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APPLICATION OF HYGROMYCIN A STRUCTURE ACTIVITY RELATIONSHIPS TO THE ANTIBIOTIC A201A.

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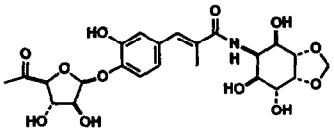
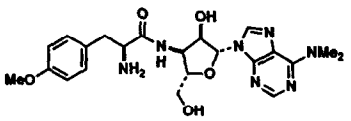
Abstract: Replacement of the disaccharide portion of A201A with an allyl ether, with concomitant 3-hydroxylation, affords an analog with biological activity comparable to that of the natural product. The response of biological activity to this modification closely parallels that observed with hygromycin A, which implies that these two natural products may share a common binding site.

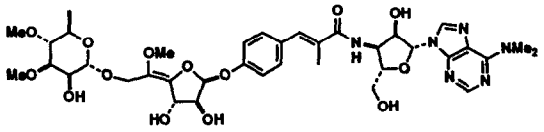
The recently discovered efficacy of hygromycin A[1] (**1**) in the treatment of swine dysentery[2] has generated renewed interest in this fermentation-derived natural product, including a total synthesis.[3] As part of our program to discover new antibacterial agents for use in animal health, we have prepared numerous analogs of **1**, including vinyl methyl and simple amide analogs,[4] aryl analogs,[5] and aminocyclitol analogs by both total synthesis[6] and semisynthesis.[7] We were intrigued by the similarities in structure between **1** and A201A (**2**), a natural product first reported[8] in 1976 and whose unambiguous structure assignment was reported in 1985.[9] A201A, in addition, bears distinct structural similarities to puromycin[10] (**3**), a nucleoside antibiotic whose mechanism of action has been well characterized. Although antibiotics **1**, **2** and **3** are all inhibitors of protein synthesis, significant differences in the specific mechanisms of action of A201A and puromycin have been observed.[11] Our interest in hygromycin A led us to explore the possibility that compounds **1** and **2** might bind similarly to the ribosome/peptide complex, and if so, that this information could be used in the design of new and more potent antibacterial agents. This report describes the preparation of a set of analogs which apply the knowledge we have learned about structure activity relationships in the hygromycin A series to the A201A series.



Hygromycin A (1)

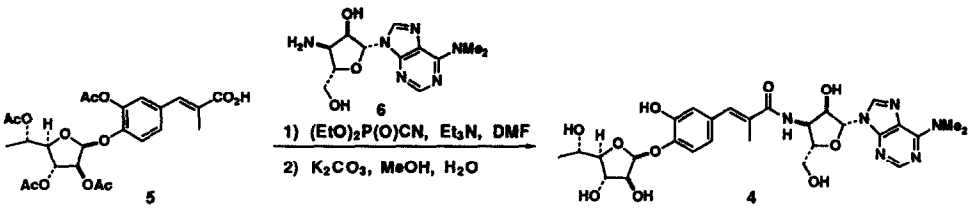


Puromycin (3)



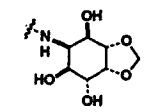
A201A (2)

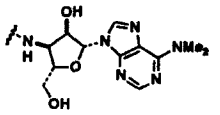
Our first approach was to prepare a hybrid structure (**4**) in which the aminonucleoside portion of A201A is coupled to the glycosyl cinnamate portion of hygromycin A. We have previously described the preparation[4] from hygromycin A of cinnamate **5**, which was converted to a variety of simple amide analogs of **1**. Coupling of compound **5** with the commercially available (Sigma) puromycin aminonucleoside **6**, followed by deacetylation, affords hybrid structure **4**.

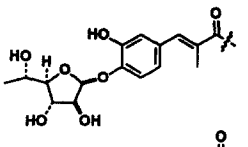
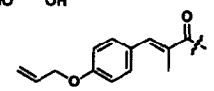
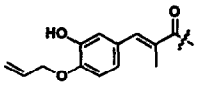
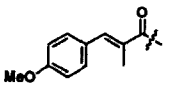


5 6 4

In addition to compound **4**, we also prepared (by similar amide coupling with the appropriate carboxylic acid) a set of analogs exploring the effect of removal of the sugar moiety entirely. It had previously been established in these laboratories that the sugar of hygromycin A could be replaced by an allyl ether (compound **10**) without loss of activity,[12] and that removal of the phenolic hydroxy group (compound **8**) causes loss of some, though not all, activity.[5] This same set of analogs was prepared in the A201A series in order to assess whether the activities of these two natural products respond similarly to structural modification. The two organisms for which we report antibacterial activity[13] are *Serpulina (Treponema) hyodysenteriae* (the causative organism of swine dysentery) and *Pasteurella multocida* (an important animal respiratory pathogen). Results for this set of A201A analogs, compared with the corresponding analogs in the hygromycin A series, are shown below.





	MIC (μg/ml) *S. hyodysenteriae*	MIC (μg/ml) *P. multocida*		MIC (μg/ml) *S. hyodysenteriae*	MIC (μg/ml) *P. multocida*
Hygromycin A (1)	0.78	0.78		--	--
A201A (2)	--	--		3.13	3.13
	7: 3.13	6.25		4: 200	200
	8: 6.25	50		9: 50	>200
	10: 0.78	3.13		11: 1.56	6.25
	12: >200	>200		13: 50	>200

Our first analog, compound **4**, has rather disappointing activity, especially given how similar its structure is to that of A201A. This result did not bode well for making more significant changes in the A201A structure. However, replacement of the entire sugar moiety by an allyl group (compound **9**) affords slightly improved activity (at least against *S. hyodysenteriae*), and addition of the 3-hydroxyl group (compound **11**) provides a compound whose activity is comparable to that of A201A itself! It is interesting to note that a minor metabolite in the fermentation of A201A, designated A201C, is also hydroxylated in the 3-position of the aryl ring.[9]

Although we were excited by these results, we entertained the possibility that the activity of compound **11** is due to its structural similarity to puromycin. To test this hypothesis, *p*-methoxy analog **13** was prepared, with the rationale that if compound **11** were a puromycin analog, then one would expect compound **13** to show reasonable activity as well, since compound **13** is more like puromycin than compound **11**. On the other hand, in the hygromycin series, *p*-methoxy substitution affords no activity (compound **12**). As shown in the table, compound **13** is inactive, and therefore is probably not acting by a puromycin-like mechanism.

Having established that the sugar portion of the A201A structure could be greatly simplified without loss of biological activity, we set out to determine if the aminonucleoside portion could also be simplified. In particular, we desired to know if aminoribose analogs lacking the nucleotide would retain biological activity. Toward this end, we prepared analog **14**, which replaces the nucleotide with a methoxy group.

The early steps of the preparation of the requisite aminoribose derivative follow the route described by Azhayev *et al.*[14] Thus, selective esterification of commercially available 1,2-O-isopropylidenexylose affords toluyl derivative **15**. Formation of the triflate followed by sodium azide displacement provides protected azide **16**. Methanolysis of the acetonide using methanol/Amberlite affords a 3:1 (separable) mixture of anomers, favoring the desired 1,2-*trans* methyl furanoside **17**. The toluyl ester is removed with ammonium hydroxide in methanol, providing intermediate **18**. Reduction of azide **18** with hydrogen over palladium on carbon affords amine **19**, which upon coupling with carboxylic acid **20** affords the desired analog **14**. Unfortunately, this compound showed no activity against the two organisms of interest.

The lack of activity of compound **14** indicates that the A201A structure can not be further simplified beyond the modifications found in compound **11** without loss of activity. The fact that both hygromycin A and A201A respond similarly to certain major structural changes indicates that the binding sites of these two antibiotics may share a region of overlap (*i.e.*, the portion which binds the aryl region). In any event, it is remarkable that the disaccharide portion of A201A can be replaced by a simple allyl ether, as in the hygromycin A series.

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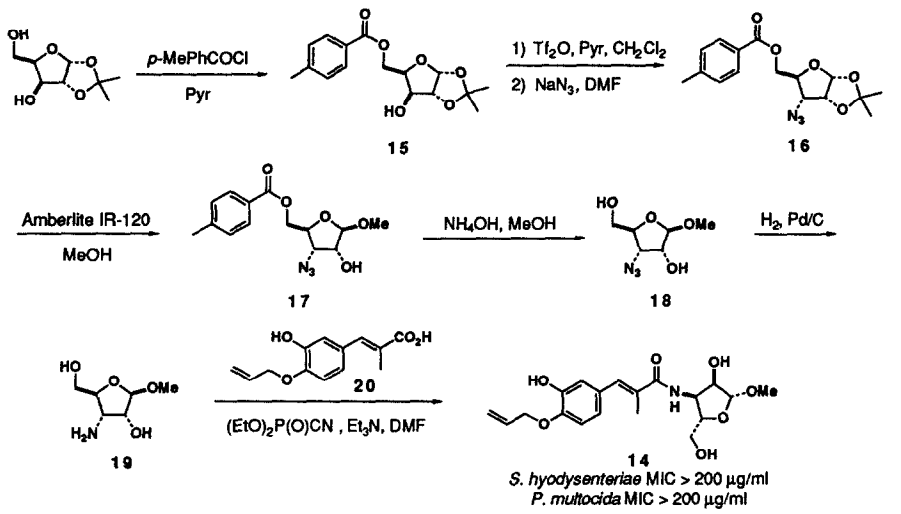

References and Notes:

1. Pittenger, R.C.; Wolfe, R.N.; Hoehn, M.M.; Marks, P.N.; Daily, W.A.; McGuire, J.M. *Antibiot. Chemother.*, **1953**, *3*, 1268; Mann, R.L.; Gale, R.M.; van Abeele, F.R. *ibid.*, **1953**, *3*, 1279.
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**.
4. Hecker, S.J.; Minich, M.L.; Werner, K.M. *BioMed. Chem. Lett.*, **1992**, *2*, 533.
5. Hecker, S.J.; Cooper, C.B.; Blair, K.T.; Lilley, S.C.; Minich, M.L.; Werner, K.M., submitted for publication.
6. Hecker, S.J.; Lilley, S.C.; Werner, K.M. *BioMed. Chem. Lett.*, **1992**, *2*, 1043.
7. Hecker, S.J.; Lilley, S.C.; Minich, M.L.; Werner, K.M. *BioMed. Chem. Lett.*, **1992**, *2*, 1015.
8. Kirst, H.A.; Dorman, D.E.; Occolowitz, J.L.; Szymanski, E.F.; Paschal, J.W. Abstracts of Papers of 16th Intersci. Conf. on Antimicrob. Agents Chemother., No. 61, Chicago, 1976.
9. Kirst, H.A.; Dorman, D.E.; Occolowitz, J.L.; Jones, N.D.; Paschal, J.W.; Hamill, R.L.; Szymanski, E.F. *J. Antibiotics*, **1985**, *38*, 575.
10. Gale, E.F.; Cundliffe, E.; Reynolds, P.E.; Richmond, M.H.; Waring, M.J. *The Molecular Basis of Antibiotic Action*, John Wiley and Sons, London, 1981.
11. Epp, K.; Allen, N.E.; Abstracts of Papers of 16th Intersci. Conf. on Antimicrob. Agents Chemother., No. 63, Chicago, 1976.
12. Jaynes, B.H.; Elliott, N.C.; Schicho, D.L., *J. Antibiotics*, in press.
13. *P. multocida* MICs were measured in a liquid microtiter serial dilution assay; *S. hyodysenteriae* MICs were determined as in Weber, F.H.; Earley, D.L. *Antimicrob. Agents Chemother.*, **1991**, *35*, 2012.
14. Ozols, A.M.; Azhayev, A.V.; Dyatkina, N.B.; Krayevsky, A.A. *Synthesis*, **1980**, 557.