Synthesis of the Ezomycin Octosyl Nucleoside

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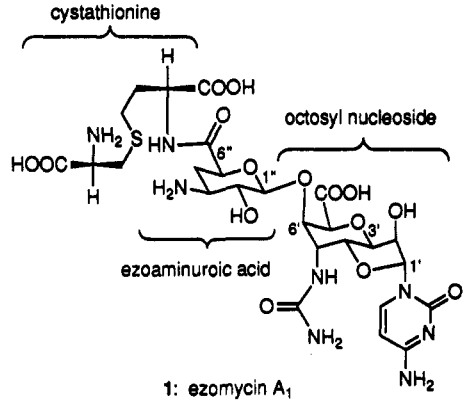
Summary: Efficient chain extension of β-D-galactopyranosyl cyanide, cyclization to a bicyclic octose, and then attachment of cytosine affords the ezomycin octosyl nucleoside **17** in a form suitable for elaboration to the ezomycins (*e.g.*, **1**).

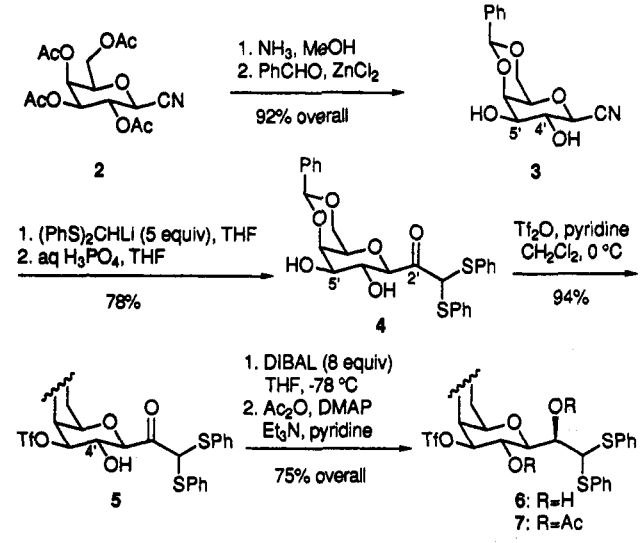
The ezomycins are a class of complex nucleoside antibiotics[1] isolated from a *Streptomyces* fermentation broth. Several representatives exhibit antimicrobial activity against certain species of phytopathogenic fungi, such as *Sclerotinia* and *Botritis*.[2] Following degradation studies and spectroscopic characterization of the components,[3] one of the active members, ezomycin A_1 (**1**), was shown to consist of a bicyclic octosyl nucleoside glycosylated at C-6′ by a glucuronic amino acid, ezoaminuronic acid, which is further linked at C-6″ to the pseudodipeptide cystathionine. Simpler members lack the cystathionine (ezomycin A_2), or have uracil instead of cytosine as the pyrimidine base (ezomycin B_1 and B_2).



1: ezomycin A_1

While most of the challenges associated with the synthesis of the cystathionine[4] and ezoaminuronic acid components[5] have now been met, the octosyl nucleoside has proven to be much more of an obstacle.[6–11] We report here the efficient and stereocontrolled synthesis of an octosyl nucleoside **17** that is appropriately functionalized for further transformation into **1** and its relatives.

Scheme 1. Chain Elongation of β-D-Galactopyranosyl Cyanide



2 **3** **4** **5** **6**: R=H **7**: R=Ac

We have viewed the ezomycin octosyl nucleoside as a chain-extended D-gulopyranoside rather than a chain-extended cytidine, requiring the addition of two (rather than three) carbons and control of two off-pyranose (rather than three off-furanose) stereocenters. Scheme 1 shows the elaboration of tetra-*O*-acetyl-β-D-galactopyranosyl cyanide (**2**), which can itself be made by treating galactose pentaacetate with trimethylsilyl cyanide and boron trifluoride,[12] into the octose dithioacetal **7**. Ammonolysis of the acetates and installation of a benzylidene protecting group gave the cyano diol **3**, which was chain-extended by addition of excess [bis(phenylthio)methyl]lithium and then hydrolysis of the intermediate imine.[13] The excess organolithium reagent serves to deprotonate the hydroxyls at C-5′ and C-4′ (ezomycin numbering), thus protecting the latter *in situ* against elimination.[14] Several other approaches that involved protecting the hydroxyls as silyl ethers led instead to an α,β-unsaturated nitrile product.

The requirement for stereoselective reduction of the C-2′ ketone **4** to the (*R*)-carbinol stimulated the devel-

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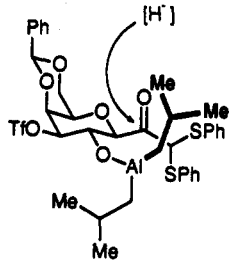
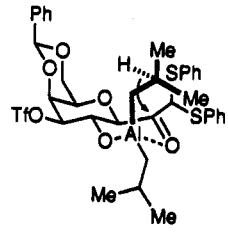
opment of a unique solution. Initial attempts to block the front face of the carbonyl with a protecting group at C-4′ so as to favor approach of a reducing agent from the back face[15] were thwarted by the low reactivity of the C-4′ hydroxyl toward electrophiles and by the tendency of the group at C-4′ to eliminate under basic conditions. However, as a reflection of the much greater reactivity of the C-3 hydroxyl in β-galactopyranosyl 2,3-diols toward electrophiles,[16] keto diol **4** could be selectively monoprotected at C-5′. In a rare example of the use of trifluoromethanesulfonyl as a blocking group, **4** was efficiently converted[17] to the monotriflate **5**, which not only left the C-4′ hydroxyl available for reaction but also set the stage for eventual S_N2 displacement at C-5′ by a nitrogen nucleophile. Reaction of **5** with excess diisobutylaluminum hydride[18] at −78 °C gave exclusively the required (*R*)-carbinol **6**, and acetylation of **6** gave its diacetate **7** (the stereochemistry at C-2′ was certified later in the synthesis). The stereoselective reduction can be formulated as occurring through a C-4′ aluminate derivative **8A**, which effectively shields the front face of the carbonyl, at least when C-1′ is in the extended conformation shown. Whereas intermolecular delivery of hydride to a C-4′ *O*-(hydridoaluminate)carbonyl-chelated intermediate[18] **8B** ought to lead



8A

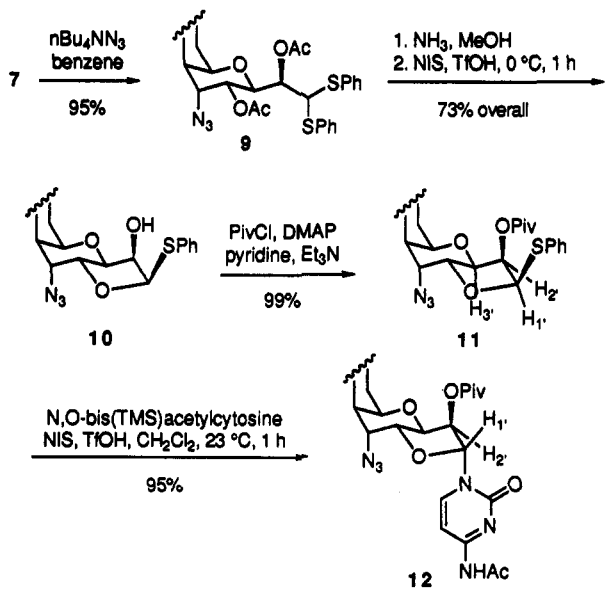


8B

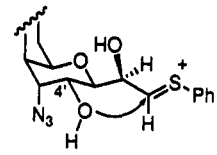
to the other stereoisomer, the alternative intramolecular delivery of hydride from the isobutyl carbon β to aluminum in **8B** (with complexation of the carbonyl oxygen to aluminum and loss of isobutylene[19]) could also account for the observed stereoselectivity. By comparison, reduction of keto diol **4** with DIBAL gave poorer stereoselectivity, and sodium borohydride reduction of **5** gave a 1:1 mixture of epimeric diols. The use of fewer equivalents of DIBAL led to incomplete conversion of **5** to **6**.

Scheme 2 shows the preparation of the bicyclic nucleoside. Treatment of triflate **7** with tetra-*n*-butylammonium azide[20] gave the azido diacetate **9** in high yield. Although some pyranoside diol monotriflates can be converted to the corresponding azido alcohols by direct S_N2 displacement with azide,[21] protection of the hydroxyls was necessary in this case to block competing ring contraction of the (cis-fused) 4,6-*O*-benzylidenepyranoside.[21] Ammonolysis of the acetates and then treatment

Scheme 2. Preparation of the Bicyclic Nucleoside



of the resulting diol dithioacetal with *N*-iodosuccinimide and triflic acid[22] gave the bicyclic thioglycoside **10** as a single isomer. Protection of the hydroxyl with pivaloyl, an excellent C-2′ participating group,[23,24] led to **11**. The stereochemistry assigned to **11**, and thereby also its precursors, is based on comparison of the vicinal proton coupling constants observed for H-1′/H-2′ (4.4 Hz) and H-2′/H-3′ (4.6 Hz) with values calculated for the four possible C-1′/2′ isomers by the MacroModel program (Amber forcefield parameter).[25] The stereochemical result upon ring closure at C-1′ therefore cannot be accommodated by formation of an intermediate 1′*R*,2′*R* epoxide[26] followed by inversion at C-1′. However, it can be rationalized by assuming the formation of an *S*-phenylthionium intermediate in an extended conformation (**13**), which suffers attack by the C-4′ hydroxyl at the more accessible π face.



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Attachment of acetylcytosine was accomplished by using the procedure developed earlier for the synthesis of nucleosides from thioglycosides.[27] Thus, treatment of a mixture of **11**, *N*(4),*O*-bis(trimethylsilyl)-*N*(4)-acetylcytosine, and *N*-iodosuccinimide with trifluoromethanesulfonic acid led to the nucleoside **12** as a single isomer within 1 h at room temperature. Assignment of **12** as the β-nucleoside follows from chemical precedent[24,27] and from

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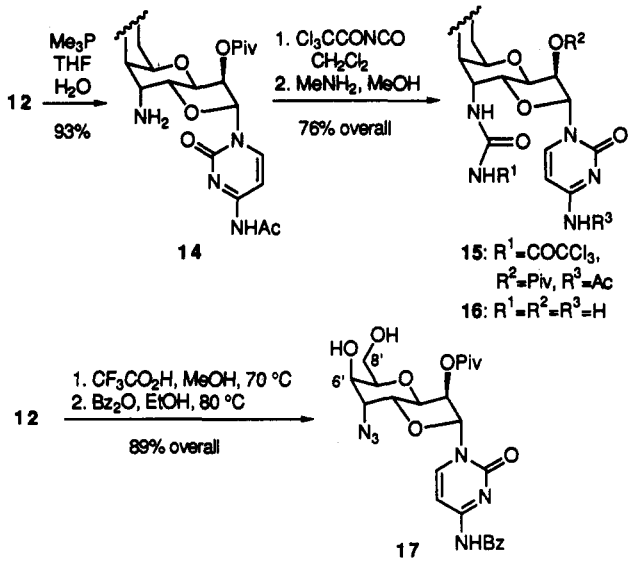
Scheme 3. Further Octose Modifications



the absence of an observable H-1′/H-2′ coupling constant, which indicates an HCCH dihedral angle of about 83° (compare **11**, ~43°). The yield for this N-glycosylation (95%) represents an improvement over previous examples of pyrimidine attachment to higher sugars.[1,6,10,11,28] The overall yield for the synthesis of nucleoside **12** from **2** is about 33% (~12 steps).

Some further modifications of the nucleoside **12** that are relevant to the synthesis of **1** and/or its congeners are displayed in Scheme 3. Conversion of the azido group to the ureido substituent present in the natural products was accomplished by trimethylphosphine-mediated reduction[29] of **12**, followed by condensation with trichloroacetylisocyanate.[30] Methanolic methylamine[31] removed the three acyl protecting groups from **15** to furnish the ureido octosyl nucleoside **16**. Alternatively, the 6,8-*O*-benzylidene protecting group was removed by acidic methanolysis,[32] which also deacetylated N-4. Reprotection of N-4 as the benzamide[33] provided diol **17**, a possible candidate for protection at the C-8′ hydroxyl and subsequent glycosylation at C-6′.

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Supplementary Material Available: Experimental procedures and spectral data for the preparation of new compounds through **17** (6 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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