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Total Synthesis of Tunicamycin V

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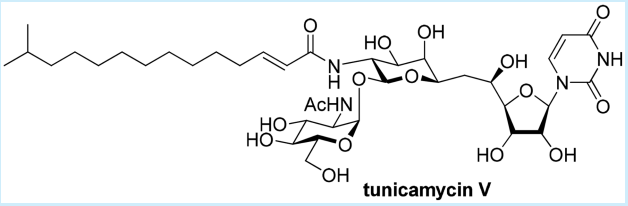
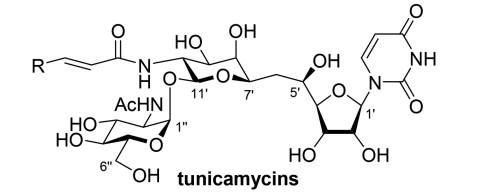
Supporting Information

ABSTRACT: The total synthesis of tunicamycin V is described. This strategy is based on the initial construction of tunicaminyluracil, which is regarded to play an important role in the observed biological activities. The key to the synthesis was a Mukaiyama aldol reaction followed by a furan-oxidation to construct the undecose skeleton, a [3,3] sigmatropic rearrangement of a cyanate, and a highly selective trehalose-type glycosylation.



Tunicamycins[1−3] (Figure 1) are nucleoside natural products isolated from the fermentation broths of *Streptomyces*

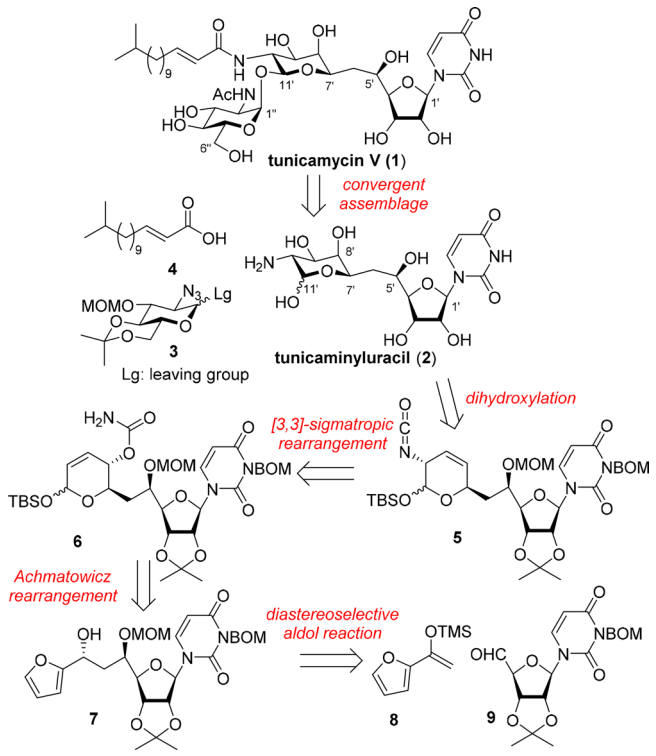


I: R = $(CH_3)_2CH(CH_2)_7$
II: R = $(CH_3)_2CH(CH_2)_8$
III: R = $CH_3(CH_2)_{10}$
IV: R = $C_{12}H_{25}$
V (**1**): R = $(CH_3)_2CH(CH_2)_9$
VI: R = $(CH_3)_2CH(CH_2)_{11}$
VII: R = $(CH_3)_2CH(CH_2)_{10}$
VIII: R = $CH_3(CH_2)_{12}$

Figure 1. Structure of tunicamycins.

lysosuperficus in 1971 and exhibit a variety of biological properties including antibacterial, antiviral, antifungal, and antitumor activities. Tunicamycins strongly inhibit UDP-*N*-acetylglucosamine (GlcNAc): polyprenol phosphate translocase, the enzyme responsible for the first *N*-acetylglucosamination of the *N*-linked glycopeptide in endothelial reticulum (ER).[4,5] Treatment of eukaryotic cells with tunicamycins results in a complete truncation of the oligosaccharides from *N*-linked glycopeptides causing ER stress and ultimately cell death. Tunicamycins also inhibit prokaryotic phospho-*N*-acetylmuraminic acid (MurNAc)-pentapeptide translocase (MraY) responsible for the biosynthesis of peptidoglycan and undecaprenyl-phosphate α-*N*-acetylglucosaminyl 1-phosphate transferase (WecA) for lipopolysaccharide and enterobacterial common antigen synthesis. MraY catalyzes the reaction between UDP-MurNAc-pentapeptide (Park's nucleotide) and undecaprenyl monophosphate, providing lipid I. MraY is an essential enzyme in bacteria and a good target for antibacterial drug discovery.[6] Their chemical structure is divided into three moieties that include GlcNAc, an amide-linked fatty acyl side chain, and tunicaminyluracil (**2**, Scheme 1), where a uracil base is attached to an

Scheme 1. Retrosynthetic Analysis of Tunicamycin V (1)



aminoundecose constructing a linked ribofuranosylgalactopyranosamine. The tunicaminyluracil and GlcNAc are connected by an 11′-β-1″-α-trehalose-type linkage. It is suggested that the structure of tunicamycins closely resembles the transition state of the transfer reaction of UDP-sugar onto a phospholipid catalyzed by the translocases. Therefore, the tunicaminyluracil moiety is

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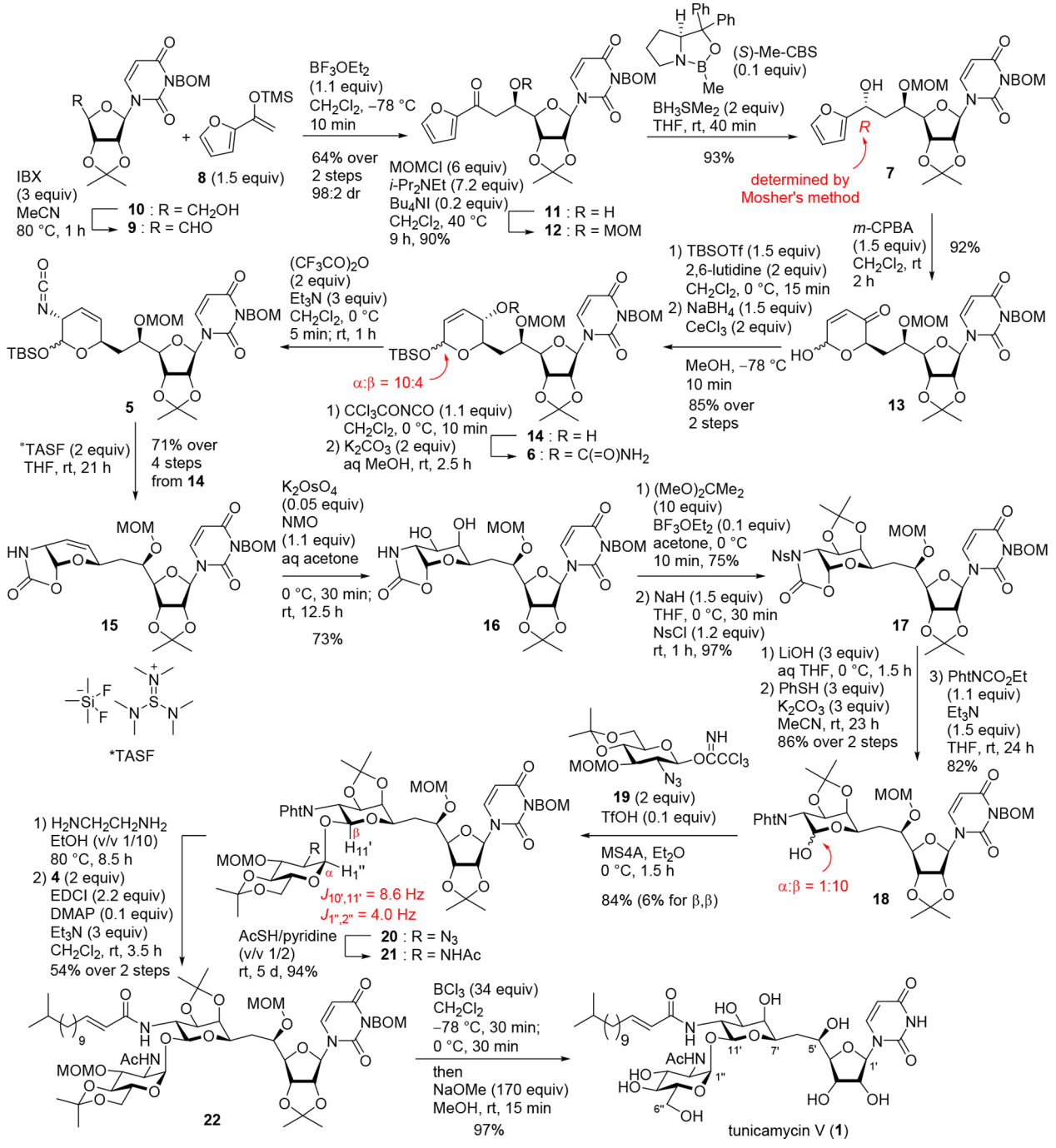
Scheme 2. Total Synthesis of Tunicamycin V (1)



regarded as a key scaffold of tunicamycins in biological properties, and the fatty acyl side chain as well as the GlcNAc moiety act as accessory motifs. Their structural complexity renders them worthy targets for organic synthesis. The total synthesis of tunicamycins has been accomplished by the groups of Suami,[7] Myers,[8] and Li,[9] and other synthetic studies of related compounds have also been reported.[10−15] In spite of much effort to synthesize tunicamycins, there are only a few studies regarding their analogue synthesis, and their structure−activity relationships have yet to be properly explored.[16] In all the previous total syntheses of **1**, the galactosamine moiety was derived from D-galactose.[7−9] We planned *de novo* construction of the galactosamine moiety, which could provide novel access to both functionality and stereochemistry unavailable in past syntheses. Here, we describe an efficient total synthesis of tunicamycin V (**1**). Our retrosynthetic analysis of **1** is shown in Scheme 1. Considering the tunicaminyluracil moiety acting as a mimic of UDP, it was desirable to first construct **2** and subsequently install the GlcNAc moiety **3** and the fatty acyl side chain **4**[8] in the final stages of the synthesis from the viewpoint of medicinal chemistry. We planned to construct the galactosamine moiety from furyl alcohol **7** via oxidative reconstruction of the dihydropyran (Achmatowicz rearrangement[17]) followed by [3,3] sigmatropic rearrangement of the allylcyanate generated from allylcarbamate **6**. The furan **7** was disconnected to the silyl enol ether **8**[18] and uridine-5′-aldehyde derivative **9**.

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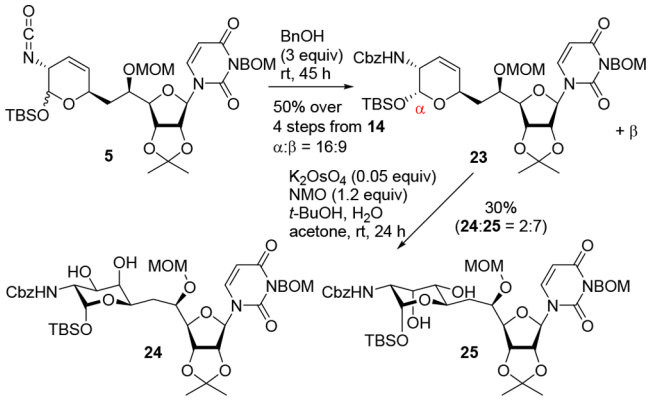
The synthesis of **1** is shown in Scheme 2. IBX oxidation of the 5′-hydroxy group of the suitably protected uridine derivative **10**[19] provided the corresponding aldehyde **9**. Mukaiyama-type aldol reaction of **9** was conducted with the silyl enol ether **8** and $BF_3{\cdot}OEt_2$ in CH_2Cl_2 at −78 °C to afford the desired 5′*R*-product **11** in 64% yield over two steps with high stereoselectivity (5′*R*/5′*S* = 98:2). Presumably, the Mukaiyama-type aldol reaction of **8** and **9** promoted by $BF_3{\cdot}OEt_2$ proceeded via an open transition state of the Felkin−Ahn model.[20] In the case of the reaction using $SnCl_4$ as a Lewis acid, the undesired 5′*S*-product was obtained as a major product (75% yield, 5′*R*/5′*S* = 4:96, see Supporting Information (SI)). It should be noted that a Mukaiyama-type aldol reaction was utilized to form the C5′−C6′ bond in the Li's synthesis.[9] Interestingly, the reaction with $SnCl_4$ provided the desired 5′*R*-product with their substrates, in contrast with our findings. Although *anti*-reduction[21] of the 7′-oxo group of **11** was first conducted by a treatment with $NaBH(OAc)_3$ in CH_2Cl_2, the desired *anti*-diol was not obtained because of the decomposition of the substrate. Extensive efforts were conducted to find that after protection of the 5′-hydroxy group by the MOM group, the 7′-oxo group of **12** could be stereoselectively reduced by (*S*)-Me-CBS[22] and $BH_3{\cdot}SMe_2$ in THF to give the *anti*-product **7** in 93% as a sole product. At this stage, the absolute stereochemistry at the 7′-position was determined by the Mosher's method for **7** (for details, see SI). Oxidation of the furan ring in **7** by *m*-CPBA cleanly proceeded to provide the lactol **13** in 92% yield, and protection of the resulting 11′-hydroxyl group by the TBS group followed by Luche reduction[23] of the enone gave allyl alcohol **14** in 85% yield over two steps as a mixture of anomers at the 11′-position. The resulting allyl alcohol **14** was converted to the carbamate **6** by way of *N*-trichloroacetylcarbamate (CCl_3CONCO, CH_2Cl_2, 0 °C) upon deacylation (K_2CO_3, aq. MeOH).[24] Dehydration of **6** resulted in clean conversion to the corresponding allylcyanate ($(CF_3CO)_2O$, Et_3N, CH_2Cl_2, 0 °C), which underwent [3,3] sigmatropic rearrangement to give the 10′-isocyanate **5**. To achieve a high facial selectivity in the following dihydroxylation, a construction of the *cis*-cyclic carbamate-fused pyranose was planned to hinder the α-face of the pyrane ring. Entrapment of the isocyanate in **5** by the adjacent hydroxyl group upon removal of the TBS group by tris(dimethylamino)sulfonium difluorotrimethylsilicate (TASF) afforded **15** in 71% yield over four steps from **14**. The following dihydroxylation proceeded stereoselectively at the convex face to give the diol **16** (K_2OsO_4, NMO, aq. acetone, 71%). The stereochemistry of **16** was determined by its conversion to the known heptaacetate of tunicaminyluracil[8] (see SI).

Dihydroxylation of the *N*-benzylcarbamate **23**, which was obtained by treating **5** with BnOH, was also conducted. However, its facial selectivity was poor, and the desired diastereomer **24** was obtained as a minor product (Scheme 3). As expected, the observed high facial selectivity in the dihydroxylation of **15** was induced by the *cis*-cyclic carbamate moiety, which hinders the α-face of the pyran ring. The diol and the carbamate in **16** were sequentially protected with the isopropylidene (2,2-dimethoxypropane, $BF_3{\cdot}OEt_2$, acetone, 75%) and Ns[25] groups (NsCl, NaH, THF, 97%), respectively, to give **17**. Hydrolysis of the cyclic carbamate of **17** (LiOH, aq. THF) followed by protecting group manipulation (PhSH, K_2CO_3, MeCN, 86% over two steps, *N*-EtO_2C-phthalimide, Et_3N, THF, 82%) gave an orthogonally protected tunicaminyluracil **18**.

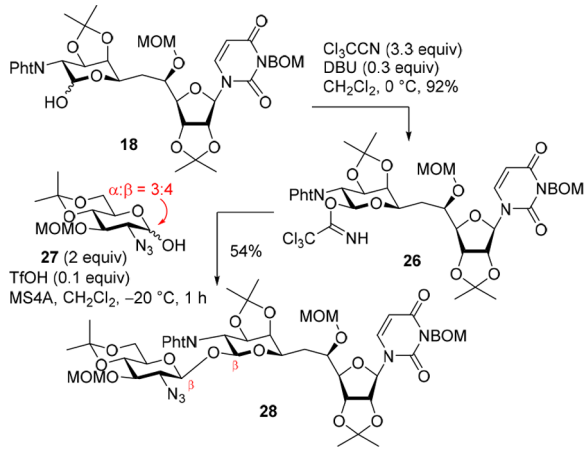
With **18** in hand, the union of three components to complete the total synthesis of **1** was investigated. Selective construction of

Scheme 3. Dihydroxylation of 23



the 11′-*β*-1″-*α*-trehalose-type linkage out of four possible anomers is a challenging task. Tunicaminyluracil derivative **18** exists as an equilibrium mixture of anomers at the 11′-position, and the 11′-*β*-anomer, which is the same stereochemistry as **1**, is predominant in $CDCl_3$ (*α*/*β* = 1:10). Thus, **18** was used as a glycosyl acceptor and reacted with a suitably protected 1-*O*-trichloroacetimido-2-azidoglucose derivative **19** as a glycosyl donor to construct the 11′-*β*-glycoside bond via neighboring group participation and the 1″-*α*-glycoside bond via anomeric effect as previously investigated by Myers.[8] Optimization of the glycosylation conditions was investigated (see SI). The use of Et_2O as a solvent improved the selectivity and treatment of **18** and **19** with TfOH in Et_2O at 0 °C provided the desired 11′-*β*-1″-*α*-glycoside **20** in 84% yield in a highly stereoselective manner (11′-*β*-1″-*α*/11′-*β*-1″-*β* = 14:1). The observed stereoselectivity in this study was the highest among previously reported total synthesis of **1**.[7−9] Alternatively, reversal of a glycosyl donor/acceptor was also investigated to construct the 11′-*β*-1″-*α*-trehalose-type linkage (Scheme 4). The hemiacetal **18** was

Scheme 4. Alternative Glycosylation



transformed to a trichloroacetimidate **26**, which is a glycosyl donor (Cl_3CCN, DBU, MeCN). The trichloroacetimidate **26** was reacted with a suitably protected 2-azidoglucose **27** (*α*/*β* = 3:4 in $CDCl_3$) in the presence of TfOH in CH_2Cl_2. However, the undesired 11′-*β*-1″-*β* glycoside **28** was obtained in 54% yield, and the desired **20** was not observed at all. The azide group of **20** was transformed to an acetamide group (AcSH, pyridine, 94%) to give **21** with clean conversion. The phthaloyl group at the GalNAc moiety of **21** was removed by ethylenediamine in EtOH,

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and the liberated amine was acylated with **4**[8] using EDCI and DMAP in CH_2Cl_2 to give **22** in 54% yield over two steps. Finally, global deprotection of six acid labile protecting groups by BCl_3 in CH_2Cl_2 followed by quenching with NaOMe resulted in clean conversion and successfully afforded **1** in 97% yield. The analytical data for synthetic **1** were in good agreement with the previously reported data.[8,9]

In conclusion, a total synthesis of tunicamycin V has been accomplished. The key to the synthesis was a diastereoselective Mukaiyama aldol reaction followed by furan-oxidation to construct an undecose skeleton, cyclic carbamate formation by [3,3] sigmatropic rearrangement of a cyanate followed by intramolecular entrapment of the resulting isocyanate, and stereoselective construction of the 11′-β-1″-α-trehalose-type linkage. Tunicamycin V is readily accessible via the longest linear sequence of 24 synthetic steps from uridine and commercially available simple materials, in an overall chemical yield of 3.9%. The complex structure with tunicamycins binding to MraY from *Clostridium bolteae* has recently been elucidated,[26] and this could be utilized for the development of analogues. Our strategy is based on initial construction of tunicaminyluracil, which is regarded as a key scaffold, playing an important role in mediating a variety of biological activities.[9] Alterations to the uridine moiety will be difficult by this strategy, but replacing the GlcNAc and the lipid moieties in the last stage of the synthesis could provide a range of new analogues of the tunicamycins. The synthesis and biological evaluation of such analogues are currently underway.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.7b03623.

Detailed experimental procedures and characterization of new compounds (PDF)

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Notes

The authors declare no competing financial interest.

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