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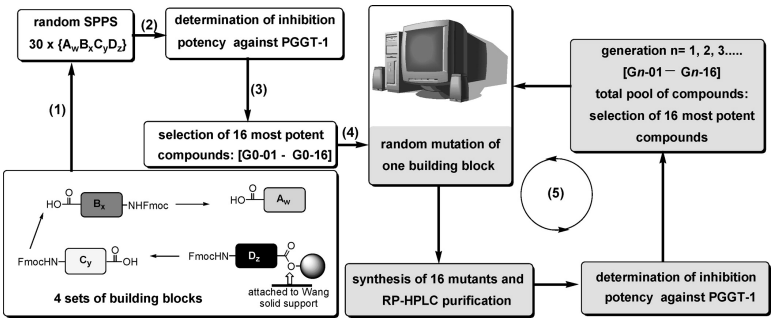

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A Combinatorial Approach toward the Generation of Ambiphilic Peptide-Based Inhibitors of Protein:Geranylgeranyl Transferase-1

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A combinatorial synthesis of oligopeptide analogues and their evaluation as protein:geranylgeranyl transferase inhibitors is presented. The combinatorial strategy is based on the random mutation, in each new generation, of one of any of the four amino acid building blocks of which the most effective compounds of the previous generation are assembled. In this way, a progressive improvement of the average inhibitory activity was observed until the fifth generation. The most active inhibitors were found to inhibit PGGT-1 in the low micromolar range (IC_{50}: 3.8−8.1 μM).

Protein isoprenylation, or the posttranslational modification of specific cysteine residues in nascent proteins with either a farnesyl group or a geranylgeranyl group, is a key event in the regulation of many biological processes.[1] Of particular interest is the finding that isoprenylation of pro-Ras proteins,[2] small GTPases that are instrumental in triggering many signal transducing pathways, is a prerequisite for their functioning. Oncogenic Ras, with the intrinsic GTPase activity impaired,[3] are found in at least 40% of human tumors, and it is for this reason that many research laboratories, in academia and industry alike, have focused on the development of compounds that can interfere with Ras isoprenylation.[4]

The natural isoprenyl group found on Ras proteins is the farnesyl lipid, transferred from farnesyl pyrophosphate (FPP) to consensus cysteine residues through the action of the enzyme protein:farnesyl transferase (PFT).[5] As a consequence, most research activities to date have focused on the development of PFT inhibitors.[6] However, the enzyme protein:geranylgeranyl transferase-1 (PGGT-1)[7] has emerged as an important alternative target for several reasons. First, there is the observation that upon blocking PFT, *N*-Ras and the most abundant human oncogenic Ras protein *K*-RasB are geranylgeranylated through the action of PGGT-1.[8] This indicates that blocking the action of PGGT-1, next to PFT, may prove equally important in the development of antitumor agents aimed at disabling Ras functioning.[8e,9] In addition, PGGT-1 inhibitors have been shown to be potential valuable agents for the treatment of smooth muscle hyperplasia,[10a] multiple sclerosis,[10b] parasitic infections,[10cd] osteoporosis,[10ef] atherosclerosis/restenosis,[10gh] and hepatitis C virus infection.[10i]

In this framework, we have recently embarked on a program aimed at the development and evaluation of potential PGGT-1 inhibitors.[11] In our search for an alternative class of compounds that could inhibit PGGT-1, we noted that (1) the action of PGGT-1 is highly reminiscent of that of PFT[7] and (2) effective ambiphilic peptidic PFT inhibitors, having a polar head, that are assembled from simple building blocks connected through amide bonds have been reported.[12] These observations led us to design a combinatorial strategy aimed at the generation of ambiphilic oligopeptides as potential PGGT-1 inhibitors,[13] based on the use of commercially available building blocks. Our strategy, which further includes a random optimization item,[14] can be summarized as follows (Schemes 1 and 2). An initial pool of 30 ambiphilic oligopeptides is assembled by standard Fmoc-based SPPS in a parallel fashion, from four sets of building blocks A−D (Figure 1). After release from the solid support and purification, the oligopeptides are screened for their propensity to inhibit PGGT-1, after which the 16 most potent compounds are selected. In the next round, in each of the 16 oligopeptides, one arbitrarily chosen building block is replaced by a new randomly chosen building block (Scheme 2). The resulting 16 mutant compounds are then synthesized and assayed, after which the 16 most active compounds from both generations are selected and the procedure is repeated.

The construction of the initial pool of 30 ambiphilic peptides entails the random selection of a diverse set of ABCD combinations, affording compounds with a polar headgroup (C-terminal carboxyl group) and a hydrophobic tail. The hydrophobic N-terminal and hydrophilic C-terminal subunits (A_w and D_z, respectively) were selected for this purpose. Mainly aromatic building blocks were selected for the hydrophobic N-terminal part (A_w set). Next to acidic

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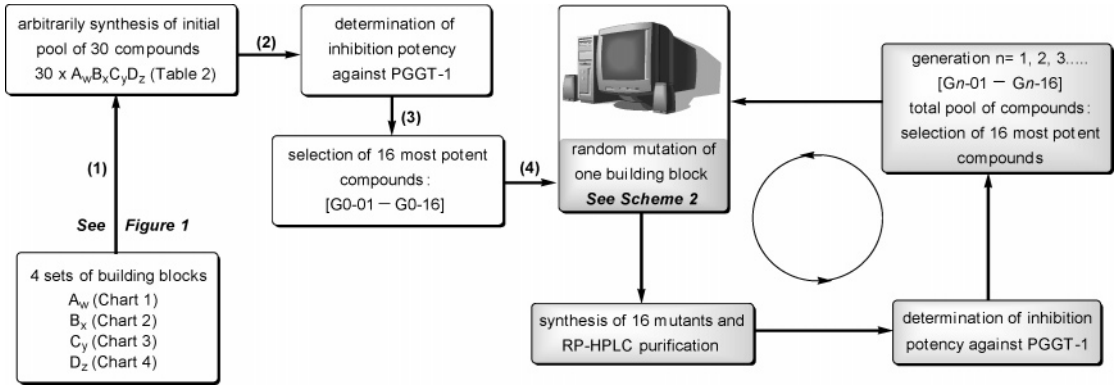
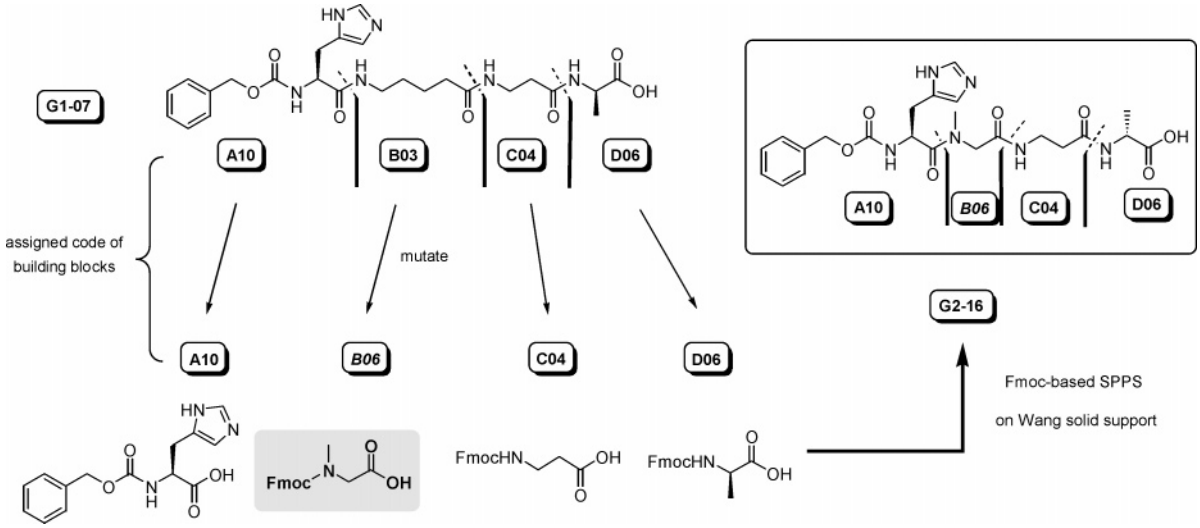
Scheme 1. Schematic Presentation of the Followed Optimization Procedure



Scheme 2. Schematic Example of One Building Block Mutation Procedure[a]



[a] G1-07: compound from generation 1 (G1) ranked #7 according to inhibitory potency.

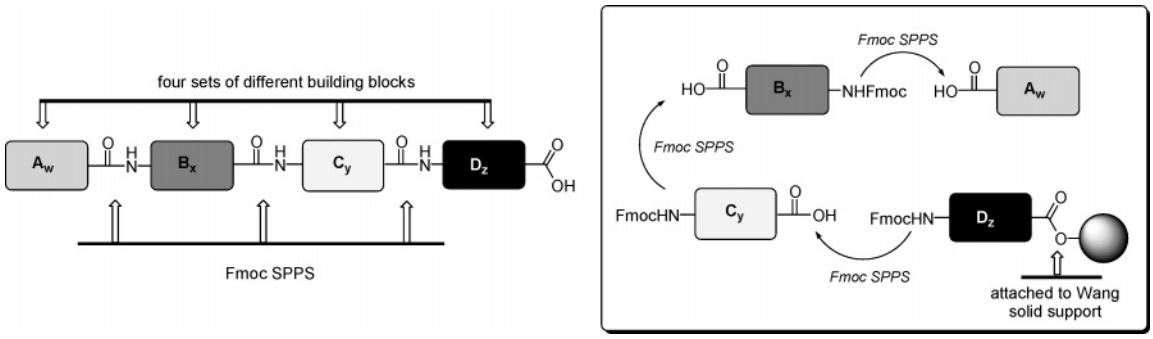


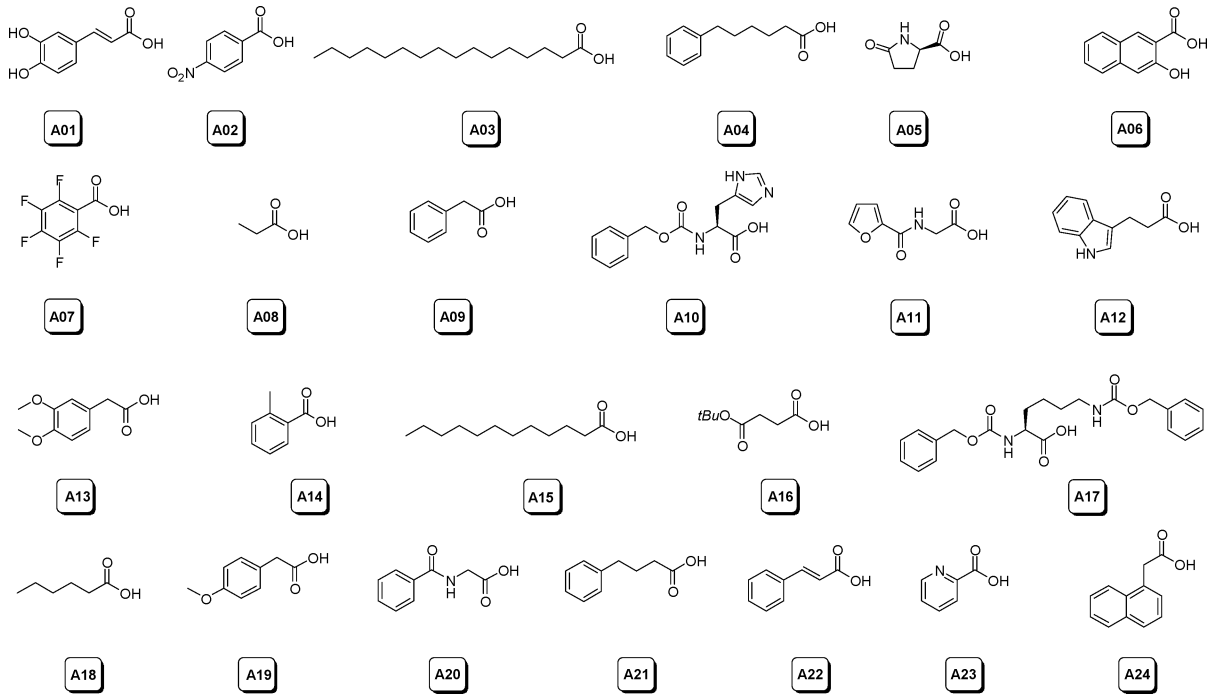
Figure 1. Ambiphilic peptides as potential PGGT-1 inhibitors.

residues, some neutral and basic residues were included in the D pool (Chart 4). Twenty-three building blocks make up set A_w (Chart 1), and 18 building blocks make up set D_z (Chart 4). On the basis of the assumption that the length of the hydrophobic tail is important with regard to inhibitory potency,[7] spacer molecules B/C that vary in length and conformational restriction were selected. In addition, by allowing the option to omit one or both spacer molecules (empty position B01 and C01), an additional possibility to vary the length of the target compounds was introduced.[15] Twenty-one building blocks make up set B_x (Chart 2) and 24 building blocks make up set C_y (Chart 3).

The efficacy of the iterative optimization procedure was evaluated by calculation of the average inhibitory percentage of the 16 best inhibitors of each generation. As can be seen in Figure 2, the average inhibitory percentage increases gradually in the first few optimization rounds. Already in the second generation (Table 1), compound **A03B02C14D16** (Scheme 3) is found to inhibit PGGT-1 for ~95% at 100 μM concentration. After five generations (Table 1), no significant improvement is observed.

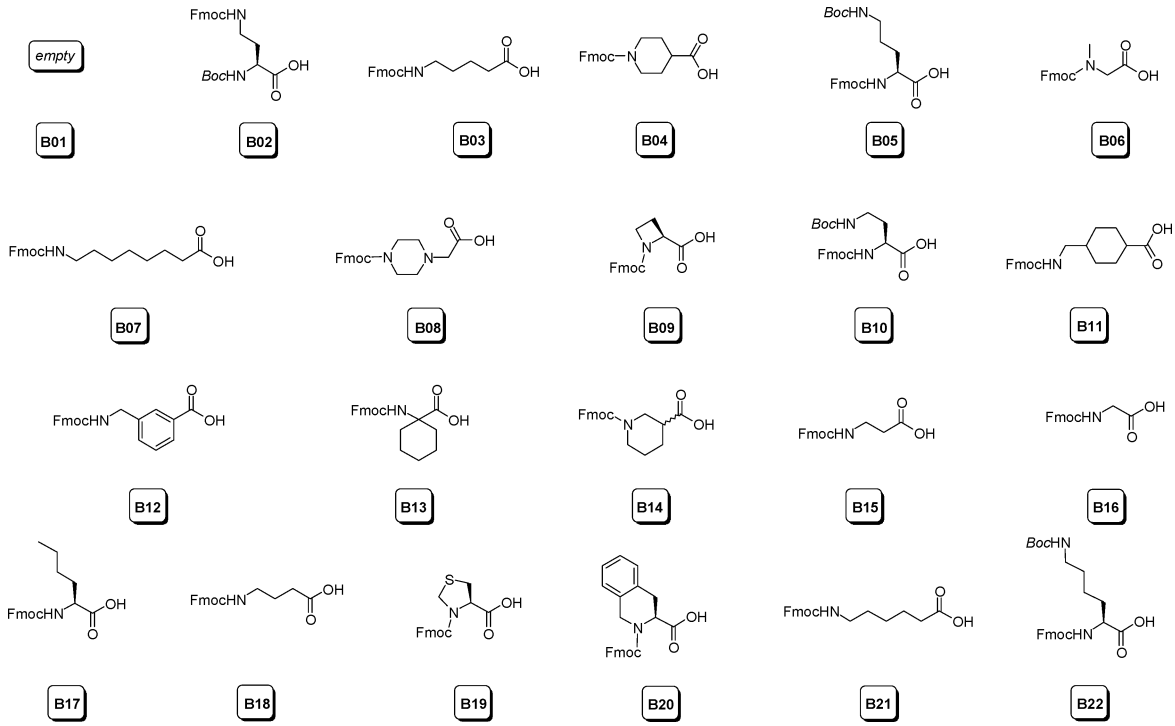
A different ranking of the 16 best inhibitors of generation 5 is obtained by looking at the percentage of inhibition at the 10 μM concentration data points (Table 1). The slightly more potent inhibitor **A03B10C14D16** (Scheme 3) now holds first place in this ranking, with 97% inhibition of enzyme activity, with **A03B02C14D16** being second at 81% inhibition of PGGT-1 activity. The IC_{50} values for these two

Chart 1. Set of A Building Blocks (A01−A24)[a]



[a] Protective groups which are removed during the TFA mediated release of the product from the solid support are depicted in italic form.

Chart 2. Set of B Building Blocks (B01−B22)[a]

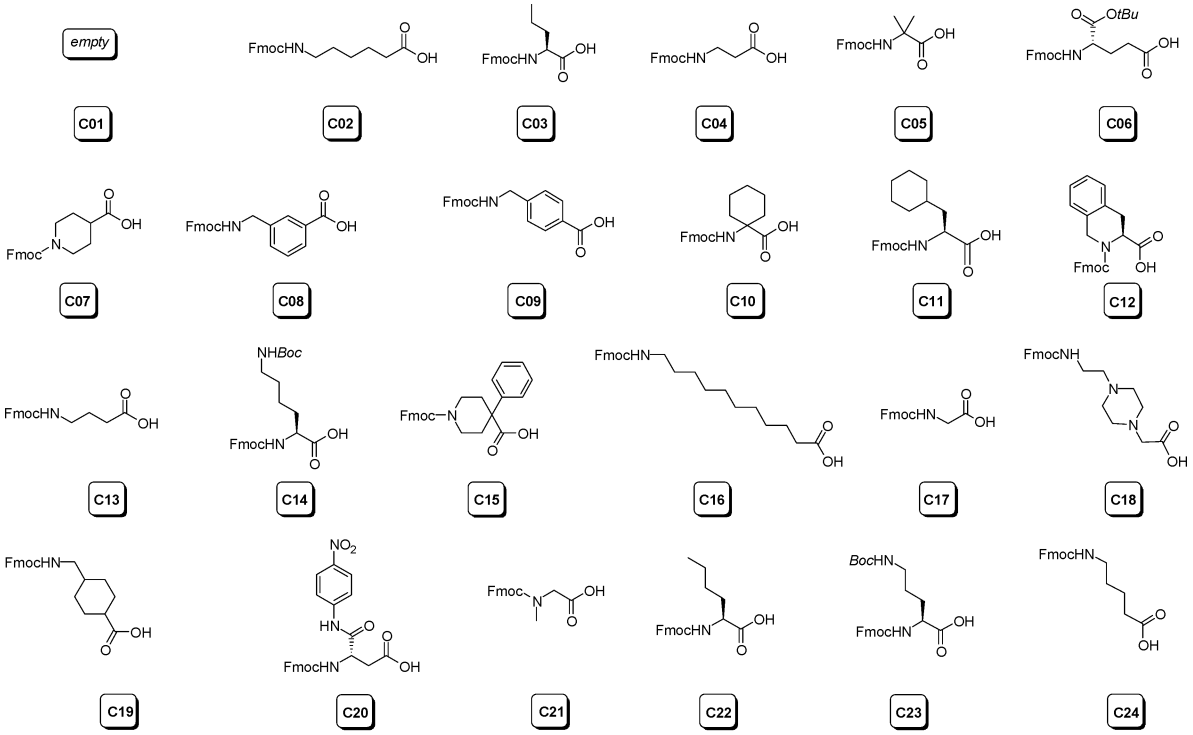


[a] Protective groups which are removed during the TFA mediated release of the product from the solid support are depicted in italic form.

most effective PGGT-1 inhibitors were 8.1 ± 1.2 and 3.8 ± 0.9 μM, respectively.[16] Scheme 3 depicts the mutational pathway to these two compounds.

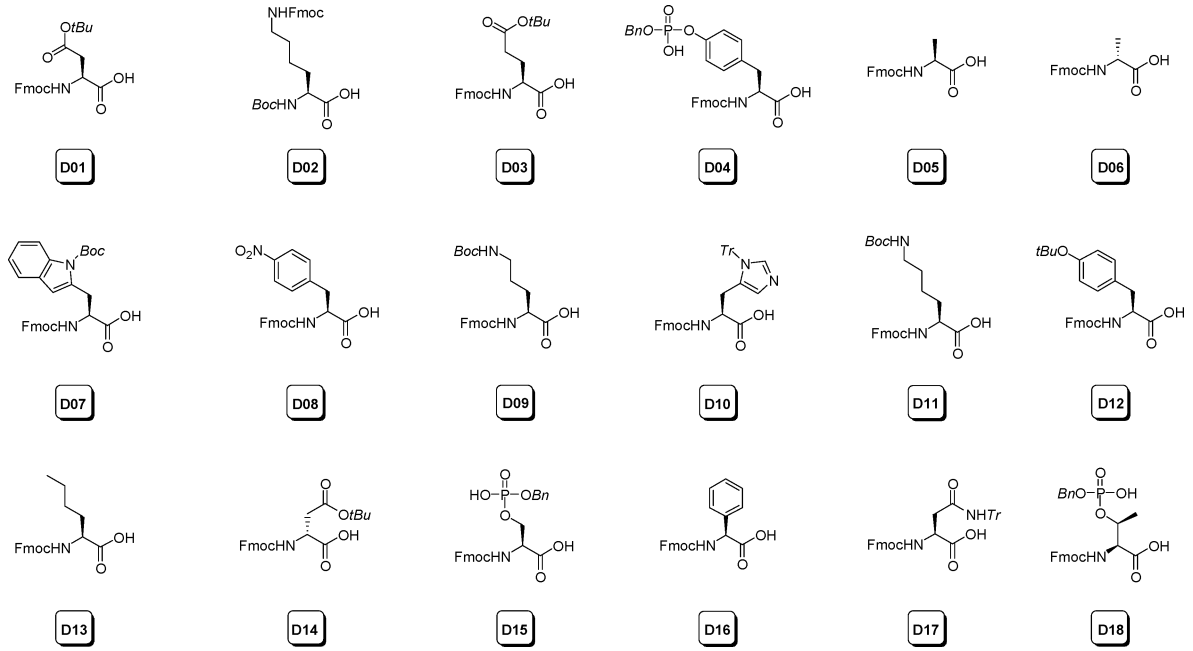
In conclusion, we have demonstrated that, using standard Fmoc-based SPPS and using commercially available building blocks, effective PGGT-1 inhibitors with IC_{50} values in the low micromolar range can be readily obtained. Obviously, it cannot be excluded that more potent inhibitors can be assembled from the four sets of building blocks; however, we feel that our random mutation strategy enables the facile identification of the potency range enclosed within a given set of combinatorial building blocks. Furthermore, our strategy may have impact both on the generation of potential PGGT-1 inhibitors and on the rapid identification of bioactive compounds, assembled from building blocks from combinatorial pools, and directed against biological targets of an altogether different nature. Current research activities are focused on the elucidation of the precise mode of action of

Chart 3. Set of C Building Blocks (C01−C24)[a]



[a] Protective groups which are removed during the TFA mediated release of the product from the solid support are depicted in italic form.

Chart 4. Set of D Building Blocks (D01−D18)[a]



[a] Protective groups which are removed during the TFA-mediated release of the product from the solid support are depicted in italic form.

the inhibitory potential of the here-presented oligopeptide-based PGGT-1 inhibitors.[16]

Experimental Section

General. ^{1}H NMR and ^{13}C NMR spectra were recorded with a Bruker Avance-400 (^{1}H = 400 MHz, ^{13}C = 100 MHz) or a Bruker DMX-600 (^{1}H = 600 MHz, ^{13}C = 150 MHz). Chemical shifts are given in parts per million (δ) relative to tetramethylsilane as internal standard (δ = 0 ppm). Mass spectra were recorded with a Perkin-Elmer/SCIEX API 165 mass instrument, and HR-MS spectra were recorded with an API QSTAR Pulsar (Applied Biosystems). Reversed-phase HPLC analysis was performed on a Jasco HPLC system (detection simultaneously at 214 and 254 nm) equipped with an Alltima C18 100-Å, 5-μm column (4.6 × 150 mm). Purifications were performed on a BioCad Vision (Applied Biosystems) HPLC system equipped with an Alltima C18 100-Å, 5-μm column (10 × 150 mm). The

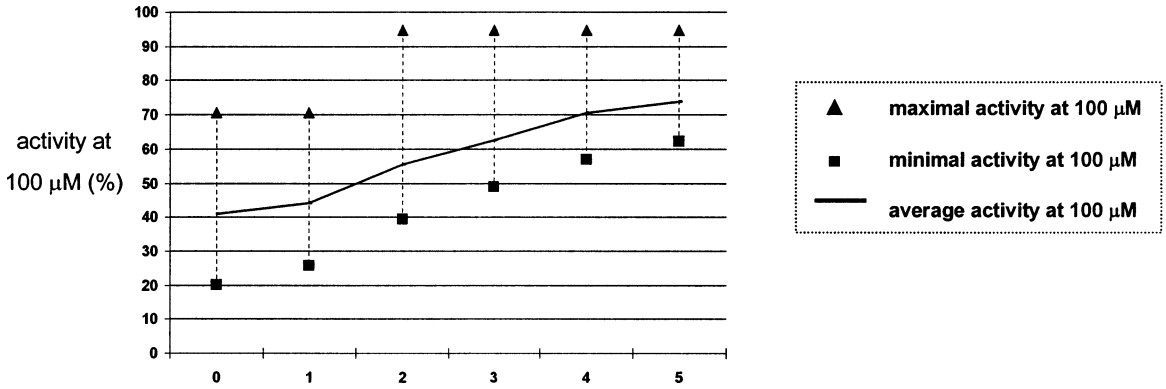


Figure 2. Development of average inhibitory activity at 100 μM of compound, expressed as percent of control activity, of the 16 best inhibitors per generation. ▲, inhibitory percentage value of best inhibitor; ■, inhibitory percentage value of worst inhibitor.

applied buffer system was A, H_2O; B, CH_3CN; and C, 1% aq TFA (effective 0.1%). In the case of compounds containing building blocks **D04**, **D15**, or **D18**, the best results were obtained by using A, H_2O; B, CH_3CN; and C, 0.1 M NH_4Ac (effective 0.01 M). All solvents were of HPLC quality (Biosolve). All employed building blocks (A−D, Charts 1−4) were purchased from commercial suppliers and were of the highest quality available. The solid-phase peptide synthesis (SPPS) was performed on a LaMOSS2 (Labotec Modular Organic Synthesis System 2) robotic synthesizer using standard Fmoc chemistry and Wang solid support (loading 0.5−1.1 mmol g^{-1}, NovaBiochem, 100−200 mesh, product no. 01-64-0014). Abbreviations used in this paper are as follows: BOP = benzotriazole-1-yloxytri(dimethylamino)phosphonium hexafluorophosphate; DCM = dichloromethane; DIC = *N*,*N*′-diisopropylcarbodiimide; DIPEA = *N*,*N*-diisopropylethylamine; DMAP = 4-(dimethylamino)pyridine; DMF = *N*,*N*-dimethylformamide; DMSO = dimethyl sulfoxide; DTT = dithiotreitol; FPP = farnesyl pyrophosphate; GGPP = geranylgeranyl pyrophosphate; GTP = guanosine triphosphate; HOBt = 1-hydroxybenzotriazole; NMP = *N*-methyl-2-pyrrolidinone; PFT = protein:farnesyl transferase; PGGT-1 = protein:geranylgeranyl transferase-1; SDS = sodium dodecyl sulfate; SPPS = solid-phase peptide synthesis; and TFA = trifluoroacetic acid.

General Procedure 1. Manual Coupling of Building Blocks D01, **D03**, **D04**, **D15 and D18.** A 1.0-g portion of Wang resin (0.81 mmol) was coevaporated 3× with anhydrous 1,4-dioxane (10 mL) and treated with a solution of the amino acid (2.0 equiv, 1.6 mmol) in DCM/DMF (3/1, v/v; c = 0.1−0.15 M), DIC (2.4 equiv, 1.9 mmol, 0.3 mL), and DMAP (0.04 equiv, 5 mg). After shaking the mixture under argon for 6 h, the resin was washed with DCM; DMF; MeOH; DCM; and, finally, Et_2O. A second coupling step was performed employing 1.0 equiv of amino acid, and this time, the reaction mixture was shaken for 16 h. Subsequently, the resin was washed (DCM and DMF), capped (0.5 M Ac_2O, 0.125 M DIPEA, and 0.015 M HOBt in NMP), washed (DMF, MeOH, DCM, and Et_2O), and dried in vacuo. The loading of the resin (0.3−0.5 mmol/g) was determined as follows: To 1−2 mg of resin in a volumetric flask (10 mL) was added a solution of piperidine/DMF (1/4, v/v, 1.0 mL), and the mixture was left for 15 min. The volume was adjusted to 10 mL by addition of EtOH (HPLC grade), and the UV absorption was measured at 300 nm. The loading could then be calculated using formula A with A_{300} = absorption at 300 nm (EtOH as reference), V = volume of sample (10 mL), and wt = weight of employed resin (1−2 mg).

$$\text{loading (mmol g}^{-1}) = \frac{A_{300} \times V}{7.8 \times \text{wt}} \qquad \text{(A)}$$

General Procedure 2. General Synthetic Protocol LaMOSS2 Robot. (1) Coupling Building Block D. Wang resin (50 μmol) was swelled with 2 × 2 mL DCM and treated with 5.0 equiv of building block D (0.25 M solution in NMP, 1.0 mL), 5.0 equiv DIC (0.5 mL, 0.5 M solution in DCM), and 0.25 equiv DMAP (0.5 mL, 0.025 M solution in NMP). The reaction mixture was flushed with N_2 for 3 h, after which the reagents were removed. This procedure was repeated; however, this time the reaction mixture was allowed to react for 16 h instead of 3 h. After washing with NMP (1 × 3 and 3 × 2 mL), the resin was capped with 2 mL of 0.5 M Ac_2O, 0.125 M DIPEA, and 0.015 M HOBt in NMP (2 × 5 min) and washed with NMP (1 × 3 and 3 × 2 mL).

(2) Removal Fmoc.[17] The resin was treated with 2 mL of 20% piperidine in NMP (4 × 2 min) and washed with NMP (1 × 3 and 3 × 2 mL).

(3) Coupling Building Block B and C. To the resin were added 5.0 equiv of a building block B or C (0.25 M solution in NMP, 1.0 mL), 5.0 equiv of BOP/HOBt (1/1, 0.5 mL, 0.5 M solution in NMP), and 10 equiv of DMAP (0.5 mL, 1.0 M solution in NMP). The reaction mixture was flushed with N_2 for 45 min, after which the reagents were removed. This coupling procedure was repeated in the case of building blocks which are known to be difficult to couple (e.g., **B06** or **C05**). The resin was washed (1 × 3 and 3 × 2 mL NMP); capped with 2 × 2 mL of 0.5 M Ac_2O, 0.125 M DIPEA, and 0.015 M HOBt in NMP; and washed (1 × 3 and 3 × 2 mL NMP).

(4) Coupling Building Block A. To the resin were added 5.0 equiv of a building block A (0.25 M solution in NMP, 1.0 mL), 5.0 equiv of BOP/HOBt (1/1, 0.5 mL, 0.5 M solution in DCM), and 10 equiv of DMAP (0.5 mL, 1.0 M solution in NMP). The reaction mixture was flushed with N_2 for 45 min, after which the reagents were removed. The

Table 1. Results of the One-Building Mutation Procedure for Generations 1−5

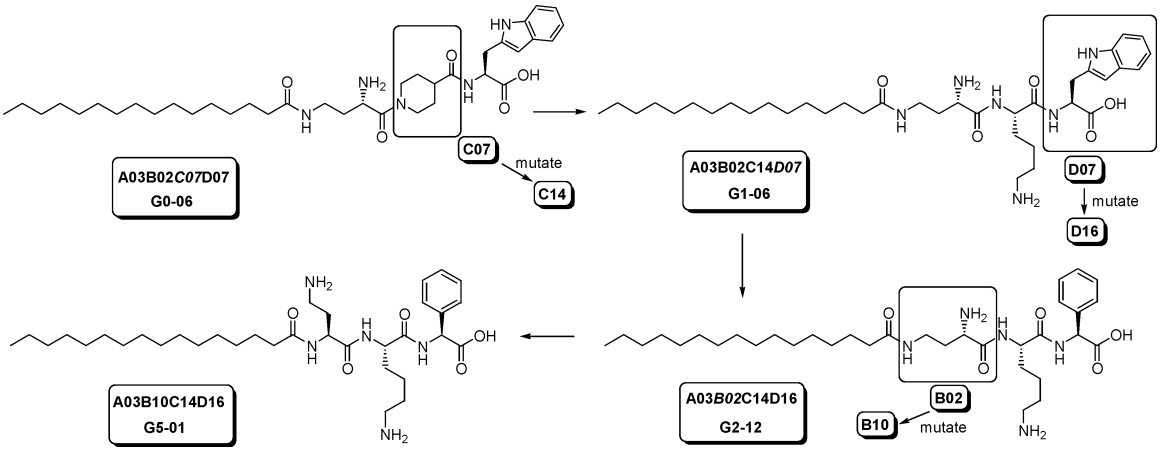
code	ABCD code	A[a] (%) at 100 μM		code	ABCD code	A[a] (%) at 100 μM
	Best 16 of Generation 0				Mutants (G1)	
G0-01	**A21B21C01D15**	71	→	G1-01	**A21B21C01*D09***	0
G0-02	**A04B07C05D11**	63	→	G1-02	**A04*B17*C05D11**	10
G0-03	**A19B12C01D15**	55	→	G1-03	**A19B12C01*D12***	24
G0-04	**A02B07C24D01**	50	→	G1-04	**A02*B17*C24D01**	0
G0-05	**A02B06C21D01**	49	→	G1-05	***A18*B06C21D01**	7
G0-06	**A03B02C07D07**	49	→	G1-06	**A03B02*C14*D07**	39
G0-07	**A10B03C04D15**	48	→	G1-07	**A10B03C04*D06***	26
G0-08	**A21B01C02D18**	41	→	G1-08	**A21*B22*C02D18**	0
G0-09	**A21B21C01D04**	40	→	G1-09	**A21B21*C24*D04**	48
G0-10	**A07B07C07D01**	30	→	G1-10	**A07B07C07*D08***	0
G0-11	**A16B11C21D11**	29	→	G1-11	**A16*B05*C21D11**	32
G0-12	**A07B11C24D05**	25	→	G1-12	**A07B11*C22*D05**	0
G0-13	**A04B03C05D11**	23	→	G1-13	**A04B03*C17*D11**	11
G0-14	**A15B03C10D01**	21	→	G1-14	**A15B03*C05*D01**	19
G0-15	**A10B04C05D03**	20	→	G1-15	**A10B04C05*D08***	41
G0-16	**A24B01C23D01**	20	→	G1-16	**A24B01C23*D08***	0
	Best 16 after 1 Generation				Mutants (G2)	
G0-01	**A21B21C01D15**	71	→	G2-01	**A21B21*C20*D15**	62
G0-02	**A04B07C05D11**	63	→	G2-02	**A04B07*C21*D11**	22
G0-03	**A19B12C01D15**	55	→	G2-03	**A19B12C01*D09***	0
G0-04	**A02B07C24D01**	50	→	G2-04	**A02B07*C12*D01**	22
G0-05	**A02B06C21D01**	49	→	G2-05	**A02B06*C15*D01**	29
G0-06	**A03B02C07D07**	49	→	G2-06	**A03B02*C12*D07**	53
G0-07	**A10B03C04D15**	48	→	G2-07	**A10*B13*C04D15**	0
G1-09	**A21B21C24D04**	48	→	G2-08	**A21*B02*C24D04**	0
G1-15	**A10B04C05D08**	41	→	G2-09	**A10*B14*C05D08**	75
G0-08	**A21B01C02D18**	41	→	G2-10	***A05*B01C02D18**	51
G0-09	**A21B21C01D04**	40	→	G2-11	**A21*B20*C01D04**	4
G1-06	**A03B02C14D07**	39	→	G2-12	**A03B02C14*D16***	95
G1-11	**A16B05C21D11**	32	→	G2-13	**A16B05*C03*D11**	0
G0-10	**A07B07C07D01**	30	→	G2-14	**A07B07*C10*D01**	18
G0-11	**A16B11C21D11**	29	→	G2-15	**A16B11*C09*D11**	0
G1-07	**A10B03C04D06**	26	→	G2-16	**A10*B06*C04D06**	0
	Best 16 after 2 Generations				Mutants (G3)	
G2-12	**A03B02C14D16**	95	→	G3-01	**A03*B08*C14D16**	57
G2-09	**A10B14C05D08**	75	→	G3-02	**A10*B11*C05D08**	20
G0-01	**A21B21C01D15**	71	→	G3-03	**A21*B11*C01D15**	0
G0-02	**A04B07C05D11**	63	→	G3-04	**A04B07C05*D01***	59
G2-01	**A21B21C20D15**	62	→	G3-05	**A21*B10*C20D15**	21
G0-03	**A19B12C01D15**	55	→	G3-06	**A19B12C01*D16***	44
G2-06	**A03B02C12D07**	53	→	G3-07	***A10*B02C12D07**	73
G2-10	**A05B01C02D18**	51	→	G3-08	**A05B01*C04*D18**	22
G0-04	**A02B07C24D01**	50	→	G3-09	**A02B07C24*D02***	0
G0-05	**A02B06C21D01**	49	→	G3-10	**A02B06C21*D18***	2
G0-06	**A03B02C07D07**	49	→	G3-11	**A03*B16*C07D07**	20
G0-07	**A10B03C04D15**	48	→	G3-12	**A10*B16*C04D15**	62
G1-09	**A21B21C24D04**	48	→	G3-13	**A21B21C24*D02***	0
G1-15	**A10B04C05D08**	41	→	G3-14	***A07*B04C05D08**	0
G0-08	**A21B01C02D18**	41	→	G3-15	**A21B01C02*D10***	65
G0-09	**A21B21C01D04**	40	→	G3-16	**A21B21*C12*D20**	62
	Best 16 after 3 Generations				Mutants (G4)	
G2-12	**A03B02C14D16**	95	→	G4-01	**A03*B13*C14D16**	0
G2-09	**A10B14C05D08**	75	→	G4-02	**A10B14*C04*D08**	73
G3-07	**A10B02C12D07**	73	→	G4-03	**A10B02*C07*D07**	41
G0-01	**A21B21C01D15**	71	→	G4-04	**A21*B20*C01D15**	71
G3-15	**A21B01C02D10**	65	→	G4-05	**A21*B05*C02D10**	0
G0-02	**A04B07C05D11**	63	→	G4-06	***A24*B07C05D11**	7
G2-01	**A21B21C20D15**	62	→	G4-07	***A13*B21C20D15**	87
G3-12	**A10B16C04D15**	62	→	G4-08	**A10*B14*C04D15**	87
G3-16	**A21B21C12D04**	62	→	G4-09	***A20*B21C12D04**	0
G3-04	**A04B07C05D01**	59	→	G4-10	***A22*B07C05D01**	0
G3-01	**A03B08C14D16**	57	→	G4-11	**A03*B22*C14D16**	0
G0-03	**A19B12C01D15**	55	→	G4-12	**A19B12*C16*D15**	67
G2-06	**A03B02C12D07**	53	→	G4-13	**A03B02C12*D03***	26
G2-10	**A05B01C02D18**	51	→	G4-14	**A05B01*C16*D18**	0
G0-04	**A02B07C24D01**	50	→	G4-15	**A02B07*C06*D01**	0
G0-05	**A02B06C21D01**	49	→	G4-16	**A02*B19*C21D01**	21

Table 1 (Continued)

code	ABCD code	A (%) at 100 μM		code	ABCD code	A (%) at 100 μM	code	ABCD code	A (%) at 100 μM	A(%) at 10 μM
Best 16 after 4 Generations					Mutants (G5)		Best 16 after 5 Generations			
G2-12	**A03B02C14D16**	95	→	G5-01	**A03*B10*C14D16**	68	G2-12	**A03B02C14D16**	95	81
G4-07	**A13B21C20D15**	87	→	G5-02	***A17*B21C20D15**	72	G4-07	**A13B21C20D15**	87	53
G4-08	**A10B14C04D15**	87	→	G5-03	**A10B14C04*D09***	0	G4-08	**A10B14C04D15**	87	26
G2-09	**A10B14C05D08**	75	→	G5-04	**A10B14*C15*D08**	73	G5-07	**A02B20C01D15**	80	60
G3-07	**A10B02C12D07**	73	→	G5-05	***A09*B02C12D07**	58	G2-09	**A10B14C05D08**	75	37
G4-02	**A10B14C04D08**	73	→	G5-06	**A10*B10*C04D08**	1	G5-04	**A10B14C15D08**	73	0
G4-04	**A21B20C01D15**	71	→	G5-07	***A02*B20C01D15**	80	G3-07	**A10B02C12D07**	73	22
G0-01	**A21B21C01D15**	71	→	G5-08	**A21*B10*C01D15**	48	G4-02	**A10B14C04D08**	73	26
G4-12	**A19B12C16D15**	67	→	G5-09	**A19*B04*C16D15**	50	G5-02	**A17B21C20D15**	72	64
G3-15	**A21B01C02D10**	65	→	G5-10	**A21B01*C03*D10**	17	G4-04	**A21B20C01D15**	71	39
G0-02	**A04B07C05D11**	63	→	G5-11	**A04B07*C19*D11**	10	G0-01	**A21B21C01D15**	71	40
G2-01	**A21B21C20D15**	62	→	G5-12	**A21B21*C04*D15**	43	G5-01	**A03B10C14D16**	68	97
G3-12	**A10B16C04D15**	62	→	G5-13	**A10B16*C18*D15**	35	G4-12	**A19B12C16D15**	67	32
G3-16	**A21B21C12D04**	62	→	G5-14	**A21*B05*C12D04**	54	G3-15	**A21B01C02D10**	65	18
G3-04	**A04B07C05D01**	59	→	G5-15	**A04B07C05*D07***	0	G0-02	**A04B07C05D11**	63	40
G3-01	**A03B08C14D16**	57	→	G5-16	***A20*B08C14D16**	16	G2-01	**A21B21C20D15**	62	29

[a] A = activity of enzyme (PGGT-1) at 100 or 10 μM of compound, expressed as percent of control activity.

Scheme 3. Mutational Development of **A03B02C14D16** (G2-12) and **A03B10C14D16** (G5-01)



resin was washed (1 × 3 and 3 × 2 mL NMP), capped (2 × 2 mL of 0.5 M Ac_2O/0.125 M DIPEA/0.015 M HOBt in NMP), and washed (2 mL of DCM; 2 mL of MeOH (3 ×); 1 × 3 mL and 3 × 2 mL of DCM).

(5) Cleavage from Resin. To the resin was added 3 mL of TFA/H_2O/^{i}Pr_3SiH (95/4/1, v/v/v) under N_2 flushing. After 2 h, the TFA solution was collected in a tube, and the resin was rinsed with TFA/H_2O/^{i}Pr_3SiH (95/4/1, v/v/v, 2 × 2 mL).

(6) Workup Procedure. The filtrate is concentrated in vacuo, dissolved in 4 mL of H_2O/CH_3CN/^{t}BuOH (1/1/1, v/v/v), analyzed by LC/MS, and purified by RP-HPLC (Tables 3−5 list LC/MS data for compounds of generations 1−5).

Spectroscopic and Spectrometric Data of Compounds Representative for the Synthesized Library. A03B02C14D16 (G2-12). ^{1}H NMR (400 MHz, DMSO-d_6): 8.86 (d, 1H, J = 7.6 Hz), 8.73 (m, 1H), 8.03 (d, J = 4.0 Hz), 7.81 (m), 7.36 (m, 5H), 7.07 (d, 1H, J = 8.0 Hz), 6.96 (d, 1H, J = 8.4 Hz), 6.82 (d, 1H, J = 6.8 Hz), 6.62 (d, 1H, J = 8.4 Hz), 5.36 (d, 1H, J = 7.6 Hz), 5.29 (d, 1H, J = 7.2 Hz), 4.47 (m, 1H), 4.16−3.96 (m, 6H), 3.72 (m, 1H), 3.32 and 3.12 (2 × s), 2.76−2.67 (2 × m), 2.49 (s), 2.06 (dd, 2H, J = 6.8 and 7.2 Hz), 1.76 (m, 3H), 1.56 and 1.50 (2 × m), 1.22 (bs), 1.10 (s), 0.86 (apparent t, 3H, J = 6.0 and 6.8 Hz). Purity >95%, 20.4 mg (66% yield). LC/MS analysis: t_R = 12.7 min (linear gradient B 05 → 90%, 26 min), (ESI) m/z 618.6 $(M + H)^+$. HR-MS: calcd for $[C_{34}H_{59}N_5O_5 + H]^+$, 618.45945; found, 618.45972.

A03B10C14D16 (G5-01). ^{1}H NMR (400 MHz, DMSO-d_6): 8.80 (d, 1H, J = 8.0 Hz), 8.66 (d, 1H, J = 7.2 Hz), 8.07 (m, 4H), 7.81 (m), 7.35 (m, 5H), 7.07 (d, 1H, J = 8.0 Hz), 6.96 (d, 1H), 6.82 (d, 1H, J = 7.2 Hz), 6.64 (d, 1H, J = 8.4 Hz), 5.35 (d, 1H, J = 8.0 Hz), 5.27 (d, 1H, J = 7.2 Hz), 4.37 (m, 2H), 4.17−3.96 (m, 3H), 3.34 and 3.16 (2 × s), 2.88−2.67 (m, 4H), 2.49 (s), 2.09 (m, 2H), 1.90−1.71 (m, 3H), 1.54−1.36 (m), 1.22 (bs), 1.10 (s), 0.84 (apparent t, 3H, J = 6.0 and 6.8 Hz). Purity >95%, 23.5 mg, (76% yield). LC/MS analysis: t_R = 17.5 min (linear gradient B 5 → 90%, 26 min), (ESI) m/z 618.6 $(M + H)^+$. HR-MS: calcd for $[C_{34}H_{59}N_5O_5 + H]^+$, 618.45945; found, 618.45953.

A10B11C05D08 (G3-02). ^{1}H NMR (400 MHz, DMSO-d_6): 8.93 (s), 8.10 (d, J = 8.4 Hz), 7.94 (apparent t, J = 5.6 and 6.0 Hz), 7.71 (s), 7.60 (dd, J = 8.0 and 8.4 Hz), 7.47 (d, J = 8.8 Hz), 7.38−7.28 (m), 5.00 (2 × d, 2H, J = 12.8 Hz), 4.47 (dt, J = 4.8, 8.0 and 8.4 Hz), 4.30 (m), 3.23 (dd, J = 4.4 and 4.8 Hz), 3.09 (m), 2.96−2.85 (m), 2.02 (apparent bt, J = 11.6 and 12.0 Hz), 1.63 (apparent bt, J = 14.4 and 15.2 Hz), 1.26 (s), 1.17 (m), 1.11 (s), 0.78 (dd, J = 12.4

Table 2. Initial Pool of Compounds and Their Inhibition Potency against PGGT-1[a]

ABCD code	A[b] (%) at 100 μM	ABCD code	A[b] (%) at 100 μM	ABCD code	A[b] (%) at 100 μM
A21B21C01D15	71	**A16B11C21D11**	29	**A24B21C04D18**	10
A04B07C05D11	63	**A07B11C24D05**	25	**A04B13C24D01**	9
A19B12C01D15	55	**A04B03C05D11**	23	**A04B03C19D11**	9
A02B07C24D01	50	**A15B03C10D01**	21	**A01B03C24D01**	4
A02B06C21D01	49	**A10B04C05D03**	20	**A21B12C01D03**	3
A03B02C07D07	49	**A24B01C23D01**	20	**A19B15C02D10**	0
A10B03C04D15	48	**A21B16C09D01**	19	**A15B02C21D01**	0
A21B01C02D18	41	**A21B03C24D01**	16	**A07B11C05D13**	0
A21B21C01D04	40	**A16B03C19D11**	16	**A12B03C10D03**	0
A07B07C07D01	30	**A10B02C24D03**	14	**A24B01C08D03**	0

[a] This set of 30 compounds was synthesized according to general procedure 2 (see building blocks, Charts 1−4). All compounds have been analyzed by LC/MS and purified by RP-HPLC (≥95% purity). [b] A = activity of enzyme at 100 μM of compound: expressed as percent of control activity (without test compound).

Table 3. LC/MS Data (t_R and $[M + H]^+$) of Compounds from Generations 1 and 2

compound	$[M + H]^+$	t_R (min)[a]	yield (%)[b]	compound	$[M + H]^+$	t_R (min)[c]	yield (%)[b]
A21B21C01D09	392.2	8.2	42	**A21B21C20D15**	680.4	10.2	1
A04B17C05D11	519.4	9.8	54	**A04B07C21D11**	533.4	12.2[a]	98
A19B12C01D12	463.2	9.0	56	**A19B12C01D09**	414.2	8.6	94
A02B17C24D01	495.3	10.2	89	**A02B07C12D01**	583.5	13.6	34
A18B06C21D01	374.1	7.9	28	**A02B06C15D01**	541.4	10.9	79
A03B02C14D07	671.8	13.8	38	**A03B02C12D07**	702.6	25.9[a]	22
A10B03C04D06	531.3	10.6	38	**A10B13C04D15**	853.6	6.9	1
A21B22C02D18	587.5	8.9	1	**A21B02C24D04**	607.4	8.0	35
A21B21C24D04	463.2	10.2	1	**A10B14C05D08**	678.4	11.5	60
A07B07C07D08	657.7	12.4	45	**A05B01C02D18**	424.1	2.3	28
A16B05C21D11	432.2	2.4	98	**A21B20C01D04**	567.2	9.3	14
A07B11C22D05	536.2	11.5	49	**A03B02C14D16**	618.6	12.7[a]	66
A04B03C17D11	477.3	8.5	42	**A16B05C03D11**	460.2	2.1	89
A15B03C05D01	500.4	20.5	68	**A07B07C10D01**	594.4	15.5	38
A10B04C05D08	678.3	8.4	48	**A16B11C09D11**	519.4	7.9	71
A24B01C23D08	493.3	8.8	43	**A10B06C04D06**	503.3	8.5	94

[a] Linear gradient B 05 → 90%, 26 min. [b] Nonoptimized yields. All compounds were ≥ 95% pure as determined by LC/MS. [c] Unless stated otherwise: linear gradient B 05 → 50%, 26 min.

Table 4. LC/MS Data (t_R and $[M + H]^+$) of Compounds from Generations 3 and 4[a]

compound	$[M + H]^+$	t_R (min)	yield (%)[b]	compound	$[M+H]^+$	t_R (min)	yield (%)[b]
A03B08C14D16	644.5	18.8	30	**A03B13C14D16**	643.3	22.6	98
A10B11C05D08	706.4	13.1	81	**A10B14C04D08**	664.2	12.2	98
A21B11C01D15	471.3	11.8	6	**A10B02C07D07**	687.5	11.2	60
A04B07C05D01	534.3	17.5	89	**A21B20C01D15**	491.2	13.0	70
A21B10C20D15	667.3	13.1	4	**A21B05C02D10**	529.5	9.9	98
A19B12C01D16	433.1	15.6	62	**A24B07C05D11**	541.3	12.7	98
A10B02C12D07	735.4	12.6	37	**A13B21C20D15**	712.5	11.1	6
A05B01C04D18	382.1	1.9	35	**A10B14C04D15**	639.2	8.8	31
A02B07C24D02	536.2	12.4	98	**A20B21C12D04**	695.4	11.7	98
A02B06C21D18	491.1	9.0	34	**A22B07C05D01**	490.2	13.9	98
A03B16C07D07	615.5	27.0	64	**A03B22C14D16**	646.5	16.7	54
A10B16C04D15	585.2	9.0	14	**A19B12C16D15**	650.3	12.0	13
A21B21C24D02	505.3	11.8	51	**A03B02C12D03**	645.4	20.1	80
A07B04C05D08	601.3	16.9	81	**A05B01C16D18**	494.2	10.4	15
A21B01C02D10	416.1	12.0	37	**A02B07C06D01**	553.3	12.6	98
A21B21C12D20	680.4	13.9	48	**A02B19C21D01**	469.1	11.6	37

[a] Linear gradient B 05 → 90%, 26 min. [b] Nonoptimized yields. All compounds were ≥95% pure as determined by LC/MS.

and 12.8 Hz). Purity >95%, 28.6 mg (81% yield). LC/MS analysis: t_R = 12.2 min (linear gradient B 05 → 90%, 26 min), (ESI) *m*/*z* 706.4 $(M + H)^+$.

A21B21C24D02 (G3-13). ^{1}H NMR (400 MHz, DMSO-d_6): 7.76 (m), 7.27 (m), 7.17 (m), 6.54 (s), 3.00 (m), 2.52 (m), 2.04 (m), 1.77 (m), 1.45 (m), 1.37 (m), 1.20 (m). Purity >95, 12.9 mg (51% yield). LC/MS analysis: t_R = 11.8 min (linear gradient B 05 → 90%, 26 min), (ESI) *m*/*z* 505.3 $(M + H)^+$.

A07B04C05D08 (G3-14). ^{1}H NMR (400 MHz, DMSO-d_6): 8.10 (dd, 2H, *J* = 1.6 and 2.0 Hz), 7.85 (d, 1H, *J* = 5.6 Hz), 7.62 (dd, 1H, *J* = 8.0 and 12.8 Hz), 7.47 (dd, 2H, *J* = 3.2 and 8.4 Hz), 4.50−4.39 (m, 2H), 3.53 (d, 1H, *J* = 13.6 Hz), 3.22 (m, 1H), 3.08 (m, 2H), 2.91 (m, 1H), 2.44 (m,

Table 5. LC/MS Data (t_R and $[M + H]^+$) and Yields of Compounds from Generation 5[a]

compound	$[M + H]^+$	t_R (min)	yield (%)[b]	compound	$[M + H]^+$	t_R (min)	yield (%)[b]
A03B10C14D16	618.6	17.5	76	**A19B04C16D15**	628.5	14.9	7
A17B21C20D15	930.5	13.7	4	**A21B01C03D10**	401.1	10.6	98
A10B14C04D09	586.3	9.6	98	**A04B07C19D11**	601.5	14.0	97
A10B14C15D08	780.4	15.0	96	**A21B21C04D15**	516.2	11.4	10
A09B02C12D07	582.3	14.1	17	**A10B16C18D15**	683.4	8.9	26
A10B10C04D08	653.6	11.3	98	**A21B05C12D04**	681.4	11.1	18
A02B20C01D15	494.1	10.8	7	**A04B07C05D07**	605.5	19.2	13
A21B10C01D15	432.1	9.2	11	**A20B08C14D16**	567.3	8.7	98

[a] Linear gradient B 05 → 90%, 26 min. [b] Nonoptimized yields. All compounds were ≥95% pure as determined by LC/MS.

1H), 1.78 (bd, $J = 13.2$ Hz), 1.63 (bt, $J = 10.4$ Hz), 1.44−1.30 (m, 8H), 1.10 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): 174.4, 173.6, 172.9, 156.2, 146.6, 131.1, 123.5, 56.2, 53.3, 46.3, 41.6, 41.5, 36.8, 29.3, 28.5, 25.5, 25.2. Purity >95%, 24.3 mg (81% yield). LC/MS analysis: $t_R = 16.9$ min (linear gradient B 05 → 90%, 26 min), (ESI) m/z 601.3 $(M + H)^+$.

A02B17C24D01 (G1-04). ^{1}H NMR (400 MHz, DMSO-d_6): 8.76 (d, 1H, $J = 8.0$ Hz), 8.30 (d, 2H, $J = 7.2$ Hz), 8.12 (m, 3H), 8.00 (t, 1H, $J = 5.6$ Hz), 4.49 (dd, 1H, $J =$ 6.4 and 6.8 Hz), 4.38 (m, 1H), 3.10−3.01 (m, 2H), 2.65 (2 × d, 1H, $J = 6.0$ Hz), 2.50 (m), 2.10 (t, 2H, $J = 7.2$ Hz), 1.74 (m, 2H), 1.51−1.20 (m, 9H), 0.85 (apparent t, 3H, $J =$ 6.0 and 6.8 Hz). ^{13}C NMR (100 MHz, DMSO-d_6): 173.0, 172.2, 171.8, 165.2, 149.5, 140.4, 129.6, 123.8, 54.2, 49.0, 38.8, 36.7, 35.1, 31.9, 29.0, 28.4, 23.0, 22.3, 14.4. Purity >95%, 22.0 mg (89% yield). LC/MS analysis: $t_R = 10.2$ min (linear gradient B 05 → 90%, 26 min), (ESI) m/z 495.3 $(M + H)^+$.

A16B11C09D11 (G2-15). ^{1}H NMR (400 MHz, DMSO-d_6): 8.53 (d, 1H, $J = 8.0$ Hz), 8.33 (t, 1H, $J = 6.0$ Hz), 7.82 (d, 3H, $J = 8.4$ Hz), 7.70 (bs, 3H), 7.30 (d, 2H, $J =$ 8.0 Hz), 4.35 (m, 1H), 4.30 (d, 2H, $J = 5.6$ Hz), 2.89 (apparent t, 2H, $J = 6.0$ and 6.4 Hz), 2.78 (bs, 2H), 2.41 (apparent t, 2H, $J = 6.4$ and 7.2 Hz), 2.31 (apparent t, 2H, $J = 6.4$ and 7.2 Hz), 2.12 (bt, 1H, $J = 12.0$ Hz), 1.79 (m, 6H), 1.59 (m, 2H), 1.52−1.25 (m, 6H), 0.85 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): 174.4, 174.2, 171.3, 166.8, 144.0, 132.8, 128.0, 127.1, 52.7, 45.2, 44.5, 42.0, 37.6, 30.5, 30.1, 29.7, 29.3, 27.0, 23.3. Purity >95%, 18.4 mg (71% yield). LC/MS analysis: $t_R = 7.9$ min (linear gradient B 05 → 50%, 26 min), (ESI) m/z 519.4 $(M + H)^+$.

A15B03C05D01 (G1-14). ^{1}H NMR (400 MHz, DMSO-d_6): 7.92 (s, 1H), 7.73 (apparent t, 1H, $J = 4.8$ and 5.2 Hz), 7.52 (d, 1H, $J = 8.0$ Hz), 4.42 (d, 1H, $J = 5.6$ Hz), 3.00 (m, 2H), 2.60 (m, 2H), 2.04 (m, 4H), 1.46 (m, 5H), 1.40−1.10 (m), 0.86 (apparent t, 3H, $J = 6.0$ and 6.8 Hz). ^{13}C NMR (100 MHz, DMSO-d_6): 174.1, 173.0, 172.6, 172.4, 56.1, 48.9, 38.6, 35.9, 35.5, 31.8, 29.5, 29.2, 25.8, 25.1, 23.0, 22.6, 14.1. Purity >95%, 17.0 mg (68% yield). LC/MS analysis: $t_R = 20.5$ min (linear gradient B 05 → 90%, 26 min), (ESI) m/z 500.4 $(M + H)^+$.

A16B05C21D11 (G1-11). ^{1}H NMR (400 MHz, DMSO-d_6): 8.44 (d, 1H, $J = 6.0$ Hz), 8.23 (dd, 2H, $J = 8.0$ and 8.4 Hz), 7.76 (bs), 4.71 (m, 1H), 4.60 (m, 1H), 4.37 (d, 1H, $J =$ 17.2 Hz), 4.17 (m, 2H), 4.10 (d, 1H, $J = 16.0$ Hz), 3.88 (dd, 2H, $J = 16.0$ and 17.2 Hz), 3.02 (s, 3H), 2.38 (m), 1.76−1.20 (m). Purity >95%, 21.1 mg (98% yield). LC/MS analysis: $t_R = 2.4$ min (linear gradient B 05 → 90%, 26 min), (ESI) m/z 432.2 $(M + H)^+$.

Procedure Pilot Assay.[18] Determination of PGGT-1 activity was performed by using a sepharose-coupled octapeptide as substrate. The amino acid sequence of the peptide was Met-Gly-Leu-Pro-Cys-Val-Val-Leu containing the C-terminal Ca_1a_2L motif, which is the consensus sequence for geranylgeranylation by PGGT-1. This substrate has been designated as pepCsep. PepDsep, another sepharose-coupled peptide which is nonisoprenylatable by replacing Cys with Ala, was used as a control to measure nonspecific association of radiolabeled GGPP. A partial purified PGGT-1 enzyme preparation, isolated from bovine brain according to Yokoyama et al.[19] was used in the assay. The incubation mixture (25 μL) contained 2.5 μL of pepCsep or pepDsep (1 nmol of peptides), 3 μL of bovine brain enzyme, 1 μM [^{3}H]-GGPP (specific radioactivity 15 Ci/mmol, American Radiolabeled Chemicals), 50 μM $ZnCl_2$, 0.5 mM $MgCl_2$, 1 mM DTT, 0.004% Triton X-100, and 50 mM Tris−HCl (pH 7.4). For the determination of the inhibitory potencies of the various compounds, three different concentrations were used (in duplo) in the mixture (for generations 0−4: 10, 100, and 1000 μM; generations 5−7: 3, 10, and 100 μM). The incubation was performed at 37 °C for 40 min under continuous shaking. The reaction was terminated by addition of 1 mL of 2% (w/v) SDS, and the beads were spun down and washed successively 3 times with 2% (w/v) SDS under shaking for 45 min at 50 °C. The remaining adhering radioactivity was counted in a Liquid Scintillation Counter. For the calculation of PGGT-1 activity, the ^{3}H counts bound to pepDsep were subtracted from the counts bound to pepCsep. For the determination of the IC_{50} values of the test compounds, the assay was repeated 2 times in the presence of the various concentrations of the compounds, and the concentration at 50% inhibition was determined using a mathematical function fitting to the concentration/inhibition curves.

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Supporting Information Available. NMR spectra of 20 library compounds, together with some details of the Lamoss2 synthesizer. This material is available free of charge via the Internet at http://pubs.acs.org.

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