

**Preparation of the 4-Ethylamino Sugar of Calicheamicin:
 Assignment of Absolute Configuration.**

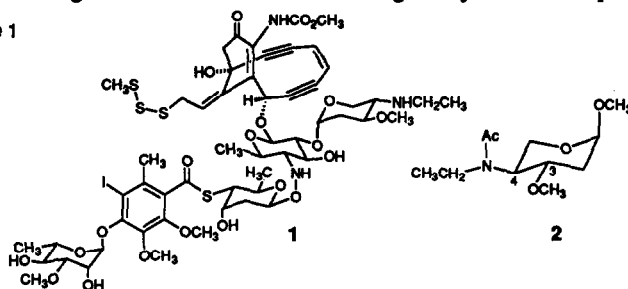
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Summary: The absolute configuration of the 4-ethylamino sugar of calicheamicin is established to be 3*S*,4*S* by comparison with material obtained from asymmetric synthesis from L-serine.

Calicheamicin γ 1 (1, Figure 1) is a potent antitumor antibiotic that cleaves DNA site specifically.¹ The cleavage mechanism is thought to involve a thiol mediated Bergman cyclization that produces a phenylene

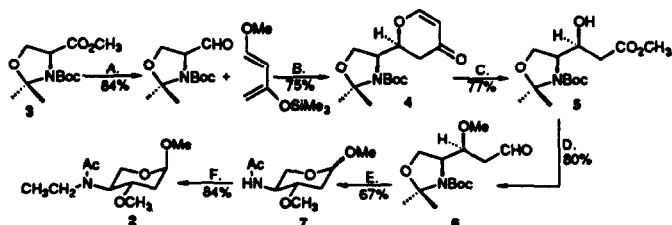
Figure 1



diradical which abstracts hydrogen atoms from the DNA backbone.^{1,2} While the origin of the cleavage specificity is not understood, there is mounting evidence that the oligosaccharide portion of calicheamicin plays an important role^{1b,3} and this has stimulated interest in its construction. However, the initial structural studies on calicheamicin did not establish the absolute configuration of the 4-ethylamino sugar.⁴ We have therefore determined the configuration of this sugar to be 3*S*,4*S* by comparison of a degradation product (2) of calicheamicin⁴ with material obtained by synthesis from L-serine.

Our route, which is patterned on the work of Danishefsky⁵ and Garner⁶ for the construction of 1,2 amino alcohols, begins with the readily available L-serine derivative 3 (Scheme 1). Diels-Alder reaction of

Scheme 1



A. DIBAL-toluene, -78 °C. B. ZnCl₂-CH₂Cl₂. C. a) NaIO₄-RuO₄ (cat.)-CCl₄:CH₃CN:H₂O = 2:2:3 b) 1. K₂CO₃-MeOH 2. 1N HCl c) CH₂N₂-Et₂O. D. a) CH₃OTf-2,6-di-*tert*-butyl-4-methylpyridine-CH₂Cl₂, 80 °C b) DIBAL-CH₂Cl₂, -78 °C. E. a) TsOH-MeOH-ZnCl₂, 60-70 °C b) Ac₂O-Pyr. F. Et₃NH-KOH-DMSO

Danishefsky's diene with the aldehyde obtained from DIBAL reduction of **3** produced dihydropyrone **4**. Oxidative degradation, hydrolysis of the formyl group and esterification with diazomethane cleanly produced β -hydroxy ester **5**. Methylation of the secondary alcohol followed by DIBAL reduction of the ester produced the β -methoxy aldehyde derivative **6**. Subsequent deprotection in acidic methanol produced a 3:1 (axial : equatorial) mixture of methyl pyranosides which were isolated as the N-acylated derivatives **7**.

After separation of the anomeric mixture of **7**,⁷ the α (axial) isomer was alkylated to give monosaccharide **2**. The proton NMR spectrum of this synthetic material was identical to natural material obtained from degradation of calicheamicin.⁸ The optical rotation of synthetic **2** in chloroform was determined to be $[\alpha]^{20}_{\text{D}} -96.0^{\circ}$, c 0.9, CHCl_3 . The β (equatorial) isomer of **7** was also alkylated and the optical rotation was determined to be $[\alpha]^{20}_{\text{D}} +99.2$, c 1.15, CHCl_3 .⁹

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7. The α (axial) and β (equatorial) methyl glycosides of **7** were separated by flash chromatography on silica gel (5% MeOH-EtOAc, $R_f \alpha = 0.3$, $R_f \beta = 0.2$). Separation at this stage is necessary because **2** and its corresponding β epimer cannot be separated.
8. a) All new compounds were characterized by ^1H and ^{13}C NMR and high resolution mass spectral analysis; b) α -methyl glycoside **2** as well as the corresponding β isomer showed two sets of proton resonances due to restricted rotation around the amide bond; ^1H NMR data for **2** (δ , CDCl_3 , 270 MHz): 4.78 (s, 1H), 4.06-4.02 (br t, 1H), 3.70-3.08 (m, 11H), 2.39-2.29 (br t, 1H), 2.14, 2.10 (2 s, 3H), 1.56-1.49 (m, 1H), 1.22-1.09 (2 t, 3H). ^{13}C NMR data for **2** (δ , CDCl_3 , 270 MHz): 171.38, 170.93, 99.02, 98.96, 72.83, 71.95, 59.97, 59.87, 59.32, 58.04, 58.01, 56.41, 55.52, 54.92, 54.71, 42.12, 36.86, 35.41, 22.26, 15.35, 14.71.
9. Insufficient material of *natural 2* (i.e., the pure α anomer used for the NMR studies) was obtained to take an optical rotation. However, a 7:3 mixture of *natural 2* and its corresponding β anomer obtained from a large scale degradation of calicheamicin gave a specific rotation of $[\alpha]^{20}_{\text{D}} -40^{\circ}$, c 0.627, CHCl_3 . This is identical to the rotation calculated for a 7:3 mixture using the rotations of the pure *synthetic* anomers.

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