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# Synthesis of dehydroalanine fragments as thiostrepton side chain mimetics

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**Abstract**—Syntheses of dehydroalanine derivatives via a solid-support route, starting from selenocystein, and via conventional solution phase chemistry are described along with initial biological testing. The target compounds were designed as mimetics of the dehydroalanine side chain of the macrocyclic antibiotic thiostrepton that acts on the bacterial ribosome.


Thiostrepton is a cyclic peptide antibiotic that inhibits bacterial translation by blocking the function of the ribosomal GTPase center (Fig. 1A).[1] The macrocycle interacts with 23S rRNA at the GTPase-associated domain of the 50S ribosomal subunit and shuts down GTP-dependent reactions during translation. The insoluble nature of thiostrepton has prevented its broad use as an antibiotic and complicated structural studies to elucidate its interaction with the ribosomal target. Despite the fact that a total synthesis of thiostrepton has been published,[2] its complex chemical structure hampers selective derivatization. Previous synthetic efforts were focused on fragments of the macrocycle as potential lead structures for antibacterial discovery targeting the ribosome.[3] While emerging structural data suggest that the thiostrepton macrocycle might be interacting with both ribosomal S23 RNA and the L11 protein,[4] the role of the bis(dehydroalanine) side chain remains elusive. To provide model compounds for the study of the biological activity of the thiostrepton side chain, we synthesized dehydroalanine fragments **1** and **2** as mimetics of the bis(dehydroalanine) moiety (Fig. 1).

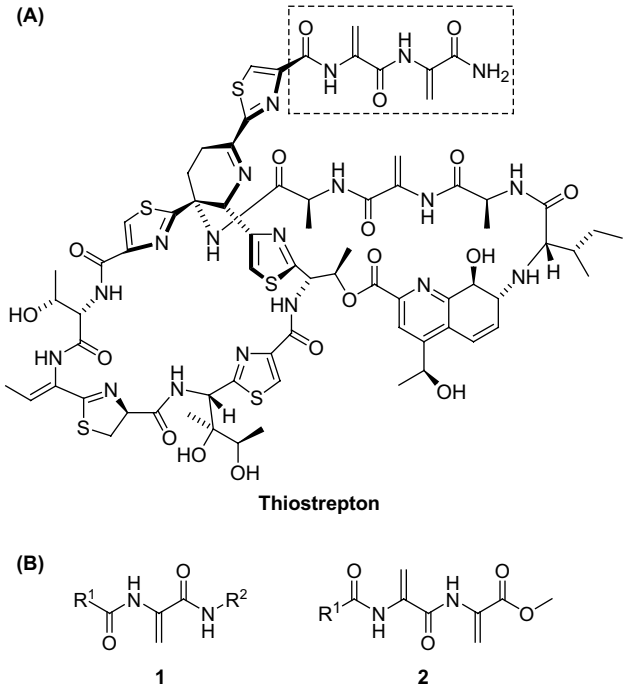


**Figure 1.** (A) Thiostrepton, a ribosome-binding cyclic peptide antibiotic first isolated from *Streptomyces azureus*. (B) Mimetics **1** and **2** of the thiostrepton bis(dehydroalanine) side chain (boxed in **A**), whose synthesis and preliminary biological testing is described in this communication.

Established routes for the synthesis of dehydroalanine and derivatives thereof include (a) conversion of masked

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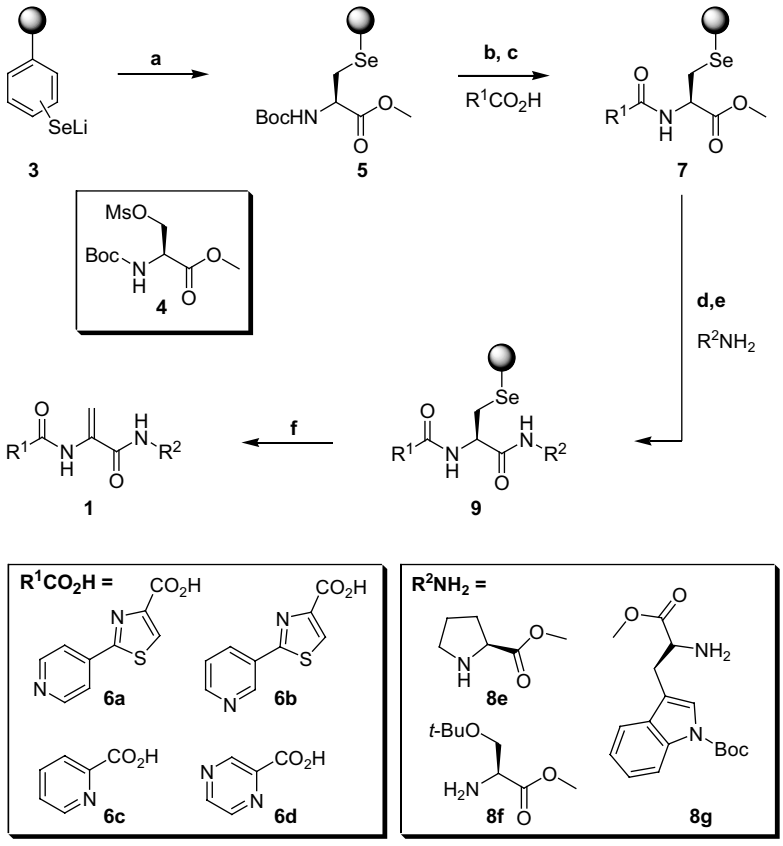
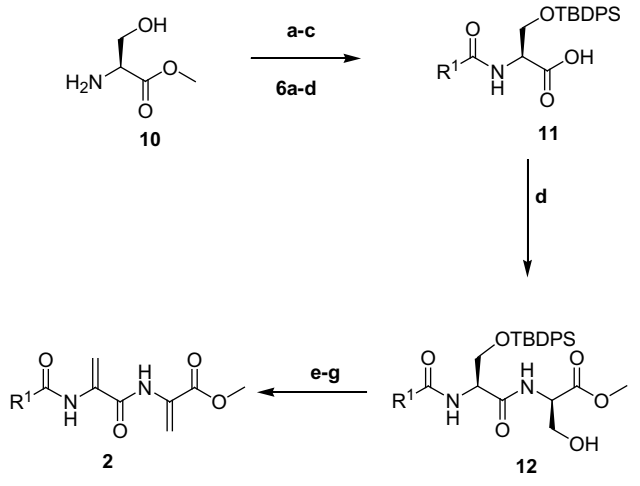



**Scheme 1.** Reagents and conditions: (a) **4** (2.0 equiv), THF (0.22 M), 2 h, 23 → 50 °C; (b) 55% TFA in $CH_2Cl_2$, 2 h, 23 °C; (c) DIC (6.0 equiv), HOBt (7.0 equiv), $R^1CO_2H$ (**6a**–**d**, 4.0 equiv), DMF (0.07 M), 20 h, 23 °C; (d) LiOH (10.0 equiv), THF/$H_2O$ (5:1, 0.47 M); (e) DIC (5.0 equiv), HOBt (5.0 equiv), $R^2NH_2$ (**8e**–**g**, 3.0 equiv), DMF (0.07 M), 20 h, 23 °C; (f) $H_2O_2$ (30% aq, 6.0 equiv), $Me_2S$ (10.0 equiv), 1 h, 23 °C. THF = tetrahydrofuran; TFA = trifluoroacetic acid; HOBt = 1-hydroxybenzotriazole; DMF = *N*,*N*-dimethyl-formamide; DIC = 1,3-diisoproylcarbodiimide.

serine and threonine residues, either as mesyloxy intermediates or halides,[5] (b) direct elimination of β-hydroxy groups,[6] via asparagine residues, and (c) Hoffmann elimination of 2,3-diaminopropionic acids.[7] Few examples have been reported using phenyl selenocysteine to promote site specific and chemoselective introduction of dehydroalanine residues.[8] In this communication, we report the use of selenocysteine as a solid phase selenium linker for peptide synthesis that, upon oxidative cleavage from the solid support, yields the dehydroalanine functionality by elimination.

Selenium-functionalized resin **3**, obtained following a protocol reported by Nicolaou and co-workers,[9] was alkylated with 2-amino-3-methanesulfonyloxy-propionic acid methyl ester **4** to yield solid-supported selenocysteine precursor **5** (Scheme 1).

Methyl ester **4** was synthesized from L-serine methyl ester **10** (Scheme 2) by treatment with di-*tert*-butyl dicarbonate followed by mesylation of the serine hydroxy group.[10] The efficiency of resin loading was determined by oxidative cleavage ($H_2O_2$/$Me_2S$) of an aliquot of the alkylated intermediate **5**, followed by NMR quantitation using 2,5-dimethylfuran as internal reference.[11] Acidic removal of the Boc protecting group, followed by coupling with various acids (**6a**–**d**) proceeded under standard conditions (DIC, HOBt) to produce amides




**Scheme 2.** Reagents and conditions: (a) $R^1CO_2H$ (1.0 equiv), BOP (1.1 equiv), *i*-$Pr_2NEt$ (2.2 equiv), $CH_2Cl_2$ (0.12 M), 3 h, 23 °C; (b) TBDPSCl (1.2 equiv), imidazol (2.2 equiv), THF (0.07 M), 3 h, 0 → 23 °C; (c) LiOH (2.0 equiv), MeOH (0.11 M), 1.5 h, 23 °C; (d) **10** (1.2 equiv), BOP (1.2 equiv), *i*-$Pr_2NEt$ (2.2 equiv), $CH_2Cl_2$ (0.12 M), 3 h, 23 °C; (e) MsCl (6.0 equiv), $Et_3N$ (10.0 equiv), $CH_2Cl_2$ (0.04 M), 3 h, 23 °C; (f) TBAF (1.2 equiv), THF (0.05 M), 3 h, 23 °C; (g) MsCl (6.0 equiv), $Et_3N$ (10.0 equiv), $CH_2Cl_2$ (0.04 M), 3 h, 23 °C. BOP = benzotriazol-l-yl-oxy-tris-(dimethylamino)phosphonium hexafluorophosphate; TBDPSCl = 1,1-dimethylethyl-diphenylsilyl chloride; TBAF = tetrabutylammonium fluoride; for reagent abbreviations see also legend to Scheme 1.

**7**. After the C-terminal ester was hydrolyzed with lithium hydroxide, the resulting carboxylic acids were reacted with amines **8e–g** to furnish the corresponding dipeptides **9**. The final dehydroalanine products **1** were liberated from the resin by oxidative cleavage with $H_2O_2/Me_2S$. This method resulted in the best overall yields (8–32%) among all others tested, including $NaIO_4$, *tert*-butyl peroxide, and aqueous $H_2O_2$. The dehydroalanine derivatives **1** exhibited satisfactory NMR and LC–MS spectra.

The relatively low overall yields of the solid-phase synthesis were attributed to the sensitivity of the double bond in **1** under the oxidative cleavage conditions. This effect became more profound in the synthesis of the bis(dehydroalanine) analogs **2** under the same conditions, as expected from the presence of two olefinic functionalities. Specifically, a variety of oxidized products were identified by LS–MS after removal of the final compounds from the solid support. In view of these results, we decided to proceed with the synthesis of **2** through a stepwise solution phase synthesis. Consequently, the route to the bis(dehydroalanine) compounds **2** commenced with the commercially available L-serine methyl ester **10** (Scheme 2). *O*-Silylation of **10** with TBDPSCl, followed by standard coupling (BOP, *i*-$Pr_2NEt$) with acids **6a–d** (Scheme 1) and subsequent hydrolysis of the ester functionality gave access to the carboxylic acids **11**. Standard peptide coupling, as described above, afforded dipeptides **12** as the sole product. Attempts were made to subject **12**, after TBAF-mediated silyl deprotection, to a one-pot per-*O*-mesylation followed by double β-elimination of the resulting mesyloxy groups to furnish **12**. This resulted in various combinations of the mono- and di-mesylated as well as β-elimination products, which were difficult to isolate using conventional methods. Eventually, *O*-mesylation of **12** in the presence of a slight excess of weak base ($Et_3N$) resulted in β-elimination of the corresponding mesyloxy group. Cleavage of the silyloxy group with TBAF, followed by mesylation and β-elimination afforded the final products **2** in good overall yield. The bis(dehydroalanine) derivatives **2** exhibited satisfactory NMR and LC–MS spectra.

To assess the biological activity of the synthesized dehydroalanine derivatives, they were tested for their activity as inhibitors of bacterial in vitro translation (Table 1).[12] All but one of the bis(dehydroalanine) compounds **2** had very poor solubility in 5% DMSO, which prevented their testing in the translation assay. Ten of the dehydroalanine derivatives **1** were soluble enough to allow testing of their inhibitory activity at one compound concentration. The limited solubility prevented collection of dose–response data at higher concentrations for the determination of $IC_{50}$ values.

Among the tested compounds only **1**–(**6b/8g**) showed any appreciable activity as translation inhibitor with an estimated $IC_{50}$ of 40 μM. Interestingly, the related derivative **1**–(**6c/8g**), which was missing the thiazole spacer between the dehydroalanine and aryl groups, was about 10-fold less active. The pyrazine derivative

**Table 1.** Activity of dehydroalanine fragments as in vitro translation inhibitors

| Compounds **1** | | % Inhibition[a] | Concentration (μM) |
|---|---|---|---|
| $R^1$ | $R^2$ | | |
| **6a** | **8f** | 5 | 150 |
| **6b** | **8f** | 30 | 400 |
| **6b** | **8e** | 20 | 400 |
| **6b** | **8g** | 20 | 15 |
| **6c** | **8e** | 4 | 400 |
| **6c** | **8f** | 40 | 400 |
| **6c** | **8g** | 20 | 150 |
| **6d** | **8e** | 25 | 400 |
| **6d** | **8f** | 15 | 400 |
| **6d** | **8g** | 10 | 150 |
| Compound **2**, $R^1$ = **6d** | | 30 | 400 |
| Thiostrepton | | 50 | 0.17 |

[a] Inhibition calculated as means of three replicate experiments for each compound (±10%).

**1**–(**6d/8g**) showed about 20-fold less activity. The fact that relatively subtle variations in the substituent at $R^1$ cause significant changes in compound activity suggests that further chemical exploration at this site might lead to more potent dehydroalanine translation inhibitors.

In conclusion, we have described the synthesis of two series of dehydroalanine fragment series, **1** and **2**, as mimetics of the linear side chain of the peptide antibiotic thiostrepton. A solid-supported synthetic route, starting from selenocysteine, was used to produce the dehydroalanine derivatives **1**, and conventional solution-phase synthesis afforded the bis(dehydroalanine) series **2**. Similar to thiostrepton itself, the dehydroalanine derivatives displayed low solubility in aqueous media, which interfered with a full assessment of their biological activity. Nevertheless, one compound was discovered that showed activity as an inhibitor of bacterial translation. Future efforts might aim at improving solubility of the thiostrepton side chain mimetics starting with the active translation inhibitor **1**–(**6b/8g**) as a lead structure.

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