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Total Synthesis of Nucleoside Antibiotic A201A

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Supporting Information

ABSTRACT: A201A, a unique nucleoside antibiotic with potent antibacterial activities, has been synthesized for the first time in a total of 47 steps in a highly modular and linear manner, highlighting the elaboration/incorporation of an unprecedented hexofuranoside unit bearing an exocyclic enol ether moiety.

A201A (**1**) is a structurally unique nucleoside antibiotic,[1] which consists of five units, i.e., 6-dimethylaminopurine (A), 3-amino-3-deoxyribose (B), α-methyl-*p*-coumaric acid (C), an unnamed hexofuranose (D), and 3,4-di-*O*-methyl-D-rhamnose (E), connected linearly via one amide and three glycosidic linkages. This compound was originally isolated from a strain of *Streptomyces capreolus* in 1976 and was found to be highly active against Gram-positive bacteria and most Gram-negative anaerobic bacteria.[2] Recently, it was rediscovered by Ju et al. unexpectedly from the fermentation of a new strain of *Actinomycetes thermotolerans* collected from a deep-sea sediment.[3] To date, the only natural products known to be structurally relevant to A201A are puromycin[4] and hygromycin A,[5] which in fact bear only minor fragments of A201A (Figure 1). These two compounds also show potent antibacterial activities. Puromycin, in particular, has been well-known as a mimic of the 3′-end of aminoacyl-tRNA, which enters the A-site of the ribosome and inhibits translation by attacking the carbonyl group of the nascent peptidyl chain on the P-site.[6] Hygromycin A and A201A are also believed to bind to the ribosome so as to inhibit the protein synthesis.[7]

To understand the structure−activity relationships (SAR) and to develop drug candidates as well as probes for investigating the translation process, many efforts have been given to the chemical synthesis of puromycin and hygromycin A derivatives.[8−11] Especially, the total synthesis of hygromycin A has been achieved by Ogawa et al. in 1989 and by Donohoe et al. in 2009, respectively,[9b,10] and a synthesis of the 1,2-*trans*-furanoside analogue of hygromycin A was accomplished by Trost et al.[11] In contrast, study toward the synthesis of A201A, which demands incorporation/elaboration of the unique hexofuranose D unit with an exocyclic enol ether moiety, has never been reported. Here, we communicate the first synthesis of this complex nucleoside antibiotic (**1**).

A linear approach, with the shelf-stable compounds **2**−**6** as building blocks, was planned for the total synthesis of A201A, so that en route adjustment of the coupling sequence and protecting groups would be flexible. In addition, the linear approach would ensure divergent assembly of the congeners of A201A to facilitate the SAR studies.[12] A major concern in designing the building blocks was on the unprecedented unit D, which bears the acid-labile enol ether moiety and is embedded via two glycosidic linkages between the units E and C. It is known that a phenol 1,2-*cis*-furanosidic linkage (such as the D → C linkage) is extremely difficult to construct in a highly stereoselective manner.[13] In fact, both the Ogawa and Donohoe synthesis of hygromycin A exploited the Mitsunobu glycosylation to effect the relevant phenol 1,2-*cis*-furanosides.[9,10] Especially, employing a furanose donor installed with a triisopropylsilyl (TIPS) group at the 2-OH (thus, a 1,2-*trans*-activation and subsequent S_N2 substitution would be favored), Donohoe et al. managed to obtain the desired 1,2-*cis*-anomer predominantly under optimized conditions.[10] The stage for elaboration of the vulnerable enol methyl ether was scheduled after the glycosidation, so as to avoid tedious screening of the anomeric protecting group and its selective removal conditions. Thus, furanose **5** was designed, in that the C-6 was masked as a carboxylic ester to ensure the enolization of a later C-5 ketone derivative to take place only at C-4. The terminal rhamnose E

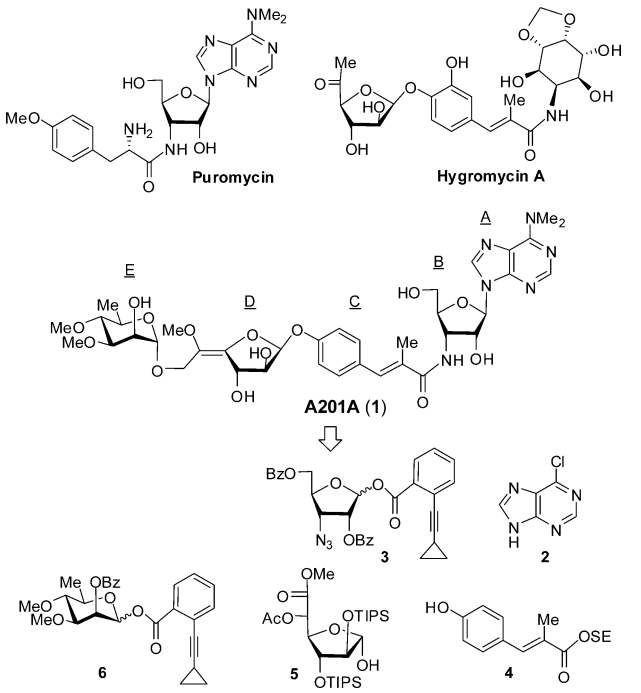

Figure 1.

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unit was designed as an *ortho*-alkynylbenzoate (i.e., **6**), which is capable of glycosylation under neutral conditions,[14] so as to not affect the enol ether. Since glycosylation of purine derivatives with glycosyl *ortho*-alkynylbenzoates has already been proved to be an efficient protocol,[15] the coupling of *ortho*-alkynylbenzoate **3** and 6-chloro-purine (**2**) turned out to be a certain choice to build the AB fragment.

The synthesis commenced with the preparation of the desired furanose building block **5** (Scheme 1). Thus, D-arabinose, which possesses the required configurations at C-2 and C-3, was converted into 5-aldehyde-furanoside **7** in nine transformations with a high 21% overall yield.[16] Subjection of aldehyde **7** to a THF solution of tris-(phenylthio)methyllithium at −78 °C led to the phenylthioorthoester adduct,[17] which was found unstable. Immediate treatment of the crude product with $CuO/CuCl_2$ in a mixed solvent of $MeOH/H_2O/CH_2Cl_2$ at room temperature (rt) afforded methyl ester **8** as a single isomer in 78% yield.[18] The nascent 5-OH, which is assumed to be at *R* configuration,[17] was protected with an acetyl group. Subsequent cleavage of the anomeric *p*-methoxyphenyl (MP) group was achieved with ceric ammonium nitrate (CAN) as an oxidant in aqueous MeCN, furnishing **5** in good yield (71−85%). Interestingly, the quinone adduct **9** was isolated as a byproduct (5%∼21%).

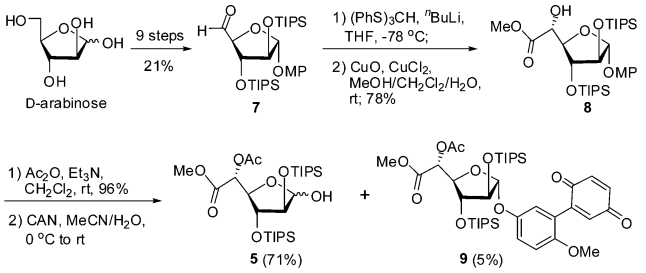
As expected, condensation of phenol **4**[16] with furanose **5** under the optimized conditions (diisopropyl azodicarboxylate (DIAD), Ph_3P, toluene, 60 °C)[10] afforded the desired glycoside **10** in a high yield (79%) and an excellent stereoselectivity (β/α = 10:1; Scheme 2). Selective removal of the 5′-*O*-acetyl group with K_2CO_3 in aqueous MeOH led to alcohol **11** (82%). The resulting 5′-OH was then oxidized successfully with *o*-iodoxybenzoic acid (IBX) in DMSO at 80 °C, leading to *α*-keto-ester **12** (95%).[19] Now the stage is set for elaboration of the enol methyl ether. In fact, we first examined the relevant transformations on simple substrates, including the methyl, *p*-methoxyphenyl, and benzyl glycoside analogues of **12** (i.e., **S10**, **S12**, and **S14**).[16] The yields and *Z*/*E* ratios of the resulting enol methyl ethers (**S11**, **S13**, and **S15**) were found to be highly dependent on the base and the methylating agent, as well as the reaction conditions.[16,20] The optimal conditions were the combination of Cs_2CO_3 and Me_2SO_4 in MeCN at rt, leading to the corresponding enol methyl ethers in constantly >73% yield and >3.5/1 *Z*/*E* ratio. Applying this set of conditions to the transformation of **12** led to enol methyl ethers **13**(*Z*/*E*) in an acceptable 66% yield, albeit in a moderate *Z*/*E* ratio of 1.6:1. Gratifyingly, we managed to convert **12** into **13**(*Z*/*E*) in a better yield of 69% (86% brsm) and *Z*/*E* ratio of 5.4:1 under modified conditions (Me_2SO_4 was added 10 min after the addition of Cs_2CO_3, so that the *Z* and *E* enols would reach at an equilibrium). At this stage, the trace *α*-isomer that had resulted from the Mitsunobu glycosylation step could be easily separated. Subjection of ester **13**(*Z*/*E*) to reduction with $LiBH_4$ (THF, 0 °C to rt) led to the corresponding 1,6′-diols (**S16**(*Z*/*E*)); NOE analysis of this pair of isomers confirmed unambiguously the assigned *Z*/*E* configurations.[16] Attempts at selective cleavage of the trimethylsilylethyl (SE) ester in **13**, without affecting the enol ether and the TIPS ethers, were not successful. Alternatively, the SE and TIPS groups in **13** were cleaved with tris-(dimethylamino)sulfonium difluorotrimethylsilicate (TASF) in DMF at rt to afford **14** (85%),[10,21] which was a ready precursor for the subsequent amide bond formation between the C and B units.

The required glycosyl *ortho*-alkynylbenzoates **3** and **6** were prepared conveniently from D-xylose and D-mannose, respectively.[16] We then set on the final assembly of A201A (Scheme 3). Thus, glycosylation of 6-chloro-purine (**2**) with ribofuranosyl *ortho*-alkynylbenzoate **3** under the catalysis of $Ph_3PAuNTf_2$ (0.1 equiv) in CH_2Cl_2 at rt provided the *β*-glycoside **15** cleanly (86%).[15] Treatment of **15** with aqueous dimethylamine in refluxing ethanol led to replacement of the 6-chloride together with cleavage of the 2′,5′-*O*-benzoyl groups;[22] subsequent protection of the resulting diol with TES group afforded **16** (76% for two steps). Azide **16** was then converted into the 3′-amino-3′-deoxyadenosine **17** (Ph_3P, THF, H_2O, 50 °C, 92%), which has been the key precursor in the synthesis of puromycin derivatives.[8]

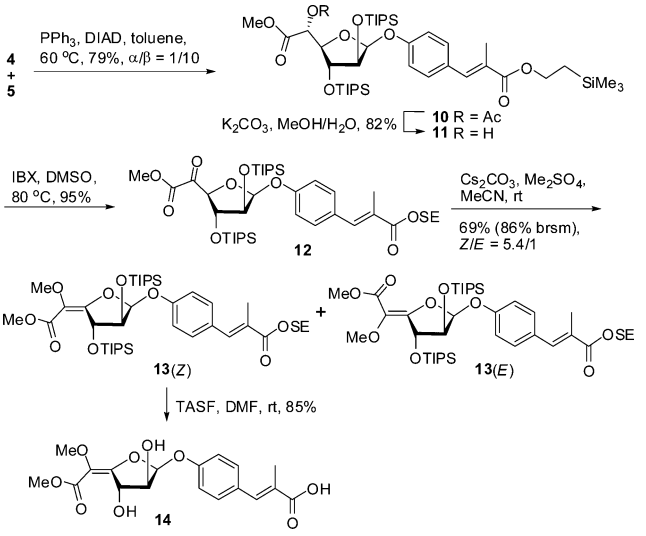
The condensation of amine **17** with acid **14**, which contains two hydroxyl groups, was effected in the presence of benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP) and $^iPr_2NEt$ in DMF at rt,[10,23] furnishing the desired amide in 78% yield. Subsequent protection of the two hydroxyl groups with TES group gave **18**. Reduction of the terminal ester in **18** succeeded with $LiBH_4$ (THF, −40 °C to rt, 78%) to afford the desired alcohol **19**.[24] Reduction with DIBAl-H led to cleavage of the amide linkage.

The complex alcohol **19** was then subjected to the final assembly. As planned, we performed the glycosylation of **19** with glycosyl *o*-alkynylbenzoate **6**. However, under conventional conditions (0.1 equiv $Ph_3PAuOTf$ or $Ph_3PAuNTf_2$, CH_2Cl_2, 4 Å MS, rt), the reaction proceeded sluggishly. The mechanistic studies have revealed that the gold(I)-catalyzed glycosylation reaction requires a protodeauration step to

Scheme 1



Scheme 2



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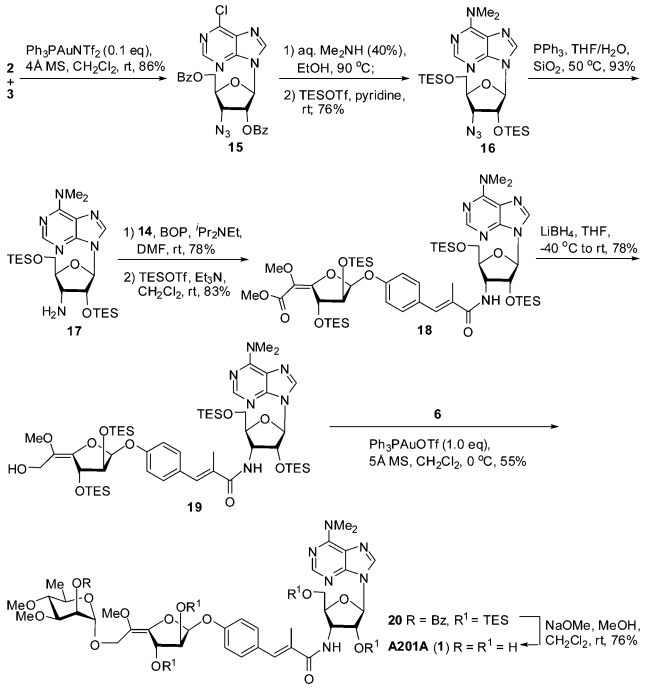
Scheme 3



regenerate the gold(I) catalyst; thus, the basic nitrogen atoms in **19** could intercept the proton in situ and stop the catalytic cycle.[14c,d] Therefore, the condensation of **19** and **6** was promoted with stoichiometric amount of $Ph_3PAuOTf$; the reaction proceeded smoothly (CH_2Cl_2, 5 Å MS, 0 °C) to lead to the desired α-rhamnoside **20** in 55% isolated yield. We have also tried the glycosylation of **19** with the relevant imidate donors (i.e., 2-*O*-benzoyl-3,4-di-*O*-methyl-D-rhamnopyranosyl trichloroacetimidate and *N*-phenyl trifluoroacetimidate).[25] Not surprisingly, under the action of TMSOTf, enol ether **19** underwent decomposition easily, while with $BF_3{\cdot}OEt_2$ as the promoter, the reaction hardly took place before decomposition. Finally, the benzoyl group and the four TES groups on **20** were removed with NaOMe in $HOMe/CH_2Cl_2$, furnishing A201A (**1**) in 76% yield. By the same token, we also synthesized the *E*-isomer of A201A (**S28**) starting from **13**(*E*).[16] The analytical data of **1** were in full agreement with those obtained for the natural product.[16]

Summarizing, the total synthesis of A201A, a unique nucleoside antibiotic, has been achieved for the fist time in a total of 47 steps starting from cheap materials, i.e., D-xylose, D-mannose, D-arabinose, 6-chloro-purine, and α-methyl-*p*-coumaric acid. The synthetic challenge of incorporation/elaboration of the unique furanoside unit bearing an exocyclic enol ether moiety has been addressed. The glycosylation of the complex alcohol (i.e., **19**) containing the acid-labile enol ether and basic nitrogen atoms was realized with the glycosyl *ortho*-alkynylbenzoate as donor under the promotion of stoichiometric $Ph_3PAuOTf$. Given its highly modular and linear nature, the present synthesis offers the prospect of being able to access numerous congeners of A201A and, thus, facilitates in-depth studies on the biological activities of this unique type of natural products.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental details, characterization data, and NMR spectra for new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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