Total Synthesis

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Synthesis of (−)-Hygromycin A: Application of Mitsunobu Glycosylation and Tethered Aminohydroxylation**

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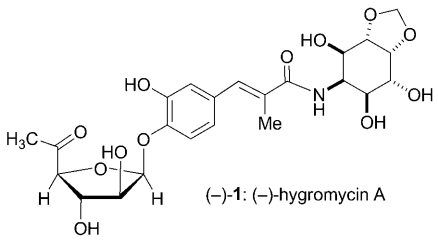
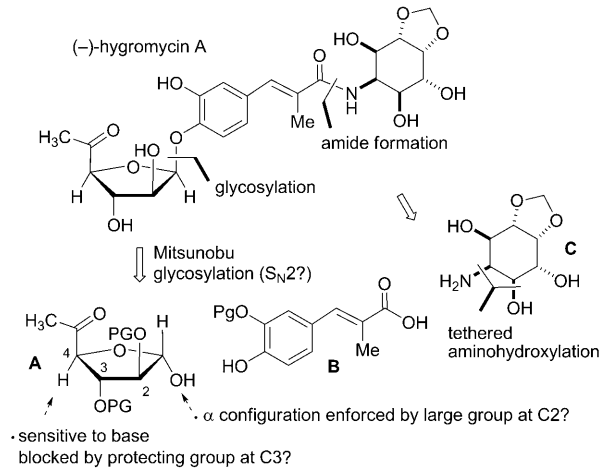
The natural product (−)-hygromycin A, (−)-**1**, was isolated from the fermentation of *Streptomyces hygroscopicus* in 1953 and shown to be a broad-spectrum antibiotic which also exhibits immunosuppressant activity.[1] The mode of antibacterial action of the natural product has been shown to involve inhibition of the ribosomal peptidyl transferase activity, and hygromycin A binds to the ribosome in a manner that is closely related, but not identical, to that of chloroamphenicol.[2] Recent interest from the pharmaceutical industry has centered around the possibility of using this compound, or analogues thereof, in the control of swine dysentery and other infectious diseases of economic importance in animal health.[3]



Despite its biological activity and interesting structure, there has been only one successful synthesis of this product, by Ogawa et al. in 1991,[4] although note should be made of the elegant synthesis of C-2-epihygromycin A by Trost et al. in 2002.[5] Although Ogawa's synthesis was inspiring, several areas could be improved upon. Most notably the formation of the *cis* sugar linkage between the phenol and furanose was essentially nonselective, and the sequence was lengthy with low overall yield. Our interest in the molecule was originally sparked by the aminocyclitol moiety which was a challenging target on which to test our recently developed tethered aminohydroxylation (TA) methodology.[6] In addition, we felt that the sequence to the natural product itself could be shortened and that the key glycosylation reaction could be made selective for the desired β anomer.

Our retrosynthesis of this molecule consisted of disconnection into three portions, namely the furanose sugar **A**, the aromatic linker **B**, and the aminocyclitol **C**. The key challenges were thought to be the formation of the sterically crowded β-configured sugar linkage to the phenol and the avoidance of epimerization at C4 of the sugar adjacent to the sensitive ketone unit.[7] While our earlier studies had culminated in a short synthesis of cyclitol **C**, we anticipated that we could improve this further and generate material suitable for completion of the synthesis of (−)-**1**. One other noteworthy point about our proposed route is the importance of the protecting groups at C2 and C3 of the furanose sugar **A**. We anticipated that installation of a bulky group here (we chose to use a triisopropylsilyl (TIPS) group) might have two beneficial effects on the synthesis. Firstly, the large group at C2, adjacent to the anomeric position, might exhibit a bias on the diastereoisomeric ratio of the anomeric hydroxy group that is a precursor to sugar coupling (favoring the α anomer, see **A**; Scheme 1). According to the innovative work of Smith



Scheme 1. Retrosynthetic analysis of (−)-hygromycin A. PG = protecting group.

et al. and then Roush et al.,[8] and assuming that S_N2 displacement is faster than anomerization, then such a bias may well manifest itself in a β-selective glycosylation reaction with a phenol acceptor under Mitsunobu-type coupling conditions.

Secondly, it has been noted in previous studies that the natural product and its precursors are sensitive towards epimerization at C4 (in Ogawa's synthesis this sensitivity

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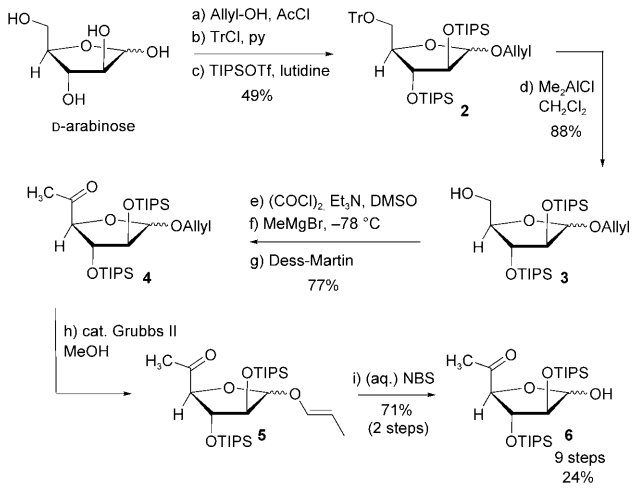
required the introduction of a ketal protecting group for the ketone).[7] We hoped that a bulky protecting group at C3 would hinder the vulnerable proton and thus enable us to carry the kinetically stable ketone through the synthesis without protection.

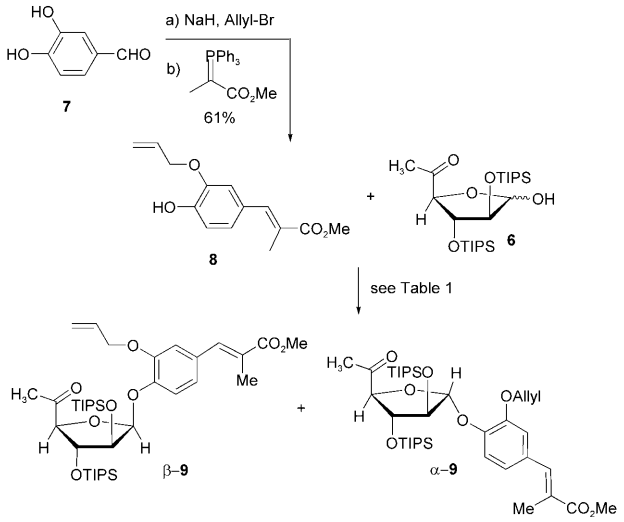
Our synthesis began with commercially available and inexpensive D-arabinose (drawn in the furanose form for clarity), which was substituted with an allyl group at the anomeric position before ensuing tritylation of the primary hydroxy group and TIPS protection of the two secondary alcohols (see **2**, Scheme 2). Then, selective cleavage of the



Scheme 2. Preparation of the furanose segment. DMSO = dimethyl sulfoxide, NBS = *N*-bromosuccinimide, py = pyridine, Tf = trifluoromethanesulfonyl, Tr = triphenylmethyl (trityl).

trityl ether gave alcohol **3**, which was oxidized to the aldehyde, reacted with MeMgBr, and then oxidized with the Dess–Martin reagent to yield ketone **4** in 68% yield from **2**. Finally the allyl protecting group at C1 was removed by a two-step procedure that began with alkene isomerization under the action of RuH generated in situ by the decomposition of the Grubbs II catalyst.[9] The enol ether (**5**) thus formed was cleaved by reaction with aqueous NBS to generate the free alcohol **6** in 71% yield from allyl ether **4**. This sequence concluded a nine-step preparation of the sugar unit of hygromycin A that proceeded in 24% overall yield.

The pivotal glycosylation reaction was then investigated using phenol **8** (made in two steps from **7**) as the nucleophile. Given the acidic nature of the pronucleophile, we decided to examine the Mitsunobu glycosylation reaction, searching for conditions that favored the more hindered β isomer, which we required (Scheme 3). Table 1 shows that there was a significant variation in the α/β ratio depending upon the solvent that was used for the coupling. Toluene was clearly the solvent of choice, and further optimization of the reaction conditions led us to perform the reaction at 60 °C with slow addition of diisopropylazodicarboxylate (DIAD) (Table 1, entry 6). Pleasingly, this procedure furnished the product in 83% yield and with 90:10 selectivity for β-**9** (note that the diastereoisomers were not separable). In general terms there was only a broad correlation between the α/β ratio of



Scheme 3. Selective Mitsunobu glycosylation.

Table 1: Mitsunobu glycosidation of **6** leading to **9**.

Entry	Conditions[a]	**6** (α/β ratio)[b]	Yield of **9** (α/β)[c]
1	DIAD, PPh_3, THF, RT	[D_8]THF (83:17)	47% (40:60)
2	DIAD, PPh_3, CH_2Cl_2, RT	CD_2Cl_2 (86:14)	68% (29:71)
3	DIAD, PPh_3, toluene, RT	C_6D_6 (86:14)	80% (25:75)
4	DIAD, PPh_3, toluene, 60 °C	C_6D_6 (86:14)	75% (17:83)
5	DIAD, PPh_3, toluene, 60 °C	C_6D_6 (86:14)	83% (10:90)[c]
6	ADDP, PBu_3, toluene, RT		69% (100:0)

[a] The ratio of diazo compound/phosphine/**6** was 1.6:1.6:1 throughout. [b] The relative configurations were determined from NMR spectra recorded in the solvent given. [c] The relative configurations were assigned by examination of NOE data and $J_{H1,H2}$ coupling constants for α-**9** and β-**9**.[4b,10] [c] Slow addition of DIAD. ADDP = 1,1′(azodicarbonyl)dipiperidine.

the starting material **6** and the α/β ratio of the coupled products, which shows that other processes (such as S_N1 or anomerization) can compete with S_N2 attack; these results are not as clear-cut as those reported with pyranose sugar donors.[8] Interestingly, complete α-selectivity was achieved with Bu_3P and ADDP, and this was interpreted in terms of prior ionization of the anomeric hydroxy group to an oxocarbenium ion, which was then intercepted by the nucleophile in a stereoselective manner.

The inositol unit of the natural product was then prepared using a route developed in our laboratories, starting from commercially available dienone **10** (Scheme 4). The key step in the sequence was the tethered aminohydroxylation reaction of the primary carbamate derived from alcohol **11**. However, we have recently shown that significant improvements can be made to the tethered aminohydroxylation protocol by embedding the re-oxidant into the substrate and preparing N,O-acylated derivatives (ROCONHOCOAr), rather than the parent carbamates ($ROCONH_2$- which must be chlorinated in situ).[6b] Therefore, alcohol **11** was activated (CDI) and then quenched with hydroxylamine to provide a N-OH carbamate, which was subsequently esterified on the free hydroxy group by reaction with mesitoyl chloride (Scheme 4).

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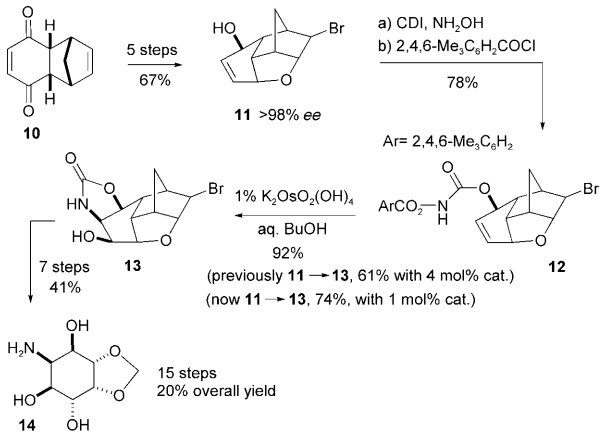


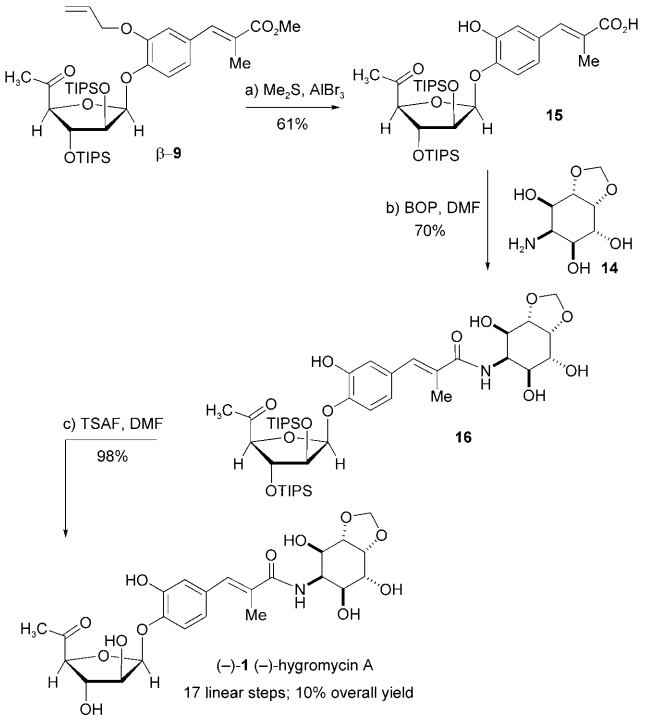
Scheme 4. Synthesis of the aminocyclitol moiety of hygromycin A. CDI = 1,1′-carbonyldiimadazole.

Ensuing aminohydroxylation of **12** was accomplished by reaction with potassium osmate (1 mol%) in aqueous butanol at room temperature; no halogenating agent was required and the yields and catalyst loadings are far improved over those of the original procedure. The original protocol transformed **11** into **13** in 61% yield using 4 mol% of the osmium catalyst; now we could accomplish the same transformation in 74% using 1 mol% of the catalyst. The oxazolidinone thus produced was converted into aminocyclitol **14** in a further seven steps to constitute a 15-step route with 20% overall yield; this compares well with the yields of the two routes reported in the literature (Ogawa group: 20 steps, 2.7% overall yield; Trost group: 13 steps, 10% overall yield from benzoquinone or 10 steps, 23% yield from conduritol B tetraacetate).

The final sequence required deprotection of the aromatic linker. The two protecting groups on the arene unit were removed by reaction with aluminum tribromide and dimethylsulfide,[11] yielding free acid **15** in 61% yield (Scheme 5). (Note that at this point the minor diastereoisomer from the sugar coupling could be separated by chromatography.) This free acid was then coupled with aminocyclitol **14** (see Scheme 4), furnishing amide **16** in 70% yield after chromatography. Pleasingly, no epimerization was detected at C4 during either of these two steps, suggesting that the OTIPS group at C3 was acting as planned. Finally, the two TIPS groups were removed using a mild source of fluoride (tris(dimethylamino)sulfonium difluorotrimethylsilicate (TSAF))[12] at 0 °C. (At this point care must be taken to ensure that there is no epimerization at C4.) This sequence gave synthetic (−)-hygromycin A (−)-**1**; its spectroscopic data closely matched that reported in the literature (^{1}H/^{13}C NMR, m.p., $[\alpha]_D$).

In conclusion, we have synthesized (−)-hygromycin A (−)-(**1**) by a sequence that is noteworthy for its brevity and efficiency with just 17 linear steps and 10% overall yield. This route compares well with the only previous synthesis of this compound by Ogawa (24 linear steps and < 1% yield).

Key points are the successful Mitsunobu glycosylation strategy which can be tuned to produce good selectivity for the desired anomer (and the unnatural anomer if desired). In addition, recent improvements to the reagents used for the tethered aminohydroxylation mean that the synthesis of the aminocyclitol ring is improved considerably.



Scheme 5. Completion of the synthesis. BOP = 1-benzotriazolyloxytris-(dimethylamino)phosphonium hexafluorophosphate.

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