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SYNTHESIS AND *IN VITRO* ANTIBACTERIAL ACTIVITY OF HYGROMYCIN A ANALOGS MODIFIED AT THE $C_{4'}$ ARYL POSITION

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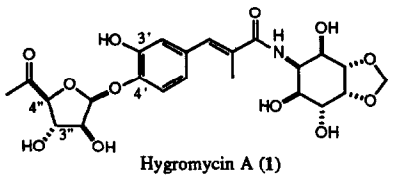
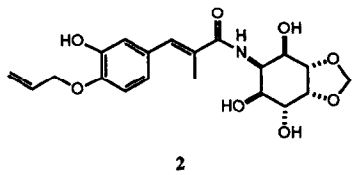
Abstract: A variety of hygromycin A analogs are described which contain modifications of or replacements for the natural sugar. Antibacterial activities against *Serpulina (Treponema) hyodysenteriae*, an important animal health pathogen, are reported and indicate that small lipophilic $C_{4'}$ substituents serve as useful sugar surrogates in the hygromycin A class.

Hygromycin A (**1**) is a fermentation-derived natural product first isolated from *Streptomyces hygroscopicus* in 1953.[1] Although known to possess moderate antibacterial activity, it was only recently discovered to have excellent *in vitro* potency[2] against *Serpulina* (*Treponema*) *hyodysenteriae*, the causative agent of swine dysentery, an economically significant muco-hemorrhagic disease of swine. More importantly, the antibiotic also demonstrated efficacy in the treatment of induced dysentery infection in pigs at levels of 5-20 g/ton feed.[3] The renewed interest in hygromycin A has led to increased synthesis activities, including a recent total synthesis.[4] Additional reports from our laboratories have detailed structure-activity relationships (SAR) about the aromatic ring,[5] the enamide,[6] and the aminocyclitol.[7] In addition, we described the remarkable functional equivalence of simple lipophilic alkyl ethers in place of the *arabino*-furanosyl aryl substituent of hygromycin A, represented by allyl ether **2**.[8] This paper further describes the range of accepted groups at the $C_{4'}$ position, including modifications of the natural sugar, and oxygen, nitrogen, and carbon-linked substituents.



Hygromycin A (**1**)



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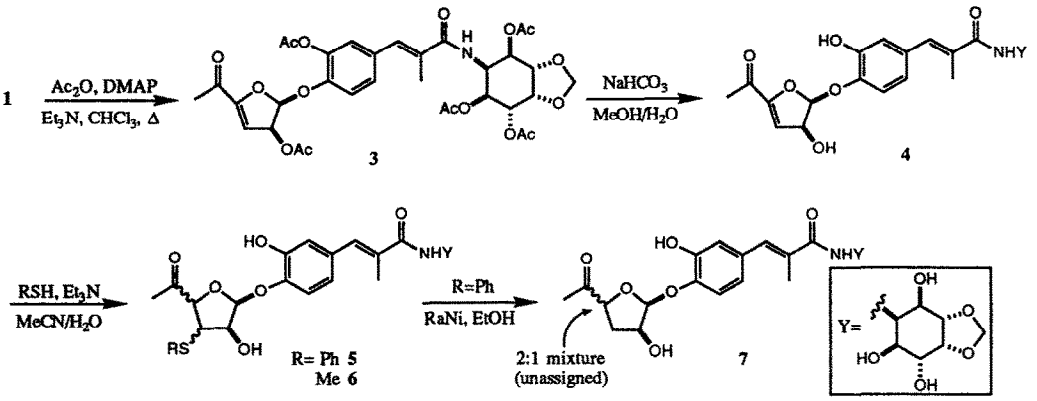
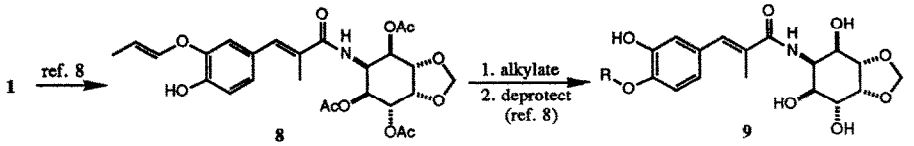
Initial semisynthetic studies on hygromycin A's furanose moiety suggested that this region's SAR may be very sensitive to minor alterations in structure. By acetylation of the natural product with concomitant elimination, enone **3** is produced (Scheme 1); acetate cleavage with wet $NaHCO_3$/MeOH affords dihydrofuran **4** which retains only moderate *in vitro* antibacterial activity[9] (*S. hyodysenteriae* MIC = 25 μg/mL). In order to reintroduce sp^3 hybridization at $C_{3''}$ and $C_{4''}$, phenyl and methyl thiolate anions were added in a conjugate fashion but failed to restore *in vitro* activity[10] (MICs of **5** and **6**: >100 μg/mL). Subsequent desulfurization was accomplished with Raney nickel to provide an inactive mixture of the 3''-deshydroxy hygromycin A epimers **7**[10] (MIC of **7**: >100 μg/mL), indicating that inactivity of the sulfides is not due to simple steric repulsion at $C_{3''}$, and implicating an absolute requirement for the $C_{3''}$ hydroxyl group.

With this surprising stringency uncovered in the furanose region, we set about to determine if complete replacement of the *arabino*-furanose, patterned after successful efforts in the antiviral acyclic nucleoside field,[12] would yield analogs less sensitive to variation. We sought an acyclic sugar surrogate which would alleviate potential $C_{4''}$ epimerization problems (hygromycin A is isolated as a mixture of epimers) and provide the minimum structural requirements for antibacterial activity. Methodology was developed to cleave the sugar of hygromycin A and to derivatize selectively the resultant $C_{4'}$ phenolic hydroxyl group.[8] Through intermediate **8**, a variety of ethers were prepared by halide displacement or Mitsunobu reaction with an alcohol, followed by deprotection (Scheme 2). *In vitro* activities of some of these analogs are listed in Table 1.

Scheme 1

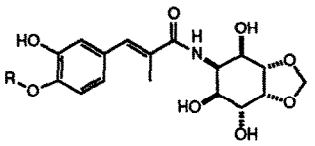


Scheme 2



The *in vitro* results in Table 1 suggest that small lipophilic groups (less than five methylene groups, in general) lead to the most active $C_{4'}$ substituted analogs. (And this has been shown to translate to *in vivo* activity in both mice[8] and swine.[13]) Analogs bearing longer aliphatic chains at $C_{4'}$ are generally less potent (**9k**, **9l**), although activity may be restored through the introduction of polar groups to the sidechain (compare pentyl ether **9l** with methyl sulfide **9o** and ketone **9p**). Hydroxyl groups, designed to overlay with those at $C_{2''}$ and $C_{3''}$ of the natural furanose, do not improve potencies (entries **9t**, **9u**, **9v**). Steric hindrance influences antibacterial activity as branched chain substituents decrease potency (**2** versus **9g** and **9h**) while cyclic structures bearing a greater number of carbon atoms retain activity (**9m**, **9q**, **9r**). Interestingly, "typical" antiviral sidechains[12] (**9s**, **9t**, **9u**, **9v**) do not serve as effective sugar surrogates for hygromycin A and activity actually *decreases* as hydroxyl groups are added, a remarkable finding considering the parent structure's hydrophilic furanose.

Table 1. 4' ether analogs



Compound	R	*S. hyodysenteriae* MIC (μg/mL)	Method of preparation
Hygromycin A (1)	---	0.78-1.56	---
2	CH_2=$CHCH_2$	0.78	ref. 14, 15
9a	OR = H	>200	ref. 16
9b	H	200	ref. 15, 17
9c	CH_3	12.5	ref. 18
9d	CH_3CH_2	1.56	ref. 18
9e	FCH_2CH_2	6.25	ref. 19
9f	$CH_3CH_2CH_2$	0.78-1.56	ref. 18
9g	CH_2=$CHCH(CH_3)$	25	ref. 19
9h	CH_2=$C(CH_3)CH_2$	3.13	ref. 19
9i	propargyl	6.25	ref. 19
9j	cyclopropylCH_2	0.39-0.78	ref. 19
9k	$CH_3CH_2CH_2CH_2$	1.56	ref. 18
9l	$CH_3CH_2CH_2CH_2CH_2$	>200	ref. 18
9m	cyclopentyl	6.25	ref. 19
9n	NH_2COCH_2	>200	ref. 20
9o	$CH_3SCH_2CH_2CH_2$	0.78-1.56	ref. 19
9p	$CH_3(CO)CH_2CH_2CH_2$	12.5-25	ref. 19
9q	$PhCH_2$	25	ref. 19
9r	thiopheneCH_2	3.13-6.25	ref. 19
9s	$CH_3CH_2OCH_2$	25	ref. 21
9t	$HOCH_2CH_2$	50-100	ref. 22
9u	$HOCH_2CH_2OCH_2$	25	ref. 14, 25
9v	$HOCH_2CH(OH)CH_2$	25	ref. 26

To study the importance of the linking ether oxygen at $C_{4'}$, several nitrogen and carbon-linked analogs were prepared. In general, these analogs were synthesized from the appropriately substituted benzaldehyde by olefination using a three-step sequence initiated with a stabilized Wittig reaction or a one-step phosphonate procedure (Scheme 3).[5] The surprising tolerance for simple lipophilic side chains is also observed when the oxygen at $C_{4'}$ is replaced with nitrogen or carbon as seen in examples from Table 2. Although the MICs are not as good as in the oxygen-linked series, similar trends are observed with the various lipophilic substituents.

Previously published work described the tolerance of the $C_{3'}$ position for proton, amino and fluoro substitution in addition to the natural hydroxy functionality.[5] Indeed, the $C_{4'}$ structure-activity relationships are maintained when the 3-position hydroxyl group is replaced with a hydrogen (**13g**, **13k**, and **13l**) or fluorine atom (**13h** and Table 3) in the $C_{4'}$ oxygen, nitrogen, and carbon-linked series.

Scheme 3

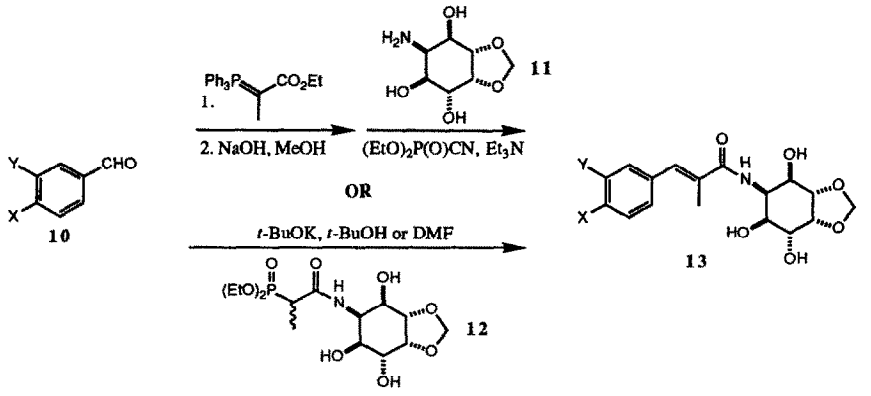
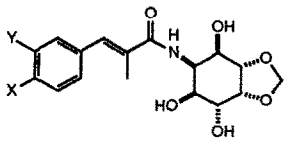


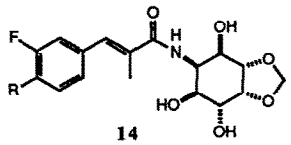
Table 2. 4' amino and carbon analogs



Compound	X	Y	*S. hyodysenteriae* MIC (μg/mL)	Method of preparation
13a	NH_2	OH	>200	ref. 27
13b	CH_3CH_2NH	OH	100	ref. 29
13c	$CH_3CH_2CH_2NH$	OH	12.5-25	ref. 29
13d	CH_2=$CHCH_2NH$	OH	12.5-25	ref. 29
13e	$CH_3CH_2CH_2(Et)N$	OH	>200	ref. 29
13f	CH_2=$CHCH_2(Ac)N$	OH	>200	ref. 30
13g	pyrrolidin-1-yl	H	6.25	ref. 16, 31
13h	pyrrolidin-1-yl	F	6.25	ref. 16, 31
13i	$CH_3CH_2CH_2CH_2$	OH	3.13-6.25	ref. 16, 32
13j	$PhCH_2$	OH	>200	ref. 16
13k	$CH_3CH_2CH_2CH_2$	H	12.5-25	ref. 16, 32
13l	Ph	H	12.5	ref. 16

In summary, despite dramatic differences from the natural furanose of hygromycin A, analogs possessing small lipophilic $C_{4'}$ substituents, whether linked through an oxygen (**9b-v**, **14a-c**), nitrogen (**13a-h**), or carbon atom (**13i-l**), maintain comparable *in vitro* activity relative to the natural product as measured against *S. hyodysenteriae*. Several of these *in vitro* actives have been shown to possess *in vivo* activity similar to hygromycin A in mouse and swine *S. hyodysenteriae* infection models.[8,13] In contrast, analogs incorporating hydroxyl groups on the $C_{4'}$ side chain designed to overlay with the natural furanose alcohols, and thus bind similarly to a molecular target,[34] fail to retain *in vitro* antibacterial activity. In fact, minor modifications that employ the furanose as a template for semisynthesis lead to a decrease in potency.

Table 3. 3' fluoro analogs



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Compound	R	*S. hyodysenteriae* MIC (μg/mL)	Method of preparation
14a	CH_3O	6.25-12.5	ref. 16, 33
14b	CH_2=$CHCH_2O$	1.56	ref. 5
14c	propargyl-O	6.25	ref. 16, 33
14d	F	>200	ref. 16

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8. Jaynes, B.H.; Elliott, N.C.; Schicho, D.L. *J. Antibiot.* **1992**, *45*, 1705.

9. Hygromycin A MIC (minimum inhibitory concentration) = 0.78-1.56 μg/mL. *S. hyodysenteriae* MICs were determined as in Weber, F.H.; Earley, D.L. *Antimicrob. Agents Chemother.* **1991**, *35*, 2012.

10. All possible diastereomers were obtained for analogs **5**, **6**, and **7**, but not separated; the known $C_{4''}$ epimer of hygromycin,[11] epihygromycin A, has an MIC against *S. hyodysenteriae* of 6.25 μg/mL.

11. Wakisaka, Y.; Koizumi, K.; Nishimoto, Y.; Kobayashi, M.; Tsuji, N. *J. Antibiot.* **1980**, *33*, 695.

12. For example, see: Chu, C.K.; Cutler, S.J. *J. Heterocyclic Chem.* **1986**, *23*, 289.

13. Illyes, E.F.; Kamicker, B.J.; Jaynes, B.H.; LeMay, M.A.; Shively, J.E., manuscript in preparation.

14. Prepared as outlined in reference 8, by alkylation with the appropriate bromide.

15. An improved procedure for the preparation of hygromycin A aglycone (**9b**) and allyl ether analog **2** will be reported elsewhere.

16. Prepared from the appropriately substituted benzaldehyde as illustrated in Scheme 3.[5]

17. Mann, R.L.; Woolf, D.O. *J. Am. Chem. Soc.* **1957**, *79*, 120.

18. Prepared as outlined in reference 8, by alkylation with the appropriate iodide.

19. Prepared as outlined in reference 8, by Mitsunobu reaction with the appropriate alcohol.

20. Prepared as outlined in reference 8, by Mitsunobu reaction with methyl glycolate. In the acetate removal step (NH_3/MeOH), the methyl ester was converted to primary amide **9n**.

21. Prepared as outlined in reference 8, by alkylation of the protected aglycone with chloromethyl ethyl ether and N,N-diisopropylethylamine.

22. Compound **9t** was prepared from 3-(allyloxy)-4-hydroxybenzaldehyde[23] by (1) ethylene sulfate opening with the sodium anion (NaH, THF) followed by *in situ* sulfate hydrolysis,[24] (2) olefination with (carbethoxyethylidene)triphenylphosphorane in CH_2Cl_2, (3) allyl group removal,[8] (4) ester saponification (aq. NaOH, MeOH), (5) hydroxyl group protection with Ac_2O, DMAP, and Et_3N, (6) coupling with aminocyclitol **11** ($(EtO)_2P(O)CN$ and Et_3N in DMF), and (7) deacetylation (K_2CO_3, MeOH).

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25. (2-Acetoxyethoxy)methyl bromide was prepared from acetyl bromide and 1,3-dioxolane: Robins, M.J.; Hatfield, P.W. *Can. J. Chem.* **1982**, *60*, 547.

26. Compound **9v** was prepared from 4-(allyloxy)-3-hydroxybenzaldehyde[23] by (1) catalytic *cis*-hydroxylation (OsO_4, NMO, THF, H_2O), (2) olefination with (carbethoxyethylidene)-triphenylphosphorane in THF, (3) ester saponification (aq. NaOH, MeOH), (4) hydroxyl group protection with Ac_2O, DMAP, and Et_3N, (5) coupling with aminocyclitol **11** ($(EtO)_2P(O)CN$ and Et_3N in DMF), and (6) deacetylation (K_2CO_3, MeOH).

27. Compound **13a** was prepared from 3-hydroxy-4-nitrobenzaldehyde by (1) allylation (allyl bromide, K_2CO_3, DMF), (2) olefination with (carbethoxyethylidene)triphenylphosphorane in CH_2Cl_2, (3) reduction to the aniline ($SnCl_2$, HCl), (4) allyl isomerization ($Ir(COD)(MePh_2P)_2PF_6$, THF),[28] (4) ester saponification (KOH, EtOH), (5) coupling with aminocyclitol **11** (EEDQ, DMF), and (6) enol ether hydrolysis (TFA, CH_2Cl_2, MeOH).

28. Oltvoort, J.J.; van Boeckel, C.A.A.; de Koning, J.H.; van Boom, J.H. *Synthesis* **1981**, 305.

29. Analogs **13b-e** were prepared in a manner identical to that used for **13a** with an alkylation step immediately before or after allyl isomerization.

30. Compound **13f** was prepared from **13d** by (1) peracetylation (Ac_2O, DMAP, Et_3N) and (2) de-*O*-acetylation ($NaHCO_3$, aq. MeOH).

31. 4-Pyrrolidinobenzaldehyde and 3-fluoro-4-pyrrolidinobenzaldehyde were prepared by reaction of pyrrolidine with 4-fluorobenzaldehyde and 3,4-difluorobenzaldehyde, respectively.

32. 4-Butyl-3-hydroxybenzaldehyde was prepared from vanillin by (1) triflate formation ($(Tf)_2O$, pyridine), Stille coupling (Bu_4Sn, $(CH_3CN)_2PdCl_2$, DMF), and (3) demethylation (BBr_3, CH_2Cl_2). 4-Butylbenzaldehyde was prepared similarly from 4-hydroxybenzaldehyde.

33. 3-Fluoro-4-methoxybenzaldehyde and 3-fluoro-4-propargyloxybenzaldehyde were prepared by reaction of sodium methoxide and sodium propargyloxide, respectively, with 3,4-difluorobenzaldehyde.

34. Mode of action studies have revealed a ribosomal peptidyltransferase inhibition mechanism for hygromycin A: Guerrero, M. del C.; Modolell, J. *Eur. J. Biochem.* **1980**, *107*, 409.