Expedited Articles

Potent, Highly Selective, and Non-Thiol Inhibitors of Protein Geranylgeranyltransferase-I

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The design, synthesis, and biological evaluation of a family of peptidomimetic inhibitors of protein geranylgeranyltransferase-I (PGGTase-I) are reported. The inhibitors are based on the C-terminal CAAL sequence of many geranylgeranylated proteins. Using 2-aryl-4-aminobenzoic acid derivatives as mimetics for the central dipeptide (AA), we have attached a series of imidazole and pyridine derivatives to the N-terminus as cysteine replacements. These non-thiol-containing peptidomimetics show exceptional selectivity for PGGTase-I over the closely related enzyme protein farnesyltransferase (PFTase). This selectivity is retained in whole cells where the inhibitors were shown to block the geranylgeranylation of Rap-1A without affecting the farnesylation of small GTP-binding proteins such as Ras.

Introduction

Protein geranylgeranyltransferase-I (PGGTase-I) is an enzyme that plays a critical role in a range of cellular processes.[1] It is a member of the protein prenyltransferase family that catalyzes the transfer of prenyl groups from prenylpyrophosphates to cysteine residues at the C-termini of a specific set of proteins in eukaryotic cells. Several small G-proteins require this prenylation for proper cellular localization and regulation of signal transduction. PGGTase-I attaches a C_{20} geranylgeranyl group to the cysteine residue of proteins that contain the C-terminal motif CAAX, where C is cysteine, A is an aliphatic amino acid, and X is leucine or phenylalanine. A separate enzyme, protein farnesyltransferase (PFTase), attaches a farnesyl (C_{15}) group to proteins terminating in the CAAX sequence, where X is methionine, serine, glutamine, alanine, cysteine, or possibly other residues.[2] The third prenyltransferase is protein geranylgeranyltransferase-II (PGGTase-II) which attaches two C_{20} geranylgeranyl groups to newly synthesized Rab proteins.[3] The enzymological characterization of these three enzymes has demonstrated that, in principle, it should be possible to selectively inhibit one with little or no effect on the others.

In the past several years major emphasis has been placed on the design of inhibitors of PFTase as potential antitumor agents.[4] However, geranylgeranylated proteins have recently been shown to be required for cells to proceed from G1 to S phase in the cell cycle.[5] Known substrates of PGGTase-I include the γ subunits of brain heterotrimeric G-proteins and Ras-related small GTP-binding proteins such as Rho A, Rho B, Rho C, and CDC42. We have recently shown that inhibitors of PGGTase-I can block cells in their G1 phase[6] and inhibit the growth of human tumors in nude mouse xenografts.[7] Furthermore, PGGTase-I inhibitors suppress the growth of and induce apoptosis in smooth muscle cells[8] and also cause a superinduction of nitric oxide synthase-2 after IL-1β treatment.[9] As a result there is a growing recognition of the potential application of PGGTase-I inhibitors in several clinical areas, including novel therapies for cancer and cardiovascular diseases. However, the few PGGTase-I inhibitors available are not particularly selective for GGTase-I.[10] This has spurred us to search for highly active PGGTase-I inhibitors that show much greater selectivity over other prenyltransferase enzymes (particularly PFTase) and that can act as probes of the effect of protein geranylgeranylation in normal and oncogenic cell growth.[11]

Chemistry

Since PGGTase-I is a bisubstrate enzyme, the design of inhibitors can be approached by mimicking either the isoprenyl substrate geranylgeranylpyrophosphate (GGPP) or the C-terminal sequence CAAL. Recently, GGPP analogues have been shown to inhibit the geranylgeranylation of Rap in cell cultures.[12] A more attractive and potentially selective strategy is the peptidomimetic approach, in which the central aliphatic dipeptide unit in CAAL is replaced by a hydrophobic spacer, such as a substituted 4-aminobenzoyl group. We had earlier used a related approach to prepare highly potent peptidomimetic inhibitors of PFTase (e.g., FTI-276) which also have excellent antitumor properties.[13] Extrapolation of this strategy to PGGTase-I afforded potent but weakly selective inhibitors (e.g., **1**, GGTI-286; **3**, GGTI-297; only

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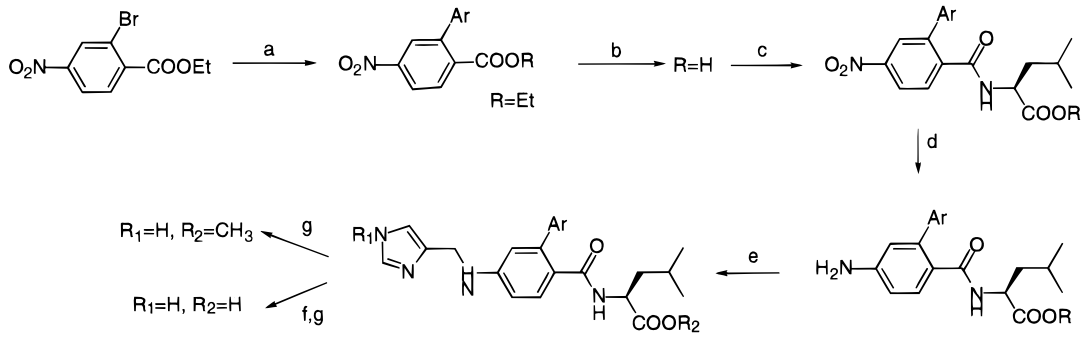
Scheme 1[a]



[a] Reagents: (a) $Pd(PPh_3)_4$, arylboronic acid, K_2CO_3, reflux, 24 h, 75%; (b) NaOH, MeOH, reflux, 85%; (c) L-leucine methyl ester hydrochloride, EDCI, HOBT; (d) $SnCl_2$; (e) tritylimidazole-4-carboxaldehyde, $NaCNBH_3$, rt; (f) LiOH, THF/H_2O; (g) TFA/triethylsilane.

2−4-fold for PGGTase-I over PFTase).[7,14] These peptidomimetics were further hampered by the presence of a free and metabolically unstable thiol functionality. We have investigated a series of N-terminal groups to replace the cysteine thiol and potentially coordinate to the putative Zn^{2+} ion in the active site of PGGTase-I.[15] In this paper we report the synthesis of a series of highly potent, non-thiol-containing inhibitors that show remarkable selectivity for PGGTase-I over PFTase.

Replacement of the mercaptan functional group by an imidazole has been successfully employed in the design of inhibitors for several zinc-containing enzymes, including angiotensin-converting enzyme (ACE)[16] and PFTase.[17] Since Zn^{2+} has been implicated in the mechanism of action of PGGTase-I,[15] a similar modification to our PGGTase-I inhibitors would be expected to confer increased metabolic stability as well as probe the importance of having a metal-coordinating ligand at the N-terminal. Using 2-aryl-4-aminobenzoic acid derivatives as mimetics for the central dipeptide in the CAAX sequence, we have attached a series of imidazole and pyridine derivatives to the N-terminus. The syntheses of these compounds followed the general route outlined in Scheme 1.[18]

Suzuki coupling of the arylboronic acid with ethyl 2-bromo-4-nitrobenzoate followed by ester hydrolysis afforded the appropriately substituted nitrobenzoic acid. An alternative route involved aryl−aryl coupling of 2-bromo-4-nitrotoluene with the arylboronic acid followed by oxidation of the methyl group. Coupling of the benzoic acid with L-leucine methyl ester hydrochloride using 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDCI)/1-hydroxybenzotriazole (HOBT), followed by reduction of the nitro group, afforded the corresponding aniline derivative**.** Reductive amination with the heterocyclic aldehyde (with trityl protection for imidazole derivatives) afforded the fully protected peptidomimetic. Saponification of the methyl ester (followed, when necessary, by TFA removal of the trityl group) afforded the unprotected inhibitors. The compounds prepared in this study are listed in Chart 1.

Results and Discussion

The in vitro inhibition assays of PGGTase-I and PFTase were carried out by measuring the [^{3}H]GGPP and [^{3}H]FPP incorporated into H-Ras-CVLL and H-Ras-CVLS, respectively, using the same procedure described before.[6] Representative dose-respose curves are shown in Figure 1. The in vivo inhibition of geranylgeranylation and farnesylation was determined based on the level of inhibition of Rap1A and H-Ras processing, respectively.[7] Briefly, oncogenic H-Ras-transformed NIH 3T3 cells were treated with various concentrations of peptidomimetics, and the cell lysates were separated on 12.5% SDS−PAGE. The separated proteins were transferred to nitrocellulose and immunoblotted using an anti-Ras antibody (Y13-258) or an anti-Rap1A antibody (SC-65; Santa Cruz Biotechnology). Antibody reactions were visualized using either peroxidase-conjugated goat

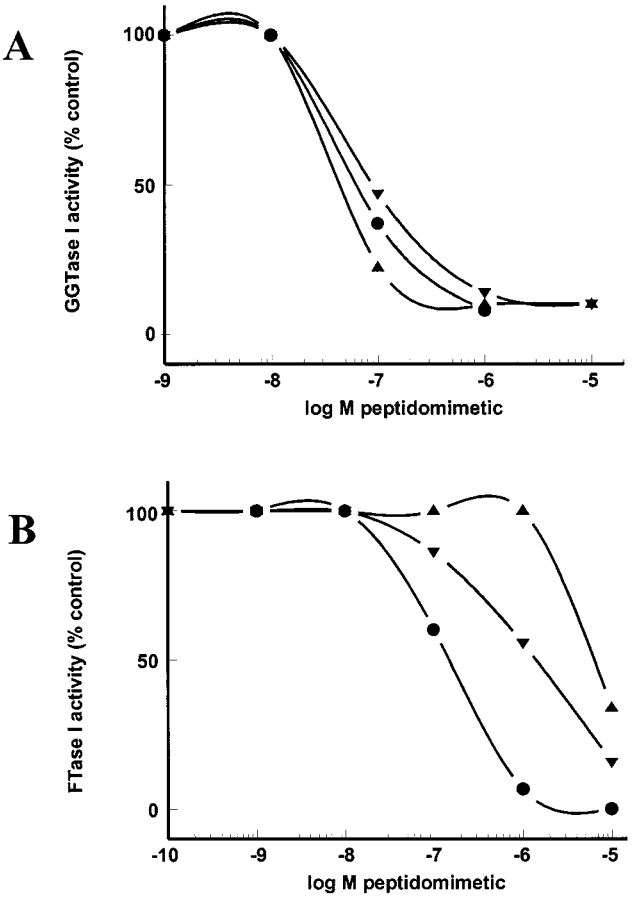
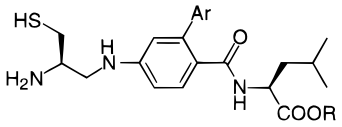
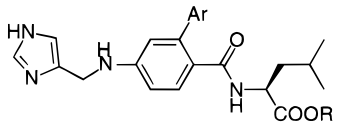


Figure 1. In vitro inhibition of PGGTase-I and PFTase by non-thiol-containing CAAL peptidomimetics. Human FTase and GGTase-I were incubated in the presence of various concentrations of peptidomimetics, and the ability of the compounds to inhibit the transfer of (A) geranylgeranyl from [^{3}H]GGPP to recombinant Ras-CVLL or (B) farnesyl from [^{3}H]-FPP to recombinant Ras-CVLS was determined: GGTI-297 (●), GGTI-2132 (▼), GGTI-2133 (▲).

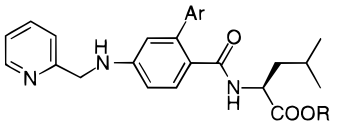
Chart 1



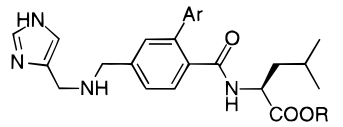
1. (GGTI-287) R = H, Ar = phenyl
2. (GGTI-286) R = CH_3, Ar = phenyl
3. (GGTI-297) R = H, Ar = 1-naphthyl
4. (GGTI-298) R = CH_3, Ar = 1-naphthyl



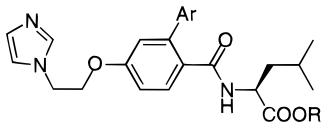
5. (GGTI-2132) R = H, Ar = phenyl
6. (GGTI-2146) R = CH_3, Ar = phenyl
7. (GGTI-2133) R = H, Ar = 1-naphthyl
8. (GGTI-2147) R = CH_3, Ar = 1-naphthyl
9. (GGTI-2144) R = H, Ar = 3,5-dimethylphenyl



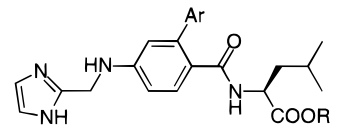
10. (GGTI-2145) R = H, Ar = 1-naphthyl



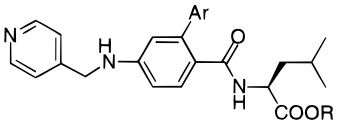
11. (GGTI-2151) R = H, Ar = 1-naphthyl
12. (GGTI-2152) R = CH_3, Ar = 1-naphthyl



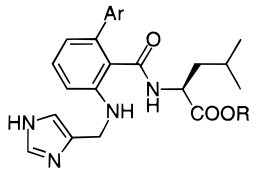
13. (GGTI-2157) R = H, Ar = 1-phenyl
14. (GGTI-2158) R = CH_3, Ar = 1-phenyl



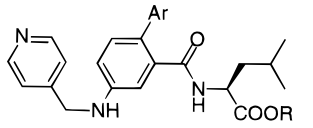
15. (GGTI-2159) R = H, Ar = 1-naphthyl
16. (GGTI-2160) R = CH_3, Ar = 1-naphthyl



17. (GGTI-2164) R = H, Ar = 1-naphthyl
18. (GGTI-2165) R = CH_3, Ar = 1-naphthyl



19. (GGTI-2163) R = H, Ar = phenyl



20. (GGTI-2139) R = H, Ar = phenyl

anti-rat IgG or goat anti-rabbit IgG and an enhanced chemiluminescence detection system. An example of this Western blot analysis for several key compounds is shown in Figure 2.

In most cases replacement of the cysteine residue in **1** (GGTI-287) and **3** (GGTI-297) led to diminished inhibition of both PFTase and PGGTase-I (Table 1). However, the effect was much more pronounced for PFTase leading to a family of highly selective inhibitors of PGGTase-I. For example, Figure 1 shows that 4-imidazolemethylene derivative **7** (GGTI-2133) has an IC_{50} for PGGTase-I of 38 nM and a 140-fold selectivity over FTase (Table 1). The corresponding methyl ester **8** (GGTI-2147) retained this selectivity in whole cells, blocking Rap1A processing at concentrations as low as 1 μM with no effect on Ras processing at 30 μM (Figure 2). Compound **8** (GGTI-2147) inhibited the geranylgeranylation of Rap1A with an IC_{50} value more than 60-fold lower than that required to disrupt the farnesylation of H-Ras (Figure 2). Modification to the 2-imidazolemethylene derivative **15** (GGTI-2159) gave less in vitro selectivity (Table 1), but the corresponding methyl ester **16** (GGTI-2160) showed similarly high potency (0.5 μM) and selectivity (>60-fold) for inhibition of Rap1A geranylgeranylation in whole cells (Figure 2).

Highest selectivity was seen with the aminomethyl homologue **11** (GGTI-2151), but the corresponding methyl ester was much less active. The basic imidazole N appears more important than the hydrogen-bonding NH center since N-alkylated **13** (GGTI-2157) retained potent in vitro activity. However, replacement of the imidazole by differently substituted pyridine groups led to loss of inhibition activity (cf. **10**, **17**, **20**). Similarly, in a probe of the shape demands of the PGGTase-I active site, the 4-substituted aminobenzoate spacer was found to be much more effective than the related 2- or 3-substituted derivatives (**19** and **20**).

In summary, we have developed a highly potent series

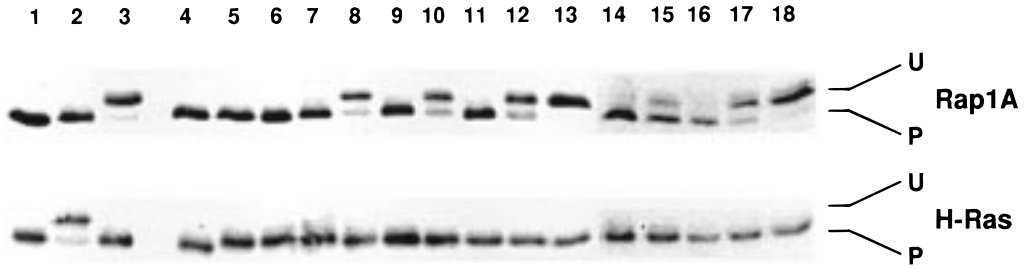


Figure 2. Effect of non-thiol-containing peptidomimetics on processing of H-Ras and Rap1A in NIH 3T3 cells (H-Ras 61L). Cells were treated on each of two successive days with peptidomimetics, then harvested, and subjected to Western blot analysis to demonstrate the inhibition of geranylgeranylated Rap1A or farnesylated H-Ras as seen in the band shift from processed (P) protein to unprocessed (U) protein: lane 1, vehicle control; lane 2, 5 μM FTI-277; lane 3, 15 μM GGTI-298; lanes 4−5, 1 and 10 μM GGTI-2132; lanes 6−8, 0.1, 1, and 10 μM GGTI-2146; lanes 9−10, 1 and 10 μM GGTI-2133; lanes 11−13, 0.1, 1, and 10 μM GGTI-2147; lanes 14−15, 1 and 10 μM GGTI-2159; lanes 16−18, 0.1, 1, and 10 μM GGTI-2160.

Table 1. IC_{50} Values for Enzyme Inhibition, Rap1A or H-Ras Processing, and Relative Selectivity for PGGTase and PFTase Inhibitors **1**−**20**

	IC_{50} (nM)			IC_{50} (μM)		
inhibitor	PGGTase	PFTase	PFTase/PGGTase	RapIA	H-Ras	H-Ras/RapIA
1 (GGTI-286)[14b]	240 ± 65	183 ± 104	0.76	10	>30	>3
2 (GGTI-287)[14b]	7.3 ± 2.6	17 ± 13	2.3	nd	nd	
3 (GGTI-297)[14b]	55 ± 19	203 ± 17	3.7	10	>30	>3
4 (GGTI-298)[14b]	nd	nd		5	>20	>4
5 (GGTI-2132)	90	1300	14.4	>10	>10	
6 (GGTI-2146)	nd	nd		10	>30	>3
7 (GGTI-2133)	38 ± 9	5400 ± 750	140	10	>30	>3
8 (GGTI-2147)	nd	nd		0.5	>30	>60
9 (GGTI-2144)	150	900	6	>100	>100	
10 (GGTI-2145)	4000	100000	25	nd	nd	
11 (GGTI-2151)	44 ± 10	10000	230	>100	>100	
12 (GGTI-2152)	nd	nd		10	>10	>1
13 (GGTI-2157)	32 ± 11	303 ± 57	9.5	>10	>10	
14 (GGTI-2158)	nd	nd		>10	>10	
15 (GGTI-2159)	210	8000	38	20	>30	>1.5
16 (GGTI-2160)	nd	nd		0.5	>30	>60
17 (GGTI-2164)	1600	58000	36	>30	>30	
18 (GGTI-2165)	nd	nd		20	>30	>1.5
19 (GGTI-2163)	6100	46000	7.5	>100	>100	
20 (GGTI-2139)	400	50000	125	>100	>100	

of inhibitors for PGGTase-I that shows unprecedented selectivity over the closely related prenyltransferase, PFTase. Importantly, these compounds do not contain thiol groups and are able to selectively block the processing of a known geranylgeranylated protein, Rap1A, at submicromolar concentrations in whole cells. We are at present investigating the antitumor activity of these compounds in a range of animal models.

Experimental Section

Nuclear magnetic resonance spectra were acquired using Bruker AM-300 series spectrometers (300 MHz for ^{1}H, 75 MHz for ^{13}C) and are reported in δ units. All synthesized final compounds (amine hydrochloride or amine trifluoroacetate salts) were checked for purity by analytical high-pressure liquid chromatography which was performed using a Rainin HP controller and a Rainin UV-C detector with a Rainin 250- × 4.6-mm, 5-μm Microsorb C18 column. Preparative HPLC was performed on a Waters 600E controller and a Waters 490E multiwavelength UV detector with a 25- × 10-cm Delta-Pak C-18 300-Å cartridge column inside a Waters 25- × 10-cm Radial compression module. Solvents consisting of 20−80% acetonitrile and 0.1% TFA in water were used with a flow rate of 15 mL/min in 40 min.

Synthesis of GGTI-2132 (5). 4-[[*N*-(1-Tritylimidazol-4-yl)methyleneamino]-2-phenylbenzoyl]leucine Methyl Ester. To a solution of 2-phenyl-4-aminobenzoylleucine methyl ester hydrochloride[14b] (0.25 g, 0.74 mmol) in methanol was added tritylimidazole-4-carboxaldehyde (0.25 g, 0.74 mmol) followed by 1 mL of glacial acetic acid, and the reaction was stirred for 30 min. This was followed by the addition of $NaCNBH_3$ (0.046 g, 0.74 mmol), and the reaction was stirred at room temperature for 2 h. The solvent was evaporated, and the residue was dissolved in ethyl acetate (~3 mL) and chromatographed on a silica gel column (1.05" × 23") to afford a white solid (0.30 g, 61%): ^{1}H NMR (300 MHz, $CDCl_3$) 0.79 (dd, 6 H), 1.10−1.35 (m, 3 H), 3.64 (s, 3 H), 4.28 (d, 2 H), 4.51 (m, 1 H), 4,66 (br s, 1 H), 5.45 (d, 1 H), 6.49 (s, 1 H), 6.64 (dd, 1 H), 6.74 (s, 1 H), 7.12 (m, 6 H), 7.09−7.27 (m, 15 H), 7.48 (s, 1 H), 7.69 (d, 1 H).

The above compound (0.30 g, 0.45 mmol) was dissolved in 15 mL of THF/H_2O (3:2), and LiOH (0.035 g, 0.91 mmol) was added. The reaction was stirred at room temperature for 2 h. The THF was evaporated, the residue was dissolved in 15 mL of water, and 1 N HCl was added to lower the pH to 2. The compound was then extracted with ethyl acetate (3 × 25 mL) and dried, and the solvents were evaporated to afford the free carboxylic acid as a white solid (0.25 g, 84%).

The above compound (0.25 g, 0.38 mmol) was dissolved in 10 mL of dichloromethane and 3 mL of trifluoroacetic acid followed by the addition of 1.5 mL of triethylsilane, and the reaction was stirred at room temperature for 2 h. The solvents were evaporated, and ether was added followed by the addition of 6 N HCl in ether to precipitate the desired compound (GGTI-2132) which was collected by rapid filtration (0.12 g, 77%): ^{1}H NMR (300 MHz, DMSO-d_6) 0.76 (dd, 6 H), 1.38 (m, 2 H), 1.55 (m, 1 H), 4.19 (m, 1 H), 4.42 (d, 2 H), 6.63 (s, 1 H), 6.73 (d, 1 H), 7.31−7.36 (m, 6 H), 7.63 (s, 1 H), 8.23 (d, 1 H), 9.09 (s, 1 H); HRMS (FAB) *m*/*z* calcd for $C_{23}H_{26}N_4O_3 \cdot H^+$ 407.2083, found 407.2083.

Synthesis of GGTI-2133 (7). 4-[[*N*-(Imidazol-4-yl)methyleneamino]-2-(1-naphthyl)benzoyl]leucine. The synthesis followed a similar procedure to that for GGTI-2132, using 2-(1-naphthyl)-4-aminobenzoylleucine methyl ester hydrochloride (0.59 g, 1.5 mmol), tritylimidazole-4-carboxaldehyde (0.61 g, 1.80 mmol), and $NaCNBH_3$ (0.34 g, 5.4 mmol) to afford a white solid (0.72 g, 67%): ^{1}H NMR (300 MHz, $CDCl_3$) 0.13 (m, 0.8 H), 0.44 (m, 5.2 H), 0.57 (m, 2 H), 0.93 (m, 1 H), 3.40 (s, 1 H), 3.57 (s, 2 H), 4.24 (m, 1 H), 5.37−5.47 (dd, 1 H), 6.49 (s, 1 H), 7.08 (m, 2 H), 7.09−7.11 (m, 6 H), 7.30−7.32 (m, 11 H), 7.49−7.57 (m, 5 H), 7.80 (m, 2 H), 8.08 (d, 1 H).

The above compound (0.24 g, 0.34 mmol) was dissolved in 10 mL of THF/H_2O (2:1), and LiOH (0.029 g, 0.68 mmol) was added. The reaction was stirred at room temperature for 2 h and following a similar workup gave a white solid (0.20 g, 84%). This compound (0.20 g, 0.29 mmol) was dissolved in 10 mL of dichloromethane and 3 mL of trifluoroacetic acid followed by the addition of 1.5 mL of triethylsilane, and the reaction was stirred at room temperature for 2 h. The solvents were evaporated, and ether was added followed by the addition of 6 N HCl in ether to precipitate the desired compound (GGTI-2133) which was collected by rapid filtration (0.096 g, 74%): ^{1}H NMR (300 MHz, DMSO-d_6) 0.37 (m, 3 H), 0.65 (m, 3 H), 1.08 (m, 2 H), 1.25 (m, 1 H), 3.89 (m, 1 H), 4.37 (d, 2 H), 6.53 (s, 1 H), 6.79 (m, 1 H), 7.01 (m, 1 H), 7.42−7.62 (m, 8 H), 7.86−7.94 (m, 2 H), 8.86 (s, 1 H); HRMS (FAB) *m*/*z* calcd for $C_{27}H_{28}N_4O_3 \cdot H^+$ 457.2240, found 457.2245.

Synthesis of GGTI-2139 (20). 5-[[*N*-(4-Pyridyl)methyleneamino]-2-phenylbenzoyl]leucine Methyl Ester. This was prepared by a similar route to GGTI-2132, using 2-phenyl-5-aminobenzoylleucine methyl ester hydrochloride (0.50 g, 1.47 mmol), pyridine-4-carboxaldehyde (0.164 g, 1.53 mmol), and $NaCNBH_3$ (0.16 g, 2.56 mmol) to afford a yellowish solid (0.40 g, 63%): ^{1}H NMR (300 MHz, $CDCl_3$) 0.76 (m, 6 H), 1.10−1.17 (m, 2 H), 1.27−1.32 (m, 1 H), 3.62 (s, 3 H), 4.43−4.53 (overlapping m & s, 3 H), 5.60 (d, 1 H), 6.61−6.65 (dd, 1 H), 6.97 (d, 1 H), 7.13 (d, 1 H), 7.25−7.35 (m, 8 H), 8.55 (m, 2 H).

The above compound (0.22 g, 0.51 mmol) was dissolved in 10 mL of THF/H_2O (2:1) and cooled to 0 °C, and LiOH (0.04 g, 1.02 mmol) was added. The reaction was stirred at 0 °C for 1 h, followed by stirring at room temperature for 2 h. The solvents were evaporated, and the residue was passed through a bed of silica gel and eluted with CH_2Cl_2:CH_3OH (9:1) to afford the desired GGTI-2139 as a white solid (0.19 g, 90%): ^{1}H NMR (300 MHz, DMSO-d_6) 0.79 (t, 6 H), 1.19 (m, 2 H), 1.42 (m, 1 H), 4.06 (m, 1 H), 4.36 (d, 2 H), 6.53 (dd, 1 H), 6.81 (m, 2 H), 7.04 (d, 1 H), 7.17−7.31 (m, 6 H), 7.59 (d, 1 H), 8.46 (d, 2 H); HRMS (FAB) *m*/*z* calcd for $C_{25}H_{27}N_3O_3 \cdot H^+$ 418.2131, found 418.2128.

Synthesis of GGTI-2145 (10). 4-[[*N*-(2-Pyridyl)methyleneamino]-2-(1-naphthyl)benzoyl]leucine Methyl Ester. This was prepared by a similar route to GGTI-2132, using 2-(1-naphthyl)-4-aminobenzoylleucine methyl ester hydrochloride (0.20 g, 0.51 mmol), pyridine-2-carboxaldehyde (0.06 g, 0.51 mmol), and $NaCNBH_3$ (0.05 g, 0.77 mmol) to give the protected derivative as a yellow solid (0.15 g, 61%): ^{1}H NMR (300 MHz, $CDCl_3$) 0.13 (m, 0.8 H), 0.48 (m, 5.2 H), 0.57−0.64 (m, 2 H), 0.96−1.1 (m, 1 H), 3.4 (s, 1 H), 3.61 (s, 2 H), 4.21 (m, 1 H), 4.50 (m, 2 H), 5.40 (m, 1 H), 5.48 (br t, 1 H), 6.57 (d, 1 H), 6.82 (dd, 1 H), 7.23−7.52 (m, 7 H), 7.85 (m, 1 H), 7.92−8.04 (m, 3 H), 8.57 (d, 1 H).

The above compound (0.15 g, 0.31 mmol) was dissolved in 10 mL of THF/H_2O (2:1) and cooled to 0 °C, and LiOH (0.03 g, 0.62 mmol) was added. The reaction was stirred at 0 °C for 1 h, followed by stirring at room temperature for 2 h. The solvents were evaporated, and the residue was passed through a bed of silica gel and eluted with $CHCl_3$:CH_3OH (9:1) to afford the desired GGTI-2145 as a white solid (0.13 g, 86%): ^{1}H NMR (300 MHz, DMSO-d_6) 0.45 (m, 7 H), 0.81 (m, 1 H), 1.08 (m, 1 H), 3.77 (m, 1 H), 4.39 (s, 2 H), 6.22 (d, 1 H), 6.50 (m, 1 H), 6.72 (d, 1 H), 7.15 (br s, 1 H), 7.18−7.51 (m, 8 H), 7.81 (m, 1 H), 7.88 (m, 2 H), 8.48 (m, 1 H); HRMS (FAB) *m*/*z* calcd for $C_{29}H_{29}N_3O_3 \cdot H^+$ 468.2287, found 468.2282.

Synthesis of GGTI-2146 (6). 4-[[*N*-(Imidazol-4-yl)methyleneamino]-2-phenylbenzoyl]leucine Methyl Ester. 4-[[*N*-(1-Tritylimidazol-4-yl)methyleneamino]-2-phenylbenzoyl]leucine methyl ester (0.45 g, 0.68 mmol) was dissolved in 10 mL of dichloromethane, and 3 mL of TFA was added, followed by the addition of 1.5 mL of triethylsilane. The colorless solution was stirred at room temperature for 2 h, following which the solvent was evaporated and the residue was dissolved in ethyl acetate and washed with saturated $NaHCO_3$ (25 mL). The organic layer was dried and evaporated to afford GGTI-2146 as a white solid (0.24 g, 84%): ^{1}H NMR (300 MHz, DMSO-d_6) 0.76 (d, 3 H), 0.81 (d, 3 H), 1.38 (m, 2 H), 1.52 (m, 1 H), 3.60 (s, 3 H), 4.19 (m, 1 H), 4.42 (d, 2 H), 6.61 (s, 1 H), 6.67 (dd, 1 H), 7.22−7.35 (m, 6 H), 7.55 (s, 1 H), 8.18 (d, 1 H), 9.04 (s, 1 H); HRMS (FAB) *m*/*z* calcd for $C_{24}H_{28}N_4O_3 \cdot H^+$ 421.2239, found 421.2240.

Synthesis of GGTI-2147 (8). 4-[[*N*-(Imidazol-4-yl)methyleneamino]-2-(1-naphthyl)benzoyl]leucine Methyl Ester. This was prepared by a similar route to GGTI-2146, using 4-[[*N*-(1-tritylimidazol-4-yl)methyleneamino]-2-(1-naphthyl)-benzoyl]leucine methyl ester (0.40 g, 0.56 mmol), 3 mL of TFA added, and 1.5 mL of triethylsilane to afford GGTI-2147 as a white solid (0.21 g, 81%): ^{1}H NMR (300 MHz, DMSO-d_6) 0.38−0.74 (m, 6 H), 0.91 (m, 2 H), 1.11 (m, 1 H), 3.46 (s, 3 H), 3.94 (m, 1 H), 4.41 (d, 2 H), 6.55 (s, 1 H), 6.79 (m, 1 H), 7.34−7.57 (m, 8 H), 7.83−7.94 (m, 3 H), 9.05 (s, 1 H); HRMS (FAB) *m*/*z* calcd for $C_{28}H_{30}N_4O_3 \cdot H^+$ 471.2396, found 471.2400.

Synthesis of GGTI-2163 (19). 6-[[*N*-(1-Tritylimidazol-4-yl)methyleneamino]-2-phenylbenzoyl]leucine Methyl Ester. To a mixture of 35 mL of acetone and 43 mL of water were added 2-bromo-6-nitrotoluene (3.42 g, 15 mmol), phenylboronic acid (1.92 g, 15.7 mmol), K_2CO_3 (5.17 g, 37 mmol), and palladium acetate (168 mg, 0.75 mmol), and the mixture was refluxed for 20 h. The solution was cooled to room temperature, then acidified to pH 3 with 1 N HCl, and extracted with ethyl ether. Drying with $MgSO_4$, followed by evaporation of the solvent, afforded a white solid (2.37 g, 74%): ^{1}H NMR ($CDCl_3$) 7.43 (d, J = 6.8 Hz, 1 H), 7.12−6.91 (m, 7 H), 1.99 (s, 3 H, CH_3); ^{13}C NMR ($CDCl_3$) 151.41, 145.03, 140.11, 133.83, 130.07, 129.23, 128.49, 127.82, 126.17, 123.05, 16.84.

The above compound (2.23 g, 11.5 mmol) was suspended in 11.5 mL of pyridine and 21 mL of water, and the mixture was heated to boiling, at which time $KMnO_4$ (9.9 g, 63 mmol) was added gradually and the mixture heated at reflux for 72 h. At that time, additional amounts of $KMnO_4$ (3.3 g, 21 mmol) were added, and the mixture was refluxed for 96 h. The mixture was cooled and filtered through Celite, following which the filtrate was acidified with 6 N HCl and the product was extracted with ethyl acetate to afford a yellow solid (1.28 g, 51%): ^{1}H NMR (300 MHz, $CDCl_3$) 8.02 (d, J = 6.27 Hz, 1 H, H-5), 7.61−7.53 (m, 2 H, H-3, H-4), 7.37−7.32 (m, 5 H, phenyl).

The nitro compound (1.22 g, 5 mmol) was suspended in anhydrous dichloromethane (20 mL). To this solution were added L-leucine methyl ester hydrochloride (0.90 g, 5 mmol), triethylamine (0.69 mL, 5 mmol), 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide (1.00 g, 5.2 mmol), and 1-hydroxybenzotriazole (0.67 g, 5 mmol) at 0 °C, and the mixture stirred at 0 °C for 1 h and then at room temperature for 12 h. The mixture was then washed with 1 N HCl, and the organic layer was dried and evaporated to affford 2-phenyl-6-nitrobenzoylleucine methyl ester (1.38 g, 75%).

The above compound was obtained as a 2:3 mixture of diastereomers. No attempt was made to separate the individual rotational isomers: ^{1}H NMR (minor isomer) ($CDCl_3$) 8.30 (m, 1 H), 8.04 (d, 2 H), 7.79 (m, 3 H), 7.21 (m, 2 H), 6.65 (d, 1 H), 4.81 (m, 1 H), 3.73 (s, 3 H), 1.33−1.29 (m, 2 H), 1.27 (m, 1 H), 0.93 (m, 6 H); ^{1}H NMR (major isomer) ($CDCl_3$) 7.39 (m, 5 H), 7.66 (m, 3 H), 6.13 (d, 1 H), 4.57 (m, 1 H), 3.60 (s, 3 H), 1.73 (m, 2 H), 0.91 (m, 1 H), 0.77 (t, 6 H).

The above compound (1.25 g, 3.24 mmol) was suspended in ethyl acetate (50 mL), $SnCl_2$ (3.67 g, 16.2 mmol) was added, and the mixture was heated to reflux for 3 h. The mixture was cooled and washed with saturated $NaHCO_3$; the combined organic extracts were dried and evaporated to afford the amino

compound as a yellow solid (0.99 g, 90%). The ^{1}H NMR indicated the presence of two diastereomers.

The amine (0.5 g, 1.47 mmol) was suspended in 15 mL of methanol, and tritylimidazole-4-carboxaldehyde (0.49 g, 1.47 mmol) was added. After stirring for 0.5 h, glacial acetic acid (1 mL) was added followed by $NaCNBH_3$ (0.18 g, 2.94 mmol). The mixture was stirred for 1 h, then an additional amount of tritylimidazole (0.25 g, 0.74 mmol) was added, and the mixture was stirred for another 1 h. The solvents were evaporated, and the mixture was purified by column chromatography on silica gel using ethyl acetate:hexanes:NH_4OH (1:1:0.1) as the eluant to afford 6-[[*N*-(1-triphenylmethylimidazol-4-yl)methyleneamino]-2-phenylbenzoyl]leucine methyl ester as a white solid (0.48 g, 50%): ^{1}H NMR ($CDCl_3$) 7.38 (m, 4 H), 7.29 (m, 12 H), 7.12 (m, 6 H), 6.76 (s, 1 H), 6.70 (d, 1 H), 6.65 (d, 1 H), 6.07 (t, 1 H), 5.75 (d, 1 H), 4.36 (overlapping m and d, 3 H), 3.55 (s, 3 H), 1.24 (m, 2 H), 0.92 (m, 1 H), 0.71 (m, 6 H).

The coupled product (0.40 g, 0.60 mmol) was dissolved in 10 mL of a 3:2 mixture of THF/water, and LiOH was added. The mixture was stirred at 0 °C for 1 h and then at room temperature for 4 h. The mixture was acidifed with 1 N HCl and the solvents were evaporated and extracted with ethyl acetate to afford a white solid (0.31 g, 81%).

This solid was dissolved in 10 mL of dichloromethane, and 2 mL of TFA was added, followed by the dropwise adddition of triethylsilane until the yellow coloration disappeared. The solvents were evaporated, and anhydrous ether was added to precipitate a white solid. This solid was sonicated in 15 mL of ether for 1 h, following which the supernatant was decanted. This procedure was repeated two times, and after the third wash, the solid was dried to afford pure GGTI-2163 (0.17 g, 72%): mp 168−170 °C; ^{1}H NMR (DMSO-d_6) 8.59−8.54 (m, 2 H), 7.36−7.21 (m, 7 H), 6.73 (d, 1 H, J = 8.3 Hz), 6.60 (d, 1 H, J = 7.53 Hz), 5.85 (br s, 1 H), 4.34 (s, 2 H), 4.08 (m, 1 H), 1.49−1.29 (m, 1.7 H), 1.10−1.06 (m, 1.3 H), 0.73 (d, 3 H, J = 6.42 Hz), 0.63 (d, 3 H, J = 6.33 Hz); HRMS (FAB) *m*/*z* calcd for $C_{23}H_{26}N_4O_3 \cdot H^+$ 407.2083, found 407.2083.

Synthesis of GGTI-2160 (16). 4-[[*N*-(1*H*-Imidazol-2-yl)-methyleneamino]-2-(1-naphthyl)benzoyl]leucine Methyl Ester. This compound was obtained via the reductive amination of 2-(1-naphthyl)-4-aminobenzoyl-L-leucine methyl ester hydrochloride and imidazole-2-carboxaldehyde, following similar procedures to those described for GGTI-2132, to afford after purification a pale yellow solid (46%): mp 167−169 °C; ^{1}H NMR ($CDCl_3$) 9.10 (br s, 1 H, imidazole-NH), 7.89−7.80 (m, 3 H, aryl), 7.55−7.45 (m, 3 H, aryl), 7.35−7.28 (m, 2 H), 6.90 (s, 2 H), 6.58 (d, 1 H, J = 8.7 Hz), 6.51 (d, 1 H, J = 2 Hz), 5.62 (m, 1 H, NH), 4.43 (d, 2 H, CH_2), 4.14 (m, 1 H, leu α-CH), 3.50 (s, 2 H, $COOCH_3$), 3.36 (s, 1 H, $COOCH_3$), 1.25−1.18 (m, 1 H), 1.03 (m, 2 H), 0.56 (d, 2 H), 0.40 (m, 4 H); HRMS (FAB) *m*/*z* calcd for $C_{28}H_{30}N_4O_2 \cdot H^+$ 471.2396, found 471.2391.

Synthesis of GGTI-2159 (15). 4-[[*N*-(1*H*-Imidazol-2-yl)-methyleneamino]-2-(1-naphthyl)benzoyl]leucine. This compound was obtained via the deprotection of the above methyl ester using LiOH in a 3:2 mixture of THF/H_2O, following procedures described for the synthesis of GGTI-2146, to afford a white solid, after lyophilization (88%): mp 110−114 °C (softens); ^{1}H NMR (DMSO-d_6) 7.92−7.77 (m, 3 H, aryl), 7.57−7.13 (m, 9 H, aryl), 6.84 (m, 1 H), 6.56 (s, 1 H), 4.73 (d, 2 H, CH_2), 3.86 (m, 1 H, leu α-CH), 1.32−1.02 (m, 3 H), 0.65 (d, J = 6.2 Hz, 1.5 H), 0.56 (d, J = 6.2 Hz, 1.5 H), 0.45 (d, J = 6.09 Hz, 1.5 H), 0.36 (d, J = 6.2 Hz, 1.5 H); HRMS (FAB) *m*/*z* calcd for $C_{27}H_{28}N_4O_3 \cdot H^+$ 457.2240, found 457.2241.

Synthesis of GGTI-2164 (17) and GGTI-2165 (18). 4-[[*N*-(4-Pyridyl)methyleneamino]-2-(1-naphthyl)benzoyl]leucine. This compound was synthesized in a manner similar to that described for the synthesis of GGTI-2145, via the reductive amination of 2-(1-naphthyl)-4-aminobenzoylleucine methyl ester and pyridine-4-carboxaldehyde in methanol to afford, after purification, GGTI-2165 as a white solid (64%): ^{1}H NMR (DMSO-d_6) 0.71 (d, J = 5.45 Hz, 3 H), 0.82 (d, J = 5.13 Hz, 3 H), 1.20−1.27 (m, 2 H), 1.29−1.32 (m, 1 H), 3.72 (s, 2 H), 3.79 (s, 1 H), 4.15 (m, 1 H, leu α-CH), 4.41 (d, 2 H, CH_2), 5.52 (d, 1 H, NH), 6.67−6.75 (dd, 1 H), 6.89 (d, 1 H), 7.23 (m, 2 H), 7.25−7.35 (m, 8 H), 8.55 (m, 2 H); HRMS (FAB) *m*/*z* calcd for $C_{30}H_{31}N_3O_3 \cdot H^+$ 482.2444, found 482.2446.

Saponification of the methyl ester afforded the desired GGTI-2164 as a white solid (82%): ^{1}H NMR (DMSO-d_6) 8.46 (d, 2 H), 7.55 (d, J = 8.3 Hz, 1 H), 7.31−7.21 (m, 7 H), 7.01 (m, 1 H), 6.83−6.79 (m, 2 H), 6.72 (d, J = 6.6 Hz, 1 H), 4.22 (d, J = 5.7 Hz, 2 H), 4.01 (m, 1 H, leu α-CH), 1.47−1.32 (m, 3 H, leu CH_2, CH), 0.77 (d, J = 5.5 Hz, 6 H, leu CH_3); HRMS (FAB) *m*/*z* calcd for $C_{29}H_{29}N_3O_3 \cdot H^+$ 468.2287, found 468.2287.

Synthesis of GGTI-2151 (11) and GGTI-2152 (12). Methyl 4-Nitro-2-(1-naphthyl)benzoate. Methyl 2-bromo-4-nitrobenzoate (3.86 g, 14.85 mmol) was coupled with 1-naphthylboronic acid (3.06 g, 17.79 mmol) in DMF in the presence of K_2CO_3 (3.0 equiv) and $Pd(PPh_3)_4$ (0.03 equiv) at 110 °C for 15 h. After flash column chromotography purification, methyl 4-nitro-2-(1-naphthyl)benzoate was obtained as a pale yellow oil (3.86 g, 85%): ^{1}H NMR ($CDCl_3$) 8.34 (d, J = 8.5 Hz, 1 H), 8.28 (s, 1 H), 8.14 (d, J = 8.5 Hz, 1 H), 7.92 (m, 2 H), 7.47−7.56 (m, 2 H), 7.41 (d, J = 3.8 Hz, 2 H), 7.34 (d, J = 6.8 Hz, 1 H), 3.40 (s, 3 H); ^{13}C NMR ($CDCl_3$) 166.4, 149.2, 142.7, 137.2, 136.8, 133.2, 131.3, 131.0, 128.6, 128.4, 126.6, 126.2, 126.0, 125.0, 124.7, 122.3, 52.3.

Methyl 4-Iodo-2-(1-naphthyl)benzoate. This compound was prepared from the nitro derivative. A catalytic amount of Pd/C was added, and the mixture was hydrogenated at 40 psi for 1 h. After removal of the catalyst, the reduced product was obtained. This amine was dissolved in 7 mL of concentrated hydrochloric acid (12 N), 1 mL of water, and 3 mL of acetic acid. To this clear solution was added sodium nitrite in 4 mL of water at 0 °C. The mixture was stirred at 0 °C for 25 min. Then a solution of KI (1.5 equiv) in 4 mL of 2 N HCl was added. The mixture was warmed to 60 °C and then extracted with ethyl acetate. The ethyl acetate solution was washed with sodium bicarbonate. After evaporation of solvents, the residue was purified by flash column chromatography to give a pale yellow oil (54%): ^{1}H NMR ($CDCl_3$) 7.85−7.90 (m, 3 H), 7.73−7.80 (m, 2 H), 7.31−7.53 (m, 4 H), 7.28 (t, J = 6.8 Hz, 1 H), 3.37 (s, 3 H); ^{13}C NMR ($CDCl_3$) 166.7, 142.8, 140.3, 137.7, 136.5, 132.8, 131.4, 131.2, 130.5, 128.0, 127.7, 125.9, 125.6, 125.5, 124.9, 124.8, 98.8, 51.6.

Methyl 4-Cyano-2-(1-naphthyl)benzoate. This compound was prepared by dissolving the above iodo derivative in 10 mL of THF. Then $Pd(PPh_3)_4$ (2.2% equiv) and powdered KCN (1.5 equiv) were added. The mixture was refluxed for 12 h. GC/MS showed all the starting material was converted to the product. The mixture was extracted with ethyl acetate and water. After evaporation of solvents, the residue was purified by flash column chromatography (10:1 hexane/ethyl acetate) to give an oily product (50%): ^{1}H NMR ($CDCl_3$) 8.10 (d, J = 8.1 Hz, 1 H), 7.92 (d, J = 8.2 Hz, 2 H), 7.81 (d, J = 8.1 Hz, 1 H), 7.75 (s, 1 H), 7.46−7.56 (m, 2 H), 7.38 (d, J = 3.7 Hz, 2 H), 7.32 (d, J = 7.0 Hz, 1 H), 3.40 (s, 3 H); ^{13}C NMR ($CDCl_3$) 165.9, 141.5, 136.5, 135.2, 134.7, 132.7, 131.0, 130.7, 130.1, 128.0, 126.1, 125.8, 125.5, 124.7, 124.3, 117.3, 114.7, 51.7.

4-Cyano-2-(1-naphthyl)benzoylleucine Methyl Ester. The above ester (780 mg, 2.72 mmol) was dissolved in 8.0 mL of THF. To this solution were added 0.5 N LiOH (6.5 mL, 1.2 equiv) and 4.0 mL of methanol. The mixture was refluxed for 2 h. TLC showed the disappearance of the starting material. Solvents were evaporated, and the residue was first acidified with 1 N HCl and then extracted with ethyl acetate. After the evaporation of solvents, a white solid was obtained (752 mg, 100%). This acid (741 mg, 2.71 mmol) was coupled with L-leucine methyl ester hydrochloride (542 mg, 1.10 equiv) using EDCI and HOBT. After purification by flash column chromatography (2:1 hexane/ethyl acetate), 4-cyano-2-(1-naphthyl)-benzoylleucine methyl ester was obtained (775 mg, 72%): ^{1}H NMR ($CDCl_3$) 8.10 (d, J = 8.0 Hz, 0.6 H), 7.89−7.97 (m, 2.4 H), 7.80 (m, 1 H), 7.67 (s, 1 H), 7.36−7.59 (m, 5 H), 5.68 (d, J = 7.9 Hz, 0.5 H), 5.60 (d, J = 7.9 Hz, 0.5 H), 4.25 (m, 1 H), 3.55 (s, 1.5 H), 3.50 (s, 1.5 H), 1.02−1.05 (m, 0.5 H), 0.87−0.95 (m, 0.5 H), 0.73−0.76 (m, 0.5 H), 0.40−0.58 (m, 6.5 H), 0.18−0.25 (m, 1 H); ^{13}C NMR ($CDCl_3$) 172.2, 172.0, 166.3, 165.6, 140.0, 139.0, 138.7, 135.6, 135.0, 134.6, 134.5, 133.3,

131.3, 131.2, 131.1, 130.1, 129.5, 129.0, 128.8, 128.3, 128.2, 127.0, 126.9, 126.6, 126.4, 126.1, 125.1, 124.6, 124.4, 117.6, 117.5, 114.2, 113.4, 51.8, 50.4, 40.3, 23.6, 23.5, 22.2, 20.9, 20.8.

4-(*N*-Boc-aminomethyl)-2-(1-naphthyl)benzoylleucine Methyl Ester. The above nitrile (700 mg, 1.75 mmol) was dissolved in 1 mL of THF and 12 mL of methanol. To this solution was added $CoCl_2 \cdot 6H_2O$ (835 mg, 3.5 mmol). The mixture was vigorously stirred, and then $NaBH_4$ (670 mg, 17.5 mmol) was added in several portions (H_2 gas evolved and color turned to black). The black mixture was stirred at room temperature for 3 h. The solvents were evaporated, and the residue was extracted with ethyl acetate. After evaporation of solvents, the residue (550 mg) was dissolved in 10 mL of methylene chloride. To this solution was added di-*tert*-butyl dicarbonate (445 mg, 1.5 equiv), and the mixture was stirred for 10 h. After evaporation of solvents, the residue was purified by flash column chromatography (1.3:1 hexane/ethyl acetate) to give a colorless oil (300 mg, 34% for two steps): ^{1}H NMR ($CDCl_3$) 8.00 (d, J = 8.0 Hz, 0.6 H), 7.84−7.92 (m, 2.4 H), 7.55 (d, J = 8.0 Hz, 1 H), 7.35−7.50 (m, 5 H), 7.23 (s, 1 H), 5.63 (d, J = 7.9 Hz, 0.6 H), 5.55 (d, J = 7.9 Hz, 0.4 H), 5.07 (br s, 1 H), 4.38 (br s, 2 H), 4.21−4.29 (m, 1 H), 3.52 (s, 1.8 H), 3.44 (s, 1.2 H), 1.42 (s, 9 H), 1.01−1.10 (m, 0.4 H), 0.84−0.93 (m, 0.6 H), 0.71−0.77 (m, 0.5 H), 0.46−0.55 (m, 3.3 H), 0.39−0.45 (m, 3.7 H), 0.14−0.19 (m, 0.6 H).

4-[*N*-(1*H*-Imidazol-4-yl)methylaminomethyl]-2-(1-naphthyl)benzoylleucine Trifluoroacetate (GGTI-2151). The above Boc-protected derivative was dissolved in 7 mL of methylene chloride. To this solution was added 4 mL of 4 N HCl in ether. The mixture was stirred at room temperature for 15 min. TLC showed all starting material had disappeared. The solvents were evaporated, and the amine hydrochloride salt was obtained in quantitative yield: ^{1}H NMR ($CDCl_3$) 8.30 (br s, 3 H), 7.80 (m, 1 H), 7.57 (d, J = 8.0 Hz, 1 H), 7.24 (m, 4.5 H), 7.07 (d, J = 7.4 Hz, 0.5 H), 6.26 (d, J = 6.9 Hz, 1 H), 4.55 (m, 1 H), 4.04 (br s, 2 H), 3.60 (s, 3 H), 2.13 (s, 1.5 H), 2.00 (s, 1.5 H), 1.99 (m, 5 H), 1.80−1.88 (m, 1 H), 1.57−1.64 (m, 1 H).

The above hydrochloride salt was extracted with ethyl acetate and 5% aqueous ammonium hydroxide. After evaporation of solvents, a corresponding amine was obtained. This amine was dissolved in 4 mL of THF. To this solution were added *N*-tritylimidazol-4-ylaldehyde (1.25 equiv) and titanium isopropoxide (1.25 equiv). The mixture was stirred at room temperature for 1 h. This reaction mixture was diluted with 6 mL of anhydrous methanol, and then 2.0 mL of 0.43 N $NaBCNH_3$ (0.67 equiv) in THF was added dropwise. The mixture was stirred at room temperature for 4 h, and then solvents were evaporated. The residue was extracted with ethyl acetate and 5% ammonium hydroxide. The ethyl acetate layer was filtered to remove the white precipitate. The solvents were evaporated, and the residue was dissolved in 3 mL of methylene chloride. To this solution was added TFA (2 mL), and then triethylsilane was added dropwise until the deep brown color disappeared. The mixture was stirred at room temperature for 1 h. The solvents were evaporated, and the residue was dried under vacuum. The white residue was washed with ether. The white precipitate was filtered, dried, and dissolved in 1 mL of methanol and then 2 mL of 1 N NaOH was added. The mixture was stirred at room temperature for 30 min. The solvents were evaporated, and the residue was lyophilized. The crude mixture was purified by reverse-phase preparative HPLC (20% for four steps): ^{1}H NMR (CD_3OD) 8.66 (s, 1 H), 7.88−7.93 (m, 2 H), 7.80 (d, J = 7.9 Hz, 0.5 H), 7.68−7.75 (m, 1.5 H), 7.66 (s, 1 H), 7.46−7.62 (m, 4.5 H), 7.34−7.42 (m, 1.5 H), 4.45 (s, 2 H), 4.39 (s, 2 H), 4.00−4.11 (m, 1 H), 1.04−1.26 (m, 2 H), 0.59 (d, J = 6.3 Hz, 1.5 H), 0.51 (br s, 2 H), 0.43−0.50 (m, 0.5 H), 0.29−0.34 (m, 3 H); HRMS (FAB) m/z calcd for $C_{28}H_{30}N_4O_3 \cdot H^+$ 471.2396, found 471.2400.

4-[*N*-(1*H*-Imidazol-4-yl)methylaminomethyl]-2-(1-naphthyl)benzoylleucine Methyl Ester Trifluoroacetate (GGTI-2152). This compound was prepared by the same method as described in the preparation of GGTI-2151 except that the methyl ester was not hydrolyzed. The final product was purified by reverse-phase preparative HPLC (20%, three steps): ^{1}H NMR (CD_3OD) 8.54 (s, 1 H), 7.91 (br d, J = 5.2 Hz, 2 H), 7.69−7.79 (m, 2 H), 7.38−7.58 (m, 7 H), 4.40 (br s, 4 H), 4.03−4.14 (m, 1 H), 3.59 (s, 1.3 H), 3.54 (s, 1.7 H), 1.11−1.21 (m, 2 H), 0.60 (m, 1.7 H), 0.52 (br s, 2.3 H), 0.31−0.33 (m, 3 H); HRMS (FAB) m/z calcd for $C_{29}H_{32}N_4O_3 \cdot H^+$ 485.2553, found 485.2551.

Synthesis of GGTI-2157 (13) and GGTI-2158 (14). Methyl 4-Methoxy-2-phenylbenzoate. Methyl 2-hydroxy-4-methoxybenzoate (11.0 g, 60.4 mmol) was dissolved in 30 mL of pyridine. To this solution was slowly added trifluoromethanesulfonic anhydride (20 g, 70.8 mmol) at 0 °C. The mixture was stirred at room temperature for 20 h. After evaporation of pyridine, the residue was extracted with ether and water. The ether solution was washed with 1 N HCl. After evaporation of solvent, the residue was purified by flash column chromatography to give the desired triflate (16.5 g, 87%). GC/MS showed this compound was pure (m/z = 314): ^{1}H NMR ($CDCl_3$) 8.06 (d, J = 8.8 Hz, 1 H), 6.94 (d, J = 8.8 Hz, 1 H), 6.77 (s, 1 H), 3.93 (s, 3 H), 3.88 (s, 3 H).

This aryltriflate (5.02 g, 16.0 mmol) was reacted with phenylboronic acid in DMF in the presence of $Pd(PPh_3)_4$ (0.03 equiv) and potassium carbonate (6.62 g, 3.0 equiv). After stirring at 100 °C for 7 h, the mixture was worked up and purified by flash column chromatography to give methyl 4-methoxy-2-phenylbenzoate (3.53 g, 91%). GC/MS showed the compound was pure (m/z = 242): ^{1}H NMR ($CDCl_3$) 7.90 (d, J = 8.7 Hz, 1 H), 7.30−7.43 (m, 5 H), 6.92 (d, J = 8.7 Hz, 1 H), 6.86 (s, 1 H), 3.86 (s, 3 H), 3.63 (s, 3 H); ^{13}C NMR ($CDCl_3$) 167.4, 161.2, 144.7, 141.1, 131.8, 127.8, 127.3, 126.6, 122.0, 115.8, 111.8, 54.6, 50.8.

Methyl 4-(2-Bromoethoxy)-2-phenylbenzoate. $AlCl_3$ (4.0 g, 30 mmol) was dissolved in 6 mL of methylene chloride and 9.5 mL of EtSH at 0 °C. To this solution was added methyl 4-methoxy-2-phenylbenzoate (2.42 g, 10 mmol) in 10 mL of methylene chloride. The mixture was stirred at 0 °C for 2 h and at room temperature for 30 min. This mixture was poured into ice water and neutralized with 3 N aqueous HCl. The mixture was extracted with ethyl acetate. After evaporation of solvents, a pale pink oil was obtained (2.20 g). This material was pure as shown by TLC. The oily compound (2.20 g, 10 mmol) was dissolved in 60 mL of acetone. To this solution were added 1,2-dibromoethane (7.52 g, 40 mmol) and potassium carbonate (5.52 g, 40 mmol). The mixture was refluxed for 2 days. After workup, the crude material was purified by flash column chromatography (12% ethyl acetate in hexane) to give methyl 4-(2-bromoethoxy)-2-phenylbenzoate as a pale yellow oil (1.69 g, 52%): ^{1}H NMR ($CDCl_3$) 7.89 (d, J = 8.7 Hz, 1 H), 7.36−7.40 (m, 3 H), 7.26−7.30 (m, 2 H), 6.92 (d, J = 8.7 Hz, 1 H), 6.86 (s, 1 H), 4.35 (t, J = 6.2 Hz, 2 H), 3.66 (t, J = 6.2 Hz, 2 H), 3.62 (s, 3 H); ^{13}C NMR ($CDCl_3$) 167.9, 160.1, 145.3, 141.3, 132.3, 128.1, 127.8, 127.2, 123.2, 116.8, 113.0, 67.8, 51.6, 28.6.

Methyl 4-[2-(Imidazol-1-yl)ethoxy]-2-phenylbenzoate. The above bromoethyl derivative (1.62 g, 4.83 mmol) was dissolved in 20 mL of THF. To this solution were added imidazole (656 mg, 9.64 mmol) and triethylamine (1.38 mL, 10 mmol). The mixture was refluxed overnight. After evaporation of solvents, the crude material was purified by flash column chromatography (7% methanol in methylene chloride) to give unreacted starting material (530 mg) and the desired compound as a colorless oil (748 mg, 71%): ^{1}H NMR ($CDCl_3$) 7.87 (d, J = 8.6 Hz, 1 H), 7.59 (s, 1 H), 7.35−7.42 (m, 3 H), 7.26−7.28 (m, 2 H), 7.05 (d, J = 12 Hz, 2 H), 6.87 (d, J = 8.6 Hz, 1 H), 6.81 (s, 1 H), 4.36 (t, J = 5.0 Hz, 2 H), 4.27 (t, J = 4.9 Hz, 2 H), 3.61 (s, 3 H); ^{13}C NMR ($CDCl_3$) 167.4, 159.6, 144.8, 140.7, 137.0, 131.9, 128.9, 127.7, 127.4, 126.8, 122.8, 118.9, 116.3, 112.4, 66.8, 51.2, 45.6.

4-[2-(Imidazol-1-yl)ethoxy]-2-phenylbenzoylleucine Methyl Ester (GGTI-2158). The above ester (2.29 mmol) was dissolved in 5 mL of methanol. To this solution was added 4.5 mL of 1 N NaOH solution, and the mixture was refluxed for 5 h. The solution was filtered, and the filtrate was acidified with 1 N aqueous HCl to pH 4.2. After cooling in an ice bath, the carboxylic acid was collected and coupled with L-leucine

methyl ester in methylene chloride using EDCI and HOBT. After workup with sodium bicarbonate solution, the residue was purified by flash column chromatography (6% methanol in methylene chloride) to give GGTI-2158 as a white foam (93%): ^{1}H NMR ($CDCl_3$) 7.72 (d, J = 8.6 Hz, 1 H), 7.57 (br s, 1 H), 7.33−7.43 (m, 5 H), 7.02 (br s, 2 H), 6.86 (d, J = 8.6 Hz, 1 H), 6.75 (s, 1 H), 5.64 (d, J = 7.7 Hz, 1 H), 4.47 (ddd, J = 3.1, 5.1, and 7.7 Hz, 1 H), 4.32 (t, J = 4.9 Hz, 2 H), 4.23 (t, J = 4.8 Hz, 2 H), 3.61 (s, 3 H), 1.28−1.38 (m, 1 H), 1.07−1.18 (m, 2 H), 0.76 (t, J = 6.4 Hz, 6 H); ^{13}C NMR ($CDCl_3$) 172.7, 168.1, 158.6, 141.5, 139.7, 137.1, 130.5, 128.9, 128.2, 127.9, 127.5, 119.1, 115.8, 112.7, 67.0, 51.7, 50.7, 45.9, 40.5, 24.0, 22.4, 21.4; HRMS (FAB) m/z calcd for $C_{25}H_{29}N_3O_4 \cdot H^+$ 436.2236, found 436.2230.

4-[2-(Imidazol-1-yl)ethoxy]-2-phenylbenzoylleucine Trifluoroacetate (GGTI-2157). This compound was prepared from the hydrolysis of GGTI-2158. The final product was purified by preparative HPLC (85%): ^{1}H NMR (CD_3OD) 9.07 (s, 1 H), 7.75 (s, 1 H), 7.58 (s, 1 H), 7.48 (d, J = 8.4 Hz, 1 H), 7.34−7.39 (m, 5 H), 6.99 (d, J = 8.5 Hz, 1 H), 6.94 (s, 1 H), 4.69 (t, J = 4.6 Hz, 2 H), 4.45 (t, J = 4.6 Hz, 2 H), 4.33 (t, J = 7.4 Hz, 1 H), 1.46 (t, J = 7.1 Hz, 2 H), 1.15−1.24 (m, 1 H), 0.80 (dd, J = 6.5 Hz, 6 H); HRMS (FAB) m/z calcd for $C_{24}H_{27}N_3O_4 \cdot H^+$ 422.2080, found 422.2082.

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(18) The exceptions were ethers **13** and **14** and aminomethylenes **11** and **12** which were prepared from the corresponding 4-hydroxy- and 4-cyanobenzoic acid derivatives, respectively.

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