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# Synthesis of L-Carbafuranomycin, an Unnatural Analogue of the Antibiotic Amino Acid Furanomycin†,‡

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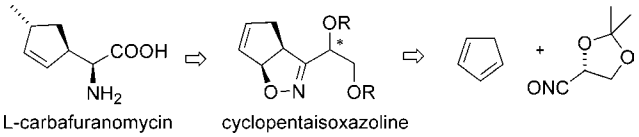
## ABSTRACT




**L-(+)-Carbafuranomycin is a novel analogue of L-(+)-furanomycin, an unusual antibiotic α-amino acid that attracted great interest due to its activity as an isoleucine antagonist. We present here a concise and efficient asymmetric synthesis of this carba-analogue starting with the 1,3-dipolar cycloaddition of a chiral nitrile oxide with cyclopentadiene. Notably, the methyl group was introduced by an $S_N2'$ cuprate substitution with high stereo- and regioselectivity.**

Furanomycin **1** is a naturally occurring antibiotic α-amino acid that was isolated from metabolites of *Streptomyces threomyceticus* in 1967 by Katagiri et al.[1] This unusual α-amino acid suppresses the growth of several bacterial species such as *E. coli*, *Bacillus subtilis*, or *Shigella* and *Salmonella* strains.[1]

Furanomycin **1** competitively inhibits the isoleucyl aminoacyl tRNA synthetase. In vitro experiments have shown that furanomycin is incorporated in proteins instead of isoleucine.[2] It is further remarkable that furanomycin is a substrate for isoleucyl tRNA synthetase, considering that the structures of isoleucine and furanomycin differ considerably. NMR studies showed, however, that the conformation of enzyme-bound furanomycin is very similar to that of isoleucine.[2] These types of translatable amino acids are of great interest for the preparation of peptides or proteins containing unusual, i.e., nonproteinogenic amino acids.[3,4]

Until now, several syntheses of **1**, both as a racemic mixture and as pure enantiomer, have been published. The starting points for most of the enantioselective syntheses were either substituted furans[5] or carbohydrates.[6] The latest reports include a Ag(I)-catalyzed cyclization of an allenic alcohol[4] and a 1,3-dipolar cycloaddition of a chiral nitrile oxide with methylfuran, respectively.[7] Apart from this, several strategies for routes to stereoisomers of **1** like 5-*epi*-furanomycin have likewise employed substituted furans[6a] or carbohydrate precursors.[8,9]

† Syntheses via Isoxazolines, Part 27. For Part 26, see ref 7b.

‡ Dedicated to Professor Günther Helmchen on the occasion of his 65th birthday.

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|| Bayer HealthCare AG.

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Concerning furanomycin analogues, syntheses of norfuranomycin,[10] cyclopentylglycine,[11] cyclopentenyl-glycine,[12] and cyclohexenylglycine[12b,13] and their biological activities have been disclosed. Norfuranomycin showed antibiotic activity against *E. coli* and several species of *Pseudomonas* strains. The other compounds also inhibited the growth of *E. coli*, but details were not reported in these papers. Recently, syntheses of a series of racemic furanomycin analogues such as norfuranomycin, the 3-, 4-, and α-methyl regioisomers of furanomycin, cyclopentylglycine, and cyclopentenylglycine were published, based on aldol additions and ring-closing metathesis or ester-enolate Claisen rearrangements.[14] According to the biological evaluation, no activities were found apart from the case of norfuranomycin, however.[14] In contrast to this, 1-cyclobutenylglycine was shown to substitute valine and isoleucine, but not leucine, in the translation of GFP in vitro.[4b]

In view of these structure−activity studies, which showed a very narrow activity profile of the natural structure, we reasoned that carbafuranomycin **2** would present only a minute and tolerable change (Figure 1).

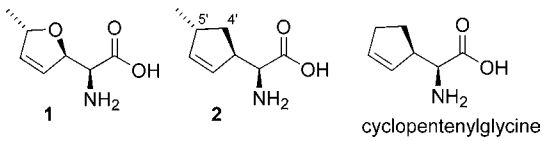
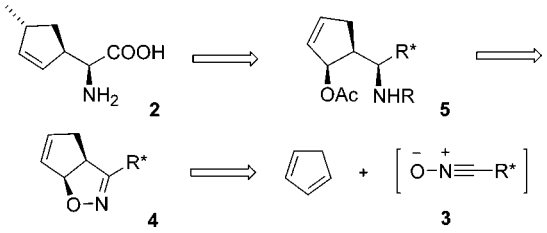



**Figure 1.** L-Furanomycin and carba-analogues.

This paper describes an enantioselective synthesis of **2** where the dihydrofuran oxygen atom has been replaced by a $CH_2$ unit, along with preliminary tests of its biological activity.

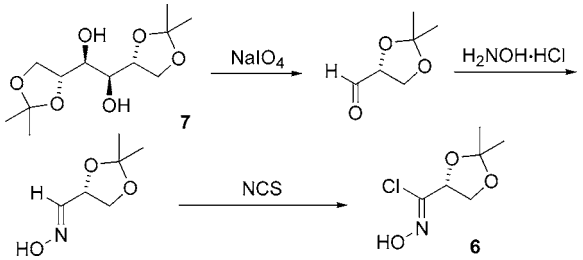
A main feature of this synthesis (Scheme 1) is the 1,3-dipolar cycloaddition of cyclopentadiene and the chiral nitrile oxide **3**. On one hand, the cyclopentene moiety of carbafuranomycin **2** is supplied by cyclopentadiene; on the other hand, the chiral cyclopentaisoxazoline **4** contains two stereogenic centers in the carbocycle, from which the two others, in the ring and in the side-chain of the target structure, were to be elaborated. Thus, the stereoselective reduction of **4** would lead to the cyclopentenol derivative **5**, the precursor for the crucial step, the introduction of the methyl group by an $S_N2'$ reaction.

**Scheme 1.** Retrosynthesis of Carbafuranomycin **2**



The required nitrile oxide precursor, the hydroximoyl chloride **6**, was obtained according to earlier work[15,16] from the mannitol bis(acetonide) **7** by periodate cleavage, oximation, and subsequent chlorination with *N*-chloro-succinimide (NCS)[16b] in 75% overall yield (Scheme 2).

**Scheme 2.** Synthesis of the Hydroximoyl Chloride **6**



The nitrile oxide was released in situ in low concentration by slow addition of triethylamine to **6**, in order to minimize furoxan formation (dimerization). In the presence of excess, freshly distilled cyclopentadiene, the dipole underwent cycloaddition to provide a diastereomeric mixture of **8** and **9** in 80% yield (dr 56:44).

Separation of **8** and **9** proved to be difficult, so diol protection was switched from the acetonide to benzyl in **10** and **11**, which were separable by MPLC. Thus, the mixture of **8** and **9** was treated with dilute trifluoroacetic acid in methanol and water (4:1) to give the diols, which were benzylated with benzyl bromide and sodium hydride in DMF in 78% yield for the two steps. At this stage, the configuration of **10** and **11** was assigned as (3a*S*,6a*S*,1′*S*)- ("anti") and (3a*R*,6a*R*,1′*S*)- ("syn"), respectively, on the basis of the specific rotations amounting to $[\alpha]_D^{20} = -36.9$ and $+135.3$.[17]

Next, the stereoselective reduction of the isoxazoline to the β-amino alcohol was envisaged, a reliable transformation well known from earlier work.[7,18] The cyclopentaisoxazoline **10** was reduced to the amino alcohol by action of lithium aluminum hydride in diethyl ether. Due to the bicyclic, bowl-shaped structure, hydride attack occurred preferentially from the sterically less hindered *exo*-side, as expected.[7,18] The free

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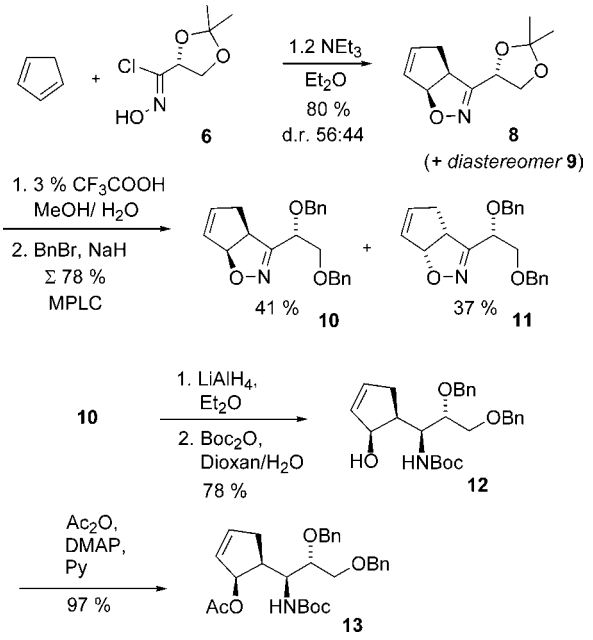
amino group was then protected with $Boc_2O$ in dioxane and water (3:1) to give the cyclopentenol **12**, plus a minor product (probably a diastereoisomer, ratio = 90:10), which was separated by MPLC (87% yield of **12** from **10**). To act as a leaving group, the hydroxy group was acetylated with acetic anhydride in pyridine with a catalytic amount of DMAP to provide the cyclopentenyl acetate **13** in 97% yield (Scheme 3).

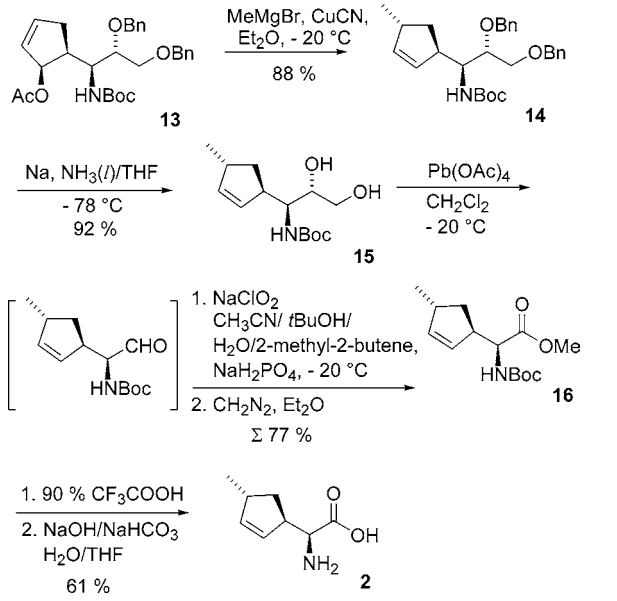
**Scheme 3.** Synthesis of the Cyclopentenyl Acetate **13** Starting with 1,3-Dipolar Nitrile Oxide Cycloaddition




For the next step, the key introduction of the methyl group, use of an organocuprate seemed promising. Organocuprates[19] are known to react with suitable cyclic systems in an $S_N2'$ fashion, i.e., nucleophilic attack at the position $\gamma$ to the leaving goup, with relocation of the double bond.[20] Furthermore, anti attack is stereochemically favored.[20] On this basis, we assumed that the required methylcyclopentenyl moiety might be obtained with the correct regio- and stereochemical preference.

The acetate **13** was added dropwise to the methyl cuprate,[21] prepared from 5 equiv of CuCN and 10 equiv of MeMgBr in diethyl ether, and provided compound **14** in 88% yield with a ratio of 88:12 (Scheme 4). The minor product,

**Scheme 4.** Synthesis of Carbafuranomycin **2**




probably the regioisomer from the competing $S_N2$ reaction, was not identified. The isomer ratio was improved from 88:12 to >95:5 by recrystallizing the diol **15** (vide infra). For this substitution, 5 equiv of the "higher order" cyanocuprate were required, probably because of the additional functional groups in **13** with complexing ability. Experiments with less cuprate proceeded too slowly or with incomplete reaction.

For the next step, twofold O-debenzylation, catalytic hydrogenation was presumed to be incompatible with the

(17) (a) Such large, consistent differences in optical rotation had been noted earlier with each one of some 60 5- and 4,5-cis-substituted isoxazolines whose absolute configuration had been established by chemical correlation and/or crystal structure analyses; see refs 7, 16a, 18b, and 18c. For example, in the furanomycin series, the respective furoisoxazolines (methylfuran adducts of the nitrile oxide generated from **6** in a 60:40 ratio) showed $[\alpha]_D^{20} = -171$ for the (3a*S*,6a*S*,1′*S*)-isomer ("anti"), corresponding to **10**, and $[\alpha]_D^{20} = +199$ for the (3a*R*,6a*R*,1′*S*)-diastereomer ("syn"), corresponding to **11**; see ref 7. (b) Müller, R. Dissertation, Universität Würzburg, Würzburg, Germany, 1992. (c) Leibold, T. Dissertation, Universität Stuttgart, Stuttgart, Germany, 1995.

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(21) To a solution of 5 equiv of CuCN in diethyl ether at −20 °C was added 10 equiv of $CH_3MgBr$ in diethyl ether (3.0 M). After 20 min at −20 °C, the acetate **13** in diethyl ether was added slowly and kept for 1.5 h at −20 °C.

C−C double bond present; therefore, sodium metal in liquid ammonia was used. Thus, at −78 °C and short exposure,[22] the benzyl ether was transformed to the diol **15** in 92% yield. The diol moiety served as a latent carboxyl group as had been exploited earlier.[23] The diol **15** was cleaved by lead tetraacetate in $CH_2Cl_2$ to afford the intermediate aldehyde, which was directly oxidized to the carboxylic acid by means of sodium chlorite ($NaClO_2$) in a phosphate buffer.[24] Despite these very weakly acidic conditions, some epimerization was observed (probably at the α-amino aldehyde stage), which required immediate conversion to the diastereomerically pure ester **16** using diazomethane (77% yield over three steps). Finally, the Boc group was removed with 90% trifluoroacetic acid, and the ester was hydrolyzed under mildly basic conditions (pH 10.5). After ion-exchange column purification (Dowex 50WX8, $H^+$ form), L-carbafuranomycin **2** was obtained in analytically and spectroscopically pure form in 61% yield from the *N*-Boc methyl ester **16**.

The structure of **2**, with the methyl substituent at C5′, and its configuration were confirmed notably by $^1$H NMR coupling constants and chemical shifts. The chemical shift difference for 4′-$H_a$ and 4′-$H_b$ in **2** was a mere 0.26 ppm, characteristic for trans-3,5-disubstituted cyclopentenes.[20k,25] The optical rotation of **2** was found to be $[\alpha]_D^{20} = +164$ ($c = 0.08$, $H_2O$), mp 215−217 °C. Antibacterial tests with **2** were performed with several bacterial species such as *E. coli*, *S. aureus*, and *B. subtilis*; no significant activity was detected, however.[26,27] The minimal inhibitory concentration (MIC) of **2** was >100 μM, with the exception of the efflux pump-deficient *E. coli* HN818 (MIC = 25 μM (12.5 μM in the presence of membrane permeabilizer polymycin B nonapeptide)).[27] This again confirms the narrow structure−activity relationship of furanomycin.[27] In summary, an efficient synthesis of the new carba-analogue of L-furanomycin is presented, featuring 1,3-dipolar nitrile oxide cycloaddition and $S_N2'$ organocuprate substitution as key steps. This strategy also would give access to the enantiomer of **2** using the minor cycloadduct **11** and to the respective cyclopentenylglycines.

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**Supporting Information Available:** Detailed experimental procedures, spectral data ($^1$H and $^{13}$C NMR, IR, elemental analysis, and MS), $^1$H NMR spectra of **10**, **12**−**16**, and **2**, and $^{13}$C NMR of **10**, **13**−**15**, and **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(22) **14** in THF was added to liquid ammonia at −78 °C, and then 10 equiv of Na was added in small pieces. The reaction was quenched after a permanent blue color appeared (ca. 5 s later). This kind of careful handling was necessary to avoid coreduction of Boc-group, observed on a small scale (<0.1 mmol).

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(26) Biological evaluation was done at Bayer HealthCare AG, Wuppertal; cf. ref 14.

(27) The lack of toxicity of the carbafuranomycin does not mean nontranslatability; this may be elicited through further testing of aminoacyl tRNA synthetase assays. We are grateful to one of the referees for this comment/suggestion.