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Stereocontrolled Total Synthesis of Muraymycin D1 Having a Dual Mode of Action against *Mycobacterium tuberculosis*

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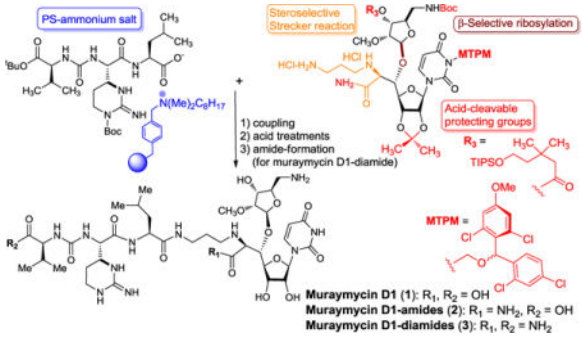
Abstract

A stereocontrolled first total synthesis of muraymycin D1 (**1**) has been achieved. The synthetic route is highly stereoselective, featuring 1) selective β-ribosylation of the C2-methylated amino ribose, 2) selective Strecker reaction, 3) ring-opening reaction of a diastereomeric mixture of a diaminolactone to synthesize muraymycidine (*epi*-capreomycidine). The acid-cleavable protecting groups for *secondary* alcohol and uridine ureido nitrogen are applied for simultaneous deprotections with the Boc and ^{t}Bu groups. Muraymycin D1 (**1**) and its amide derivatives (**2** and **3**) exhibited growth inhibitory activity against *Mycobacterium tuberculosis* (MIC_{50} 1.56–6.25 μg/mL) and strong enzyme inhibitory activities against the bacterial phosphotransferases (MurX and WecA) (IC_{50} 0.096−0.69 μM).

Graphical abstract



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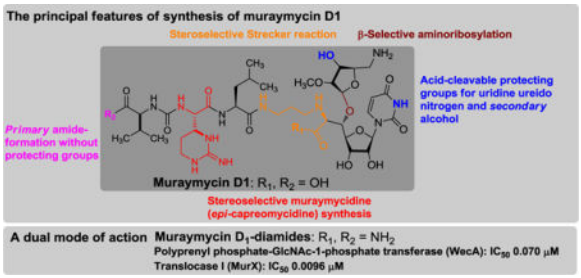
Supporting Information

The Supporting Information is available free of charge on the ACS Publication website at http://pubs.acs.org. Complete experimental details, compound characterization data, and biological evaluation and data (PDF)

Notes

The authors declare no competing financial interests.



INTRODUCTION

Muraymycins belong to aminoribosyl-uridyl peptides that were isolated from *Streptomyces spp*. by McDonald et al.[1] To date, 19 muraymycin congeners (muraymycin A1–5, B1–7, C1–4, D1–3) have been isolated. Their structural diversity is observed in the lipid moiety (R_2) and the appended C5′-aminoribose unit (R_1) (Figure 1). Muraymycin A1 is one of the most active members of this family and showed bactericidal activity against both Gram-positive and Gram-negative bacteria. Notably, muraymycin A1 demonstrated efficacy in the *Staphylococcus aureus* infected mice models (ED_{50} 1.1 mg/kg).[1] The muraymycins are structurally related to the other uridyl peptide antibiotics such as the liposidomycins, mureidomycins, pacidamycins, and tunicamycin.[2] This class of natural products is reported to exhibit strong inhibitory activities against translocase I (MraY/MurX), essential peptidoglycan biosynthesis enzymes that catalyze the formation of lipid I from Park's nucleotide (UDP-MurNAc-pentapeptide) with polyprenyl phosphate.[3] Besides muraymycin A1, *in vitro* properties of the other muraymycin congeners have been poorly characterized. The difficulties in isolating the muraymycins in their pure form via reverse-phase HPLC as well as inaccessibility of the muraymycin-producing strain preclude biological evaluation. Recently, muraymycin D2 (R_1, R_2 = H in Figure 1) was reported to show no significant antibacterial activity, even though it has strong MraY enzyme inhibitory activity (IC_{50} 0.01 μM).[4] In addition, some structure-activity relationship (SAR) studies were also described based on the structure of muraymycins.[5] Muraymycin D1 (**1**) is synthetically more challenging than other members of the muraymycin D series. Because **1** lacks only the lipophilic side chain appended in the L-leucine moiety of muraymycin A1, achievement of synthesis of **1** will make a promising step toward the total synthesis of muraymycin A1. Therefore, we desired to establish an efficient synthesis of **1** and thoroughly evaluate the efficacy of **1** *in vitro*. Several groups have reported synthetic efforts on muraymycins including a total synthesis of muraymycin D2.[4,6] Although remarkable accomplishments have been documented in the reported syntheses, more efficient strategies that minimize generations of diastereomers and protecting group manipulations will accelerate the development of new analogs for multi-drug resistant (MDR) bacterial infections. Herein, we report a highly stereocontrolled total synthesis of muraymycin D1 (**1**), its amide analogs (**2** and **3**), and their evaluation against the bacterial phosphotransferases.

RESULTS AND DISCUSSION

Our retrosynthesis for muraymycin D1 (**1**) is illustrated in Scheme 1; the challenging synthetic outcomes are highlighted in the structure of **1**. Muraymycin D1 is retrosynthetically divided into the left- and right-half segments. We envisioned that the 3-aminopropyl amino acid moiety (C6′,7′-positions) of **1** could be constructed via Strecker reaction of the aldehyde **8** with the mono-protected 1,3-diaminopropane in the presence of an appropriate CN source. We have extensively studied ribosylations via non-anchimeric assistance of the C2-position and found that β-selective ribosylations can be achieved when the ribose-donors possess a bulky ester group at the C3″-postion.[7] The muraymycins are vulnerable to strong bases and give rise to complex mixtures upon exposure. In order to achieve facile deprotection of the acyl group under acidic conditions, we planned to introduce the 3,3-dimethyl-5-(triisopropylsilyloxy)pentanoate protecting group for the alcohol at the C3″-position of the amino ribose (see **9**).[7] Construction of the *R*-configuration at C5′-position relies on Carreira's asymmetric alkynation.[8] Presence of (2*S*,3*S*)-muraymycidine (*epi*-capreomycidine) is one of the characteristics of the muraymycins.[9] We have previously investigated lactone-opening reactions to synthesize 2*S*,3*S*-ureido-muraymycidine **7** through a diastero-mixture of **13**.[10] In addition, a unique selective-deprotection method to remove the 2-(trimethylsilyl)ethanol group of **7** followed by capturing the carboxylate using the polymer-supported fluoride (PS-F) is applied to facilitate the synthesis of the left-half segment (the ureido-tripeptide carboxylic acid).[11] Coupling of the right- and left-half segments, global deprotections of all acid labile groups including monomethoxytetrachlorodiphenylmethoxymethyl (MTPM)[12], followed by hydrolysis of the amide group are envisioned to furnish **1** in a single step.

The synthesis of **1** commenced with the left-half segment **7** (Scheme 2). We previously reported a scalable synthesis of (2*R*,3*S*)- and (2*S*,3*S*)-diaminolactones from (2*S*)-2-amino γ-butyrolactone.[10] Extensive studies of the opening of **13** with a wide range of amino acids revealed that the undesired 2*R*-configuration of **13** is completely epimerized to the desired (2*S*,3*S*)-**13** by treatment with 2(1H)-pyridinone at 70 °C. Interestingly, nucleophilic attacks of (2*R*,3*S*)-**13** with *C*-protected amino acids did not take place, while (2*S*,3*S*)-**13** underwent thermal amide-forming reaction. Taking advantage of these observations, a one-pot epimerization/lactone-opening reaction with the hydrazide **14** gave rise to the dipeptide **15**. The overall yield of the transformation from **13** to **15** was determined to be >80% after acetylation of the *primary* alcohol of **15**. Although the phenylhydrazide could serve as an appropriate *C*-protecting group to accomplish the synthesis of the left-half segment **23**, deprotection of the phenylhydrazide group in **22** required multiple time-consuming purifications via reverse-phase HPLC (CH_3OH-0.1% TFA = 50 : 50) to provide **23** in its pure form. In order to facilitate the synthesis of **23**, we revised the orthogonal protection strategy. The hydrazide group of **16** was converted to the trimethylsilylethyl (TMSE) ester **17** in 95% yield by using *N*-bromosuccinimide (NBS)/$NaHCO_3$ in anhydrous CH_2Cl_2. Hydrogenations of **17** provided the free-amine, which was then subjected to the urea-forming reaction with the imidazole-carboxamido derivative **18** to furnish **19** in 65% overall yield. The Boc group of **19** was removed with 4*N* HCl, and the generated HCl-amine salt was coupled with *N,N*′-di-*tert*-butoxycarbonyl-*S*-methyl isothiourea in the presence of Et_3N

and $HgCl_2$ to afford **20** in 75% overall yield.[13] $[^tBu_2Sn(OH)Cl]_2$-catalyzed deacetylation[14] of **20** followed by tosylation of the *primary* alcohol provided the intermediate **21** which subsequently underwent intramolecular cyclization and concomitant deprotection of the Boc group of the imino-*N*, yielding the ureido-muraymycidine tripeptide **7** in 85% overall yield.

We have introduced the monomethoxytetrachlorodiphenylmethoxymethyl (MTPM) protecting group because it has significant advantages over ordinal protecting groups (e.g. BOM) for uridine ureido nitrogen; the MTPM group is stable under hydrogenation conditions and to a wide range of acids, but it can be deprotected by solvolytic cleavage with 30% TFA.[12,15] The MTPM-protected uridine **11**[15] was subjected to a modified Swern oxidation to provide the corresponding aldehyde in quantitative yield which was then subjected to Carreira's asymmetric alkynation reaction using (+)-*N*-methylephedrine,[8] yielding the (*S*)-propargyl alcohol **10** in 80% yield with *S*/*R* = >98:2 selectivity. Without the chiral controller, the 1,2-addition of the zinc acetylide species provided a mixture of the propargyl alcohols in 75% yield with *S*/*R* = 1.7:1.0 selectivity (Scheme 3). The stereochemistry of the *secondary* alcohol of **10** generated via Carreira's alkynation was unequivocally determined by the advanced Mosher's method.[16] NIS-$AgBF_4$ promoted ribosylation of **10** with the thioglycoside **9** furnished the β-glycoside **24** exclusively in 91% isolated yield.[17] It is worth mentioning that the ribosylation demonstrated with **9** is an unusual observation in that the C2-ether-protected ribose donor provided β-glycoside without contamination of the α-glycoside. This observation may be attributable to C3-acyl group participation in the oxocarbenium ion transition state, leading exclusively to β-ribosylation of **9**.[7] The azido group of **24** was reduced with Zn metal in the presence of aq. NH_4Cl, and the generated free-amine was protected with $(Boc)_2O$ to furnish **25** in 90% overall yield. The alkyne moiety of **25** was converted to the aldehyde via a standard three step procedure including partial reduction with a Lindlar's catalyst, osmylation, and oxidative cleavage with $Pb(OAc)_4$. The crude aldehyde **8** was subjected to a thiourea-catalyzed Strecker reaction[18] with the Cbz-protected 1,3-diaminopropane to provide the desired aminonitrile **26** in 60% overall yield from **25**. In this reaction, the undesired (*R*)-diastereomer was not observed by LC-MS, TLC or ^{1}H-NMR analyses of the reaction mixture; thus, selectivity of the Strecker reaction of **8** was determined to be >25/1. Later, we realized that same transformation (from **8** to **26**) could be catalyzed by $MgSO_4$, producing **26** with the yield and *S*/*R*-selectivity comparable to the reaction catalyzed by the thiourea. The nitrile group of **26** was hydrated with $HgCl_2$-aldoxime to furnish the amide **6** in 70% yield. The stereogenic center (C6′) generated via the Strecker reaction (**8**→**26**) was confirmed by the ^{1}H-NMR analyses of **6** where the value of the coupling constant between H-5′ and H-6′ ($J_{5',6'}$ = 3.4 Hz) was in good agreement with the reported *J* value for (2S, 3S)-2-amino-3,4-dihydroxybutyric acid derivatives (*J* = 3.2–8.0 Hz) and the muraymycin D2 synthetic intermediate (*J* = 3.5 Hz).[1a,4,19,20] The Cbz group of **6** was removed under hydrogenation conditions[21] followed by treatment with 1*N* HCl to provide the diamine HCl salts **27** in quantitative yield.

The 2-(trimethylsilyl)ethanol (TMSE) group of the left-half segment **7** was selectively cleaved with PS-F[11] to furnish the PS-ammonium salt **28** (Scheme 4). The purity of **28** was determined to be >92% by ^{1}H-NMR and HPLC analyses of the protonated form **23** (see

Scheme 2). Decomplexation of the PS-ammonium complex **28** was not observed under neutral conditions; conveniently, **28** could be dissociated under the peptide-forming reaction conditions (Glyceroacetonide-Oxyma (GOx, **29**), EDCI, $NaHCO_3$ in DMF-H_2O),[22] and the coupling reaction with **27** was complete in 3h to afford the protected muraymycin D1, **4** with >90% purity after water work-up and filtration. Global deprotection of **4** to form muraymycin D1 (**1**) was performed in two steps in a one-pot procedure with 84% overall yield; the MTPM, Boc, ^{t}Bu and acyl (R_1) groups were first removed via 30% TFA/CH_2Cl_2, then addition of 50% TFA/H_2O removed the acetonide and amide groups leading to **1**. Similarly, muraymycin D1-amide (**2**) was synthesized via treatment with 50% TFA/CH_2Cl_2 followed by addition of H_2O. *Primary* amide-formation of the *C*-terminus of **2** was accomplished via our standard coupling conditions (**29**, EDCI, $NaHCO_3$ in DMF-H_2O)[22] with excess NH_4Cl to give rise to **3** in 75% yield. Muraymycin D1 and its amide analogs were synthesized in their pure forms as determined by C_{18} reverse-phase HPLC analyses (retention time of **1**, **2**, and **3**: 10.0, 17.5, and 18.0 min, respectively; solvent system: MeOH: 0.1% TFA/H_2O = 25 : 75, flow rate : 2.0 mL/min, UV: 254 nm).[23]

Antibacterial activity of some muraymycins is believed to be solely due to inhibition of MraY/MurX. The other bacterial phosphotransferase, polyprenyl phosphate-GlcNAc-1-phosphate transferase (WecA), has never been investigated as a potential mechanism of action for the muraymycins. WecA is an essential enzyme for the growth of *M. tuberculosis*. Inhibition of WecA blocks the entire biosynthesis of essential cell wall components of *M. tuberculosis* in both replicating and non-replicating states, making this enzyme a target for development of novel TB drugs.[24] The synthetic molecules (**1**, **2**, and **3**) were evaluated in MurX and WecA assays (Table 1). Muraymycin D1 (**1**) and muraymycin D1-amide (**2**) exhibited equal enzyme inhibitory activities against the bacterial phosphotransferases (MurX and WecA) and their IC_{50} values were in low μM range. Inhibition of WecA enzyme of muraymycin D1-diamide (**3**) was ~10 times greater than that of **1** and **2**. Extensive bacterial growth inhibitory assays of **1**, **2**, and **3** against Gram-positive and -negative bacteria including *Mycobacterium spp*. revealed that **1**–**3** exhibited bacteriostatic activity against *M. tuberculosis*; the MIC_{50} values are comparable to UT-01320 (a selective WecA inhibitor)[24,25], capuramycin (a selective MurX inhibitor), and tunicamycin (a non-selective phosphotransferase inhibitor). However, **1**–**3** did not show antibacterial activity against Gram-negative bacteria (*E. coli, K. pneumoniae, P. aeruginosa* and *A. baumannii*) and Gram-positive bacteria (*S. aureus*, *C. difficile*, and *E. faecium*) even at >100 μg/mL concentrations. Unlike tunicamycin, **1**–**3** did not exhibit cytotoxicity against mammalian cells such as Vero cells even at 300 μg/mL concentration (see SI).

CONCLUSIONS

In summary, a highly stereocontrolled first total synthesis of muraymycin D1 (**1**) has been achieved from the reported intermediates.[10,12] The principal features of this synthesis include 1) stereoselective synthesis of the ureido-muraymycidine tripeptide, 2) β-selective glycosylation of the C2-methyl ether of the amino-ribose, and 3) *syn*-selective Strecker reaction to construct the 3-aminopropyl α-amino acid moiety in a single step. The acid-cleavable protecting groups introduced here allowed us to accomplish the synthesis of **1** with

a minimum number of protecting group manipulations. *Primary* amide-formation of the free carboxylic acid of **2** could be achieved via a GOx/EDCI based coupling condition in H_2O-containing solvents without protections of the amino and alcohol groups. We have demonstrated that the amide derivatives of **1** do not diminish MurX enzyme inhibitory activity. Muraymycin D1 and its amide derivatives are also effective in inhibiting WecA enzyme activity at low concentrations. Muraymycin D1-diamide (**3**) shows significantly greater inhibition of the WecA enzyme than its natural form. To date, only a few investigational TB drugs such as UT-01320 and CPZEN-45 have been reported to inhibit the WecA enzymes at low concentrations.[25,26] Although the activity of muraymycin A1 has been evaluated *in vitro* and *in vivo*,[1a] the antibacterial activity of the other muraymycins (B, C and D) has not been thoroughly investigated. Interestingly, we have identified that muraymycin D1 shows strong bacteriostatic activity against *M. tuberculosis* by targeting both MurX and WecA enzymes. Amide derivatives of muraymycins can be purified readily via conventional methods without the need for HPLC purification. These chemical properties will facilitate the discovery of new muraymycin analogs. Application of the synthetic strategies presented here continues for the synthesis of muraymycin A1 and its analogs in our laboratory. Efficacy of muraymycin congeners against non-replicating *M. tuberculosis* will be reported elsewhere.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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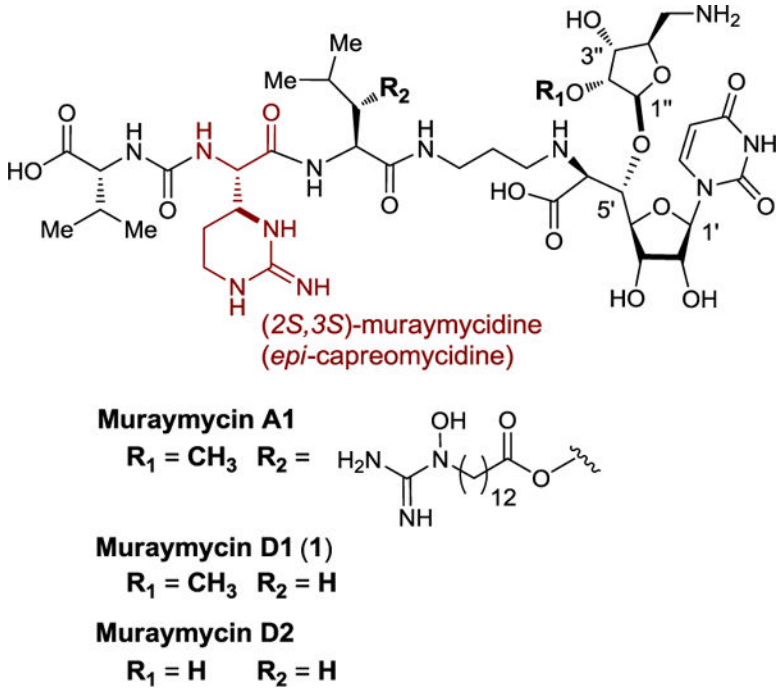
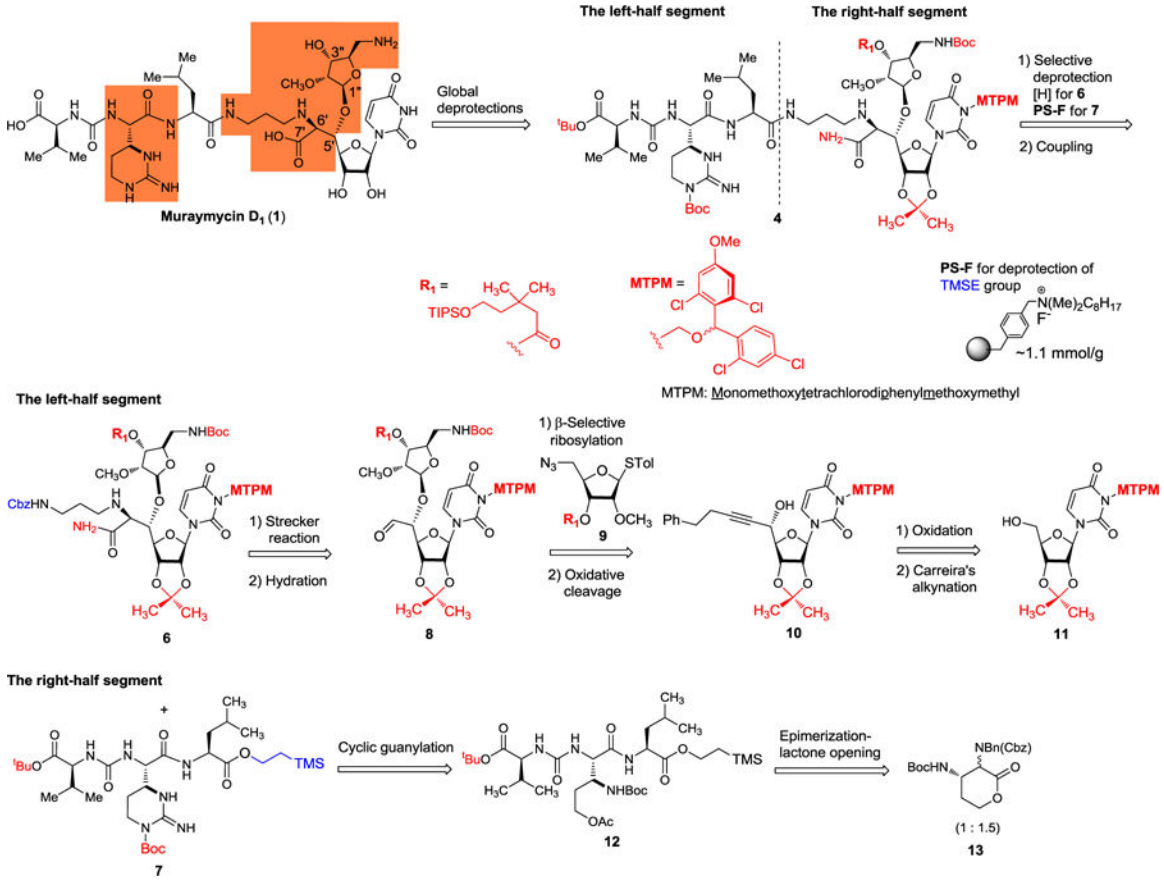
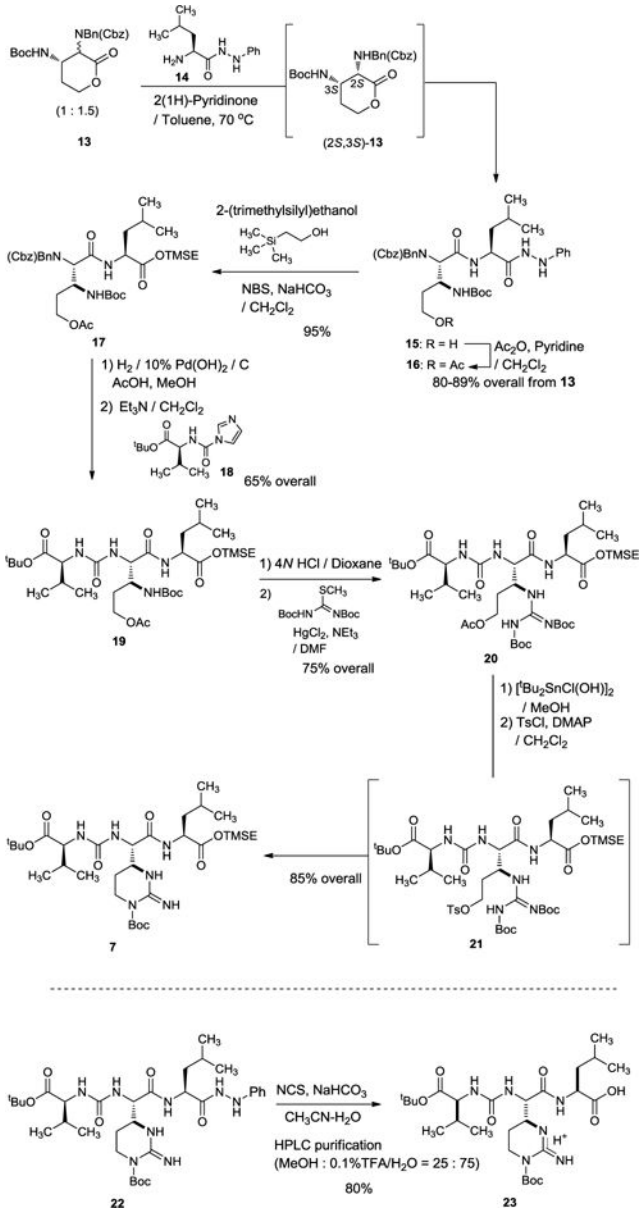


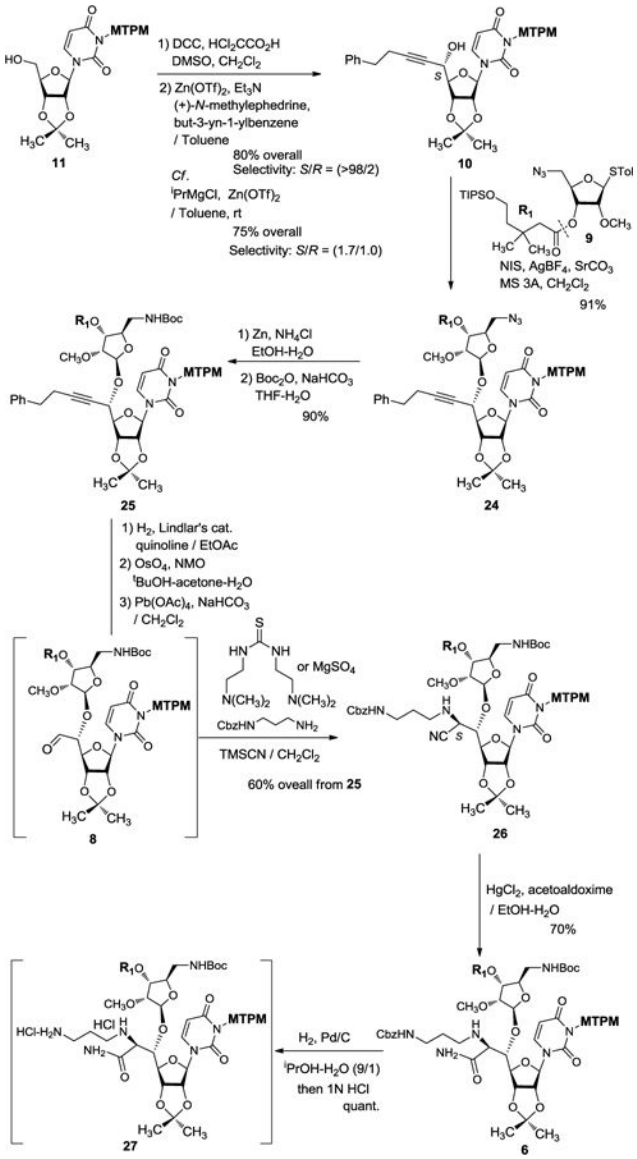
Figure 1.
Structures of representative muraymycins



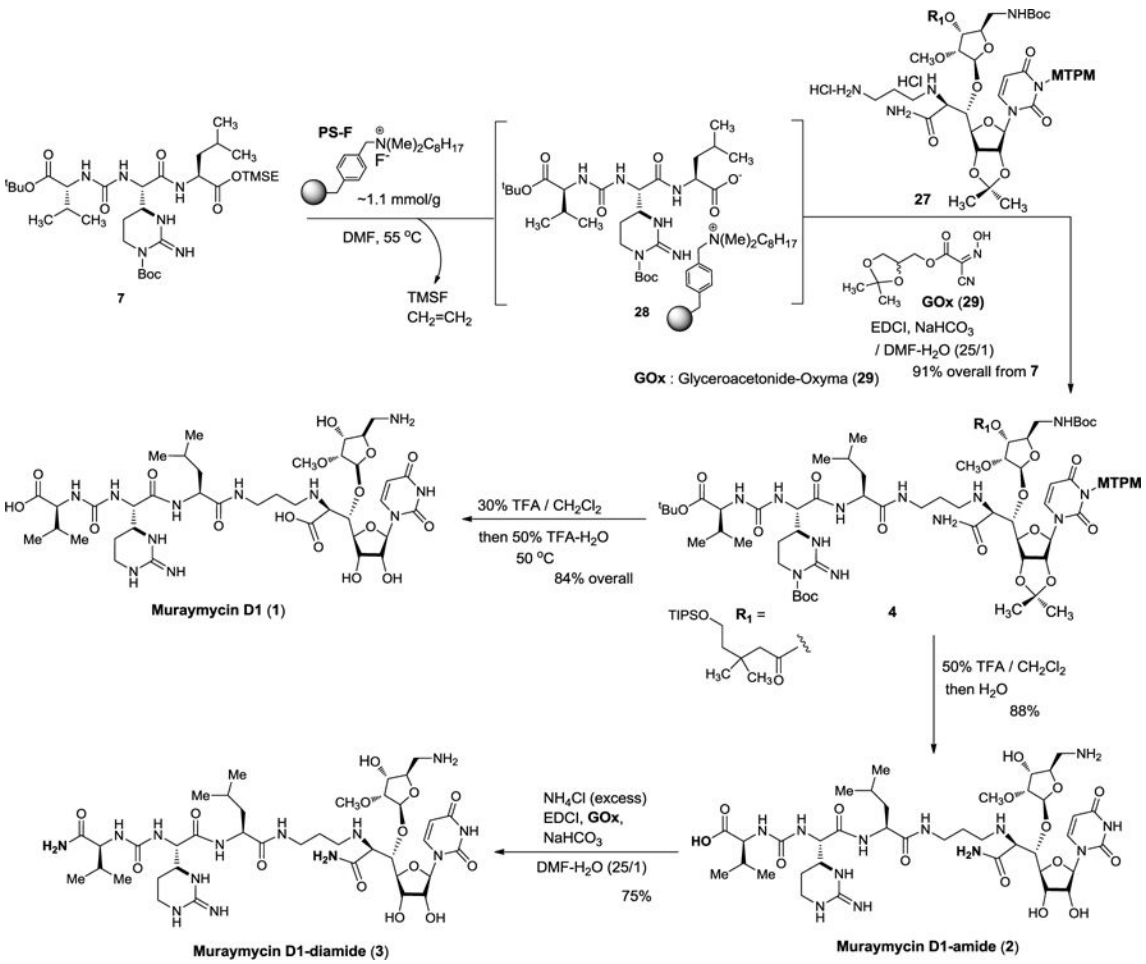
Scheme 1.
Retrosynthetic analysis of muraymycin D1



Scheme 2.
Synthesis of the left-half segment



Scheme 3.
Synthesis of the right-half segment



Scheme 4.

Synthesis of muraymycin D1 and its amide analogs

Table 1

Bacterial phosphotransferase activities and MICs against *M. tuberculosis*

Compound	WecA inhibition IC_{50} (μM)[a]	MurX inhibition IC_{50} (μM)[a]	M. *tuberculosis* growth inhibition [MIC_{50} (μg/mL)][b]
Muraymycin D1 (**1**)	0.69	0.011	1.56
Muraymycin D1-amide (**2**)	0.66	0.011	1.56
Muraymycin D1-diamide (**3**)	0.070	0.0096	6.25
Tunicamycin	0.15	3.38	3.13
Capuramycin	–	0.22	6.25
UT-01320	0.060	–	1.56

[a] WecA and MurX assays (see SI).

[b] A microdilution broth method was used. All structures in Table 1 are shown in SI.