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SYNTHETIC STUDIES TOWARD THE GENERATION OF URIDINE-AMINO ALCOHOL-BASED SMALL OPTIMIZED LIBRARIES

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Dedicated to Professor Yoshito Kishi on the occasion of his 70th birthday

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Abstract - An efficient synthesis of a versatile scaffold **3**, *anti*-2-chloro-3-hydroxy ester can be achieved *via* a boron mediated diastereofacial *anti*-aldol reaction of 2-(*N*-methylbenzyl-*N*-2,4,6-trimethylbenzyl)-amino-1-phenylpropanol chloroester **1** and the uridyl aldehyde derivative **2**. Generation of uridine-amino alcohol-based library is demonstrated by using the scaffold **3**.

INTRODUCTION

Mycobacterium tuberculosis (Mtb) causes tuberculosis (TB) and is responsible for nearly two million deaths annually. Currently, about 42 million people are HIV-infected and almost one-third are also infected with Mtb.[1] Persons infected with both HIV and Mtb are 30 times more likely to progress to active TB disease. Recent studies have shown that infection with Mtb enhances replication of HIV and may accelerate the progression of HIV infection to AIDS. The current recommended approach to TB treatment is the local directly observed treatment strategy (DOTS).[2] Even where DOTS has been established, if the multidrug-resistant (MDR) rate locally is high, first line drugs (isoniazid, rifampicin, pyrazinamide, and ethambutol) alone give an unacceptably low cure rate.[3] Moreover, if the patient remains ill the transmission rate is increased. Clinical responses of MDR-TB patient to first line drug

have been poor, and in some cases there is no response at all. Second line drugs (amikacin, cycloserine, ethionimide, kanamycin, capreomycin, and ofloxacin) are often poorly effective and tolerated.[4]

As a result of massive medicinal chemistry efforts, several promising TB drug leads such as diarylquinoline, fluoroquinolone, and nitroimidazole have been developed based upon known templates. Because of high rate of failure of promising drug leads in rate clinical trials, it is of paramount importance to increase the number of pipeline TB drug leads which affect different drug targets.

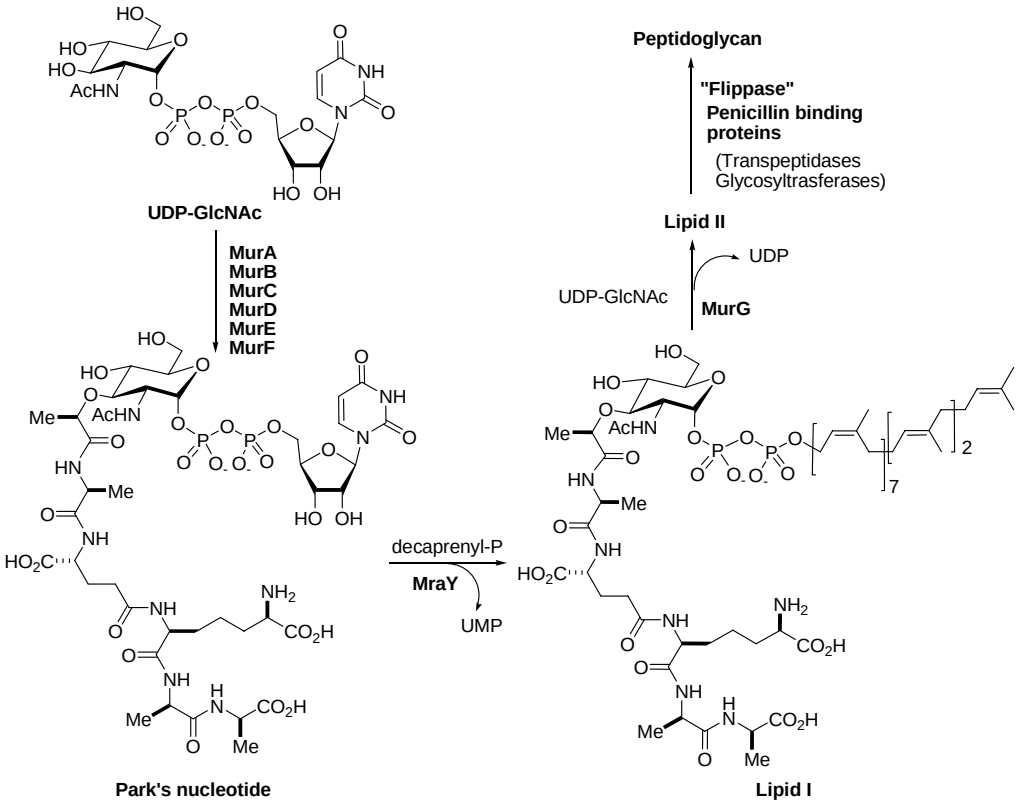


Figure 1. Biosynthesis of peptidoglycan.

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.[6] 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.[7] Three other biosynthetic steps in PG synthesis can be targeted by antibiotics in current clinical use; these antibiotics include bacitracin, D-cycloserine and fosfomycin.[8] Regrettably, all are of limited usefulness in treating TB.[9] Thus, PG synthesis appears to be a source of unexploited drug targets in mycobacterial pathogens. Although the machinery for PG biosynthesis has been considered a

potential drug target, which step of PG biosynthesis represents is the most promising has not been defined. 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.[10] However, in spite of significant efforts by pharmaceutical industries, MurA~F inhibitors have not yet yielded drug leads. 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 pursued for drug development (Figure 1).[11]

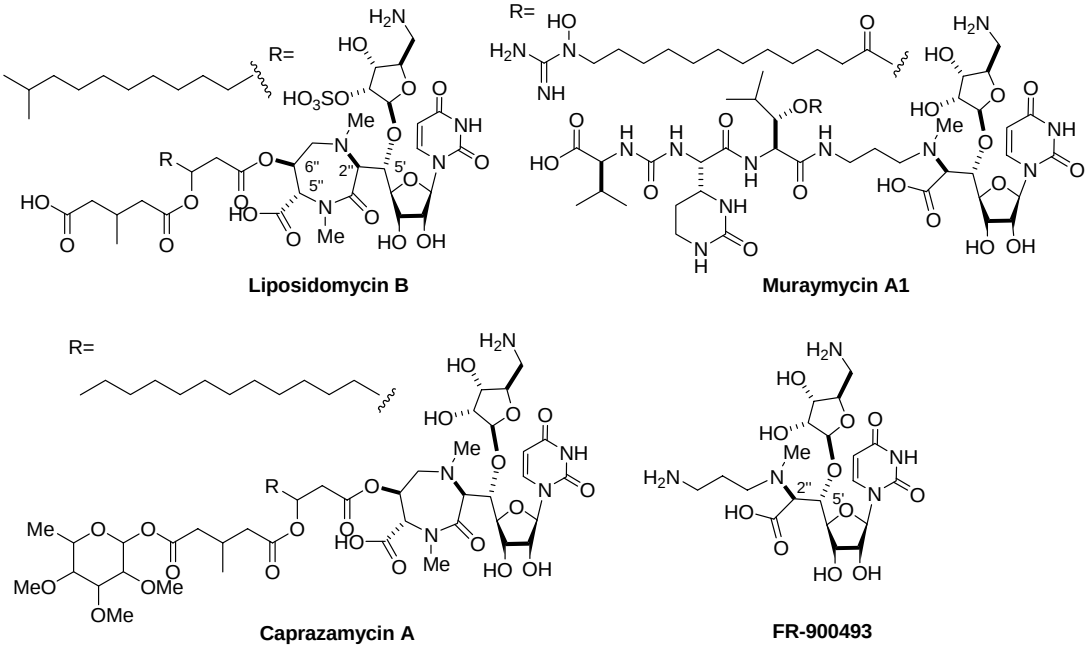
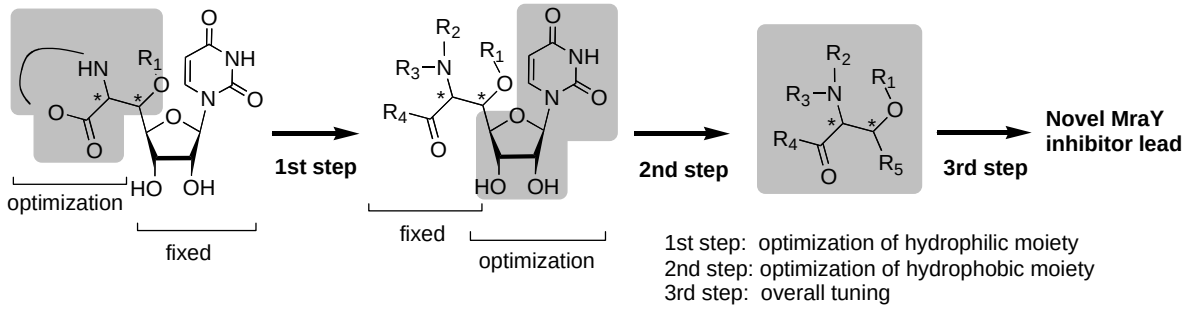


Figure 2. Representative structures of MraY inhibitors of natural product origin.

However, inhibitors of this enzyme such as nucleoside antibiotics exhibit significant antibacterial activity *in vitro*. Significantly, ribosamino-uridine-containing compounds (liposidomycins, caprazamycins, muraymycins, and FR-900493) are specific inhibitors of MraY and nontoxic; as, unlike tunicamycin, they do not inhibit glycoconjugate biosynthesis at low concentrations.[10] This low cytotoxicity translates well *in vivo*; liposidomycins were administrated intravenously up to 500 mg/kg in mice without evidence of acute toxicity.[12] On the contrary, liposidomycins showed poor pharmacokinetics because of their high hydrophilicity (poor membrane penetration). Caprazamycins exhibit *anti*-Mtb activity (MIC, 3.13 μg/mL for H37RV) and therapeutic efficacy at 1.5 mg/kg•day in a pulmonary tuberculosis infection model in mice, when administered intranasaly.[13] FR-900493 displays antistaphylococcal activity (MIC, 3.13 μg/mL) *in vitro* and *in vivo* in murine staphylococcal infection

model. The LD_{50} in mice is above 500 mg/kg (intravenous administrations).[14] Muraymycins possess an additional functionality at the amine side chain of FR-900493. Muraymycins inhibit lipid I formation and demonstrated activity against Gram-positive bacteria, methicillin-resistant *S. aureus* (MIC, 2 μg/mL). Animal efficacy studies indicated that muraymycin was active when administered intravenously in an *S. aureus* lethal-infection model (50% effective dose, 1.1 mg/kg).[15] Thus, ribosamino-uridine-containing compounds showed 1) excellent MraY inhibitory activity as well as antibacterial activity *in vitro*, and 2) good *in vivo* activity in mice models when administered intravenously. High hydrophilicity nature of ribosamino-uridine-containing MraY inhibitors (Figure 2) results in significantly poor pharmacokinetics.[10] Thus, fine tuning of physico-chemical properties is expected to provide inhibitors displaying improved biological activity. In addition, it is very important to improve properties of oral absorption and high distribution to lung tissue that are advantage for TB therapy.

The uridine moiety in MraY inhibitors of natural product origin seems to be a crucial functionality to exhibit significant biological activities.[16] Our strategy, fragment-based design of novel MraY inhibitors includes: 1) assay guided optimization of the hydrophilic moiety in which the uridine moiety is fixed in the first step, 2) optimization of hydrophobic moiety in which the hydrophilic moiety is fixed in the second step, and 3) in the third step the identified MraY inhibitor is tuned to increase microbial activity against *Mycobacterium* spp. (Scheme 1).



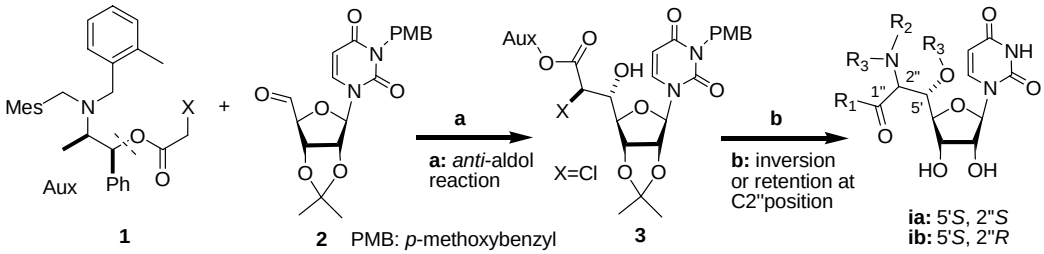
Scheme 1. Fragment-based design of novel MraY inhibitors.

In order to facilitate the structure optimization process illustrated in Scheme 1, it is indispensable to establish an efficient synthetic route to deliver library molecules and a convenient assay for synthesized library molecules. Although several synthetic efforts toward the total synthesis of caprazamycins, liposidomycins, muraymycins and their congeners have been reported, no convenient synthetic method that: 1) can deliver the diversity core structure of ribosamino-uridine natural products, and 2) is amenable to the construction of library molecules is available in the literature.[17]

We have developed an efficient synthesis-assay cycle for the identification of druggable MraY inhibitors that includes: 1) flexible synthesis of the core structure of nucleoside antibiotics, and generation of small optimized library molecules based on these core structures, 2) an assay using coupled MraY-MurG enzymes,[18] 3) an *anti*-microbial assay, and 4) a cytotoxicity test.[19] Herein, we describe a highly efficient and flexible synthesis of the core structure of ribosamino-uridine-containing MraY inhibitors of natural product origin.

RESULTS AND DISCUSSION

Although the absolute and relative chemistries of the core structures of many of ribosamino-uridine-containing natural products are still unknown, the absolute stereochemistries of the 1,4-diazepan-3-one ring of caprazamycins were unequivocally determined to be 5′*S*, 2″*S*, 5″*S*, 6″*S* by X-ray crystal analysis.[20] The extensive NMR studies suggested that muraymycins and liposidomycins possess the same stereochemistries of 5′*S*, 2″*S*. In light of these stereochemical considerations, we hypothesized that the stereochemistries of C5′ and C2″ of FR-900493 are as indicated in Figure 2.

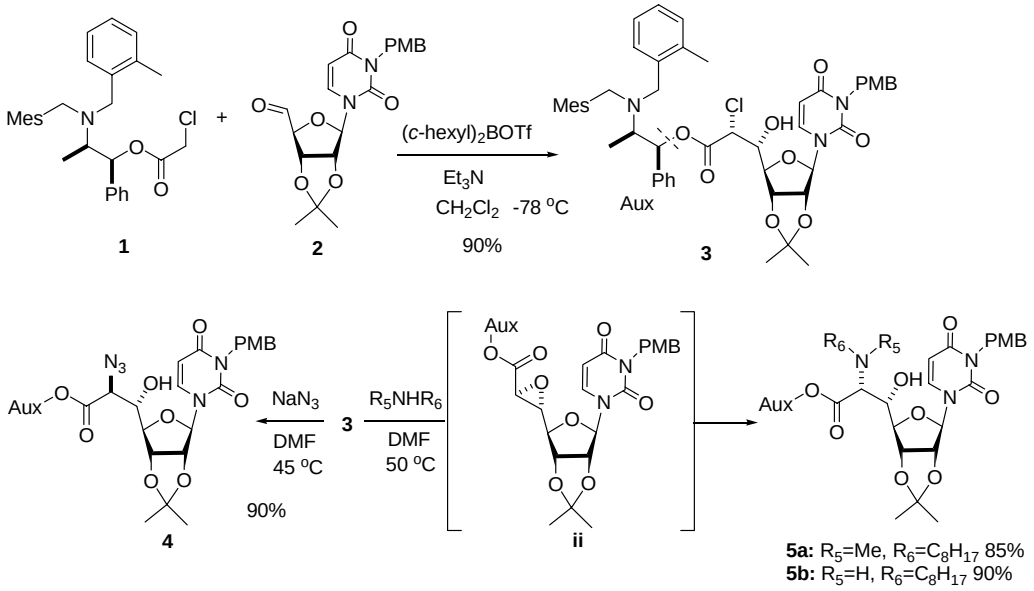


Scheme 2. Versatile strategy for the synthesis of uridine-β-hydroxylaminoesters.

We envisioned synthesizing a series of uridine-β-hydroxyaminoesters possessing 5′*S*, 2″*S* (natural) and 5′*S*, 2″*R* (unnatural) stereochemistries from the same intermediate. As illustrated in Scheme 2 (5′*S*, 2″*R*)-2″-chloro-5′-hydroxy ester **3** is an ideal intermediate to access to **ia** and **ib** by S_N2 type reactions of the 2″-chloro and the epoxy compound. The *anti*-aldol product **3** will be synthesized by diastereoselective *anti*-aldol reaction between the (–)-*N*,*N*-dibenzylnorephedrine derivative **1** and the uridyl aldehyde **2**.[21]

In spite of abundant examples of the utility of *syn*-2-chloro-3-hydroxy ester units, which is synthesized by the Evans' procedure through the diatereoselective *syn*-aldol reactions of the chiral oxazolidinones,[22] no efficient diastereoselctive or reagent controlled asymmetric *anti*-aldol reaction to construct *anti*-2-chloro-3-hydroxycarbonyl units has been reported. In addition it is required to devise hydrolytically stable ester which enables to functionalize at the C2-position with diversity structure of

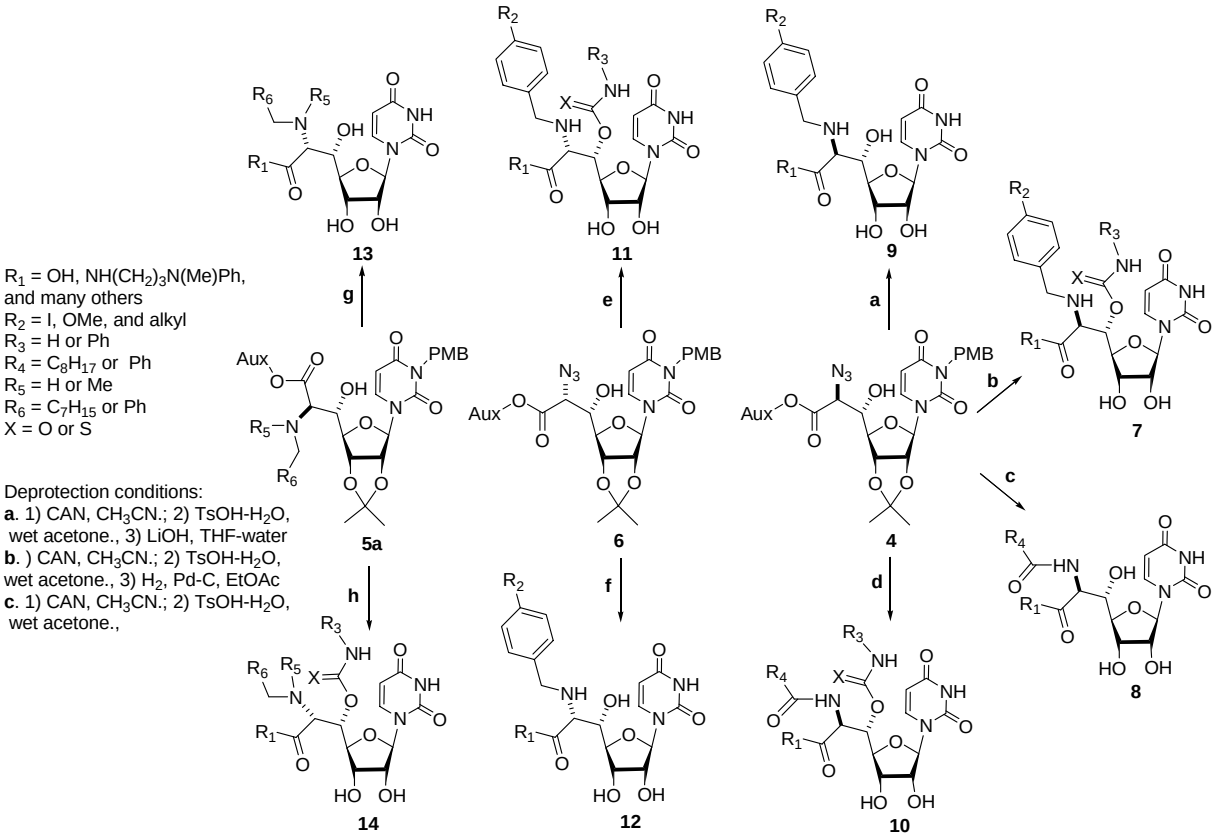
amine derivatives even at elevated temperatures without the formation of undesired by-products such as free carboxylic acid and amides. We have established highly diastereofacial *anti*-aldol reactions with 2-(*N*-2-methylbenzyl-*N*-2,4,6-trimethylbenzyl)-amino-1-phenylpropanol ester (**3**, X=alkyl in Scheme 2); in these reactions LDA-Cp_2ZrCl_2 or (*c*-hexyl)$_2$BOTf / Et_3N (Masamune's condition)[23] is utilized in order to achieve exclusive *E*(O,R)-enolate formations.[24] The *anti*-2-alkyl-3-hydroxycarboxylic acid esters generated under these conditions exhibited excellent susceptibility against *primary* and *secondary* amines at 90 °C over 12h. Through NOESY experiments and molecular modeling the origin of the excellent diastereofacial selectivity and hydrolytical stability can be attributed to the following factors. The bulky mesityl group on nitrogen is on the sterically less demanding site. Because of a significant steric interaction between the mesityl group and methyl of *o*-methylbenzyl group the methyl group of *o*-methylbenzyl locates toward the ester moiety. Thus *re*-face is completely blocked from the approach of electrophiles and nucleophiles. The LDA-Cp_2ZrCl_2 system forms *E*(O,R)-zirconium enolate of **3** (X=Me in Scheme 2). Similarly, kinetically controlled boron enolate formation of **3** using (*c*-hexyl)$_2$BOTf / Et_3N generates *E*(O,R)-boron enolate exclusively.



Scheme 3. *Anti*-aldol reaction with chloroester **1** and introduction of nitrogens at C2″ center.

Gratifyingly, the boron-mediated ((*c*-hexyl)$_2$BOTf / Et_3N) aldol reaction between **1** (X=Cl) and **2** furnished the desired *anti*-chlorohydroxy ester **3** (X=Cl) in 90% yield without other detectable diastereomers (Scheme 3).[25] On the other hand, base catalyzed (LDA-Cp_2ZrCl_2) *anti*-aldol reaction did not provide the desired product because of base sensitivity of the uridyl aldehyde derivative **2**. Although the epoxide formation of *anti*-2-halo-3-hydroxycarbonyls is generally entropically favored

process, the *anti*-2-chloro-3-hydroxyester **3** is stable under pH values of 6.0~8.5 for a prolonged time. Indeed, the nitrogen atom could be introduced to **3** *via* a S_N2 reaction of azide anion at 45 °C to furnish the azido-alcohol **4** without observation of the competitive retro-aldol reaction. On the other hand, the treatment of **3** with *primary* amines and *secondary* amines in DMF at 50 °C furnished the epoxide as an intermediate which then underwent the opening of epoxide at α-position to ester carbonyl to yield the amino-alcohol **5**. Thus, we could efficiently synthesize the uridine-β-hydroxyaminoesters possessing natural and unnatural stereochemistries from the same intermediate **3**.

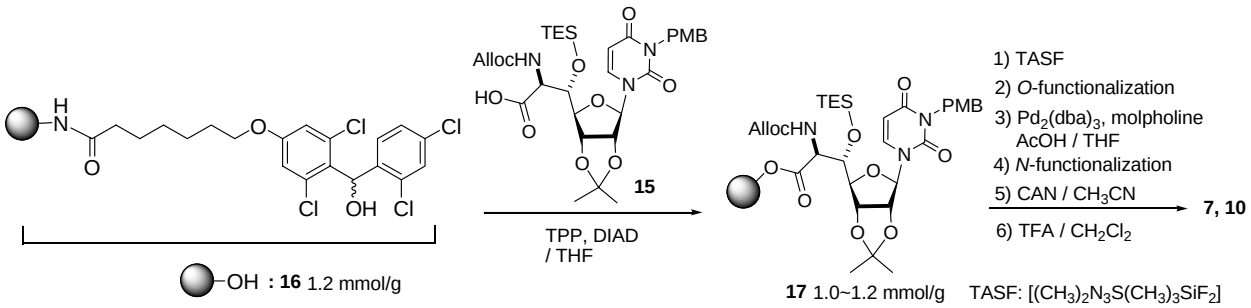


Reagents and conditions: **a**. for R_1=OH, R_2=I. 1) PMe_3, THF-water, 2) 4-I-benzaldehyde.; $Ti(O^iPr)_4$, $NaBH_4$, THF, 45% for two steps.; 3) deprotection conditions a, 40%.; **b** R_1=OH, R_2=I, R_3=H, X=O. 1) TMSNCO, THF, 98%.; 2) PMe_3, THF-water.; 3) 4-I-benzaldehyde.; $Ti(O^iPr)_4$, $NaBH_4$, THF, 40% for two steps.; 3) deprotection conditions a, 40%.; **c** for R_1= $NH(CH_2)_3N(Me)Ph$, R_4=C_8H_{17}. 1) PMe_3, THF-water.; 2) $C_8H_{17}COOH$, PyBOP, HOBt, $^iPr_2NEt$, THF, 94%.; 3) LiOH, THF-water.; 4) R_1=$NH(CH_2)_3N(Me)Ph$, PyBOP, HOBt, $^iPr_2NEt$, THF, for 85% for two steps.; 4) deprotection conditions b. 40%; **d** for R_1=OH, R_3=H, R_4=C_8H_{17}. 1) TMSNCO, THF, 98%.; 2) PMe_3, THF-water.; 3) $C_8H_{17}COOH$, PyBOP, HOBt, $^iPr_2NEt$, THF, 95%.; 4) LiOH, THF-water, 5) R_1=$NH(CH_2)_3N(Me)Ph$, PyBOP, HOBt, $^iPr_2NEt$, THF, 93%.; 5) deprotection conditions c, 60%.; **e** R_1=OH, R_2=H, R_3=H, X=O. 1) TMSNCO, THF, 98%.; 2) PMe_3, THF-water.; 3) benzaldehyde.; $Ti(O^iPr)_4$, $NaBH_4$, THF, 40% for two steps.; 3) deprotection conditions a, 40%.; **f** for R_1= $NH(CH_2)_3N(Me)Ph$, R_2=H. 1) PMe_3, THF-water.; 2) benzaldehyde.; $Ti(O^iPr)_4$, $NaBH_4$, THF, 43% for two steps.; 3) LiOH, THF-water.; 4) R_1=$NH(CH_2)_3N(Me)Ph$, PyBOP, HOBt, $^iPr_2NEt$, THF, for 85% for two steps.; 4) deprotection conditions c. 45%; **g** for R_1=$NH(CH_2)_3N(Me)Ph$, R_5=Me, R_6=C_7H_{15}. 1) LiOH, THF-water.; 2) R_1=$NH(CH_2)_3N(Me)Ph$, PyBOP, HOBt, $^iPr_2NEt$, THF, for 85% for two steps.; 3) deprotection conditions c. 40%; **h**. for R_1=OH, R3=Ph, R_5=Me, R_6=C_7H_{15}, X=S. 1) PhCSO, THF, 98%.; 2) deprotection conditions a, 40%.

Scheme 4. Generation of small optimized library.

The generation of small optimized library was demonstrated by using **4**, **6**,[25] and **5a**. As summarized in Scheme 4, the C5′ alcohol of **4** could be functionalized with isocyanates and thioisocyanates to form the corresponding urethanes and thiourethanes **7** and **10** after exhaustive deprotection followed by purification. Reduction of the azide group was accomplished by PMe_3 in aqueous THF. The generated amine could be functionalized by reductive amination and acylations. As expected the carboxylic acid group could be utilized for the formation of amide. Similarly, the epimer **6** of **4** at C2″ position could be converted to the corresponding analogs **11** and **12**. *N*,*N*-dialkyl amine **5a** was demonstrated to functionalize at C5′ and C1″ positions to furnish **13** and **14** after global deprotections. All molecules shown in Scheme 4 were purified by TLC or HPLC and their structures were confirmed by ^{1}H-NMR or LC-MS.

We recently developed a novel linker, (2-chloro-4-methoxyphenyl)(2,4-dichlorophenyl)methanol **16**, to immobilize carboxylic acids that was especially designed for applying the Boc-strategy in which the linker is stable under a reliable Boc-deprotection conditions (TsOH•H_2O/THF-CH_2Cl_2).[26] In addition, unlike photolytic (350 nm, 72h)[27] or Pd-mediated[28] or base-catalyzed cleavage of linkers, the esters of linker **16** can efficiently be cleaved *via* volatile acids such as 30% TFA/CH_2Cl_2 or HF•pyridine within 1h.[29] Thus, the progress of each step can conveniently be monitored by LC-MS after the treatment of such Brønsted acids followed by evaporation of all volatiles.



Scheme 5. Solid-phase studies of the synthesis of the uridine-amino alcohol derivatives.

In order to facilitate the small optimized library production we have established the functionalization reaction on the polymer support. As illustrated in Scheme 5 the carboxylic acid was loaded onto the polymer **16** by using Mitsunobu reaction to furnish **17** whose loading yield was established to be over 83% by the cleavage of the linker followed by ^{1}H-NMR analysis of the crude product using an internal standard. The TES group was selectively deprotected with TASF[30] and the generated alcohol was functionalized with TMSNCO in THF in quantitative yield based on ^{1}H-NMR analysis. Pd-mediated deprotection of the Alloc group to provide the free amine. *N*-functionalizations on the polymer support

were demonstrated by a reductive amination with benzaldehyde under $NaCNBH_4$ in the presence of HCO_2H in THF-MeOH and an amide-formation with nonanoic acid *via* PyBOP as a coupling reagent. Deprotection of the PMB group with CAN followed by simultaneous deprotections of the acetonide and the linker with TFA to provide **7** and **10** in 45% and 40% overall yields, respectively.

Thus, we could establish a platform for the generation of library based on uridine-amino alcohol in solution and on the polymer support. Although a relatively large membered library can be generated by the use of split-pool synthesis with a large number of commercially available building blocks, we would rather deliver small optimized library in which building blocks are evaluated by ab initio calculations to yield desired conformations, which can be superimposed on the lowest energy conformers of the caprazamycin and FR-900493 core.

CONCLUSION

To date, studies on the structure-activity relationships of MraY inhibitors in Figure 2 are limited by the complexity of those structures and difficulty in selectively modifying the desired positions. In addition, the synthetic routes for these molecules reported in the literature are rather inconvenient to apply to medicinal chemistry. Our synthetic scheme illustrated in Scheme 3 and 4, however, is flexible and amenable to deliver the library molecules. In addition, feasibility of small optimized library production based on the uridine-amino alcohol was established by the chemical transformations demonstrated in Scheme 4 and 5. We are currently screening the building blocks that mimic the hydrophilic moiety of ribosamino-uridine-containing MraY inhibitors using ab initio calculations. Generation of small optimized library using the *anti*-aldol product **3** and evaluation of the library molecules against MraY and *Mycobacterium* spp. will be reported elsewhere.

EXPERIMENTAL

General Considerations. All glassware was oven dried, assembled hot and cooled under a stream of nitrogen before use. Reactions with air sensitive materials were carried out by standard syringe techniques. Commercially available reagents were used as received without further purification. Thin layer chromatography was performed using 0.25 mm silica gel 60 (F254, Merck) plates visualizing at 254 nm, or developed with potassium permanganate solutions by heating with a hot-air gun. Specified products were purified by flash column chromatography using silica gel 60 (230-400 mesh, Merck). IR absorptions on NaCl plates were run on a Perkin Elmer FT-IR 1600. ^{1}H NMR spectral data were obtained using Varian 300, 400 or 500 MHz instruments. The residual solvent signal was utilized as an

internal reference. ^{13}C NMR spectral data were obtained using a Varian 75 or 100 or 125 MHz spectrometer. Chemical shifts were reported in parts per million (ppm) downfield from TMS, using the middle resonance of $CDCl_3$ (77.0 ppm) as an internal standard. For all NMR spectra, δ values are given in ppm and *J* values in Hz. Mass spectra were obtained at Colorado State University's Central Instrument Facility. Optical rotations were taken using Rudolph research–Autopol III, automatic polarimeter.

Synthesis of (1S,2R)-2-((2-methylbenzyl)(2,4,6-trimethylbenzyl)amino)-1-phenylpropyl-2-chloroacetate (1). 2-(*N*-2-methylbenzyl-*N*-2,4,6-trimethylbenzyl)-amino-1-phenylpropanol was synthesized from (1*R*, 2*S*)-(–)-norephedrine according to the procedure described in the literature.[23a] To a stirred solution of 2-(*N*-2-methylbenzyl-*N*-2,4,6-trimethylbenzyl)-amino-1-phenylpropanol (5 g, 12.9 mmol) in CH_2Cl_2 (30 mL) at 0 °C was added DMAP (3.15 g, 25.8 mmol) and 2-chloroacetyl chloride (2.17g, 19.4 mmol). The reaction mixture was stirred for 1h and quenched with aq. $NaHCO_3$, and extracted with EtOAc. The combined extracts were washed with brine, dried over Na_2SO_4, and concentrated in *vaccuo*. The residue was purified by silica gel chromatography (2:1, hexanes:EtOAc) to afford **1** (5.67g, 12.2 mmol, 95%). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 1.30 (d, *J* = 7.2 Hz, 3H), 2.09 (s, 9H), 2.26 (s, 3H), 3.28 (m, 1H), 3.56 (m, 2H), 3.71 (m, 2H), 4.02 (m, 2H), 6.05 (d, *J* = 7.2 Hz, 1H), 6.82 (s, 2H), 6.91 (m, 2H), 7.18 (m, 7H). ^{13}C-NMR ($CDCl_3$, 100 MHz) 9.2, 19.2, 20.1, 20.9, 41.1, 47.6, 51.2, 56.2, 79.1, 129.4, 127.0, 127.3, 127.8, 128.1, 129.0, 130.2, 130.8, 131.4, 136.4, 136.8, 137.4, 138.5, 166.4. LR-MS (ESI): $C_{29}H_{34}NO_2Cl$ ($M+H^+$) found 264.2. $[\alpha]_D = -15.1^o$ (*c* 0.8, $CHCl_3$, 25 °C).

Synthesis of the anti-chlorohydroxy ester 3. To a stirred solution of **1** (2.43 g, 5,25 mmol) was dissolved in CH_2Cl_2 (50 mL) and cooled to –78 °C. Into the reaction mixture Et_3N (1.8 mL, 13.0 mmol) followed by (*c*-hexyl)$_2$BOTf (9.15 mL, 1.21 M solution in dichloroethane) were added. After being stirred for 2 h at –78 °C, the uridine-aldehyde **2** (750 mg, 1.85 mmol) was added. The reaction mixture was stirred for 1h at the same temperature and quenched with pH 7.3 phosphate buffer. The water phase was extracted with CH_2Cl_2. The combined extracts were washed with brine, dried over Na_2SO_4, and concentrated in *vaccuo*. The residue was purified by silica gel chromatography (2:1, hexanes:EtOAc) to afford **3** (1.44 g, 1.67 mmol, 90%). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 1.35 (m,3H), 1.53 (s, 3H), 1.59 (s, 3H), 2.10 (s, 9H), 2.25 (s, 3H), 3.58 (m, 3H), 3.70 (m, 1H), 3.78 (s, 3H), 3.98 (d, *J* = 7.2 Hz, 1H), 4.24 (m, 3H), 5.01 (m, 4H), 5.45 (d, *J* = 2.1 Hz, 1H), 5.76 (d, *J* = 7.8 Hz, 1H), 6.09 (d, *J* = 6.6 Hz, 1H), 6.75 (s, 2H), 6.82 (d, *J* = 10 Hz, 2H), 6.93 (m, 2H), 7.17 (m, 8H), 7.42 (d, *J* = 10.0 Hz, 2H). ^{13}C-NMR ($CDCl_3$, 75 MHz) 9.3, 14.1, 19.2, 20.1, 20.8, 25.3, 27.2, 43.5, 47.3, 51.1, 54.9, 55.1, 56.6, 60.3, 72.5, 78.9, 79.1, 83.9, 86.1, 96.4, 102.4, 113.7, 114.6, 125.3, 126.9, 127.6, 128.0, 128.5,

129.0, 130.1, 130.6, 130.9, 131.0, 131.5, 136.3, 136.8, 137.4, 138.3, 138.4, 138.7, 140.4, 150.9, 159.1, 162.2, 167.3. LRMS(ESI) $C_{49}H_{56}N_3O_9Cl$ found 866.2. $[\alpha]_D$ = + 8.0 (*c* 0.05, $CHCl_3$, 25 ^oC).

Synthesis of the azide-alcohol 4. To a stirred solution of **3** (135 mg, 0.16 mmol) in DMF (10 mL) was added NaN_3 (52.0 mg, 0.80 mmol). The reaction mixture was stirred at 45 ^oC for 45 min and quenched with water. The water phase was extracted with EtOAc. The combined extracts were washes with brine, dried over Na_2SO_4, and concentrated in *vaccuo*. The residue was purified by silica gel chromatography (3:1, hexanes:EtOAc) to afford **4** (126 mg, 0.14 mmol, 90%). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 1.35 (m, 3H), 1.54 (s, 3H), 1.56 (s, 3H), 2.10 (s, 9H), 2.23 (s, 3H), 3.08 (d, *J* = 6.6 Hz, 1H), 3.59 (m, 3H), 3.76 (m, 1H), 3.79 (s, 3H), 3.96 (m, 1H), 4.45 (m, 1H), 4.51 (d, *J* = 2.7 Hz, 1H), 5.01 (m, 4H), 5.41 (d, *J* = 2.1 Hz, 1H), 5.76 (d, *J* = 7.8 Hz, 1H), 6.13 (d, *J* = 6.6 Hz, 1H), 6.74 (s, 2H), 6.84 (m, 2H), 6.93 (m, 2H), 7.17 (m, 8H), 7.42 (m, 2H). ^{13}C-NMR ($CDCl_3$, 75 MHz) 14.2, 19.2, 20.1, 20.8, 25.1, 27.0, 31.6, 43.5, 47.5, 51.2, 55.2, 60.4, 63.2, 71.4, 72.0, 79.4, 81.2, 83.9, 87.7, 97.9, 102.5, 113.7, 114.4, 125.4, 127.0, 127.2, 127.9, 128.1, 128.5, 129.0, 130.2, 130.6, 130.9, 131.4, 136.3, 136.8, 137.3, 138.3, 141.1, 150.6, 159.1, 162.2, 167.1, 168.1, 171.2. LRMS (ESI) $C_{49}H_{56}N_6O_9$ found 873.4 $[\alpha]_D$ = + 19.0^o (*c* 2.0, $CHCl_3$, 25 ^oC).

Synthesis of 5a. To a stirred solution of **3** (30 mg, 0.036 mmol) in DMF (2 mL) was added *N*-methyl butylamine (15.7 mg, 0.18 mmol). The reaction mixture was stirred at 50 ^oC for 12h. All volatiles were evaporated in *vaccuo*. The residue was purified by silica gel chromatography (3:1, hexanes:EtOAc) to afford **5a** (29.8 mg, 0.032 mmol, 90%). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 0.97 (t, *J* = 7.2 Hz, 3H), 1.33 (m, 7H), 1.54 (s, 3H), 1.59 (m, 3H), 2.01 (s, 9H), 2.14 (s, 3H), 2.25 (s, 3H), 2.69 (m, 2H), 3.58 (m, 3H), 3.70 (m, 1H), 3.78 (s, 3H), 4.26 (m, 3H), 4.40 (m, 1H), 5.00 (m, 4H), 5.49 (d, *J* = 2.4 Hz, 1H), 5.76 (d, *J* = 8.1 Hz, 1H), 6.04 (d, *J* = 8.4 Hz, 1H), 6.73 (m, 2H), 6.82 (m, 2H), 6.93 (m, 2H), 7.11 (m, 8H), 7.40 (m, 2H). LRMS (ESI) $C_{54}H_{68}N_4O_9$ found = 917.5. $[\alpha]_D$ = + 12^o (*c* 0.1, $CHCl_3$, 25 ^oC).

Synthesis of the azide-alcohol 6. To a stirred solution of **3** (30.0 mg, 0.035 mmol) in benzene (1.0 mL) was added DBU (10.7 mg, 0.070 mmol). After 15 min the reaction mixture was in *vaccuo* to furnish the crude epoxide. This was dissolved in DMF (1.0 mL) and added NaN_3 (11.7 mg, 0.18 mmol) and NH_4Cl (19.0 mg, 0.35mmol). The reaction mixture was stirred at 90 ^oC for 1h and quenched with water. The water phase was extracted with EtOAc. The combined extracts were washed with brine, dried over Na_2SO_4, and concentrated in *vaccuo*. The residue was purified by silica gel chromatography (3:1, hexanes:EtOAc) to afford **6** (26.0 g, 0.029 mmol, 85%). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 1.35 (m, 3H),

1.54 (s, 3H), 1.56 (s, 3H), 2.10 (s, 9H), 2.23 (s, 3H), 3.08 (d, *J* = 6.6 Hz, 1H), 3.59 (m, 3H), 3.76 (m, 1H), 3.79 (s, 3H), 3.96 (m, 1H), 4.45 (m, 1H), 4.51 (d, *J* = 2.7 Hz, 1H), 5.01 (m, 4H), 5.41 (d, *J* = 2.1 Hz, 1H), 5.76 (d, *J* = 7.8 Hz, 1H), 6.13 (d, *J* = 6.6 Hz, 1H), 6.74 (s, 2H), 6.84 (m, 2H), 6.93 (m, 2H), 7.17 (m, 8H), 7.42 (m, 2H). ^{13}C-NMR ($CDCl_3$, 75 MHz) 14.2, 19.2, 20.1, 20.8, 25.1, 27.0, 31.6, 43.5, 47.5, 51.2, 55.2, 60.4, 63.2, 71.4, 72.0, 79.4, 81.2, 83.9, 87.7, 97.9, 102.5, 113.7, 114.4, 125.4, 127.0, 127.2, 127.9, 128.1, 128.5, 129.0, 130.2, 130.6, 130.9, 131.4, 136.3, 136.8, 137.3, 138.3, 141.1, 150.6, 159.1, 162.2, 167.1, 168.1, 171.2. LRMS (ESI) $C_{49}H_{56}N_6O_9$ found 873.4 $[\alpha]_D$ = + 5.0^o (*c* 1.0, $CHCl_3$, 25 ^{o}C).

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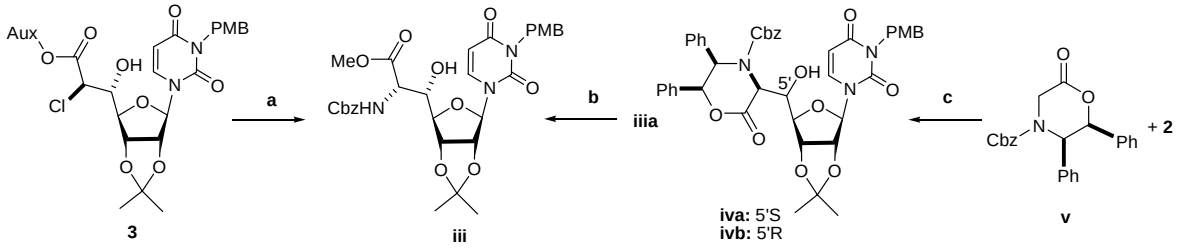
24. The stereochemistries of the *anti*-aldol product **3** was confirmed by ^{1}H- and ^{13}C-NMRs and TLC analysis of **iii**, which was transformed from **3** *via* the S_N2 addition of azide, reduction of the azide to the amine, Cbz protection, saponification, and methyl esterification, with that of the molecule synthesized through an aldol reaction of Williams' lactone **v** and **2** as summarized below, see. D. E. DeMong and R. M. Williams, *Tetrahedron Lett*., 2001, **42**, 183.



Reagents and conditions: **a**. 1) NaN_3, DMF, 95%, 2) PMe_3, THF-water.; 3) CbzCl, Na_2CO_3, dioxane-water, 90% for two steps.; 4) LiOH, THF-water.; 5) $TMSCHN_2$, $CHCl_3$-MeOH, 95% for two steps.; **b**. 1) H_2, Pd-C, EtOAc, 2) CbzCl, Na_2CO_3, dioxane-water, 3) $TMSCHN_2$, $CHCl_3$-MeOH, 45% for three steps.; **c**. Bu_2BOTf, $^{i}Pr_2NEt$, CH_2Cl_2, 0 ^{o}C, 95% (**5a**:**5b**=1:2.2) or (*c*-hexyl)$_2$BOTf, Et_3N, CH_2Cl_2, -78 ^{o}C, 90% (**5a**:**5b**=0:1).

25. Compound **6** was synthesized via an azide anion addition to the epoxide **ii** (Scheme 3) which was obtained by the treatment of **3** with DBU in benzene. see, experimental.

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