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Synthesis of coumermycin A_1

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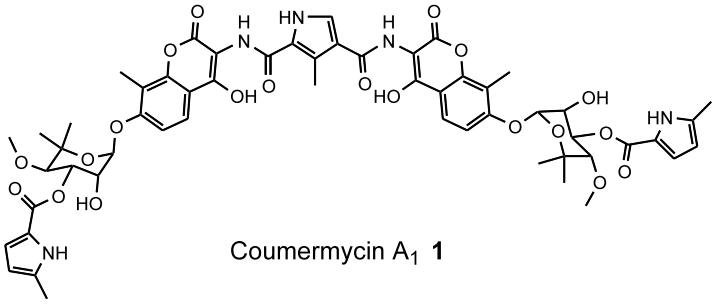
Abstract—A concise synthesis of the antibiotic coumermycin A_1, a natural product isolated from *streptomyces*, was achieved. In a key step, a selectively protected noviose sugar was prepared from novobiocin through a transglycosylation reaction with acetone.

Coumermycin A_1 (**1**) and novobiocin (**2**) are potent antibiotics isolated from *Streptomyces*[1] that bind reversibly and with high affinity to the amino-terminal 24K subdomain of the B subunit of bacterial DNA gyrase (GyrB).[2] This enzyme catalyzes the negative supercoiling of closed-circular DNA and is essential for bacterial replication and transcription. Coumermycin is nearly dimeric, and equilibrium binding and gel filtration studies have established that it binds to 2 equiv. of the GyrB protein.[2] As expected, novobiocin binds with a 1:1 stoichiometry.[2] Lately, interest in GyrB inhibitors has been renewed due to reports that coumermycin and novobiocin are active against methicillin-resistant species of staphylococci strains.[3]

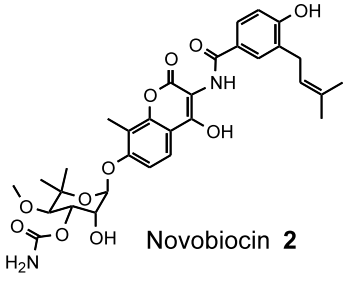
Recent work at the University of Washington and Merck has shown that coumermycin can mediate signal transduction pathways by promoting the dimerization of fusion proteins containing GyrB covalently bound to a transcriptional protein.[4,5] For example, coumermycin-induced dimerization of a chimeric protein containing GyrB linked to the Raf-1 serine/threonine kinase activated components of the MAP kinase cascade.[5] Coumermycin's pharmacokinetics, low toxicity in animal models, and lack of high-affinity eukaryotic binding targets make it a very useful tool[6] for understanding gene expression both in cell cultures and in vivo.

A prior synthesis of coumermycin was reported in 1966 by chemists at Hoffman–La Roche,[7] but the route was lengthy, and details of their work such as reagents and yields were never published. To support our biological efforts to control gene signaling, we wished to develop an efficient synthesis of coumermycin A_1. In developing our synthetic plan, we decided to leave amide bond construction until the final step to allow rapid preparation of coumermycin analogs in which the 3-methyl-1*H*-pyrrole-2,4-dicarboxylic acid linking group could be replaced with other dicarboxylic acids.[8]

The most readily available source of the noviose sugar in coumermycin is novobiocin (Sigma). Initially, we tried to directly convert novobiocin into coumermycin by cleaving the benzamide and exchanging the carbamate at the C-3′ position of the noviose sugar with 5-methyl-1*H*-pyrrole-2-carboxylate. This would give the desired PNC-amine **10**. Unfortunately, this approach was plagued by poor yields and an inability to selectively differentiate the C-2′ and C-3′ alcohols of the noviose.[9] Ultimately, we decided to cleave the glyco-



Coumermycin A_1 **1**



Novobiocin **2**

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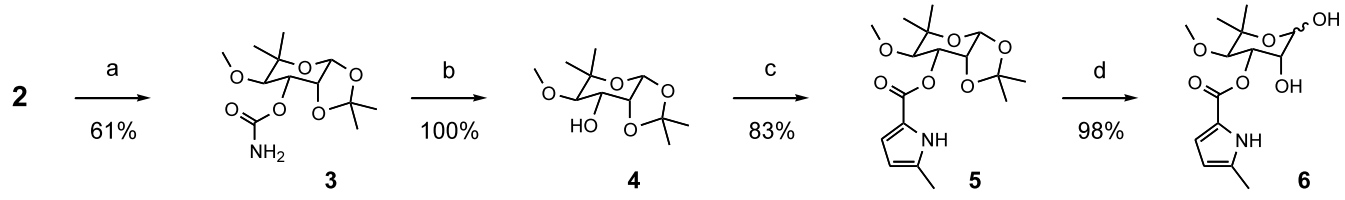
sidic linkage and protect C-2′ by linking the protecting group to the anomeric alcohol.

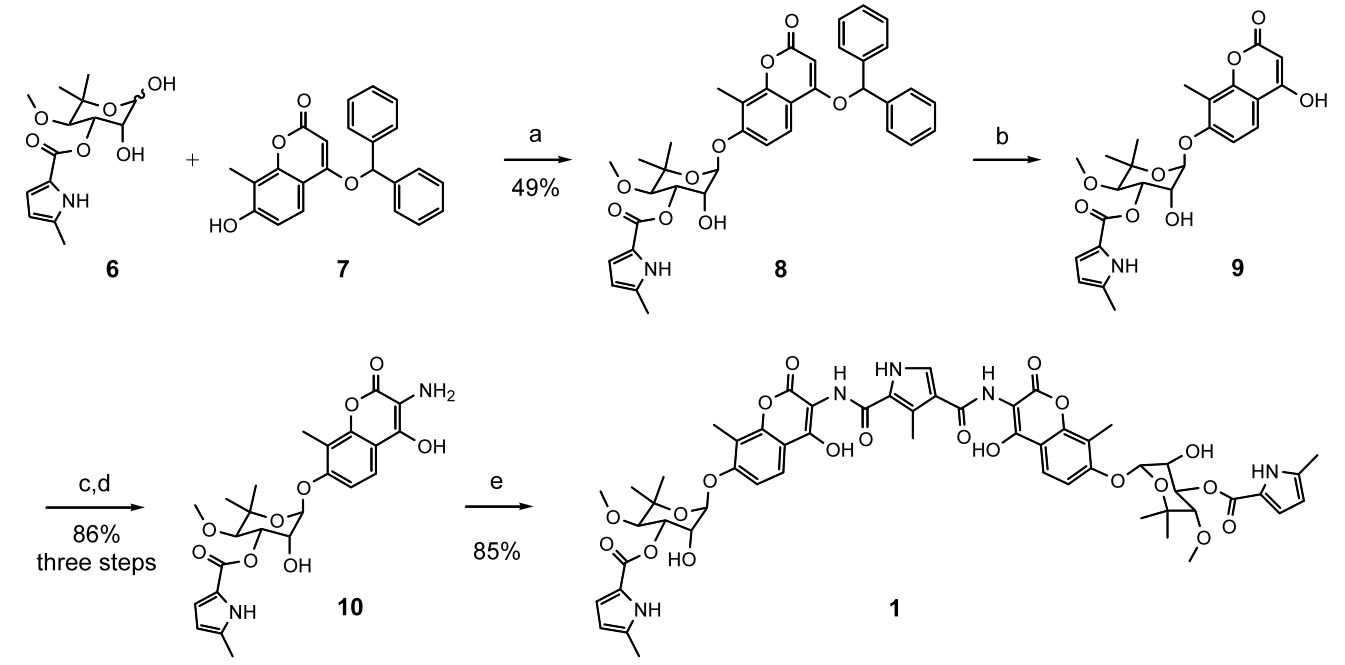
Preparation of the noviose sugar is outlined in Scheme 1. An acid-catalyzed transglycosylation of **2** with acetone provided the suitably protected noviose acetonide **3**.[10] With C-2′ and C-3′ reliably differentiated, the carbamate was hydrolyzed with lithium hydroxide to give **4**. The C-3′ hydroxyl proved to be extremely hindered, and acylation proceeded very slowly. Ethyl 5-methyl-1*H*-pyrrole-2-carboxylate was easily prepared by the method of Curran[11] and converted to the corresponding anhydride.[12]

Acylation of **4** with 5-methyl-1*H*-pyrrole-2-carboxylic anhydride proceeded in good yield in the presence of tributyl phosphine.[13] The nucleophilicity and low basicity of the tributyl phosphine made it an ideal catalyst for this transformation. More basic catalysts, particularly pyridine derivatives, were significantly inferior. Finally, the acetonide in **5** was rapidly removed with wet trifluoroacetic acid.

We surveyed a number of glycosylation reactions between the noviose and various coumarin fragments, and the most efficient was a Mitsunobu reaction between **6** and **7**[14] using the procedure of Laurin et al. (Scheme 2).[14] As expected, proton NMR studies showed that the ratio of anomers in the noviose was inversely related to the ratio of α- and β-glycoside products. Nonpolar solvents favored the β-alcohol and, thus, the desired α-glycoside. The diphenylmethyl ether protecting group proved optimal, because it ensured that the coumarin would be soluble in methylene chloride. After separating the isomers (α:β = 3:1), the desired product **8** was isolated in 49% yield. The diphenylmethyl ether was cleaved by hydrogenolysis to provide the enolic β-keto lactone **9**. Installation of the C-3 amine at an earlier stage of the synthesis hindered the glycosylation, therefore, it was introduced on the fully elaborated system by treatment of **9** with diazobenzene. PNC-amine, **10**, was prepared by a sodium dithionate reduction of the diazotized intermediate (Scheme 2).



Scheme 1. *Reagents and conditions*: (a) acetone, *p*-toluenesulfonic acid, 60°C; (b) LiOH, THF, H_2O, rt, 40°C; (c) 5-methyl-1*H*-pyrrole-2-carboxylic anhydride, Bu_3P, CH_2Cl_2, rt, 3 days; (d) trifluoroacetic acid, CH_2Cl_2, H_2O, rt.



Scheme 2. *Reagents and conditions*: (a) DEAD, PPh_3, CH_2Cl_2, rt; (b) H_2, Pd/C, THF, MeOH; (c) aniline, $NaNO_2$, NaOAc, HCl(aq), MeOH, THF; (d) $Na_2S_2O_4$, NaOAc, EtOH, H_2O, THF; (e) 3-methyl-1*H*-pyrrole-2,4-dicarboxylic acid, *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HATU), 4-methylmorpholine, DMF, Ar.

Finally, coumermycin A_1 was prepared from 3-methyl-1*H*-pyrrole-2,4-dicarboxylic acid,[15] 2 equiv. of **9**, and HATU under oxygen-free conditions. The product was purified by reverse-phase HPLC and was identical to natural coumermycin (Sigma) by NMR, HPLC, mass spectroscopy, and biological activity.

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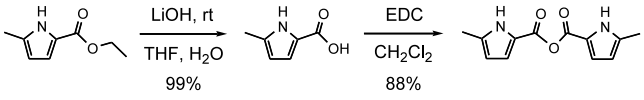

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