Efficient Preparation of Hygromycin A Aglycon and Its Antibacterial 4'-Allyl Ether, CP-111905

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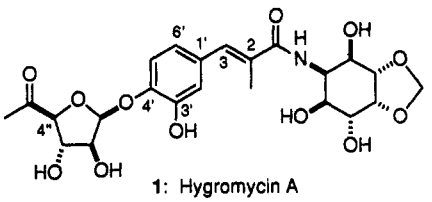
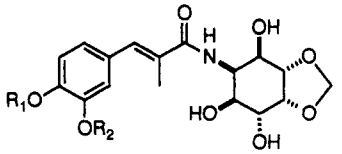
Hygromycin A (1) is an effective agent for the control of swine dysentery, a mucohemorrhagic disease of economic importance to swine producers.[1] We have identified an analog of hygromycin A, CP-111905 (3), that is potent against *Serpulina (Treponema) hyodysenteriae*, the causative agent of swine dysentery, and is at least as efficacious as hygromycin A in mouse and swine infection models.[2] In our early analog work, allyl ether 3 was synthesized from hygromycin A by a relatively inefficient route in eight steps that included several protection/deprotection sequences.[2] We now report a significant improvement upon the literature method[3] for preparation of hygromycin aglycon 2, as well as an efficient procedure for conversion of 2 to CP-111905.



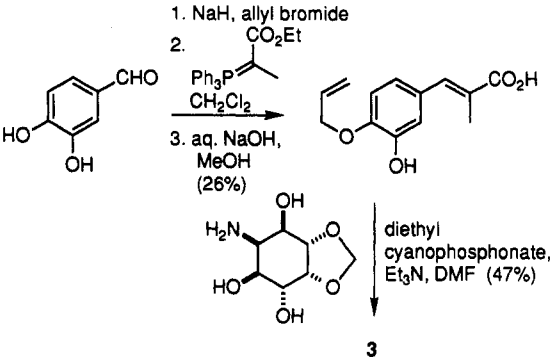
1: Hygromycin A



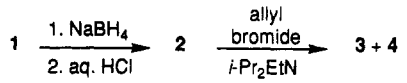
2: $R_1 = R_2 = H$ (aglycon)
3: R_1 = allyl, $R_2 = H$ (CP-111905)
4: $R_1 = H$, R_2 = allyl

Our original semisynthetic route relied on initial protection of the C3' and aminocyclitol hydroxyl groups, sugar removal, alkylation, and several deprotection steps.[2] The sequence permitted preparation of a variety of analogs but was not amenable to multigram synthesis. Although aglycon 2 seemed like an ideal intermediate, we initially avoided it since its reported preparation[3] from hygromycin A was inefficient, the subsequent alkylation was not highly regioselective (~3:1 in favor of 3), and the resulting mixture was not separated readily by standard silica gel chromatography. Early attempts to improve the synthesis employed the more selective alkylation of 3,4-dihydroxybenzaldehyde with allyl bromide;[4] alkylation with NaH/DMSO provides a mixture of ~7:1 in favor of the C4' allyl ether (Scheme 1). The mixture is olefinated with (carbethoxyethylidene)triphenylphosphorane and the resulting esters are saponified and crystallized from benzene, which removes the minor C3'-alkylated material. Synthesis of the analog CP-111905 is completed by coupling the activated acid (diethyl cyanophosphonate, triethylamine) with the natural aminocyclitol[5] obtained by degradation of hygromycin A.[6] Although this route provides allyl ether 3, we were not satisfied with the number of steps required and the inefficient utilization of the fermentation-derived hygromycin A. We returned our attention to the use of aglycon 2 as an intermediate that uses the natural product most effectively.

Scheme 1



Scheme 2



Degradation of hygromycin A to aglycon 2 by mercaptanolysis has been reported in the literature[3] but proceeds in low yield and requires the use of ethanethiol/HCl. After several acidic sugar hydrolysis procedures were tried, a one-pot, two-step process was developed, requiring only a filtration for workup (Scheme 2). Thus, hygromycin A is reduced with $NaBH_4$ in water;[7] the reaction solution is acidified with aqueous HCl which leads to precipitation of aglycon 2. Allylation of 2 is accomplished with allyl bromide and *N,N*-diisopropylethylamine in chloroform/methanol to afford a ~3:1 mixture in favor of C4' *O*-alkylation.

Separation of regioisomers 3 and 4 proved to be straightforward by recrystallization from water; pure 3 is obtained in 27% yield from aglycon 2. Improved yields can be obtained by HPLC separation of the two isomers. Reverse phase chromatography (C_{18} column, 65/35 water/methanol mobile phase) provides a dramatic difference in elution time for 4 (9.5 min) and 3 (15.4 min) and allows purification of 30-g samples on preparatory scale columns.

Recrystallization from water provided crystalline CP-111905 suitable for X-ray diffraction analysis.[8] In addition to confirming the regiochemistry of the allylation, two interesting features are revealed by the X-ray structure. First, the enamide moiety is nearly coplanar with the

(1) Nakagawa, A.; Fujimoto, T.: Omura, S.; Walsh, J. C.; Stotish, R. L.; George, B. *J. Antibiot.* **1987**, *40*, 1627.
(2) Jaynes, B. H.; Elliott, N. C.; Schicho, D. L. *J. Antibiot.* **1992**, *45*, 1705.
(3) Mann, R. L.; Woolf, D. O. *J. Am. Chem. Soc.* **1957**, *79*, 120.
(4) Kessar, S. V.; Gupta, Y. P.; Mohammad, T.; Goyal, M.; Sawal, K. K. *J. Chem. Soc., Chem. Commun.* **1983**, 400.
(5) The three-step sequence from a benzaldehyde to a cinnamide is analogous to the route used in Ogawa's total synthesis of hygromycin A: Chida, N.; Ohtsuka, M.; Nakazawa, K.; Ogawa, S. *J. Org. Chem.* **1991**, *56*, 2976.
(6) Kakinuma, K.; Sakagami, Y. *Agric. Biol. Chem.* **1978**, *42*, 279.
(7) As with the mercaptanolysis,[3] ketone reduction is required for clean conversion to aglycon.
(8) The author has deposited atomic coordinates for 3 with the Cambridge Crystallagraphic Data Centre. The coordinates can be obtained, on request, from the Director, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB2 1EZ, U.K.

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aromatic ring in contrast to the twisting predicted by molecular modeling minimizations (gas phase). A consequence of aryl-enamide planarity is distorted sp^2 bond angles (C2–C3–C1′ = 133.5°; C3–C1′–C6′ = 116.4°) owing to the proximity of the α-methyl group and the C2′ *ortho* hydrogen. Second, as suggested by hygromycin A solution phase ^1H NMR coupling constants,[6] the aminocyclitol adopts a rigid twist-boat conformation with the amide nitrogen pseudoequatorially disposed. Previously published studies indicate that this conformation is preferred for potent *in vitro* antibacterial activity.[9]

In summary, a short and efficient preparation of the antibacterial agent CP-111905 from hygromycin A is described. The synthetic route relies on an improved degradation to aglycon **2** and an effective separation of regioisomers **3** and **4** by recrystallization or reverse phase HPLC and has allowed the synthesis of multigram quantities of CP-111905. This synthesis also provides a general route to other C4′ ether analogs of hygromycin A.

Experimental Section

^1H and ^{13}C NMR spectra were recorded on a 300-MHz NMR spectrometer using methanol-d_4 as solvent.

(*E*)-5-Deoxy-5-[[3-[3,4-dihydroxyphenyl]-2-methyl-1-oxo-2-propenyl]amino]-1,2-*O*-methylene-D-*neo*-inositol (Aglycon 2). To a mechanically-stirred solution of hygromycin A (**1**, 110 g, 206 mmol, 3:1 mixture of diastereomers at C4″ as obtained from fermentation) in 850 mL of water was added a sodium borohydride solution (8.6 g, 227 mmol, in 150 mL water) over a 3-h period at ambient temperature. Some MeOH was added to the reaction to decrease surface foaming. After an additional hour of stirring, the pH was carefully adjusted to neutrality with concentrated HCl, followed by addition of 40 mL of concentrated HCl. The mixture was stirred at 40 °C for 16 h, cooled to ambient temperature, and filtered. The white solid was slurried in acetone, filtered, and dried under vacuum to afford 40.5 g (54%) of the title aglycon. Spectroscopically, this material was identical to that prepared by a lengthier literature method.[3]

(9) Hecker, S. J.; Lilley, S. C.; Werner, K. M. *BioMed. Chem. Lett.* **1992**, *2*, 1043.

(*E*)-5-Deoxy-5-[[3-[4-(2-propenyloxy)-3-hydroxyphenyl]-2-methyl-1-oxo-2-propenyl]amino]-1,2-*O*-methylene-D-*neo*-inositol (CP-111905, 3). To a solution of **2** (40 g, 109 mmol) and *N,N*-diisopropylethylamine (95 mL, 550 mmol) in chloroform and methanol (300 mL each) at ambient temperature was added a solution of allyl bromide (10.4 mL, 120 mmol) in chloroform (25 mL). The solution was then stirred for 16 h at 50 °C and cooled, and the solvent was evaporated. The residue was dissolved in water (250 mL) and extracted with chloroform (3 × 300 mL); the combined organic layers were washed with water (200 mL) and then the aqueous layers were combined and evaporated to dryness. After silica gel chromatography (10% MeOH/$CHCl_3$), a ~3:1 mixture of 4′-*O*-allyl:3′-*O*-allyl ethers was obtained. Recrystallization from chloroform and then water yielded pure title compound (first crop, 10.5 g, >99.5% purity by HPLC; second crop, 1.5 g, 94.8% by HPLC; total yield 27%; HPLC conditions—Beckman Ultrasphere ODS 5 mm 4.6 × 250 mm column, premixed 65% water/35% MeOH, 272 nm wavelength, 1 mg/mL concentration, 1 mL/min flow rate).

Alternatively, the isomers can be separated by preparative HPLC. A typical run involved loading the mixture onto a Waters Prep 500 (four C_{18} cartridges) and running a methanol/water gradient (20% to 25% water; total volume, 46 L). Eleven 1-l fractions contained pure **3**; concentration and lyophilization afforded 15.9 g of >99.5% pure material (34% from 42.6 g of **2**): mp 171–172 °C (water); ^1H NMR (CD_3OD) δ 7.24 (1H, br s,), 6.92 (2H, m), 6.84 (1H, dd, J = 1.9, 8.4), 6.08 (1H, m), 5.40 (1H, br d, J = 17), 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), 3.81 (1H, t, J = 2.8), 2.11 (3H, d, J = 1.3); ^{13}C NMR (CD_3OD) δ 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 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.

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Supplementary Material Available: ^1H NMR spectra of **2**, **3**, and **4** and ORTEP of **3** (4 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.