Design, Synthesis, and Evaluation of Sugar Amino Acid Based Inhibitors of Protein Prenyl Transferases PFT and PGGT-1

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Abstract: Eleven analogues of the C-terminal Ca_1a_2X motif found in natural substrates of the prenyl transferases PFT and PGGT-1 were synthesized and evaluated for their inhibition potency and selectivity against PFT and PGGT-1. Replacement of the central dipeptide part a_1a_2 by a benzylated sugar amino acid resulted in a good and highly selective PFT inhibitor (**8**, $IC_{50} = 250 \pm 20$ nM). The methyl ester of **8** (**13**) selectively inhibited protein farnesylation in cultured cells.

Protein farnesyl transferase[1] (PFT) and protein geranylgeranyl transferase-1[2] (PGGT-1) (both protein prenyl transferases) catalyze the transfer of isoprenoid lipids from the corresponding pyrophosphates to cysteine residues at the C termini of precursor proteins that in the mature state are key factors in various biological processes. One of the most studied prenylated proteins is Ras, a small GTP-binding protein that is an important switch in signal transduction pathways that mediate growth factor stimulated cell proliferation. The wide interest in Ras is based on the existence of various forms of mutated Ras proteins that are present in (and partially responsible for the proliferation of) 30−40% of all human tumors.[3] Isoprenylation is the first and essential step in a series of post-translational modification events that lead to mature Ras. Inhibition of isoprenylation of oncogenic Ras prevents localization at the cell membrane, thereby prohibiting cell transformation.

PFT attaches a farnesyl group to proteins that contain the C-terminal Ca_1a_2X motif (C = cysteine, a_1a_2 = any aliphatic amino acid, X = methionine or serine), while PGGT-1 catalyzes the geranylgeranylation of the same motif but with X as leucine. Initially, PFT was considered as the main pharmacological target aimed at disabling Ras functioning for the development of antitumor agents. However, it was recently demonstrated that upon inhibion of PFT the most abundant human oncogenic Ras protein, Ras K-4B, can be geranylgeranylated by PGGT-1,[4] resulting in a modified protein with comparable transforming potential as the farnesylated protein.[5] This finding underscores the importance of PGGT-1, next to PFT, as a therapeutic target in cancer research.[1,6]

The discovery that simple tetrapeptides based on Ca_1a_2X can function as substrate analogue inhibitors of PFT and PGGT-1[7] initiated many research activities. This has resulted in the development of an array of inhibitors of PFT and, to a lesser extent, PGGT-1.[8] At the moment four PFT inhibitors are in clinical development (phases I−III).[1e,f] It was found that the introduction of hydrophobic aromatic residues at the a_2 position has a beneficial effect on the inhibitory potency against both enzymes.[9a−f] For PFT this is exemplified by the potent and competitive inhibitor CVFM,[10] in which the presence of phenylalanine at the a_2 position is of importance. In line with these results, it was reported[9d,e,11] that Ca_1a_2L analogues in which the a_1a_2 portion was replaced by dipeptide isosteric 2-aryl-4-aminobenzoic moieties were effective inhibitors of PGGT-1. The replacement of amide linkages by amine connections in certain Ca_1a_2X analogues not only has a beneficial effect on the stability against proteolytic degradation but also influences the selectivity of inhibition.[12]

As part of a program aimed at the development of novel inhibitors of both PFT and PGGT-1, we recently reported[13,14] a new type of Ca_1a_2X analogue in which a sugar amino acid (SAA) is incorporated as a replacement of the central dipeptide a_1a_2. By virtue of the intrinsic properties of SAAs[15a−c] (i.e., inducing a β-turn[15d] or an extended conformation) and the number and spatial orientation of the functionalities appended to the sugar core, the inhibitory action can be substantially enhanced. In line with this, a new set of inhibitors can be obtained by the introduction of an aromatic functionality on the peptidomimetic part. Moreover, our recently developed[16] synthesis of amino acid/carbohydrate conjugates via the Fukuyama−Mitsunobu glycosylation of amino acid derived *o*-nitrobenzenesulfonamides offered the opportunity to evaluate the inhibitory potency and selectivity of Ca_1a_2X analogues having an amine bond between the peptidomimetic and the C-terminal residue.

We report the synthesis of novel inhibitors of the prenylating enzymes PFT and PGGT-1 containing benzylated SAA having a 1,5-trans (i.e., α) or a 1,5-cis (i.e., β) substituted sugar core. The Ca_1a_2L analogues (**1**−**4** and the controls **11** and **12**,[13b] Scheme 3) were designed to inhibit PGGT-1, while the corresponding Ca_1a_2M analogues (**5**−**8**) were projected to inhibit PFT. Sulfoxides **9** and **10** were obtained as side products in a deprotection step. All compounds were evaluated for their inhibitory potency and selectivity in a PFT and PGGT-1 enzyme bioassay. The effect of **13** (methyl ester of **8**) on the prenylation of proteins was investigated in cultured cells.

Epimeric alcohols **16α** and **16*β***, required for the preparation of the here-reported Ca_1a_2X analogues, are readily available from tri-*O*-acetyl-D-glycal.[17] The synthesis of the fully protected amine precursors (**19α,*β*** and **20α,*β***) is exemplified by the conversion of **16α** into **19α** and **20α** (Scheme 1). Thus, treatment of **16α** with *o*-Ns-Leu-OMe (**14**)[18a] or *o*-Ns-Met-OMe (**15**)[18b] and PPh_3/DEAD gave, after removal of the nosyl group, the dimer **17α** or **18α**, respectively, in good yield. The Boc group was removed, and the corresponding amine was condensed with Fmoc-Cys(StBu)-OH, furnishing Ca_1a_2X

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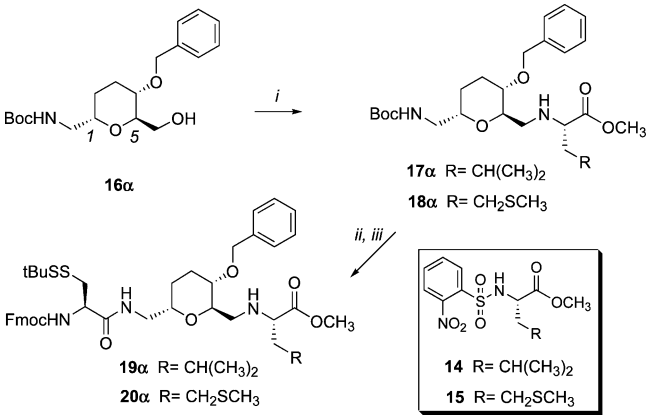
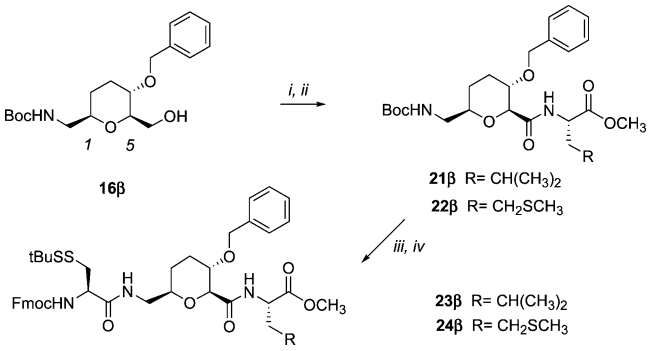
Scheme 1. Synthetic Scheme for **19α** and **20α**[a]



[a] Reagents and conditions: (i) (a) *o*-Ns-Leu-OMe (**14**) or *o*-Ns-Met-OMe (**15**), PPh_3, DEAD, THF; (b) K_2CO_3, PhSH, CH_3CN, 50 °C (**17α**, 65%; **18α**, 81%, two steps); (ii) TFA/DCM 1/1 v/v, *i*Pr_3SiH or Et_3SiH; (iii) Fmoc-Cys(StBu)-OH, BOP, *N*-ethylmorpholine, DMF (**19α**, 40%; **20α**, 47%, two steps).

Scheme 2. Synthetic Scheme for **23*β*** and **24*β***[a]

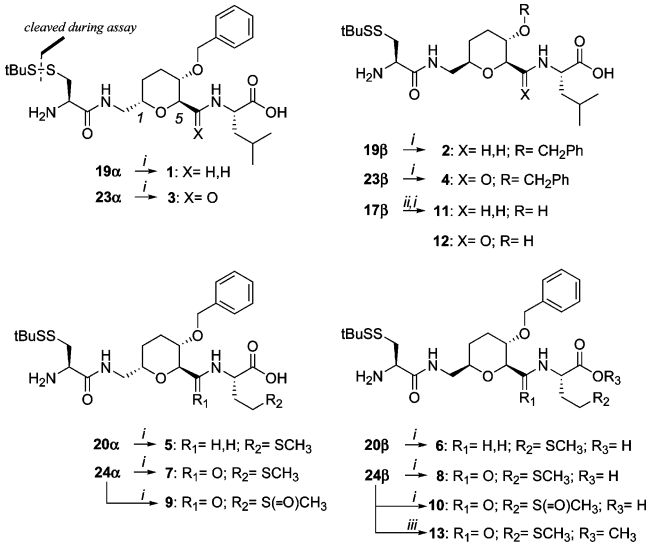


[a] Reagents and conditions: (i) $RuCl_3 \cdot 3H_2O$, $K_2S_2O_8$, 1 M KOH or TEMPO, bisiodobenzene diacetate, DCM/H_2O (SAA, 100%); (ii) HCl·H-Leu-OMe, BOP/DiPEA or HCl·H-Met-OMe, EDC, HOBt, DMF (**21*β***, 98%; **22*β***, 83%); (iii) TFA/DCM 1/1 v/v, *i*Pr_3SiH or Et_3SiH; (iv) Fmoc-Cys(StBu)-OH; for **23*β***, BOP/HOBt, DiPEA; for **24*β***, EDC/HOBt, DMF (**23*β***, 85%; **24*β***, 79%, two steps).

analogues **19α** and **20α**. Starting from **16*β***, the corresponding 1,5-*cis*-amine precursors were obtained. The synthesis of the fully protected amide precursors (**23α**,***β*** and **24α**,***β***) is exemplified by the conversion of **16*β*** into compounds **23*β*** and **24*β*** (Scheme 2). Hence, oxidation of **16*β*** with $RuCl_3 \cdot 3H_2O$[19] or TEMPO/bisiodobenzene diacetate[20] gave the corresponding SAA. Subsequent condensation with HCl·H-Leu-OMe or HCl·H-Met-OMe yielded **21*β*** and **22*β***, respectively. Deprotection of the Boc group and condensation with Fmoc-Cys(StBu)-OH afforded **23*β*** and **24*β***. Employing **16α** furnished the corresponding 1,5-*trans*-Ca_1a_2X analogues.

Target **1**−**8** were obtained by treatment of the fully protected precursors with Tesser's base (MeOH/dioxane/4 M NaOH, 15/4/1, v/v/v) to effect simultaneous hydrolysis of the ester and removal of the Fmoc protective group (Scheme 3). The crude compounds were characterized by LC−MS and purified by RP-HPLC. For **24α** and **24*β*** the applied deprotection conditions resulted in the formation of oxidized side products that were identified by NMR and mass spectrometry as the corresponding sulfoxides **9** and **10** (Scheme 3). Isolation of these products allowed evaluation of their biological activity. Fortunately, base-mediated deprotection under stringent nonaerobic conditions allowed isolation of the desired **7** and **8**.

Scheme 3. Synthesis **1**−**8**, **11**, and **13**[a]



[a] Reagents and conditions: (i) (a) MeOH/1,4-dioxane/4 M NaOH; (b) RP-HPLC purification; (ii) (a) Pd/C, H_2, EtOH/$CHCl_3$ (100%); (b) TFA/DCM 1/1 v/v, *i*Pr_3SiH; (c) Fmoc-Cys(StBu)-OH, BOP, *N*-ethylmorpholine, DMF (52%, two steps); (iii) 20% piperidine in DMF (**13**, 81%). For **7** and **8** the reaction was performed under argon with freshly distilled 1,4-dioxane (benzophenone ketyl).

Table 1. IC_{50} Values of **1**−**11** and Reference Compounds CVIM and CVIL in PFT and PGGT-1 Bioassay[a,b]

	IC_{50} (μM)[a]			IC_{50} (μM)[a]	
compd	PFT	PGGT-1	compd	PFT	PGGT-1
1	∼1000	270 ± 122	**8**	0.25 ± 0.02	>1000
2	>1000	464 ± 147	**9**	91 ± 14	521 ± 75
5	∼1000	311 ± 94	**10**	2.2 ± 0.6	>1000
6	>1000	500 ± 81	**11**	>1000	261 ± 55
3	321 ± 18	206 ± 34	CVIM	0.42 ± 0.05	nd[c]
4	57 ± 18	14 ± 6	CVIL	nd[c]	4.4 ± 0.2
7	42 ± 6	48 ± 11			

[a] IC_{50}: concentration of compound required to inhibit by 50% the PFT or PGGT-1 catalyzed incorporation of [^{3}H]FPP or [^{3}H]G-GPP, respectively. All IC_{50} values are the mean of three determinations. One determination involves performing the assay at five concentrations of compound. By use of a mathematical function fitting the concentration−inhibition curve, the IC_{50} value was determined. [b] For CVIM the reported IC_{50} for PFT in vitro varies between 150 and 200 nM. For CVIL the reported IC_{50} for PGGT-1 varies between 2 and 11 μM. [c] nd = not determined.

Removal of the benzyl group in **17*β*** (Pd/C, H_2) followed by the same sequence of reactions as described for the synthesis of **1** allowed us to evaluate amine **11** (Scheme 3) and to compare it with its previously reported amide analogue **12** (IC_{50}\{PGGT-1\} ≈ 1000 μM).[13b]

1−**11** (Scheme 3) were evaluated for their in vitro inhibitory activity against PFT and PGGT-1 following previously described procedures.[14c,d] The thio *tert*-butyl protective group in the cysteine residue of **1**−**11** is cleaved under the conditions of the assay (dithiothreitol, Tris buffer, pH 7.4). As a reference, we also evaluated the known tetrapeptides CVIM and CVIL[12] in our assay. In Table 1 all determined IC_{50} values are summarized.

8, having a 1,5-cis configuration of the SAA core, proved to be a good (IC_{50} = 250 ± 20 nM) and selective PFT inhibitor. Furthermore, **8** shows a 1000- to 4000-fold improvement in potency compared to our previously reported[13a] PFT inhibitors and was found to be slightly more active than the known[12] tetrapeptide inhibitor CVIM. When **8** and **7** (having a 1,5-trans configuration) are compared, it becomes clear that the stereochemical

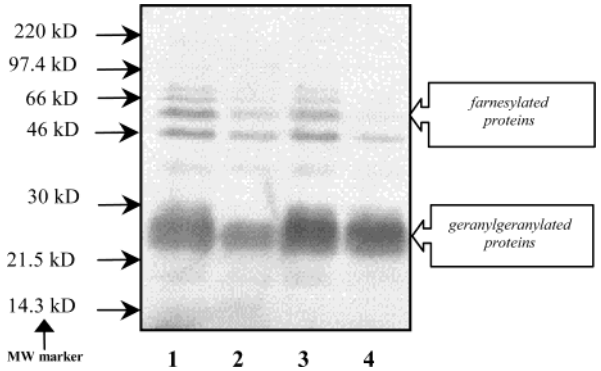
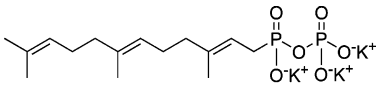


Figure 1. In vivo evaluation of **TR006**, **8**, and **13**. Met-18b-2 cells were treated with [^{3}H]mevalonate and simvastatin and in the absence of compound (lane 1): with **TR006** (lane 2, 100 μM); **8** (lane 3, 100 μM); **13** (lane 4, 100 μM). Monolayers of cells were dissolved in detergent solution and subjected to electrophoresis and autoradiography (see Supporting Information).



TR006

Figure 2. Compound **TR006**.

identity of the SAA is important with respect to potency and selectivity.

For PGGT-1, **4** was found to be the most active inhibitor (IC_{50} = 14 μM). **4**, which only differs from **8** in its X amino acid, shows no high selectivity for either enzyme.[21] Comparing **12**[13b] with **4** shows that enhanced hydrophobicity at the a_2 position has a positive effect on the inhibitory potency. Having an amine linkage between the dipeptide isostere and the X amino acid (**1**, **2**, **5**, and **6**) proves to be detrimental for the inhibition of both enzymes, in particular for PFT.[22] However, **11** is ~4 times more active against PGGT-1 than its amide analogue **12**. Although introduction of a sulfoxide functionality (**9**, **10**) leads to a decrease in inhibitory potency, this modification is more tolerated by PFT than PGGT-1.[23a] This is in analogy with results reported by Manne and co-workers[23b] who observed that replacement of the methionine by polar residues (e.g., a carboxamide or sulfone) retained inhibitory potency against PFT and to a lesser extent against PGGT-1.

To evaluate whether **8** is able to inhibit protein farnesylation in vivo (Met-18b-2 cells),[24] its methyl ester (**13**) was synthesized to facilitate cellular uptake.[25] As illustrated in Figure 1, incubation with [^{3}H]mevalonate resulted in several labeled prenylated proteins that can be roughly divided into farnesylated proteins at molecular weights of about 46−80 kDa and geranylgeranylated proteins at about 22−28 kDa.[26]

While incubation with **8** (lane 3) did not influence the prenylation pattern, **13** (lane 4) inhibited the incorporation of mevalonate into the higher molecular weight bands, indicating that protein farnesylation was strongly decreased. The 21−28 kDa bands did not change, supporting the specificity of the inhibition. As a positive control, **TR006** (Figure 2), a potent inhibitor of both PFT[14d] and GGPP synthase[14e] was shown to decrease the prenylation of both the higher and lower molecular weight proteins (lane 2).

In summary, we have synthesized novel Ca_1a_2X analogues containing benzylated SAA dipeptide isosteres of the a_1a_2 part and evaluated their inhibitory potency in a PFT and PGGT-1 bioassay. Structure−activity relationship analysis demonstrates that the stereochemistry of the SAA residue has a pronounced effect on inhibition potency and selectivity. **8**, having a 1,5-cis configuration of the SAA core and X = methionine, was shown to be the most potent and selective PFT inhibitor in this series. Diastereoisomer **7** is a modest active dual inhibitor of both enzymes. **4**, having a 1,5-cis SAA configuration and X = leucine, was also found to be a modest active dual inhibitor.[27] In addition, while **8** was not active in intact cells, the corresponding methyl ester **13** was shown to selectively inhibit protein farnesylation in intact Met-18b-2 cells.

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Supporting Information Available: Spectroscopic data and experimental procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

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