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SYNTHETIC STUDIES TOWARDS THE IDENTIFICATION OF NOVEL CAPURAMYCIN ANALOGS WITH MYCOBACTERICIDAL ACTIVITY

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Dedicated to Professor Emeritus Keiichiro Fukumoto on the occasion of his 75th birthday.

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Abstract – Expeditious syntheses of capuramycin, an effective MraY inhibitor *in vivo*, analogs are described. Synthetic schemes reported here are extremely useful for the generation of capuramycin analogs to identify minimum structure requirement to exhibit antimycobactericidal activity.

Mycobacterium tuberculosis (Mtb) causes tuberculosis (TB) and is responsible for nearly two million deaths annually.[1] In particular, people who have HIV-AIDS patients are susceptible to TB infection.[2] Moreover, the emergence of multidrug-resistant strains of Mtb seriously threatens TB control and prevention efforts.[3] Recent studies have shown that infection with Mtb enhances replication of HIV and may accelerate the progression of HIV infection to AIDS. In addition, there are significant problems present with respect to treatment of AIDS and TB co-infected patients; rifampicin (a key drug of DOTS therapy) shows significant interactions with protease inhibitors and non-nucleoside reverse transcriptase inhibitors.[4] Therefore, it is an urgent need to identify *in vivo* effective mycobactericidal drug leads which interfere with unexploited bacterial molecular targets.

Since peptidoglycan (PG) is an essential bacterial cell-wall polymer, the machinery for PG biosynthesis provides a unique and selective target for antibiotic action.[5] By far, the most commonly exploited target in PG synthesis are the penicillin binding proteins (PBPs), which are inhibited by the β-lactams and glycopeptides. Unfortunately, these compounds are of limited use in TB infections due to the interplay of β-lactamase activity and the relative impermeability of the mycolic acid layer in mycobacteria.[6] Three other biosynthetic steps in PG synthesis can be targeted by antibiotics in current clinical use; these

antibiotics include bacitracin, D-cycloserine and fosfomycin.[7] Regrettably, all are of limited usefulness in treating TB.[8] *Thus, PG biosynthesis appears to be a source of unexploited drug targets in mycobacterial pathogens.* Earlier cytoplasmic steps in peptidoglycan biosynthesis are catalyzed by highly conserved Mur enzymes. Therefore, inhibitors of any of these enzymes (MurA~F) would likely possess a broad spectrum of action. However, in spite of significant efforts by pharmaceutical industries, MurA~F inhibitors have not yet yielded drug leads.[9] Similarly, there is no antibiotic or a small molecule directed against MraY, which transfers UDP-*N*-acetylmuramyl-L-alanyl-γ-D-glutamyl-*meso*-diaminopimelyl-D-alanyl-D-alanine (Park's nucleotide) to a molecule of prenyl phosphate forming Lipid I, being developed for drugs for clinical trials. However, MraY inhibitors such as nucleoside antibiotics exhibit significant antibacterial activity *in vitro*.[10] Although many MraY inhibitors of natural product origin (i.e. liposidomycins, caprazamycins, and muraymycins)[11] are very complex and no extensive medicinal chemistry efforts with these molecules have been reported,[12] the structures of capuramycin (**1**)[13] and A-500359A (**2**[14], Figure 1), isolated from *Streptomyces* spp., are less complicated and their chemical synthesis to perform medicinal chemistry seems to be feasible.

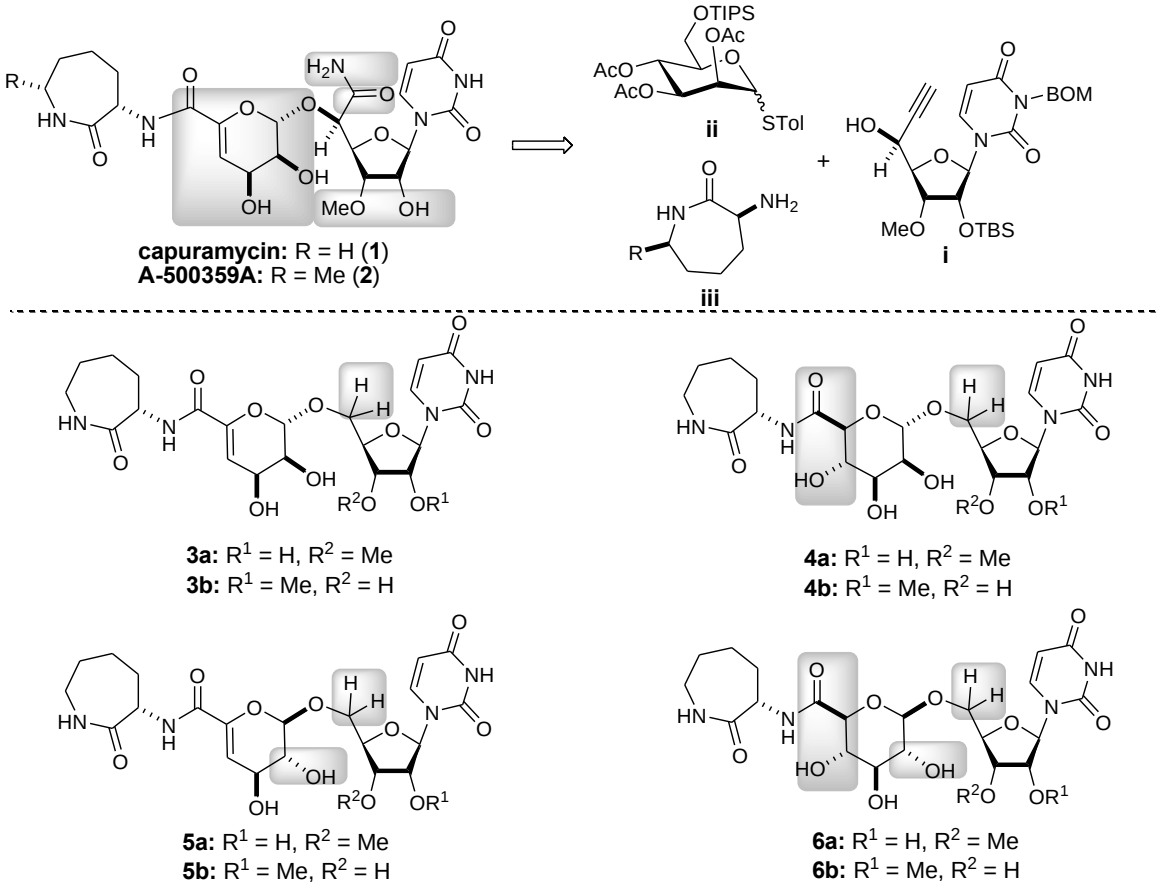


Figure 1. Structures of capuramycin (**1**) and A-500359A (**2**), the synthons (**i**, **ii**, and **iii**) for their syntheses, and designed capuramycin analogs **3a**~**6b**.

To date, however, only one total synthesis of capuramycin was reported by Knapp and Nandan.[15] Their synthesis requires 21 linear steps from diisopropylidine-D-glucofuranose and seems to be difficult to apply to the synthesis of the diversity structures of capuramycin analogs. A Sankyo group in Japan reported chemical modification of A-500359A (**2**) and biological evaluation of A-500359A analogs.[16] We have recently established a flexible synthesis of campuramycin using the synthons, **i**, **ii**, and **iii** (Figure 1), and obtained extensive knowledge of synthetic accessibility of capuramycin analogs.[17] However, our synthetic route for campuramycin relies on 1) oxidative cleavage to generate the carboxyamide, and 2) oxidation of the 6-position of mannose moiety of the coupling product (**i** + **ii**) to form the manno-pyranuronate. These additional several steps required for the synthesis of intact capuramycin core structure are not ideal for the generation of a library of capuramycin analogs in a time-efficient manner. Therefore, we envisioned identifying the indispensable functionalities of capuramycin to exhibit antimycobactericidal activity. To this end we plan to synthesize the decarboxyamide-capuramycin **3a**, and its congeners **4a**, **5a**, **6a**, and their methyl isomer **3b**, **4b**, **5b**, **6b** (Figure 1). Because the saturated analogs **4a**, **4b**, **6a**, and **6b** can be synthesized by skipping the elimination step, we, thus, focused in establishing expeditious preparations of **3a**, **3b**, **5a**, and **5b**. Herein, we describe a highly flexible synthesis of capuramycin analogs **3a**, **3b**, **5a**, and **5b** with a minimum number of protecting group manipulations.

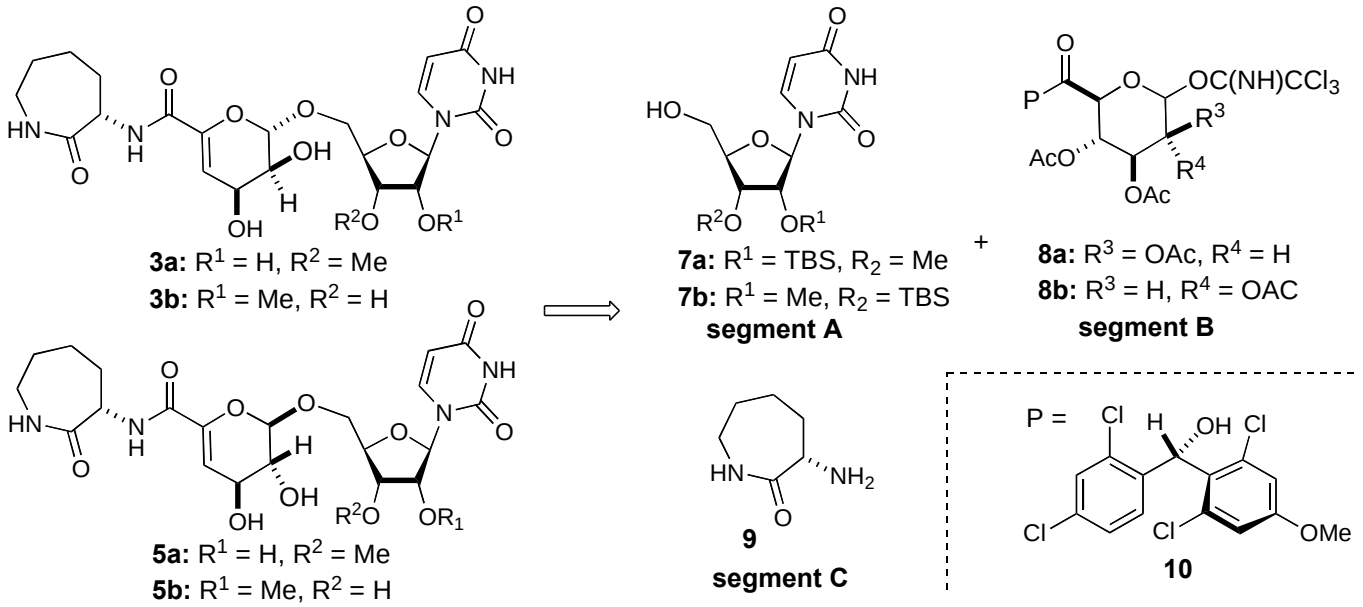
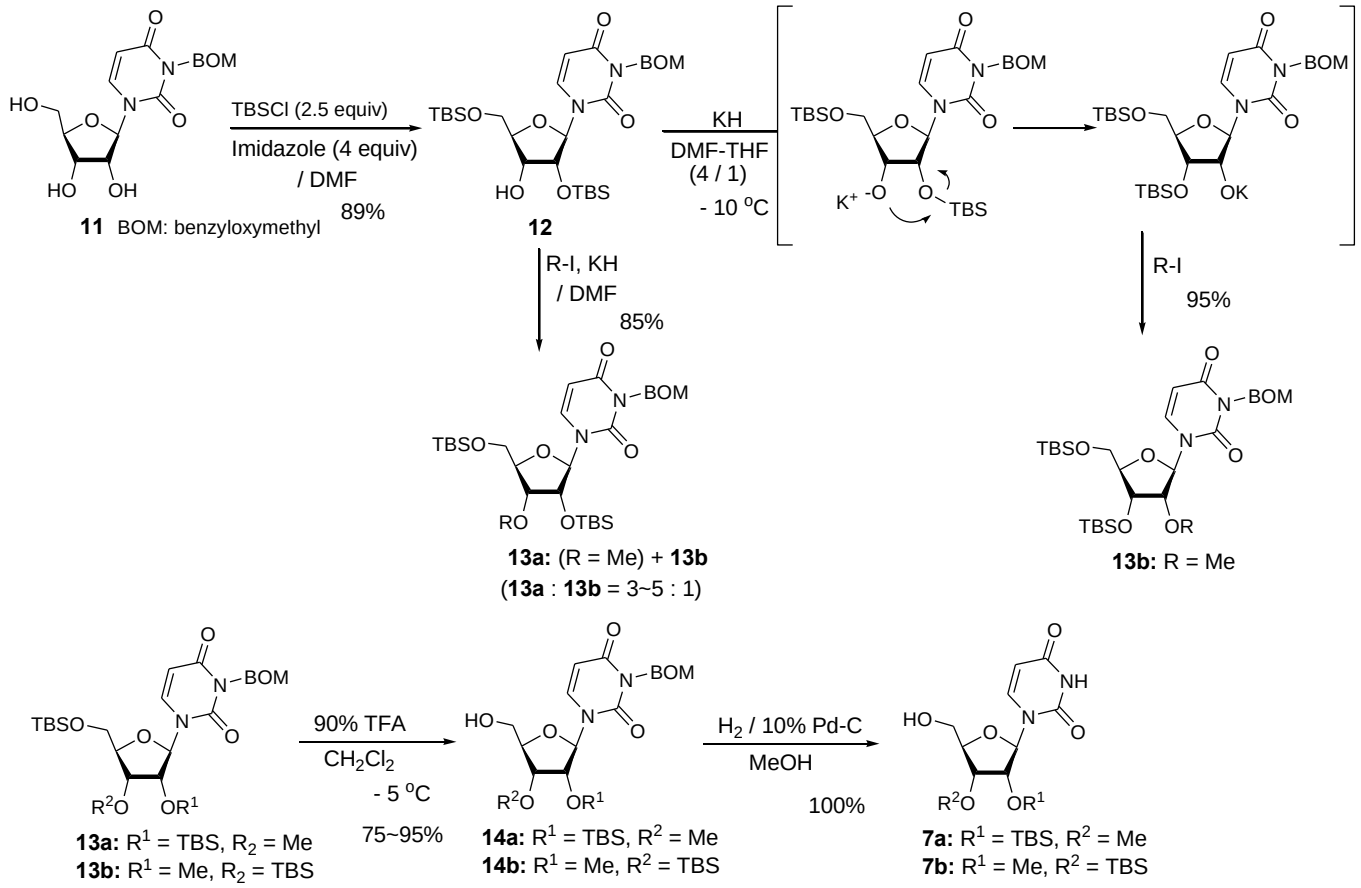


Figure 2. Revised synthetic route for capuramycin analogs **3a**~**5b**.

The revised synthetic route for **3a**, **3b**, **5a**, and **5b** includes 1) pyranulonidations of **7a** and **7b** (Segment A) with the pyranuroates donors **8a** and **8b** (Segment B), 2) the base-catalyzed elimination reactions of the coupling products (**7** + **8**) to furnish the α,β-unsaturated esters, 3) amide-forming reaction with Segment C, and 4) global deprotections in a single step (Figure 2). In order to perform pyranurosylation

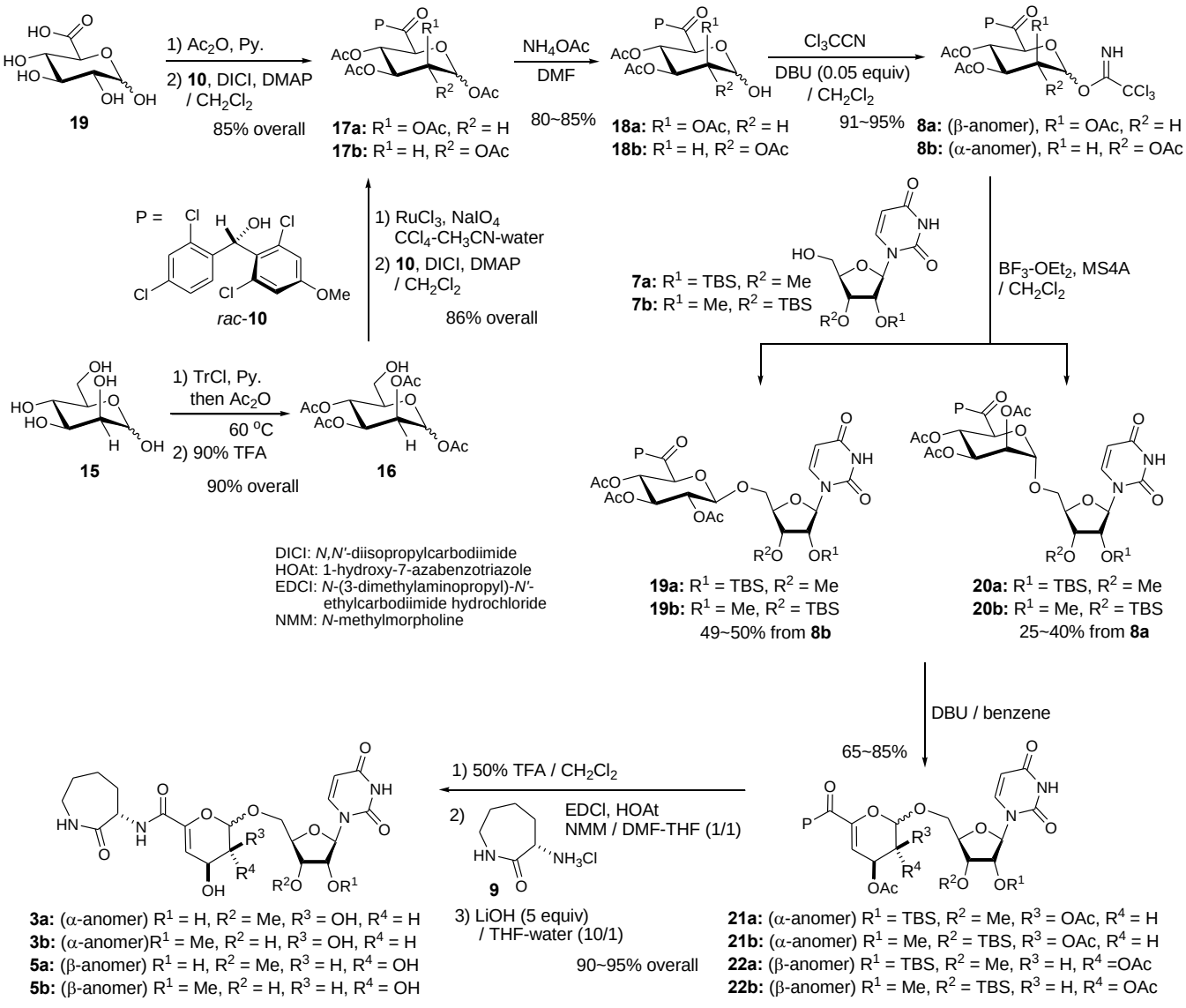
and elimination reactions with a minimum number of protecting group manipulations, choice of protecting groups for each building block (**7** and **8**) requires careful consideration. In addition, it is very important to devise a flexible synthetic route for the generation of a wide variety of capuramycin analogs from the point of medicinal chemistry.

Limited examples of glycosylations with pyranuronoates were found in literatures, and the Schmidt glycosylation conditions using Lewis acid are the standard for pyranulonidation reactions.[18] Having considered the synthetic and medicinal chemistry aspects described above and limited examples of pyranulonidations, we decided to introduce the protecting group, (2,6-dichloro-4-methoxyphenyl)(2,4-dichlorophenyl)methyl alcohol (**10**) which showed an unusual stability to Brønstead acids (i.e. 15% TFA, 30% HF, 2N HCl, and HBr/AcOH), Lewis acids (i.e. $TiCl_4$, $ZnCl_2$, $AlCl_3$, $B(C_6F_5)_3$, BCl_3, BBr_3, TMSOTf, and $La(OTf)_3$), and bases (i.e. 40% NH_4OH, 10% LiOH, and DBU).[19] Such a protecting group would enable us to accomplish the synthesis of capuramycin analogs **3a**, **3b**, **5a**, and **5b** with a minimum number of deprotection/protection manipulations.



Scheme 1. Syntheses of the functionalized uridine derivatives **7a** and **7b**.

The convenient syntheses of the glycosyl acceptors **7a** and **7b** were illustrated in Scheme 1. The *N*(3)-BOM uridine **11**[20] was subjected to double-silylation reactions with TBSCl and imidazole in DMF to furnish the 2',5'-*O*-di-TBS product **12** in 89% yield. It was realized that the treatment of **12** with KH underwent the silyl group migration to furnish the potassium 2'-alkoxide which could be alkylated with a variety of electrophiles (i.e. MeI, EtI, octyl iodide, and allyl bromide); the silyl migration of **12** was completed within 0.5 h and followed by methylation with MeI provided **13b** (R = Me) in 95% yield.[21] The structures of **13a** and **13b** were established through ^{1}H-NMR NOESY experiments. On the contrary, the treatment of **12** with KH in the presence of MeI (10 equiv) yielded a mixture of **13a** and **13b** with 3~5:1 (**13a** / **13b**) selectivity. Selective desilylation of the primary TBS group of **13** with cooled 90% TFA (–5 °C) followed by hydrogenolysis of the BOM group gave rise to the partially protected-uridines **7a** and **7b** in 75~95 % overall yield.



Scheme 2. Syntheses of the capuramycin analogs **3a**, **3b**, **5a** and **5b**.

D-*Gluco*-pyranuroate glycosyl donor **8b** was synthesized in four steps with greater than 68% overall yield from glucuronic acid (**19**). On the other hand, synthesis of D-*manno*-pyranuroate donor **8a** requires additional several steps to selectively oxidize the 6-OH group of **16**.[15] 1,2,3,4-Tetra-*O*-acetyl-D-mannopyranose (**16**) was oxidized under typical Sharpless conditions ($RuCl_3$, $NaIO_4$) and the generated carboxylic acid was esterified with *rac*-**10**.[22] Selective cleavage of the anomeric acetate of **17a** followed by the reaction of the free anomeric alcohol with Cl_3CCN in the presence of catalytic amount of DBU afforded the β-imidate **8a**; the conversion of **8a** from D-mannose was greater than 50% overall yield.

The gluculonidations of alcohols with **8b** are known to be low yielding reactions and several variations of glucuronate imidates have been devised in order to improve reactivity.[23] Nonetheless, the gluculonidations of **7a** and **7b** with **8b** were examined under typical Schmidt conditions. It was observed that TMSOTf (5~10 mol%) catalyzed gluculonidation of **7a** with **8b** at –20 °C furnished the orthoester exclusively with near quantitative yield. Rearrangement of the orthoester to the desired β-glycoside **19a** were not observed at elevated temperature or under the conditions of an increased amount of TMSOTf (100~250 mol%). Interestingly, the same reaction was successfully catalyzed by using BF_3•OEt_2 (300 mol%) at –20 °C to afford **19a** in 50% yield without the formation of the orthoester; however, this transformation under these conditions required a relatively long reaction time of 10~14 h. Similarly, **7b** could be coupled with **8b** to furnish **19b** in 49% yield. Although the desired product **20a** and **20b** could be synthesized by the reaction of **7a** and **7b** with **8a** *via* the conditions performed for the syntheses of **19**, D-*manno*-pyranulonidation resulted in lower isolation yields (25~40% yields) due to the formation of the orthoesters. In general, the orthoester formation observed using acetyl group-protected glycosyl donors is able to be diminished by using pivaloyl protecting groups, however, we could not improve D-*manno*-pyranulonidations with disarmed donors such as the benzoyl- or pivaloyl-protected D-manno-pyranuronate imidate.[24] Even though isolation yields for **19** and **20** were moderate, these products could be isolated simply by column chromatography. In addition, **8a**, **8b**, **7a**, and **7b** could be synthesized a multi-gram quantity without difficulty. Thus, further reaction optimization was not undertaken for this program. E2 elimination reactions of **19** and **20** with a stoichiometric amount of DBU furnished **21** and **22** in 65~85% yields. (2,6-Dichloro-4-methoxyphenyl)(2,4-dichlorophenyl)methyl ester and TBS groups were simultaneously deprotected with 50% TFA to provide the acid-alcohol and trifluoroacetic acid ester of **10**.[25] The resulting crude mixture was coupled with (*2S*)-aminocaprolactam (**9**) under a condition of HOAt, EDCI, and NMM to yield the ligation product in quantitative yield. The acetyl-protected decarboxyamide-capuramycin analogs were hydrolyzed by using LiOH in aq. THF to provide **3a**, **3b**, **5a**, and **5b** in 90~95% yields.[26] Thus, we demonstrated a flexible synthesis of capuramycin analogs in which the 2',3'-positions in ribose, 4-deoxy-4-hexenuronic acid, and amide

moieties can be selectively functionalized or be replaced with a wide range of physicochemically interesting building blocks. It is worthwhile mentioning that *rac*-(2,6-dichloro-4-methoxyphenyl)(2,4-dichlorophenyl)methanol (**10**) is one of very few protecting groups which 1) are stable to Brønstead bases and Lewis acids, 2) can be cleaved by a volatile acid such as TFA, and 3) are reusable. Introduction of a protecting group possessing such chemical properties in the synthesis of capuramycin analogs enable us to synthesize desired analogs with a minimum number of protecting group manipulations. We will 1) functionalize the 2'- and 3'-positions of Segment A (Figure 2) with a wide range of alkyl groups, 2) introduce the other pyranuronate and furanonate in place of D-*manno*-pyranuronate (Segment B), and 3) diversify the structure of molecules with a wide variety of *primary* and *secondary* amines (Segment C). Detailed structure antimycobactericidal activity relationship of library molecules based on capuramycin will be reported elsewhere.

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21. Because **13b** was isolated exclusively in the conversion of **12** to **13b** it was concluded that the C3-TBS silyl group did not migrate to the C2-position. Thus, no rapid equivilation of the silyl group was taking place.

22. We have not observed separation of the diastereomers caused by *rac*-**10** throughout the syntheses of **21** and **22**.
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24. Trace amounts of the desired coupling product were isolated with the pivaloyl- or benzoyl-protected imidate of D-*manno*-pyranuronate.
25. The TFA ester of **10** was saponified with NH_3 in MeOH (for 12 h) to regenerate **10** in quantitative yield.
26. Syntheses of **4a**, **4b**, **6a**, and **6b** (Figure1) were accomplished by skipping the elimination step (**19** → **22**) in Scheme 2. Representative physical data for the molecules listed in Figure 1 were listed below. **3a**: $[\alpha]^{20}_D$ +14.2 (*c* 0.1 in MeOH); IR (film) 3378, 1680, 1070 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.90 (d, *J* = 8.4 Hz, 1H), 5.94 (d, *J* = 3.2 Hz, 1H), 5.93 (d, *J* = 3.2 Hz, 1H), 5.72 (d, *J* = 8.4 Hz, 1H), 5.19 (d, *J* = 4.8 Hz, 1H), 4.57 (d, *J* = 10.2 Hz, 1H), 4.35 (t, *J* = 4.0 Hz, 1H), 4.20 (t, *J* = 4.2 Hz, 1H), 4.16-4.07 (m, 3H), 3.80 (dd, *J* = 4.4, 8.8 Hz, 2H), 3.25 (m, 2H), 2.00 (m, 2H), 1.84 (m, 2H), 1.57 (m, 1H), 1.37 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.9, 166.2, 162.7, 152.2, 143.5, 142.5, 109.8, 103.3, 103.0, 88.5, 85.0, 82.0, 71.2, 768.3, 67.8, 67.5, 58.9, 53.4, 42.5, 32.5, 29.9, 29.2; HRMS (ESI) Calcd. for $C_{22}H_{30}N_4NaO_{11}$ $(M+Na)^+$: 549.1809; found: 549.1812. **5a**: $[\alpha]^{20}_D$ +12.0 (*c* 0.5 in MeOH); IR (film) 3390, 1681, 1463, 1087 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.87 (d, *J* = 8.1 Hz, 1H), 6.05 (d, *J* = 3.9 Hz, 1H), 5.99 (d, *J* = 4.8 Hz, 1H), 5.70 (d, *J* = 8.4 Hz, 1H), 5.16 (d, *J* = 4.5 Hz, 1H), 4.56 (d, *J* = 11.1 Hz, 1H), 4.33 (t, *J* = 5.1 Hz, 1H), 4.18 (dd, *J* = 2.1, 11.1 Hz, 1H), 4.12 (t, *J* = 3.3 Hz, 2H), 4.01 (t, *J* = 4.8 Hz, 1H), 3.91 (dd, *J* = 2.7, 11.1 Hz, 1H), 3.79 (t, *J* = 4.2 Hz, 1H), 3.48 (s, 3H), 3.26 (m, 2H), 1.97 (m, 2H), 1.82 (m, 2H), 1.52-1.34 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.8, 166.3, 162.5, 152.4, 143.6, 143.0, 109.7, 103.2, 102.8, 88.4, 84.6, 84.3, 72.0, 70.4, 70.1, 67.5, 58.8, 53.5, 42.6, 32.5, 30.0, 29.2; HRMS (ESI) Calcd. for $C_{22}H_{30}N_4NaO_{11}$ $(M+Na)^+$: 549.1809; found: 549.1815.