SHORT COMMUNICATION

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Function-Oriented Synthesis of Liponucleoside Antibiotics

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Function-oriented synthesis of a class of liponucleoside antibiotics was investigated through rational simplification guided by previous structure–activity relationship studies of caprazamycins and muraymycins to address the issue associated with their molecular complexity. A lactam-fused isoxazolidine scaffold was designed, and a diverse set of lactam-fused isoxazolidines derivatives were constructed by intramolecular 1,3-dipolar cycloaddition of alkenyl nitrones. Several analogues exhibited moderate activity against a range of Gram-positive drug-resistant bacterial pathogens.

Introduction

Although natural products are a rich source for drug development,[1] many natural products possess large, complex, or labile chemical structures that may restrict chemical modifications in a structure–activity relationship (SAR) study. Function-oriented synthesis (FOS)[2] is a concept not only to be considered in terms of step economy to synthesize target molecules but also as a strategy to pursue for the design of less-complex structured targets with comparable activity. This concept is important for drug development based on natural products. Muraymycins (MRYs)[3] and caprazamycins (CPZs)[4] (Figure 1) are a class of liponucleoside antibiotics that exhibit potent antibacterial activity in vitro without significant toxicity in mice, and they inhibit peptidoglycan biosynthesis through a novel mode of action.[5] Accordingly, this class of natural products is a promising lead as a novel antibacterial agent against drug-resistant bacterial pathogens.[6] However, the 5′-*O*-aminoribosyl-5′-*C*-glycyluridine moiety as well as the diazepanone moiety, which is a seven-membered ring found in caprazamycins, are not only prone to β-elimination and epimerization, but they also require rather lengthy steps for their preparation because of their molecular complexity.[7] Herein we describe the FOS of this class of nucleoside antibiotics through rational simplification to address the issue associated with their molecular complexity. Our efforts on the SAR of MRYs and CPZs elucidated that the key functional groups essential for antibacterial activity are the uridine moiety, the amino group of the aminoribose, and a lipophilic side chain.[8–10] During the course of this study, we also found that truncated MRY analogue **1**, which has an arginine residue as an accessory group, retained antibacterial activity comparable to that of the parent MRYs (Figure 2).[10] As for the CPZs, the diazepanone ring could play a role as a scaffold, on which each of the key functional groups hang in the right orientation.[8,9] A simpler scaffold would be utilized to install the key functional groups, and we designed lactam-fused isoxazolidine scaffold **3** instead of such a complicated ring system. The choice of the lactam-fused isoxazolidine scaffold enabled us to modulate the three dimensional orientation of the key functional groups simply by changing the stereochemistry or the ring size. Step economy is also an important issue for rapid and efficient access to the target molecules. A set of diverse lactam-fused isoxazolidines **3** was readily constructed by intramolecular 1,3-dipolar cycloaddition of alkenyl nitrone **4**.[11]

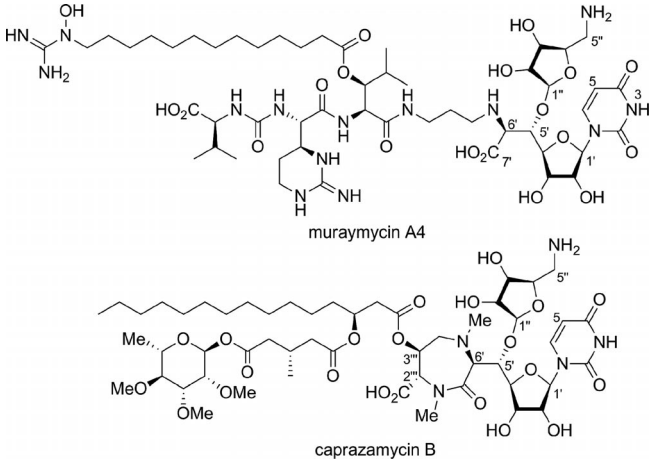

Figure 1. Structures of caprazamycins (CPZs) and muraymycins (MRYs).

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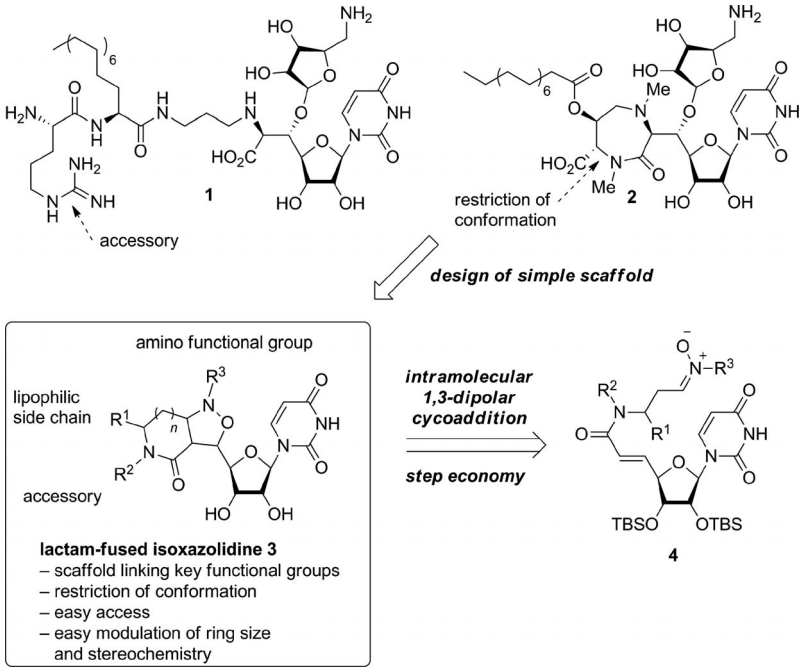
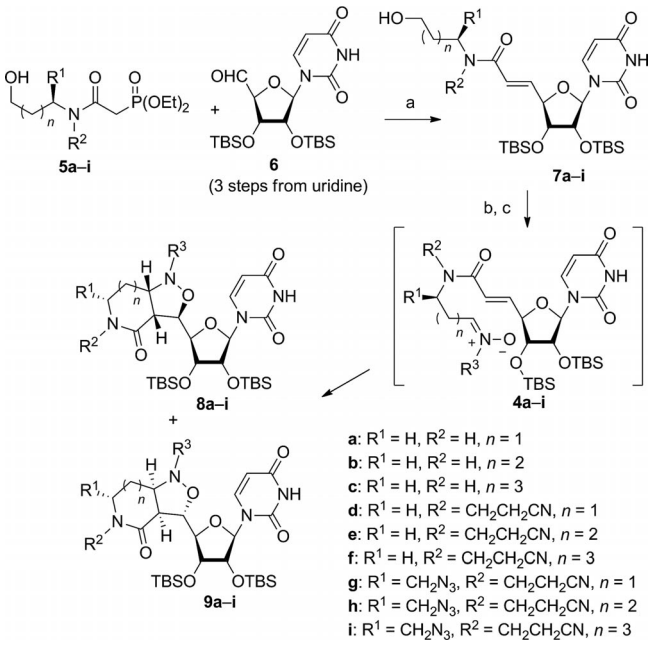


Figure 2. Function-oriented synthesis of muraymycins and caprazamycins; TBS = *tert*-butyldimethylsilyl.

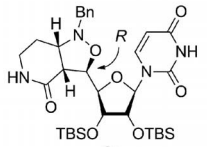
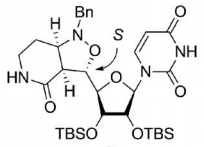
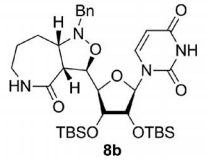
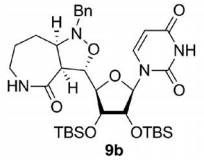
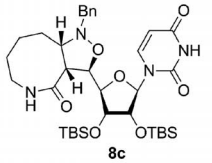
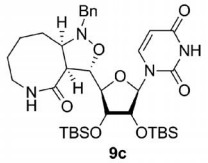
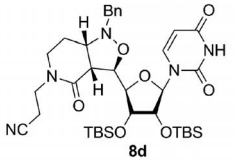
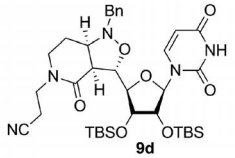
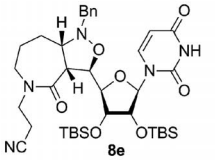
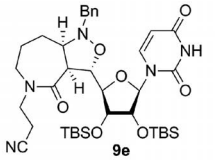
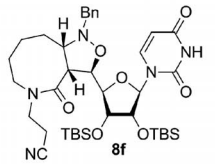
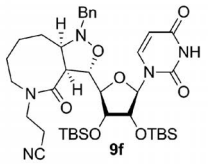
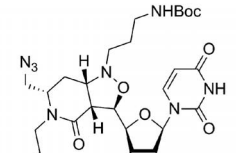
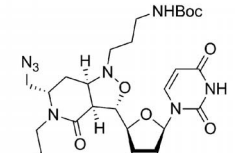
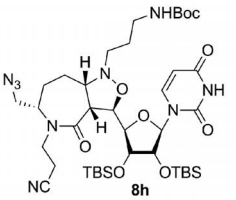
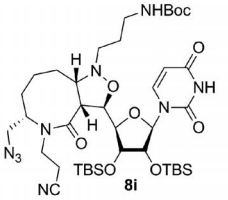
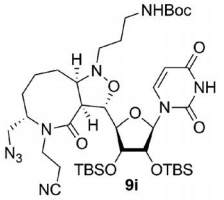
Results and Discussion

The preparation of the key lactam-fused isoxazolidine core structures is described in Scheme 1. Mild zinc-promoted Horner–Wadsworth–Emmons reaction[12] of phosphonates **5a**–**i**[13] with uridine 5′-aldehyde derivative **6**, which was obtained from uridine in three steps, gave *trans*-α,β-unsaturated amides **7a**–**i**. Then, the primary alcohol functionality of **7a**–**i** was oxidized by 2-iodoxybenzoic acid (IBX) in MeCN/DMSO, and the resulting aldehydes were subjected to a hydroxylamine in MeCN to form nitrones **4a**–**i**. Then, the intramolecular 1,3-dipolar cycloaddition was investigated, and the results are summarized in Table 1. Lactam-fused bicyclo[4.3.0]-type isoxazolidines are known structures.[11] Upon heating nitrone **4a** at reflux in MeCN, desired *cis*-fused bicyclo[4.3.0]-type isoxazolidines **8a** and **9a** were obtained in 77% yield (**8a**/**9a** = 1.4:1) over three steps from **7a** (Table 1, entry 1). In contrast, a decrease in the yield was observed upon increasing the size of the lactam ring, which has not yet been previously investigated. Thus, bicyclo[5.3.0]-type isoxazolidines **8b** and **9b** were obtained from **7b** in 34% yield (Table 1, entry 2), and no reaction occurred to give bicyclo[6.3.0]-type isoxazolidines **8c** and **9c** from **7c** (Table 1, entry 3). Generally, a carboxamide exists predominantly as the *trans* conformer, and these conformational features suggest that nitrones **4a**–**c** adopt extended conformations, in which the target olefin and the nitrone are far apart. *N*-Protection of the carboxamide to induce a conformational change is an established strategy to improve the yields of certain intramolecular reactions, especially those that provide highly strained macrocycles.[14] A cyanoethyl group was introduced at the nitrogen atom of the carboxamide moiety (i.e., **4d**–**9i**) as a removable cyclization auxiliary[15] as well as a precursor to the accessory functional group, as described later. The impact of the newly introduced cyanoethyl group on the cycloaddition proved to be immense, and the cycloaddition of **4d** proceeded even at room temperature to afford bicyclo[4.3.0]-



Scheme 1. Reagents and conditions: (a) $Zn(OTf)_2$ (1.2 equiv.), TMEDA (1.2 equiv.), Et_3N (4 equiv.), THF, r.t., 34–65%; (b) IBX (1.5 equiv.), MeCN/DMSO, r.t.; (c) BnNHOH or $BocNHCH_2CH_2CH_2NHOH$ (1.2 equiv.), 4 Å molecular sieves, MeCN, r.t. or reflux, see Table 1. TMEDA = *N*,*N*,*N*′,*N*′-tetramethylethylenediamine.

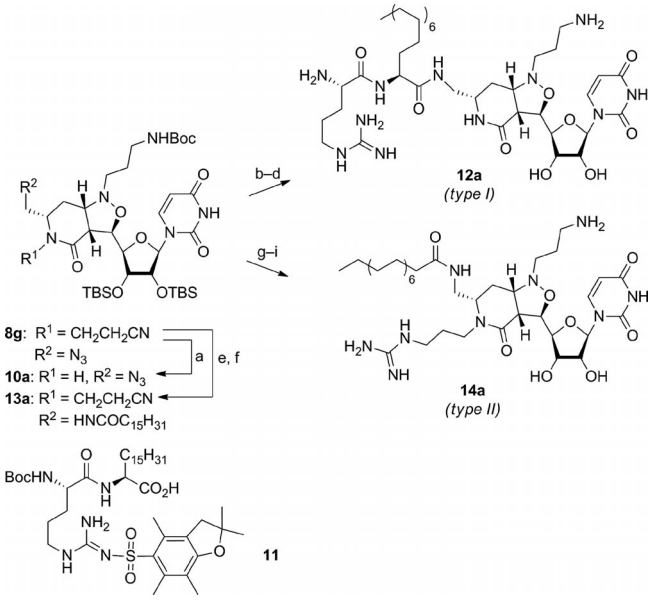
Table 1. Intramolecular 1,3-dipolar cycloaddition of alkenyl nitrones.

Entry	Temp.	Products	Yield [%] (*R*/*S* ratio)
1	reflux	 **8a**  **9a**	77 (1.4:1)
2	reflux	 **8b**  **9b**	34 (1.5:1)
3	reflux	 **8c**  **9c**	no reaction
4	r.t.	 **8d**  **9d**	82 (1.6:1)
5	reflux	 **8e**  **9e**	78 (1.6:1)
6	reflux	 **8f**  **9f**	82 (1.6:1)
7	r.t.	 **8g**  **9g**	68 (1.2:1)
8	reflux	 **8h**	78 (single diastereomer)
9	reflux	 **8i**  **9i**	49 a mixture of 4 diastereomers

type isoxazolidines **8d** and **9d** in 82% yield (Table 1, entry 4). Bicyclo[5.3.0]-type **8e**/**9e** and bicyclo[6.3.0]-type **8f**/**9f** were obtained in 78 and 82% yield, respectively, although heating was necessary for the cyclization to proceed (Table 1, entries 5 and 6). The structures of **8a** and **9a** were determined with the materials obtained from **8d** and **9d** after deprotection of the cyanoethyl group. The relative stereochemistry of *cis*-fused bicyclo[4.3.0]-type isoxazolidines **8d** and **9d** were in good accordance with those of related compounds previously reported.[11] The absolute stereochemistry was predicted by NOE experiments in conjunction with molecular modeling, because of the difficulty in obtaining the crystal structure of the cycloadducts (for details, see the Supporting Information). The structures of the other cycloadducts were assigned in a similar manner. The reaction sequence of **5g**–**i** with $BocHN(CH_2)_3NHOH$ (Boc = *tert*-butoxycarbonyl) was also successful to provide highly functionalized lactam-fused isoxazolidines **8g**–**i** and **9g**–**i** (Table 1, entries 7–9). However, the reaction of **4i** gave an inseparable mixture of four diastereomers, which was not used for further transformation.

Lactam-fused isoxazolidines **8g**,**h** and **9g** were converted into two types of analogues. One has a linear substituent linking a lipophilic side chain and the accessory arginine group (type I), and the other is a branched version with each substituent on the lactam ring (type II). These were synthesized within five steps from **8g**,**h** and **9g**. For example, the cyanoethyl group of **8g** was removed by *t*BuOK in THF in 79% yield, and the azide of **10a** was reduced to the corresponding amine, which was acylated with **11** (Scheme 2).[13] Finally, global deprotection afforded **12a** in 70% yield over three steps. The cyanoethyl group can be used as an accessory group. Namely, reduction of the azide group of **8g** was followed by palmitoylation to give **13a** in 55% yield over two steps. The cyano group of **13a** was reduced by hydrogenation catalyzed by PtO_2 in the presence of $Me_3N{\cdot}HCl$, and the liberated amine was converted into the protected guanidine with BocHNC(=NBoc)NHTf (Tf = trifluoromethylsulfonyl). Global deprotection provided **14a** in 69% yield over three steps. The other analogues were prepared in a similar manner (Scheme S4).

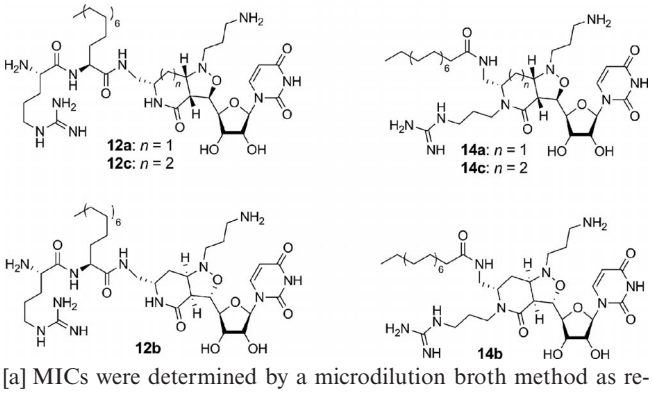
The antibacterial activity of the series of compounds was evaluated,[16] and the results are summarized in Table 2. Overall, these analogues exhibited moderate activity against a range of Gram-positive drug-resistant bacterial pathogens including *S. aureus* SR3637 (MRSA) and *E. faecium* SR7917 (VRE) with MIC values of 8–64 $\mu g\,mL^{-1}$. There is a trend that type II compounds show higher activity than type I compounds. Compound **14a** was the most potent analogue, the activity of which was comparable to that of **1** within only a 2–4-fold decrease. Compound **14a** did not exhibit significant cytotoxicity against human liver hepatocellular (HepG2) cells ($IC_{50} > 50\ \mu g\,mL^{-1}$).



Scheme 2. Reagents and conditions: (a) *t*BuOK (5 equiv.), THF, r.t., 79%; (b) H_2, Pd/C, MeOH, r.t.; (c) **11** (1 equiv.), EDCI (1.5 equiv.), HOBt (1.5 equiv.), CH_2Cl_2, r.t., (d) 80% aq. TFA, r.t., 70% over 3 steps; (e) H_2, Pd/C, MeOH, r.t.; (f) palmitoyl chloride (1.2 equiv.), Et_3N (1.5 equiv.), CH_2Cl_2, r.t., 55% over 2 steps; (g) H_2, PtO_2, $Me_3N{\cdot}HCl$ (1 equiv.), MeOH, r.t.; (h) BocHNC(=NBoc)NHTf (2 equiv.), $NaHCO_3$ (excess), THF/H_2O, r.t.; (i) 80% aq. TFA, r.t., 69% over 3 steps. EDCI = 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride, HOBt = 1-hydroxybenzotriazole. TFA = trifluoroacetic acid.

Table 2. Antibacterial activity of lactam-fused isoxazolidine analogues.

Strain	MIC (mg mL^{-1})[a]							
	1	**12a**	**12b**	**12c**	**14a**	**14b**	**14c**	VCM[b]
S. aureus ATCC 29213 (MSSA)	4	>64	>64	16	8	16	>64	1
S. aureus SR3637 (MRSA)	4	32	32	32	8	16	16	2
E. faecalis ATCC 29212	4	16	32	16	16	16	16	2
E. faecalis SR7914 (VRE)	4	32	32	16	16	16	16	>64
E. faecium SR7917 (VRE)	4	16	16	8	8	8	16	>64



[a] MICs were determined by a microdilution broth method as recommended by the National Committee for Clinical Laboratory Standards (NCCLS) with cation-adjusted Mueller–Hinton broth (CA-MHB). Serial twofold dilutions of each compound were made in appropriate broth, and the plates were inoculated with 5×10^4 CFU (CFU = colony-forming unit) of each strain in a volume of 0.1 mL. Plates were incubated at 35 °C for 20 h and then MICs were scored. [b] VCM = vancomycin.

Conclusions

In conclusion, the FOS of liponucleoside antibiotics was investigated to lead to the discovery of lactam-fused isoxazolidine derivatives as lead compounds active against drug-resistant bacterial pathogens. It is important to simplify the core structures of natural products to reduce the size of the molecules and to stabilize the chemically labile structure. Lactam-fused isoxazolidine derivatives **12** and **14** possess a simple chemical structure with a molecular weight half of that of the parent natural products, which can be easily prepared in 10 steps from uridine. This study provides a novel structure for the development of a new type of antibacterial agent effective against drug-resistant bacteria.[17,18]

Supporting Information (see footnote on the first page of this article): Experimental procedures for the synthesis and characterization of the studied compounds and antibacterial activity evaluation.

Acknowledgments

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