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Synthesis and biological evaluation of novel hygromycin A antibacterial agents

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ABSTRACT

Novel hygromycin A derivatives bearing a variety of functionalized aminocyclitol moieties have been synthesized in an effort to increase the antibacterial activity and drug-like properties of this class of agents. A systematic study of the effect of alkylation and removal of the hydroxyls of the aminocyclitol directed us to a series of alkylated aminocyclitol derivatives with improved Gram-positive activity.

The emergence of multidrug resistance among Gram-positive pathogens represents a significant challenge for medical professionals.[1] Of particular concern are methicillin-resistant *Staphylococcus aureus* (MRSA)[2,3] and vancomycin-resistant enterococci (VRE), due to a combination of increasing prevalence and recalcitrance to therapy.[4] One important strategy to address these resistance issues is the development of new classes of antibiotic drugs with activity against resistant Gram-positive pathogens.

Hygromycin A (**1**, Table 1), isolated from *Streptomyces hygroscopicus* and first reported in 1953,[5] has been demonstrated to be a bacterial protein synthesis inhibitor[6] with weak activity against Gram-positive organisms.[7] A program to develop hygromycin A derivatives for Gram-positive pathogens led to the discovery that the furanose portion of the natural product could be truncated to simple lipophilic groups such as the allyl group in **2**.[8,9] Critical in this work was the discovery that the phenolic hydroxyl group could be replaced by either one or two fluorine atoms as phenyl substituents.[10,11] This resulted in the discovery of compounds such as **3**. Compound **3** showed antibacterial activity, with MIC values in the range of 0.2–4 μg/ml against a panel of Gram-positive bacteria. However, **3** behaved sub-optimally in oral pharmacokinetic studies in rats, exhibiting low bioavailability (~20%). Because this was a low clearance, soluble compound, we expected that the low bioavailability was due primarily to poor permeability and possible active efflux (CaCO-2 values of AB = 0.9×10^{-6} cm/s, and BA = 5.9×10^{-6} cm/s).

Here we describe initial derivatizations of the aminocyclitol moiety,[12,13] with the goal of producing more potent antibacterial agents and improving permeability to produce analogs with enhanced bioavailability. A systematic study of alkylation and hydroxyl removal from the aminocyclitol moiety was undertaken to determine which position of the aminocyclitol held the most promise for further derivatization efforts.

The alkylated and deoxygenated aminocyclitol derivatives were prepared as shown in Scheme 1A. Synthesis of methylated analogs began with protection of aminocyclitol **4**, which was derived from degradation of hygromycin A.[14] Selective protection of the amine and 6′-hydroxyl as the phenyloxazoline provided a common intermediate for diversification of the remaining hydroxyls of the aminocyclitol based on selective protecting group manipulation.[12,13] The 5′-hydroxyl was selectively silylated with TBSCl to afford **5**. The free 2′-hydroxyl was subsequently alkylated with MeI; removal of all protecting groups with HF–pyridine, followed by transfer hydrogenation,[15] afforded **6**. Alternatively, intermediate **5** was deoxygenated by treatment with Ph_3P and DEAD, and similar deprotection afforded compound **7**. Synthesis of the 5′-methoxy hygromycin analog began with forcing bis-silyl protection of the oxazoline aminocyclitol with TBSCl followed by selective deprotection of the 5′-position, to afford mono-silyl diol **8**. Subsequent alkylation of the 5′-position with NaH and MeI in THF followed by deprotection produced methoxy aminocyclitol **9**. Compound **8** was also used as an intermediate to remove the 5′-hydroxyl. In this

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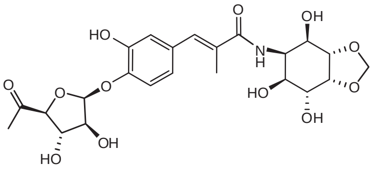
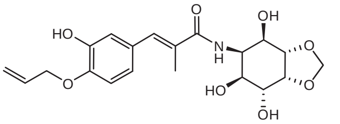
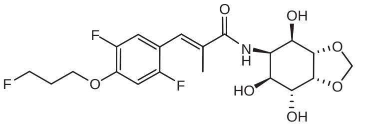
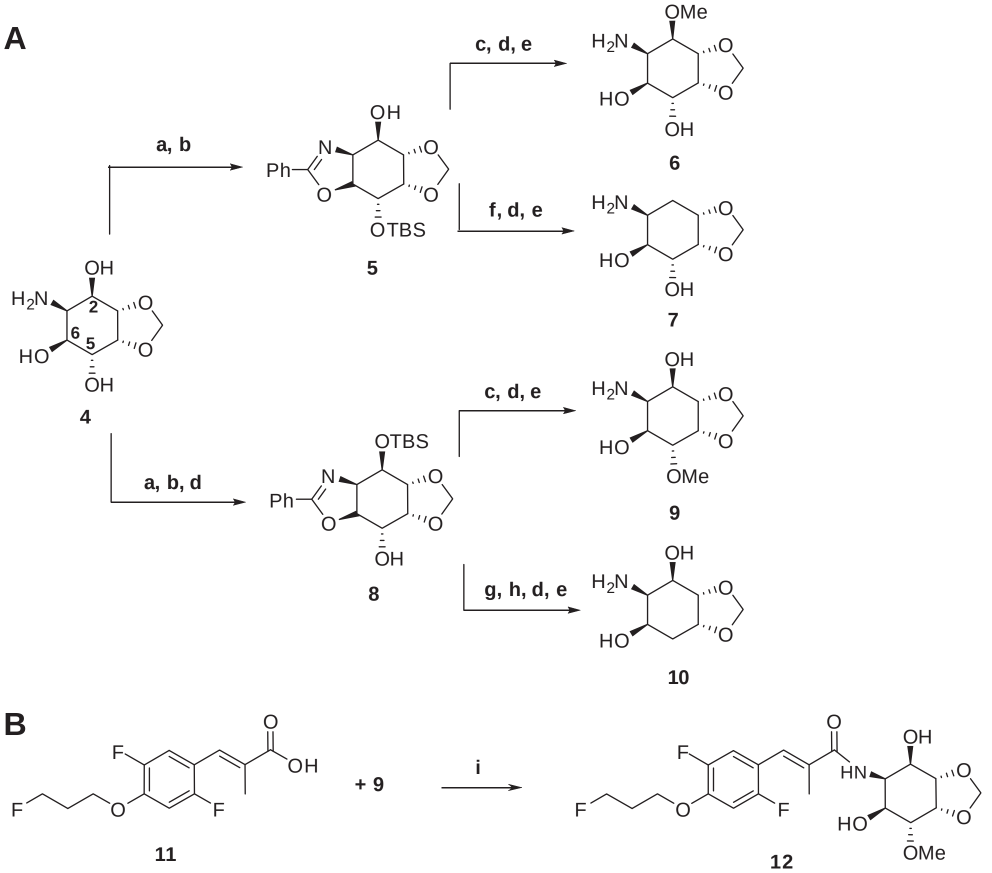
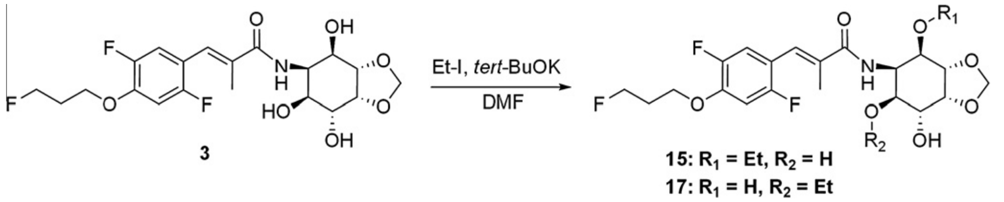
Table 1
In vitro antibacterial activity of hygromycin A, **1** [MIC (μg/ml)] and other early analogs in a panel of Gram-positive pathogens

Compound	Structure	*S. aureus* 1095	*S. aureus* 1146	*S. pneumoniae* 1046	*S. pneumoniae* 1095	*S. pyogenes* 203	*S. pyogenes* 1079
1		50	25	12.5	12.5	6.25	6.25
2		50	50	1.56	1.56	1.56	0.8
3		4	4	0.5	0.4	2	<0.2



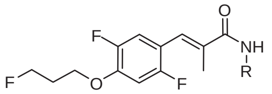
Scheme 1. (A and B) Reagents and conditions: (a) PhCN, K_2CO_3, glycerol; (b) TBSCl, imidazole, CH_2Cl_2; (c) NaH, MeI, THF; (d) HF–pyridine, THF; (e) Pd black, HCO_2NH_4, AcOH; (f) Ph_3P, DEAD; (g) NaH, CS_2, CH_3I, THF; (h) Bu_3SnH, toluene; (i) EDCI, DMAP, CH_2Cl_2.

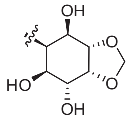
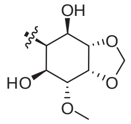
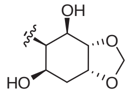
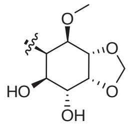
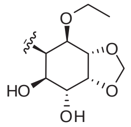
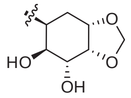
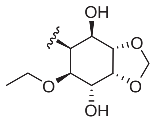
sequence, formation of the methyl xanthate with NaH, CS_2 and MeI, followed by reduction of the xanthate with tributyltin hydride and removal of the silyl and oxazoline protecting groups, gave the 5′-deoxy aminocyclitol **10**.[16] Final analogs **12**, **13**, **14**, and **16** were prepared by coupling the appropriate aminocyclitol intermediate (such as **9**) with carboxylic acid **11** using EDCI (Scheme 1B).[17] In order to more efficiently explore potential substituents at these positions, a new synthetic route (Scheme 2) was developed. This approach took advantage of a regioisomeric alkylation preference for the 2′- and 6′-position isomers (with a consistent, major preference for 2′-alkylation), relative to the rather unreactive 5′-position. Purification by reverse phase HPLC consistently separated the desired analogs for testing, but generally in only low to moderate yield.



Scheme 2. Regioisomeric alkylation of the 2′- and 6′-positions of hygromycin derivatives.

Table 2
In vitro antibacterial activity [MIC (μg/ml)] of aminocyclitol derivatives

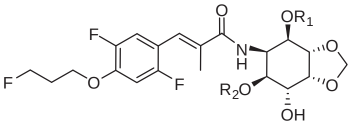


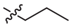
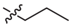
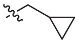
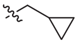
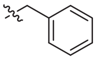
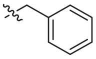
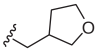
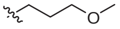
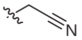
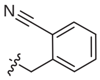
Compound	R-group	*S. aureus* 1095	*S. aureus* 1146	*S. pneumoniae* 1046	*S. pneumoniae* 1095	*S. pyogenes* 203	*S. pyogenes* 1079
3		4	4	0.5	0.39	2	<0.20
12		>100	100	25	25	100	50
13		64	32	4	8	16	8
14		6.25	3.13	0.78	1.56	1.56	1.56
15		8	8	1	4	4	1
16		16	8	ND	8	4	8
17		8	8	2	8	1	2

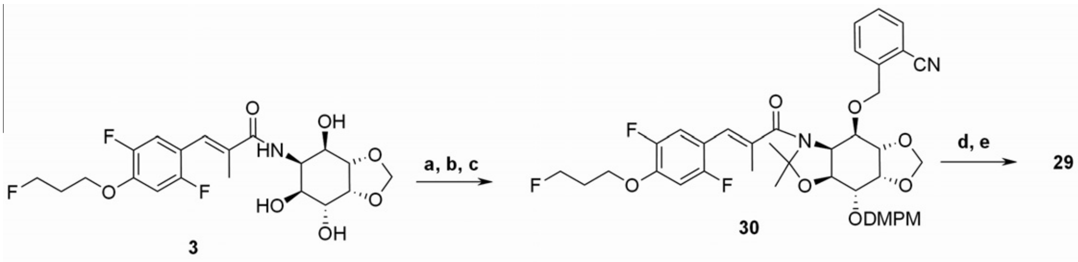
The new hygromycin A analogs were assessed for antibacterial activity against a panel of Gram-positive strains (Table 2). It immediately became clear that the 5′-hydroxyl was critical to maintain useful antibacterial activity, as the methylation of that hydroxyl (**12**) or its removal (**13**) were both very detrimental modifications, leading to significant loss in activity.[13] In contrast, the 2′-position seemed far more tolerant of manipulation, as the methyl and ethyl derivatives (compounds **14** and **15**) retained much of the potency of the parent analog. Deoxygenation at the 2′-position (**16**) decreased activity relative to the parent hydroxyl, but not to the same extent as the 5′-regioisomer (**13**). Alkylation of the 6′-position to provide the ethyl ether (**17**) was also tolerated. Compounds **14** and **17** had profiles that argued for further exploration at the 2′- and 6′-positions of the aminocyclitol.

Building on the knowledge gained in the early derivatization, a more focused set of analogs was synthesized to further elucidate the SAR of the 2′- and 6′-position alkyl analogs (Table 3). The activity of the analogs that were alkylated in the 6′-position was noticeably worse than the corresponding 2′-derivatives. While this difference was not readily apparent with the ethyl group initially used to probe these two positions (i.e., compounds **15** and **17** in Table 2), a substantial divergence in activity was observed when the alkyl group was even one carbon larger (**18** and **19**, Table 3). The bulkier 6′-position analogs **23** and **25** had almost no activity relative to their 2′-position isomers **22** and **24**. In general, a variety of motifs were tolerated in the 2′-position, including functionalized alkyl (**20**, **27**, **28**), cycloalkylmethyl (**22**), and heterocycloalkylmethyl (**26**). The simple benzyl analog (**24**) was relatively weakly active against all three organisms, and the functionalized benzyl derivative (**29**) was one of the most potent analogs produced in this program, representing a significant improvement relative to compound **3**. In all cases, the analogs were more potent versus

Table 3
In vitro antibacterial activity [MIC (μg/ml)] of advanced, alkylated aminocyclitol derivatives



Compound	R_1	R_2	*S. aureus* 1095	*S. aureus* 1146	*S. pneumoniae* 1046	*S. pneumoniae* 1095	*S. pyogenes* 203	*S. pyogenes* 1079
18		–H	4	8	1	2	4	1
19	–H		>64	>64	64	64	64	32
20		–H	2	1	0.25	0.25	0.5	0.125
21	–H		16	8	8	8	8	2
22		–H	4	4	0.5	1	1	0.5
23	–H		>64	>64	64	>64	>64	32
24		–H	16	16	4	8	16	2
25	–H		>64	64	32	32	64	32
26		–H	4	2	0.5	1	1	0.25
27		–H	16	16	4	4	2	2
28		–H	2	1	0.5	1	0.5	0.25
29		–H	1	1	<0.0625	<0.0625	0.25	<0.0625



Scheme 3. Reagents and conditions: (a) *p*TsOH, 2,2-dimethoxypropane, DMF; (b) DMPMOC(=NH)CCl_3, PPTS, CH_2Cl_2; (c) 2-chloromethylbenzonitrile, NaH, THF; (d) DDQ, CH_2Cl_2, H_2O; (e) PPTS, MeOH.

streptococci than staphylococci, which parallels the intrinsic spectrum seen in the natural product and the parent aminocyclitol analogs. In some cases this MIC difference between pathogens was only one or two dilutions, but in other cases (such as **29**) the differential was much greater.

To better enable functionalization of the 2′-position of the aminocyclitol, a new selective protection scheme was developed (Scheme 3). Protection of the 6′-hydroxyl and the amide nitrogen of the aminocyclitol was accomplished by formation of an acetonide with 2,2-dimethoxypropane and *p*-TsOH. Selective protection of the 5′-hydroxyl with a 3,5-dimethoxybenzyl group was followed by alkylation of the free 2′-hydroxyl, to afford intermediates such as **30**. Global deprotection provided alkylated hygromycin analogs, and this sequence could be carried out on multigram scale to enable advanced in vivo studies.

Beyond changes in antibacterial potency, the addition of lipophilicity to the aminocyclitol portion of the hygromycin analogs was designed to increase the permeability of the compounds. Although the effect was small in some cases, all analogs studied did show improved CaCO-2 permeability. Some alkylated compounds, such as **25**, showed a balanced permeability profile (CaCO-2 values of AB = 10×10^{-6} cm/s, and BA = 14×10^{-6} cm/s). Others were more permeable but still showed efflux potential (**29**, CaCO-2 AB = 2.5×10^{-6} cm/s, and BA = 22×10^{-6} cm/s). Based on overall profile, the 3-methoxypropyl-substituted analog (**27**) was submitted to a rat pharmacokinetic study and demonstrated improved bioavailability ($F = 90\%$), although higher in vivo clearance ultimately limited oral exposure for this compound.

In summary, we have reported the antibacterial activity of a novel series of hygromycin A analogs. A systematic study of the effect of removal and substitution of the hydroxyl groups of **3** led to further exploration of the 2′-position of the aminocyclitol. Compounds with improved antibacterial activity as compared to **1** and **3** and improved oral bioavailability have been identified. Further lead optimization details will be reported in due course.

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17. Compound **11** was prepared by the following synthetic sequence: base-promoted addition of 3-fluoropropanol to 2,4,5-trifluorobenzaldehyde afforded 2,5-difluoro-4-(3-fluoropropoxy)benzaldehyde. This intermediate was then used in a Horner–Wadsworth–Emmons olefination reaction with ethyl 2-(diethoxyphosphoryl)propanoate. The resulting ethyl ester was saponified with LiOH to provide (*E*)-3-[2,5-difluoro-4-(3-fluoropropoxy) phenyl]-2-methylacrylic acid (**11**).