# SEMISYNTHETIC HYGROMYCIN A ANALOGS: SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF DERIVATIVES LACKING THE FURANOSE MOIETY

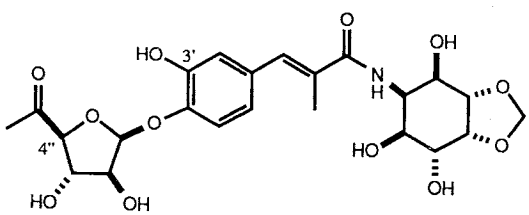
Sir:

Hygromycin A (**1**) is a fermentation-derived natural product first isolated from *Streptomyces hygroscopicus* in 1953.[1,2] Although known to possess moderate antibacterial activity, it was only recently discovered to have excellent *in vitro* potency[3] 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.[4] The renewed interest in hygromycin A has led to increased synthesis activities, including a recent total synthesis.[5] In this Communication, we wish to report a highly potent series of hygromycin A analogs in which the *arabino*-furanosyl aryl substitutent has been replaced by simple lipophilic alkyl chains.

Our objectives within a semisynthetic hygromycin A analog program were to discover a novel antibacterial with improved spectrum and potency and, since hygromycin A is usually obtained as a mixture (with *epi*-hygromycin, its C-4″ epimer), we sought simplified sugar surrogates devoid of easily epimerized centers. Towards this end, we developed methodology to remove the sugar moiety from the antibiotic and regioselectively derivatize the newly liberated phenolic hydroxyl group. Selective protection of the C-3′ phenolic hydroxyl group of hygromycin A† with allyl bromide and potassium carbonate in DMF was followed by acetylation of the remaining hydroxyl groups. When the latter reaction was carried out with acetic anhydride and triethylamine as base, acetylation was accompanied by the loss of one equivalent of acetic acid to provide unsaturated ketone **2** (S. J. HECKER, manuscript in preparation). The methyl ketone was reduced ($NaBH_4$) to a mixture of alcohols which was subjected to enol ether hydrolysis conditions ($I_2$, $H_2O$, THF) to cleave the dihydrofuran from the C-4′ aryl oxygen to yield aglycone **3**. Prior to derivatization of the phenolic hydroxyl, the C-3′ allyl protecting group was armed for subsequent deprotection by olefin migration with catalytic 1,5-cyclooctadiene-bis(methyldiphenylphosphine)-iridium hexafluorophosphate.[6]

Alkylation of phenol **4** was accomplished by either displacement of an alkyl bromide or Mitsunobu coupling[7] with an alcohol. For example, treatment with allyl bromide and $K_2CO_3$ in DMF or with allyl alcohol, triphenylphosphine, and diethyl azodicarboxylate (DEAD) in THF afforded allyl ether **5** (R=allyl), which was completely deprotected by vinyl ether hydrolysis ($I_2$, $H_2O$) and deacetylation ($K_2CO_3$, MeOH). Characterization of resultant analog **6** (R=allyl) by $^1H$ and $^{13}C$ NMR and combustion analysis supports the structure assignment: $^1H$ NMR (300 MHz, $CD_3OD$) $\delta$ 7.24 (1H, br s), 6.92 (2H, m), 6.84 (1H, dd, $J$=1.9, 8.4 Hz), 6.08 (1H, m), 5.40 (1H, br d, $J$=17 Hz), 5.25 (2H, m), 4.78 (1H, s), 4.61 (2H, m), 4.50 (1H, m), 4.19 (3H, m), 3.97 (1H, t, $J$=6.7 Hz), 3.81 (1H, t, $J$=2.8 Hz), 2.11 (3H, d, $J$=1.3 Hz); $^{13}C$ NMR (75 MHz, $CD_3OD$) $\delta$ 172.7, 147.9, 147.7, 135.4, 134.9, 131.3, 130.8, 122.8, 118.0, 117.7, 114.4, 96.2, 78.2 (2), 72.6, 71.6, 71.3, 70.9, 50.2, 14.7; *Anal* calcd

Fig. 1.




Hygromycin A (**1**)

Table 1. *In vitro* and *in vivo* antibacterial activities of hygromycin A ethers **6**.

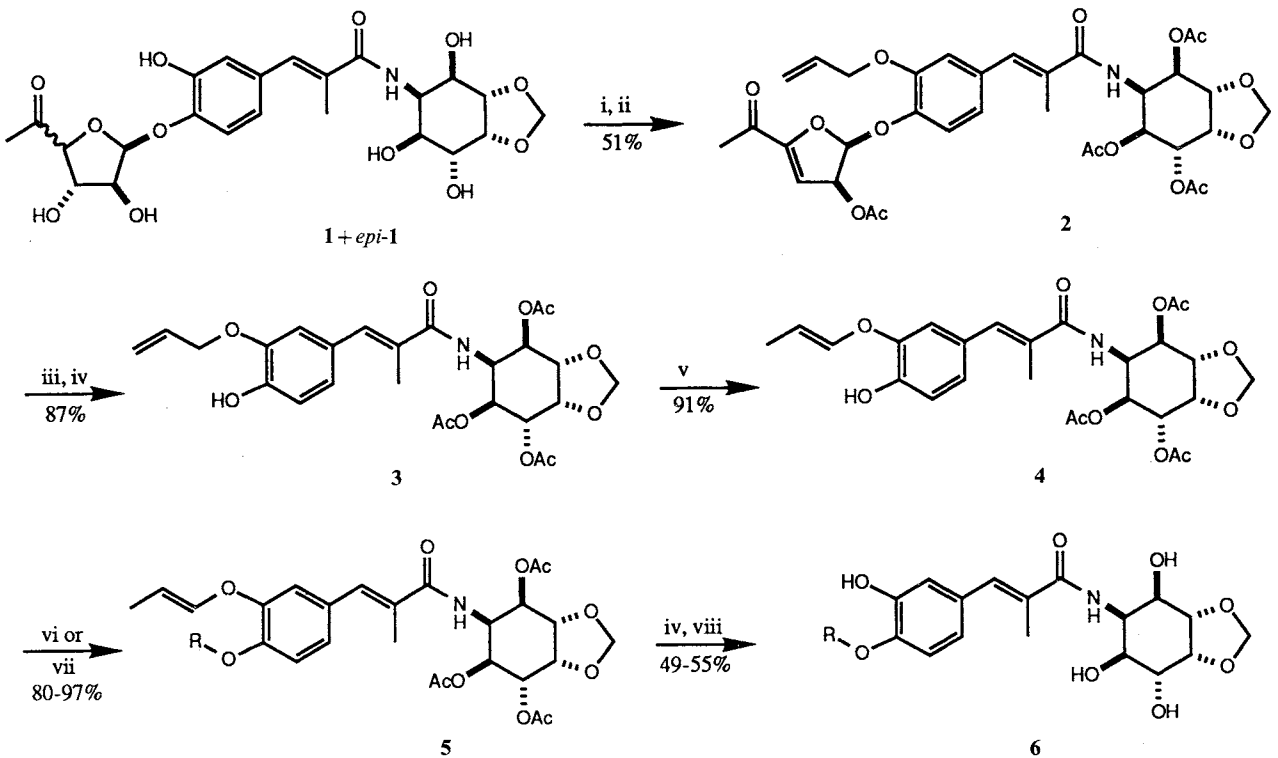
| R | *S. hyodysenteriae* MIC[a] | Mouse $ED_{50}$[b] (po) |
|---|---|---|
| Hygromycin A (**1**) | 1.56 | 0.1 |
| Propyl | 1.56 | 0.6 |
| Allyl | 0.78 | 0.2 |
| Propargyl | 6.25 | 1.5 |
| Cyclopropyl methyl | 0.78 | 0.3 |

[a] *Serpulina hyodysenteriae* 94A002 MICs (μg/ml) determined indirectly by measuring hemolytic activity in liquid medium according to WEBER and EARLEY.[8]

[b] Compound required (mg/kg/day) to eliminate *S. hyodysenteriae* 94A008 from 50% of infected mice.[8]

† Material was obtained by fermentation of *Streptomyces hygroscopicus* NRRL-2388, and consisted of hygromycin A and its C-4″ epimer in a ratio of approximately 70:30.

Scheme 1.




Reagents: i) Allyl bromide, $K_2CO_3$, DMF, ii) $Ac_2O$, DMAP, $Et_3N$, $CH_2Cl_2$, iii) $NaBH_4$, MeOH, $H_2O$, iv) $I_2$, poly(4-vinylpyridine), $H_2O$, THF, v) $Ir(COD)(MePh_2P)_2PF_6$, THF, vi) RBr, $K_2CO_3$, DMF, vii) ROH, DEAD, $Ph_3P$, THF, viii) $K_2CO_3$, MeOH.

for $C_{20}H_{25}NO_8$: C 58.96, H 6.18, N 3.44. Found: C 58.80, H 6.07, N 3.41.

Although our initial targets were to be somewhat more complex sugar replacements,† we were gratified to find that compounds **6** retain *in vitro* and *in vivo* activity despite having none of the structural elements of the hygromycin furanose. Ether **6** (R=allyl) shows *in vitro* activity against *S. hyodysenteriae* at least as potent as the parent compound; the antibacterial activities of several ether analogs are compared to hygromycin A against this pathogen in Table 1. In addition, ethers **6** demonstrate *in vivo* activity in a mouse *S. hyodysenteriae* colonization model.[8] The effective dose ($ED_{50}$) for the allyl ether is 0.2 mg/kg/day compared to 0.1 mg/kg/day for hygromycin A. In conclusion, simple lipophilic ethers bearing little structural resemblance to the natural product have been found to serve as functional sugar surrogates. Further *in vivo* studies of hygromycin A ethers and additional synthetic modifications employing intermediate **4** will be reported in the future.

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† Details will be published elsewhere.

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