overnight at room temperature. After evaporation of the solvent, the residue was dissolved in EtOAc (10 mL). The organic layer was washed with 10% citric acid and dried over $MgSO_4$. The crude yellow oil was purified by flash column chromatography with $CHCl_3$:acetone: EtOH 100:40:8 to give 2-phenyl-4-[3-*S*-(*t*-butoxy-lcarbonyl)-mercaptobenzoyl] amino-3′-carboxylbiphenyl as a yellow oil (24 mg, 44%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.25 (s, 1H, ArH), 8.10 (d, J=7.0 Hz, 1H, ArH), 7.99 (d, J=12.5 Hz, 1H, ArH), 7.90 (d, J=7.0 Hz, 1H, ArH), 7.73 (d, J=7.5 Hz, 1H, ArH), 7.67 (d, J= 8.5 Hz, 2H, Aryl H), 7.46–7.49 (m, 4H, ArH), 7.46–7.49 (m, 4H, ArH), 7.10–7.21 (m, 6H, ArH), 1.51 (s, 9H, Boc); HRMS (FAB, *m/z*) calcd for $C_{31}H_{27}NO_5S$ $(M)^+$ 526.1689, found 526.1688.

Deprotection of 2-phenyl-4-[3-*S*-(*t*-butoxylcarbonyl)mercaptobenzoyl]amino-3′-carboxylbiphenyl with 50% TFA in CH_2Cl_2 gave compound **6** as a yellow amorphous solid (100%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.00 (s, 1H, ArH), 7.86–7.95 (m, 2H, ArH), 7.60–7.78 (m, 3H, ArH), 7.08–7.45 (m, 11H, ArH), 3.57 (s, 1H, SH); HRMS (FAB, *m/z*) calcd for $C_{26}H_{19}NO_3S$ $(M)^+$ 426.1165, found 426.1164.

2-Phenyl-4-(3-mercaptoethylcarbonyl)amino-3′-carboxylbiphenyl (7). To a solution of **22** (24 mg, 0.10 mmol) and Et_3N (15 μL, 0.10 mmol) in dry THF (0.5 mL) was added isobutyl chloroformate (13.5 μL, 0.10 mmol) in THF (0.5 mL) by syringe at −15°C. After stirring for 15 min at −15°C, a solution of **20** (30 mg, 0.10 mmol) in THF (0.5 mL) was added by syringe at −15°C, and the mixture was stirred overnight at room temperature. After evaporation of the solvent, the residue was dissolved in AcOEt (50 mL). The organic layer was washed with 10% citric acid followed by brine and then dried over $MgSO_4$. The crude oil was purified by flash column chromatography eluted with $CHCl_3$(50 mL) followed by $CHCl_3$:acetone:EtOH 100:40:8 to give 2-phenyl-4-[3-*S*-(*t*-butoxylcarbonyl)-ethylcarbonyl]amino-3′-carboxylbiphenyl as an yellow oil (23 mg, 46%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.96 (s, 1H, ArH), 7.90–7.93 (m, 1H, ArH), 7.82 (br s, 1H, ArH), 7.64 (dd, J=2.0, 8.0 Hz, 1H, ArH), 7.59 (d, J=2.0 Hz, 1H, ArH), 7.39 (d, J=8.8 Hz, 1H, ArH), 7.18–7.23 (m, 5H, ArH), 7.08–7.11 (m, 2H, ArH), 3.16 (t, J=7.2 Hz, 2H, CH_2S), 2.79 (t, J=7.2 Hz, 2H, CH_2CO), 1.50 (s, 9H, Boc); HRMS (EI, *m/z*) calcd for $C_{27}H_{27}NO_5S$ $(M)^+$ 477.1610, found 477.1610.

Deprotection of 2-phenyl-4-[3-*S*-(*t*-butoxylcarbonyl)ethylcarbonyl]amino-3′-carboxylbiphenyl (23 mg, 0.048 mmol) with 50% of TFA in CH_2Cl_2 gave **7** as an yellow amorphous solid (18 mg, 100%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.72 (t, J=8.4 Hz, 1H, SH), 7.91–7.94 (m, 2H, ArH), 7.80 (s, 1H, NH), 7.63 (dd, J=2.0, 8.4 Hz, 1H, ArH), 7.53 (d, J=2.0 Hz, 1H, ArH), 7.41 (d, J=8.4 Hz, 1H, ArH), 7.08–7.26 (m, 7H, ArH), 2.90–2.95 (m, 2H, CH_2S), 2.77 (t, J=6.4 Hz, 2H, CH_2CO), 1.72 (t, J=8.4 Hz, 1H, SH); HRMS (EI, *m/z*) calcd for $C_{22}H_{19}NO_3S$ $(M)^+$ 378.1162, found 378.1164.

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