Design and Synthesis of Non-Peptide Ras CAAX Mimetics as Potent Farnesyltransferase Inhibitors

Yimin Qian,[†] Andreas Vogt,[‡] Saïd M. Sebti,*[,‡] and Andrew D. Hamilton*[,†]

Department of Chemistry, Faculty of Arts and Science, and Department of Pharmacology, School of Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania 15260

Received June 7, 1995[⊗]

Cysteine farnesylation of the ras oncogene product Ras is required for its transforming activity and is catalyzed by farnesyltransferase (FTase). The Ras carboxyl terminal tetrapeptide CAAX (C is cysteine, A is any aliphatic amino acid, X is methionine or serine) is the minimum sequence for FTase recognition. We report here the design, synthesis, and biological characterization of Ras CAAX non-peptide mimetics in which the cysteine is linked through a reduced pseudopeptide bond to 4-amino-3′-carboxybiphenyl. These non-peptide mimetics are potent inhibitors of FTase (IC_{50} = 40 nM for the most potent inhibitor) and are highly selective for FTase over GGTase I (geranylgeranyltransferase I). They are not substrates for farnesylation, do not have peptidic features, and have no hydrolyzable bonds. Structure−activity studies reveal the importance of the position of the carboxylic acid on the aryl ring as well as the reduction of the cysteine amide bond. Substitution at the 2-position of 4-amino-3′-carboxybiphenyl increases inhibitory potency, while the removal of the carboxylic acid results in a 10-fold loss of inhibitory activity.

Introduction

The mammalian *ras* genes encode a family of 21 kDa, guanine nucleotide binding proteins called Ras.[1] Ras uses an on/off cycle between its GTP (active) and a GDP (inactive) bound state to regulate the transduction of biological information from the plasma membrane to the nucleus. Ras serves as a relay between growth factor receptor tyrosine kinases and the serine/thereonine kinase network that triggers MAP (mitogen-activated protein) kinase and causes proliferation and differentiation.[2] Single amino acid substitutions at positions 12, 13, or 61 lock Ras in its GTP-bound form and result in uncontrolled growth. These mutations are found in 50% of colon and 95% of pancreatic human carcinomas.[3] Thus, blocking oncogenic Ras may provide new targets for drug design in cancer chemotherapy.[4, 10]

The crucial role played by Ras in signal transduction is absolutely dependent on its plasma membrane association.[4,5] Ras is synthesized as a cytosolic precursor and is post-translationally modified by a lipid in order to anchor itself in the membrane.[6] The first and obligatory step in this modification is the attachment of a farnesyl group to the cysteine residue of the Ras carboxyl terminal sequence CAAX, a reaction catalyzed by farnesyltransferase (FTase).[7] This farnesylation reaction is followed by peptidase removal of the AAX tripeptide and carboxymethylation of the remaining farnesylated cysteine.[8] FTase is a heterodimeric metalloenzyme composed of α and β subunits with molecular weights of 49 and 46 kDa, respectively.[9] Since farnesylation is the required step for Ras membrane localization and subsequent transforming activity, inhibition of FTase provides an attractive strategy for developing new anti-cancer drugs.[10]

Although Ras and other cellular proteins are farnesylated, most prenylated proteins are geranylgeranylated.[11] At least two classes of enzymes catalyzing the addition of a geranylgeranyl group to proteins have been identified in mammalian cells. One is geranylgeranyltransferase type-I (GGTase I), which modifies proteins with a carboxyl terminal sequence CAAX where X is leucine or isoleucine.[12] The other is geranylgeranyltransferase type-II (GGTase II), which catalyzes the modification of proteins with the carboxyl terminal sequences CC or CXC (C is cysteine).[13] As protein geranylgeranylation is more prevalent than farnesylation, inhibitors with selectivity for FTase over GGTase are expected to exhibit fewer side effects.

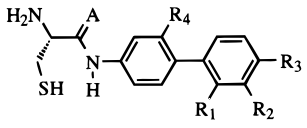
The discovery that CAAX tetrapeptides can potently inhibit FTase in vitro has played a key role in the design of FTase inhibitors.[14] However, these tetrapeptides are totally inactive in whole cells due to poor cellular uptake and metabolic instability. We[15] and others[16] have previously reported CAAX peptidomimetics and pseudopeptides as FTase inhibitors. Although these peptidomimetics showed potent FTase inhibition, they still retained several peptide characteristics, such as one or more amide bonds and an unchanged methionine residue. A highly desirable goal in this area has been to construct inhibitors without peptidic features. In this paper, we report the design, synthesis and biological activity of a series of non-peptide CAAX mimetics.

Design

A tetrapeptide such as Cys-Val-Ile-Met (**1**) can inhibit FTase in vitro (IC_{50} = 200 nM) but has no effect on whole cells due presumably to poor membrane permeability and sensitivity to proteolysis. In order to enhance cellular uptake and improve stability toward proteases, we replaced the central two amino acids in Cys-Val-Ile-Met by 3-(aminomethyl)benzoic acid and 4-aminobenzoic acid.[15] These spacers provided a hydrophobic link between cysteine and methionine residues. The most potent inhibitor in this series, Cys-4-

* To whom correspondence should be addressed.
[†] Department of Chemistry.
[‡] Department of Pharmacology.
[⊗] Abstract published in *Advance ACS Abstracts,* December 1, 1995.

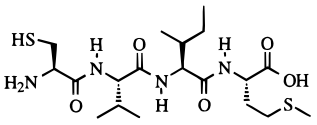
0022-2623/96/1839-0217$12.00/0



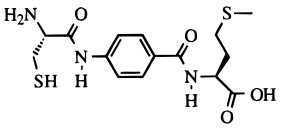
	R_1	R_2	R_3	R_4	A
3	COOH	H	H	H	H,H
4	H	COOH	H	H	H,H
5	H	H	COOH	H	H,H
6	H	COOH	H	H	O
7	H	H	H	H	H,H
8	H	CH_3	H	H	H,H
9	H	$COOCH_3$	H	H	H,H
10	H	COOH	H	OCH_3	H,H

Figure 1. Structures of compounds **3**−**10**.

aminobenzoyl-Met **2** (IC_{50} = 150 nM), retained significant



1



2

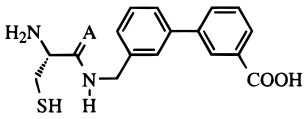
structural similarity to the original CAAX sequence, particularly with respect to the two amide bonds and the key cysteine and methionine residues. Our goal in this work was to replace one part of this structure, the methionine residue, by an extended hydrophobic subunit. To this end we designed a series of 4-amino-3′-carboxybiphenyl derivatives as mimics of the Val-Ile-Met tripeptide but with restricted conformational flexibility. We also reduced the cysteine amide bond to provide completely non-peptidic Ras CAAX mimetics. The structures of the primary CAAX mimetics in this study are shown in Figure 1.

Although the structures of these designed non-peptide CAAX mimetics are significantly different from that of the tetrapeptide Cys-Val-Ile-Met, the functional groups of free amine, thiol, and carboxylic acid are retained. Our early reported results suggested that an extended rather than a turn conformation of tetrapeptide **1** might be responsible for its inhibitory activity.[15] Therefore, we calculated an energy-minimized conformation of compound **4** by using the AMBER force field within the MacroModel program in the absence of solvent and compared it with the extended conformation of tetrapeptide **1**. Molecular modeling (Figure 2) shows that the designed non-peptide mimetic **4** has the functional groups arranged very similarly to those of the tetrapeptide **1** (SH to COOH distance about 10.5 Å). Furthermore, the hydrophobic biphenyl residue in **4** mimics the side chains of tripeptide Val-Ile-Met in **1** which provide a large hydrophobic area.

Synthesis

The basic approach used for the preparation of these compounds is illustrated in Scheme 1 with the synthesis of compound **4**. 1-Bromo-4-nitrobenzene was coupled to (3-methylphenyl)boronic acid[17] through a modified Suzuki[18] coupling to afford compound **14**.[19] Compound **14** was oxidized to carboxylic acid **15** which was converted to the acid chloride and reacted with lithium *tert*-butoxide[20] to give the *tert*-butyl ester **16**. Reduction of **16** by hydrogenation and subsequent reductive amination[21] of the resulting amine with *N*-Boc-*S*-tritylcysteinal **17**[16d,22] gave the fully protected derivative **18**, which was deprotected by trifluoroacetic acid in the presence of triethylsilane.[23] Compound **4** was purified by reverse phase HPLC and isolated as its trifluoroacetate salt by lyophilization.

Our previous results had shown that linking cysteine and methionine through a 3-(aminomethyl)benzoic acid spacer led to moderate inhibition of FTase.[15a] In an attempt to reproduce the same distance dependence in the biphenyl series we have prepared compounds **11** and **12**.



11 A = O **12** A = H,H

The synthesis of **11** and **12** is described in Scheme 2. Compound **19** was made from 3-methyl-3′-carboxybiphenyl (itself formed from aryl−aryl coupling of methyl 3-bromobenzoate with (3-methylphenyl)boronic acid followed by a sponification) via the same method as compound **16**. Bromination of **19** followed by reaction with sodium azide gave **20** which was catalytically hydrogenated to give the corresponding amine. Reaction of Boc-trityl-protected cysteine with this amine through the mixed anhydride method gave **21**, while compound **22** was made by reductive amination with Boc-trityl-protected cysteinal.

Biological Evaluation

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.[24] Enzymes were incubated with [^{3}H]FPP and recombinant H-Ras-CVLS

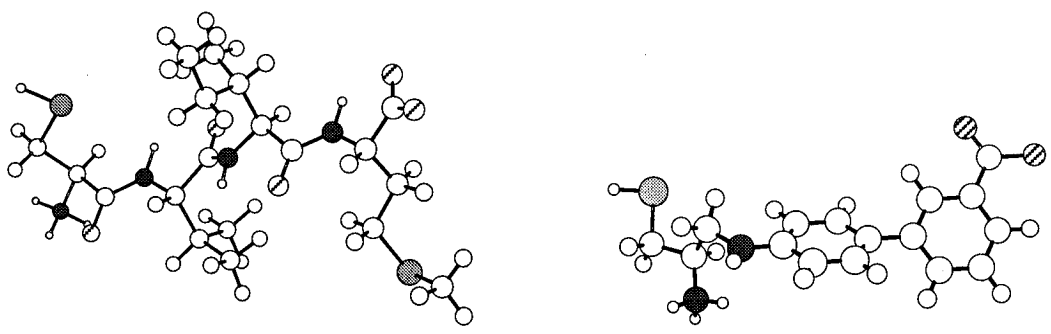
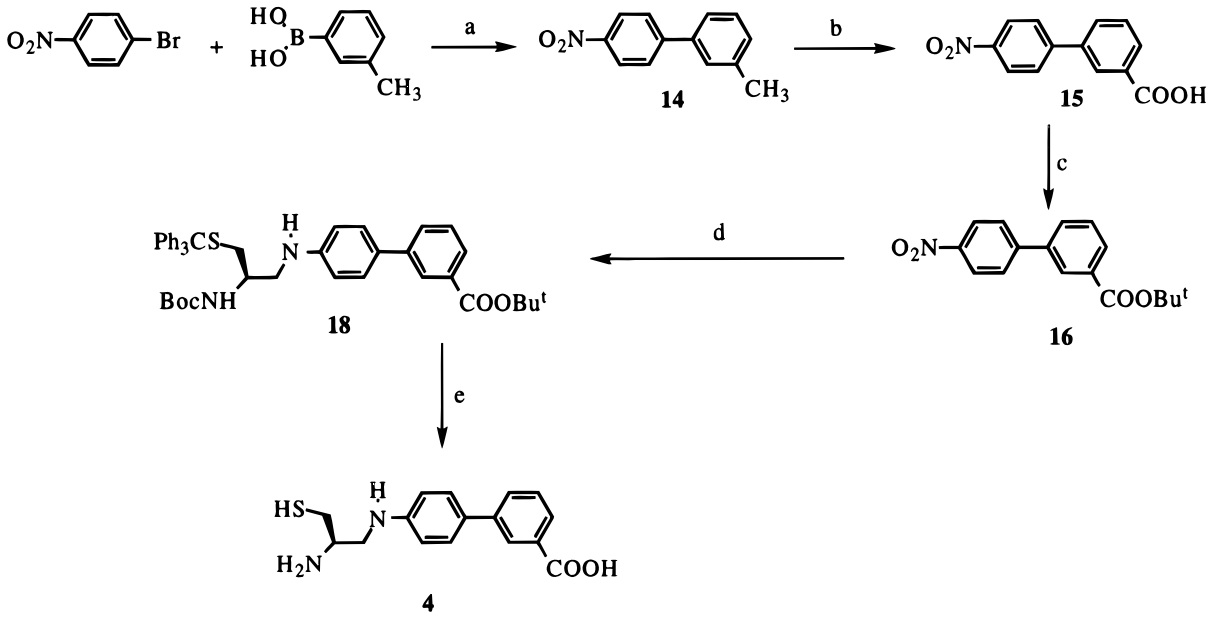


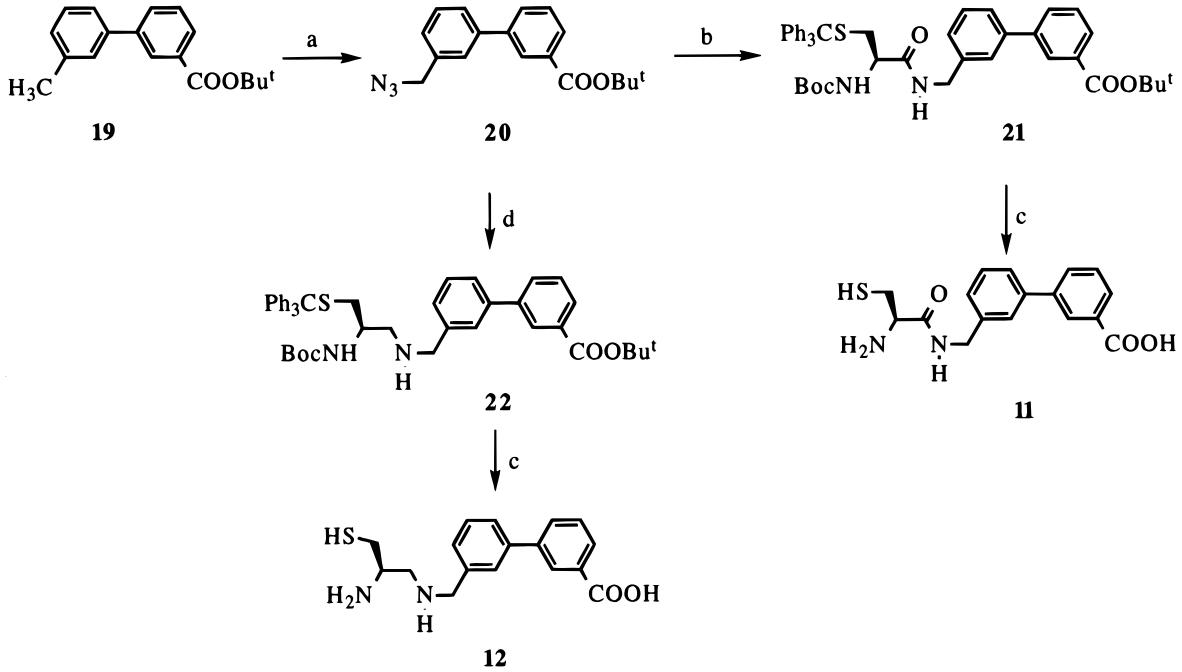
Figure 2. Energy-minimized structures of CVIM and **4** in extended conformations.

Scheme 1. Representative Synthesis of FTase Inhibitors[a]



[a] Reagents: (a) $Pd(OAc)_2$; (b) $KMnO_4$, pyridine/H_2O; (c) (1) $(COCl)_2$, (2) *tert*-butyl alcohol, *n*-BuLi; (d) (1) H_2, Pd/C, (2) *N*-Boc-*S*-tritylcysteinal **17**, (3) $NaB(CN)H_3$; (e) TFA, Et_3SiH.

Scheme 2. Synthesis of Compounds **11** and **12**[a]



[a] Reagents: (a) (1) NBS, (2) NaN_3; (b) (1) H_2, Pd/$BaSO_4$, (2) *N*-Boc-*S*-tritylcysteine, isobutyl chloroformate, Et_3N; (c) TFA, Et_3SiH; (d) (1) H_2, Pd/$BaSO_4$, (2) *N*-Boc-*S*-tritylcysteinal, $NaB(CN)H_3$.

(FTase) or [^{3}H]GGPP and H-Ras-CVLL (GGTase I) in the presence of different concentrations of inhibitors. After incubation for 30 min at 37 °C, the reaction was stopped and filtered on glass fiber filters to separate free from incorporated label, as described earlier.[9] The activity of the inhibitors is reported in Table 1 as IC_{50} values, the concentration at which FTase or GGTase I activity was inhibited by 50%. Some of the inhibitors were further characterized for their ability to serve as substrates for farnesylation by thin layer chromatography.[24]

The published sequence dependence studies on FTase have shown a strong preference for methionine in the terminal position of CAAX. Here we describe inhibitors where no methionine residue is present and the tripeptide AAX is completely replaced by a simple hydrophobic moiety. The most potent inhibitor in the CAAX series is Cys-Ile-Phe-Met[14] with an IC_{50} value of 30 nM. Peptidomimetic inhibitor **10** is as potent as CIFM despite the large difference between their structures. These results confirm our hydrophobic strategy for AAX replacement.

As seen in Table 1, the precise positioning of the free carboxylate is important for potent inhibition. Compounds **3**, **4**, and **5** differ only in their positioning of the carboxylic acid on the phenyl ring. Inhibitor **4** has the carboxylic acid positioned in correspondence with the dipeptide mimetic **2**, while this positioning in **3** and **5**

Table 1. In Vitro Activity of CAAX Mimetic Inhibitors of FTase

inhibitors	FTase IC_{50} (nM)	GGTase I IC_{50} (nM)	substrate
3	543 (3)[a]	140 000 (2)[a]	nd[b]
4	114 (10)	100 000 (6)	no
5	4 575 (4)	>100 000 (2)	no
6	13 500 (2)	100 000 (2)	nd
7	1 070 (3)	>100 000 (3)	no
8	710 (3)	>100 000 (3)	no
9	917 (3)	>100 000 (3)	no
10	40 (6)	43 600 (5)	nd
11	100 000 (2)	>100 000 (3)	no
12	11 000 (1)	35 000 (2)	nd

[a] The number inside the bracket stands for experimental times. [b] nd stands for not determined.

is either closer to or farther from the N-terminus. The effect of this changing position on inhibition potency is significant (5-fold difference between **4** and **3**, 40-fold difference between **4** and **5**). This result is consistent with our early reported study in which cysteine and methionine residues are linked through 4-aminobenzoic acid or 3-aminobenzoic acid.[15b]

We next investigated the influence of different substituents at the 3′-position of compound **4** on FTase inhibitory potency. Methylation of the carboxylic acid, as in **9**, or replacement of the carboxylic acid by methyl or hydrogen, as in **8** and **7**, resulted in inhibitors with 10-fold less activity (around 1 μM) than **4**. This suggests that in the active site of the enzyme there is a positively charged residue which interacts with the negatively charged carboxylate from the inhibitor. However, this electrostatic interaction requires the carboxylate at the 3′-position of the aromatic ring. The different activity between **7** and **5** (4-fold) suggested that the 4′-position of the aromatic ring is close to a hydrophobic region in the enzyme.

Probably the most striking result comes from reduction of the amide bond. For tetrapeptide CIFM, it has been shown by others that reduction of the N-terminal peptide bond results in loss of selectivity for FTase over GGTase I, while the reduction of the C-terminal peptide bond leads to a reduction in FTase inhibitory potency.[16d] Here we observe an increase in selectivity for FTase over GGTase I when the cysteine amide bond is reduced in our biphenyl-derived inhibitors (compare **4** to **6**). We also show that the C-terminal amide bond is not necessary for potent inhibition activity since there is no amide bond in compound **10**. In our earlier paper,[24] reduction of the cysteine amide bond of compound **2** was shown to have little effect on the activity. Here we observed a 100-fold difference in inhibition activity between **4** and **6**. One reason for this enhanced activity on reduction of the amide bond is the increased flexibility of the (*R*)-2-amino-3-mercaptopropyl unit and the consequent accessibility of alternative binding conformations.

We then focused on increasing hydrophobicity and restricting conformational flexibility of the biphenyl spacer by placing substituents at the 2-position of the aryl ring. The substitution of hydrogen by a methoxy group at the 2-position of the left hand aromatic ring increased the inhibitory activity by 3-fold. Thus, 2-methoxy-4-[N-[2(*R*)-amino-3-mercaptopropyl]amino]-3′-carboxybiphenyl has the most potent activity (IC_{50} = 20−60 nM in six independent experiments). This suggested there might be a hydrophobic interaction between the enzyme active site and the methoxy group, corresponding to the valine or isoleucine binding pocket with CVIM. It is also possible that the increased potency is due to the restricted flexibility of the biphenyl.

In contrast to our dipeptide mimetic in which cysteine and methionine were linked through 3-(aminomethyl)-benzoic acid,[15] the biphenyl derivatives of compound **11** and **12** have extremely poor activity toward FTase even though they keep the similar distance dependence when compared with compound **4**. This result suggested that the pharmacophore of (*R*)-1,2-diamino-3-mercaptopropane must be linked to a well-defined hydrophobic residue in order to obtain a potent inhibition activity.

As reported by others, the incorporation of an aromatic amino acid into the A_2 position of CA_1A_2X (such as CIFM) prevents the tetrapeptide from serving as a substrate for farnesylation.[14a] Table 1 shows that the designed non-peptide CAAX mimetics (such as compound **4**) are not substrates for farnesylation. This lack of farnesylation by FTase may be due to the inhibitor binding to the enzyme in a conformation that does not permit farnesyl transfer to the thiol group.

Since GGTase I is an enzyme closely related to FTase, and geranylgeranylation is a more common protein prenylation reaction than farnesylation,[11] it is critical for FTase inhibitors to be selective. The results from Table 1 showed that our compounds are poor inhibitors of GGTase I. The selectivity for FTase over GGTase I with compound **10** is about 1100-fold. In the tetrapeptide CAAX series, the X position directs farnesylation or geranylgeranylation. Despite removing all features of the CAAX C-terminal residue (Met or Ser) from our peptidomimetics, the high selectivity against GGTase I is retained. This suggests there is a larger difference than previously thought between the active site of FTase and that of GGTase I.

Conclusions

We have synthesized true non-peptide Ras CAAX mimetics which have no amide bonds and lack any peptidic features of the tripeptide AAX. These mimetics are potent inhibitors of FTase and are highly selective against GGTase I. The structure−activity relationship showed that a key feature for FTase recognition is the separation of a (*R*)-1,2-diamino-3-mercaptopropyl group and a carboxylic acid by a hydrophobic scaffold. The rigidity of our scaffold provides important information about the active site of FTase. These Ras CAAX mimetics have several desirable features for further drug design. They have small molecular weights, lack peptidic features, are highly potent and specific, and are not metabolically inactivated. We are currently investigating their ability to antagonize oncogenic Ras signaling and their antitumor efficacy in animal models with human tumor xenografts.

Experimental Section

^{1}H and ^{13}C NMR spectrum were recorded on a Bruker AM-300 spectrometer. Chemical shifts were reported in δ (ppm) relative to tetramethylsilane. All coupling constants were described in hertz. Elemental analyses were performed by Atlantic Microlab Inc., GA. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. Concentrations are expressed in g/mL. 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 lithium aluminum hydride. Preparative HPLC was performed using a Waters 600 E controller and a Waters 490 E Multi-Wavelength UV detector with a 25 × 10 cm Delta-Pak C-18 300 Å cartridge column inside a Waters 25 × 10 cm Radial Compression Module. Analytical HPLC was performed using a Rainin HP controller and a Rainin UV-C detector with a Rainin 250 × 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 and VG 7070 mass spectrometer. The purity of all the synthesized inhibitors was more than 98% as indicated by analytical HPLC.

4-Nitro-3′-methylbiphenyl (14). To a mixture of 4-nitrobenzene (3.0 g, 14.8 mmol) and (3-methylphenyl)boronic acid (2.06 g, 15.1 mmol) in 35 mL of acetone and 40 mL of water was added $K_2CO_3 \cdot 1.5H_2O$ (5.93 g, 37.5 mmol) and $Pd(OAc)_2$ (101 mg, 0.50 mmol). The deep black mixture was refluxed for 6 h and then cooled. The mixture was extracted with ether, and the organic layer was passed through a layer of Celite. The pale yellow solution was dried and evaporated to dryness. The residue was recrystallized from hot methanol to give pale yellow crystals (2.68 g, 85%): mp 59−60 °C; ^{1}H NMR ($CDCl_3$) δ 8.26 (d, J = 8.7 Hz, 2H), 7.70 (d, J = 8.7 Hz, 2H), 7.41 (m, 3H), 7.26 (d, J = 7.1 Hz, 1H), 2.43 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 147.6, 146.8, 138.8, 138.6, 129.6, 128.9, 128.0, 127.6, 124.4, 123.9, 21.4; LRMS (EI) for $C_{13}H_{11}NO_2$ 213 (M^+, intensity 100); HRMS (EI) calcd 213.0789, obsd 213.0778.

4-Nitro-3′-carboxybiphenyl (15). Compound **14** (2.31 g, 10 mmol) was suspended in a mixture of 10 mL of pyridine and 20 mL of water. The mixture was heated to reflux, and then $KMnO_4$ (7.9 g, 50 mmol) was added in portions. This mixture was refluxed for 1 h and then stirred at room temperature for 4 h. The hot mixture was filtered, and the black solid was washed with hot water. The filtrate was acidified with 6 N HCl. The precipitate was collected and dried (2.16 g, 89%): mp 265 °C dec; ^{1}H NMR (DMSO-d_6) δ 11.1−11.4 (br s, COOH), 8.32 (d, J = 8.7 Hz, 2H), 8.27 (s, 1H), 8.02 (m, 4H), 7.66 (t, J = 7.8 Hz, 1H); ^{13}C NMR (DMSO-d_6) δ 167.1, 148.9, 145.6, 138.2, 131.8, 131.5, 129.6 (br), 127.9, 124.2 (br); LRMS (EI) for $C_{13}H_9O_4N$ 243 (M^+, 100), 152 (60); HRMS (EI) calcd 243.0531, obsd 243.0544. Anal. ($C_{13}H_9NO_4$) C, H, N.

4-Nitro-3′-(*tert*-butoxycarbonyl)biphenyl (16). To a solution of **15** (1.215 g, 5 mmol) in 30 mL of methylene chloride was added oxalyl chloride (0.65 mL, 7.45 mmol) and one drop of DMF. The mixture was stirred until no further bubbling was observed. The clear solution was evaporated to dryness to give the crude acid chloride. To another flask containing 7.0 mL of *tert*-butyl alcohol was added *n*-BuLi (1.8 M in hexane, 2.8 mL, 5.04 mmol) under a water bath. The turbid solution was stirred for 5 min at room temperature, and then the above acid chloride in 20 mL of THF was added through a dropping funnel. The mixture was stirred overnight before the solvents were evaporated. The residue was dissolved into methylene chloride and washed with 0.5 N NaOH. The organic layer was dried over $MgSO_4$ and evaporated. The residue was recrystallized from methanol to give pale yellow crystals (851 mg, 57%): mp 110.5−111.0 °C; ^{1}H NMR ($CDCl_3$) δ 8.32 (d, J = 7.8 Hz, 2H), 8.24 (s, 1H), 8.06 (d, J = 7.7 Hz, 1H), 7.77 (m, 3H), 7.56 (t, J = 7.7 Hz, 1H), 1.63 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 165.1, 147.1, 146.5, 138.7, 132.8, 131.0, 129.6, 129.0, 128.2, 127.8, 124.0, 81.4, 28.0; LRMS (EI) for $C_{17}H_{17}O_4N$ 299 (M^+, 20), 243 (70), 266 (30), 152 (25); HRMS (EI) calcd 299.1157, obsd 299.1192. Anal. ($C_{17}H_{17}NO_4$) C, H, N.

***N*-Boc-*S*-tritylcysteinal (17).** To a solution of *N*-Boc-*S*-tritylcysteine (7.44 g, 16 mmol) in 85 mL of methylene chloride was added triethylamine (2.22 ml, 16 mmoL) and *N*,*O*-dimethylhydroxylamine hydrochloride (1.57 g, 16.1 mmol). This mixture was cooled in an ice bath, and 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride (EDCI, 3.08 g, 16.0 mmol) and HOBT (2.17 g, 16 mmol) were added. The mixture was stirred at 0 °C for 1 h and at room temperature for a further 10 h. The mixture was extracted with methylene chloride and 0.5 N HCl. The organic layer was washed consecutively with 0.5 N HCl, concentrated $NaHCO_3$, and brine. The organic layer was dried and evaporated. The residue was purified by flash column chromatography (1.5:1 = hexane:ethyl acetate) to give a white foam (7.40 g, 91%): mp 59−60 °C dec; ^{1}H NMR ($CDCl_3$) δ 7.41 (m, 6H), 7.20−7.31 (m, 9H), 5.13 (d, J = 8.9 Hz, 1H), 4.76 (br s, 1H), 3.64 (s, 3H), 3.15 (s, 3H), 2.56 (dd, J = 4.7 and 12.1 Hz, 1H), 2.39 (dd, J = 7.8 and 12.1 Hz, 1H), 1.43 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 170.7, 154.9, 144.2, 129.3, 127.6, 126.4, 79.3, 66.4, 61.2, 49.5, 33.8, 31.8, 28.1. This carboxamide (2.02 g, 4.0 mmol) was dissolved in 30 mL of ether and cooled to −10 °C. Lithium aluminum hydride (167 mg, 4.40 mmol) was added, and the mixture was stirred for 15 min under nitrogen. Then 40 mL of 0.5 N HCl was added, and the solution was extracted with ether. The ether layer was washed with 0.5 N HCl and dried. The evaporation of solvents gave a white foam (1.80 g) which was used for further reaction without purification. The ^{1}H NMR spectrum of this compound was complex. The percentage of the aldehyde was about 65−70%, which was calculated according to the integration of the sharp singlet (δ 9.17) and the trityl peak (δ 7.40, m, 6H; 7.28, m, 9H). Lowering the temperature to −45 °C did not improve the aldehyde percentage.

4-[*N*-[2(*R*)-[(*tert*-Butoxycarbonyl)amino]-3-[(triphenylmethyl)thio]propyl]amino]-3′-(*tert*-butoxycarbonyl)biphenyl (18). Compound **16** (768 mg, 2.56 mmol) was dissolved in THF. A catalytic amount of 10% Pd on activated carbon (78 mg) was added. The mixture was hydrogenated (40 psi) for 30 min. The black mixture was passed through a thin layer of Celite, and the pale yellow solution was evaporated. The residue was dissolved in 10 mL of methanol. To this solution was added 0.5 mL of acetic acid and a solution of the same equivalents of aldehyde **17** (according to the ^{1}H NMR determination) in 6 mL of methanol. Sodium cyanoborohydride (241 mg, 3.84 mmol, 1.5 equiv) was added, and the mixture was stirred overnight. After evaporation of solvents, the residue was extracted with ethyl acetate and concentrated sodium bicarbonate. The organic layer was dried and evaporated. The residue was purified by flash column chromatography (3.5:1 = hexane:THF) to give a white foam (1.09 g, 61%): mp 75.0−76.0 °C dec; $[\alpha]^{25}_D$ = −2.13 (c = 0.01, $CH_3COOC_2H_5$); ^{1}H NMR ($CDCl_3$) δ 8.14 (s, 1H), 7.86 (d, J = 7.7 Hz, 1H), 7.66 (d, J = 7.8 Hz, 1H), 7.40 (m, 9H), 7.22−7.30 (m, 9H), 6.61 (d, J = 8.5 Hz, 2H), 4.58 (d, J = 7.1 Hz, 1H), 3.83 (br m, 2H, Cys α proton and the amine), 3.12 (br m, 2H, CH_2N), 2.48 (br m, 2H, CH_2S), 1.60 (s, 9H), 1.44 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 165.9, 155.6, 147.5, 144.4, 141.2, 132.3, 130.1, 129.5, 129.2, 128.5, 128.0, 127.9, 127.1, 126.8, 112.9, 80.9, 79.7, 67.0, 49.4, 47.1, 34.3, 28.3, 28.2 (expect 14 aromatic C, observed 13). Anal. ($C_{44}H_{48}N_2O_4S \cdot 1.2H_2O$) C, H, N, S.

4-[*N*-[2(*R*)-Amino-3-mercaptopropyl]amino]-3′-carboxybiphenyl (4). Compound **18** (600 mg, 0.85 mmol) was dissolved in 2 mL of TFA and 2 mL of methylene chloride. Triethylsilane was added dropwise to the deep brown mixture until the brown color had disappeared. The mixture was then kept at room temperature for 1 h. Then solvents were evaporated, and the residue was dried under vacuum. The solid was triturated with 30 mL of ether and 3 mL of 3 N HCl in ether. The white precipitate was filtered and washed with ether to obtain a crude product (270 mg, 84%). This crude product was dissolved into 30 mL of dilute HCl solution (0.01 N) and was lyophilized. Analytical HPLC showed the purity to be 95%: mp 105−106 °C dec; $[\alpha]^{25}_D$ = +13.16 (c = 0.01 in methanol); ^{1}H NMR (CD_3OD) δ 8.18 (s, 1H), 7.89 (d, J = 7.7 Hz, 1H), 7.78 (d, J = 7.3 Hz, 1H), 7.49 (m, 3H), 6.82 (d, 8.5 Hz, 2H), 3.56 (m, 2H, CHN and CH_2N), 3.42 (dd, J = 8.9 and 15.2 Hz, 1H, CH_2N), 2.94 (dd, J = 4.9 and 14.6 Hz, 1H, CH_2S), 2.83 (dd, J = 5.6 and 14.6 Hz, 1H, CH_2S); ^{13}C NMR (D_2O and CD_3OD) δ 171.1, 147.1, 141.4, 131.9, 130.9, 130.1, 128.8, 128.5, 127.0, 115.2, 53.2, 45.4, 25.0; LRMS (FAB, glycerol) for $C_{16}H_{18}N_2O_2S$ (M + 1) 303. Anal. ($C_{16}H_{18}N_2O_2S \cdot 2HCl$) C, H, N, S. Further purification by preparative HPLC (Waters C-18, 40% acetonitrile, 60% water, 0.1% TFA, 40 min gradient) gave product **4** (120 mg) with a purity over 99.9%.

3-Methyl-3′-(*tert*-butoxycarbonyl)biphenyl (19). The coupling of (3-methylphenyl)boronic acid with 3-bromobenzoic acid methyl ester gave a 3-methyl-3′-(methoxycarbonyl)biphenyl (79% yield), which was then hydrolyzed to give a 3-methyl-

3′-carboxybiphenyl (97% yield). Compound **19** (an oil) was prepared from this acid using the same method as for the preparation of compound **16** (65% yield): ^{1}H NMR ($CDCl_3$) δ 8.21 (s, 1H), 7.95 (d, J = 7.8 Hz, 1H), 7.73 (d, J = 6.6 Hz, 1H), 7.46 (m, 3H), 7.35 (t, J = 7.5 Hz, 1H), 7.20 (d, J = 7.4 Hz, 1H), 2.43 (s, 3H), 1.62 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 165.6, 141.3, 140.2, 138.3, 132.4, 140.0, 128.7, 128.5, 128.3, 128.0, 127.9, 124.2, 81.0, 28.1, 21.4; LRMS (EI) for $C_{18}H_{20}O_2$ 268 (M^+, 35), 212 (100), 195 (20); HRMS (EI) calcd 268.1463, obsd 268.1458.

3-Azido-3′-(*tert*-butoxycarbonyl)biphenyl (20). Compound **19** (2.18 g, 8.13 mmol) and *N*-bromosuccinimide (1.70 g, 9.50 mmol) was suspended in 60 mL of CCl_4. Dibenzoyl peroxide (20 mg) was added, and the mixture was refluxed for 1.5 h. After the solid was removed, the filtrate was washed with concentrated sodium bicarbonate and dried over sodium sulfate. ^{1}H NMR showed the crude material contained 70% of monobrominated and 30% of dibrominated product. This material was dissolved in 20 mL of DMSO, and sodium azide (3.70 g, 57 mmol) was added. The mixture was heated to 80 °C for 4 h before being poured into a mixture of methylene chloride and water. The organic layer was dried and evaporated. The residue was purified by flash column chromatography (5% of ethyl acetate in hexane) to give **20** (2.14 g, 78%, two steps) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 8.22 (s, 1H), 8.00 (d, J = 7.7 Hz, 1H), 7.76 (d, J = 8.2 Hz, 1H), 7.58 (m, 2H), 7.50 (m, 2H), 7.33 (d, J = 7.6 Hz, 1H), 4.43 (s, 2H), 1.62 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 165.2, 140.5, 140.3, 135.8, 132.3, 130.7, 129.1, 128.5, 128.2, 127.8, 127.1, 126.7, 126.6, 80.9, 54.3, 27.8.

***N*-Boc-*S*-tritylcysteinyl-3-(aminomethyl)-3′-(*tert*-butoxycarbonyl)biphenyl (21).** Compound **20** (0.75 g, 2.43 mmol) was dissolved in 30 mL of methanol. A catalytic amount of 5% palladium on barium sulfate (0.30 g) was added. The mixture was hydrogenated at 1 atm for 5 h. The catalyst was removed by filtration, and the methanol was evaporated. This residue was dissolved in 40 mL of methylene chloride. *N*-Boc-*S*-tritylcysteine (1.12 g, 2.43 mmol) was added at 0 °C followed by EDCI (1 equiv) and HOBT (1 equiv). The mixture was stirred for 24 h. After workup and evaporation of solvents, the residue was purified by flash column chromatography (hexane:ethyl acetate = 3.2:1) to give **21** (570 mg, 44%): mp 84−86 °C; ^{1}H NMR ($CDCl_3$) δ 8.17 (s, 1H), 7.95 (d, J = 7.7 Hz, 1H), 7.70 (d, J = 7.7 Hz, 1H), 7.50−7.30 (m, 9H), 7.30−7.10 (m, 11H), 6.44 (br, 1H), 4.86 (br, 1H), 4.45 (d, J = 4.0 Hz, 2H, CH_2Ph), 3.87 (br, 1H, Cys α H), 2.75 (dd, J = 7.2 and 12.8 Hz, 1H, CH_2S), 2.55 (dd, J = 5.3 and 12.8 Hz, 1H, CH_2S), 1.62 (s, 9H), 1.36 (s, 9H). Anal. ($C_{45}H_{48}N_2O_5S$) C, H, N, S.

Cysteinyl-3-(aminomethyl)-3′-carboxybiphenyl (11). Compound **21** (150 mg) was deprotected using the same method as for the preparation of compound **4**. Final purification by preparative HPLC gave **11** as a white solid (42 mg, 46%): mp 88−89 °C dec; ^{1}H NMR (CD_3OD) δ 8.26 (s, 1H), 8.01 (d, J = 7.7 Hz, 1H), 7.86 (d, J = 7.7 Hz, 1H), 7.64 (s, 1H), 7.56 (m, 2H), 7.46 (t, J = 7.6 Hz, 1H), 7.35 (d, J = 7.6 Hz, 1H), 4.53 (s, 2H), 4.00 (t, J = 5.2 Hz, 1H, Cys α H), 3.06 (dd, J = 14.6 and 5.2 Hz, 1H, CH_2S), 2.97 (dd, J = 14.6 and 6.8 Hz, 1H, CH_2S); LRMS (EI) for $C_{17}H_{18}N_2O_3S$ 331 (M + 1, 8), 281 (100), 226 (75). Anal. ($C_{17}H_{18}N_2O_3S\cdot HCl\cdot 0.6H_2O$) C, H, N.

3-[[*N*-[2(*R*)-[(*tert*-Butoxycarbonyl)amino]-3-[(triphenylmethyl)thio]propyl]amino]methyl]-3′-(*tert*-butoxycarbonyl)biphenyl (22). The azide **20** (900 mg, 2.91 mmol) was dissolved in 20 mL of methanol. A catalytic amount of 5% Pd on barium sulfate (90 mg) was added. This mixture was hydrogenated at 1 atm overnight. The catalyst was removed, and the methanol was evaporated. The remaining residue was dissolved in a mixture of 0.5 N HCl (20 mL) and ether (20 mL). The aqueous phase was neutralized with 1 N NaOH and extracted into methylene chloride. After the evaporation of solvents, a viscous oil was obtained (600 mg, 73%): ^{1}H NMR ($CDCl_3$) δ 8.22 (s, 1H), 7.97 (d, J = 7.8 Hz, 1H), 7.75 (d, J = 7.7 Hz, 1H), 7.57 (s, 1H), 7.50 (m, 2H), 7.43 (t, J = 7.7 Hz, 1H), 7.33 (d, J = 7.4 Hz, 1H), 3.96 (s, 2H), 1.62 (s, 9H), 1.46 (br s, 2H, NH_2). This amine (581 mg, 2.05 mmol) was dissolved in 10 mL of methanol and 0.5 mL of acetic acid before *N*-Boc-*S*-tritylcysteinal (1 equiv, according to ^{1}H NMR determination of the aldehyde percentage) was added. Sodium cyanoborohydride (193 mg, 1.50 equiv) was added to the above solution, and the mixture was stirred at room temperature overnight. After workup, the crude residue was purified by flash column chromatography (1:1 = ethyl acetate:hexane) to give a white foam (602 mg, 41%): mp 66−68 °C dec; ^{1}H NMR ($CDCl_3$) δ 8.21 (s, 1H), 7.96 (d, J = 7.7 Hz, 1H), 7.73 (d, J = 8.0 Hz, 1H), 7.37−7.51 (m, 10H), 7.15−7.31 (m, 10H), 4.69 (br d, 1H), 3.75 (br s, 3H, $PhCH_2N$ and Cys α H), 2.68 (dd, J = 6.0 and 12.3 Hz, 1H, CH_2S), 2.56 (dd, J = 5.5 and 12.3 Hz, 1H, CH_2S), 2.47 (m, 1H, CH_2N), 2.35 (m, 1H, CH_2N), 1.62 (s, 9H), 1.42 (s, 9H), 1.12 (br s, 1H, NH).

3-[[*N*-[2(*R*)-Amino-3-mercaptopropyl]amino]methyl]-3′-carboxybiphenyl (12). Compound **22** (480 mg, 0.672 mmol) was dissolved in a mixture of 2 mL of methylene chloride and 2 mL of trifluoroacetic acid. Several drops of triethylsilane were added until the deep brown color had disappeared. This mixture was kept at room temperature for 1.5 h, then the solvents were evaporated, and the residue was dried under vacuum. The solid residue was dissolved in 1 mL of acetic acid and 2 mL of HCl (1.7 M) in acetic acid. Finally 5 mL of HCl (3 M) in ether and 10 mL of ether were added. The white precipitate was washed with dry ether and dried to give a hydrochloride salt (215 mg, 81%): ^{1}H NMR (D_2O) δ 8.16 (s, 1H), 7.94 (d, J = 7.7 Hz, 1H), 7.85 (d, J = 7.7 Hz, 1H), 7.70 (s, 2H), 7.55 (t, J = 7.8 Hz, 2H), 7.46 (d, J = 7.5 Hz, 1H), 4.36 (s, 2H, $PhCH_2$), 3.81 (m, 1H, Cys α H), 3.57 (dd, J = 5.7 and 13.7 Hz, 1H, CH_2N), 3.44 (dd, J = 6.5 and 13.7 Hz, 1H, CH_2N), 2.97 (dd, J = 5.3 and 15.1 Hz, 1H, CH_2S), 2.86 (dd, J = 5.9 and 15.1 Hz, 1H, CH_2S).

2-Methoxy-4-nitro-3′-(*tert*-butoxycarbonyl)biphenyl (23). The coupling of 1-bromo-2-methoxy-4-nitrobenzene with (3-methylphenyl)boronic acid followed by oxidation gave the 2-methoxy-4-nitro-3′-carboxybiphenyl. The reaction of the acid chloride with lithium *tert*-butoxide gave **23** (three steps, 35%): mp 88.0−88.5 °C; ^{1}H NMR ($CDCl_3$) δ 8.13 (s, 1H), 8.00 (d, J = 7.7 Hz, 1H), 7.89 (d, J = 8.3 Hz, 1H), 7.81 (s, 1H), 7.69 (d, J = 7.7 Hz, 1H), 7.48 (m, 2H), 3.90 (s, 3H), 1.60 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 165.2, 156.7, 148.0, 136.3, 136.2, 133.2, 132.0, 130.8, 130.1, 129.0, 127.9, 115.8, 106.0, 81.1, 55.9, 27.9; LRMS (EI) for $C_{18}H_{19}NO_5$ 329 (M^+, 30), 273 (100).

2-Methoxy-4-[*N*-[2(*R*)-[*N*-(*tert*-butoxycarbonyl)amino]-3-[(triphenylmethyl)thio]propyl]amino]-3′-(*tert*-butoxycarbonyl)biphenyl (24). Compound **24** was prepared using the same method as for the preparation of compound **18** (yield 63%): mp 76.0−77.0 °C dec; $[\alpha]^{25}_D$ = −11.25 (c = 0.01, $CH_3COOC_2H_5$); ^{1}H NMR ($CDCl_3$) δ 8.09 (s, 1H), 7.86 (d, J = 7.0 Hz, 1H), 7.65 (d, J = 7.0 Hz, 1H), 7.37 (t, J = 7.7 Hz, 1H), 7.43 (m, 6H), 7.21−7.32 (m, 9H), 7.11 (d, J = 8.1 Hz, 1H), 6.21 (s, 1H), 6.18 (d, J = 8.1 Hz, 1H), 4.58 (d, J = 6.1 Hz, 1H), 3.86 (br s, 1H), 3.76 (s and m, 4H), 3.14 (br d, J = 4.9 Hz, 2H), 2.49 (br d, J = 5.1 Hz, 2H), 1.59 (s, 9H), 1.43 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 165.9, 157.3, 155.5, 148.8, 144.3, 138.9, 133.3, 131.5, 131.2, 130.0, 129.4, 127.8, 127.5, 126.7, 118.7, 104.7, 96.2, 80.5, 79.4, 66.8, 55.2, 49.3, 47.0, 34.1, 28.2, 28.1. Anal. ($C_{45}H_{50}N_2O_5S$) C, H, N, S.

2-Methoxy-4-[*N*-[2(*R*)-amino-3-mercaptopropyl]amino]-3′-carboxybiphenyl (10). Compound **10** was obtained from the deprotection of compound **24**: mp 120−121 °C dec $[\alpha]^{25}_D$ = +12.62 (c = 0.01, in methanol); ^{1}H NMR (CD_3OD) δ 8.09 (s, 1H), 7.89 (d, J = 7.8 Hz, 1H), 7.67 (d, J = 7.8 Hz, 1H), 7.43 (t, J = 7.7 Hz, 1H), 7.20 (d, J = 8.1 Hz, 1H), 6.56 (s, 1H), 6.53 (d, J = 8.1 Hz, 1H), 3.81 (s, 3H), 3.60 (m, 2H, Cys α H and CH_2N), 3.48 (m, 1H, CH_2N), 2.96 (dd, J = 4.9 and 13.7 Hz, 1H, CH_2S), 2.86 (dd, J = 5.4 and 13.7 Hz, 1H, CH_2S); ^{13}C NMR (D_2O and CD_3OD) δ 171.1, 158.2, 149.3, 139.7, 135.1, 132.2, 131.1, 130.4, 129.4, 128.4, 120.5, 106.2 (broad, due to deuterium exchange), 98.8, 56.3, 53.4, 45.1, 24.9; LRMS (EI) for $C_{17}H_{20}N_2O_3S$ 332 (M^+). Anal. ($C_{17}H_{20}N_2O_3S\cdot 1.2HCl\cdot H_2O$) C, H, N, S.

Cystenyl-4-amino-3′-carboxybiphenyl (6). Compound **6** was purified through preparative HPLC. Purity was shown over 99%: mp 120.0−121.0 °C; ^{1}H NMR (CD_3OD) δ 8.25 (s, 1H), 7.98 (d, J = 7.6 Hz, 1H), 7.84 (d, J = 7.7 Hz, 1H), 7.74 (d, J = 7.0 Hz, 2H), 7.54 (t, J = 7.7 Hz, 1H), 7.66 (d, J = 8.6 Hz, 2H), 4.16 (q, J = 5.0 Hz, 1H), 3.19 (dd, J = 5.20 and 14.8 Hz, 1H), 3.07 (dd, J = 7.7 and 14.7 Hz, 1H); ^{13}C NMR (CD_3OD) δ 169.8, 166.7, 141.9, 138.7, 137.8, 132.6, 132.2, 130.2, 129.5,

128.8, 128.5, 121.6, 56.9, 26.4; LRMS (EI) for $C_{16}H_{16}O_3N_2S$ 316 (M^+, 25), 213 (100); HRMS (EI) calcd 316.0882, obsd 316.0867. Anal. ($C_{16}H_{16}N_2O_3S\cdot CF_3COOH\cdot H_2O$) C, H, N.

4-[*N*-[2(*R*)-Amino-3-mercaptopropyl]amino]-2′-carboxybiphenyl (3): mp 129−130 °C dec; $[\alpha]^{25}_D = +12.58$ ($c = 0.01$, CH_3OH); ^1H NMR (CD_3OD) δ 7.73 (d, $J = 7.6$ Hz, 1H), 7.50 (d, $J = 7.6$ Hz, 1H), 7.35 (m, 2H), 7.21 (d, $J = 8.5$ Hz, 2H), 6.86 (d, $J = 8.5$ Hz, 2H), 3.58 (m, 2H), 3.46 (m, 1H), 2.97 (dd, $J = 4.8$ and 14.6 Hz, 1H), 2.86 (dd, $J = 5.4$ and 14.6 Hz, 1H); ^{13}C NMR (D_2O and CD_3OD) δ 174.3, 146.3, 141.9, 132.8, 132.7, 131.4, 130.6, 130.2, 127.9, 115.3, 52.9, 45.8, 25.0; LRMS (FAB, glycerol) for $C_{16}H_{18}N_2O_2S$ (M+1) 303. Anal. ($C_{16}H_{18}N_2O_2S\cdot$ 1.6HCl) C, H, N, S.

4-[*N*-[2(*R*)-Amino-3-mercaptopropyl]amino]-4′-carboxybiphenyl (5): mp 260 °C dec; $[\alpha]^{25}_D = +12.20$ ($c = 0.01$, CH_3OH); ^1H NMR (CD_3OD) δ 8.03 (d, $J = 8.5$ Hz, 2H), 7.66 (d, $J = 8.4$ Hz, 2H), 7.56 (d, $J = 8.4$ Hz, 2H), 6.85 (d, $J = 8.5$ Hz, 2H), 3.57 (m, 2H), 3.45 (m, 1H), 2.98 (dd, $J = 4.8$ and 14.5 Hz, 1H), 2.85 (dd, $J = 5.7$ and 14.5 Hz, 1H); ^{13}C NMR (D_2O and CD_3OD) δ 169.8, 146.4, 146.1, 133.6, 131.4, 129.8, 129.4, 127.1, 117.0, 53.3, 47.2, 25.5; LRMS (EI) for $C_{16}H_{18}O_2N_2S$ 302 (M^+, 15), 285 (15), 226 (100), 213 (50); HRMS (EI) calcd 302.1088, obsd 302.1089. Anal. ($C_{16}H_{18}N_2O_2S\cdot$2HCl) C, H, N.

4-[*N*-[2(*R*)-Amino-3-mercaptopropyl]amino]biphenyl (7): mp 216 °C dec; $[\alpha]^{25}_D = +13.27$ ($c = 0.01$, CH_3OH); ^1H NMR (CD_3OD) δ 7.54 (m, 4H), 7.39 (m, 2H), 7.26 (m, 1H), 6.82 (br s, 2H), 3.56 (br m, 2H), 3.45 (m, 1H), 2.98 (m, 1H), 2.87 (m, 1H); ^{13}C NMR (CD_3OD) δ 144.8, 141.8, 135.7, 129.9, 129.1, 127.8, 127.4, 117.4, 53.2, 47.6, 25.5; LRMS (EI) for $C_{15}H_{18}N_2S$ 258 (M^+, 15), 182 (100); HRMS (EI) calcd 258.1190, obsd 258.1183. Anal. ($C_{15}H_{18}N_2S\cdot$1.6HCl) C, H, N, S.

4-[*N*-[2(*R*)-Amino-3-mercaptopropyl]amino]-3′-methylbiphenyl (8): ^1H NMR (CD_3OD) δ 7.50 (d, $J = 8.2$ Hz, 2H), 7.35 (m, 2H), 7.27 (t, $J = 7.6$ Hz, 1H), 7.08 (d, $J = 7.3$ Hz, 1H), 6.95 (d, $J = 8.2$ Hz, 2H), 3.60 (m, 2H), 3.46 (m, 1H), 2.99 (dd, $J = 4.9$ and 14.6 Hz, 1H), 2.88 (dd, $J = 5.5$ and 14.6 Hz, 1H), 2.37 (s, 3H); LRMS (EI) for $C_{16}H_{20}N_2S$ 272 (M^+, 15), 196 (100); HRMS (EI) calcd 272.1341, obsd 272.1347.

4-[*N*-[2(*R*)-Amino-3-mercaptopropyl]amino]-3′-methoxycarbonylbiphenyl (9): mp 86−89 °C dec; ^1H NMR (CD_3OD) δ 8.18 (s, 1H), 7.90 (d, $J = 7.7$ Hz, 1H), 7.79 (d, $J = 6.6$ Hz, 1H), 7.55 (d, $J = 8.6$ Hz, 2H), 7.49 (t, $J = 7.7$ Hz, 1H), 7.01 (d, $J = 8.6$ Hz, 2H), 3.92 (s, 3H), 3.54−3.65 (m, 2H), 3.44−3.52 (m, 1H), 2.97 (dd, $J = 4.7$ and 14.7 Hz, 1H), 2.88 (dd, $J = 5.5$ and 14.7 Hz, 1H); LRMS (EI) for $C_{17}H_{20}O_2N_2S$ 316 (M^+, 15), 299 (20), 240 (100); HRMS (EI) calcd 316.1240, obsd 316.1239. Anal. ($C_{17}H_{20}N_2O_2S\cdot$2HCl) C, H, N, S.

Acknowledgment. This work was supported by a grant from the National Institutes of Health (CA67771). We thank the Andrew Mellon Foundation for a predoctoral fellowship to Y.Q.

References

(1) Grand, B. A.; Owen, D. The biochemistry of ras p21. *Biochem. J.* **1991**, *279*, 609−631.
(2) Marshall, M. S. The effector interactions of p21ras. *Trends Biochem. Sci.* **1993**, *18*, 250-254.
(3) Barbacid, M. ras genes. *Annu. Rev. Biochem.* **1987**, *56*, 779−828.
(4) (a) Gibbs, J. B.; Oliff, A.; Kohl N. Farnesyltransferase Inhibitors: Ras Research Yields a Potential Cancer Therapeutic. *Cell* **1994**, *77*, 175−178. (b) Der, C. J.; Cox, A. D. Isoprenoid Modification and Plasma Membrane Association: Critical Fators for Ras Oncogenicity. *Cancer Cells* **1991**, *3*, 331−340.
(5) Porfiri, E.; Evans, T.; Chardin, P.; Hancock, J. F. Prenylation of Ras Proteins Is Required for Efficient hSOS1-promoted Guanine Nucleotide Exchange. *J. Biol. Chem.* **1994**, *269*, 22672−22677.
(6) Hancock, J. F.; Magee, J. E.; Marshall, C. J. All ras Proteins Are Polyisoprenylated but Only Some Are Palmitoylated. *Cell* **1989**, *57*, 1167−1177.
(7) Casey, P. J.; Solski, P. A.; Der, C. J.; Buss, J. E. p21ras is modified by the isoprenoid farnesol. *Proc. Natl. Acd. Sci. U.S.A.* **1989**, *86*, 8323−8327.
(8) Gutierrez, L.; Magee, A. I.; Marshall, C. J.; Hancock, J. F. Post-translational processing of p21ras is two-step and involves carboxylmethylation and carboxy-terminal proteolysis. *EMBO J.* **1989**, *8*, 1093−1098.
(9) (a) Reiss, Y.; Seabra, M. C.; Goldstein, J. L.; Brown, M. S. Purification of ras Farnesyl Transferase. *Methods* **1990**, *1*, 241−245. (b) Chen, W. J.; Moomaw, J. F.; Overton, L.; Kost, T.; Casey, P. J. High Level Expression of Mammalian Protein Farnesyltransferase in a Baculovirus System. *J. Biol. Chem.* **1993**, *268*, 9675−9680.
(10) Gibbs, J. B. Ras C-Terminal Processing Enzymes-New Drug Targets. *Cell* **1991**, *65*, 1−4.
(11) Casey, P. J. Biochemistry of protein prenylation. *J. Lipid Res.* **1992**, *33*, 1731−1740.
(12) Casey, P. J.; Thissen, J. A.; Moomaw, J. F. Posttranslational Modification of Ha-ras p21 by farnesyl versus geranylgeranyl isoprenoids is determined by the COOH-terminal amino acid. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 8934−8938.
(13) Sebra, M. C.; Goldstein, J. L.; Sudhof, T. C.; Brown, M. S. Rab Geranylgeranyl Transferase. *J. Biol. Chem.* **1992**, *267*, 14497−14503.
(14) (a) Goldstein, J. L.; Brown, M. S.; Stradley, S. T.; Reiss, Y.; Gierasch, L. M. Nonfarnesylated Tetrapeptide Inhibitors of Protein Farnesyltransferase. *J. Biol. Chem.* **1991**, *266*, 15575−15578. (b) Reiss, Y.; Stradley, S.; Gierasch, L.; Brown, M. S.; Goldstein, J. L. Sequence requirement for peptide recognition by rat brain p21ras protein farnesyltransferase. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 732−736.
(15) (a) Nigam, M.; Seong, C. M.; Qian, Y.; Hamilton, A. D.; Sebti, S. M. Potent Inhibition of Human Tumor p21ras Farnesyltransferase by A1A2-lacking p21ras CA1A2X Peptidomimetics. *J. Biol. Chem.* **1993**, *268*, 20695−20698. (b) Qian Y.; Blaskovich, M. A.; Saleem, M.; Seong, C. M.; Wathen, S. P.; Hamilton, A. D.; Sebti, S. M. Design and Structural Requirements of Potent Peptidomimetic Inhibitors of p21ras Farnesyltransferase. *J. Biol. Chem.* **1994**, *269*, 12410−12413. (c) Qian, Y.; Blaskovich, M. A.; Seong, C. M.; Vogt, A.; Hamilton, A. D.; Sebti, S. M. Peptidomimetic Inhibitors of p21ras Farnesyltransferase: Hydrophobic Functionalization Leads to Disruption of p21ras Membrane Association in Whole Cells. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 2579−2584.
(16) (a) Kohl, N. E.; Mosser, S. D.; deSolms, S. J.; Giuliani, E. A.; Pompliano, D. L.; Graham, S. L.; Smith, R. L.; Scolnick, E. M.; Oliff, A.; Gibbs, J. B. Selective Inhibition of ras-Dependent Transformation by a Farnesyltransferase Inhibitor. *Science* **1993**, *260*, 1934−1937. (b) James, G. L.; Goldstein, J. L.; Brown, M. S.; Rawson, T. E.; Somers, T. C.; McDowell, R. S.; Crowley, C. W.; Lucas, B. K.; Levinson, A. D.; Marsters, J. C. Benzodiazepine Peptidomimetics: Potent Inhibition of Ras Farnesylation in Animal Cells. *Science* **1993**, *260*, 1937−1942. (c) Garcia, A. M.; Rowell, C.; Ackermann, K.; Kowalczyk, J. J.; Lewis, M. D. Peptidomimetic Inhibitors of Ras Farnesylation and Function in Whole Cells. *J. Biol. Chem.* **1993**, *268*, 18415−18418. (d) Graham, S. L.; deSolms, S. J.; Giuliani, E. A.; Kohl, N. E.; Mosser, S. D.; Oliff, A. I.; Pompliano, D. L.; Rands, E.; Breslin, M. J.; Deana, A. A.; Garsky, V. M.; Scholz, T. H.; Gibbs, J. B.; Smith, R. L. Pseudopeptide Inhibitiors of Ras Farnesyl-Protein Transferase. *J. Med. Chem.* **1994**, *37*, 725−732. (e) Bhide, R. S.; Patel, D. V.; Patel, M. M.; Robinson, S. P.; Hunihan, L. W.; Gordon, E. M. Rational Design of Potent Carboxylic Acid Based Bisubstrate Inhibitors of Ras Farnesyl Protein Transferase. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 2107-2112. (f) Harrington, E. M.; Kowalczyk, J. J.; Pinnow, S. L.; Ackermann, K.; Garcia, A. M.; Lewis, M. D. Cysteine and Methionine Linked by Carbon Pseudopeptides Inhibit Farnesyl Transferase. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 2775−2780.
(17) Gardino, J.; Thompson, W. J. A General Synthesis of 5-Arylnicotinates. *J. Org. Chem.* **1984**, *49*, 5237−5243.
(18) Watanabe, T.; Miyaura, N.; Suzuki, A. Synthesis of sterically Hindered Biaryls via the Palladium Catalyzed Cross-Coupling Reactions of Arylboronic Acids or Their Esters with Haloarenes. *Synlett* **1992**, 207−210.
(19) Wallow, T. I.; Novak, B. M. Highly Efficient and Accelerated Suzuki Aryl Couplings Mediated by Phosphine-Free Palladium Sources. *J. Org. Chem.* **1994**, *59*, 5034−5037.
(20) Crowther, G. P.; Kaiser, E. M.; Woodruff, R. A.; Hauser, C. R. Esterification of Hindered Alcohols: t-Butyl p-Tolunate. *Org. Synth.* **1971**, *51*, 96−100.
(21) Fincham, C. I.; Higginbottom, M.; Hill, D. R.; Horwell, D. C.; O'Toole, J. C.; Ratcliffe, G. S.; Rees, D. C.; Roberts, E. Amide Bond Replacements Incorporated into CCK-B Selective "Dipeptoids". *J. Med. Chem.* **1992**, *35*, 1472−1484.
(22) Goel, O. P.; Krolls, U.; Stier, M.; Kesten, S. N-tert-Butoxycarbonyl-L-Leucinal. *Org. Synth.* **1989**, *67*, 69−75.
(23) Pearson, D. A.; Blanchette, M.; Baker, M. L.; Guindon, C. A. Trialkylsilanes as Scavengers for Trifluoroacetic Acid Deblocking of Protecting Groups in Peptide Synthesis. *Tetrahedron Lett.* **1989**, *30*, 2739−2742.
(24) Vogt, A.; Qian, Y.; Blaskovich, M. A.; Fossum, R. D.; Hamilton, A. D.; Sebti, S. M. A Non-peptide Mimetic of Ras-CAAX: Selective Inhibition of Farnesyltransferase and Ras Processing. *J. Biol. Chem.* **1995**, *270*, 660−664.

JM950414G