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Synthesis of desthio prenylcysteine analogs: sulfur is important for biological activity

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Abstract

N-Acetyl-*S*-farnesyl cysteine (AFC) is the minimal synthetic substrate for the enzyme Icmt, which methylates prenylated proteins. The desthio-AFC isostere **2** has been synthesized in racemic form. This analog was not an Icmt substrate, but instead was a weak inhibitor with an IC₅₀ of ~325 μM

Biological and chemical interest in protein lipidation¹ has lagged behind that in protein phosphorylation and glycosylation. As we learn more about the proteome, it is becoming clear that protein prenylation is a critically important post-translational modification,^{1,2} and it has recently been estimated that 120 human proteins are farnesylated or geranylgeranylated as shown in Figure 1.^{3,4} Protein prenylation is now an issue of significant scientific and medical importance, due in large part to the development and potential use of farnesyltransferase inhibitors (FTIs) as anti-cancer agents.^{5,6} This medical relevance has led to increased interest in other aspects of the chemical biology of protein prenylation, including the synthesis of prenylated peptides as probes of the function of protein prenylation,^{7,8} and the investigation of the enzymes Rce1 and Icmt,⁴ which are responsible for the proteolytic processing and C-terminal methylation of prenylated proteins. Inhibitors of Icmt are of particular interest, due to the recent report by Casey and coworkers that a small molecule Icmt inhibitor (cysmethylnil) exhibits significant *in vitro* antitumor activity.⁹ This report prompts us to report some of our own recent work in this area.¹⁰

A key characteristic of prenylated peptides and proteins is the biologically rare allyl sulfide moiety. In this paper, we report the synthesis of a racemic “all-carbon” analog of the minimal Icmt substrate *N*-acetyl-*S*-farnesylcysteine (**1**, AFC),¹¹ where the sulfur is replaced with a methylene (**2**). It is hypothesized that this replacement would lead to a compound that would have a significantly longer half-life in cell-based assays compared to AFC. This conclusion was drawn based on the chemically¹² and enzymatically^{13,14} labile nature of the allylic thioether moiety. The analog would have the additional advantage of being stable in the harshly acidic conditions employed in standard Fmoc peptide coupling schemes, facilitating the synthesis of prenylated peptides, which are quite useful for chemical biology studies on protein prenylation.^{7,8} Note that Waldmann and co-workers have developed elegant alternative routes for the synthesis of prenylated peptides.⁸ In the course of the synthetic studies on the preparation of **2**, we also synthesized *N*-acetylfarnesylglycine (**3**) as a model system. Herein we report the synthesis of **2** and **3** in racemic form, and their biochemical evaluation versus yeast Icmt. Note that previously Rando and coworkers had

prepared and evaluated AFC analogs bearing oxygen, selenium, sulfoxide, and amino substitutions for the allylic sulfur in **1**.¹⁵

We examined the synthesis of the model system *N*-acetylfarnesylglycine to optimize the amino acid coupling chemistry originally developed by O'Donnell and coworkers for use with an isoprenoid side chain.^{16,17} Earlier, unsuccessful attempts were made to prepare **2** using other amino acid synthesis procedures. We chose to utilize the direct stoichiometric deprotonation of **4** rather than the standard phase transfer protocol for operational simplicity. The protected glycine variant **4** (Scheme 1) was deprotonated using NaH as the base, followed by coupling with farnesyl bromide (**5**), to afford **6** in modest yield. Attempts were made to optimize the production of **6** by deprotonating **4** with potassium bis(trimethylsilyl)amide. Surprisingly, under these conditions the bisfarnesylated product was preferred over the desired monoalkylated product **6** at room temperature, despite the use of an excess of **4**. A mixture of the two products was obtained at 0 °C. Subsequent acidic deprotection of the benzophenone imine **6** afforded ester **7**. The free amine was then acylated to give **8**, and saponification of the farnesyl glycine gave a mixture of products, from which the desired farnesyl glycine analog **3** was obtained in modest yield. This unoptimized route provided a precedent for the synthesis of **2**, and also provided sufficient amounts of **3** for its evaluation versus Icmt.

The initial challenge in the synthesis of **2** was the conversion of farnesyl bromide (**5**, Scheme 2) into bis-homofarnesol (**10**). Attempts were made to employ a previously published homologation protocol using the Grignard reagent $\text{ClMgCH}_2\text{CH}_2\text{OMgCl}$,¹⁸ but this approach was not successful in our hands. The primary product formed was squalene, the farnesyl bromide dimer. Efforts to directly alkylate the farnesyl bromide with the potassium enolate of ethyl acetate to give **9** failed, leading to the O-alkylated product. This difficult first step was accomplished using the procedure developed by Kuwajima and Doi,¹⁹ and later utilized by Coates and coworkers.²⁰ The lithium-copper enolate of ethyl acetate was formed by deprotonation with LDA, followed by addition to anhydrous CuI. Farnesyl bromide addition then afforded the desired ester **9** as shown below in Scheme 2. DIBAL-H reduction of the ester produced bis-homofarnesol (**10**). The alcohol was then converted to the bromide, which was not purified but instead directly coupled to the anion of the glycine benzophenimine ethyl ester, to give **11** in 57% overall yield. Subsequently, the imine protecting group was removed under acidic conditions, and the free amine **12** was acetylated with acetic anhydride to give **13**. Saponification of the ethyl ester to the carboxylate gave the desired AFC analog **2**.²¹

The desthio-AFC analog **2** and the model system **3** were biochemically evaluated versus the well-characterized *Saccharomyces cerevisiae* Icmt variant (Ste14p).²² The previously reported vapor diffusion assay, with AFC (or **2/3**) as the prenylcysteine co-substrate, was utilized to measure Ste14p activity.²³ The biological activity of *N*-acetylfarnesylglycine (**3**) was examined first. This compound is not recognized by Icmt and has essentially no ability to act as either a substrate (maximal specific activity of ~4.5, compared to 700 for AFC) or an inhibitor ($\text{IC}_{50} > 1000 \mu\text{M}$). This finding emphasized the importance of the proper length of the isoprenoid moiety in its recognition by Icmt. The geranyl analog of AFC, with a C_{10} rather than C_{15} isoprenoid, also binds very poorly to Icmt due to the shortened length of the backbone and is not an Icmt substrate (data not shown). We expected poor activity for **3** based on the modest biological activity of the geranyl variant,²⁴ however the complete lack of activity of the longer “all-carbon” farnesylglycine is somewhat surprising.

The “all-carbon” AFC analog **2** was next examined as both a substrate for and inhibitor of Ste14p. It also has essentially no ability to act as a substrate, with a maximal specific activity of ~3, versus ~700 for AFC. Compound **2** is a weak inhibitor of Ste14p, with an

IC₅₀ value of 325 μ M (Figure 2), although this level of inhibition may be in part due to non-specific detergent effects on the activity of the membrane-bound Icmt. This is in sharp contrast to the behavior of the oxygen, selenium, and sulfoxide analogs of AFC, which all bind effectively to mammalian Icmt.¹⁵ Note that the racemic DL-AFC exhibits significant substrate activity, in sharp contrast to **2**, with the unnatural D-enantiomer exhibiting modest inhibitory potency ($K_i = 73 \mu$ M).²⁵ To provide a comparison with **2**, the structures and inhibitory potencies of four representative Icmt inhibitors are presented in Figure 3.

In summary, the poor substrate activity of the previously reported oxygen, selenium, and amine AFC analogs,¹⁵ coupled with the data from the all-carbon AFC isostere **2**, highlights the importance of the sulfur in the interaction of prenylcysteine moieties with Icmt. It is interesting to contrast this finding with recent model system studies, which have suggested that the thioether in methionine can be replaced with a methylene group with few negative consequences.²⁶ Enantiomerically enriched variants of **2** could be used for the synthesis of “all-carbon” prenylcysteine peptide analogs. Such analogs, which should possess enhanced synthetic and biological stability, may prove valuable biological probes for other prenyl binding proteins, such as RhoGDI²⁷ and RabGDI.^{8,28}

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References and Notes

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Figure 1.

Figure 2.

Inhibition of Ste14p activity by **2**. In vitro vapor diffusion methyltransferase activity was performed as previously described²⁰ with 33 μM *N*-acetyl-*S*-farnesylcysteine, 20 μM *S*-adenosyl-¹⁴C-*methy*]methionine, 5 μg of crude membrane preparation from His-Ste14p overexpressing cells, and increasing concentrations of compound **2**, all in 100 mM Tris-HCl, pH 7.5. The reaction was allowed to proceed for 30 minutes at 30°C. The IC₅₀ value calculated from the data above using GraphPad Prism 4 was 325 μM (95% confidence interval = 298 – 354 μM).

Figure 3. Selected IcmT Inhibitors. The K_i or IC_{50} values for the AFC sulfoxide,²⁵ FTA,²⁴ 3-isobutenylAFC,¹⁰ and cysmethanil⁹ are from the indicated literature references. a) IC_{50} value for IcmT by **16** under the conditions utilized by Casey and coworkers. b) IC_{50} value for IcmT by **16** under the conditions utilized in this study (see Figure 2 caption).

Scheme 1.

Scheme 2.