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SEMISYNTHETIC MODIFICATION OF HYGROMYCIN A. 3. SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF AMINOCYCLITOL ANALOGS.[1]

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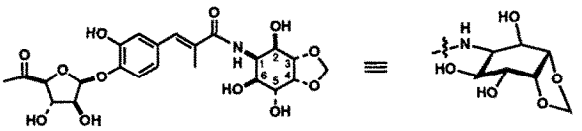
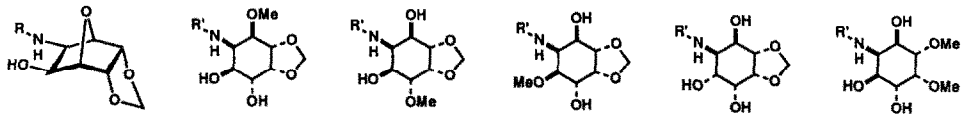
Abstract: A series of aminocyclitol-modified analogs of hygromycin A has been prepared, including three derivatives exploring the effect on biological activity of methylation of each of the three hydroxyl groups. Antibacterial activities against *Serpulina hyodysenteriae* and *Pasteurella multocida*, two important animal health pathogens, are presented.

The newly discovered efficacy of hygromycin A[2] (**1**) in the treatment of swine dysentery[3] has led to heightened interest in this fermentation-derived natural product, including a recent total synthesis.[4] As part of our program to discover new antibacterial agents for use in animal health, we sought to prepare analogs of **1** which would be useful in the treatment of swine dysentery as well as other infectious diseases. Other reports from our laboratories have described the preparation of vinyl methyl analogs,[5] simple amide analogs,[5] aryl analogs,[1] and aminocyclitol analogs lacking the methylenedioxy ring.[6] This report describes the selective functionalization of the hygromycin A aminocyclitol, allowing the preparation of a series of analogs **2**-**7** which explore the effects of substitution at each position.

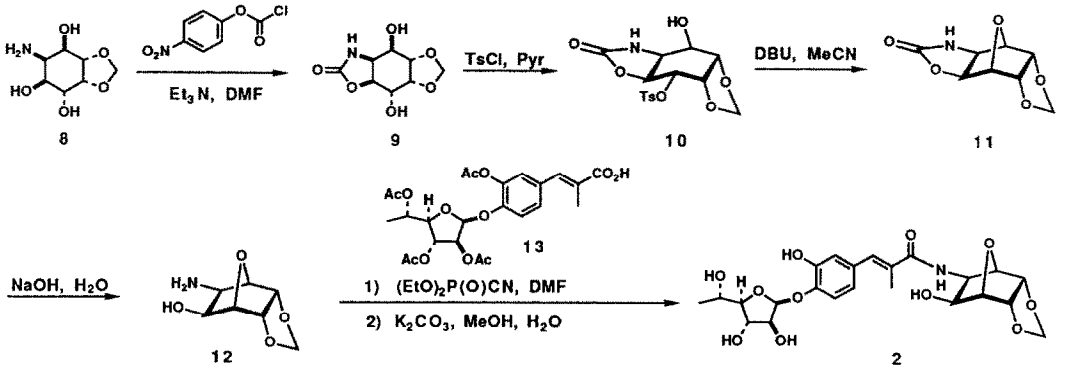


Hygromycin A (1)



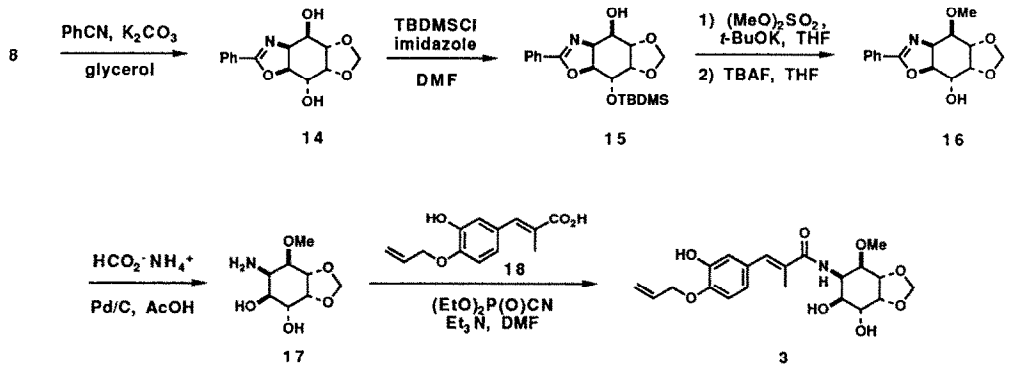
2 3 4 5 6 7

Our previous work,[6] in combination with the results of Chida *et al.* in their studies of methoxyhygromycin,[7] made clear to us the importance for biological activity of the twist-boat conformation[8] of the aminocyclitol, induced by the methylenedioxy ring. One of our early targets was oxo-bridged analog **2**, which adopts the desired conformation. The isolation of aminocyclitol **8** by degradation of hygromycin A has been described earlier.[8] Treatment of **8** with *p*-nitrophenyl chloroformate and triethylamine in DMF affords cyclic carbamate **9** with complete regioselectivity (69%).[9] Tosylation of **9** with *p*-toluenesulfonyl chloride in pyridine affords exclusively tosylate **10** (67%). Exposure of **10** to DBU in acetonitrile at room temperature causes clean cyclization, giving bridged intermediate **11** (87%). The cyclic carbamate protecting group is removed by treatment with 1*N* NaOH at 95 °C (81%). The resulting amine **12** is coupled using diethyl cyanophosphonate with carboxylic acid **13**, the preparation and use of which was described in our earlier report.[5] Treatment of the crude amide with potassium carbonate in methanol/water affords the desired derivative **2**, in 76% yield from **12**.

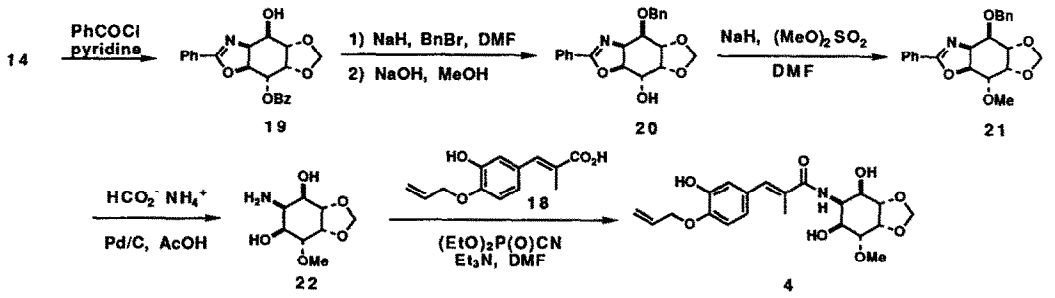


Subsequent to the preparation of compound **2**, it was discovered[10] that the aryl sugar substituent could be replaced by an allyl ether without loss of biological activity. Therefore, for ease of synthesis our later targets contained the allyl ether group. We desired to prepare a series of O-methyl derivatives of the aminocyclitol in order to explore the effects of substitution at each position.

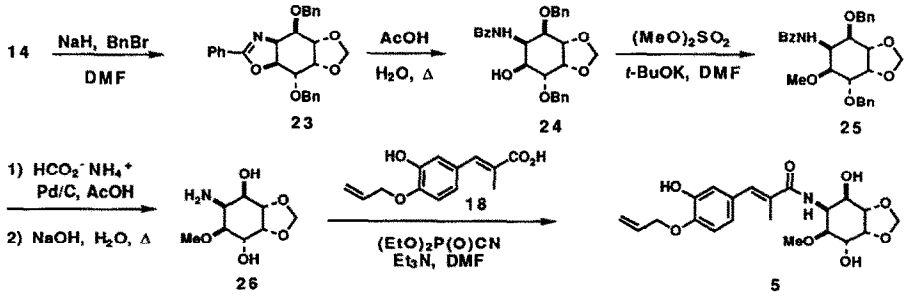
The preparation of phenyloxazoline **14** from aminocyclitol **8** is described in our earlier report.[6] Selective protection of the 5-hydroxyl as the *t*-butyldimethylsilyl ether **15** is effected using *t*-butyldimethylsilyl chloride and imidazole in DMF (67%). Methylation with dimethyl sulfate (potassium *t*-butoxide, THF; 62%) is followed by silyl group removal using tetrabutylammonium fluoride in THF, affording methyl ether **16** (96%). Removal of the phenyloxazoline protecting group is accomplished by transfer hydrogenation,[11] affording **17** (47%); coupling of **17** to carboxylic acid **18**[12] using diethyl cyanophosphonate affords 2-O-methyl analog **3** (56%).



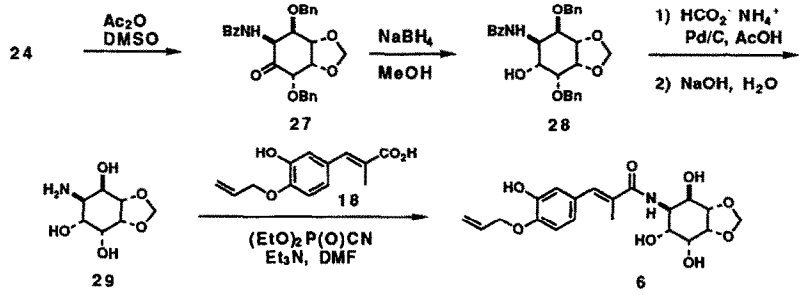
Treatment of phenyloxazoline **14** with benzoyl chloride in pyridine affords predominantly benzoate **19** (42%). Alkylation of **19** with benzyl bromide (NaH, DMF; 70%) followed by ester cleavage (NaOH, MeOH; 52%) provides 2-O-benzyl ether **20**. Alkylation with dimethyl sulfate gives methyl ether **21** (65%), which upon transfer hydrogenation affords amine **22** (51%). Coupling with diethyl cyanophosphonate provides 5-O-methyl analog **4** (46%).



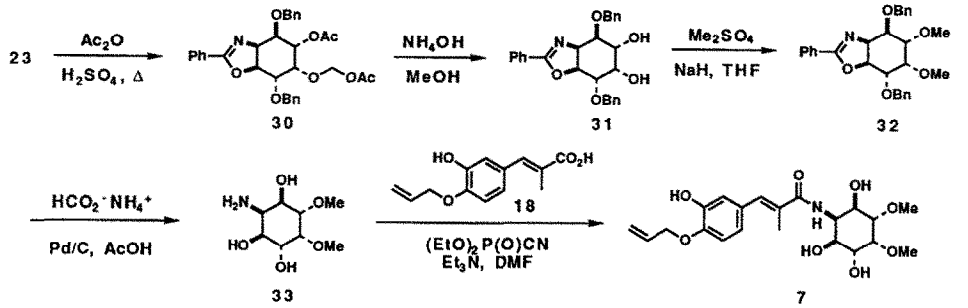
Alkylation of phenyloxazoline **14** with benzyl bromide (NaH, DMF) affords bis-benzyl ether **23** (77%). The oxazoline is hydrolyzed to benzamide **24** by heating in aqueous acetic acid (60%). Methylation of the resulting free hydroxyl group is accomplished using dimethyl sulfate (potassium *t*-butoxide, DMF), affording **25** (70%). The benzyl ether is removed by transfer hydrogenation (48%), and the benzamide is cleaved with 1*N* aqueous sodium hydroxide at reflux, providing amine **26** (60%). Coupling as before with carboxylic acid **18** affords 6-O-methyl analog **5** (29%).



Inversion of the 6-hydroxyl group is accomplished as follows. Oxidation of alcohol **24** with acetic anhydride in DMSO[13] affords ketone **27** (73%), which is reduced to inverted alcohol **28** by treatment with sodium borohydride in methanol (88%). Removal of protecting groups as described above for compound **25** provides amine **29** (44%), which upon coupling with carboxylic acid **18** affords 6-epi analog **6** (31%).



The conversion of bis-benzyl ether **23** *via* diacetate **30** to diol **31** was described in our earlier report.[6] Methylation of both free hydroxyl groups is accomplished using dimethyl sulfate (NaH, THF), giving **32** (61%). Transfer hydrogenolysis of the benzyl ether and phenyloxazoline protecting groups affords amine **33** (41%), which upon coupling with carboxylic acid **18** affords 3,4-di-O-methyl ether **7** (43%); compound **7** represents a 3-O-methyl analog of methoxyhygromycin.[7]



The minimum inhibitory concentrations (MIC's) of the cyclohexylamide analogs against *Serpulina (Treponema) hyodysenteriae* and *Pasteurella multocida* are shown in the table below.[14]

Compound	*S. hyodysenteriae* (μg/ml)	*P. multocida* (μg/ml)
1 (hygromycin A)	1.56	1.56
2	>200	>200
3	6.25	12.5
4	25	100
5	3.13	12.5
6	3.13	12.5
7	100	>400

Since compound **2** adopts the same conformation as the hygromycin A aminocyclitol, its lack of biological activity suggests that the presence of either the 2-hydroxyl or the 5-hydroxyl (or both) is required. Methylation of the 2-hydroxyl (compound **3**) results in a 4- to 8-fold loss in potency relative to **1**; methylation of the 5-hydroxyl (compound **4**) causes a 16- to 64-fold loss in potency. The 6-position is the least sensitive to modification, in that both methylation and epimerization (compounds **5** and **6**, respectively) result in only a 2- to 8-fold loss in potency. The results of compound **7** reinforce the notion (*vide supra*) that the methylenedioxy ring is required in order to induce the proper conformation of the aminocyclitol moiety.

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