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SPECIAL FEATURE: REVIEW ARTICLE

Recent advances in the biosynthesis of nucleoside antibiotics

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Abstract
Nucleoside antibiotics are a diverse class of natural products with promising biomedical activities. These compounds contain a saccharide core and a nucleobase. Despite the large number of nucleoside antibiotics that have been reported, biosynthetic studies on these compounds have been limited compared with those on other types of natural products such as polyketides, peptides, and terpenoids. Due to recent advances in genome sequencing technology, the biosynthesis of nucleoside antibiotics has rapidly been clarified. This review covering 2009–2019 focuses on recent advances in the biosynthesis of nucleoside antibiotics.

Introduction

Nucleosides are structural subunits of nucleic acid-derived macromolecules, DNA and RNA, which convey genetic information in living cells. In addition, nucleosides are also energy sources (such as ATP), signal molecules (such as cyclic AMP [cAMP] and guanosine tetraphosphate [ppGpp]), glycosyl donors for glycosylation, and coenzymes (such as NAD and *S*-adenosyl-L-methionine [SAM]). All these nucleosides are primary metabolites essential for living organisms. A large number of compounds with nucleoside structures have been reported as secondary metabolites [1]; including tunicamycin [2], which has been used as an inhibitor of *N*-linked glycosylation in biochemical studies, and polyoxins [3], which have been used as pesticides. In addition, many nucleoside analogues exhibiting antitumor and antiviral activities have been chemically synthesized [4]. However, while nucleoside antibiotics are an attractive group of natural products used as lead compounds in pharmaceutical development, their biosynthesis is poorly understood and has not been systematically studied in the way that the biosynthesis of polyketides, peptides, and terpenoids has. In the last ten years, biosynthetic gene clusters of several nucleoside antibiotics have been identified, and unique biosynthetic mechanisms have been elucidated. These developments have made it possible to discover new nucleoside antibiotics by genome mining methods.

Nucleoside antibiotics can be classified into two categories, *N*-nucleosides and *C*-nucleosides, in which a sugar and a nucleobase are linked via a C–N bond or a C–C bond, respectively. This review describes the recently revealed biosynthetic logic of nucleoside antibiotics, with particular emphasis on C5′ extension reactions used in the biosynthesis of *N*-nucleosides, the mechanisms of C–C bond formation and the modifications of the nucleobases in the biosynthesis of *C*-nucleosides (Fig. 1).

N-nucleoside antibiotics

Biosynthesis of polyoxins and nikkomycins

Polyoxins and their analogues, nikkomycins, exhibit antibacterial activities by specifically inhibiting the biosynthesis of chitin, which is a cell wall component of *Aspergillus* and *Basidiomycetes* [3]. Since its discovery, polyoxin has been an important compound in practical use as a pesticide antibiotic. Despite their important practical applications, these nucleoside antibiotics have not been biochemically studied. Prior to biochemical studies on the biosynthesis of

This study is dedicated to Dr Kiyoshi Isono with respect to his long-standing contribution to the study of nucleoside antibiotics.

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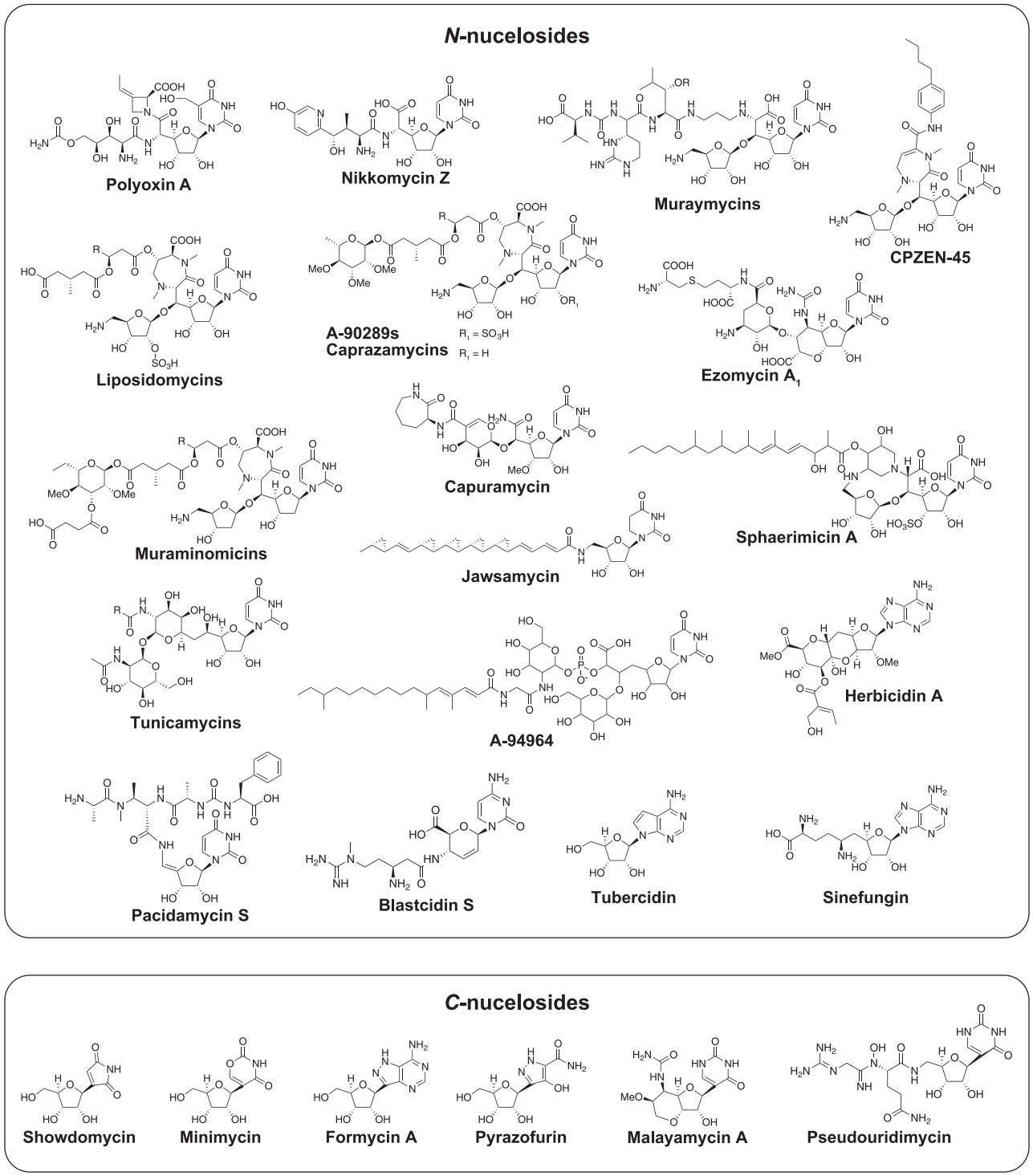

Fig. 1 Nucleoside antibiotics featured in this review

nikkomycin and polyoxin, tracer experiments with isotopically labeled precursors were only conducted [5]. The tracer experiments showed that the hexose skeleton of polyoxin is not derived from glucose but is formed by the oxidative elimination of two carbons from an eight-carbon sugar formed from five carbons from ribose and three carbons from pyruvate (Fig. 2). This result suggested that an aldol condensation of uridine-5′-aldehyde and phosphoenolpyruvate (PEP) yields the eight-carbon sugar intermediate. The *nik* (for nikkomycins) and *pol* (for polyoxins) biosynthetic gene clusters were finally identified in 2009 [6]. Recent biochemical studies revealed that the

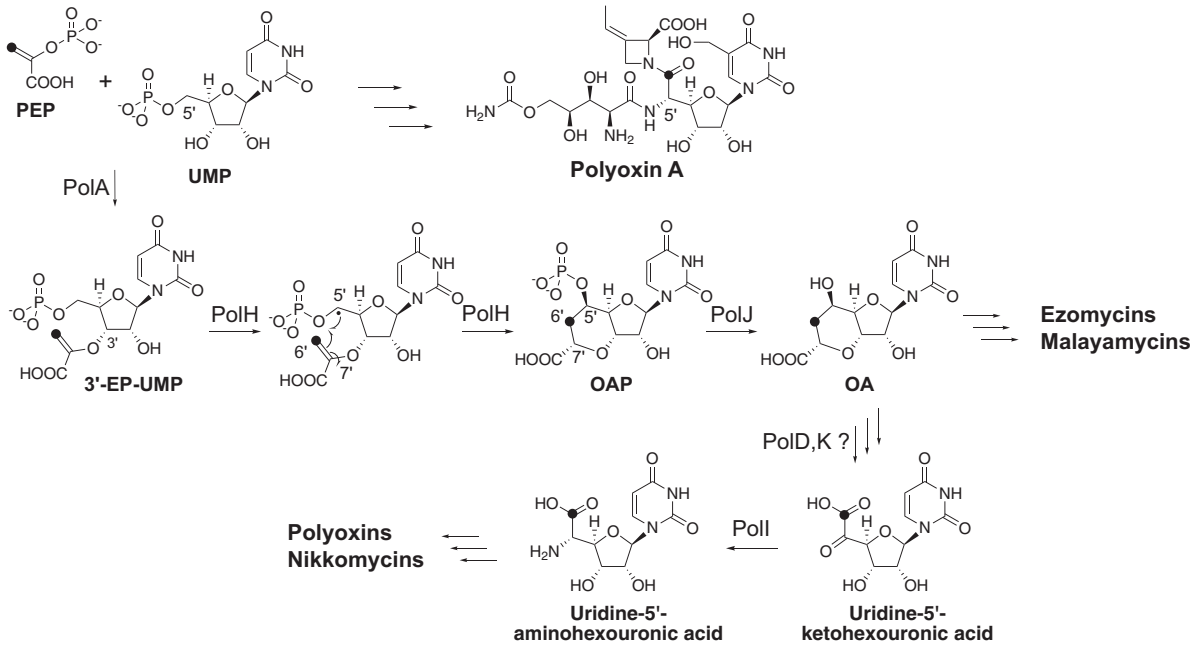

Fig. 2 Biosynthetic pathways of polyoxin and nikkomycin. Ezomycins and malayamycins are also biosynthesized via OA. 3′-EP-UMP 3′-enolpyruvyl-UMP, OAP octosyl acid phosphate, OA octosyl acid

biosynthesis proceeds via a completely different pathway from the hypothetical pathway based on the results of the tracer experiments. First, functional analysis of NikO/PolA showed that these enzymes catalyze the addition of PEP to the hydroxyl group at the 3′ position, not the 5′ position, of UMP to form 3′-enolpyruvyl-UMP (Fig. 2) [6]. More recently, it has been shown that the radical SAM enzyme NikJ/PolH catalyzes C–C bond formation between the C5′ and C6′ positions of 3′-enolpyruvyl-UMP [7, 8]. In this radical reaction, the 5′-deoxyadenosyl radical abstracts the hydrogen atom from the C5′ position of 3′-enolpyruvyl-UMP, and the resulting radical intermediate reacts with C6′ in the terminal methylene and is finally quenched by Cys209 of PolH to form octosyl acid phosphate (OAP). OAP is then dephosphorylated by PolJ to octosyl acid (OA). Although the reactions after OA are still unclear, the results of gene disruption experiments have suggested that PolD and PolK oxidases are involved in the formation of uridine-5′-ketohexouronic acid, and that PolI aminotransferase catalyzes the amination of uridine-5′-ketohexouronic acid to form uridine-5′-aminohexouronic acid (Fig. 2) [9]. The fact that OA is detected in the culture extract of polyoxin-producing bacteria also suggests that this pathway is indeed involved in the biosynthesis of polyoxin [10]. Other nucleoside antibiotics having an OA-like structure, such as ezomycins and malayamycins, are also known. These compounds are presumably biosynthesized through the same route as polyoxins and nikkomycins. In fact, it has recently been reported that malayamycin is biosynthesized through OA as an intermediate [11].

Biosynthesis of liposidomycins and caprazamycins

Liposidomycins are nucleoside antibiotics reported by Isono et al. [12]. Unlike polyoxin and nikkomycin, liposidomycins exhibit antibacterial activity by inhibiting translocase I (MraY), which is involved in the formation of lipid I, one of the key reactions in bacterial cell wall biosynthesis [13]. Following the discovery of liposidomycin, the related compound caprazamycin was isolated [14]. In particular, the semisynthetic caprazamycin derivative CPZEN-45 was reported to exhibit antibacterial activity with high selectivity against extensively drug resistant *Mycobacterium tuberculosis* (XDR-MTB) [15]. Since the structure contains a seven-carbon uronic acid linked to a nucleobase, its C5′ extension presumably involves a biosynthetic mechanism that is logically distinct from that of polyoxin.

The *cpz* (for caprazamycins), *lpm* (for liposidomycin), and *lip* (for A-90289) biosynthetic gene clusters were identified, and functional analyses were conducted [16–18]. These analyses revealed that the biosynthetic pathways of these nucleoside antibiotics start from UMP but their routes are different from those of polyoxins. In the biosynthetic pathway of these compounds, UMP is converted to uridine-5′-aldehyde (U5A) by nonheme Fe(II)- and α-ketoglutarate-dependent dioxygenase LipL (Fig. 3a) [19]. Then, the C–C bond formation between U5A and L-Thr, which is catalyzed by PLP-dependent transaldolase LipK, yields 5′-*C*-glycyluridine (GlyU) with the release of acetaldehyde (Fig. 3a) [20]. LipK catalyzes the cleavage of the Cα–Cβ bond of L-Thr, which is followed by the formation of a C–C bond

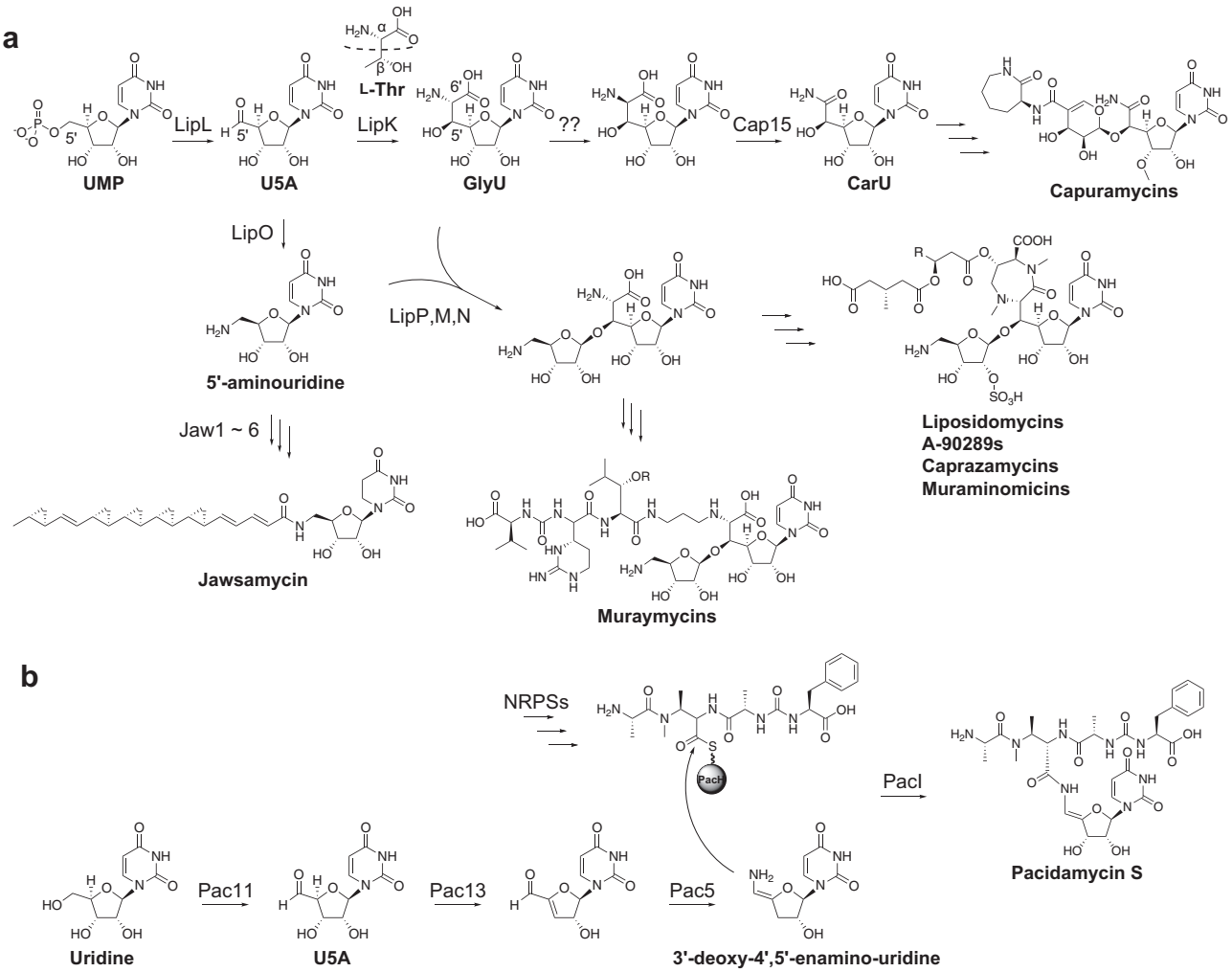

Fig. 3 Biosynthetic pathways of (**a**) liposidomycin, caprazamycin and (**b**) pacidamycin. GlyU is a common intermediate of the biosynthesis of capuramycin, lipposidomycin, A-90289, caprazamycin, muraminomicin, and muraymycin. U5A 5′-aldehyde, GlyU 5′-*C*-glycyluridine, CarU 5′-*C*-carbamoyluridine

between Cα and C5′ of U5A. The C5′ extension proceeds by an aldol condensation as originally predicted for polyoxins. Interestingly, in the biosynthesis of these compounds, U5A is also used as a precursor of amino sugars; U5A undergoes aminotransfer reaction catalyzed by LipO to afford 5′-aminouridine and is incorporated into the final product as 5-aminoribose (Fig. 3a) [21].

The genes encoding these LipK/L/O homologs are also found in the biosynthetic gene cluster of the uridine-containing nucleoside antibiotic muraymycins [22]. Presumably, muraymycins are also biosynthesized via a pathway similar to those of caprazamycin, liposidomycin, and A-90289. In addition, homologs of LipK/L are found in the biosynthetic gene cluster of capuramycin, and GlyU formed in this process is epimerized at C6′ and further converted to 5′-*C*-carbamoyluridine (CarU) by PLP-dependent decarboxylase Car15 [23]. Homologs of LipL and LipO are also present in the biosynthetic gene cluster of the nucleoside antibiotic jawsamycin [24]. In the biosynthesis of jawsamycin, these enzymes are involved in the formation of the 5′-aminouridine skeleton. Thus, LipK/L is useful as a probe for identifying new nucleoside antibiotics by genome mining methods. In fact, a novel nucleoside antibiotic, sphaerimicin was discovered by a genome mining method using LipK/L as probes [25].

Biosynthesis of pacidamycins, napsamycins, and mureidomycins

Pacidamycin and its analogues napsamycin and mureidomycin are peptidyl nucleoside antibiotics isolated from actinomycetes [26]. Like liposidomycins, pacidamycins exhibit antibacterial activity by inhibiting translocase I (MraY), which is involved in bacterial peptidoglycan biosynthesis. The biosynthetic gene cluster for pacidamycins was reported in 2010, and its biosynthetic mechanism was

subsequently analyzed in detail [27–30]. The peptide moiety is biosynthesized mainly by NRPS, which is similar to other peptide-based natural products, while the uridine skeleton is derived from the U5A [28, 30]. However, the enzyme involved in U5A formation during the biosynthesis of pacidamycins is completely different from the enzyme involved in the biosynthesis of capramycins. The flavin-dependent oxydoreductase Pac11 catalyzes the formation of U5A using uridine as a substrate rather than UMP (Fig. 3b). U5A is then converted to 3′-deoxy-4′,5′-enamino-uridine by the dehydration enzyme Pac13 and the aminotransferase Pac5 (Fig. 3b) [30, 31]. Finally, the peptide bond formation between 3′-deoxy-4′,5′-enamino-uridine and a peptide tethered to the thiotemplate PacH is catalyzed by the stand-alone C domain PacI (Fig. 3b) [29].

Biosynthesis of tunicamycin

Tunicamycin is a nucleoside antibiotic isolated from the actinomycete *Streptomyces lysosuperificus* [32]. Tunicamycin inhibits the *N*-glycosylation and palmitoylation of proteins in eukaryotes in addition to inhibiting peptidoglycan biosynthesis, similar to liposidomycins. Due to its cytotoxicity, tunicamycin has not been applied as a medical drug, but since its discovery, it has been widely used as a biochemical reagent. The structure of tunicamycin includes a unique 11-carbon amino sugar called tunicamine (Fig. 4). Tracer experiments revealed that tunicamine is biosynthesized from UDP-Glc*N*Ac and uridine [33]. Recently, its biosynthetic gene clusters were identified and analyzed [34–36]. The molecular basis of the biosynthetic pathway has thus been determined, and the biosynthesis of tunicamycin is thought to proceed through a radical process, similar to the biosynthesis of polyoxins [37]. Biochemical analysis revealed that UDP-Glc*N*Ac is converted to UDP-6-deoxy-5,6-ene-Gal*N*Ac by dehydration by TunA followed by the reaction with isomerase TunF (Fig. 4). Because the loss of tunicamycin production and the accumulation of UDP-6-deoxy-5,6-ene-Gal*N*Ac were detected in a mutant deleting the gene *tunB*, which encodes the radical SAM enzyme, TunB was suggested to catalyze the C5′ extension; however, no in vitro analyses have been reported. The formation of a radical intermediate by abstraction of the hydrogen at C5′ of the uridine and subsequent C–C bond formation between the radical intermediate and the terminal methylene of UDP-6-deoxy-5,6-ene-Gal*N*Ac has been proposed as the reaction mechanism of TunB (Fig. 4).

Unsolved mechanisms of C5′ extension

A-94964 is a uridine-containing nucleoside antibiotic that inhibits bacterial peptidoglycan biosynthesis, similar to the activities of liposidomycins and tunicamycins [38]. A-94964 has a unique 8-carbon uronic acid in its structure. Due to this structural feature, it has been speculated that the biosynthesis of A-94964 involves C5′ extension between uridine and pyruvate or lactate. However, the enzyme homologs LipK and TunB mentioned above are not present in the recently identified biosynthetic gene cluster of A-94964 [39]. Therefore, it is assumed that the C5′ extension proceeds via a pathway completely unlike the known pathways. Gene disruption experiments have suggested that lactate dehydrogenase homolog Anb1, monooxygenase Anb2, and Anb10 with unknown functions may be involved in the formation of an unknown intermediate in which pyruvate and uridine are condensed (Fig. 5a).

C5′ extension reactions in some uridine-containing nucleoside antibiotics are described above. However, C5′ extension reactions are also involved in the biosynthesis of adenosine-containing nucleoside antibiotics such as herbicidin and sinefungin [40, 41]. Herbicidin, which was isolated from the actinomycete *Streptomyces saganonensis*, is

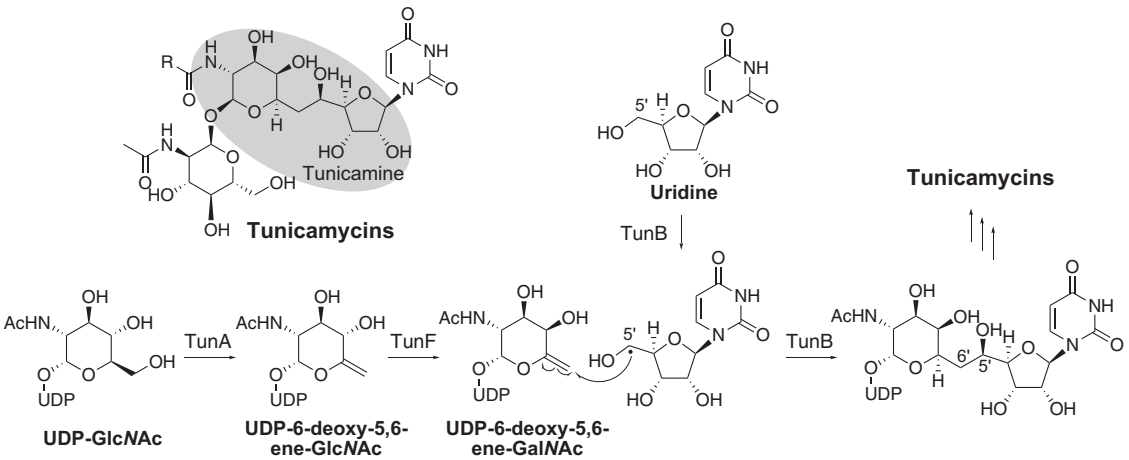

Fig. 4 Biosynthetic pathway of tunicamycin. The tunicamine skeleton in tunicamycin is highlighted in a gray oval

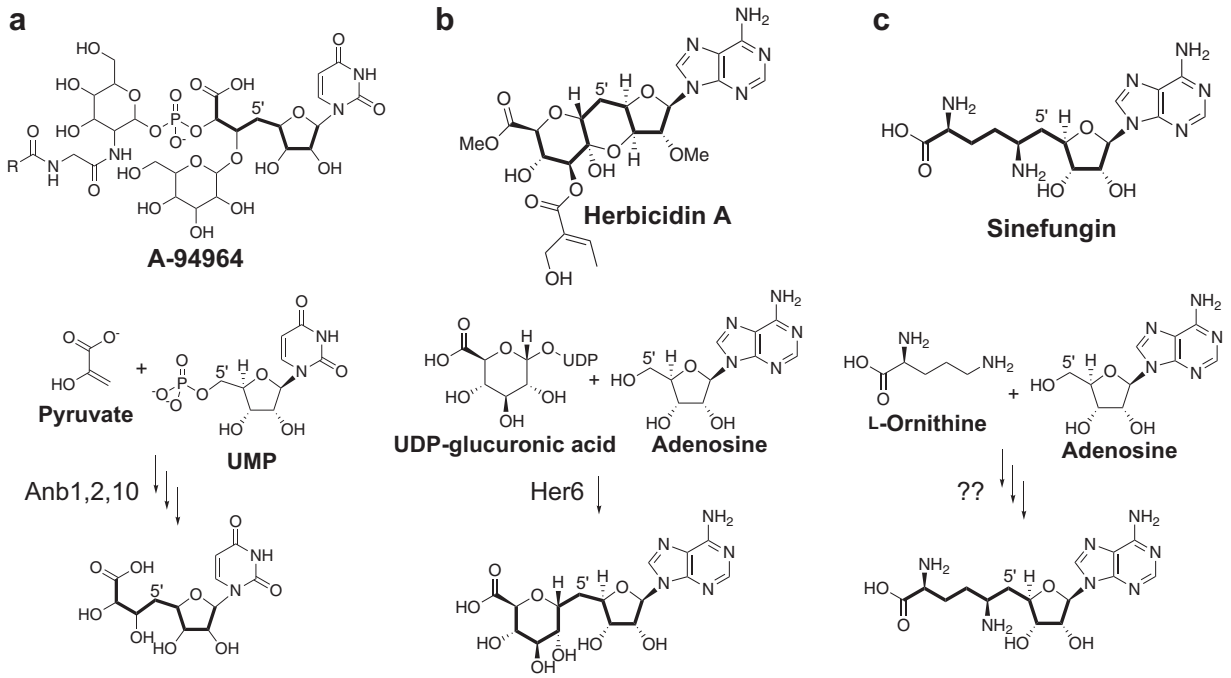

Fig. 5 Unsolved mechanisms of the C5′ extension in the biosynthesis of nucleoside antibiotics. The unique uronic acid skeletons in (**a**) A-94964, (**b**) herbicidin, and (**c**) sinefungin are represented in bold lines

a nucleoside antibiotic showing herbicidal activity. Tracer experiments have shown that the 11-carbon uronic acid structure of herbicidin is biosynthesized by the condensation of adenosine and glucose. However, the enzyme responsible for the C5′ extension reaction has not yet been identified even though the biosynthetic gene cluster of herbicidin has been identified. Similar to the biosynthesis of tunicamycin and polyoxin, the radical SAM enzyme Her6 may be responsible for C–C bond formation (Fig. 5b) [42].

Sinefungin, an adenine-containing nucleoside antibiotic produced by the actinomycete *Streptomyces griseolus*, shows antifungal and antimalarial activities. Sinefungin is presumably biosynthesized by the condensation of adenosine and ornithine. Due to its structural similarity to SAM, sinefungin is widely used as an inhibitor of SAM-dependent methyltransferases. Tracer experiments and the in vitro assays using crude enzymes have suggested that the skeleton of sinefungin is derived from adenosine and ornithine and that PLP is essential in the biosynthesis (Fig. 5c) [43, 44]. However, the biosynthetic genes responsible for sinefungin biosynthesis have not yet been identified.

Biosynthesis of blasticidin S and its related compounds

Blasticidin S is a nucleoside antibiotic isolated from the actinomycete *Streptomyces griseochromogenes* [1]. The nucleoside moiety of blasticidin S is composed of cytosine and a C6 uronic acid. Cytosine is derived from CMP by the action of the nucleotide hydrolase BlsM and C6 uronic acid is derived from UDP-glucuronic acid [45]. BlsD catalyzes the condensation of cytosine and UDP-glucuronic acid to form cytosylglucuronic acid (CGA) (Fig. 6a) [46]. The fact that homologous genes encoding these enzymes are also found in the biosynthetic gene clusters of the related compounds arginomycin and gougerotin suggests that CGA is a common intermediate of these nucleoside antibiotics [46, 47].

Similar to blasticidin S, several other nucleoside antibiotics are biosynthesized via the condensation of a unique sugar skeleton and a nucleobase. During the biosynthesis of 7-deazapurine-containing nucleoside antibiotics represented by tubercidin, the phosphoribosyltransferase homolog, TubE, catalyzes condensation between PRPP and 7-carboxy-7-deazaguanine (CDG) derived from GTP by the actions of TubA, TubB, and TubC (Fig. 6b) [48]. Similarly, it is speculated that Ari9 or its homolog MacI/MacT, which are purine nucleoside phosphorylase homologs, catalyzes the condensation of adenine and the five-membered cyclitol phosphate during the biosynthesis of aristeromycin [49, 50].

Although details have not yet been identified, the results of feeding experiments with various labeled precursors and in silico analysis of the biosynthetic gene clusters have suggested that the biosynthetic pathways for albomycins and amipurimycin are similar to that of blasticidin S. In the biosynthesis of albomycin, the condensation of the

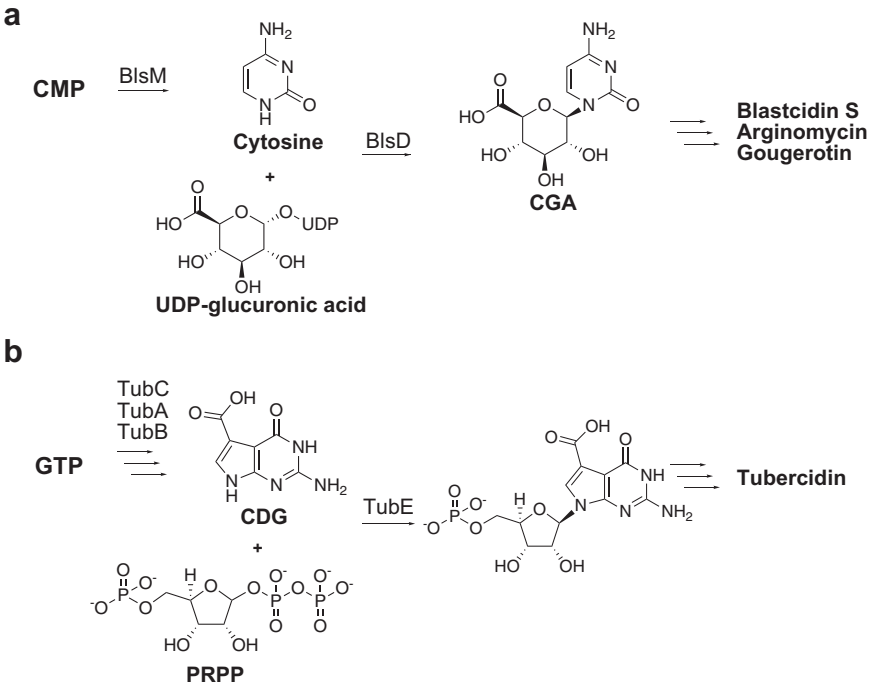

Fig. 6 Biosynthetic pathways of blasticidin S and tubercidin. **a** Biosynthetic pathway for blastcidin S. Arginomycin and gougerotin are also biosynthesized via CGA. CGA, cytidylglucuronic acid. **b** Biosynthetic pathway for tubercidin. CDG, 7-carboxy-7-deazaguanine

characteristic thiosugar and cytosine have been proposed [51]. In the biosynthesis of amipurimycin, the condensation of the unique branched sugar and 2-aminopurine has been proposed [52, 53].

C-nucleoside antibiotics

Biosynthesis of showdomycin, minimycin, formycin, and pyrazofurin

A large number of natural products containing a *C*-nucleoside, in which a ribose and a nucleobase are linked by a C–C bond, have been isolated [1]. Biosynthetic studies on some of these compounds have been conducted. Tracer experiments revealed that the nucleobases of *C*-nucleosides, such as showdomycin, minimycin, formyicin, and pyrazofurin, are all derived from glutamine, glutamate or α-ketoglutarate (α-KG) (Fig. 7a). Furthermore, in all these compounds, the carbon atom bound to the ribose comes from the carbon atom at the C4 position of glutamate. Therefore, it has been speculated that all of these compounds are biosynthesized from a common precursor.

Recently, the biosynthetic gene cluster of showdomycin was identified, and their biosynthetic pathway was proposed [54]. In showdomycin biosynthesis, it has been suggested that SdmA catalyzes the condensation of a ribose and maleimide derived from glutamate. SdmA is a homolog of *C*-ribosyltransferase AlnA encoded in the alnumycin A biosynthetic gene cluster. AlnA catalyzes the condensation of ribose-5-phosphate (R5P) and prealnumycin [55]. Therefore, it is speculated that SdmA catalyzes the condensation of R5P with maleimide in a similar mechanism to that of AlnA (Fig. 7b). Tracer experiments have suggested that minimycin is biosynthesized by a mechanism similar to that of showdomycin; however, the biosynthetic gene cluster of minimycin has not been identified.

Formycin, also called 8-aza-9-deazaadenosine, is a *C*-nucleoside antibiotic. The recently identified formycin biosynthetic gene cluster encodes enzymes similar to those involved in purine biosynthesis [44]. Of these enzymes, ForA, ForB, and ForC are involved in the latter half of the biosynthesis of formycin [56, 57]. Interestingly, PurH, which is involved in de novo purine biosynthesis and is not present in the gene cluster, also contributes to the biosynthesis of formycin. ForC and ForB catalyze the conversion of 5-aminopyrazole-4-carboxyl-1-β-D-ribofuranosyl-5′-monophosphate (CAPR) to 5-aminopyrazole-4-carboxamide-1-β-D-ribofuranosyl-5′-monophosphate (APCAR), and PurH catalyzes the transfer of a formyl group to the amino group of APCAR to form 5-formaminopyrazole-4-carboxamide-1-β-D-ribofuranosyl-5′-monophosphate (FAPCAR). Next, ForH, encoded in the gene cluster, catalyzes the cyclization of FAPCAR to form formycin B-5′-phosphate (Fig. 7c). The *C*-ribosylation in the biosynthesis of formycin may be catalyzed by ForT, which shows no sequence similarity to SdmA. ForT is a homolog of β-ribofuranosyl-aminobenzene 5′-phosphate (RFAP) synthase, which catalyzes the decarboxylative condensation of PRPP and *p*-aminobenzoate to form RFAP in the biosynthesis of methanopterin, a coenzyme in

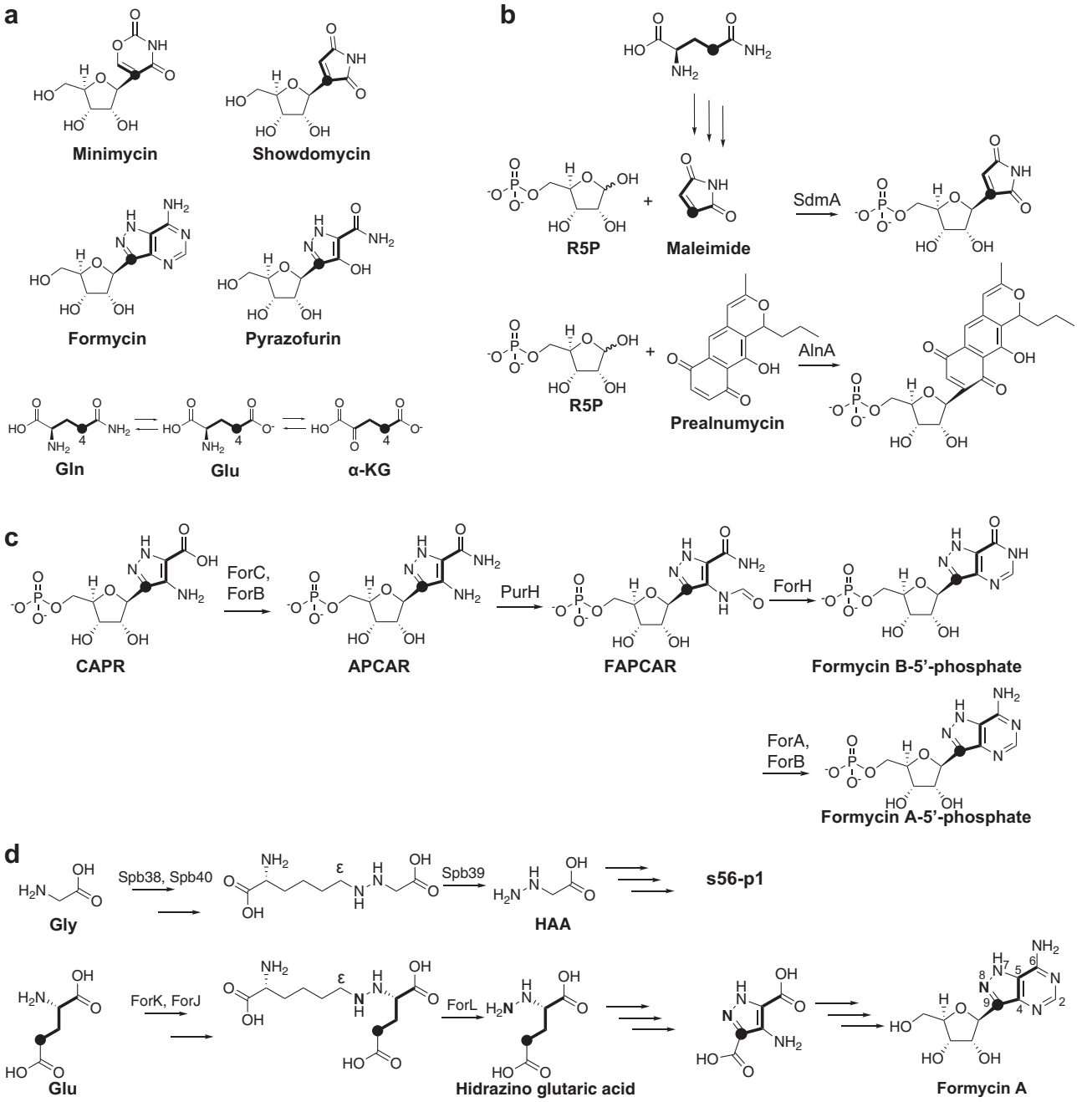

Fig. 7 Biosynthetic pathways of the *C*-nucleoside antibiotics. Dots represent the carbon atoms that come from the carbon atom at C4 of glutamate. The N atom derived from the ε-amino group of Lys is shown in bold. **a** Structures of the *C*-nucleoside antibiotics, minimycin, formycin, showdomycin and pyrazofurin. **b** SdmA-catalyzed reaction in showdomycin biosynthesis and AlnA-catalyzed reaction in alnumycin A biosynthesis. R5P, ribose-5-phosphate. **c** Late steps of formycin biosynthesis. CAPR, 5-aminopyrazole-4-carboxyl-1-β-D-ribofuranosyl-5′-monophosphate; APCAR, 5-aminopyrazole-4-carboxamide-1-β-D-ribofuranosyl-5′-monophosphate; FAPCAR, 5-formaminopyrazole-4-carboxamide-1-β-D-ribofuranosyl-5′-monophosphate. **d** HAA biosynthesis and early steps of formycin biosynthesis

methanogenesis [58, 59]. Therefore, ForT is proposed to be involve in the condensation of PRPP and 4-aminopyrazole-3,5-dicarboxylic acid to form a formycin intermediate via a mechanism similar to that of methanopterin biosynthesis. The mechanism of the formation of the characteristic deazaaminoadenine structure of formycin has been inferred as follows based on tracer experiments and bioinformatics analysis of the biosynthetic gene clusters [57, 60]. Tracer experiments revealed that the C9–C4–C5–C6 carbon chain and N8 nitrogen center of formycins are derived from glutamate and the ε-amino group of lysine, respectively (Fig. 7a, d). Bioinformatics analysis revealed that ForK/J/L in the formycin gene cluster show sequence similarity to Spb38/40/39, which

generate hydrazino acetic acid (HAA) from Lys and Gly [61]. In HAA biosynthesis, Spb38 activates the ε-amino group of Lys by hydroxylation, and Spb40 subsequently catalyzes the formation of an N–N bond between the resulting ε-amino group of lysine and the amino group of glycine. Spb39 then catalyzes the cleavage of the bond between Cε and the N derived from lysine to form HAA. Finally, HAA is incorporated into the natural product, s56-p1 (Fig. 7d) [62]. Thus, it is speculated that ForK and ForJ form an N–N bond between the α-amino group of Glu and the ε-amino group of Lys via a pathway similar to that of HAA and then ForL catalyzes the cleavage of the C–N bond derived from lysine to form hydrazino glutaric acid (Fig. 7d). Although the reaction order is unknown, 4-aminopyrazole-3,5-dicarboxylic acid would be subsequently formed by modification reactions including cyclization and amino group addition to hydrazino glutaric acid.

Biosynthesis of pseudouridimycin

Recently, a *C*-nucleoside antibiotic, pseudouridimycin, which inhibits bacterial RNA polymerase, was isolated from a culture broth of *Streptomyces* spp [63]. Pseudouridimycin exhibits antibacterial activity against drug-resistant pathogenic bacteria. Since its activity is based on a different mechanism of action from those of other RNA polymerase inhibitors, pseudouridimycin is expected to be a novel lead compound for antibacterial therapy. Recently, the biosynthetic gene cluster for pseudouridimycin was identified, and the mechanism for the formation of the *C*-nucleoside scaffold has also been elucidated [64]. Pseudouridine is involved in the posttranscriptional modification mechanism of tRNA in various organisms. In *E. coli*, it is known that the post-transcriptional modification of tRNA involves TruA, TruB, RsuA, RluA, and TruD. In the biosynthetic gene cluster of pseudouridimycin, PumJ showed 28% sequence homology with TruD. Gene disruption experiment for *pumJ* and a feeding experiment with pseudouridine suggested that PumJ is involved in the formation of pseudouridine [64].

Furthermore, genome mining has indicated that genes encoding PumJ-like enzymes are found in various bacteria and have been suggested to be involved in the biosynthesis of various secondary metabolites [64]. A gene encoding an enzyme homologous to PumJ has also been found in the biosynthetic gene cluster for malayamycin, which is also a *C*-nucleoside [11]. It has been suggested that pseudouridine is formed by the same mechanism utilized during the biosynthesis of malaymycin. Therefore, although the substrate and the detailed reaction mechanism of PumJ have not yet been identified, the *pumJ* gene may serve as a probe to identify novel *C*-nucleoside antibiotics by genome mining methods.

Perspective

This review has introduced the recently described biosynthetic mechanisms, especially the C5′ extension reactions of nucleoside antibiotics, which have previously eluded biochemical studies. As described above, the C5′ extension reactions, which are often the first step in the biosynthesis of nucleoside antibiotics, are diverse and unique. In the biosynthesis of nucleoside antibiotics, a collinearity rule, which is clearly applicable in the biosynthesis of polyketides, peptides, and terpenes, has not yet been found. Therefore, more studies are needed to comprehensively understand the biosynthesis of nucleoside antibiotics. Such studies would lead to the discovery of new nucleoside antibiotics and new enzymatic tools.

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Compliance with ethical standards

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

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