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**SPECIAL FEATURE: REVIEW ARTICLE**

# Tunicamycin: chemical synthesis and biosynthesis

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## Abstract
Tunicamycins are nucleoside natural products and show antibacterial, antiviral and antitumor activities, which are attributed to their inhibition of enzymatic reactions between polyisoprenyl phosphate and UDP-GlcNAc or UDP-MurNAc-pentapeptide. Because of their various intriguing biological activities, tunicamycins have potential as therapeutic agents for infectious diseases or cancers. Structurally, tunicamycins have a unique structure composed of an undecodialdose skeleton, a lipid chain and a GlcNAc fragment linked by a 1,1-β,α-trehalose-type glycosidic bond. In this mini review, we summarize the total chemical syntheses and biosynthetic studies of tunicamycins.

## Introduction

Tunicamycins (Fig. 1) [1–7] are nucleoside natural products that were first isolated from the fermentation broths of *Streptomyces lysosuperficus* and later *Streptomyces chartreusis*. Tunicamycins inhibit enzymatic reactions catalyzed by bacterial phospho-*N*-acetylmuramyl-pentapeptide transferase (MraY), which is responsible for the biosynthesis of peptidoglycan, and undecaprenyl-phosphate α-*N*-acetylglucosaminyl 1-phosphate transferase (WecA), which is responsible for the synthesis of lipopolysaccharide and enterobacterial common antigen [8–16]. These mechanisms are modes of antibacterial activity of tunicamycins. MraY is an essential enzyme for bacterial survival and a good target for antibacterial drug discovery [17–19]. Tunicamycins also strongly inhibit human UDP-*N*-acetylglucosamine-dolichyl phosphate *N*-acetylglucosamine-phosphotransferase (GPT), which is responsible for the first *N*-acetylglucosamination of *N*-linked glycopeptides in the endothelial reticulum [20].

Tunicamycins are broadly used as biological tools in studies of the *N*-glycosylation of proteins.

Tunicamycins have a unique structure comprising an undecodialdose core, referred to as tunicamine (**2**), decorated with uracil, D-*N*-acetylglucosamine (GlcNAc) which attached to the core by a 1,1-β,α-trehalose-type glycosidic bond, and an amide-linked fatty acid. Streptovirudins [21], corynetoxins [22], MM19290 [23], mycospocidin [24] and antibiotic 24010 [25] share the same pseudotrisaccharide core, and quinovosamycins [26] are a deoxy congener. These unique structural features and various biological activities have attracted the attention of many researchers to attempt their challenging chemical synthesis or revealed their biosynthetic pathways. Here, we review previous total chemical syntheses and biosynthetic analyses of tunicamycins. The total synthesis of tunicamycin V (**1**) has been accomplished by Suami's [27–29], Myers' [30], Yu's [31] and our groups [32], and many other synthetic studies have been conducted [33–38]. Of all the synthetic studies, this review focuses only on the total syntheses.

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This manuscript is dedicated to Dr Kiyoshi Isono's 88 years' anniversary and his long-standing contribution to the study of antibiotics, especially nucleoside antibiotics, polyoxins.

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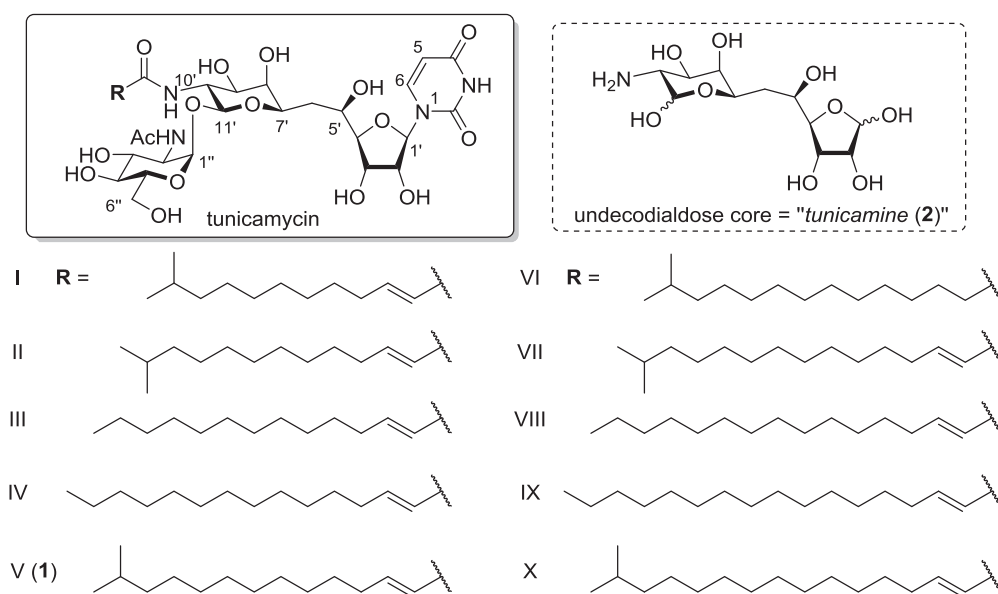
## Total synthesis

The construction of the tunicamine (**2**) core by a C5′–C6′ bond formation and a 1,1-β,α-trehalose-type glycosylation, which could result in four stereoisomers, are challenging tasks in total synthesis of tunicamycin. Suami and co-workers completed the first total synthesis of **1** in 1985 (Scheme 1) [27–29]. They achieved the C5′–C6′ bond formation to afford product **7** *via* the Henry reaction between 5-deoxy-5-nitro-D-ribose derivative **4** and an aldehyde **6**, which was prepared

**Fig. 1** Structures of tunicamycins. The structure shown in a box with broken line is called to tunicamine (**2**), which is composed of a D-ribose and D-galactosamine. These antibiotic congeners bear a various length of fatty acyl side chains

from *N*-acetylgalactosamine derivative **5**. Coupling product **7** was converted to a suitably protected tunicamine derivative **9** *via* **8**, and subsequent protecting group manipulation provided hexaacetate **10**. Then, a uracil moiety was introduced to **10** with bis(trimethylsilyloxy)pyrimidine in the presence SnCl$_4$, and the substitution of the 11′-OMe group with Cl *via* a two-step reaction provided glycosyl chloride **11**.
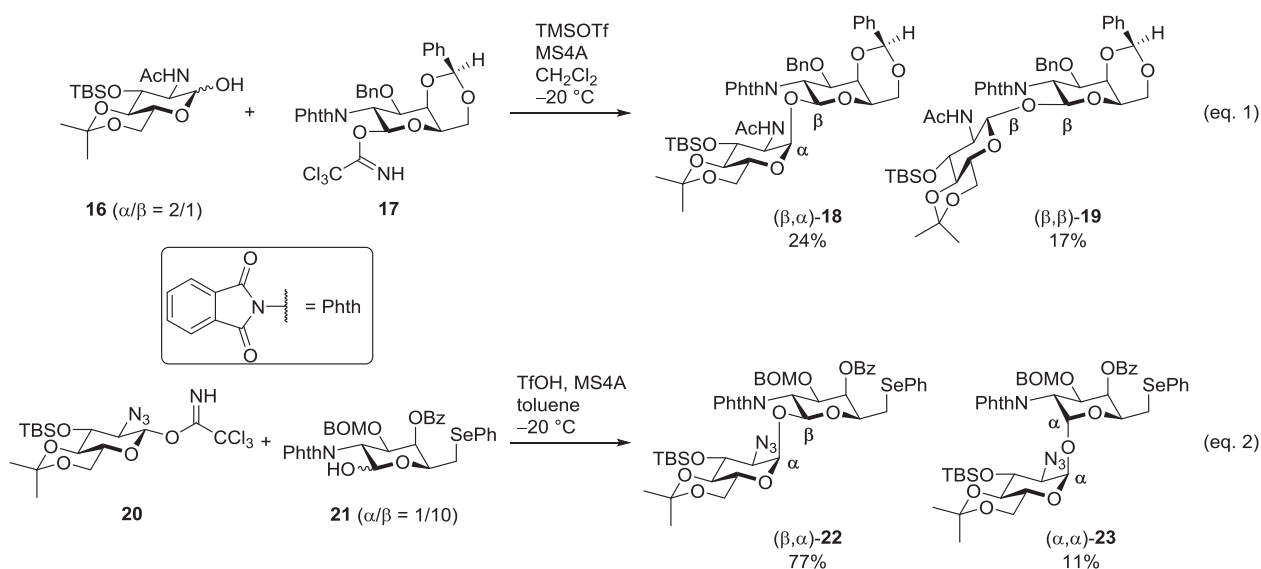
The challenging trehalose-type glycosylation using glycosyl chloride **11** and acceptor **12** was conducted in the presence of two silver salts and provide desired (11′-β,1″-α)-product **13**, but undesired (11′-β,1″-β)-product **14** was also obtained in addition to **13** and no selectivity was observed (~1:1) despite the α-configuration of the anomeric hydroxyl group of the GlcNAc moiety. Glycoside **13** was then converted into tunicamycin V (**1**) *via* four additional transformations, including the installation of the fatty acyl side chain.

The second total synthesis was achieved by Myers and co-workers [30]. In their synthetic strategy, the trehalose-type linkage was constructed first, and a C5′–C6′ bond was formed in the last stage of the synthesis. In this type of glycosylation, the choice of glycosyl donor and acceptor played a very important role in the selectivity. The glycosylation of glycosyl acceptor **16** and donor **17** in the presence of TMSOTf as a promoter in CH$_2$Cl$_2$ provided desired glycoside (β,α)-**18** and undesired glycoside (β,β)-**19** (Scheme 2, eq. 1). The β-selectivity at the anomeric position on the galactosamine side was controlled by neighboring group participation of the phthaloyl (Phth) protecting group on donor **17**. However, no selectivity at the anomeric position of the GlcNAc moiety was observed due to the α-dominance of acceptor **16** (α/β = 2/1). On the other hand, the glycosylation of donor **20** and Phth-protected acceptor **21** preferentially provided desired
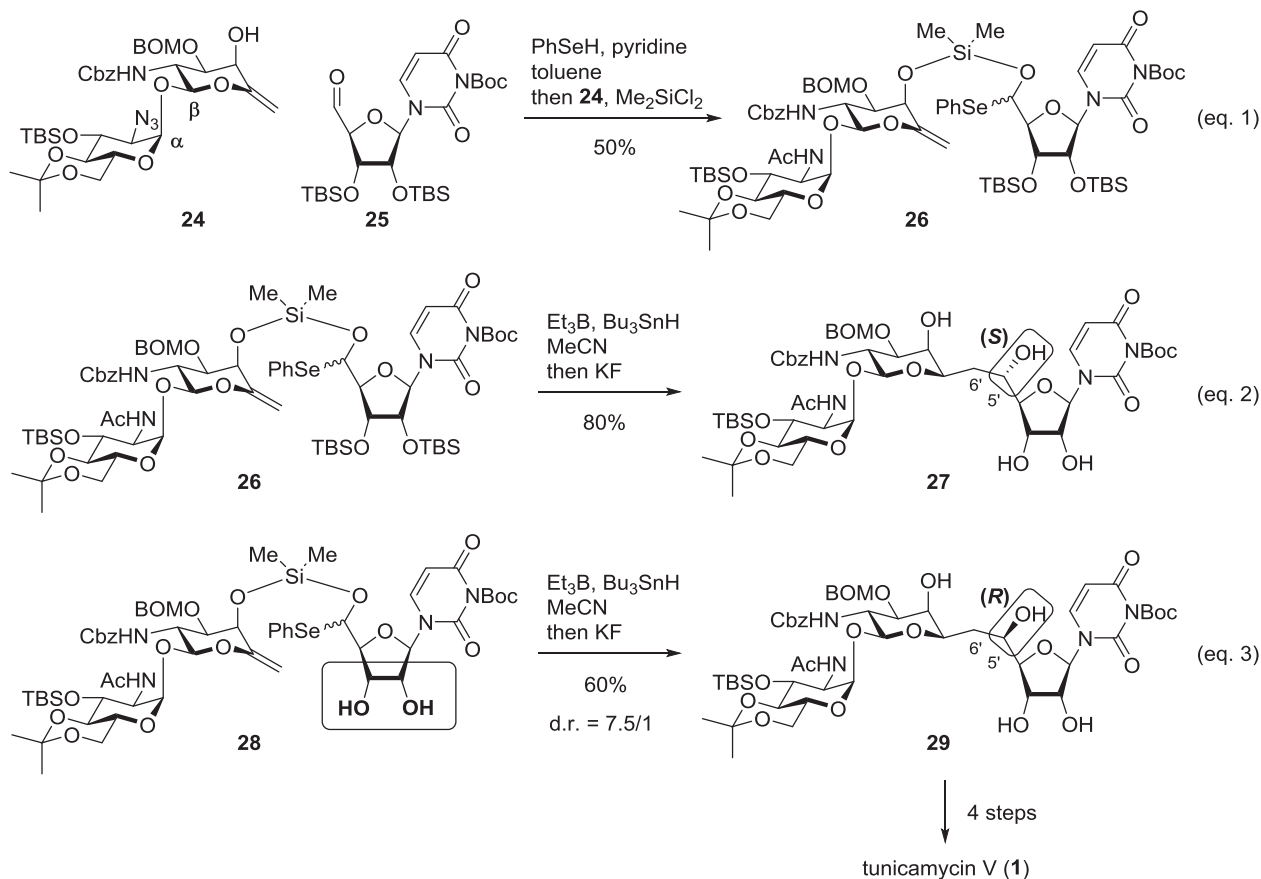
glycoside (β,α)-**22** (**22**/**23** ratio = 7/1; eq. 2). In this case, an anomeric hydroxyl group of acceptor **21** was β-dominant (α/β = 1/10) due to the steric effect of the Phth group. The high α-selectivity at the anomeric position of 2-azidoglucose putatively resulted from the restricted transition state conformation of donor **20** in or an S$_N$2-like mechanism in the glycosylation using an imidate donor [39, 40].

To achieve the C5′–C6′ bond formation between the disaccharide unit and uridine, they studied intramolecular radical cyclization (Scheme 3). Glycoside **22** was converted to allyl alcohol **24** over several steps, and the formation of a silylene bridge with uridine 5′-aldehyde derivative **25** provided radical cyclization precursor **26** (eq. 1). The intramolecular radical cyclization of **26** proceeded cleanly in the presence of Et$_3$B/Bu$_3$SnH, but undesired (5′S)-product **27** was obtained (eq. 2). On the other hand, precursor **28** without a TBS groups on the uridine moiety preferentially provided desired (5′R)-product **29**, which is consistent with the stereochemistry of tunicamycins (eq. 3). After introducing the fatty acyl chain and global deprotection, the total synthesis of tunicamycin V (**1**) was accomplished.
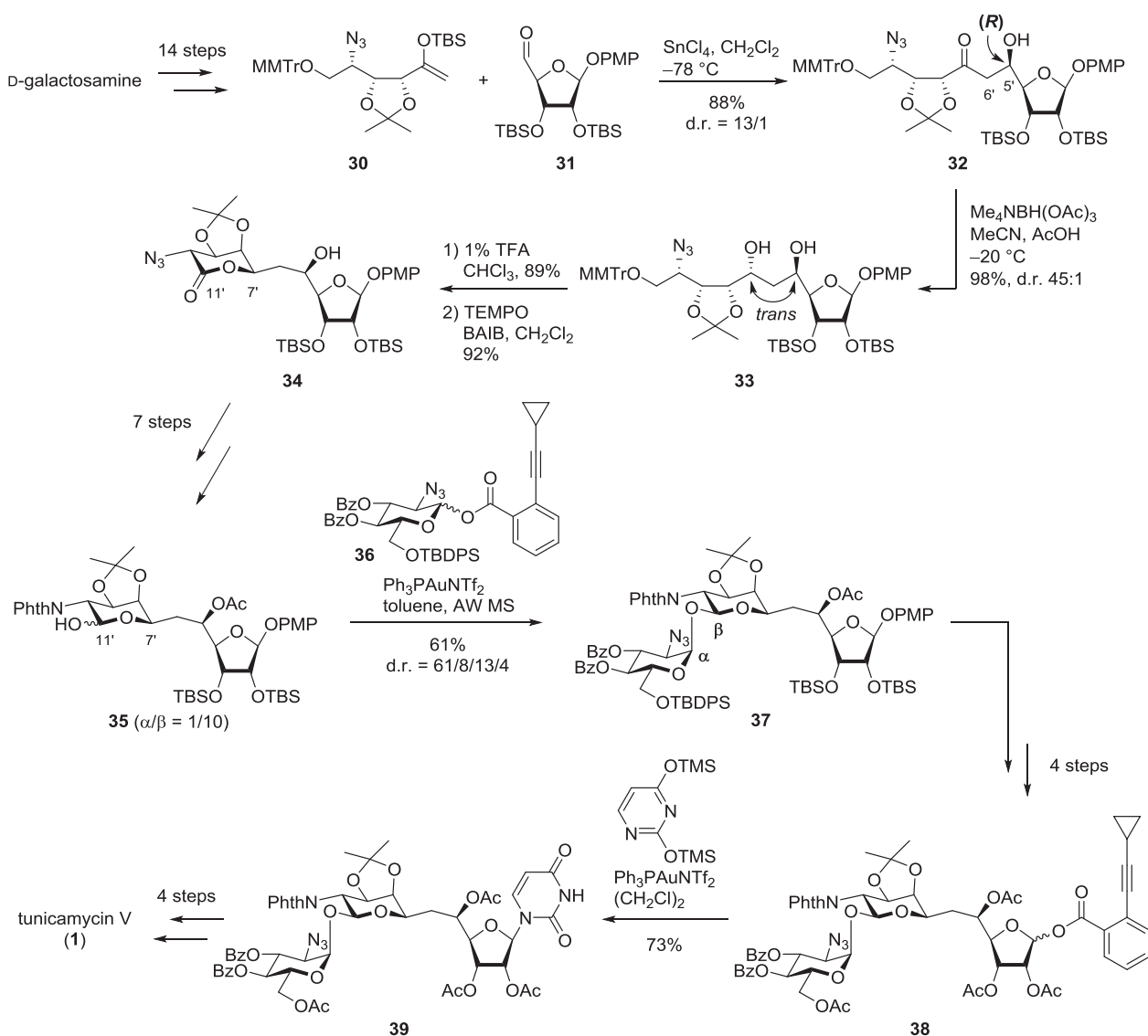
The total synthesis of tunicamycin V (**1**) by Yu and co-workers [31] was reported almost two decades after the Myers' report. Their strategy included an aldol reaction to realize the C5′–C6′ bond formation and two in-house-developed Au-catalyzed glycosylation to introduce the uracil and GlcNAc units (Scheme 4). Linear enol silyl ether **30**, used as the C6′–C11′ unit in tunicamycin, was prepared from D-galactosamine in 14 steps. Mukaiyama aldol reaction of **30** and **31** was conducted in the presence of SnCl$_4$, leading to (5′R)-product **32** with high diastereoselectivity (d.r. = 13/1). The resulting (5′R)-hydroxyketone **32** was converted to Phth-

**Scheme 1** Total synthesis of tunicamycin V by Suami and co-workers. Ac acetyl, Cbz benzyloxycarbonyl, DCC dicyclohexylcarbodiimide, MS4A molecular sieves 4A, TMS trimethylsilyl

protected glycosyl acceptor **35** over several steps, including a 1,3-*trans*-selective ketone reduction of **32** and formation of lactone **34**. Next, they studied a stereoselective trehalose-type glycosylation to introduce the GlcNAc moiety. Glycosylation between 2-azidoglucosyl alkynylbenzoate **36** and acceptor **35** proceeded in the presence of a catalytic amount of Ph₃PAuNTf₂ in toluene as a solvent at room temperature to give desired pseudotrisaccharide **37** with moderate selectivity in addition to the other three isomers. After the conversion of **37** to alkynylbenzoate **38** over four steps, the second Au-catalyzed glycosylation provided uracil-bearing product **39**. Finally, the conversion of the azide group to the corresponding acetamide and the introduction of the fatty acyl chain provided tunicamycin V (**1**).

Recently, we accomplished the total synthesis of tunicamycin V (**1**) with a de novo sugar synthesis strategy (Scheme 5) [32]. The Mukaiyama aldol reaction of enol silyl ether **40** and aldehyde **41** in the presence of BF₃·OEt₂ as a promotor provided desired (5′R)-C5′–C6′ coupling product **42** with very high diastereoselectivity (d.r.>98/2). On the other hand, the use of SnCl₄ as a promotor gave the undesired (5′S)-product (d.r. = 96/4). Surprisingly, this stereoselectivity was in contrast to that observed in Yu's synthesis (Scheme 4, **32**). This diastereoselectivity may reflect the difference in the conformation of the ribose ring or in the substituent at the 1′-position [41, 42]. A multistep transformation involving an oxidative furan ring rearrangement of **43** and a stereoselective reduction of enone **44** afforded allyl carbamate **45** and set the

**Scheme 2** Studies of 1,1-trehalose-type glycosylation in total synthesis by Myers and co-workers. Bn benzyl, Bz benzoyl, BOM benzyloxymethyl, Phth phthaloyl, TBS *tert*-butyldimethylsilyl, Tf trifluoromethanesulfonyl



**Scheme 3** Total synthesis of tunicamycin V *via* radical cyclization by Myers and co-workers. Boc *tert*-butyloxycarbonyl
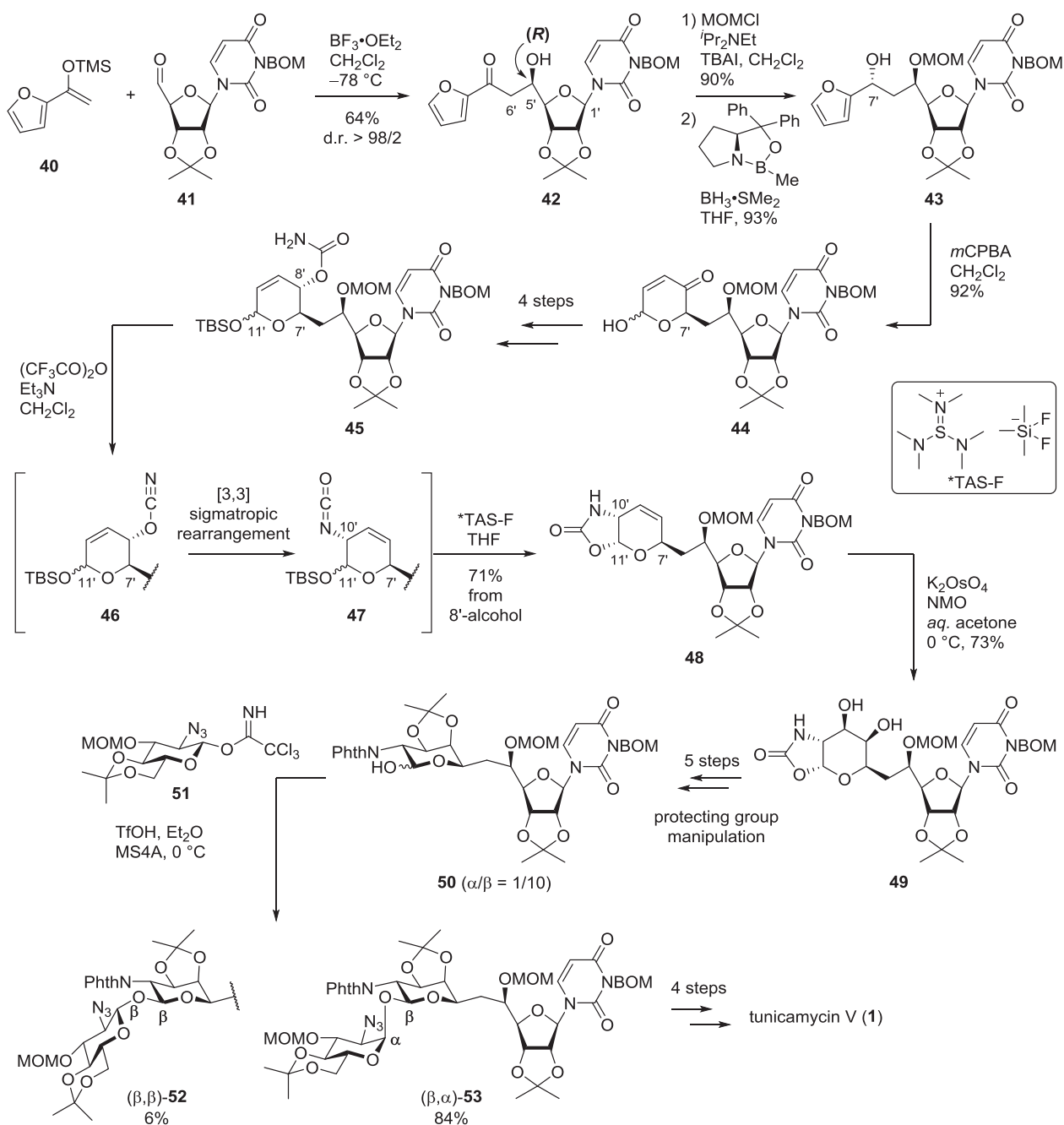
**Scheme 4** Total synthesis of tunicamycin V by Yu and co-workers. AW MS acid washed molecular sieves, BAIB bis-(acetoxy) iodobenzene, MMTr monomethoxytrytyl, PMP *p*-methoxyphenyl, TBDPS *tert*-butyldiphenylsilyl, TEMPO 2,2,6,6-tetramethyl-1-piperidinyloxyl, TFA trifluoroacetic acid

stage for the allylcyanate rearrangement to introduce the 10′-amino functional group. Dehydration of the carbamate group provided cyanate **46**, which smoothly underwent a [3,3] sigmatropic rearrangement to give **47**. The sequential treatment of **47** with TAS-F afforded cyclic carbamate **48**. The facial selectivity of the dihydroxylation of olefin in **48** was controlled by the *cis*-fused structure. After the transformation of diol **49** to Phth-protected glycosyl acceptor **50**, the trehalose-type glycosylation between **50** and 2-azidoglucosyl imidate **51** in the presence of a catalytic amount of TfOH in Et₂O was conducted according to Myers' reports [30]. As a result, desired β,α-glycoside **53** was provided in a higher selectivity than was observed in previous reports (β,α-**53**/β,β-**52** = 14/1). Finally, introducing the fatty acyl chain and removing the

protection group accomplished the total synthesis of tunicamycin V (**1**).
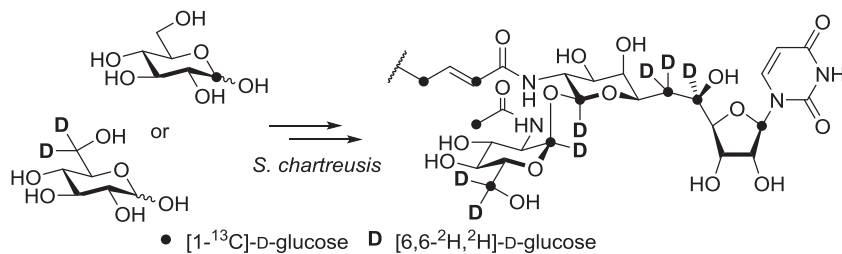
## Biosynthesis

The Tunicamycin family has a unique and curious structure that contains a C5′–C6′-linked disaccharide, which is unparalleled in nature in terms of biosynthesized compounds. Although numerous studies on the chemical synthesis of tunicamycins have been reported since shortly after its isolation in 1971, biosynthetic studies were not reported until the 2000's. The biosynthetic study of tunicamycins by Price and co-workers reported a new tunicamycin-producing

**Scheme 5** Total synthesis of tunicamycin V by our group. *m*CPBA *m*-chloroperbenzoic acid, MOM methoxymethyl, NMO *N*-methylmorpholine-*N*-oxide, TAS-F tris(dimethylamino)sulfonium difluorotrimethylsilicate, TBAI tetrabutylammonium iodide



**Fig. 2** Labeling site by tunicamycin production experiment using labelled D-glucose
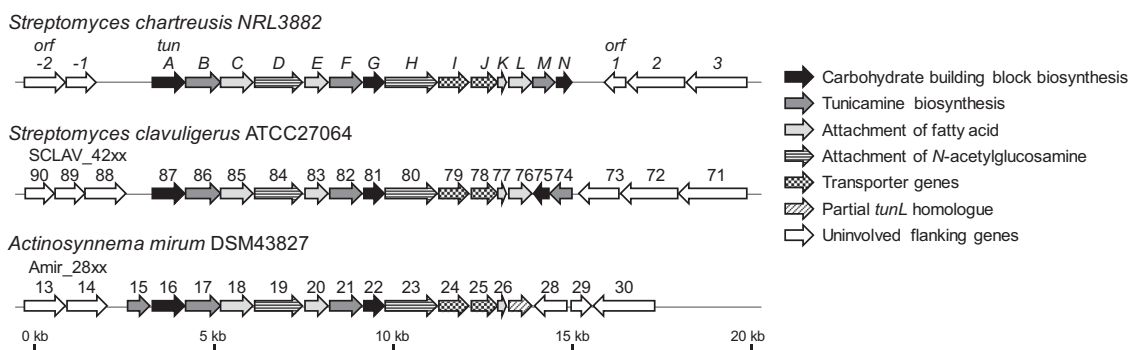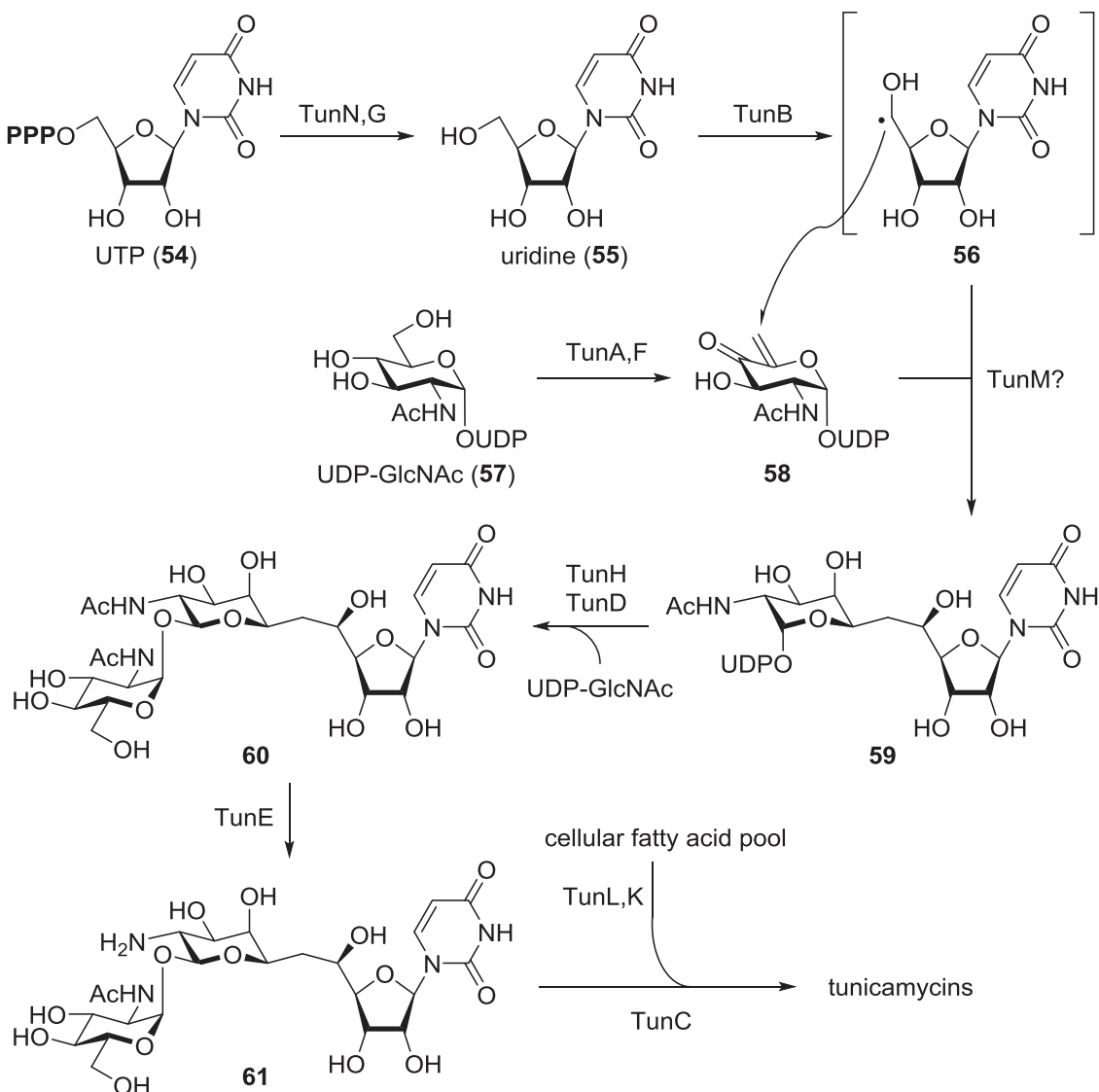
● [1-$^{13}$C]-D-glucose    D [6,6-$^2$H,$^2$H]-D-glucose

**Fig. 3** Genetic organization of the tunicamycin biosynthetic gene cluster. The figure was modified from *Chem. Sci.* 2010 [22]



**Scheme 6** Proposed biosynthetic pathway by Davis and co-workers in 2010

microorganism (*S. chartreusis*), and the metabolic origin of tunicamine was revealed [43]. LC-ESI-CD-MS analyses of the stable isotope-labeled tunicamycin indicated that both the D-GlcNAc and D-galactosamine moieties originated from D-glucose (or UDP-GlcNAc) *via* the sugar metabolic pathway (Fig. 2), which suggested the presence of UDP-GlcNAc 4′-epimerase to produce the galactosamine moiety.

In addition, a minimal gene cluster in tunicamycin biosynthesis was identified by Davis and co-workers in 2010 (Fig. 3) [44]. Because tunicamycins are not polyketides or peptide natural products biosynthesized by polyketide synthase or non-ribosomal peptide synthase (NRPS) with highly conserved sequences, they utilized bioinformatics
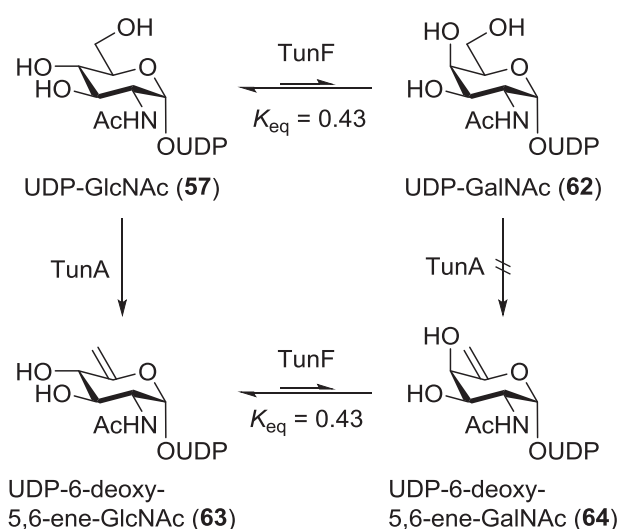
tools and searched genes involving $1 \rightarrow 1$-linking glyco-syltransferases, *N*-acetylhexosamine *N*-deacetylases, lipid-processing proteins and NDP-hexose epimerase/dehydrase, which were expected to be involved in the construction of the unique substructure of tunicamycins. Through these analyses, *tun* gene clusters were identified from several microorganisms containing *S. chartreusis*, which was shown to be a tunicamycin-producing microorganism in a previous report [43]. Considering the putative functions of *tun* gene products, it was suggested that a TunB/M-cata-lyzed, enol-aldehyde reductive C5′-C6′ bond formation in the radical process formed the tunicamine scaffold ($56 + 58 \rightarrow 59$, Scheme 6), and this was a previously unknown enzymatic reaction, and TunD formed the 11′-β,1″-α trehalose-type glycosidic bond ($59 +$ UDP-GlcNAc $\rightarrow 60$, Scheme 6). Additionally, the diversity of fatty acyl chains in the tunicamycin family is attributed to the use of lipids present in the cells because of the lack of a fatty acid synthase in the *tun* gene cluster. These insights were consistent with a previous report [44], in which D-glucose (or UDP-GlcNAc) was used as a biosynthetic precursor.

Although tunicamycin-producing gene clusters were identified and the biosynthetic pathway was proposed, the details of the C–C radical coupling mechanism remained unclear. Davis and co-workers elucidated the true precursor of the C–C radical coupling in 2012 (Fig. 4) [45]. A detailed analysis of the enzymatic reactions revealed that TunF catalyzes the epimerization of the *equatorial*-hydroxyl groups at the 4-positions in UDP-GlcNAc (**57**) and UDP-6-deoxy-5,6-ene-GlcNAc (**63**) to give UDP-GalNAc (**62**) and UDP-6-deoxy-5,6-ene-GalNAc (**64**), respectively. In addition, TunA catalyzes the 5,6-dehy-dration of **57** but not **62**. These results indicated that **57**



**Fig. 4** The enzymatic experiments of TunA, F
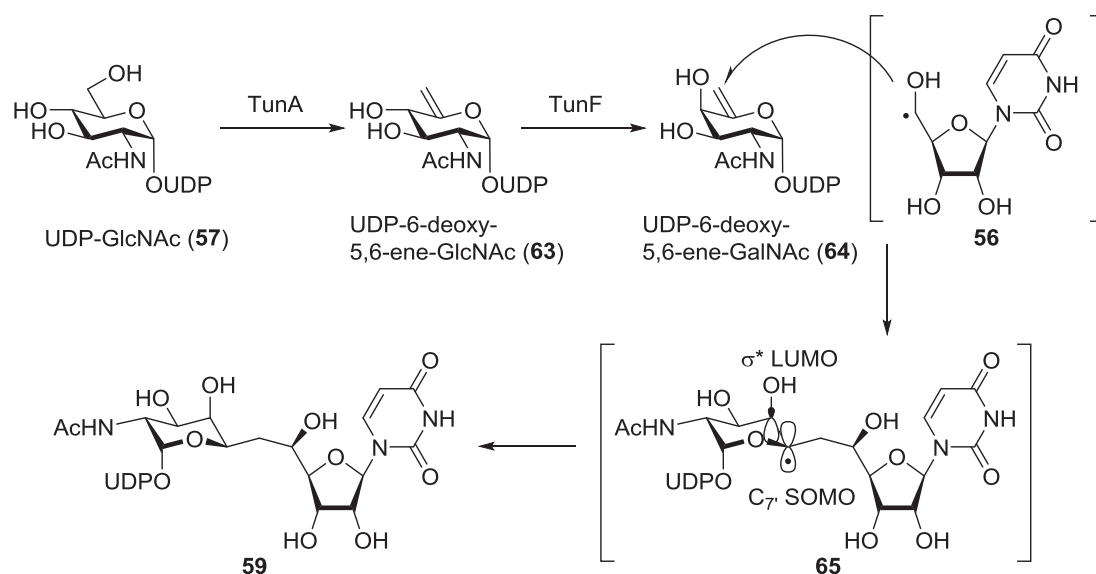


**Scheme 7** Revised biosynthetic pathway by Davis and co-workers in 2012

was dehydrated by TunA to give 5,6-ene-product **63**, which was subsequently epimerized by TunF to give **64** in the biosynthesis of tunicamycin. Therefore, as shown in the revised biosynthetic pathway (Scheme 7), the C–C radical coupling is predicted to occur between uridine 5′-carbon radical **56** and allyl alcohol **64**, instead of unsaturated ketone (**58**, Scheme 6), which directly gives tunicamine scaffold **59**. Interestingly, the total synthesis by Myers' group [30] mimicked this revised biosynthetic pathway, especially in the construction of the tunicamine scaffold, 18 years before Davis' study.

In recent studies [46], mutant experiments of a *tun* gene cluster suggested that six genes (*tunABCDEH*) are essential for tunicamycin production, but the functions of the five gene products (TunFGKLN) could be replaced by other primary metabolic enzymes. It has also been shown that the deletion of *tunMIJ* genes induced an increase in tunicamycin sensitivity in the producing bacterium, meaning that the *tunMIJ* gene products are involved in self-resistance mediated by deactivation *via* methylation (*tunM*) and the expulsion (*tunIJ*) of tunicamycins.

## Conclusion

Tunicamycins have various biological activities, including antimicrobial, antivirus and antitumor activities, which result from the inhibition of MraY in prokaryotes and GPT in eukaryotes. Tunicamycins also have a unique C5′-C6′-linked undecodialdose core, which is rare in nature. This has attracted the attention of many researchers, and many chemical synthetic and biosynthetic studies have been conducted. In the total syntheses of tunicamycin V (**1**), the C5′–C6′ bond formation has been accomplished with a nitroaldol reaction, an intramolecular radical cyclization and a Mukaiyama aldol reaction. On the other hand, biosynthetic studies indicated that the tunicamine core was produced *via* a radical coupling between uridine and UDP-6-deoxy-5,6-ene-GalNAc, which is a new type of enzymatic reaction in biosynthesis.

The usefulness of tunicamycins as antibacterial agents is limited by their off-target inhibition of human-GPT. To decrease this off-target effect, structural modifications of tunicamycins to achieve target selectivity is required. Recently, the structures of tunicamycin complexed with MraY and GPT were revealed [47–49]. According to the co-crystal structure of MraY and GPT, the recognition of GlcNAc moiety in tunicamycin is quite different. Therefore, a GlcNAc-modified analogue was designed, and this analogue inhibited only MraY but not GPT ($IC_{50} = 640$ nM vs 15 μM) [48]. As just described, it is expected that the various synthetic methods of tunicamycins in conjunction with the structural information provided by the ligand-protein complexes will facilitate rational drug design to improve

selectivity among enzymes, biological activities, metabolic stabilities and so on to improve the applicability of these compounds as antibacterial or anticancer agents.

## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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## References

1. Takatsuki A, Arima K, Tamura G. Tunicamycin, a new antibiotic. I. J Antibiot. 1971;24:215–23.
2. Takatsuki A, Tamura G. Tunicamycin, a new antibiotic. II. J Antibiot. 1971;24:224–31.
3. Takatsuki A, Tamura G. Tunicamycin, a new antibiotic. III. J Antibiot. 1971;24:232–8.
4. Takatsuki A, Tamura G. Effect of tunicamycin on the synthesis of macromolecules in cultures of chick embryo fibroblasts infected with new castle disease virus. J Antibiot. 1971;24:785–95.
5. Ito T, et al. The structure of tunicaminyl uracil, a degradation product of tunicamycin. Agric Biol Chem. 1977;41:2303–5.
6. Takatsuki A, et al. The structure of tunicamycin. Agric Biol Chem. 1977;41:2307–9.
7. Ito T, Takatsuki A, Kawamura K, Sato K, Tamura G. Isolation and structures of components of tunicamycin. Agric Biol Chem. 1980;44:695–8.
8. Anderson JS, Matsuhashi M, Haskin MA, Strominger JL. Lipid-phosphoacetylmuramyl-pentapeptide and lipid-phosphodisaccharide-pentapeptide: presumed membrane transport intermediates in cell wall synthesis. Proc Natl Acad Sci USA. 1965;53:881–9.
9. Struve WG, Neuhaus FC. Evidence for an initial acceptor of UDP-NAc-muramyl-pentapeptide in the synthesis of bacterial mucopeptide. Biochem Biophys Res Commun. 1965;18:6–12.
10. Lehrman MA. A family of UDP-GlcNAc/MurNAc: polyisoprenol-P GlcNAc/MurNAc-1-P transferases. Glycobiology. 1994;4:768–71.
11. BrandishP. E, et al. Modes of action of tunicamycin, liposidomycin B, and mureidomycin A: inhibition of phosphor-*N*-acetylmuramyl-pentapeptide translocase from *Escherichia coli*. Antimicrob Agents Chemother. 1996;40:1640–4.
12. Boyle DS, Donachie WD. *mraY* is an essential gene for cell growth in *Escherichia coli*. J Bacteriol. 1998;180:6429–32.

13. Schmidt G, Myer H, Mäkelä PH. Presence of *rfe* genes in *Escherichia coli*: their participation in biosynthesis of O antigen and enterobacterial common antigen. J Bacteriol. 1976;127:755–62.

14. Anderson MS, Eveland SS, Price NPJ. Conserved cytoplasmic motifs that distinguish sub-groups of the polyprenol phosphate:*N*-acetylhexosamine-1-phosphate transferase family. FEMS Microbiol Lett. 2000;191:169–75.

15. Lehrer J, Vigeant KA, Tatar LD, Valvano MA. Functional characterization and membrane topology of *Escherichia coli* WecA, a sugar-phosphate transferase initiating the biosynthesis of enterobacterial common antigen and O-antigen lipopolysaccharide. J Bacteriol. 2007;189:2618–28.

16. Dabbagh BA, Lecreulx DM, Bouhss A. Purification and characterization of the bacterial UDP-GlcNAc:undecaprenyl-phosphate GlcNAc-1-phosphate transferase WecA. J Bacteriol. 2008;190:7141–6.

17. Kimura K, Bugg TDH. Recent advances in antimicrobial nucleoside antibiotics targeting cell wall biosynthesis. Nat Prod Rep. 2003;20:252–73.

18. Bugg TDH, Lloyd AJ, Roger DI. Phospho-MurNAc-pentapeptide translocase (MraY) as a target for antibacterial agents and antibacterial proteins. Infect Dis Drug Targets. 2006;6:85–106.

19. Winn M, Goss RJM, Kimura K, Bugg TDH. Antimicrobial nucleoside antibiotics targeting cell wall assembly: recent advances in structure-function studies and nucleoside biosynthesis. Nat Prod Rep. 2010;27:279–304.

20. Lehrman MA. Biosynthesis of *N*-acetylglucosamine-P-P-dolichol, the committed step of asparagine-linked oligosaccharide assembly. Glycobiology. 1991;1:553–62.

21. Thrum H, et al. Streptovirundins, new antibiotics with antibacterial and antiviral activity. I. Culture taxonomy, fermentation and production of streptovirudin complex. J Antibiot. 1975;28:514–21.

22. Vogel P, et al. Isolation of a group of glycolipid toxins from seedheads of annual ryegrass (Lolium rigidum Gaud.) infected by Corynebacterium rathayi. Aust J Exp Biol Med Sci. 1981;59:455–67.

23. Kenig M, Reading C. Holomycin and an antibiotic (MM 19290) related to tunicamycin, metabolites of Streptomyces clavuligerus. J Antibiot. 1979;32:549–54.

24. Nakamura S, Arai M, Karasawa K, Yonehara H. An antibiotic, mycospocidin. J Antibiot. 1957;10:248–53.

25. Mizuno M, Shimojima Y, Sugawara T, Takeda I. An antibiotic 24010. J Antibiot. 1971;24:896–9.

26. Price NPJ, et al. Quinovosamycins: new tunicamycin-type antibiotics in which the α,β-1″,11′-linked *N*-acetylglucosamine residue is replaced by *N*-acetylquinovosamine. J Antibiot. 2016;69:637–46.

27. Suami T, Sasai H, Matsuno K. Synthesis of methyl hexaacetyltunicaminyl uracil. Chem Lett. 1983;12:819–22.

28. Suami T, et al. Synthetic approach toward antibiotic tunicamycins–VI Total synthesis of tunicamycins. Tetrahedron Lett. 1984;25:4533–6.

29. Suami T, Sasai H, Matsuno K, Suzuki N. Total synthesis of tunicamycin. Carbohydr Res. 1985;143:85–96.

30. Myers AG, Gin DY, Rogers DH. Synthetic studies of the tunicamycin antibiotics. Preparation of (+)-tunicaminyluracil, (+)-tunicamycin-V, and 5′-*epi*-tunicamycin-V. J Am Chem Soc. 1994;116:4697–718.

31. Li J, Yu B. A modular approach to the total synthesis of tunicamycins. Angew Chem Int Ed. 2015;54:6618–21.

32. Yamamoto K, Yakushiji F, Matsumaru T, Ichikawa S. Total synthesis of tunicamycin V. Org Lett. 2018;20:256–9.

33. Danishefsky SJ, DeNinno SL, Chen SH, Boisvert L, Barbachyn M. Fully synthetic stereoselective routes to the differentially protected subunits of the tunicamycins. J Am Chem Soc. 1989;111:5810–8.

34. Ramza J, Zamojski A. New convenient synthesis of tunicamine. Tetrahedron. 1992;48:6123–34.

35. Karpiesiuk W, Banaszek A. Highly stereoselective synthesis of α, β-linked, nonreducing disaccharides related to tunicamycin. Carbohydr Res. 1994;261:243–53.

36. Karpiesiuk W, Banaszek A. Stereoselective syntheses of the *O*, *N*-protected subunits of the tunicamycins. Carbohydr Res. 1997; 299:245–52.

37. Sarabia F, Martin-Ortiz L, López-Herrera FJ. Synthetic studies towards the tunicamycins and analogues based on diazo chemistry. Total synthesis of tunicaminyl uracil. Org Biomol Chem. 2003;1:3716–25.

38. Ichikawa S, Matsuda A. Synthesis of tunicaminyluracil derivatives. Nucleosides, Nucleotides Nucleic Acids. 2004;23:239–53.

39. Schmidt RR. New methods for the synthesis of glycosides and oligosaccharides – Are there alternatives to the Koenigs-Knorr method? Angew Chem Int Ed. 1986;25:212–35.

40. Zhu X, Schmidt RR. New principles for glycoside-bond formation. Angew. Chem. Int. Ed. 48, 1900–34 (2009).

41. Danishefsky SJ, DeNinno MP, Phillips GB, Zelle RE, Lartey PA. On the communication of chirality from furanose and pyranose rings to monosaccharide side chains: anomalous results in the glucose series. Tetrahedron. 1986;42:2809–19.

42. Chen X, Wiemer DF. α-Phosphono lactone analogues of cytidine and cytosine arabinoside diphosphates: synthesis via ring closing metathesis. J Org Chem. 2003;68:6597–604.

43. Tsvetanova BC, Kiemle DJ, Price NPJ. Biosynthesis of tunicamycin and metabolic origin of the 11-carbon dialdose sugar, tunicamine. J Biol Chem. 2002;277:35289–96.

44. Wyszynski FJ, Hesketh AR, Bibb MJ, Davis BG. Dissecting tunicamycin biosynthesis by genome mining: cloning and heterologous expression of a minimal gene cluster. Chem Sci. 2010;1:581–9.

45. Wyszynski FJ, et al. Biosynthesis of the tunicamycin antibiotics proceeds via unique exo-glycal intermediates. Nat Chem. 2012;4:539–46.

46. Widdick D, et al. Analysis of the tunicamycin biosynthetic gene cluster of *Streptomyces chartreusis* reveals new insights into tunicamycin production and immunity. Antimicrob Agents Chemother. 2018;62:e00130–18.

47. Hakulinen JK, et al. MraY-antibiotic complex reveals details of tunicamycin mode of action. Nat Chem Biol. 2017;13:265–7.

48. Yoo J, et al. GlcNAc-1-P-transferase-tunicamycin complex structure reveals basis for inhibition of N-glycosylation. Nat Struct Mol Biol. 2018;25:217–24.

49. Dong YY, et al. Structures of DPAGT1 explain glycosylation disease mechanisms and advance TB antibiotic design. Cell. 2018;175:1045–58.