Bioorganic & Medicinal Chemistry Letters, Vol.2, No.9, pp. 1043-1046, 1992
Printed in Great Britain

0960-894X/92 $5.00 + .00

HYGROMYCIN A: PREPARATION OF AMINOCYCLITOL ANALOGS DEFINING THE MINIMUM FUNCTIONALITY REQUIRED FOR BIOLOGICAL ACTIVITY

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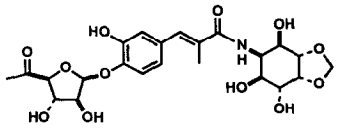
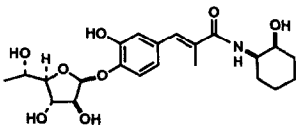
(*Received* 5 *June* 1992)

Abstract: Analogs of hygromycin A in which the aminocyclitol is replaced by dihydroxycyclohexylamine and trihydroxycyclohexylamine (with stereochemistry matching that of the natural product) have been prepared. The latter was prepared both in racemic form (starting with 1,3-cyclohexadiene) and as a single enantiomer (by degradation of hygromycin A.) Antibacterial activities against key animal health pathogens are reported.

Hygromycin A (**1**) is a fermentation-derived natural product[1] with modest antibacterial activity, which only recently was discovered to be efficacious in the treatment of swine dysentery.[2] The heightened interest generated by this latter 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. This report describes our efforts to define the minimum cyclohexylamide functionality required to afford biological activity.



Hygromycin A (1)



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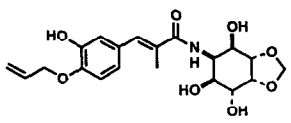

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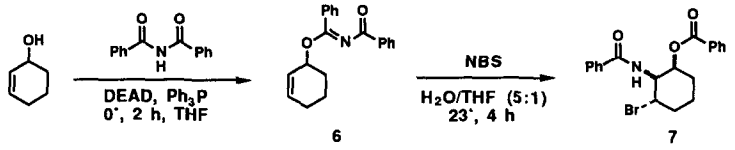
Earlier studies in our laboratory[4] established that *cis*-2-hydroxycyclohexylamide analog **2** was devoid of activity against the the target organisms (*vide infra*). Subsequent work carried out by our colleagues[5] demonstrated that the aryl sugar substituent could be replaced by an allyl ether (**3**) without loss of activity. Therefore, our initial target was *cis,cis*-2,6-dihydroxycyclohexylamide **4**. The key aminodiol was prepared by a modification of the method of Sammes, *et al.*[6] Thus, Mitsunobu reaction of racemic cyclohex-2-enol with dibenzamide affords N-acylimidate **6** (84%), which undergoes bromocyclization and subsequent hydrolysis to bromide **7** upon treatment with NBS in H_2O/THF (46%). Heating of **7** in H_2O/DMF causes intramolecular bromide displacement followed by hydrolysis of the intermediate oxazoline, affording aminodibenzoate salt **8**. Coupling of **8** with carboxylic acid **9**[5] using diethyl cyanophosphonate, followed by benzoate hydrolysis, affords dihydroxycyclohexylamide analog **4** (39% from **7**).[7]



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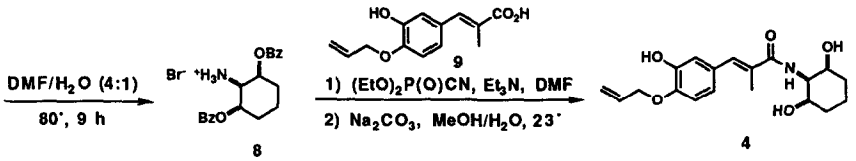
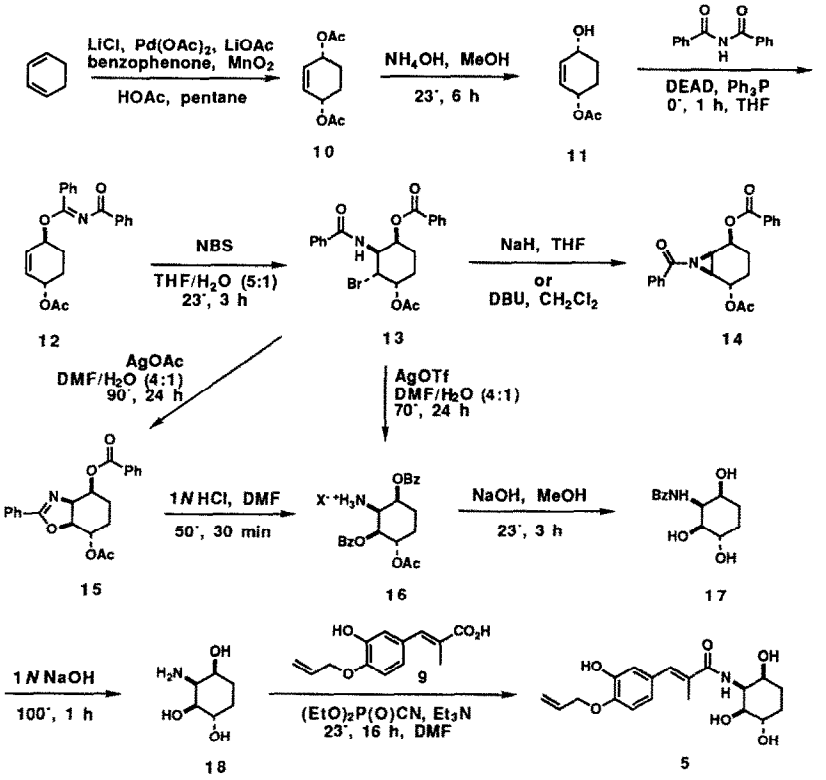
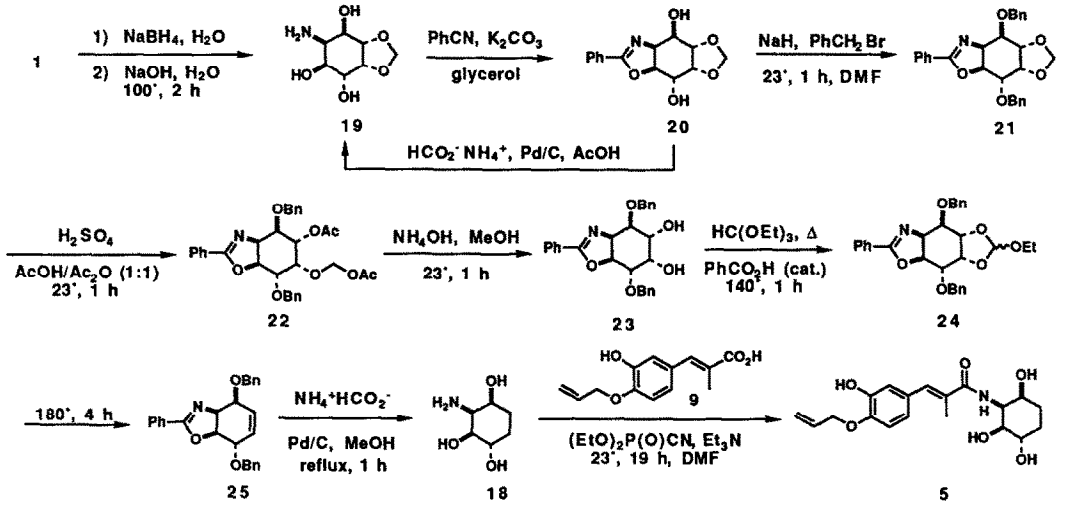


8 4

We next set about construction of trihydroxycyclohexylamide **5**, which contains the third free hydroxyl group found in the hygromycin A aminocyclitol; our first approach targeted this material in racemic form. *Cis*-1,4-diacetoxycyclohex-2-ene (**10**) is readily available from 1,3-cyclohexadiene by the Bäckvall procedure.[8] Ammonolysis affords a mixture from which is isolated monoacetate **11** in 50% yield. Mitsunobu reaction of **11** with dibenzamide provides acylimidate **12** (62%), which is converted to bromide **13** upon treatment with NBS in H_2O/THF (82%). The conditions described above for intramolecular bromide displacement on compound **7** (heating in DMF/H_2O) fail in this case, probably because the additional electron-withdrawing acetate group slows solvolysis of the bromide. Deprotonation of the amide nitrogen of **13** with either sodium hydride/THF or DBU/dichloromethane causes attack by nitrogen rather than oxygen, affording acylaziridine **14**. On the other hand, use of either silver tetrafluoroborate or silver acetate to assist bromide solvolysis of **13** causes conversion to oxazoline **15**, which can be hydrolyzed to aminotriester **16** by treatment with aqueous HCl in DMF. Use of a more acidic counterion for silver (silver trifluoroacetate, silver nitrate or silver triflate) allows direct conversion of bromide **13** to aminotriester **16**. Deacylation is best accomplished in two steps; treatment with sodium hydroxide in methanol at room temperature affords benzamide **17**, which is purified at this stage by silica gel chromatography (44% from **13**). Subsequently, amide **17** is subjected to aqueous sodium hydroxide at reflux; purification by ion-exchange chromatography on Amberlite IR-120 resin affords aminotriol **18** (45%). Compound **18** is coupled with carboxylic acid **9** using diethyl cyanophosphonate to provide trihydroxycyclohexylamide analog **5** (34%).[7]



We have also prepared analog **5** in optically pure form by semisynthetic modification of the hygromycin A aminocyclitol **19**. The isolation of **19** by hydrolysis of hygromycin A with 1*N* sodium hydroxide at reflux, followed by ion-exchange chromatography, has been described previously.[9] Selective protection of the amino and 6-hydroxyl groups is accomplished by formation of phenyloxazoline **20**; we found that this protecting group can be conveniently removed by transfer hydrogenation with ammonium formate and palladium on carbon in acetic acid.[10] Since we were in need of substantial quantities of compound **20**, we developed a 3-step, 2-pot, 1-purification procedure providing this material directly from hygromycin A. Thus, a solution of hygromycin A in water is reduced with sodium borohydride. When the reduction is complete, solid sodium hydroxide (to make a 1*N* solution) is added, and the mixture is heated at reflux for an hour. The water is removed by lyophilization, and the resulting powder is heated in glycerol with benzonitrile and potassium carbonate.[11] The product is extracted into chloroform and purified by column chromatography, providing oxazoline **20** in 53% yield.

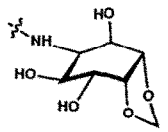


Oxazoline **20** is alkylated to afford bis-benzyl ether **21** (87%). Cleavage of the methylenedioxy ring of compound **21** with sulfuric acid/acetic anhydride affords intermediate **22** (77%),[12] which upon acetate hydrolysis with ammonium hydroxide in methanol affords diol **23** (74%). Compound **23** is converted to a mixture of orthoesters **24** by heating in triethylorthoformate with catalytic benzoic acid; pyrolysis[13] of **24** in dichlorobenzene (180°, 4 h) provides olefin **25** (53% from **23**). Transfer hydrogenation of compound **25** effects reduction of the olefin as well as removal of the benzyl ether and oxazoline protecting groups, affording aminotriol **18** (61%). Compound **18** is coupled with carboxylic acid **9** using diethyl cyanophosphonate, providing trihydroxycyclohexylamide analog **5** in enantiomerically pure form (40%).[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] As stated earlier, compound **2** lacks activity against these organisms; compound **4**, having an additional hydroxyl group, has modest activity. The presence of a third hydroxyl group in racemic **5** improves activity further; however, *compound 5 as a single enantiomer derived from 1 has poorer activity than either compound 4 or racemic 5!*

Compound	*S. hyodysenteriae* (µg/ml)	*P. multocida* (µg/ml)
1 (hygromycin A)	1.56	1.56
2	>200	>200
3	0.78	1.56
4	12.5	100
5 (racemate)	6.25	25
5 (single enantiomer)	100	>200

The fact that racemic **5** is significantly more potent than homochiral **5** implies that the enantiomer opposite to that derived from hygromycin A is responsible for most of the activity. A rationalization of this curious result lies in analysis of the conformations of the hygromycin A aminocyclitol and our analogs. Earlier workers have shown[9] by NMR studies that the hygromycin A aminocyclitol adopts a twist-boat conformation, induced by the presence of the methylenedioxy ring. Our analogs, lacking the constraints imposed by a fused ring, presumably exist in a chair conformation. Therefore, the positions in space occupied by the hydroxyl groups of the two enantiomers of **5** are very different from those of the hydroxyl groups of **1**, and thus it is impossible to make an accurate prediction of their relative biological activity.[15]



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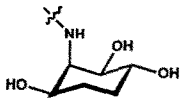
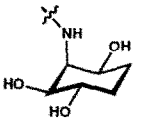


5 (active enantiomer)



5 (inactive enantiomer)

The fact that analogs **4** and **5** have significantly poorer antibacterial activity than compounds **1** or **3** indicates that the conformation induced by the methylenedioxy ring of hygromycin A is important for attaining optimal activity; this is in accord with the findings of Chida *et al.* in studies of methoxyhygromycin and its 5-epimer.[16] Subsequent studies in our laboratories have been directed toward semisynthetic modification of aminocyclitol **19** in order to further define the requirements for biological activity. These efforts will be the subject of a separate publication from our laboratory.[17]

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

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7 Compound 4: ^{1}H NMR (CD_3OD): δ 1.5-1.9 (m, 6), 2.13 (s, 3), 3.94 (m, 2), 3.98 (m, 1), 4.63 (d, 2, J=5), 5.24 (dd, 1, J=1,10), 5.39 (dd, 1, J=1,17), 6.06 (m, 1), 6 8-6.95 (m, 3), 7.22 (s, 1). Compound **5**: ^{1}H NMR (CD_3OD): δ 1.45 (m, 1), 1.72 (m, 1), 1 9-2 1 (m, 2), 2.15 (s, 3), 3.74 (m, 1), 3.90 (m, 1), 3.97 (m, 1), 4.30 (t, 1, J=4), 4.63 (d, 2, J=5), 5.23 (dd, 1, J=1,10), 5.39 (dd, 1, J=1,17), 6.08 (m, 1), 6.8-6 95 (m, 3), 7 22 (s, 1).
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12. The regiochemistry of this methylenedioxy group cleavage is assigned by analogy to the structure of the product obtained by performing this reaction on compound **3**, whose NMR spectra indicate the presence of a plane of symmetry in the aminocyclitol ring.
13 Eastwood, F.W.; Josan, J.S *Aust J Chem*, **1968**, *21*, 2013.
14. *P multocida* MIC's were measured in a liquid microtiter serial dilution assay; *S. hyodysenteriae* MIC's were determined as in Weber, F.H.; Earley, D.L. *Antimicrob Agents Chemother*, **1991**, *35*, 2012.
15. This rationalization if purely speculative; a firmer understanding would require (1) a molecular modeling study to assess the energy cost of compound **5** adopting a twist-boat conformation, and (2) testing of the enantiomers of **5** in a cell-free protein synthesis inhibition assay, to determine if differential cellular uptake contributes to their disparate activities.
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