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A Short Total Synthesis of (+)-Furanomycin

Michael Pete VanBrunt and Robert F. Standaert*

Texas A&M University, Department of Chemistry, P.O. Box 30012, College Station, Texas 77842-3012

standaert@mail.chem.tamu.edu

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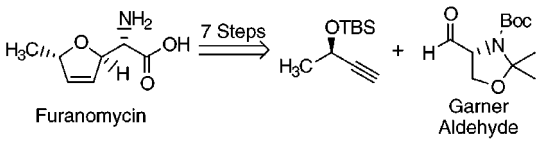
ABSTRACT



Furanomycin is a *Streptomyces* metabolite that substitutes for isoleucine in protein translation. We report a concise and modular synthesis starting from the Garner aldehyde and proceeding in seven steps to furanomycin. The key steps include a stereoselective acetylide addition and the Ag^{+}-mediated cyclization of an α-allenic alcohol to construct the *trans*-2,5-dihydrofuran. The efficiency (12% overall yield) and flexibility of the route will provide ample quantities of furanomycin and analogues for protein engineering.

Furanomycin (**1**) is an isoleucine analogue isolated by Katagiri from *Streptomyces* L-803 (Figure 1).[1] It is a substrate

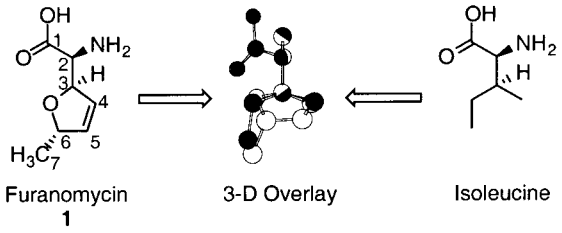


Figure 1. Superposition of furanomycin (white) and isoleucine (black).

of the isoleucyl aminoacyl tRNA synthetase ($AARS^{Ile}$) and substitutes for isoleucine in protein translation in vitro.[2] Its ability to substitute in vivo is inferred from the in vitro findings, as well as the observation that it inhibits the growth of *Escherichia coli* and a number of other bacteria but that growth inhibition is antagonized by isoleucine.[1]

Translatable amino acid analogues of this type are of great interest for the preparation of proteins containing unusual amino acids. Their incorporation into proteins, requiring simply that they be added to the cellular growth medium, has been studied for decades,[3] and in a rapidly growing number of cases, analog-bearing proteins have been isolated and have proven valuable. For instance, proteins labeled with selenomethionine in place of methionine are useful as heavy-atom derivatives for X-ray crystallography.[4] Human epidermal growth factor containing norleucine in place of its sole methionine has also been prepared,[5] as have mutants containing a variety of phenylalanine analogues.[6] Many fluorinated aromatic amino acids substitute readily and are widely employed as spectroscopic[7] and mechanistic probes (for example, by exploiting enhanced acidity and hydrogen bond donor strength of fluorinated tyrosines[8]).

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Recently, Tirrell and co-workers have begun systematic studies of translatable analogues for the preparation of designed polypeptides.[9] In addition to producing new materials, their efforts have significantly expanded the pool of useful analogues. Of particular importance is their demonstration that non-native functional groups can be installed via translatable analogues and subsequently employed for unique reactions. For instance, a repetitive polypeptide substituted with 3-thienylalanine (90% replacement for phenylalanine) was electrochemically polymerized,[9d] and another, substituted with γ,δ-dehydroproline (>90% replacement for proline) was brominated and dihydroxylated.[9f] New translatable analogues with novel functionality have the potential to allow unprecedented types of transformations to be performed on microbially expressed polypeptides.

In a similar fashion, we seek to exploit the use of furanomycin and its analogues for the mutagenesis and engineering of native proteins (as opposed to designed polypeptides). One limitation in the use of translatable analogues is a limited pool of suitable monomers. This work, by providing practical access to furanomycin and potentially many analogues, makes a significant addition to the pool.

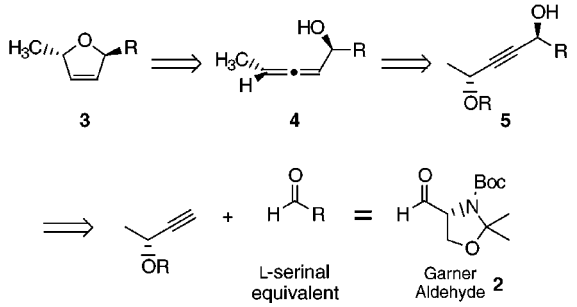
A second limitation is site selectivity. Translatable analogues substitute at all positions ordinarily filled by their cognate coded amino acids. Therefore, site-selective installation requires a background free from the cognate amino acid. In the future, this limitation may be overcome through the invention of novel tRNAs and AARSs (expansion of the genetic code).[10] For the present, it may be circumvented by careful choice of application; targets for modification may be designed, as in the work of Tirrell, or they may be selected.

In the case of furanomycin, proteins to which it may be applied for site-specific modification must be free of isoleucine. This restriction, while eliminating most proteins, leaves many interesting targets. Notable among these is human hemoglobin, in which there remains great general interest, as well as specific interest in preparing novel forms suitable for use as blood substitutes. For the latter, furanomycin and its analogues might prove particularly valuable in that their unique functionality could allow for site-specific chemical transformations not possible with coded amino acids.

In seeking to develop furanomycin as a useful tool for protein engineering, we required large quantities of furanomycin itself, as well as facile access to analogues of it. There have been three successful enantioselective syntheses of furanomycin and its isomers. Joullié reported the first synthesis, starting from acetone glucose and proceeding in nine steps to **1**, in 1980;[11] this work also provided the correct absolute and relative stereochemistry for the first time. Recently, Kang reported a 20-step synthesis starting from dimethyl L-tartrate,[12] and Clive reported an 18-step synthesis starting from L-xylose.[13] For our work, we desired a shorter synthesis, and especially one suited to the production of analogues. We wish to report the realization of these goals as a concise, efficient, modular synthesis of furanomycin in seven linear steps (eight total steps) from (*R*)-Garner aldehyde (**2**),[14] producing (+)-furanomycin in 12% overall yield.

Our analysis of furanomycin (Scheme 1) centers around its most challenging feature, the *trans*-2,5 dihydrofuran (**3**). The number of methods for preparing dihydrofurans stereoselectively is limited. In previous syntheses of furanomycin, the dihydrofuran was introduced by elimination from a substituted tetrahydrofuran. In considering alternative approaches, our attention became focused on the cyclization of allenic alcohols to dihydrofurans catalyzed by $AgNO_3$ (**4** → **3**), Scheme 1).[15] This reaction proceeds in high yield under mild conditions, is stereospecific,[16] and has been employed extensively by Marshall for the synthesis of dihydrofurans.[16,17]

Scheme 1. Retrosynthesis of Furanomycin



The lynchpin of the approach thus becomes the availability of an α-allenic alcohol **4**; we anticipated that the requisite compound could be derived via hydroxyl-directed metal

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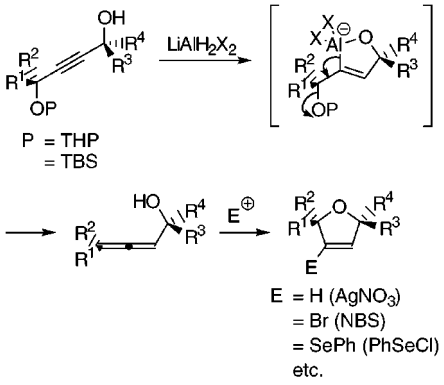
hydride reduction of a propargyl ether (**5** → **4**). Reductions of this type using $LiAlH_4$ were introduced by Landor[18] and Claesson.[19] Subsequently, Claesson showed that the reaction is highly stereoselective; it proceeds via *trans*-hydrometalation followed by *anti*-elimination of the alkoxide (Scheme 2) and affords allenic alcohols of ≥90% ee from chiral propargyl ethers.[20] With this approach to the dihydrofuran, the synthetic problem reduces to a stereoselective addition of an acetylene to a synthetic equivalent of L-serinal, for which we chose the well-studied and commercially available (*R*)-Garner aldehyde **2**.

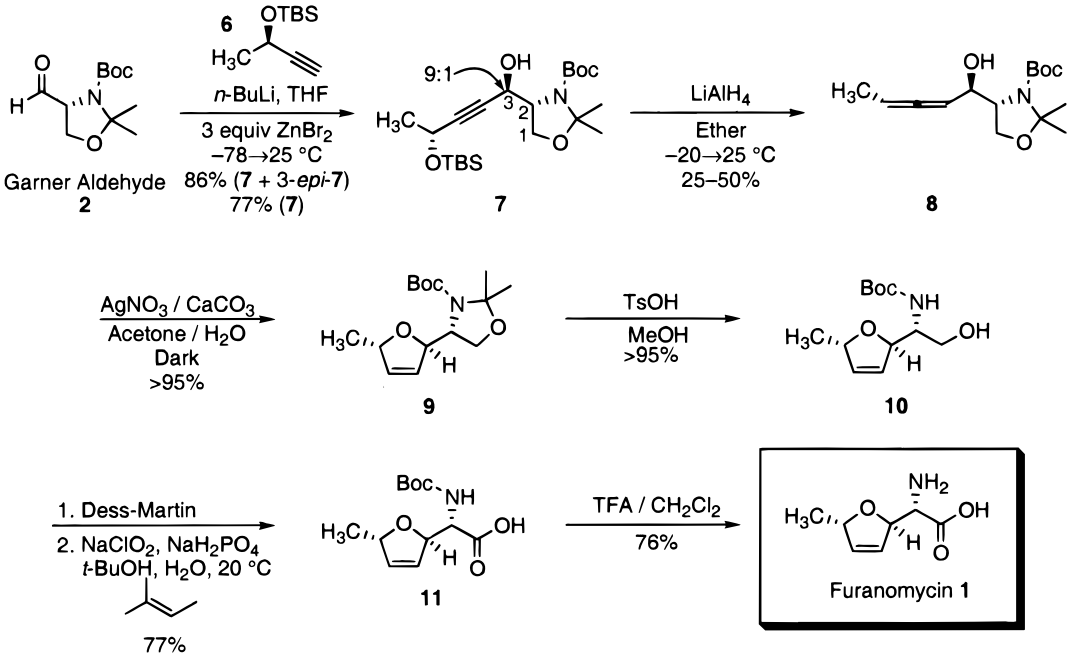
Scheme 2. Mechanism of Directed Reduction−Elimination



With this strategy, the synthesis was executed as follows (Scheme 3). Silyl propargylic ether **6** was obtained in >95% yield by silylation of commercial (*R*)-3-butyn-2-ol (Aldrich, 99% ee) with TBS−Cl/imidazole.[21] Lithium acetylide additions to Garner aldehydes proceed with high selectivity in the Felkin−Anh sense;[22,23] in the present case, the opposite selectivity was required to give the proper stereochemistry at C3. Herold[23] and others[24,25] have demonstrated that certain Lewis acids, notably zinc halides, lead to apparent chelation control and reverse the intrinsic preference of lithium acetylides for Felkin−Anh addition. Of particular relevance is the demonstration by Jurczak and co-workers[25] that TBS-protected propargyl alcohol adds to Garner aldehyde in this manner with high selectivity. Thus, acetylene **6** was lithiated (*n*-BuLi) and treated with 3 equiv of anhydrous $ZnBr_2$ at −78 °C[23] followed by Garner aldehyde to afford a 9:1 mixture of diastereomers, from which the major isomer **7** could be isolated by chromatography in 77% yield. The stereochemistry of the isomers was assigned on the basis of Jurczak's precedent, supported by close correspondence of the ^1H chemical shifts and coupling constants for the C1 and C3 protons between our two adducts and Jurczak's.[25] Subsequent conversion of the major diastereomer **7** to (+)-furanomycin confirms the assignments conclusively.

The next step was the hydroxyl-directed reduction, which proved to be the only problematic step in the synthesis. Both $LiAlH_4$ and $LiAlH_2(OCH_2CH_2OCH_3)_2$ (RedAl) were tested in several stoichiometries, but the yield never exceeded 50% and was typically closer to 25%. Best results were obtained when **7** was treated with 1.5 equiv of $LiAlH_4$ (6 equiv of hydride) in ether at room temperature. When the reaction was run in THF instead of ether, the reaction failed entirely, producing only unidentifiable products. Although the yield is less than ideal, it is in line with precedent from the literature, large amounts of material can be processed, and the product is essentially one stereoisomer as judged by ^1H NMR. The stereochemistry of the allenic alcohol was not

Scheme 3. Synthesis of Furanomycin



determined directly but was proved by its conversion to furanomycin.

Cyclization of the allenic alcohol **8** with $AgNO_3/H_2O$−acetone[15] in the dark proceeded smoothly, affording dihydrofuran **9** in 97% yield. Selective deprotection of the *O*,*N*-acetonide with TsOH/MeOH afforded in 97% yield *N*-Boc furanomycinol **10**, which was oxidized to *N*-Boc furanomycin **11** in 77% yield using the two-step sequence of Dess−Martin reagent[26] followed by $NaClO_2$,[27] in direct analogy to the procedure reported by Clive for oxidation of *N*-Cbz furanomycinol.[13] The crude *N*-Boc furanomycin, which was contaminated with 15−20% of an unidentified impurity, was deprotected with CF_3CO_2H/CH_2Cl_2. Purification of the crude amino acid by silica gel chromatography, anion-exchange chromatography (Amberlite IRA 67), and recrystallization from acetone−water provided homogeneous (+)-furanomycin in 76% yield. The 500-MHz ^{1}H NMR, melting point, and optical rotation of the final product were identical to those of the natural material; the ^{13}C NMR spectrum of furanomycin has not previously been reported. This information firmly establishes the identity of the final product, as all diastereomers of furanomycin have been prepared and can easily be distinguished from the natural product on the basis of one or more of these data.[11,28]

This synthesis of furanomycin is suited to the production of multigram quantities. With adequate material in hand, we are exploring the use of furanomycin as a translatable amino acid analogue for protein engineering. In addition, the synthesis is modular and provides independent control of all stereocenters; therefore, it can easily be adapted to many furanomycin derivatives. Inspection of the three-dimensional superposition of furanomycin and isoleucine (Figure 1) suggests C5 and C7 as particulary opportune sites for modification. The latter position may be varied at will through use of a suitable acetylene in the first step. At C5, halogens and other substituents may be installed through the use of appropriate electrophiles to induce cyclization of the allenic alcohol (Scheme 2).[16a,29]

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Supporting Information Available: Detailed experimental procedures, tabulated spectral data (^{1}H and ^{13}C NMR, IR, MS), and ^{1}H NMR spectra for all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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