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SEMISYNTHETIC MODIFICATION OF HYGROMYCIN A. 1. SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF VINYL METHYL AND AMIDE ANALOGS.

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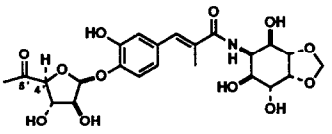
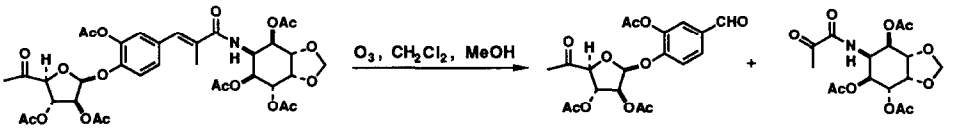
Abstract: Formation of the peracetate of hygromycin A, followed by sodium borohydride reduction and oxidative cleavage, affords an aldehyde which serves as a key intermediate for preparation of vinyl methyl and amide analogs. Several analogs of each type were prepared and tested for activity against *Serpulina hyodysenteriae*, the causative organism of swine dysentery.

Hygromycin A (**1**) is a fermentation-derived natural product[1] with modest antibacterial activity, which was only recently discovered to be efficacious in the treatment of swine dysentery.[2] The heightened interest generated by this finding has led to a recent total synthesis.[3] 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. Our overall approach was directed at variation of the sugar, aryl cinnamide, and aminocylitol portions of **1**; this report describes the preparation and biological activity of hygromycin A analogs that are modified at the vinyl methyl group and aminocyclitol amide.



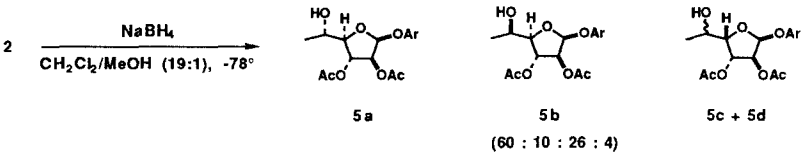
Hygromycin A (1)

Hygromycin A (**1**), together with its C-4' epimer,[4] was obtained by fermentation of *Streptomyces hygroscopicus* NRRL 2388. The conversion of **1** to hexa-acetate **2**, and subsequent ozonolysis to afford aldehyde **3** and ketoamide **4**, has been described previously.[5] We intended to use aldehyde **3** for the preparation of the desired analogs; however, all attempts to remove the acetate protecting groups from **3** resulted in ß-elimination to generate an enone. Since we had previously observed that the sodium borohydride reduction product of hygromycin A retains potent activity against the causative organism of swine dysentery, *Serpulina (treponema) hyodysenteriae*, we chose to prepare C-5'-reduced analogs of **1** in order to avoid the problem of ß-elimination.

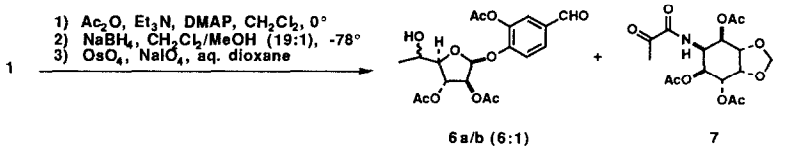


2 **3** **4**

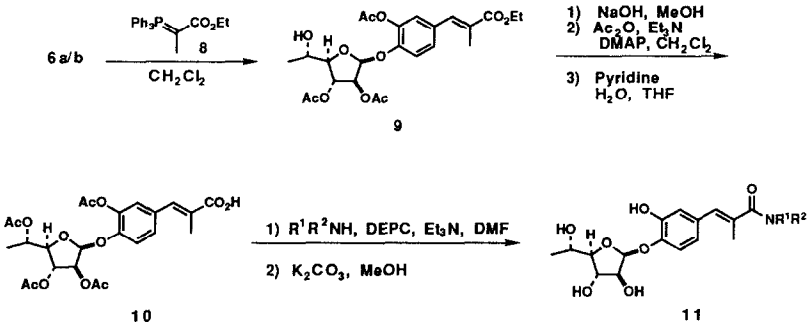
Low temperature sodium borohydride reduction (to prevent acetate loss) of hygromycin peracetate **2** (70:30 mixture of diastereomers) affords four diastereomers of product **5** in a ratio of about 60:10:26:4 (**5a:5b:5c:5d**). Silica gel chromatography on this mixture allows isolation of a mixture of **5a/5b** in a ratio of about 6:1.[6] Upon deacetylation, compound **5a** is converted to a material identical with the major diastereomer obtained from sodium borohydride reduction of **1**, which is known to have the L-*fuco* configuration.[7]



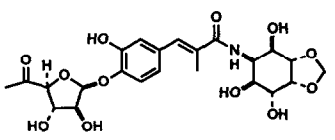
Although the conditions for oxidative cleavage in this series were originally optimized using purified **5a/b**, we ultimately found that the most efficient sequence proceeds without purification until after the cleavage reaction. Thus, hygromycin A is acetylated to give **2**, and reduced with sodium borohydride to afford mixture **5**; crude **5** is subjected to oxidative cleavage with osmium tetraoxide/sodium periodate,[8] giving a mixture of aldehyde diastereomers **6** and ketoamide **7**. Column chromatography on this mixture succeeds in isolating **6a/b** (6:1) in 42% yield, and ketoamide **7** in 56% yield, based on unprotected hygromycin A as starting material (3 steps).



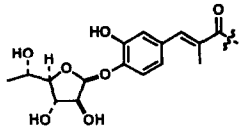
Condensation of aldehyde **6a/b** (6:1) with (carbethoxyethylidene)triphenylphosphorane (**8**) cleanly affords a 6:1 mixture (at C-5') of *E*-cinnamates, from which the major diastereomer **9** can be obtained in pure form by column chromatography in 56% yield. Saponification of ester **9** with sodium hydroxide in methanol affords the fully deprotected carboxylic acid. Reacetylation with acetic anhydride (triethylamine, DMAP, dichloromethane) proceeds with formation of varying amounts of mixed anhydride; brief treatment of the crude product with pyridine/water/THF cleanly generates fully acetylated carboxylic acid **10** (79% from **9**). Compound **10** can be coupled efficiently with primary or secondary amines using diethylphosphonocyanate (DEPC)[9] in DMF to provide the protected amides; deacetylation to afford analogs **11** is cleanly effected with potassium carbonate in MeOH.



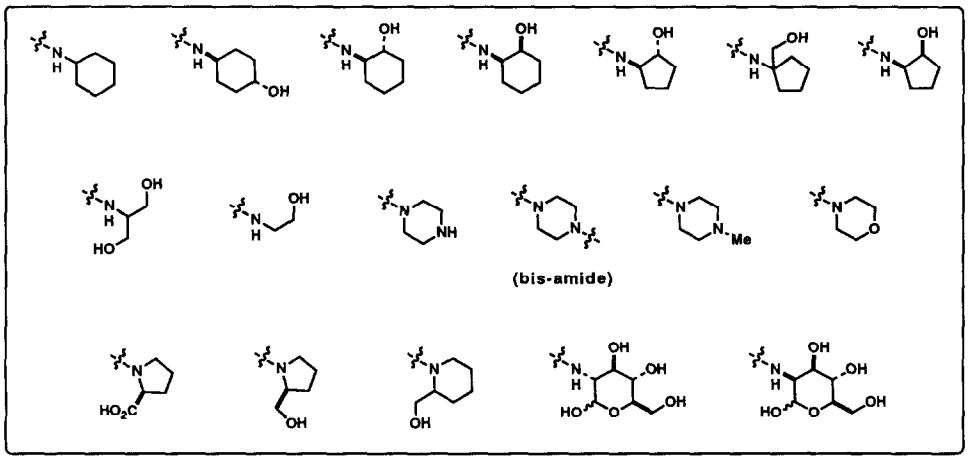
The amide analogs prepared by this method are shown below; they include a variety of hydroxy-substituted cyclic and acylic alkylamides, as well as two analogs which are amides of aminosugars. Unfortunately, all of these compounds were inactive against *S. hyodysenteriae* at concentrations up to 200 μg/ml (hygromycin A displays a minimum inhibitory concentration (MIC) of 0.78 μg/ml against this organism, while that of its sodium borohydride reduction product is 3.13 μg/ml.[10]) The low tolerance for variation of the aminocyclitol portion of hygromycin A has been noted previously by Chida *et al.* in studies of methoxyhygromycin and its 5-epimer.[11]



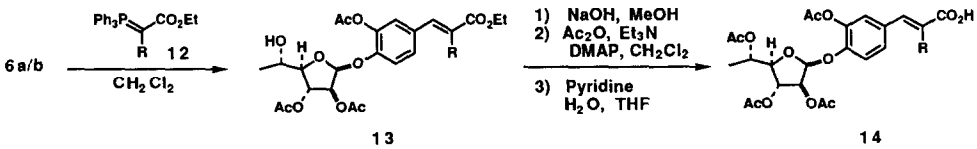
Hygromycin A (1)

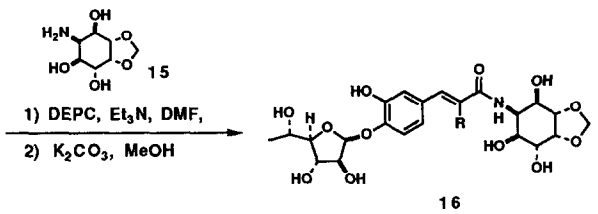


Amide analogs



Variation of the phosphorane reagent **8** in the chemistry described above was useful in the preparation of a series of compounds in which the vinylic methyl group of hygromycin A is replaced by other alkyl and halogen substituents. The appropriate phosphoranes **12** where R is ethyl, *n*-propyl, allyl, propargyl, chloro and bromo were prepared by literature routes;[12] condensation with aldehyde **6a/b** followed by column chromatography affords diastereomerically pure esters **13**. Saponification and reacetylation proceed as above to provide carboxylic acids **14**. Compounds **14** are coupled with aminocyclitol **15** (obtained by degradation of hygromycin A[13]), using DEPC; deacetylation with potassium carbonate in methanol provides analogs **16**.





R	*S. hyodysenteriae* MIC (μg/ml)
H	100
Me	3.13
Et	3.13
Pr	>200
allyl	>200
propargyl	100
Cl	6.25
Br	3.13

The *in vitro* biological activity of this series of compounds against *S. hyodysenteriae* is shown above. It appears that there is a fairly strict steric requirement for a substituent similar in size to a methyl or ethyl group, since activity decreases markedly with either hydrogen or propyl substitution. Replacement of alkyl by halogen is consistent with good activity, but offers no improvement over alkyl substitution.

In summary, we have developed a practical and efficient route for the preparation of amide and vinyl methyl analogs of hygromycin A. Synthesis of a variety of other types of hygromycin A analogs will be reported in due course.

Acknowledgements: We are grateful to Mr. W. Cullen and Mr. J. Oscarson for providing hygromycin A from fermentation, and to Dr. S. Froshauer, Dr. S. Hayashi, Ms. A. Silvia and Mr. S. Seibel for microbiological testing.

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1. Pittenger, R.C.; Wolfe, R.N.; Hoehn, M.M.; Marks, P.N.; Daily, W.A.; McGuire, J.M. *Antibiotics Ann.*; Welch, H. and Ibanez, F.M., *Eds.*, Medical Encyclopedia, Inc., New York, **1953/54**, pp. 157-166.

2. Nakagawa, A.; Fujimoto, T.; Omura, S. *J. Antibiotics*, **1987**, *40*, 1627.

3. Chida, N.; Ohtsuka, M.; Nakazawa, K., Ogawa, S. *J. Org. Chem.* **1991**, *56*, 2976, and refs. therein.

4. Pure hygromycin A rapidly epimerizes at C-4' at pH 10 or above; the material provided to us consisted of hygromycin A and its epimer in a ratio of approximately 70:30.

5. This transformation is found in ref. 14 of Chida, N.; Ohtsuka, M.; Ogawa, S. *Chem. Lett.* **1988**, 969.

6. The minor diastereomer (**5b**) which is isolated with **5a** is assigned 4'-β stereochemistry on the basis of significant similarities in the sugar ring resonances of the proton NMR spectra of **5a** and **5b**, and substantial differences in the proton NMR spectra of this pair with spectra of **5c** and **5d**. The stereochemistry at C-5' for **5c** and **5d** is unassigned.

7. Mann, R.L; Woolf, D.O. *J. Am. Chem. Soc.* **1957**, *79*, 120. In these early structure studies on hygromycin, workers reported that treatment of sodium borohydride reduced hygromycin with ethanethiol and 6*N* HCl affords in 34% yield a dithioacetal identical to that obtained from L-fucose. We have deacetylated compound **5a** and repeated this degradation procedure; the high-field ^{13}C and ^{1}H NMR spectra of the mercaptanolysis product are identical to those of an authentic sample of the dithioacetal prepared from L-fucose.

8. Pappo, R.; Allen, D.S., Jr.; Lemieux, R.U.; Johnson, W.S. *J. Org. Chem.*, **1956**, *21*, 478.

9. Yamada, S.; Kasai, Y.; Shioiri, T. *Tetrahedron Lett.*, **1973**, 1595.

10. MIC's were determined as in Weber, F.H.; Earley, D.L. *Antimicrob. Agents Chemother.*, **1991**, *35*, 2012.

11. Chida, N.; Nakazawa, K.; Ohtsuka, M.; Suzuki, M.; Ogawa, S. *Chem. Lett.*, **1990**, 423.

12. Compounds **12** where R = Et and Pr were prepared by Ph_3P displacement of the corresponding bromoester; compounds **12** where R = allyl and propargyl were prepared by alkylation of ethyl (triphenylphosphoranylidene)acetate (ETPPA) with allyl and propargyl bromide (Mali, R.S.; Tilve, S.G.; Yeola, S.N.; Manekar, A.R. *Heterocycles*, **1987**, *26*, 121); compound **12** where R = Cl was prepared by the action of *t*-butyl hypochlorite on ETPPA (Denney, D.B.; Ross, S.T. *J. Org. Chem.*, **1962**, *27*, 998); compound **12** where R = Br was prepared by the action of bromine on ETPPA (*Bull. Soc. Chim. France*, **1979**, 559.)

13. Kakinuma, K.; Sakagami, Y. *Agric. Biol. Chem.*, **1978**, *42*, 279.