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Synthesis of Stable Analogues of Geranylgeranyl Diphosphate Possessing a (*Z*,*E*,*E*)-Geranylgeranyl Side Chain, Docking Analysis, and Biological Assays for Prenyl Protein Transferase Inhibition

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*Herein, we report the synthesis of novel stable analogues of geranylgeranyl diphosphate (GGPP), in which the "natural" all-trans geranylgeranyl portion has been replaced by a (Z,E,E)-geranylgeranyl chain. The change in configuration and consequent change in the relative position of the polar portion with the lipophilic side chain did not improve the properties of the E,E,E analogues in their inhibition of geranylgeranyl protein transferase I (GGTase I). However, a significant level of GGTase I inhibition and selectivity for GGTase I over farnesyl transferase (FTase) was maintained the unsubstituted phosphonoacetamidoxy derivative **4a**. This has shed light on the relative importance of the configuration at the C2=C3 double bond among GGPP derivatives. Moreover, the biological activities of all the compounds reported herein, in particular the preferential FTase inhibitory activity shown by compound **6**, were in good agreement with the results of docking analysis.*

Introduction

A specific set of heterotrimeric GTP-binding proteins in eukaryotic cells, including Ras and Ras-like GTPases (Rho, Rac, Rab, and Cdc42 proteins), contain covalently attached carboxy-terminal prenyl groups (15-carbon farnesyl and 20-carbon geranylgeranyl) as a result of posttranslational modification.[1,2] The prenyltransferases, which attach isoprenoid moieties (farnesyl or geranylgeranyl groups) derived from mevalonic acid metabolism to the cysteine residues near or at the carboxyl terminus of proteins, have been well-characterized and termed, respectively, farnesyl protein transferase (FTase) and geranylgeranyl protein transferase type I and II (GGTase I and II).[3]

The regulatory activity of Ras-like GTPases in cellular functions such as growth, cell motility, and invasion is critically dependent on targeting proteins to the proper cellular membrane; indeed, protein prenylation is the first and necessary step that leads to membrane binding and acquisition of biological activity of these proteins. Aberrant signalling through Ras pathways occurs in several types of cancer, in which mutated Ras accumulates in its GTP-bound active form and causes uncontrolled cell proliferation.[4] For these reasons, the development of specific therapeutic agents that interfere with the function of these proteins is an appealing goal as a potential treatment of tumor pathologies. A large body of work has focused on the inhibition of FTase, the enzyme that post-translationally activates Ras, for the clear role the Ras oncoprotein plays in human malignancies. Recent research, however, has shown that when farnesylation of Ras-like peptides, including

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Rho, Rac, and Cdc42 is blocked, geranylgeranylation mediated by GGTases may occur and restore uncontrolled cell proliferation.[5] Therefore, in an attempt to block the geranylgeranylation activation pathway, many research groups have developed new GGTase I inhibitors.[6–11]

Our research group has developed some new geranylgeranyl diphosphate-like ("GGPP-like") GGTase inhibitors, the structures of which contain stable polar groups (phosphonoacetamido, phosphonoacetamidoxy, diphosphono, and 3-(phosphono)propionamido) in place of the metabolically unstable diphosphate moiety of GGPP. Some of them (**1a**,**b**, **2**, and **3**, Figure 1) have shown good GGTase inhibitory properties and remarkable GGTase/FTase selectivity levels.[12,13]

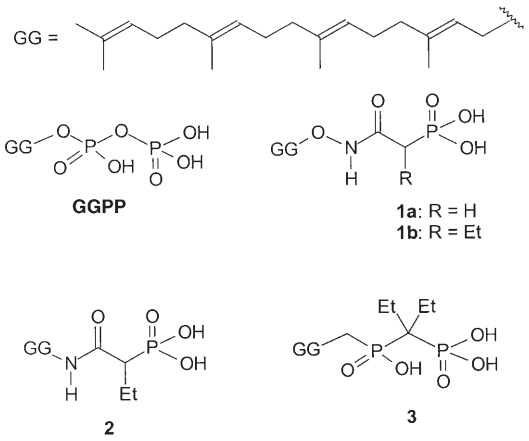


Figure 1. Structures of GGPP and its analogues **1a**,**b**, **2**, and **3**.

We subsequently focused our attention on modifying the lipophilic side chain of this class of molecules. In particular, we wanted to observe whether a change in configuration about the C2=C3 double bond of the geranylgeranyl portion could influence the inhibitory activity toward GGTase or FTase. For this purpose, we synthesized the new compounds **4a**,**b**, **5,** and **6** (Figure 2), analogues of **1a**,**b**, **2**, and **3**, respectively, in which the geranylgeranyl side chain shows a *Z* configuration about the C2=C3 double bond. These compounds were then subjected to enzyme inhibition assays.

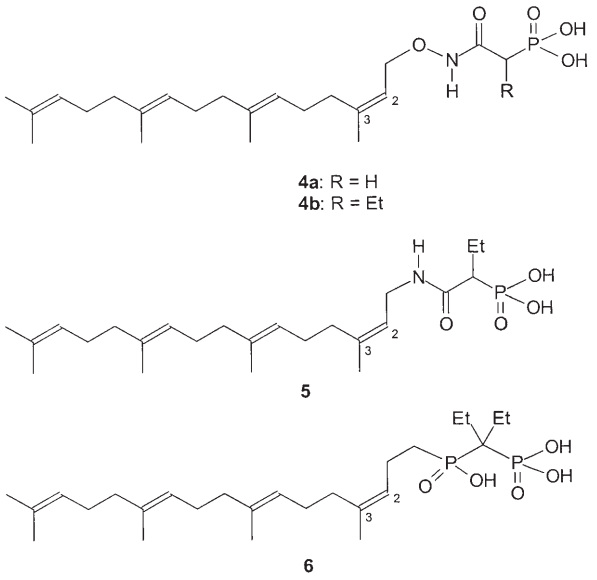
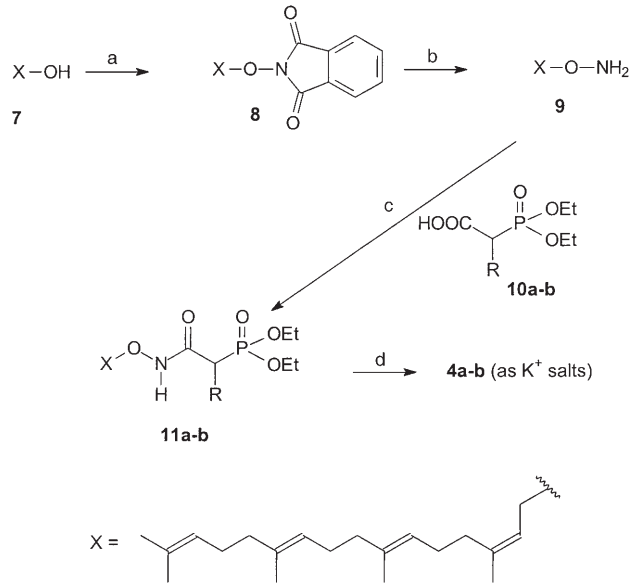


Figure 2. Structures of compounds **4a**,**b**, **5**, and **6**.



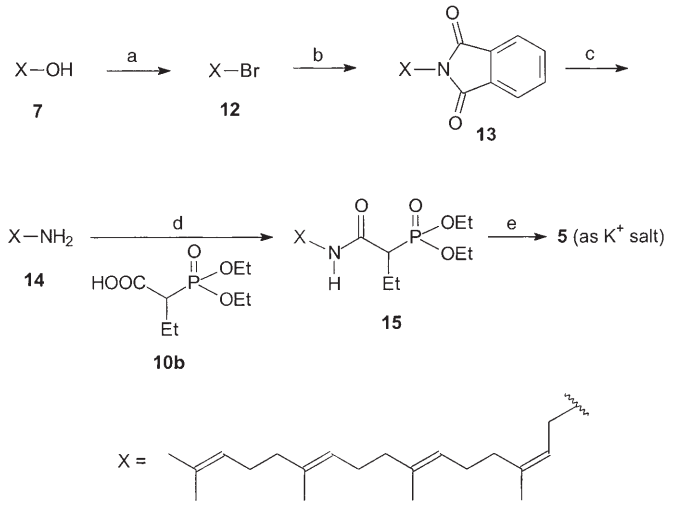
Scheme 1. Reagents and conditions: a) *N*-hydroxyphthalimide, Ph_3P, DEAD, THF, RT; b) NH_2-NH_2·H_2O, EtOH, RT; c) 1-hydroxybenzotriazole, EDC, THF, RT; d) 1. TMS-Br, 2,4,6-collidine, CH_2Cl_2, RT; 2. 1 N aqueous KOH. DEAD = diethylazodicarboxylate, EDC = 3-(3-dimethylaminopropyl)-1-ethylcarbodiimide hydrochloride.

Results and Discussion

Chemical synthesis

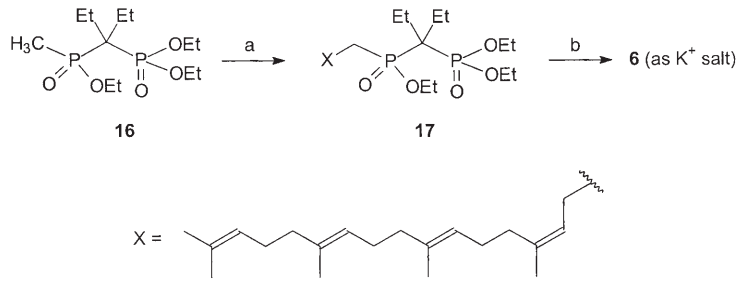
All of the synthetic routes for the preparation of compounds **4a**,**b**, **5**, and **6** started from (*Z*,*E*,*E*)-geranylgeraniol **7**, which was obtained from commercially available farnesyl bromide as previously reported.[14,15] Compounds **4a**,**b** were prepared as shown in Scheme 1. (*Z*,*E*,*E*)-Geranylgeraniol **7** was subjected to a Mitsunobu reaction with *N*-hydroxyphthalimide to give intermediate **8**. Hydrazinolysis of the phthalimido portion afforded free oxyamine **9**, which was then subjected to a condensation reaction with the appropriate acid **10a**,**b**.[13] The diethyl phosphonates thus obtained (**11a**,**b**) were subjected to hydrolysis; treatment with bromotrimethylsilane, 2,4,6-collidine, and aqueous potassium hydroxide, gave compounds **4a**,**b** as the dipotassium salts.

The synthesis of compound **5** is shown in Scheme 2. (*Z*,*E*,*E*)-Geranylgeraniol **7** was treated with phosphorus tribromide[16] to give compound **12**, which was then used to alkylate potassium phthalimide. The phthalimido derivative **13** thus obtained was subjected to hydrazinolysis to afford free amine **14**, which was then condensed with acid **10b**.[13] Final hydrolysis of the diethyl phosphonate **15** afforded the target compound **5** as the dipotassium salt.



Scheme 2. Reagents and conditions: a) PBr_3, THF, −10 °C; b) potassium phthalimide, DMF, RT; c) NH_2-NH_2·H_2O, EtOH, RT; c) 1-hydroxybenzotriazole, EDC, THF, RT; d) 1. TMS-Br, 2,4,6-collidine, CH_2Cl_2, RT; 2. 1 N aqueous KOH. DMF = *N*,*N*-dimethylformamide, TMS = trimethylsilyl.

Compound **6** was prepared as shown in Scheme 3. Compound **16**[17] was deprotonated on the terminal methyl portion



Scheme 3. Reagents and conditions: a) 1. *n*BuLi, THF, −78 °C; 2. (*Z*,*E*,*E*)-geranylgeranyl bromide **12**, THF, −78 °C; b) 1. TMS-Br, 2,4,6-collidine, CH_2Cl_2, RT; 2. 1 N aqueous KOH.

by using *n*-butyllithium and then alkylated with bromide **12** to give the intermediate **17**. Subsequent hydrolysis of the ethyl phosphonate group was carried out by initial treatment with bromotrimethylsilane and 2,4,6-collidine followed by aqueous potassium hydroxide to give compound **6** as the potassium salt.

Enzyme assays

The in vitro inhibition assays of FTase and GGTase I were carried out by measuring the degree of [^{3}H]GGPP and [^{3}H]FPP incorporation into H-Ras-CVLL and H-Ras-CVLS, respectively, as described in the Experimental Section. The inhibitory activities of the newly synthesized compounds **4a**,**b**, **5**, and **6**, together with those of **1a**,**b**, **2**, and **3**,[13] are reported in Table 1 as IC_{50} values (concentrations at which GGTase I and FTase activities were inhibited by 50%).

Table 1. Inhibitory activities toward GGTase I and FTase.

Compound	IC_{50} [nM][a]		Selectivity[b]
	GGTase I	FTase	
1a[c]	66 ± 8	3500 ± 700	53
1b[c]	146 ± 20	> 10000	> 68
2[c]	566 ± 75	> 10000	> 18
3[c]	500 ± 80	> 10000	> 20
4a	255 ± 35	> 10000	> 39
4b	1000 ± 130	> 10000	> 10
5	764 ± 73	> 10000	> 13
6	5000 ± 650	1250 ± 160	0.25

[a] Values are reported as the mean ± SD of 2–3 independent experiments. [b] Fold (GGTase I/FTase). [c] Reference [13].

In general, compounds **4a**,**b**, **5**, and **6**, the structures of which include a geranylgeranyl side chain with a *Z* configuration at the C2=C3 bond, showed a decrease in GGTase I inhibition and in GGTase I/FTase selectivity with respect to the corresponding reference compounds **1a**,**b**, **2**, and **3**. Among the newly synthesized compounds, **4a**, which possesses an unsubstituted phosphonoacetamidoxy moiety, proved to be the most active toward GGTase I (IC_{50} = 255 nM) with an appreciable GGTase I/FTase selectivity (> 39-fold). The diethyl substituted analogue **4b** was observed to be less active than **4a** on GGTase I. A slight improvement in GGTase I inhibition, albeit smaller than that of compound **4a**, was eventually achieved by the removal of the "linking oxygen" atom from the polar portion of **4b**, as is observed for compound **5**. The combination of the (*Z*,*E*,*E*)-geranylgeranyl side chain together with the diethyl-substituted diphosphonate group, as in compound **6**, caused a considerable drop in GGTase I inhibitory activity (10-fold lower than that of the all-*trans* analogue **3**) and an unexpected improvement in FTase inhibitory activity, which switched the selectivity ratio in favour of FTase.

Docking studies

To investigate the binding interactions between the synthesized molecules and the two enzymes (GGTase I, FTase) and, in particular, to analyze the different selectivity exhibited by **6**, an automated docking procedure using AutoDock 3.0[18] was applied to the compounds reported in Table 1. The parameters chosen in AutoDock (see Experimental Section) were tested for their ability to reproduce the crystallized binding geometry of geranylgeranyl diphosphate (GGPP) and farnesyl diphosphate (FPP) into GGTase I (PDB code: 1N4P)[19] and FTase (PDB code: 1FT2),[20] respectively. In both cases, AutoDock easily found the binding geometry corresponding to the crystallized complex among the solutions with the lowest energy, which has the rms deviation between the docked and crystallized geometries in the limit of the crystallographic resolution (the rms is less than 1.3 Å, whereas the crystallographic resolution is greater than 2.6 Å).

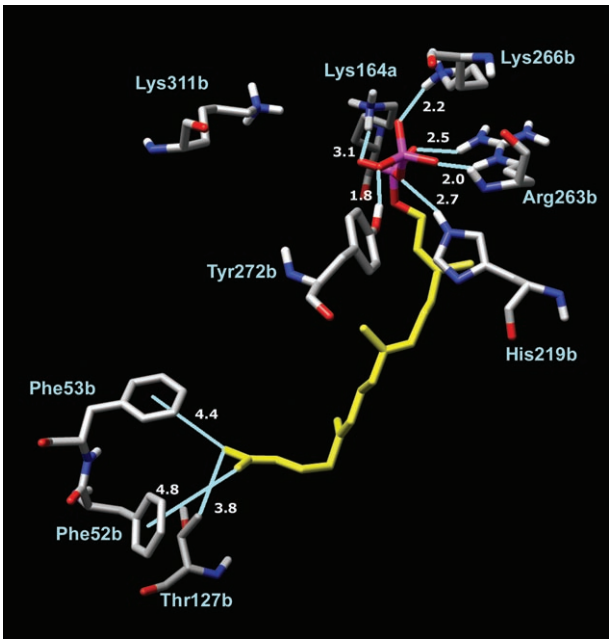


Figure 3. GGPP docked into GGTase I. The main interatomic distances (in Å) are reported in sky blue.

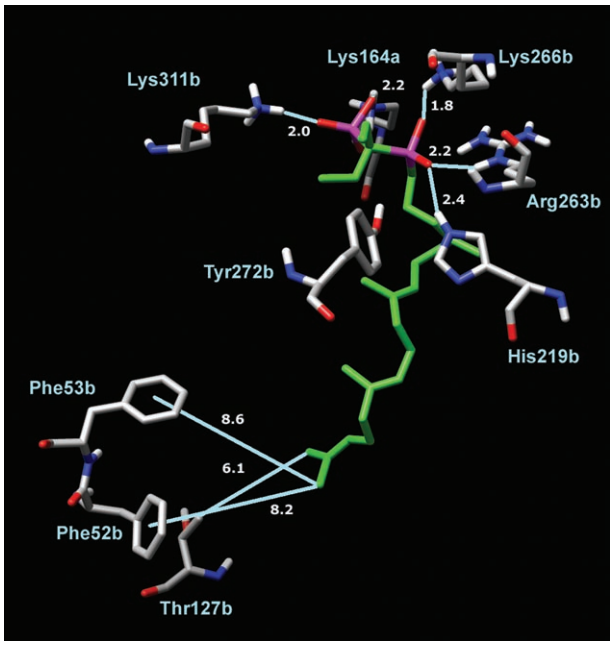


Figure 4. Compound **6** docked into GGTase I. The main interatomic distances (in Å) are reported in sky blue.

Figure 3 shows GGPP complexed with GGTase I; the substrate interacts with Lys 164α, His 219β, Arg 263β, Lys 266β, and Tyr 272β through the diphosphate group while the end of the lipophilic side chain is principally stabilized by the interactions with Phe 52β, Phe 53β, and Thr 127β. Among the ligands tested, compound **1 a** is the only one that shows a binding interaction with GGTase I similar to that of GGPP; all of the other compounds present the terminal phosphonic group in a position whereby it is able to interact with Lys 164α and Lys 311β, whereas the variable portion bound to this group is stabilized by the interaction with His 219β, Arg 263β, and Lys 266β. As for the binding interaction of the lipophilic side chain, all the ligands docked, with the exception of compound **6**, show a disposition similar to that observed for GGPP (Figure 5 a). Compound **6**, which is the least active toward GGTase I, presents a different rearrangement of the lipophilic side chain which results in the loss of interaction with Phe 52β, Phe 53β, and Thr 127β (Figure 4).

With regard to the docking of the ligands into FTase, compounds **3** and **6** place the diphosphonic substituent in a position similar to that of the diphosphate group of FPP and, consequently, this group efficiently interacts with Lys 164α(164), His 248β(219), Arg 291β(263), Lys 294β(266), and Tyr 300β(272). All the other compounds interact through the terminal anionic group with Lys 164α(164) and Lys 294β(311). (Numbers in brackets refer to the respective sequence positions of GGTase 1.)

The lipophilic side chain of most compounds considered herein appears to be too long to fit into the lipophilic cavity delimited by Trp 102β(49) and Tyr 365β(324), and this fact induces a refolding of the fourth isoprene unit outside the cavity. Like FPP, compound **6**, which is the most active, is the only one that is able to interact inside this lipophilic environment (see Figure 5 b). A possible explanation for this behaviour can be obtained by comparing the lipophilic cavity of the two proteins. As shown in Figure 5 a in the case of GGTase I, the position of the fourth isoprene unit of the docked compounds is placed near residue Thr 49β, which is not conserved in FTase (as shown in Figure 5 b) where it is substituted by tryptophan (Trp 102β). This residue, together with the presence of the Tyr 365β (instead of Phe 324β in GGTase 1), fills the space in the FTase molecule where the fourth isoprene unit binds in GGTase I,[19] therefore causing a decrease in the inhibitory activity. In light of these results, compound **6** presents the diphosphonic group and the shorter lipophilic side chain with the *Z* configuration on the C2=C3 double bond. Among all of the compounds tested, this combination is the only one that allows the insertion of the side chain into the lipophilic environment of FTase, which could explain the inhibitory activity of **6** on this enzyme.

In conclusion, we have synthesized new stable analogues of GGPP in which the "natural" all-*trans* geranylgeranyl portion is replaced by a (*Z,E,E*)-geranylgeranyl chain. This change in configuration and consequent change in the relative position of the polar portion and the lipophilic side chain generally does not improve the properties of the *E,E,E* analogues in their inhibition of GGTase I. Nevertheless, a good level of GGTase I inhibition and GGTase I/FTase selectivity was still maintained in the unsubstituted phosphonoacetamidoxy derivative **4 a**, showing that, in this case, the double-bond configuration is less critical than it is for the other differently substituted derivatives (**4 b**, **5**, and **6**). Moreover, the biological activities of all the compounds reported herein, in particular the preferential FTase inhibitory activity shown by compound **6**, were in good agreement with the results of docking analysis.

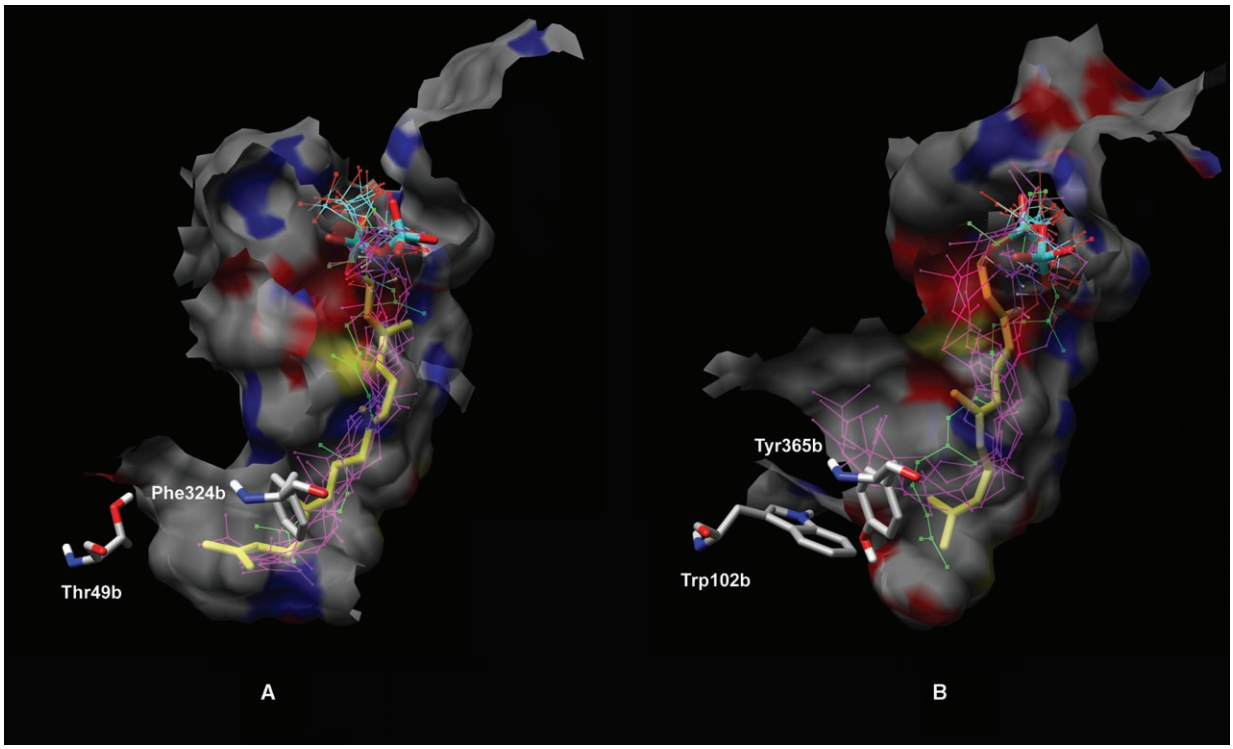


Figure 5. Superposition of the compounds docked into GGTase I (A) and FTase (B) and surface analysis of the two binding sites. GGPP and FPP are indicated in yellow and compound **6** is indicated in green.

Experimental Section

Chemistry: Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. ^{1}H NMR spectra of all compounds were obtained with a Varian Gemini-200 instrument operating at 200 MHz; the data are reported as follows: chemical shift (in ppm) from the Me_4Si line as the external standard, multiplicity (br = broad, s = singlet, d = doublet, t = triplet, m = multiplet). Mass spectra were recorded on a VG 70–250S mass spectrometer or an HP-5988 A spectrometer. Analytical TLC was carried out on silica gel plates (layer: 0.25 mm) containing a fluorescent indicator; spots were detected under UV light (254 nm). Column chromatography was performed using 230–400 mesh silica gel. Sodium sulfate was used as the drying agent. Evaporations were performed in vacuo (rotating evaporator). Commercially available chemicals were purchased from Sigma–Aldrich.

8: *N*-hydroxyphthalimide (1.12 g, 6.88 mmol), triphenylphosphine (1.81 g, 6.88 mmol) and diethylazadicarboxylate (1.19 mL, 7.57 mmol) were added to a solution of (*Z*,*E*,*E*)-geranylgeraniol **7** (2.00 g, 6.88 mmol) in anhydrous THF (60.0 mL). The resulting solution was stirred at room temperature for 18 h followed by the incorporation of additional triphenylphosphine (0.45 g, 1.7 mmol) and diethylazadicarboxylate (0.3 mL, 2 mmol). The solution was stirred at room temperature for an additional 5 h and the solvent was evaporated under vacuum. The residue was purified by column chromatography on silica gel by eluting with *n*-hexane/CH_2Cl_2 (1:1). The appropriate fractions were combined and evaporated to give pure intermediate **8** (1.84 g, 4.23 mmol, 62% yield) as a solid; mp: 39–40 °C. ^{1}H NMR ($CDCl_3$): δ = 1.57 (s, 9H), 1.66 (s, 3H), 1.76 (s, 3H), 1.90–2.22 (m, 12H), 4.68 (d, 2H, J = 7.2 Hz), 4.92–5.21 (m, 3H), 5.52 (t, 1H, J = 7.2 Hz), 7.62–7.93 ppm (m, 4H).

9: Hydrazine monohydrate (0.38 mL, 8.0 mmol) was added to a solution of the phthalimido derivative **8** (1.75 g, 4.02 mmol) in absolute ethanol (100 mL) and the solution was stirred at room temperature for 18 h. The mixture was then filtered and the solvent evaporated under decreased pressure. The crude residue was triturated with diethyl ether to afford oxyamine **9** as an oil (1.10 g, 3.58 mmol, 89% yield) that was used for the next step without further purification. ^{1}H NMR ($CDCl_3$): δ = 1.59 (s, 9H), 1.68 (s, 6H), 1.88–2.21 (m, 12H), 4.12 (d, 2H, J = 7.2 Hz), 4.95–5.23 (m, 3H), 5.29 ppm (t, 1H, J = 7.2 Hz). MS: *m/z* 306 [M^++H].

11a,b: A solution of oxyamine **9** (0.40 g, 1.3 mmol) in anhydrous THF (20 mL) was treated with the appropriate α-substituted phosphonoacetic acid (**10a** or **10b**)[13] (1.4 mmol) in the presence of 1-hydroxybenzotriazole (0.27 g, 2.0 mmol) and 1-[3-(dimethylamino)-propyl]-3-ethylcarbodiimide hydrochloride (0.30 g, 1.6 mmol). The resulting reaction mixture was stirred at room temperature for 18 h. The solvent was then removed under vacuum, and the crude products were purified by silica gel column chromatography by eluting with *n*-hexane/EtOAc (1:1) to obtain phosphonoacetamidoxy esters **11a** and **11b. 11a**: (0.54 g, 1.1 mmol, 86% yield). ^{1}H NMR ($CDCl_3$): δ = 1.18–1.35 (m, 6H), 1.58 (s, 9H), 1.68 (s, 6H), 1.88–2.41 (m, 12H), 2.88 (d, 2H, J = 20 Hz), 3.80–4.32 (m, 4H), 4.38 (d, 2H, J = 7.2 Hz), 4.92–5.28 (m, 3H), 5.38 ppm (t, 1H, J = 7.2 Hz). MS (FAB$^+$): *m/z* 484 [M^++H]. **11b**: (0.58 g, 1.1 mmol, 87% yield). ^{1}H NMR ($CDCl_3$): δ = 0.98 (t, 3H, J = 7.2 Hz), 1.11–1.42 (m, 6H), 1.58 (s, 9H), 1.66 (s, 3H), 1.75 (s, 3H), 1.83–2.18 (m, 14H), 2.55 (dt, 1H, J = 22 Hz, 7.2 Hz), 3.85–4.27 (m, 4H), 4.39 (d, 2H, J = 7.2 Hz), 4.89–5.20 (m, 3H), 5.39 ppm (t, 1H, J = 7.2 Hz). MS (FAB$^+$): *m/z* 512 [M^++H].

12: A solution of phosphorus tribromide (0.4 mL, 4 mmol) in anhydrous THF (10 mL) was added dropwise to a cooled (−10 °C) solution of (*Z*,*E*,*E*)-geranylgeraniol **7** (3.0 g, 10 mmol) in anhydrous THF

(10 mL) and the resulting mixture was stirred at the same temperature for 15 min. The solvent was evaporated under decreased pressure, and the residue was treated with a mixture of diisopropyl ether/*n*-hexane (1:1, 30 mL) and extracted with 5% aqueous $NaHCO_3$. The organic layer was then washed with brine, dried, and evaporated to give the intermediate **12** (3.06 g, 8.65 mmol, 84% yield), which was used for the next step without further purification.

13: Potassium phthalimide (1.15 g, 6.23 mmol) was added to a solution of bromide **12** (2.00 g, 5.66 mmol) in anhydrous DMF (20 mL) and the resulting mixture was stirred under argon at room temperature for 48 h. Potassium phthalimide (0.57 g, 3.1 mmol) was added again and the mixture was stirred at room temperature for an additional 18 h. The solvent was then evaporated at decreased pressure and the crude residue was purified by silica gel column chromatography (*n*-hexane/dichloromethane 7:3) to afford pure product **13** (1.32 g, 3.16 mmol, 56% yield). ^{1}H NMR ($CDCl_3$): $\delta = 1.59$ (s, 9H), 1.66 (s, 6H), 1.80–2.31 (m, 12H), 4.24 (d, 2H, $J =$ 7.2 Hz), 4.89–5.36 (m, 4H), 7.52–7.87 ppm (m, 4H). MS (FAB^+): *m/z* 420 [M^++H].

14: Hydrazine monohydrate (0.29 mL, 6.0 mmol) was added to a solution of compound **13** (1.26 g, 3.02 mmol) in absolute EtOH (72 mL) and the mixture was stirred at room temperature for 18 h. The white solid formed was filtered off and the solvent was evaporated in vacuo. Trituration of the crude residue with diethyl ether afforded compound **14** as an oil (0.66 g, 2.3 mmol, 76% yield), which was used for the next step without further purification. ^{1}H NMR ($CDCl_3$): $\delta = 1.59$ (s, 9H), 1.69 (s, 6H), 1.82–2.15 (m, 12H), 3.23 (d, 2H, $J =$ 7.2 Hz), 4.98–5.38 ppm (m, 4H). MS (FAB^+): *m/z* 290 [M^++H].

15: A solution of amine **14** (0.50 g, 1.7 mmol) in anhydrous THF (25 mL) was treated with the phosphonoacetic acid **10b** (0.43 g, 1.9 mmol) in the presence of 1-hydroxybenzotriazole (0.35 g, 2.6 mmol) and 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (0.40 g, 2.1 mmol). The reaction mixture was stirred at room temperature for 18 h. The solvent was then removed under vacuum and the crude residue was purified by silica gel column chromatography by eluting with *n*-hexane/EtOAc (1:1) to obtain phosphonoacetamidoxy ester **15** (0.72 g, 1.5 mmol, 84% yield). ^{1}H NMR ($CDCl_3$): $\delta = 1.00$ (t, 3H, $J =$ 7.2 Hz), 1.12–1.46 (m, 6H), 1.59 (s, 9H), 1.67 (s, 6H), 1.83–2.18 (m, 14H), 2.28–2.81 (m, 1H), 3.72–4.29 (m, 6H), 4.91–5.29 ppm (m, 4H). MS (FAB^+): *m/z* 496 [M^++H].

17: *n*-Butyllithium (1.6 M in hexane, 1.8 mL, 2.9 mmol) was added dropwise to a stirred solution of compound **16** (0.74 g, 2.4 mmol) in anhydrous THF (20 mL), cooled at −78 °C under argon. After stirring at −78 °C for 30 min, the mixture was treated dropwise with a solution of bromide **12** (1.0 g, 2.8 mmol) in anhydrous THF (3 mL) and then stirred for an additional 1 h at the same temperature. The reaction mixture was allowed to warm to room temperature and was quenched with an aqueous solution of acetic acid (10%, 15 mL). The aqueous phase was extracted repeatedly with dichloromethane, and the organic layers were combined, dried, and evaporated under decreased pressure. The residue thus obtained was purified by silica gel column chromatography by eluting with *n*-hexane/AcOEt (3:7) to afford compound **17** as an oil (0.32 g, 0.54 mmol, 23% yield). ^{1}H NMR ($CDCl_3$): $\delta = 0.85$–1.46 (m, 18H), 1.58–1.79 (m, 15H), 1.81–2.18 (m, 14H), 3.88–4.36 (m, 4H), 4.91–5.25 ppm (m, 4H). MS (FAB^+): *m/z* 597 [M^++H].

General procedure for 4a–b, 5, and 6: Bromotrimethylsilane (0.46 mL, 3.5 mmol) was added to a stirred solution of the appropriate ester **11a**, **11b**, **15**, **17** (0.70 mmol) and 2,4,6-collidine (0.19 mL, 1.4 mmol) in anhydrous dichloromethane (8 mL). The resulting mixture was stirred at room temperature for 18 h. After evaporation of the solvent, the residue was treated with an aqueous solution of KOH (1 N, 5.3 mL) and the mixture was stirred at room temperature for 3 h. After removal of water under high vacuum, the crude residue was purified by column chromatography on reversed-phase silica gel (Merck Lichroprep RP-18) by eluting with MeOH/H_2O (2:1 for **4a** and **5**; 3:2 for **4b**; 2.5:1 for **6**). The appropriate fractions were combined, evaporated, lyophilized, and pump-dried to give final products **4a–b**, **5**, and **6** as the dipotassium salts. **4a**: (0.180 g, 0.36 mmol, 51% yield). ^{1}H NMR (D_2O): $\delta =$ 1.59 (s, 9H), 1.66 (s, 3H), 1.74 (s, 3H), 1.88–2.21 (m, 12H), 2.30 (d, 2H, $J =$ 18 Hz), 4.46–5.45 ppm (m, 6H). MS (FAB^+): *m/z* 504 [M^+ +H]. **4b**: (0.138 g, 0.26 mmol, 37% yield). ^{1}H NMR (D_2O): $\delta = 0.91$ (t, 3H, $J =$ 7.2 Hz), 1.59 (s, 9H), 1.64 (s, 3H), 1.76 (s, 3H), 1.91–2.23 (m, 14H), 2.24–2.69 (m, 1H), 4.33 (d, 2H, $J =$ 7.2 Hz), 4.91–5.29 (m, 3H), 5.39 ppm (t, 1H, $J =$ 7.2 Hz). MS (FAB^+): *m/z* 532 [M^++H]. **5**: (0.24 g, 0.47 mmol, 67% yield). ^{1}H NMR (MeOD): $\delta = 0.91$ (t, 3H, $J =$ 7.2 Hz), 1.59 (s, 9H), 1.67 (s, 3H), 1.74 (s, 3H), 1.82–2.18 (m, 14H), 2.22–2.63 (m, 1H), 3.82 (d, 2H, $J =$ 7.2 Hz), 4.93–5.41 ppm (m, 4H). MS (FAB^+): *m/z* 516 [M^++H]. **6**: (0.36 g, 0.59 mmol, 84% yield). ^{1}H NMR (D_2O): $\delta = 0.93$ (t, 6H, $J =$ 7.2 Hz), 1.46–1.58 (m, 6H), 1.59–2.02 (m, 17H), 2.02–2.20 (m, 12H), 4.89–5.19 ppm (m, 4H). MS (FAB^+): *m/z* 617 [M^++H].

Biological assays: [^{3}H]GGPP (specific activity 22 Ci mmol^{-1}) and [^{3}H]FPP (specific activity 16 Ci mmol^{-1}) were purchased from Perkin–Elmer Life Science; GGTase I and FTase were purchased from Sigma–Aldrich Corporation; H-Ras-CVLL, H-Ras-CVLS (wild-type), and Zwittergent 3-12 were purchased from Calbiochem. All other reagents were obtained from commercial sources.

GGTase I and FTase activity assays: In vitro inhibition studies were performed as previously described,[21,22] with some modifications. Briefly, GGTase I (250 ng) and FTase (20 ng) were incubated in Tris-HCl (50 mM, pH 7.7), $ZnCl_2$ (25 μM), KCl (20 mM), $MgCl_2$ (5 mM), DTT (1 mM) and Zwittergent 3-12 (0.5 mM), in the presence of different concentrations of inhibitors in a final volume of 50 μL. The reactions were incubated at 30 °C for 30 min with recombinant H-Ras-CVLL (2.5 μM) and [^{3}H]GGPP (0.1 μM) for GGTase I, and recombinant H-Ras-CVLS (2.5 μM) and [^{3}H]FPP (0.6 μM) for FTase. After incubation the reaction was stopped and filtered on glass-fibre filters to separate free from incorporated label. The activity of the inhibitors is reported in Table 1 as IC_{50} values, the concentration at which GGTase I and FTase activity was inhibited by 50%.

Computational details: *Amino acid numbering:* For GGTase I and FTase, the reference numbering used was that from PDB structure codes 1N4P and 1FT2, respectively; furthermore, for FTase, the corresponding GGTase I numbering is indicated in parentheses.

Methods: The crystal structures of GGTase I (PDB code: 1N4P)[19] and FTase (PDB code: 1FT2)[20] were taken from the Protein Data Bank.[23] The alignment between the two proteins was performed with CLUSTAL W,[24] using the Blosum series as a matrix, with a gap-open penalty of 10 and a gap-extension penalty of 0.05. The two proteins were energy-minimized by using the Amber 8 programme[25] and the parm94 force field. The minimization algorithms used were Steepest Descent, followed by Conjugated Gradient until a convergence value of 0.05 kcal Å^{-1} mol^{-1} and a distance-dependent dielectric constant of 4.0 were reached.

The conformational analysis of the ligands was performed with the MACROMODEL programme;[26] all compounds were subjected to a conformational search of 1000 steps with an energy window of 10 kJ mol^{-1} for saving the structure. The algorithm used was the

Monte Carlo method with MMFFs as the force field, a distance-dependent dielectric constant of 1.0, and water as solvent (using the analytical generalized-Born/surface-area model). The best conformation for each ligand was then minimized by using the Conjugated-Gradient method until a convergence value of 0.05 kcal $Å^{-1}$ mol^{-1} was reached; this was used as the starting structure for the automated docking procedure.

Automated docking was carried out with the AUTODOCK 3.0[18] programme; AUTODOCK TOOLS[27] was used to identify the torsion angles in the ligands and to add the solvent model. The ligand charges were calculated with the AM1-BCC method as implemented in the Antechamber suite of Amber 8.[25] The regions of interest used by AUTODOCK were defined by considering GGPP and FPP as the central groups of GGTase I and FTase, respectively; in particular, a grid of 50, 48, and 48 points in the *x*, *y*, and *z* directions, respectively, was constructed with the centre of mass of each of the two substrates as the origin. A grid spacing of 0.375 Å and a distance-dependent function of the dielectric constant were used for the energetic map calculations.

The Lamarckian Genetic Algorithm was used; all docked compounds were subjected to 100 runs of the AUTODOCK search, in which the default values of the other parameters were used. Cluster analysis was performed on the docked results by using a rms tolerance of 1.0 Å, and the best-docked conformation was used for the analysis. All graphic manipulations and visualizations were performed with Chimera.[28]

Keywords: ab initio calculations · docking · *E*/*Z* configuration · enzyme catalysis · inhibitors · prenyltransferases

[1] R. A. Gibbs, T. J. Zahn, J. S. Sebolt-Leopold, *Curr. Med. Chem.* **2001**, *8*, 1437 – 1466.
[2] D. J. Mackay, A. Hall, *J. Biol. Chem.* **1998**, *273*, 20685 – 20688.
[3] M. C. Seabra, *Cell. Signalling* **1998**, *10*, 167 – 172.
[4] J. L. Bos, *Cancer Res.* **1989**, *49*, 4682 – 4689.
[5] M. F. Olson, A. Ashworth, A. Hall, *Science* **1995**, *269*, 1270 – 1272.
[6] F. P. Coxon, M. H. Helfrich, B. Larijani, M. Muzylak, J. E. Dunford, D. Marshall, A. D. McKinnon, S. A. Nesbitt, M. A. Horton, M. C. Seabra, F. H. Ebetino, M. J. Roger, *J. Biol. Chem.* **2001**, *276*, 48213 – 48222.
[7] Y. Q. Mu, L. M. Eubanks, C. D. Poulter, R. A. Gibbs, *Bioorg. Med. Chem.* **2002**, *10*, 1207 – 1219.
[8] T. J. Zahn, J. Whitney, C. Weinbaum, R. A. Gibbs, *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1605 – 1608.
[9] B. S. Gibbs, T. J. Zahn, Y. Q. Mu, J. S. Sebolt-Leopold, R. A. Gibbs, *J. Med. Chem.* **1999**, *42*, 3800 – 3808.
[10] A. Vasudevan, Y. Qian, A. Vogt, M. A. Blaskovich, J. Ohkanda, S. M. Sebti, A. D. Hamilton, *J. Med. Chem.* **1999**, *42*, 1333 – 1340.
[11] C. M. Marson, A. S. Rioja, G. Brooke, R. C. Coombes, D. M. Vigushin, *Bioorg. Med. Chem. Lett.* **2002**, *12*, 255 – 259.
[12] M. Macchia, N. Jannitti, G. Gervasi, R. Danesi, *J. Med. Chem.* **1996**, *39*, 1352 – 1356.
[13] F. Minutolo, S. Bertini, L. Betti, R. Danesi, G. Gervasi, G. Giannaccini, C. Papi, G. Placanica, S. Rapposelli, M. Macchia, *Bioorg. Med. Chem. Lett.* **2003**, *13*, 4405 – 4408.
[14] L. Ruzicka, G. Firmenich, *Helv. Chim. Acta* **1939**, *22*, 392 – 396.
[15] S. Klinge, M. Demuth, *Synlett* **1993**, *10*, 783 – 784.
[16] T. Fukuda, S. Kobayashi, H. Yukimasa, S. Terao, M. Fujino, T. Shiba, I. Saiki, I. Azuma, Y. Yamamura, *Bull. Chem. Soc. Jpn.* **1981**, *54*, 3530 – 3535.
[17] M. Prashad, J. C. Tomesch, J. R. Wareing, T. Scallen, *Eur. J. Med. Chem.* **1993**, *28*, 527 – 531.
[18] G. M. Morris, D. S. Goodsell, R. S. Halliday, R. Huey, W. E. Hart, R. K. Belew, A. J. Olson, *J. Comput. Chem.* **1998**, *19*, 1639 – 1662.
[19] J. S. Taylor, T. S. Reid, K. L. Terry, P. J. Casey, L. S. Beese, *EMBO J.* **2003**, *22*, 5963 – 5974.
[20] S. B. Long, P. J. Casey, L. S. Beese, *Biochemistry* **1998**, *37*, 9612 – 9618.
[21] F. L. Zhang, J. F. Moomaw, P. J. Casey, *J. Biol. Chem.* **1994**, *269*, 23465 – 23470.
[22] Y. Quian, M. A. Blaskovich, M. Saleem, C. M. Seong, S. P. Wathen, A. D. Hamilton, S. M. Sebti, *J. Biol. Chem.* **1994**, *268*, 12410 – 12413.
[23] H. M. Berman, J. Westbrook, Z. Feng, G. Gilliland, T. N. Bhat, H. Weissig, I. N. Shindyalov, P. E. Bourne, *Nucleic Acids Res.* **2000**, *28*, 235 – 242.
[24] J. D. Thompson, D. G. Higgins, T. J. Gibson, *Nucleic Acids Res.* **1994**, *22*, 4673 – 4680.
[25] D. A. Case, T. A. Darden, T. E. Cheatham III, C. L. Simmerling, J. Wang, R. E. Duke, R. Luo, K. M. Merz, B. Wang, D. A. Pearlman, M. Crowley, S. Brozell, V. Tsui, H. Gohlke, J. Mongan, V. Hornak, G. Cui, P. Beroza, C. Schafmeister, J. W. Caldwell, W. S. Ross, P. A. Kollman, Amber version 8.0, University of California, San Francisco, USA; http://amber.scripps.edu.
[26] Macromodel version 8.5, Schrödinger Inc., **1999**.
[27] http://www.scripps.edu/~sanner/python/adt
[28] E. F. Pettersen, T. D. Goddard, C. C. Huang, G. S. Couch, D. M. Greenblatt, E. C. Meng, T. E. Ferrin, *J. Comput. Chem.* **2004**, *25*, 1605 – 1612.

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