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## The Biosynthesis of Nitrogen-, Sulfur-, and High-carbon Chain-containing Sugars<sup>†</sup>

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### Abstract

Carbohydrates serve many structural and functional roles in biology. While the majority of monosaccharides are characterized by the chemical composition: (CH<sub>2</sub>O)<sub>n</sub>, modifications including deoxygenation, C-alkylation, amination, O- and N-methylation, which are characteristic of many sugar appendages of secondary metabolites, are not uncommon. Interestingly, some sugar molecules are formed *via* modifications including amine oxidation, sulfur incorporation, and “high-carbon” chain attachment. Most of these unusual sugars have been identified over the past several decades as components of microbially produced natural products, although a few high-carbon sugars are also found in the lipooligosaccharides of the outer cell walls of Gram-negative bacteria. Despite their broad distribution in nature, these sugars are considered “rare” due to their relative scarcity. The biosynthetic steps that underlie their formation continue to perplex researchers to this day and many questions regarding key transformations remain unanswered. This review will focus on our current understanding of the biosynthesis of unusual sugars bearing oxidized amine substituents, thio-functional groups, and high-carbon chains.

### 1. Introduction

Carbohydrates are essential biomolecules for all living organisms and exhibit great diversity in their chemical structures. The name, carbohydrate, originally stems from their chemical composition, which is normally (CH<sub>2</sub>O)<sub>n</sub>. However, a wide variety of deoxysugars are found in lipopolysaccharides, glycoproteins, glycolipids, and numerous secondary metabolites. In many cases, these unusual sugars have been shown to be indispensable for the activity of the parent molecules. A great number of gene clusters dedicated to the production of deoxysugars have been identified and the pathways leading to their formation have been extensively studied over the past few decades.<sup>1,2</sup> Generally, the monosaccharides are first activated at C-1 by the substitution of nucleoside diphosphate (NDP), which functions as a handle for recognition by subsequent biosynthetic enzymes. Except for 2-deoxyribose, which forms the backbone of DNA, most naturally occurring deoxysugars are 6-deoxyhexoses. They are derived from a common precursor, NDP-4-keto-6-deoxy- $\alpha$ -D-

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hexose, which is further modified by a varied combination of reduction, isomerization, methylation or transamination reactions to give a diverse array of unusual sugars.

The complexity seen in unusual sugar structures is greatly enhanced by the incorporation of heteroatoms, such as nitrogen and sulfur. The intrinsic chemical properties of these unusual sugars can be significantly altered by the installed heteroatom-containing substituents. Moreover, interconversion between the oxidation states of these heteroatom-containing functional groups attenuates the electronegativity, hydrophilicity and steric hindrance of the overall structures. In addition to the substituents they carry, some unusual sugars are distinguished by a longer than usual carbon chain length. Such “high-carbon” sugars have been identified among microbially-derived antibiotic compounds and in the outer cell walls of some Gram-negative bacteria.

Since many natural products are glycosylated, modifying the structure and/or composition of the sugar components holds promise for varying and/or enhancing the biological activities of the parent systems. Thus, it is critical to have a full understanding of the biosynthesis of these unusual sugars so that suitable strategies to control, mimic, or alter their formation can be developed. Monosaccharides that contain oxidized amines, C-S linkages, or high carbon numbers are rare. In many cases, the key biosynthetic transformations leading to these unusual sugars remain obscure or only partially understood to date. This review will cover what is known regarding their biosynthesis and what work remains to be done to achieve a complete understanding.

## 2. Sugars Containing Oxidized Amines

Deoxyaminosugars comprise an important class of deoxysugars, among which 2-*N*-acetylamino sugars are widespread. However, many deoxyaminosugars having a primary amino group at either C-3, C-4, or C-6 are also known. The amino groups in these sugars are universally introduced into the corresponding ketosugar substrates *via* 5'-phosphate (PLP)-dependent transamination reactions. The biosynthesis of deoxyaminosugars and the catalytic mechanism utilized by sugar aminotransferases (SATs) have been recently reviewed.<sup>3,4</sup> Despite the abundance of deoxyaminosugars, monosaccharides carrying nitrogen-functional groups with higher oxidation states are rarely observed in nature. In a review highlighting aminosugar diversity and biosynthesis Timmons and Thorson noted that the existence of oxidized aminosugars extends the diversity of glycosylated natural products.<sup>5</sup> Coincidentally, after this review was published, several groups demonstrated the *in vitro* characterization of aminosugar *N*-oxygenase, which was considered a major breakthrough in the study of deoxyaminosugar biosynthesis. In this section of the review, we summarize current understanding regarding the biosynthesis of sugars containing hydroxylamino-, nitroso- and nitro groups, with a focus on recent progress in the enzymatic oxidation of aminosugars.

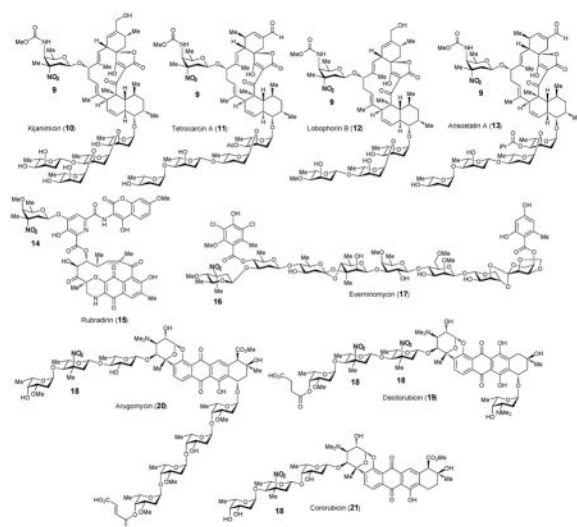
### 2.1. Occurrence and biosynthesis of hydroxyaminosugars

A hydroxyaminosugar is present in the structures of both calicheamicin (**1**) from *Micromonospora echinospora ssp. Calichensis*<sup>6</sup> and esperamicin (**2**) from *Actinomadura verrucosopora*.<sup>7,8</sup> These antibiotics belong to the 10-membered enediyne ring family and

display potent antitumor activity. Calicheamicin and esperamicin share closely related chemical structures that include a rare 4-thiosugar (**3**) in addition to the hydroxyaminosugar moiety (**4**). The elucidation of the gene clusters for the biosynthesis of calicheamicin<sup>9</sup> and esperamicin<sup>10</sup> have led to proposals regarding the formation of hydroxyaminosugars. Based on well-established studies of deoxyaminosugar biosynthesis<sup>3</sup> and an example of P450 *N*-oxygenase in nocardicin A biosynthesis (NocL),<sup>11</sup> Johnson and Thorson proposed two P450-dependent enzymes (CalE10 and CalO2) as candidates for the *N*-oxygenases in calicheamicin biosynthesis<sup>12</sup>. Subsequent *in vitro* studies of recombinant CalE10 and CalO2 using a series of putative thymidine diphosphate (TDP)-sugar substrates demonstrated that CalE10 is indeed an aminosugar *N*-oxygenase (no substrate binding or oxidation was observed with CalO2). As shown in Scheme 1, CalE10 catalyzes the oxidation of TDP-4-amino-4,6-dideoxy- $\alpha$ -D-glucose (**6**) to afford two products: the major hydroxyaminosugar (**7**) and the minor nitrosugar (**8**).<sup>12</sup> This enzymatic reaction shows regioselectivity toward C-4 amino groups and the oxidation occurs at the NDP-sugar stage prior to glycosyltransfer to the enediyne scaffold. Although the nitrosugar is the minor product in this case, CalE10 remains the only known sugar *N*-oxygenase with demonstrated capability to catalyze an overall 6-e<sup>-</sup> transfer to fully oxidize an amino group to a nitro group.

## 2.2. Occurrence of nitrososugars and nitrosugars

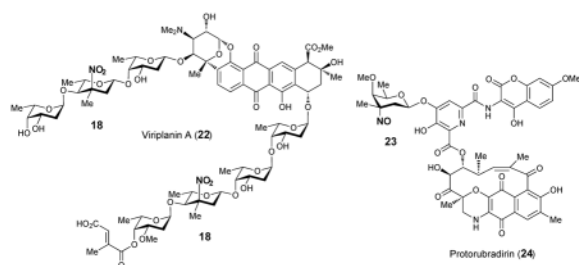
Only four naturally occurring nitrosugars have been identified.<sup>13</sup> These include D-kijanose (**9**), D-rubranitrose (**14**), L-evernitrose (**16**) and L-decilonitrose (**18**). D-Kijanose (**9**) is a highly functionalized nitrosugar that was first found in kijanimicin (**10**) from *Actinomadura kijaniata*.<sup>14</sup> The occurrence of D-kijanose is thus far limited to a family of spirotetronate natural products including kijanimicin (**10**),<sup>14</sup> tetrocarcin (**11**),<sup>15,16</sup> lobophorin (**12**)<sup>17</sup> and arisostatin (**13**).<sup>18</sup> D-Rubranitrose (**14**) is the sugar component of rubradirin (**15**), an antibiotic belonging to the ansamycin family produced by *Streptomyces achromogenes* var. *rubradiris*.<sup>19</sup> The first structural report of rubradirin identified rubranitrose as an L-sugar.<sup>20</sup> It was later revised to be a D-sugar when compared to chemically synthesized L- and D-rubranitrose.<sup>21,22</sup> The L-isomer of rubranitrose is also called L-evernitrose (**16**) as it was originally identified in the structure of everninomycins (**17**) from *Micromonospora carbonaceae*.<sup>23–25</sup> The last example, L-decilonitrose (**18**) was found in decilorubicin (**19**), an anthracycline antibiotic from *Streptomyces virginiae*.<sup>26,27</sup> It also exists in several structurally related natural products including arugomycin (**20**),<sup>28,29</sup> cororubicin (**21**),<sup>30</sup> and viriplanin A (**22**).<sup>31,32</sup> Interestingly, these anthracycline-type antibiotics all contain a bicyclo-aminosugar moiety that is attached to the aglycone *via* a C-glycosidic bond in addition to the typical O-glycosidic linkage.



### 2.3. Biosynthesis of nitrosugars

Two basic routes for the biosynthesis of nitro-containing natural products, including nitrosugars, have been identified: sequential *N*-oxidation of the respective amines and direct nitration of appropriate precursors.<sup>33</sup> In the former case, generation of the nitrated natural product is usually accompanied by the co-production of minor metabolites containing an unoxidized or a partially oxidized amino group. For example, derivatives of kijanimicin (**10**) bearing a 3-amino-D-kijanose rather than kijanose (**9**) have been identified.<sup>34</sup> A rubradirin analogue, protorubradirin (**24**), containing a 3-nitroso-D-rubranitrose (**23**) also exists in nature<sup>35</sup>.

These findings strongly suggest that the nitro moiety in these sugars is biosynthesized *via* consecutive *N*-oxidation of the corresponding aminosugars. In the case of **14**, the nitrososugar (**23**) generated as an intermediate may be prematurely released and then incorporated into **24**.<sup>35</sup> The isolation and sequencing of the gene clusters for the nitrosugar-containing natural products, kijanimicin (**10**),<sup>34</sup> tetrocarcin (**11**),<sup>36</sup> rubradirin (**15**)<sup>37</sup> and everminomycin (**17**)<sup>38</sup> allowed the postulation of the biosynthetic pathways for their corresponding nitrosugars (see Scheme 2). While minor variations exist for the proposed pathways, the key *N*-oxidation step is believed to be catalyzed by a flavin-dependent enzyme, encoded by a gene that is conserved among the above gene clusters for nitrosugar-containing secondary metabolites. These include KijD3 of the kijanimicin cluster of *A. kijaniata*,<sup>34</sup> TcaB10 of the tetrocarcin cluster of *Micromonospora chalcea*,<sup>36</sup> RubN8 of the rubradirin cluster of *S. achromogenes* var. *rubradiris*,<sup>37</sup> and ORF36 (or EvdC) of the everminomycin cluster of *M. carbonaceae* var. *Africana*.<sup>38</sup>



#### 2.4. Characterization of enzymes involved in the biosynthesis of nitrosugars

The biological roles of RubN8 and ORF36 as sugar *N*-oxygenases were first studied by Bachman and co-workers using chemically synthesized TDP-*L*-*epi*-vancosamine (**27**) as the substrate surrogate.<sup>39</sup> The incubation was carried out in the presence of FAD, NADPH, and flavin reductase, with either RubN8 or ORF36. Time course experiments revealed that the amino group of **27** was oxidized to hydroxylamine (**29**) and then nitroso (**30**) in sequence (Scheme 3). In a separate experiment, the corresponding 4-ketosugar (**26**) was shown to be oxidized only to the 3-hydroxylamino-4-ketosugar (**31**).<sup>40</sup> An analogous result was also reported for KijD3, in which partial *N*-oxidation of a 4-ketosugar substrate (**25**) to yield a hydroxyamino sugar product (**32**) was noted (Scheme 3). Crystal structures of both ORF36 and KijD3 reveal a similar protein fold shared with class D flavin-containing monooxygenases.<sup>40,41</sup>

Based on these biochemical and structural data, a mechanism for nitrososugar (**30**) formation has been proposed (Scheme 4).<sup>40</sup> The reaction is initiated by the reduction of the flavin coenzyme in the active site of *N*-oxygenase by a flavin reductase. In the *kij* gene cluster, the *kijD6* gene, which is located near *kijD3* (the *N*-oxygenase) in the *kij* gene cluster, encodes a putative NAD(P)H-dependent flavin reductase.<sup>34</sup> The gene product of *kijD6* was assigned to play such a role in the biosynthesis of kijanose (**9**). However, the *kijD6* homologue is absent in the rubradirin (**15**) and everninomycin (**17**) clusters and exogenous flavin reductases may be required. The reduced flavin coenzyme then reacts with molecular oxygen to generate a flavin-C<sub>4a</sub>-hydroperoxy species (**33**), which is well known for its ability to hydroxylate a nucleophilic functional group. After transferring the electrophilic hydroxyl group to the amino group of **27**, the hydroxylamine product (**29**) is released and the flavin coenzyme is regenerated. A second round of oxidation of the resultant hydroxylaminosugar (**29**) along with dehydration of water yields the nitrososugar (**30**).

It should be noted that ORF36 and RubN8 convert their respective aminosugar substrate (**27**) to the corresponding nitrososugar (**30**) instead of the anticipated nitrosugars (**28**) during *in vitro* assays,<sup>39</sup> and prolonged incubation under aerobic conditions did not make a difference. Similarly, protonubradirin (**24**) is produced at a significant level during fermentation, and the nitroso group of its sugar component **23** is readily oxidized to the nitro group (as in **14**) upon exposure to ambient light and air.<sup>35</sup> It is therefore possible that nitrososugars are the immediate products generated by enzymatic *N*-oxidation during D-rubranitrose and L-evernitrose biosynthesis and that they are further oxidized to nitrosugars *via* a latent photochemical process or by reacting with endogenous reactive oxygen species.

### 3. Thiosugars

Sulfur is the fifth most abundant element in living organisms, after oxygen, carbon, hydrogen and nitrogen. Sulfur-containing compounds are widely distributed in nature and include amino acids, enzyme cofactors, antioxidants, nucleotides, metal clusters, and numerous secondary metabolites.<sup>42</sup> These natural products exhibit a remarkable degree of structural diversity and many of them have potent biological activities. The term, thiosugar, generally describes a carbohydrate moiety that carries a sulfur-containing functional group or is connected to other molecule *via* a sulfide linkage. Only a few naturally occurring thiosugars have been identified and their relative scarcity has hindered investigation aimed at elucidating their biosynthetic pathways and studying their biological activities.<sup>43,44</sup> In this review, the known thiosugars are categorized into four groups based on the chemical nature of their functional groups: sugars containing thiols, sulfides, sulfonium ions, and sulfonic acids. The biosynthetic pathways leading to their formation and the enzymes catalyzing the key sulfur incorporation step will be discussed based on the data that is currently available.

#### 3.1. Group I: Thiosugars carrying a free thiol group

The only members of group I are the angucycline-type antibiotics rhodocardin A (**34**) and B isolated from *Nocardia* sp. No. 53.<sup>45</sup> In rhodocardin A, the benz[*a*]anthraquinone skeleton is decorated with three sugar units, rhodinose (**35**), 2-thioglucose (**36**), and glucose, which are joined *via* *O*-glycosidic bonds to C-12b, C-5, and C-4a, respectively. Rhodocardin B contains only the first two sugars. The biosynthesis of rhodocardins has not been studied.

#### 3.2. Group II: Thiosugars containing sulfur as part of a sulfide linkage

Sulfides, or thioethers, are compounds containing C-S-C connectivity. The sulfur atom of a thioether can be alkylated to form a sulfonium ion or oxidized to a sulfoxide and/or sulfone under amiable conditions. However, compared to the free thiols, which display a much higher tendency toward oxidation and stronger nucleophilicity, sulfides are more resistant to chemical reactions and are, therefore, more stable. For this reason, most of the known naturally occurring thiosugars belong to group II. In this class of compounds, the sulfur atom either serves as a linkage between the sugar molecule and the aglycone, or replaces the endocyclic ring oxygen of the parent sugar structure. Compounds within group II are further divided into five subgroups according to their overall chemical structure.

**3.2.1. Thiosugars containing a thiocycle ring**—The first example of this class of compounds, 5-thio-D-glucose, is a synthetic product reported in 1962.<sup>46</sup> Since then, a number of 5-thiopyranoses and 4-thiofuranoses have been prepared as inhibitors against enzymes that utilize the parent natural saccharides as substrates.<sup>43</sup> Isolated from the marine sponge *Clathria pyramida* in 1987, 5-thio-D-mannose (**37**) is the only known free thiocycle-containing monosaccharide identified in nature so far.<sup>47</sup> All other thiosugars of this class are found as part of unusual nucleosides or glycopeptides. It has been speculated that 5-thio-D-mannose may be produced by a sulfate reducing bacterial symbiont of *C. pyramida*, rather than by *C. pyramida*, itself.



The albomycin antibiotics  $\delta_1$ ,  $\delta_2$  and  $\epsilon$  (**38a**, **38b**, and **38c**), isolated from *Streptomyces subtropicus* and several other *Streptomyces* species, contain a 6-amino-6-deoxy-4-thio-heptofuranose uronic acid nucleoside (**39**) and a ferrichrome siderophore moiety (**40**).<sup>48,49</sup> These two subunits are linked to one another *via* a serine residue. The iron-chelating siderophore can be recognized by the bacterial siderophore-dependent iron acquisition system. This allows albomycins to be actively transported across the membranes leading to their designation as “Trojan horse” antibiotics.<sup>50</sup> Next, the albomycin is hydrolyzed by cellular peptidases, such as PepN in *Escherichia coli*, releasing the bioactive nucleoside moiety.<sup>51</sup> The seryl-linked 6-amino-6-deoxy-4'-thio-heptofuranose-5'-methyl-4'-imino-cytosyluronic acid fragment, named SB-217452 (**41**), has been demonstrated to be a potent inhibitor against bacterial seryl-tRNA synthetase (SerRS).<sup>52</sup> It was proposed that this peptide-thioribose-nucleoside conjugate (**41**) mimics an intermediate in the SerRS-catalyzed reaction, thereby inhibiting the aminoacylation activity and disrupting protein biosynthesis. The presence of sulfur in **38** is essential for the observed antimicrobial activity, because the oxygen ring analogue of albomycin is totally inactive.<sup>53</sup>

Chen and co-workers recently reported the identification of the albomycin biosynthetic gene cluster in *Streptomyces* sp. ATCC 700974 (*abmA-R*).<sup>54</sup> Gene deletion and complementation experiments indicated that AbmE catalyzes the  $N^4$ -carbamoylation of cytidine during albomycin biosynthesis. Moreover, AbmI, annotated as a SAM-dependent methyltransferase, was heterologously expressed in *E. coli* and was shown to be a novel cytidine  $N^3$ -methyltransferase.

While the cytidine modification steps have been verified and the biosynthesis of the siderophore moiety is also expected to occur *via* known chemistry, how the 4-thio-heptofuranose uronic acid moiety is biosynthesized is not obvious. Ideas regarding the biosynthesis of the uronic acid moiety itself will be discussed in section 4.8. It is possible that the key sulfur incorporation step proceeds *via* a mechanism analogous to that employed by biotin (**45**) synthase (BioB) during biotin biosynthesis (see Scheme 5).<sup>55,56</sup> BioB is a member of the radical SAM enzyme superfamily.<sup>57</sup> Radical SAM enzymes harbor a [4Fe-4S] cluster that is typically bound by a conserved CX<sub>3</sub>CX<sub>2</sub>C motif. In the active +I redox state, the [4Fe-4S] cluster affects the reductive cleavage of SAM to give methionine and 5'-deoxyadenosyl radical (**42**). The latter is a powerful oxidant that can abstract a hydrogen atom from substrates to yield substrate radicals, which then undergo transformation *via* radical-mediated mechanisms. In BioB, 5'-deoxyadenosyl radical (**42**) abstracts hydrogen from dethiobiotin (**43**) and the resulting substrate radical (**44**) abstracts sulfur from a second [2Fe-2S] cluster that is bound to a conserved CX<sub>2</sub>CX<sub>5</sub>C motif. Since *abmM* appears to encode a radical SAM enzyme with both the [4Fe-4S] and [2Fe-2S] cluster binding motifs, the same sulfur insertion mechanism may be operative in AbmM (**46** → **47**, pathway a). In an alternative hypothetical mechanism, the sulfur is provided by AbmD (a homologue of cysteine desulfhydrase) and incorporated into place by AbmJ, which is also predicted to be a radical SAM enzyme (see Scheme 5, pathway b).

**3.2.2. BE-7585A**—BE-7585A (**48**) from *Amycolatopsis orientalis* subsp. *Vinaria* is a constitutional isomer of the aforementioned type I thiosugar-containing rhodocardin A

(**34**).<sup>58</sup> Both compounds contain the same benz[*a*]anthraquinone core and carry the same three sugar subunits. However, in BE-7585A, 2-thioglucose (**36**) and glucose are joined together as a 1,1'-*O*-linked disaccharide that is attached to the C-5 of aglycone *via* a thioether linkage. Since the strains that produce rhodonocardins and BE-7585A are both *Actinobacteria*, it is possible that the same mechanism of sulfur incorporation is operative during the production of both.

The BE-7585A biosynthetic gene cluster (designated as *bex*) was recently cloned and sequenced.<sup>59</sup> Among all genes in the *bex* cluster, only the *bexX* gene appears to be involved in thiosugar biosynthesis since its encoded protein exhibits sequence similarity to thiazole synthase ThiG (38% identity to ThiG from *Bacillus subtilis* subsp. *Subtilis* str 168). Because ThiG catalyzes the key sulfur incorporation reaction to form the thiazole moiety (**49**) during thiamine biosynthesis (see Scheme 6),<sup>60,61</sup> BexX is expected to perform an analogous function in the construction of 2-thioglucose in the BE-7585A pathway (Scheme 7). This proposal is supported by trapping experiments using recombinant BexX expressed heterologously in *E. coli*.<sup>62</sup> Upon reduction with NaBH<sub>4</sub>, the as-isolated BexX was found to form a reduced dehydration adduct with a hexose monophosphate substrate (derived from **51a–51c**), later determined to be D-glucose-6-phosphate (**50**, D-G6P), *via* an active site lysine residue (K110). When the BexX–D-G6P complex (**51c**) was further derivatized by the carbonyl-specific labeling reagent, hydroxylamine, the appearance of the corresponding imine BexX–D-G6P–NH<sub>2</sub>OH was detected by ESI-MS. These results clearly indicate that BexX is the enzyme responsible for priming the sugar to accept a nucleophilic sulfur donor (**51c** → **52**, Scheme 7). However, no potential sulfur delivery protein, such as ThiS, which works along with ThiG in thiazole biosynthesis (see Scheme 6), is encoded in the *bex* gene cluster. It is possible that one of the sulfur carrier proteins utilized in the biosynthesis of primary metabolites may be recruited as the partner protein of BexX to make 2-thioglucose in the biosynthesis of BE-7585A (**48**).

Since the C-5 position of the angucycline core (**53**) of BE-7585A is highly electrophilic, formation of the thioether linkage may result from a simple Michael addition of the 2-thio group to C-5 of the aglycone followed by a re-aromatization and autoxidation to give **48** (Scheme 8). Such C-S bond formation has been demonstrated *in vitro* during the conversion of urdamycin A to urdamycin E.<sup>63</sup> Interestingly, the 2-thioglucose in rhodonocardin A (**34**) is attached to C-5 of the aglycone *via* an *O*-glycosidic bond. Since a thiol group is a better nucleophile than a hydroxyl group, to avoid the competing thio-addition to the electrophilic C-5 site we propose that sulfur incorporation into rhodonocardin A may occur after the *O*-glycosylation at C-5. As depicted in Scheme 9, starting from **54**, the sulfur incorporation step may follow a similar pathway as seen in the BexX-catalyzed reaction.<sup>59,62</sup>

**3.2.3. Lincosamide-containing antibiotics: lincomycin, celesticetin, and Bu-2545**—Lincomycin A (**55**), isolated from *Streptomyces lincolnensis* culture media,<sup>64,65</sup> displays antibiotic activity against Gram-positive bacteria.<sup>66,67</sup> It has been suggested that lincomycin A blocks protein synthesis by binding to the peptidyltransferase domain of the 50S ribosomal subunit due to its structural resemblance to the 3' end of L-Pro-Met-tRNA and deacylated-tRNA.<sup>68</sup> Lincomycin A contains a 1-thiooctose core known as lincosamine



(**56**),<sup>69</sup> which is also found in Bu-2545 (**57**) from *Streptomyces* strain H230-5<sup>70</sup> and celesticetin (**58**) from *Streptomyces caelestis*.<sup>71,72</sup> For many years, lincomycin A (**55**) and its semi-synthetic chlorinated derivative, clindamycin, were heavily used to treat Gram-positive bacterial infections.<sup>66</sup> Although they are rarely prescribed today due to adverse effects, including diarrhea and colitis, they are still used for patients who are allergic to penicillin and when the infection is caused by bacteria that have developed resistance.<sup>73</sup>

The genes required for lincomycin A biosynthesis have been identified and sequenced in *S. lincolnensis* strains 78–11<sup>74</sup> and ATCC 25466<sup>75</sup> (designated as *lmb*). When *S. coelicolor* was transformed with a cosmid containing the *lmb* cluster from *S. lincolnensis* ATCC 25466, heterologous lincomycin A production was observed.<sup>75</sup> Hybridization analysis using probes designed according to the lincomycin A biosynthetic gene cluster allowed the identification of the celesticetin biosynthetic gene cluster in *S. caelestis* ATCC 1584 (designated as *ccb*).<sup>76</sup>

The two structural components of lincomycin A (**55**), methylthiolincosamide (**59**, MTL) and propylproline (**60**), have been proposed to be biosynthesized separately. Thus far, three enzymes involved in the construction of propylproline unit, LmbB1,<sup>77</sup> LmbB2<sup>77</sup> and LmbJ,<sup>78</sup> have been characterized *in vitro*. Progress has also been made in learning the origin of the lincosamine octose core.<sup>79</sup> The available data regarding the biosynthesis of the octose core will be discussed in section 4.7. Despite these efforts, how the sulfur atom is incorporated into MTL (**59**) is still poorly understood. It has been proposed that the thiomethyl substituent of MTL might be transferred from 5'-thiomethyladenosine (MTH), a side product of polyamine biosynthesis derived from SAM.<sup>80</sup> However, no obvious gene encoding an enzyme capable of catalyzing the transfer of the thiomethyl group can be found in the lincomycin A biosynthetic gene cluster. Thus, the mechanism of C-1 thiol incorporation remains a challenging problem waiting to be resolved.

**3.2.4. Thiosugars found in endiayne-type natural products**—Several unusual 4-thiosugars (**3**, **61**, **62**) have been found as structural components of four endiayne-containing natural products: calicheamicin (**1**) from *M. echinospora* ssp. *Calichensis*,<sup>6</sup> esperamicin (**3**) from *A. verrucosospora*,<sup>7,8</sup> namenamicin (**63**) from *Polysyncraton lithostrotum*,<sup>81</sup> and shishijimicin (**64**) from *Didemnum proliferum*.<sup>82</sup> While the first two are bacterial secondary metabolites, the latter two are isolated from marine invertebrates. On the basis of the analysis of the calicheamicin biosynthetic gene cluster, the translated product of the *calS4* (or *calK*) gene, which encodes a putative C-S lyase, has been proposed to catalyze the conversion of a TDP-2,6-dideoxy-4-ketosugar (**65**) to the corresponding 4-thiosugar (**66**, see Scheme 10).<sup>9,83</sup> However, this hypothesis has not been verified and it is unclear whether other proteins are also required for the sulfur incorporation step.

**3.2.5. Glucosinolates**—Glucosinolates (**67**) are 1-thiosugar-containing natural products produced by plants and are utilized for defense against insects and pathogens.<sup>84</sup> Generally, damage to the plant tissues will trigger the hydrolysis of the C-S glycosidic bond of glucosinolates by myrosinases to release unstable thiohydroximate-*O*-sulfates.<sup>85,86</sup> The sulfate moiety is then lost *via* a spontaneous Lossen rearrangement resulting in the formation

of various sulfur-containing compounds including isothiocyanates, which are toxic to the invaders.

The production of glucosinolates is limited to species of the order *Brassicales*, which include economically important *Brassica* crops, such as cabbage, and the model organism *Arabidopsis*.<sup>84</sup> Structurally, glucosinolates are  $\beta$ -thioglucoside *N*-hydroxysulfates containing a side chain that is derived from an amino acid. The biosynthesis of glucosinolates has been extensively investigated and reviewed.<sup>85–88</sup> As depicted in Scheme 11, the initial step is the oxidation of the amino acid precursor (**68**) by a cytochrome P450 monooxygenase of the CYP79 family. A second oxidation of the resulting aldoxime (**69**) catalyzed by an enzyme of the CYP83 family generates activated nitrile oxide (**70**) or *aci*-nitro (**71**) intermediates. The critical sulfur incorporation step has been proposed to occur non-enzymatically with cysteine as the sulfur donor. Subsequent cleavage of the thio-conjugate (**72**) by PLP-dependent *C-S* lyase SUR1 yields thiohydroximate (**73**), which is then glycosylated by UDP-*S*-glucosyltransferase to give **75**. A final sulfonation reaction catalyzed by a sulfotransferase completes the biosynthesis of the glucosinolate core (**67**).

Although cysteine has long been considered to be the most probable sulfur donor in glucosinolate biosynthesis, recent reports have indicated that glutathione (GSH) might be a more plausible sulfur donor.<sup>89,90</sup> One piece of evidence supporting this possibility was provided by experiments using mutants impaired with respect to GSH biosynthesis. These mutants accumulated a reduced level of glucosinolates upon herbivore challenging and fungal attack, which was restored upon feeding with GSH.<sup>89</sup> Moreover, assuming cysteine serves as the sulfur donor, the resulting thio-conjugate (**72**) would contain a free amino group, which is required for the subsequent PLP-dependent cleavage by *C-S* lyase SUR1 (**72** → **73**). On the other hand, if GSH serves as the sulfur donor, the  $\gamma$ -glutamyl moiety of the conjugate (**76**) would have to be hydrolyzed to yield a free amino group prior to cleavage by *C-S* lyase SUR1. In fact, engineering experiments of *Nicotiana benthamiana* have shown that co-transformation of  $\gamma$ -glutamyl peptidase 1 (GGP1) together with the genes for glucosinolate biosynthesis reduces GSH-conjugate accumulation and increases the production of glucosinolates.<sup>91</sup> The hypothesis that GSH is the sulfur donor has prompted the search for a specific GSH-*S*-transferase (GST) that might catalyze the sulfur incorporation step.<sup>92</sup>

### 3.3. Group III: Thiosugars containing a sulfonium ion

Several well-known sulfonium-containing compounds including SAM, *S*-methylmethionine, and dimethylsulfoniopropionate (DMSP) play crucial roles in living systems.<sup>93</sup> For example, DMSP from marine algae serves not only as the major source of reduced sulfur for marine microorganisms, but also as the precursor of dimethylsulfide, the primary sulfur source in the global atmosphere.<sup>94</sup>

Salacinol (**77**)<sup>95</sup> and kotalanol (**78**)<sup>96</sup> isolated from the medicinal herb *Salacia reticulata* are both naturally occurring thiosugars that contain a sulfonium ion moiety. Several analogues, including ponkolanol (**79**) and salaprinol (**80**),<sup>97</sup> were later found from other *Salacia* species. The unique zwitterionic structure of salacinol (**77**) and its analogues includes a 1-

deoxy-4-thio-D-arabinofuranosyl cationic core and 1-deoxy-aldosyl-3-sulfate as the anionic counter ion. These compounds exhibit high  $\alpha$ -glucosidase inhibitory activity and have consequently emerged as anti-diabetic drug candidates.<sup>95–97</sup> While extensive structure-activity relationship studies have been carried out,<sup>43,98</sup> the steps involved in the biosynthetic formation of these sulfonium-containing sugars remain unknown. Since methionine serves as a precursor for the biosynthesis of SAM, S-methylmethionine, and DMSP, it is possible that methionine is also a precursor in the biosynthesis of salacinol, kotalanol, ponkolanol and salaprinol.<sup>93</sup>

### 3.4. Group IV: Thiosugars containing a sulfonic acid group

In 1959, Benson and coworkers carried out a series of radioisotope labeling experiments in higher plants leading to the discovery of sulfoquinovosyl diacylglycerol (**81**, SQDG) as a minor component in chloroplast membranes.<sup>99,101</sup> SQDG contains an unusual sulfosugar moiety and comprises about 4–7% of total lipids in most photosynthetic organisms including algae, plants and cyanobacteria.<sup>101,102</sup> Subsequent studies using [<sup>35</sup>S]-sulfate traced the sulfolipid biosynthetic pathway to a nucleotide-activated sulfoquinovose intermediate (**82**, see Scheme 12).<sup>103</sup> Through genetic analysis of the sulfolipid-producing purple bacteria, *Rhodobacter sphaeroides*,<sup>104,105</sup> a small gene cluster with only four genes, *sqdA*, *sqdB*, *sqdC* and *sqdD*, was recently identified as the likely, the long-sought after sulfolipid biosynthetic gene cluster. Orthologues of *sqdB* are universally found in other SQDG-producing organisms. It was proposed that SqdB catalyzes the synthesis of NDP-sulfoquinovose (**82**) based on its sequence similarity to other NDP-sugar modification enzymes.<sup>106</sup> The gene sequence of *sqdB* was utilized to isolate cDNA of the orthologous *SQD1* gene in *Arabidopsis thaliana*.<sup>107</sup> SQD1 that had been recombinantly expressed in *E. coli* could catalyze the insertion of inorganic sulfite into UDP-glucose (**74**). In addition, an attempt to purify the native SQD1 protein from spinach led to the finding that SQD1 forms a tight complex with ferredoxin-dependent glutamate synthase (FdGOGAT) *in vivo*.<sup>108</sup> The exact function of this large SQD1-FdGOGAT complex and the detailed mechanism of sulfonyl incorporation awaits further investigation.

## 4. Compounds Containing High-carbon Sugars

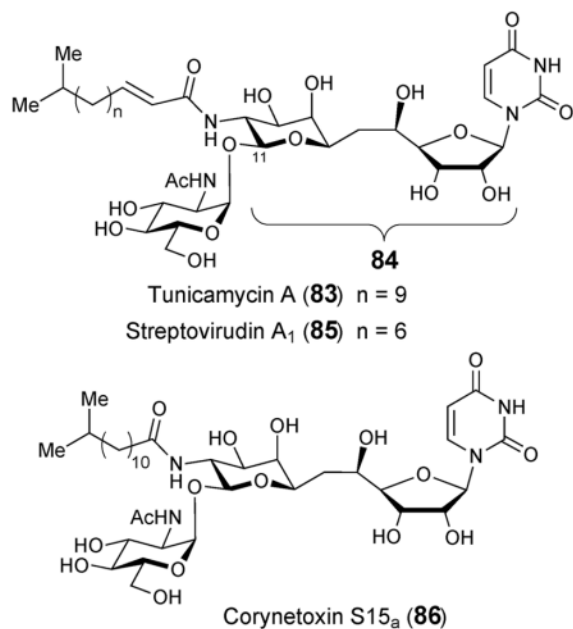
Unusual sugars with high carbon numbers are found in an array of microbially produced antibiotics<sup>109</sup> and in the outer walls of some Gram-negative bacteria. They are formed *via* condensation reactions between the C-5 and C-6 sugars of primary metabolism, or derivatives thereof, and an exogenous carbon source. The known mechanisms employed for condensation during the biosynthesis of high-carbon sugars are as varied as the mechanisms for biological C–C bond formation in general. In many cases, the mechanism of sugar chain elongation remains poorly understood.

### 4.1. Tunicamine containing nucleoside antibiotics

Tunicamycin (**83**) is a nucleoside antibiotic that was first isolated from culture media of *Streptomyces lysosuperificus* and subsequently *Streptomyces chartreusis* NRRL 3882.<sup>110,111</sup> Tunicamycin is extracted as a complex composed of closely related components having a general structure that includes an unusual 11-carbon aminodialdlose core, known as

tunicamine (**84**), joined to *N*-acetylglucosamine (GlcNAc), uracil, and an amide-linked fatty acid.<sup>112</sup> Streptovirudins (**85**) and corynetoxins (**86**), isolated from *Streptomyces griseoflavus*<sup>113</sup> and *Corynebacterium rahayi*,<sup>114</sup> respectively, are structurally homologous to tunicamycin as are antibiotics MM19290,<sup>115</sup> mycospocidin,<sup>116</sup> and antibiotic 24010.<sup>117</sup> The latter three, however, were isolated as complexes composed of multiple components for which detailed structures remain uncharacterized.

Tunicamycin (**83**) and related antibiotics disrupt assembly of the bacterial cell wall via inhibition of phospho-*N*-acetylmuramoyl-pentapeptide transferase (MraY), an enzyme involved in the biosynthesis of the peptidoglycan precursor lipid I.<sup>118</sup> The fact that tunicamine-containing compounds can also block *N*-linked glycoprotein synthesis by inhibiting UDP-*N*-acetylglucosamine: dolichol phosphate GlcNAc-1-P transferase (also known as GlcNAc-1-P translocase or GPT) in eukaryotes has precluded their use in clinical settings.<sup>119</sup> In this case, The  $\alpha,\beta$ -1,1-glycosidically-linked GlcNAc moiety of tunicamycin is thought to resemble the transferred GlcNAc-1-phosphate during the transition state of the GPT reaction.<sup>120</sup> Interestingly, tunicamycin has also been shown to block palmitoylation of acyl proteins by inhibition of palmitoyl transferase.<sup>121</sup>



The biosynthesis of the tunicamycin-like antibiotics is only partially understood. The steps leading to the unique tunicamine core structure (**84**) are of particular interest as they are predicted to include an unusual C–C bond coupling reaction. Labeling studies with [2-<sup>14</sup>C]-uridine, [6,6-<sup>2</sup>H<sub>2</sub>]-D-glucose, and D-[1-<sup>13</sup>C]-glucose showed that the tunicamine core is derived from uridine and UDP-GlcNAc (**87**).<sup>122</sup> This led to the proposal of a tentative biosynthetic pathway for tunicamine in which uridine 5'-aldehyde (**90**) undergoes condensation with UDP-4-keto-6-deoxy-GlcNAc-5,6-ene (**88**, see Scheme 13A).

The *S. chartreusis* genome was recently sequenced and a gene cluster for tunicamycin biosynthesis was identified by comparative bioinformatics.<sup>123</sup> Subsequent introduction of

the gene cluster into *Streptomyces coelicolor* confirmed heterologous production. The clustered genes include 14 open reading frames, designated *tunA-N*, which the authors claimed to comprise the minimal set of genes required for tunicamycin biosynthesis. A similar gene cluster is present in the MM19290 producer *S. clavuligerus* and in *Actinosynnema mirum*, although the latter strain has not been identified as a producer of tunicamine-containing compounds. Within the gene cluster, TunB is identified as a putative member of the radical SAM enzyme superfamily. It was proposed to catalyze the oxidation of uridine (**89**) to uridine-5'-aldehyde (**90**, see Scheme 13A).<sup>123</sup> TunA and F, which are homologous to NAD-dependent epimerase/dehydratase and UDP-GlcNAc 4-epimerase, respectively, are likely responsible for the conversion of UDP-GlcNAc (**87**) to UDP-4-keto-6-deoxy-GlcNAc-5,6-ene (**88**). TunM, a SAM-dependent methyltransferase homologue, was postulated to catalyze the subsequent condensation of this product (**88**) with uridine-5'-aldehyde (**90**) to yield UDP-*N*-acetyltunicamine-uracil adduct (**91**, Scheme 13A).

The *S. chartreusis* tunicamycin biosynthetic gene cluster was independently identified using a "high-throughput heterologous expression" approach in which culture media from many strains of *Streptomyces lividans* transformed with plasmids from a *S. chartreusis* genomic library were screened for antibiotic activity.<sup>124</sup> In this study the authors identified *tunA-L* (excluding *tunM* and *N*) as the minimal set of genes required for tunicamycin biosynthesis. This led to a more reasonable proposal that TunB catalyzes the condensation of uridine (**89**) with UDP-6-deoxy-GalNAc-5,6-ene (**93**) to yield UDP-*N*-acetyltunicamine-uracil (**91**, Scheme 13B). This hypothesis is supported by the finding that a TunB knockout strain accumulates UDP-6-deoxy-GlcNAc-5,6-ene in culture media.

The *in vitro* activities of purified recombinant proteins, TunA and TunF, from *S. chartreusis* were recently reported.<sup>125</sup> TunA is homologous to TDP-glucose 4,6-dehydratase, which carries out the conversion of TDP-glucose to TDP-4-keto-6-deoxy-glucose (**5**) during the biosynthesis of 6-deoxyhexoses.<sup>1,2</sup> However, unlike a typical 4,6-dehydratase-catalyzed reaction involving an internal hydrogen transfer from C-4 to C-6, the TunA catalytic cycle is concluded by a 1,2-hydride addition to the 4-keto group of the 4-keto-6-deoxy-GlcNAc-5,6-ene intermediate (**88**), rather than 1,4-hydride addition to the adjacent C5-C6 double bond, to give UDP-6-deoxy-GlcNAc-5,6-ene (**92**) as the product (Scheme 13B).

A 1.9 Å crystal structure of TunA in complex with UDP-GlcNAc (**87**) and NAD<sup>+</sup> was also determined.<sup>125</sup> Comparisons were drawn between the crystal structures of TunA and other 4,6-dehydratases, which all appear highly similar. It appears that the orientation of the nicotinamide ring in TunA, which places C-4 of NAD<sup>+</sup> toward C-4 of UDP-GlcNAc and away from C-6, serves as a "regioselective switch" that determines the identity of the product. On the basis of these results, a biosynthetic pathway was put forward in which UDP-6-deoxy-GalNAc-5,6-ene (**93**), produced from UDP-GlcNAc (**87**) by the successive oxidation and epimerization reactions of TunA and TunF, is joined with 5'-uridine radical (**94**) by TunB (with possible involvement of TunM) to give UDP-*N*-acetyltunicamine-uracil (**91**, Scheme 13B).<sup>125</sup> It was also proposed that epimerization of UDP-6-deoxy-GlcNAc-5,6-ene (**92**) to UDP-6-deoxy-GalNAc-5,6-ene (**93**) by TunF is needed to place  $\sigma^*(\text{C}8'-\text{O}8')$  LUMO to be parallel to C7' SOMO in 7'-UDP-*N*-acetyltunicamine-uracil, thereby stabilizing this transient radical intermediate (**95**).

## 4.2. Liposidomycin, caprazamycin, muraymycin, A-90289A and B, and FR900493

Liposidomycin (**96**),<sup>126</sup> caprazamycin (**97**),<sup>127,128</sup> muraymycin (**98**),<sup>129</sup> A-90289A (**99**) and B,<sup>130</sup> and FR900493 (**100**)<sup>131</sup> belong to a class of structurally and biosynthetically related antibiotics that contain a seven-carbon 5'-C-glycyluridine core (**101**). Like tunicamycins (**83**), they are potent inhibitors of *MraY*. Gene clusters have recently been identified for the biosynthesis of liposidomycin in *Streptomyces* sp. SN1061M (*lpmA-Y*),<sup>132</sup> caprazamycin in *Streptomyces* sp. MK730-62F2 (*cpz9-31*),<sup>133</sup> muraymycin in *Streptomyces* sp. NRRL 30471 (*mur11-36*),<sup>134</sup> and A-90289 in *Streptomyces* sp. SANK 60405 (*lipA-B1*).<sup>130</sup> A gene likely encoding serine hydroxymethyltransferase (SHMT) is found in all four clusters. The well established function of SHMT is to catalyze the reversible formation of  $\beta$ -hydroxy- $\alpha$ -amino acids from an aldehyde and glycine.<sup>135</sup> Thus, these SHMT homologues (*lpmL*, *cpz14*, *mur17*, and *lipK*) are thought to catalyze condensation between uridine 5'-aldehyde (**90**) and the PLP-bound glycine to give **101** (Scheme 14).

It was previously proposed that uridine is oxidized to uridine 5'-aldehyde by the alcohol dehydrogenase homologues encoded by *lpmW*, *cpz25*, *lipV* (the corresponding gene is absent in the muraymycin gene cluster). Surprisingly, however, LipL, an iron(II)-dependent  $\alpha$ -ketoglutarate dioxygenase, was found to be the actual enzyme catalyzing the oxidation of UMP to uridine 5'-aldehyde (**90**).<sup>136</sup> Iron,  $\alpha$ -ketoglutarate, and oxygen are necessary for the activity of this enzyme. Thus, *LpmM*, *Cpz15*, *Mur16*, which are LipL homologues, likely serve an analogous role in each respective pathway (Scheme 14). Interestingly, the gene clusters for the 5'-C-carbamoyluridine-containing *MraY* inhibitors, A-500359 from *Streptomyces griseus* SANK 60196 (*orf1-38*)<sup>130</sup> and A-503083 from *Streptomyces* sp. SANK 62799 (*capA-W*),<sup>130</sup> also harbor genes encoding LipL homologues (*Orf7* and *CapA*). This observation suggests that 5'-C-glycyluridine (**101**) could be an intermediate in the biosynthesis of these antibiotics as well. Additional high-carbon nucleoside antibiotics including A-94964 from *Streptomyces* sp. SANK 60404<sup>137</sup> and A-102395<sup>138</sup> from *Amycolatopsis* sp. SANK 60206, for which genetic data are currently unavailable, are likely produced by similar pathways.

## 4.3. Octosyl acids and ezomycins

Octosyl acids A (**102**), B (**103**), and C (**104**) are bicyclic, anhydrooctose uronic acid nucleosides isolated from media of *Streptomyces cacaoi* var. *asoensis*.<sup>139</sup> This strain also produces polyoxins (**105**, **106**), a class of nucleoside antibiotics that are active against phytogetic fungi *via* inhibition of chitin synthase.<sup>140</sup> Isotopic labeling studies suggested that octosyl acids and polyoxins may be derived from a common octofuranulose uronic acid nucleoside intermediate (**107**). Experiments in which *S. cacaoi* was fed [U-<sup>14</sup>C]-uridine or [1-<sup>13</sup>C]-glucose revealed that uridine is incorporated intact during the biosynthesis of polyoxin C (**105**) and that glucose is not a direct precursor.<sup>141</sup> In addition, C-3 of pyruvate and glycerate were shown to be the source of C-6'. When [1-<sup>13</sup>C]- or [6-<sup>13</sup>C]-glucose was fed to an *S. cacaoi* mutant that accumulates polyoxin C and octosyl acid, <sup>13</sup>C enrichment patterns for both compounds were similar.<sup>142</sup> Taken together, these observations led to a hypothesis that the biosynthesis of both polyoxins and octosyl acids involves a condensation reaction between phosphoenolpyruvate (PEP, **108**) and a uridine derivative (possibly uridine 5'-aldehyde (**90**), see Scheme 15) to yield the proposed octofuranulose uronic acid



nucleoside (**107**). In this case, cyclization followed by reduction at C-7' would yield the bicyclic anhydrooctose uronic acid of octosyl acids. Carbons derived from C-1 and C-2 of PEP are lost during polyoxin biosynthesis.

Nikkomycins (**109–111**) comprise a class of chitin synthase inhibitors produced by *Streptomyces tendae* and *Streptomyces ansochromogenes* that are biosynthetically and structurally related to polyoxins.<sup>143</sup> Four nikkomycin homologues, nikkomycin S<sub>Z</sub>, So<sub>Z</sub>, S<sub>X</sub>, and So<sub>X</sub> isolated from *S. tendae* culture media contain the same bicyclic anhydrooctose uronic acid found in octosyl acid A (**102**).<sup>144</sup> Therefore, the octosyl acids and nikkomycins S<sub>Z</sub>, So<sub>Z</sub>, S<sub>X</sub>, and So<sub>X</sub> have been regarded as the “shunt metabolites” of the biosynthetic pathways for polyoxin and nikkomycin biosynthesis, respectively.

Biosynthetic gene clusters involved in the production of polyoxins in *S. cacaoi* (*pol*) and nikkomycins in *S. tendae* (*nik*) and *S. ansochromogenes* (*san*) have recently been identified.<sup>145,146</sup> The orthologous enzymes encoded by *nikO*<sup>147,148</sup> and *polA*<sup>145</sup> have been shown to catalyze the formation of 3'-enoylpyruvoyl-UMP from PEP (**108**) and UMP in assays with purified recombinant proteins. Gene knockout experiments demonstrated that *nikO*<sup>149</sup> and *sanX*<sup>146</sup> are required for the biosynthesis of nikkomycins and that *polA* is required for the biosynthesis of polyoxins.<sup>145</sup> Presumably the corresponding orthologs are also involved in the biosynthesis of octosyl acids and nikkomycins S<sub>Z</sub>, So<sub>Z</sub>, S<sub>X</sub>, and So<sub>X</sub>. Although PEP was previously shown to be the source of C6' in polyoxin C (**105**),<sup>141</sup> the demonstrated activity of NikO and PolA came as a surprise given the hypothesis that PEP undergoes condensation with uridine 5'-aldehyde (**90**). The biosynthetic steps responsible for the conversion of 3'-enoylpyruvoyl-UMP (**112**) to the aminohexuronic acid moiety in nikkomycins and polyoxins remain to be demonstrated.<sup>150</sup> Furthermore, it is also unclear whether the octosyl acids and nikkomycins S<sub>Z</sub>, So<sub>Z</sub>, S<sub>X</sub>, and So<sub>X</sub> are shunt metabolites or precursors to polyoxins and nikkomycins.

Ezomycins (**113**) comprise another set of antibiotics that contain octosyl acid.<sup>151–153</sup> They were isolated from culture filtrates of a species similar to *Streptomyces kitazawaensis*, and are toxic to the phytopathogenic fungus *Sclerotinia sclerotiorum*. To date, no information is available regarding the biosynthesis of ezomycins.

#### 4.4. Mildiomycin

Mildiomycin (**114**) is an antimicrobial peptidyl nucleoside antibiotic produced by *Streptovorticillium rimofaciens* that inhibits protein synthesis by blocking the peptidyltransferase site on the large ribosomal subunit in both eukaryotic and prokaryotic organisms.<sup>154,155</sup> It is structurally similar to the antibiotics blasticidin S (**115**),<sup>156</sup> arginomycin (**116**),<sup>157</sup> gougerotin (**117**),<sup>158</sup> and bagougeramines A (**118**) and B,<sup>159</sup> which all share a common core structure. Mildiomycin, however, contains a unique decose carbon skeleton. The identification and subsequent sequencing of the blasticidin S biosynthetic gene cluster in *Streptomyces griseochromogenes* (*blsA-S*)<sup>160</sup> laid the foundation for the discovery of the mildiomycin biosynthetic gene cluster (*milA-Q*).<sup>161</sup> Several initial steps regarding the biosynthesis of mildiomycin have been demonstrated (see Scheme 16). MilA was previously shown to catalyze the hydroxymethylation of C-5 in CMP in the presence of formaldehyde

and tetrahydrofolate to give hydroxymethylcytosine (**119**) and MilB could hydrolyze the *N*-glycosidic bond in CMP to give **120**.<sup>162</sup> Assays with purified, recombinant MilC further demonstrated its ability to catalyze the formation of cytosylglucuronic acid (CGA, **121**) from cytosine and UDP-glucuronic acid (**123**).<sup>161</sup> MilG, a member of the radical SAM enzyme superfamily, is thought to be involved in the formation of the 2,3-deoxy-4-keto-2,3-ene-sugar moiety (**124**) of mildiomycin, since the *milG* disruption mutant accumulated 5-hydroxymethyl-CGA (**122**) in the fermentation broth. *In vitro* labeling experiments using [U-<sup>13</sup>C]-arginine or [guanidino-<sup>13</sup>C]-4-hydroxyarginine showed that the former, but not the latter, was incorporated into mildiomycin. Therefore, the 5-guanidino-2,4-dihydroxyvalerate moiety in mildiomycin is derived from arginine (**125**). MilM and MilN are homologous to aspartate aminotransferase and dihydropicolinate synthetase, respectively. MilM is proposed to catalyze the deamination of arginine (**125**) to give  $\alpha$ -keto- $\delta$ -guanidinovalerate (**126**), which is subsequently coupled to the nucleoside by MilN. Finally, the hydroxylation of mildiomycin at C-8' may occur after the formation of the decose sugar (see Scheme 16).

#### 4.5. Griseolic acids

Griseolic acids A (**127**), B (**128**) and C (**129**) are cyclic AMP analogues that contain an unusual nine-carbon bicyclic sugar, dicarboxylate glycone.<sup>163,164</sup> They were identified and isolated from culture media of *Streptomyces griseoaurantiacus* during a search for inhibitors of cyclic nucleotide phosphodiesterase. A ring-opened griseolic acid analogue (**130**) lacking the inhibitory activity was subsequently isolated from *S. griseoaurantiacus* media.<sup>165</sup> Feeding experiments showed that adenosine (**131**) is a biosynthetic precursor for griseolic acid A.<sup>166</sup> Incorporation of <sup>13</sup>C from [2-<sup>13</sup>C]-acetate into C-6', 7', 8', and 9' of **127** suggests that these carbons could originate from a dicarboxylate intermediate derived from the citric acid cycle. Since cells fed [1,4-<sup>13</sup>C]-succinate incorporated <sup>13</sup>C at the carboxylate carbons, oxaloacetate (**132**) has been proposed as a possible donor of the four-carbon dicarboxylic acid in griseolic acid A (see Scheme 17).

#### 4.6. Sinefungin and dehydrosinefungin

The *S*-adenosyl-L-methionine analogues sinefungin (A9145) (**133**) and dehydrosinefungin (A9145C) (**134**), were first isolated from the culture media of *Streptomyces griseolus*.<sup>167,168</sup> Sinefungin displays antifungal and antiviral activity as well as activity against trypanosomal pathogens including malaria.<sup>169–171</sup> Both radiotracer feeding experiments and cell-free assays have provided preliminary clues regarding sinefungin biosynthesis. However, the genes and enzymes involved in the process remain unidentified.

Feeding experiments in which a panel of <sup>14</sup>C-labeled metabolites was added to *S. griseolus* culture indicated that sinefungins are derived from ornithine (**135**) and an adenosine derivative.<sup>172</sup> Cell-free assays with *Streptomyces incarnatus* NRRL 8089, which also produces sinefungin, led to the observation that radiolabel from ATP or adenosine and arginine (**125**), rather than ornithine, are incorporated most efficiently into sinefungin (the arginine guanidino group was not incorporated).<sup>173</sup> Addition of pyridoxal 5'-phosphate, Mg<sup>2+</sup>, and dithiothreitol enhanced sinefungin production during this assay. Further *S. griseolus* feeding experiments with site-specifically labeled ornithine showed that the C-6' and the 6' amino group of sinefungin (**133**) are derived from the C-5 and the C-5 amino

group of ornithine (**135**, see Scheme 18).<sup>174</sup> In this study, the authors observed equal label incorporation with both arginine and ornithine as substrates and pointed out that ornithine is a precursor for arginine *in vivo*. Tritium from [5*R*-<sup>3</sup>H, 5-<sup>14</sup>C]-L-ornithine but not [5*S*-<sup>3</sup>H, 5-<sup>14</sup>C]-L-ornithine was incorporated into sinefungin during feeding experiments. Since the configuration at C-6' of sinefungin is *S*, the C-C bond formation at C-5 of ornithine to form sinefungin must occur with inversion of configuration (Scheme 18). This conclusion places sinefungin biosynthesis among a small number of PLP-dependent processes that do not proceed with retention of configuration.

When *S. griseolus* cell lysate was incubated with a blend of [5' (*RS*)-5'-<sup>3</sup>H]-ATP and [8-<sup>14</sup>C]-ATP, together with Mg<sup>2+</sup> and PLP, both labels were incorporated into sinefungin.<sup>175</sup> The same result was obtained with a blend of [5' (*RS*)-5'-<sup>3</sup>H]-adenosine and [U-<sup>14</sup>C]-adenosine or [1-<sup>14</sup>C]-ribose and [8-<sup>3</sup>H]-adenine. This suggests that adenosine is catabolized by a nucleoside hydrolase or nucleoside phosphorylase prior to sinefungin biosynthesis. When the assay was conducted with a blend containing a 4:1 ratio of [5' (*RS*)-5'-<sup>3</sup>H]-adenosine to [1-<sup>14</sup>C]-adenosine, the same ratio of <sup>3</sup>H:<sup>14</sup>C was observed in the product. This result implies that the ribose moiety of adenosine is incorporated intact into sinefungin and that tritium is not lost from ribose C-5' during sinefungin formation, thereby ruling out dehydrosinefungin (**134**) as a plausible biosynthetic intermediate. When cell-free assays were carried out with added PLP and either [1-<sup>14</sup>C]-ribose or [U-<sup>14</sup>C]-arginine, but neither ATP, adenosine, nor adenine, radiolabel was incorporated into an unknown compound designated "X". Radiolabeled sinefungin was observed when compound "X" was added to cell lysate that had been supplemented with adenine, suggesting that "X" could be a sinefungin precursor (see Scheme 18).

More recently, a rifamycin-resistant mutant strain of *S. incarnatus* that overproduces sinefungin was generated by UV irradiation.<sup>176</sup> The strain was designated rif-400. The production of sinefungin by rif-400 was further enhanced by adding L-arginine to the culture media, but not D-arginine or L-ornithine. Addition of urea diminished the yield of sinefungin. Addition of L-arginine, but not L-ornithine, to rif-400 culture media, resulted in the accumulation of ornithine- $\delta$ -lactam in the media.<sup>176</sup> An enzyme that catalyzes the conversion of L-arginine to ornithine- $\delta$ -lactam, with release of urea, was purified to homogeneity from rif-400 lysate. The enzyme did not utilize D-arginine or L-ornithine as substrates. It did, however, convert NAD<sup>+</sup> to NADH in the presence of alanine, consistent with *N*-terminal amino acid sequencing results, which suggested that the enzyme is homologous to L-alanine dehydrogenase. It remains unclear whether or not ornithine- $\delta$ -lactam is truly an intermediate in sinefungin biosynthesis.

#### 4.7. Lincosamide-containing antibiotics: lincomycin, celesticetin, and Bu-2545

The lincosamide-containing antibiotics were introduced earlier in section 3.2.3. Here, the currently available data regarding the construction of the octose core will be discussed. Feeding experiments were conducted in order to identify the biosynthetic origin of the octose sugar in lincomycin A (**55**). When *S. licolnensis* was grown with a blend of unlabeled and [<sup>13</sup>C<sub>6</sub>]-glucose as the sole carbon source, the <sup>13</sup>C labeling pattern of the resulting  $\alpha$ -methylthiolincosamide (MTL, **56**) suggested that MTL is formed *via* the condensation of a

three-carbon (C<sub>3</sub>) and a five-carbon (C<sub>5</sub>) unit derived from glucose.<sup>79</sup> This led to a proposal that the C<sub>5</sub> unit might be a pentose 5-phosphate from the pentose phosphate pathway and that the C<sub>3</sub> unit might be derived from D-sedoheptulose 7-phosphate (**137**) and joined to the C<sub>5</sub> unit by a transaldolase-catalyzed reaction analogous to that involved in the pentose phosphate pathway. Interestingly, both the lincomycin (**55**) and celesticetin (**58**) biosynthetic gene clusters encode a putative transaldolase (LmbR and CcbR) that may catalyze an intermolecular aldol condensation to produce the octose sugar precursor to lincosamine (**56**).<sup>47–76</sup> This hypothesis has been substantiated by a recent experiment in which the octose intermediate is shown to be formed *via* a transaldol reaction catalyzed by LmbR using D-fructose 6-phosphate (**136**) or D-sedoheptulose 7-phosphate (**137**) as the C<sub>3</sub> donor and D-ribose 5-phosphate (**138**) as the C<sub>5</sub> acceptor (Scheme 19). Subsequent 1,2- isomerization catalyzed by LmbN converts the resulting octulose 8-phosphate (**139**) to octose 8-phosphate (**140**). These results provide, for the first time, *in vitro* evidence revealing the biosynthetic origin of the eight-carbon backbone of MTL (**56**).<sup>177</sup>

#### 4.8. Albomycin

Albomycin (**38**) is first mentioned in section 3.2.1. Although currently there is no biochemical data regarding the biosynthesis of the 4-thioheptouronic acid moiety in albomycin, three possible pathways have been proposed based on sequence analysis of the albomycin biosynthetic gene cluster.<sup>54</sup> As illustrated in Scheme 20, in pathway A the reaction is initiated by the nucleophilic addition of cysteine sulfur to C-3 of PEP (**108**) catalyzed by the cysteine desulfurase homologue AbmD to form a thioether adduct (**141**). This is then followed by cyclization, oxidative decarboxylation, and deamination *via* the combined actions of AbmM and J, both of which are members of the radical SAM superfamily, and AbmF and L, both of which are hypothetical proteins of unknown function, to give 1-phospho-4-thio-*ribo*-pentodialdose (**142**). The aldehyde would then undergo aldol condensation with glycine catalyzed by AmbH, which is a SHMT homologue, to yield 6-amino-6-deoxy-1-phospho-4-thio-heptofuranose uronic acid (**47**) as the product.<sup>135</sup> The final step is similar to the condensation reaction proposed for the SHMT homologues LpmL, Cpz14, Mur17, and LipK in the biosynthesis of liposidomycin, caprazamycin, muraymycin, and A-90289, respectively (**90** → **101**, see Scheme 14). An analogous reaction sequence is also proposed for pathway B where homocysteine instead of cysteine is used as substrate in an AbmD-catalyzed reaction. In the third proposed pathway, either serine or glycine is joined to threose or ribose, respectively, *via* an intermolecular aldol condensation reaction catalyzed by AbmH. This is followed by sulfur insertion by AbmD and AbmJ, and cyclization by the combined actions of AbmF, M, and L to give 6-amino-6-deoxy-4-thio-heptofuranose uronic acid (**143**), which could then be phosphorylated at C-1 by the deoxynucleoside kinase homologue AbmG to give **47**.

#### 4.9. Apramycin

Apramycin (**144**) is an aminoglycoside antibiotic that contains an unusual bicyclic octose sugar (**145**).<sup>178,179</sup> Labeling studies have indicated that the carbon atoms from C-1' to C-6' of the octose moiety are derived from glucose while C-7' and C-8' may originate from C-2 and C-3 of pyruvate.<sup>180</sup> Gene clusters for the biosynthesis of apramycin in

*Streptoalloteichus tenebrarius* DSM 40477T and *Streptoalloteichus hindustanus* DSM 44523T have been identified and sequenced (designated *apr*). During the biosynthesis of the aminoglycoside antibiotics neomycin and butirosin, the C-6' hydroxyl group of the common biosynthetic intermediate, paromamine (**146**), is oxidized to an aldehyde (**147**) by a FAD-dependent dehydrogenase (Neo11 and BtrQ). The resulting aldehyde is then converted to an amine (**148**) by a PLP-dependent transaminase (Neo18 and BtrB, see Scheme 21).<sup>181</sup> While the apramycin biosynthetic gene cluster encodes the homologous protein of paromamine 6'-dehydrogenase (AprQ), the gene encoding the potential transaminase is absent as expected. It is possible that the resulting C-6' aldehyde (**149**) may undergo an aldol condensation with a two-carbon unit to give the octose sugar (**150**, Scheme 21).<sup>182</sup> However, the precise origin of C-7' and C-8' and the mechanism of the octose formation remain unknown.

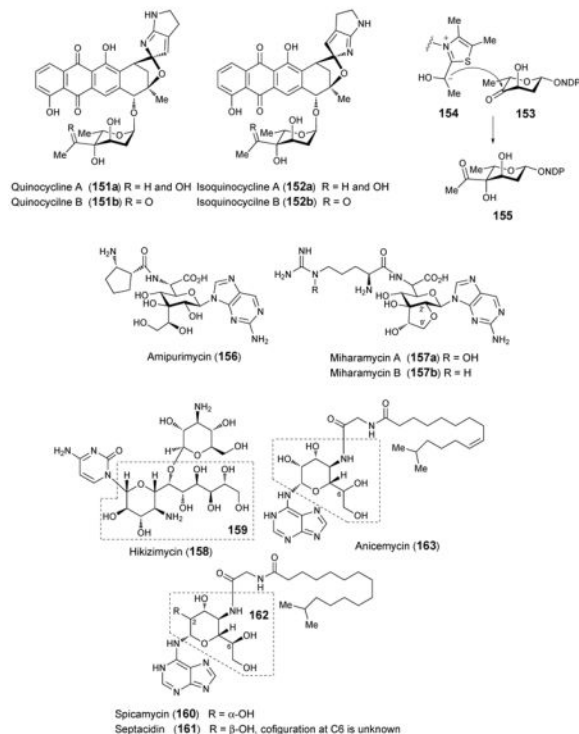
#### 4.10. Quinocyclines

Quinocyclines A and B (**151a** and **151b**) and isoquinocyclines A and B (**152a** and **152b**) are a complex of antitumor antibiotics from *Streptomyces aurofaciens*.<sup>183</sup> Each contains a branched octose sugar substituent.<sup>184</sup> Quinocycline B (konosinostatin) and isoquinocycline B were also found in culture media of *Micromonospora* sp. TP-A0468.<sup>185</sup> Feeding experiments demonstrated that [2-<sup>14</sup>C]-pyruvate was incorporated more efficiently into the branched octose than [1-<sup>14</sup>C]-acetate.<sup>186</sup> Thus, a mechanism in which an NDP-linked 4-keto-2,6-dideoxy-L-erythropryanose (**153**) undergoes condensation at C-4 with the hydroxyethyl group from hydroxyethyl-TPP (**154**) was proposed for the production of the branched octose (**155**). A similar transformation has been observed in the biosynthesis of yersinose A (**171**, see Scheme 23).<sup>187</sup> Furthermore, the efficient reduction of quinocycline B to quinocycline A by cultured *S. aurofaciens* strongly suggests that (iso)quinocycline B is the biosynthetic precursor of (iso)quinocycline A.

#### 4.11. Other antibiotics with high-carbon sugars

Several additional antibiotic compounds carrying high-carbon sugars with no information regarding their biosynthetic pathways or the genes involved will only be briefly mentioned here. Amipurimycin (**156**), isolated from the culture media of *Streptomyces novoguineensis*, was shown to display antibiotic activity against *Pyricularia oryzae*, a fungal phytopathogen that causes sheath blight in rice plants.<sup>188</sup> The structure of amipurimycin includes an unusual branched nonofuranose uronic acid sugar.<sup>189</sup> Miharamycins A and B (**157a** and **157b**) are adenine-containing nucleosides isolated from cultures of *Streptomyces miharaensis* SF-4890, and are active against rice blast disease.<sup>190</sup> The structures of the miharamycins, reported 15 years after their initial discovery,<sup>191</sup> include a branched, nonofuranose uronic acid similar to that found in amipurimycin. In miharamycins, however, the sugar is bicyclic, containing an ether linkage between C-2' and C-9'. Hikizimycin (**158**), also named as anthelmecin, was isolated from the culture media of *Streptomyces* sp., strain A-5 and exhibits antibiotic activity against phytopathogenic fungi,<sup>192</sup> which might be related with inhibition of transpeptidation during protein synthesis.<sup>193</sup> Hikizimycin is a cytosine nucleoside analogue containing hikosamine (**159**), a 4-amino-4-deoxy-undecose sugar.<sup>194-196</sup> The antitumor compounds septacidin (**161**) from *Streptomyces fimbriatus*<sup>197</sup>

and its C-2 epimer spicamycin (**160**) from *Streptomyces alanosinicus* 87-MT<sub>3</sub>198 were both isolated as mixtures of similar compounds carrying normal and iso-chain fatty acyl substituents of variable lengths. The structures of both septacidin and spicamycin contain 4-amino-4-deoxyheptose (**162**) as does anicemycin (**163**) from *Streptomyces* sp. TP-A0648, which was identified recently.<sup>199</sup> Herbicidins A, B, C, E, F and G are antibiotics with herbicidal activity produced by *Streptomyces sagamonensis*.<sup>200–202</sup> The herbicidins are adenosine nucleoside analogues that contain a tricyclic dodecose scaffold. The array of herbicidins produced by a series of *S. sagamonensis* mutants led to a proposal that herbicidin A (**166**) is derived from herbicidin F (**165**), of which the precursor is herbicidin G (**164**).<sup>203</sup> Unfortunately, there is no information regarding the biosynthesis of the dodecose sugar.



#### 4.12. High-carbon sugars from bacterial cell walls

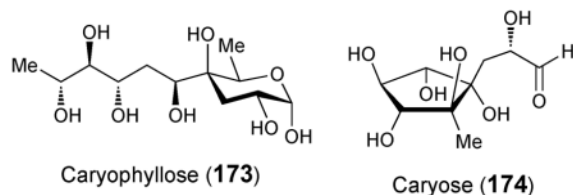
In addition to the high-carbon sugar antibiotics described above, a number of high-carbon sugars have been identified as components of bacterial cell walls. Perhaps the most widely recognized high-carbon sugar is *N*-acetyl-neuraminic acid (NeuNAc, **168**). This nine-carbon sugar is a key component of the eukaryotic extracellular matrix, and is also found to a lesser extent in bacteria. In bacterial systems, it is formed *via* an aldol condensation between *N*-acetyl-mannosamine (ManNAc, **167**) and either PEP (**108**) or pyruvate by *N*-acetyl-neuraminic acid synthase or aldolase, respectively (Scheme 22).<sup>204,205</sup> KDO (**170**), 3-deoxy-D-manno-oct-2-ulosonic acid, is an eight-carbon sugar found in the lipopolysaccharides of Gram-negative bacteria.<sup>206</sup> KDO is biosynthesized *via* an aldol condensation between arabinose-5-phosphate (**169**) and PEP (**108**) catalyzed by KDO-8-phosphate synthase (Scheme 22).<sup>207</sup> In addition to neuraminic acid and KDO, heptoses, especially those have a *glycero*-D-manno configuration, are essential components of the lipopolysaccharides. These



bacterial heptoses are biosynthetically derivatized from D-sedoheptulose 7-phosphate (**137**) and their biosynthetic pathways have been extensively studied and reviewed.<sup>208,209</sup>

Yersinirose A (**171**) is a branched chain octose found in the lipopolysaccharides of *Yersinia pseudotuberculosis* cultivar VI.<sup>210</sup> Early feeding experiments suggested that the two-carbon branch is derived from pyruvate<sup>186,211</sup> and a gene cluster dedicated to the biosynthesis of yersinirose in *Y. pseudotuberculosis* cultivar VI was identified.<sup>187</sup> The *yer* gene cluster contains eight genes and the *yerE* and *yerF* gene products were proposed to be essential for the formation of the two-carbon branch in yersinirose A (Scheme 23). Further investigation using the purified YerE expressed heterologously in *E. coli* showed that YerE indeed catalyzes the TPP-dependent attachment of the two-carbon branch using pyruvate and CDP-3,6-dideoxy-4-hexulose (**172**) as substrates (Scheme 23). A similar reaction had been proposed in the biosynthesis of the quinocycline compounds (**153** → **155**)<sup>186</sup>. YerE is a flavoprotein and displays homology to the large subunit of acetolactate synthase, an FAD-binding TPP-dependent enzyme.<sup>212</sup> When the YerE reaction is conducted under anaerobic conditions in the presence of sodium dithionite, the preservation of YerE activity suggest that flavin is not directly involved in the reaction mechanism. Thus, FAD might only play a structural role in reaction catalyzed by YerE.<sup>213</sup>

Caryophyllose (**173**), a branched 12-carbon sugar,<sup>214</sup> and caryose (**174**), a nine-carbon sugar containing a carbocycle,<sup>215</sup> were identified in the lipopolysaccharide fraction of the phytopathogenic bacterium *Pseudomonas (Burkholderia) caryophylli*. Caryophyllose, a 3,6,10-trideoxy-4-*C*-[(*R*)-1-hydroxyethyl]-*D*-erythro-*D*-gulo-decose, exists as an oligomer with  $\alpha$ -(1→7)-linkages known as caryophyllan, and caryose, a 4,8-cyclo-3,9-dideoxy-*L*-erythro-*D*-ido-nonose, exists as an oligomer with  $\beta$ -(1→7)-linkages known as caryan.<sup>216</sup> Although little is known about the biosynthesis of either sugar, two genes in *Mycobacterium marinum*, which also produces caryophyllose,<sup>217</sup> have been implicated in caryophyllose incorporation into lipooligosaccharides. When the two genes, *MMAR\_2332* and *MMAR\_2333*, were disrupted by transposon mutagenesis, caryophyllose was absent from *M. marinum* lipooligosaccharides.<sup>218</sup> The former is thought to encode a glycosyltransferase that may be responsible for the incorporation of caryophyllose into lipooligopolysaccharides.<sup>219</sup> The latter appears to encode a putative TPP-dependent carboxylase, suggesting that a TPP-dependent enzyme, which may be similar to that established for yersinirose formation, could be involved in the biosynthesis of this C-4 branched sugar (**173**) as well.



## 5. Conclusions

As described in this review, sugars with oxidized amines, C–S linkages, and high-carbon chain lengths, although rare, are broadly distributed throughout nature. Microbially-derived natural products with antibiotic activity are especially, although not exclusively, rich sources

of these unusual sugars. P<sub>450</sub>-dependent aminosugar-*N*-oxygenases are involved in the biosynthesis of *N*-hydroxyaminosugars with nitrosugars as a minor product. Flavin-dependent aminosugar-*N*-oxygenases have been demonstrated to yield nitrososugars. It is thought that non-enzymatic oxidation is responsible for the conversion of the nitrososugar product to the corresponding nitrosugar.

Many questions surrounding the biosynthesis of thiosugars remain unanswered. There is no genetic or biochemical data available regarding the biosynthesis of rhodonocardins A (**34**) and B or 5-thio-D-glucose. Based on the predicted function of several enzymes encoded within the albomycin (**38**) biosynthetic gene cluster, it is possible that a radical-mediated mechanism is involved in sulfur incorporation during albomycin biosynthesis, but this remains pure speculation. The thiazole synthase (ThiG) homologue, BexX, encoded within the BE-7585A (**48**) biosynthetic gene cluster forms a covalent adduct with glucose 6-phosphate, in accordance with its predicted function. However, the remaining steps involved in sulfur incorporation into BE-7585A are unclear. Although the gene clusters for lincomycin (**55**) and celesticetin (**58**) biosynthesis have been identified, they provide no obvious clues regarding the mechanism of sulfur incorporation into this class of antibiotics. The gene cluster for the biosynthesis of calicheamicin (**1**) encodes a C–S lyase but its function and role remain to be demonstrated. The biosynthetic pathways for glucosinolates (**67**) are fairly well characterized, but it is unclear whether the incorporated sulfide is derived from cysteine or glutathione. Nothing is known about the biosynthesis of the sulfonium containing plant products salacinol (**77**), kotalanol (**78**), ponkolanol (**79**) and salaprinol (**80**), but based on structural considerations these compounds may be derived, in part, from methionine. SQD1 from *Arabidopsis thaliana* catalyzes the incorporation of inorganic sulfite into UDP-glucose during the biosynthesis of sulfolipids (**81**).

Elucidation of the various mechanisms involved in the biosynthesis of high-carbon sugars is an active research topic, and limited advances have been made in several systems. According to recent *in vitro* experiments, octulose-8-phosphate, an intermediate in lincosamine (**56**) biosynthesis, is formed *via* a transaldol reaction between D-ribose 5-phosphate and either D-fructose 6-phosphate or D-sedoheptulose 7-phosphate catalyzed by LmbR. LmbN then catalyzes the isomerization of octulose-8-phosphate to octose-8-phosphate. The 5′-*C*-glycyluridine moiety of liposidomycin (**96**), caprazamycin (**97**), muraymycin (**98**), A-90289A (**99**) and B, and FR-900493 (**100**) is probably formed in two sequential steps: an α-ketoglutarate dioxygenase homologue oxidizes UMP to uridine 5′-aldehyde, which then undergoes subsequent condensation with glycine *via* the PLP-dependent action of an enzyme homologous to serine hydroxymethyl transferase. The first step has been demonstrated but the second has not. The mechanism behind sugar chain elongation in octosyl acids (**102–104**) and ezomycins (**113**) remains to be elucidated. However, 3′-enoylpyruvoyl-UMP has been identified as a biosynthetic intermediate in polyoxin (**106**) and nikkomycin (**109–111**) production and it could, therefore, serve as an intermediate in the formation of octosyl acids and ezomycins as well. The TPP-dependent addition of a branched carbon chain during yersiniose (**171**) biosynthesis has been demonstrated *in vitro* and similar reactions have been proposed for the branched octose sugars in quinocyclines (**155**) and the branched dodecose sugar caryophyllose (**173**).

Despite of the above progress, there is much that remains undiscovered in this area. It has been proposed that the radical SAM enzyme TunB might catalyze the formation of a C–C bond between C-6 of UDP-6-deoxy-GalNAc-5,6-ene and 5'-uridine radical to give the contiguous C-11 chain in tunicamine (**84**) but this remains a hypothesis. The gene cluster for mildiomycin (**114**) biosynthesis has been identified and MilM, an aspartate aminotransferase homologue, is proposed to catalyze the deamination of arginine to give  $\alpha$ -keto- $\delta$ -guanidinovalerate, which is subsequently coupled to the nucleoside by MilN, a dihydropicolinate synthetase homologue. Activity has yet to be demonstrated for either of these enzymes. Although radiotracer data regarding griseolic acid (**127**) biosynthesis has been reported, no additional genetic or biochemical data is available at present. Similarly, while cell-free assays have implied that sinefungin (**133**) is derived from ribose, and arginine, with subsequent addition of adenine, no genes or enzymes involved in this process have been identified. Several pathways have been proposed for the biosynthesis of the 4-thioheptouronic acid core (**47**) of albomycin (**38**) based on the albomycin gene cluster but they are purely hypothetical. The source and mechanism of incorporation of C-7 and C-8 into the bicyclic octose sugar moiety remain to be established despite the availability of sequence information on the apramycin (**144**) gene cluster from two organisms. Moreover, nothing is known regarding the biosynthesis of the high-carbon sugars in hikizimycin (**158**), amipurimycin (**156**), the miharamycins (**157**), spicamycin (**160**), septacidin (**161**), anicemycin (**163**), the herbicidins (**164–166**), or caryose (**174**).

This review is intended to provide an update of current research in the biosynthesis of several classes of “rare” unusual sugars along with a summary of work that remains to be done. It is our hope that this review will inspire more scientists to get involved in this underexplored field to study the biosynthesis of unusual sugars. The challenge is not only to elucidate the complex biosynthetic pathways but also to investigate the mechanisms of intriguing enzyme-catalyzed transformations. The opportunities to find new pathways and new enzymes are numerous, and the likelihood of discovering new and exciting chemistry is high. In addition, future research in this field offers tremendous potential to develop an array of glycosylated metabolites that possess useful biological activities.

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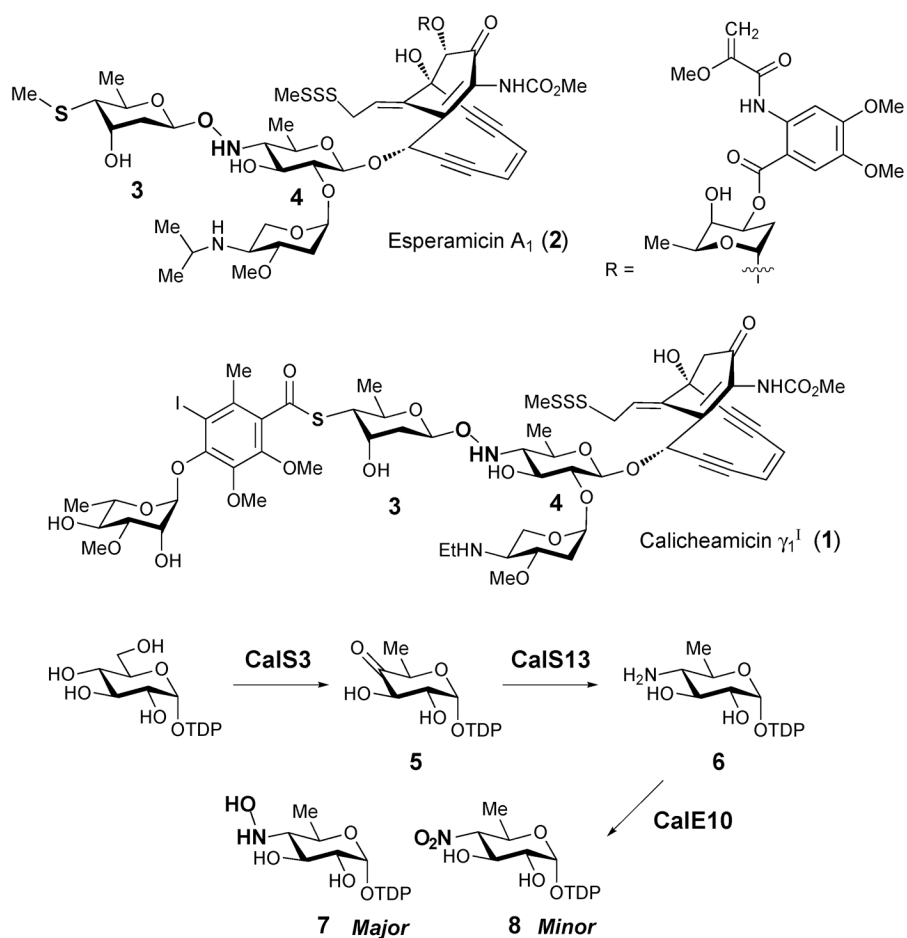


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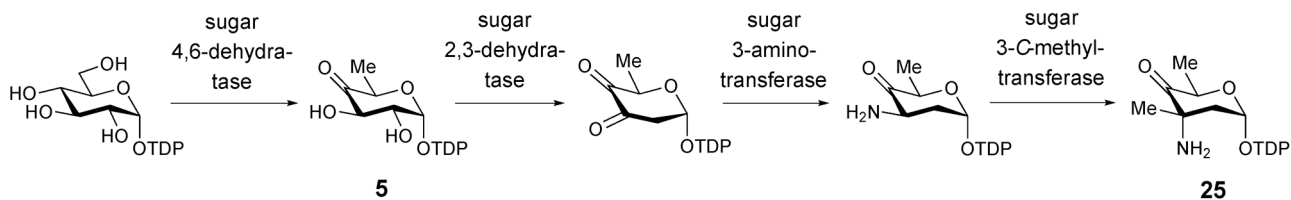
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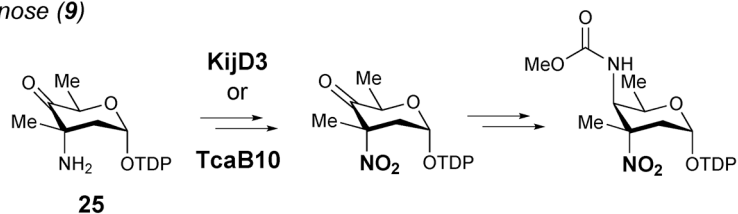
**Scheme 1.**

Proposed pathway for the biosynthesis of hydroxyaminosugar

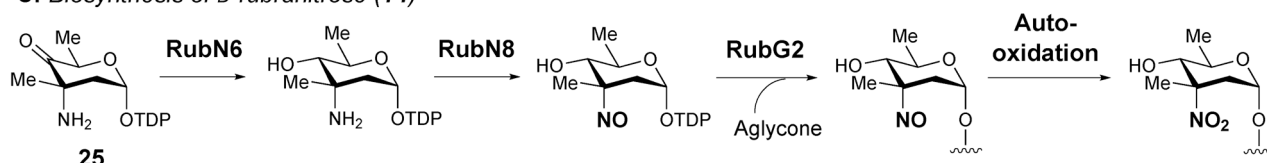
**A. Construction of the key intermediate (25)**



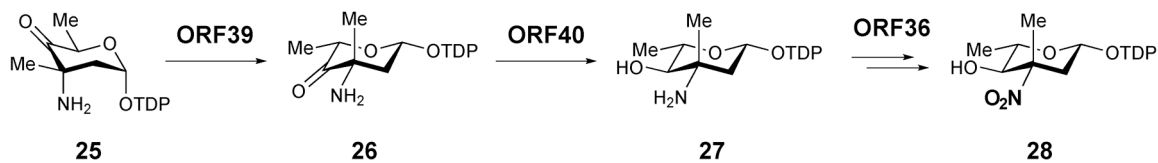
**B. Biosynthesis of *D*-kijanose (9)**



**C. Biosynthesis of *D*-rubranitrose (14)**



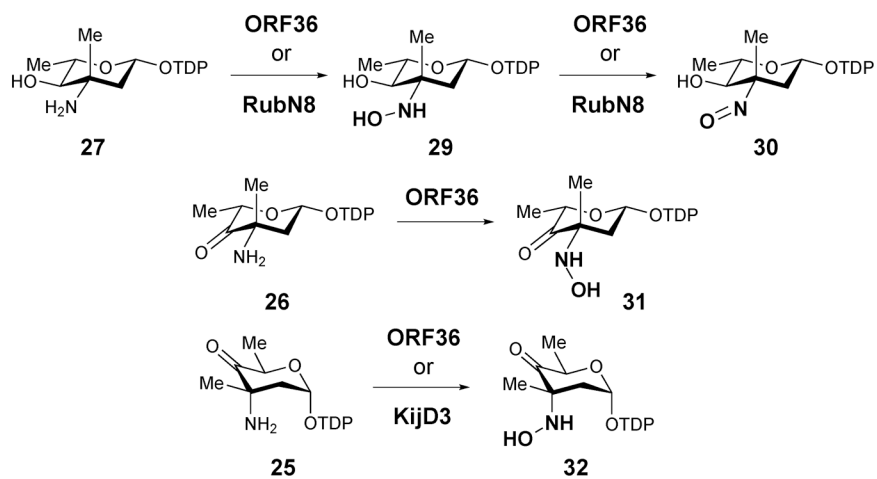
**D. Biosynthesis of *L*-evermitrose (16)**

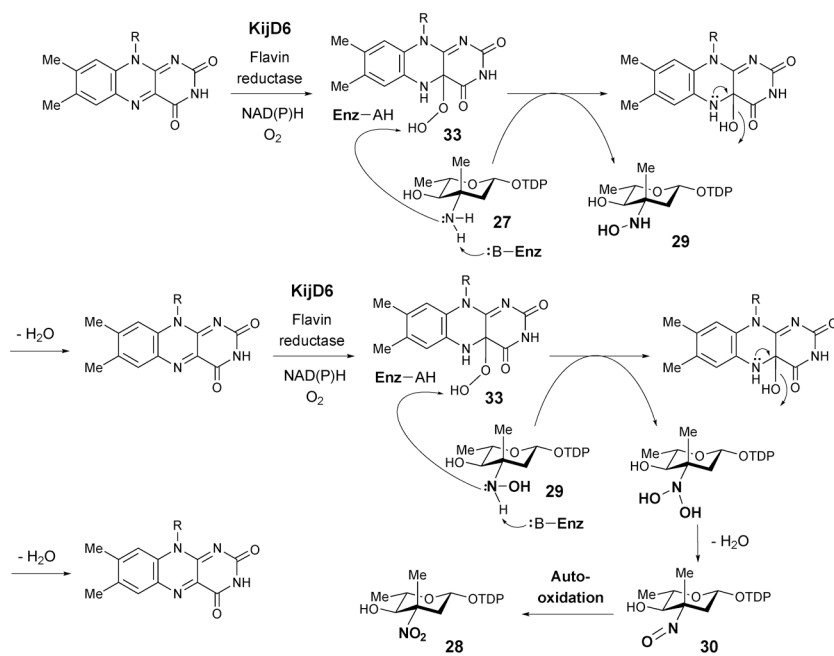


**Scheme 2.**

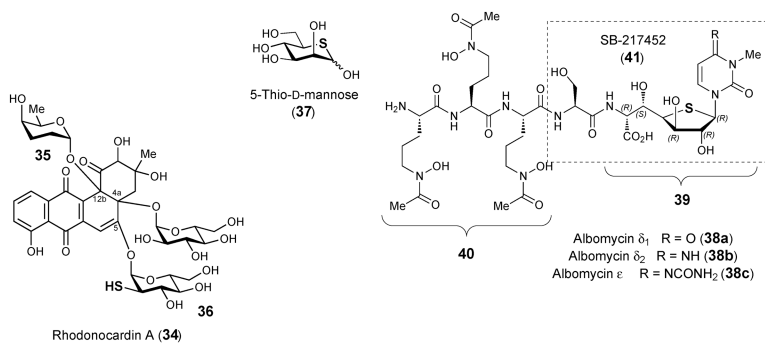
Proposed pathways for the biosynthesis of nitrosugars



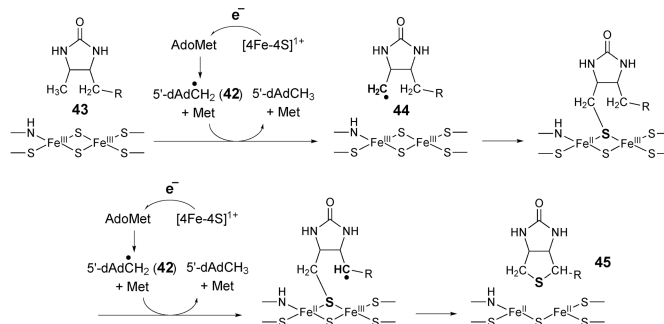
**Scheme 3.**Reactions catalyzed by *N*-oxygenases

**Scheme 4.**

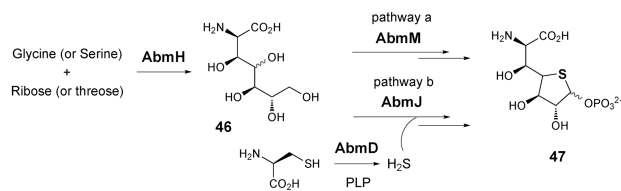
Proposed mechanism for ORF36-catalyzed N-oxidation



**A. Proposed mechanism for BioB-catalyzed sulfur incorporation in biotin biosynthesis**

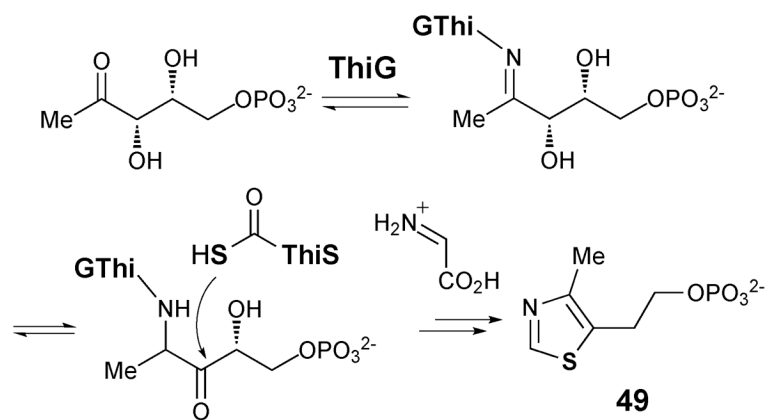


**B. Proposed mechanism for sulfur incorporation in albomycin biosynthesis**

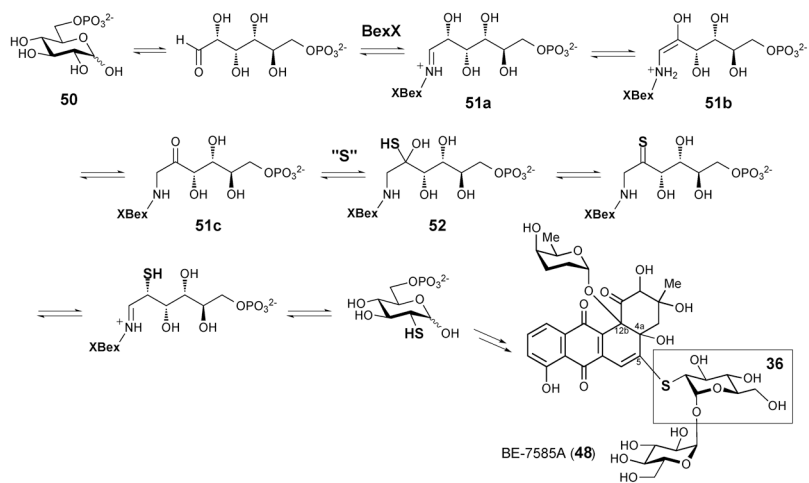


**Scheme 5.**

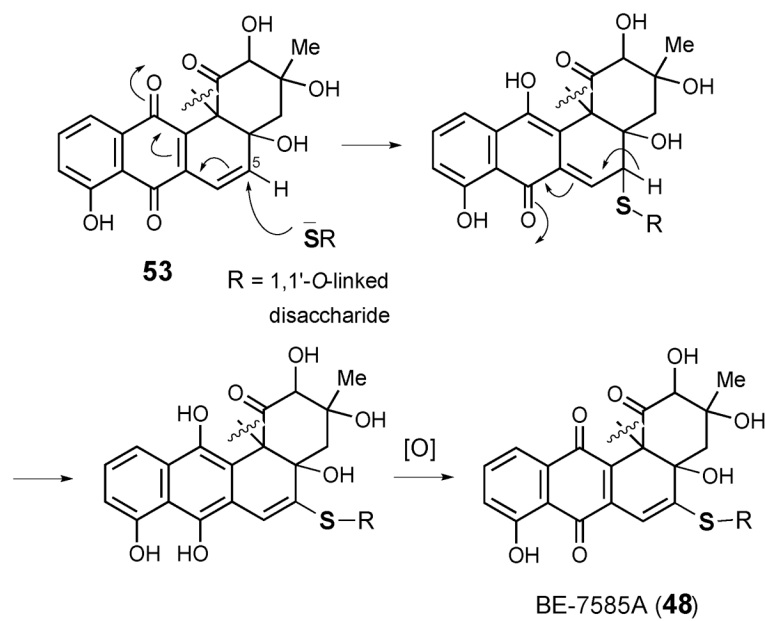
Possible mechanism of sulfur incorporation into albomycin catalyzed by radical SAM dependent enzymes



**Scheme 6.**  
ThiG-catalyzed thiazol formation

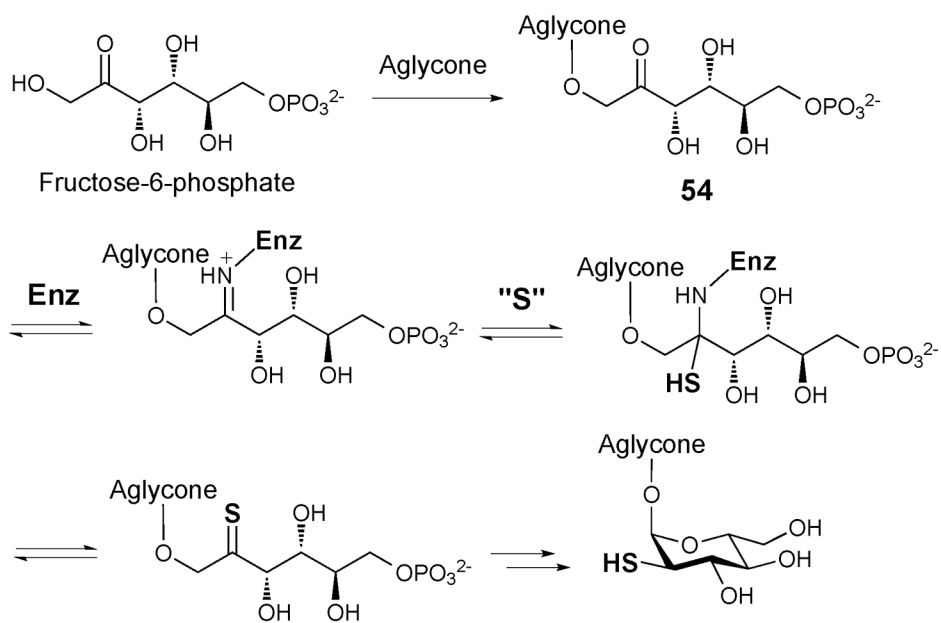


**Scheme 7.**  
Biosynthesis of 2-thioglucose in BE-7585A

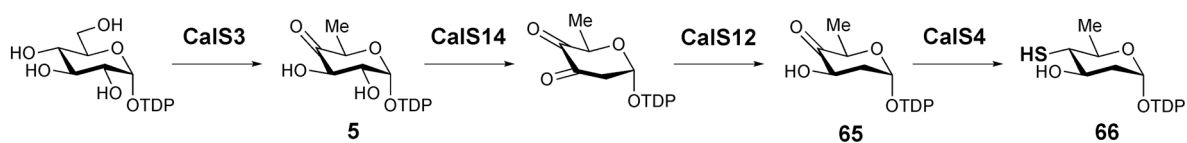
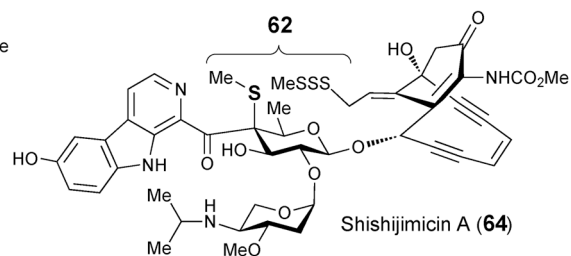
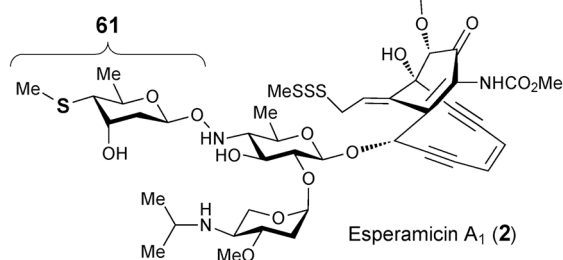
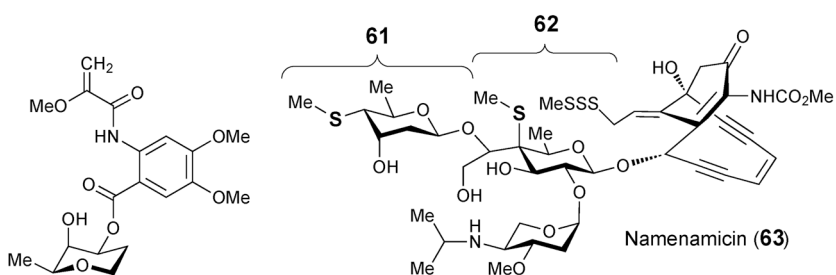
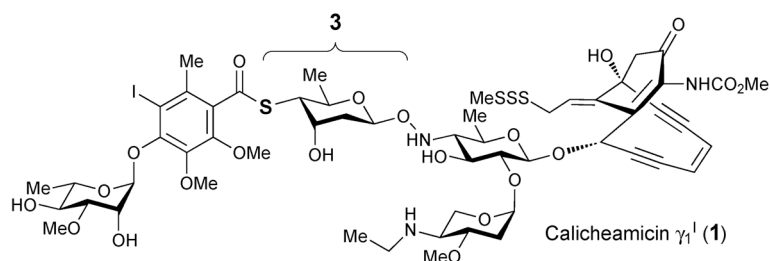
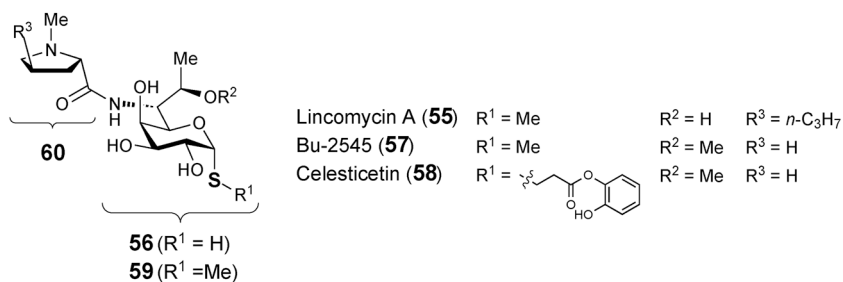
**Scheme 8.**

Possible mechanism for the formation of the thioether linkage in BE-7585 A



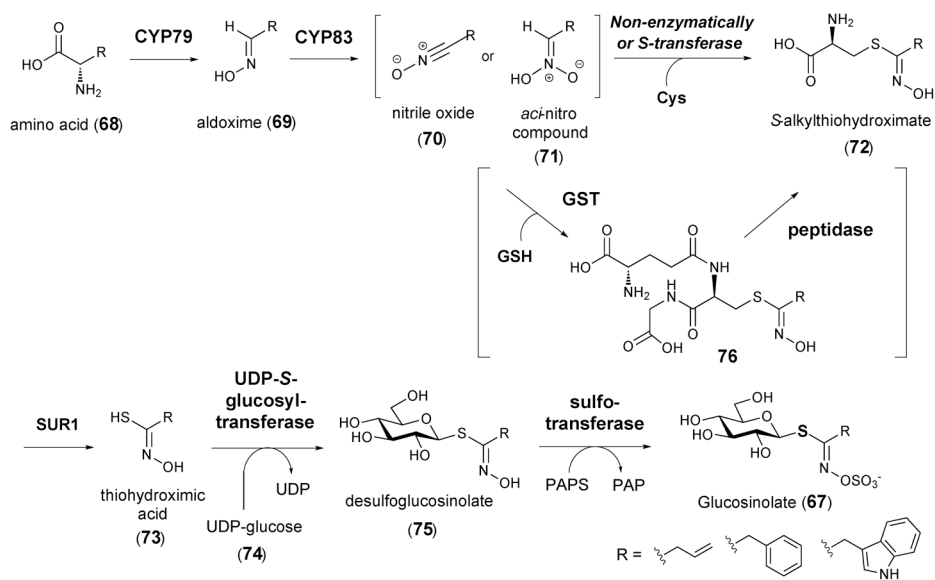


**Scheme 9.**  
Proposed biosynthesis of 2-thioglucose in rhodonocardin

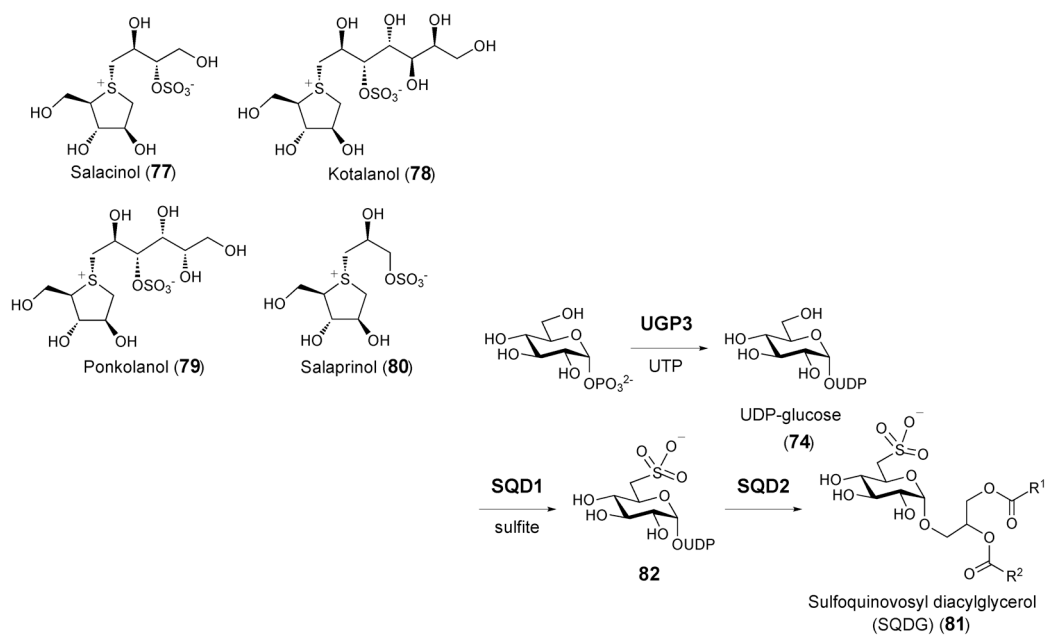


Scheme 10.

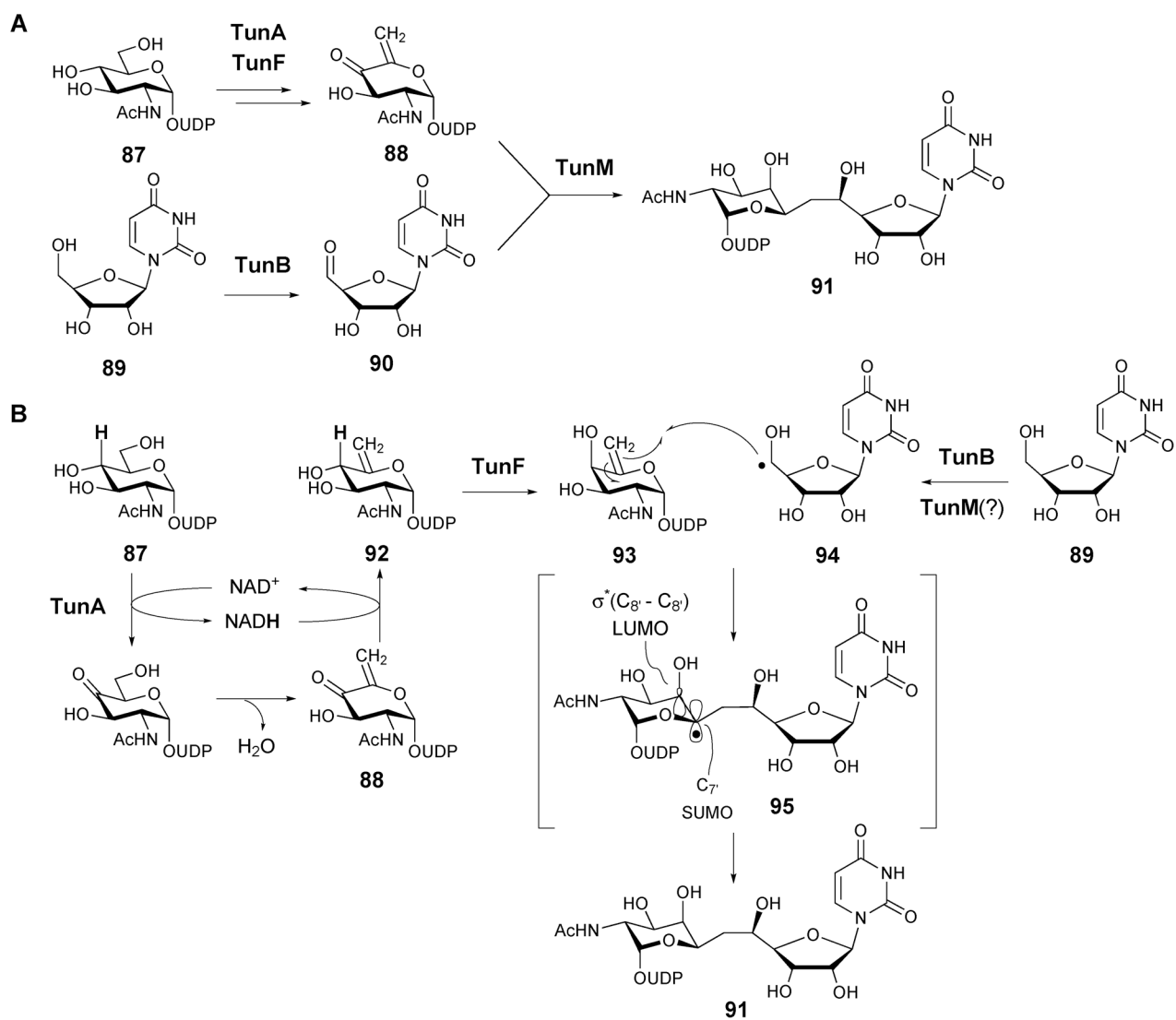
Proposed pathway for the biosynthesis of 4-thioglucose in calicheamicin

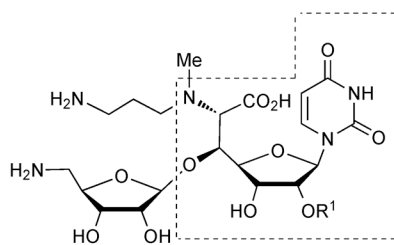
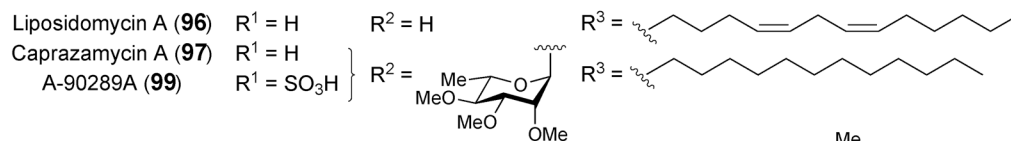
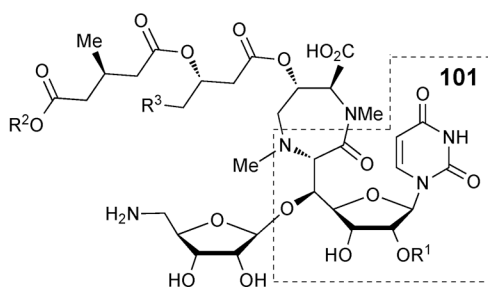
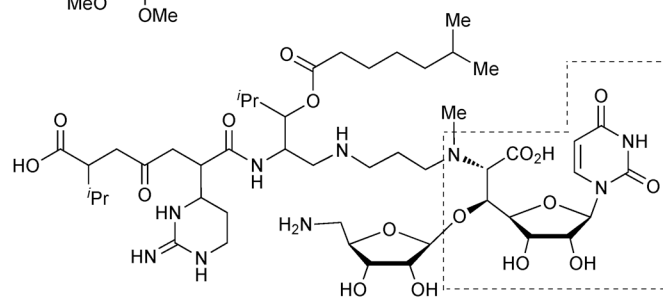
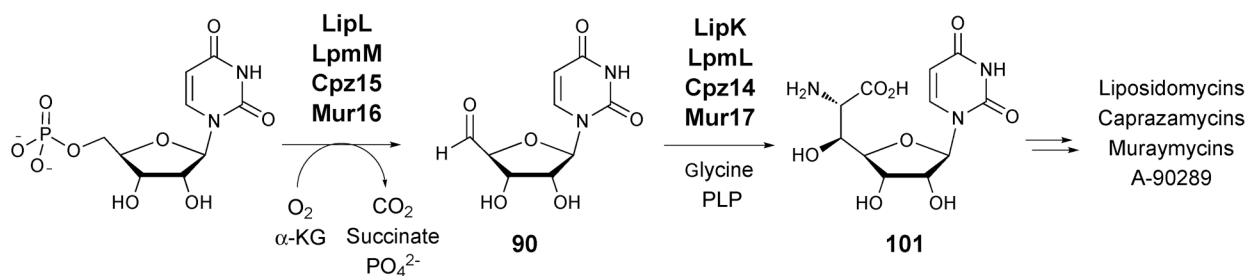


**Scheme 11.**  
Proposed pathway for the biosynthesis of glucosinolates

**Scheme 12.**

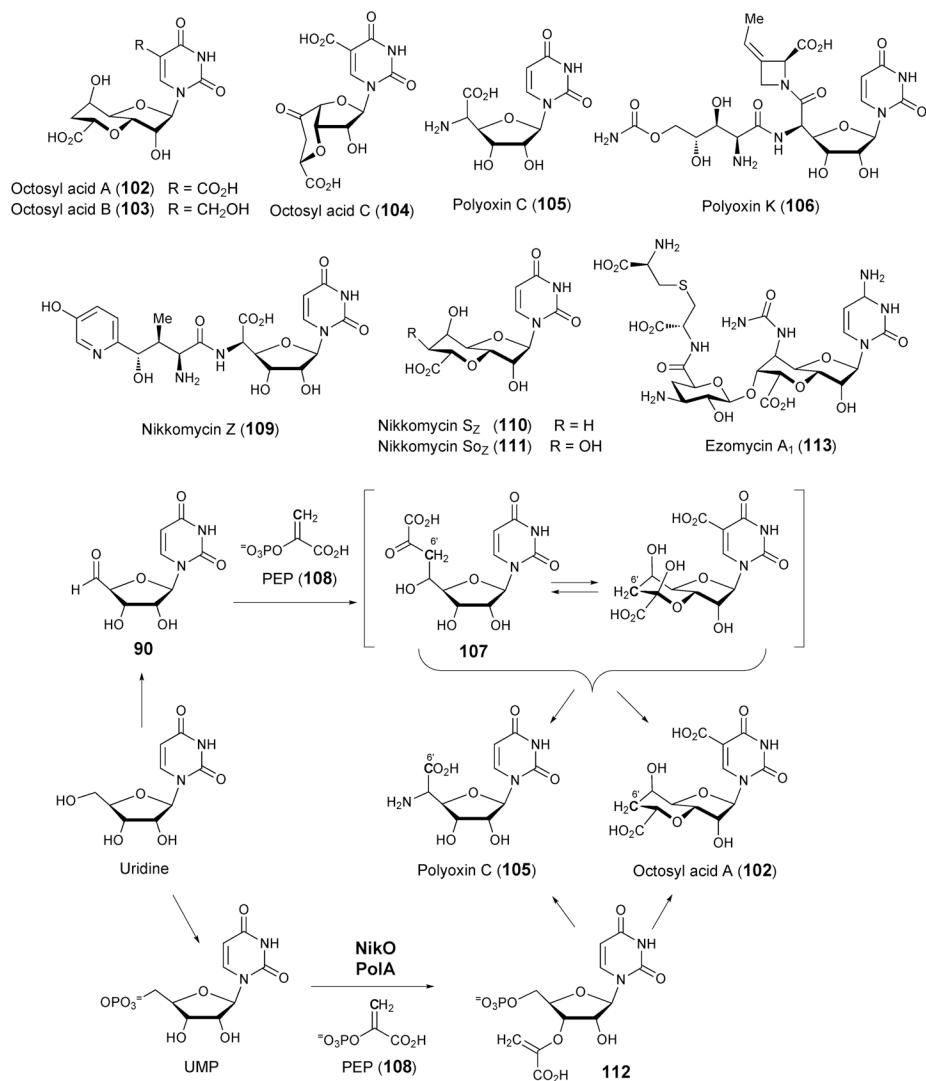
Proposed pathway for the biosynthesis of sulfoquinovosyl diacylglycerol



FR-900493 (**100**)Muraymycin (**98**)

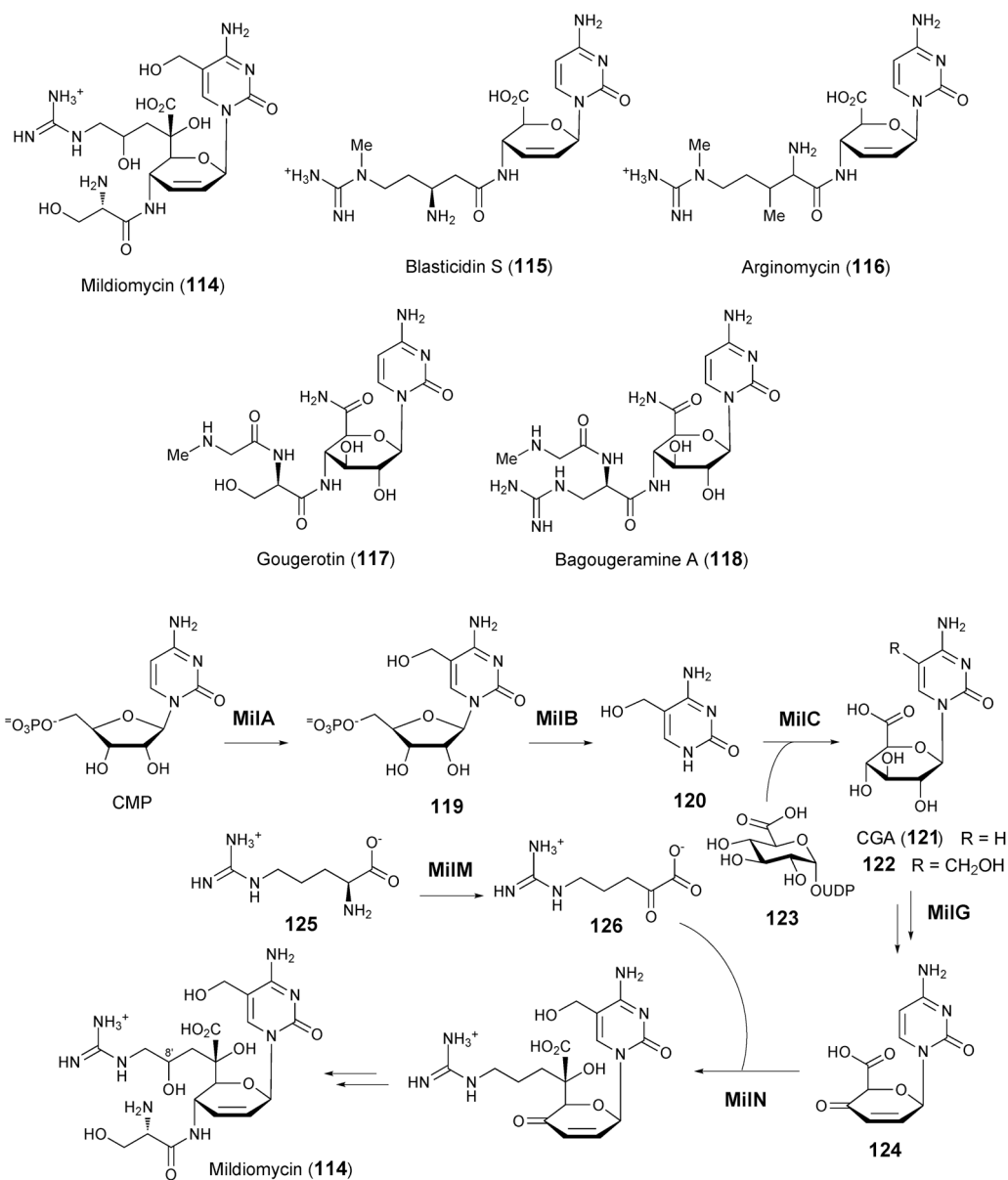
**Scheme 14.**  
Proposed biosynthesis of 5'-C-glycyluridine core



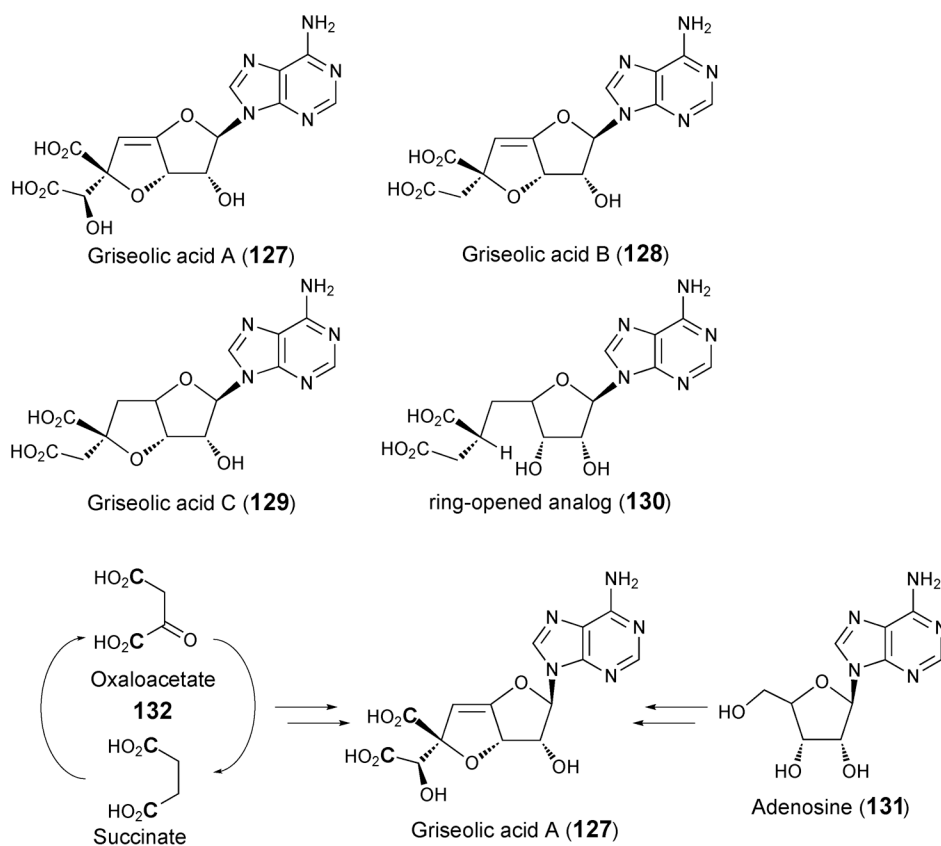


Scheme 15.

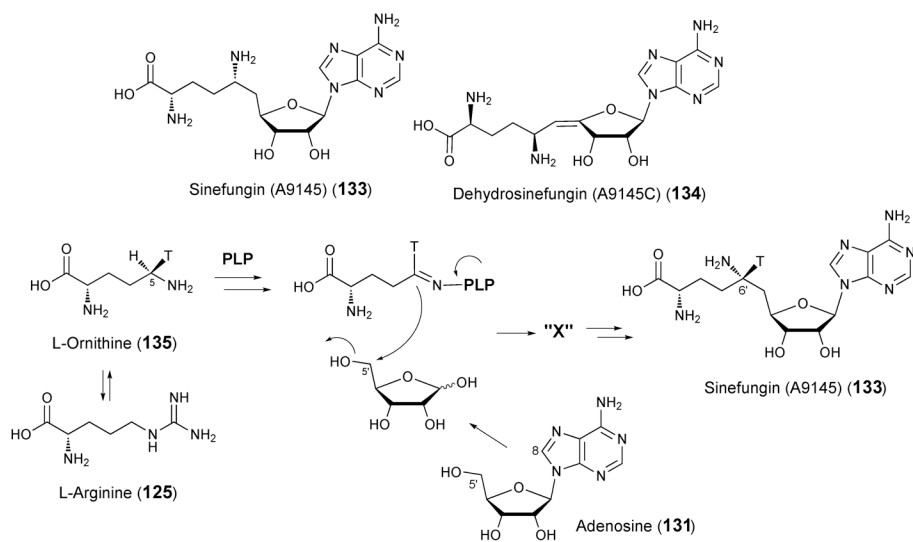
Proposed pathways for the biosynthesis of octosyl acids, polyoxins and nikkomycins



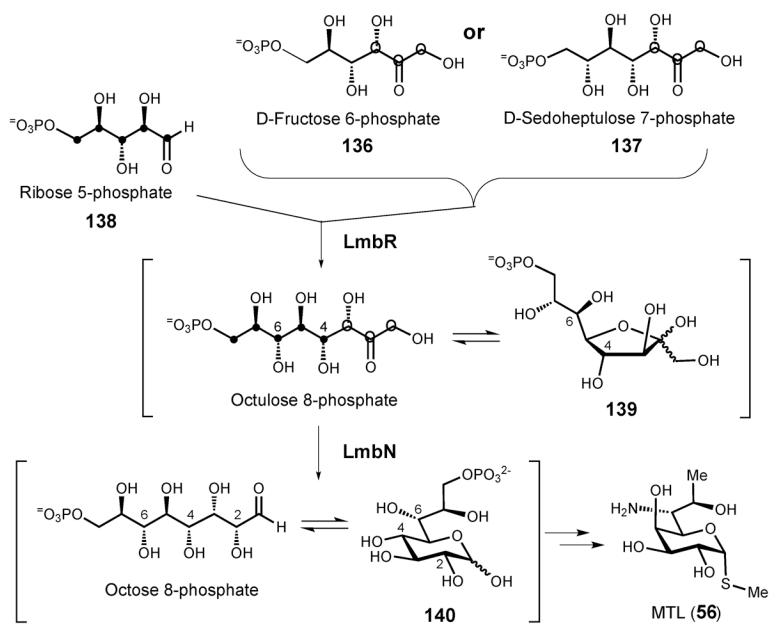
**Scheme 16.**  
Proposed pathway for the biosynthesis of mildiomycin



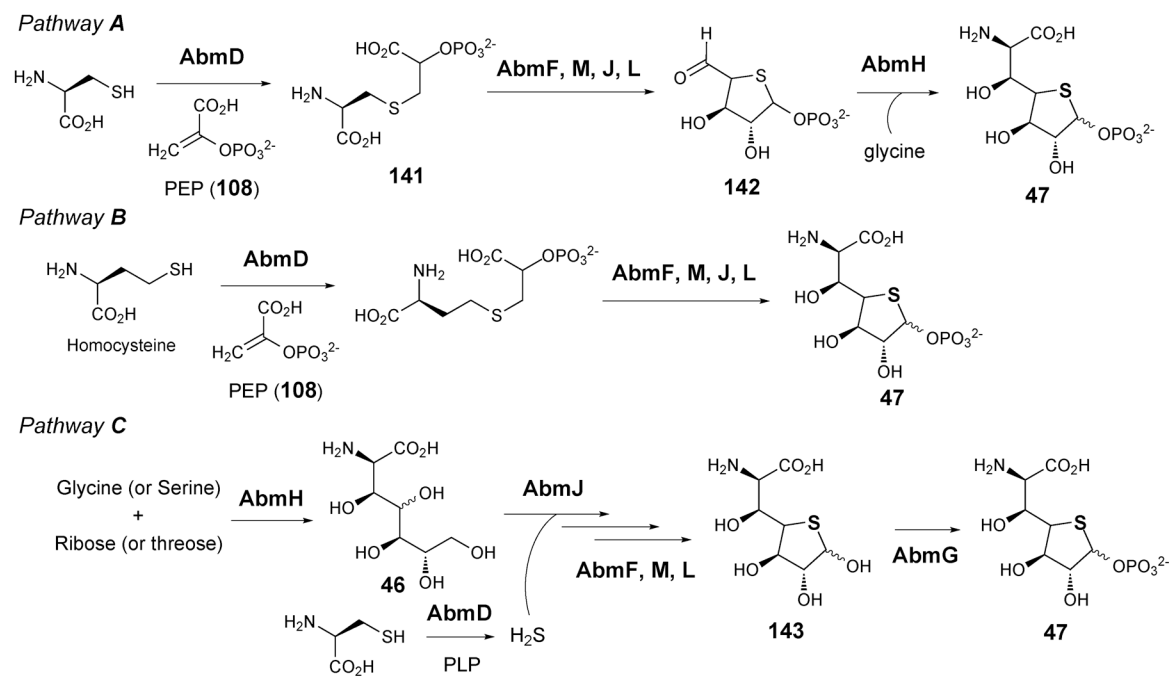
**Scheme 17.**  
Proposed biosynthetic precursors for griseolic acid A

**Scheme 18.**

Proposed biosynthetic precursors for sinefungin (A9145)

**Scheme 19.**

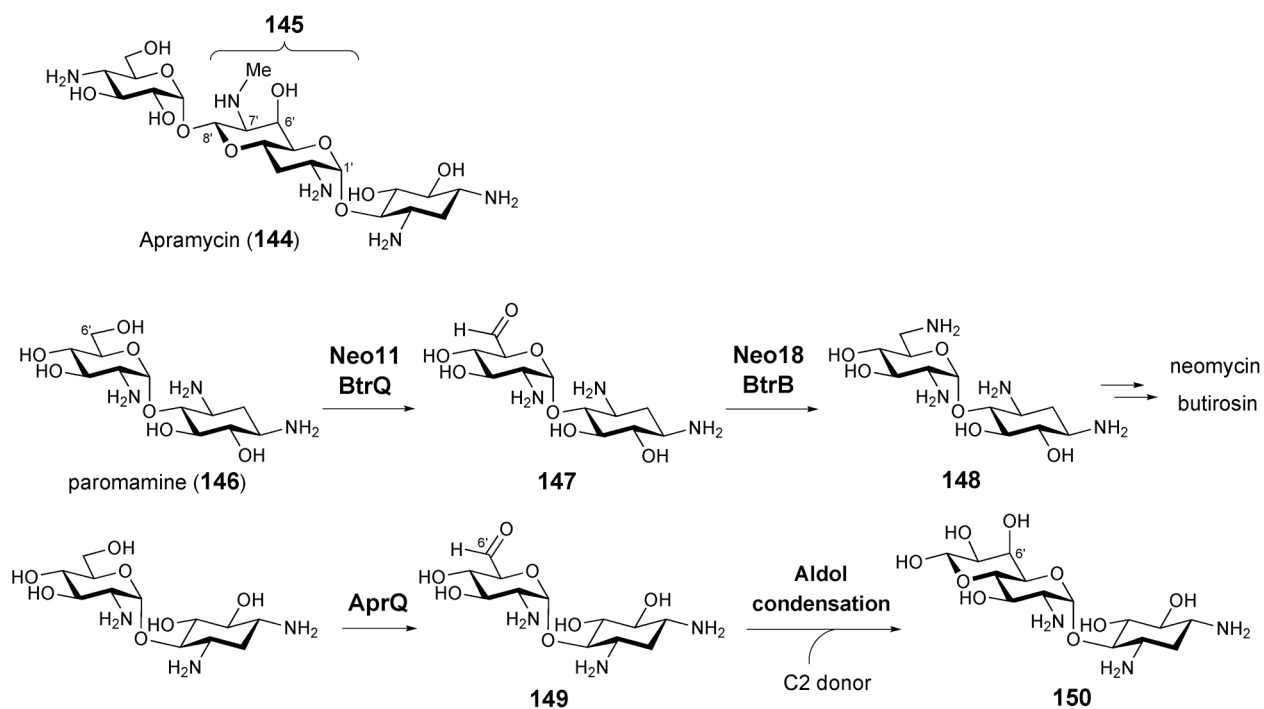
Reactions catalyzed by LmbR and LmbN in the biosynthesis of MTL



Scheme 20.

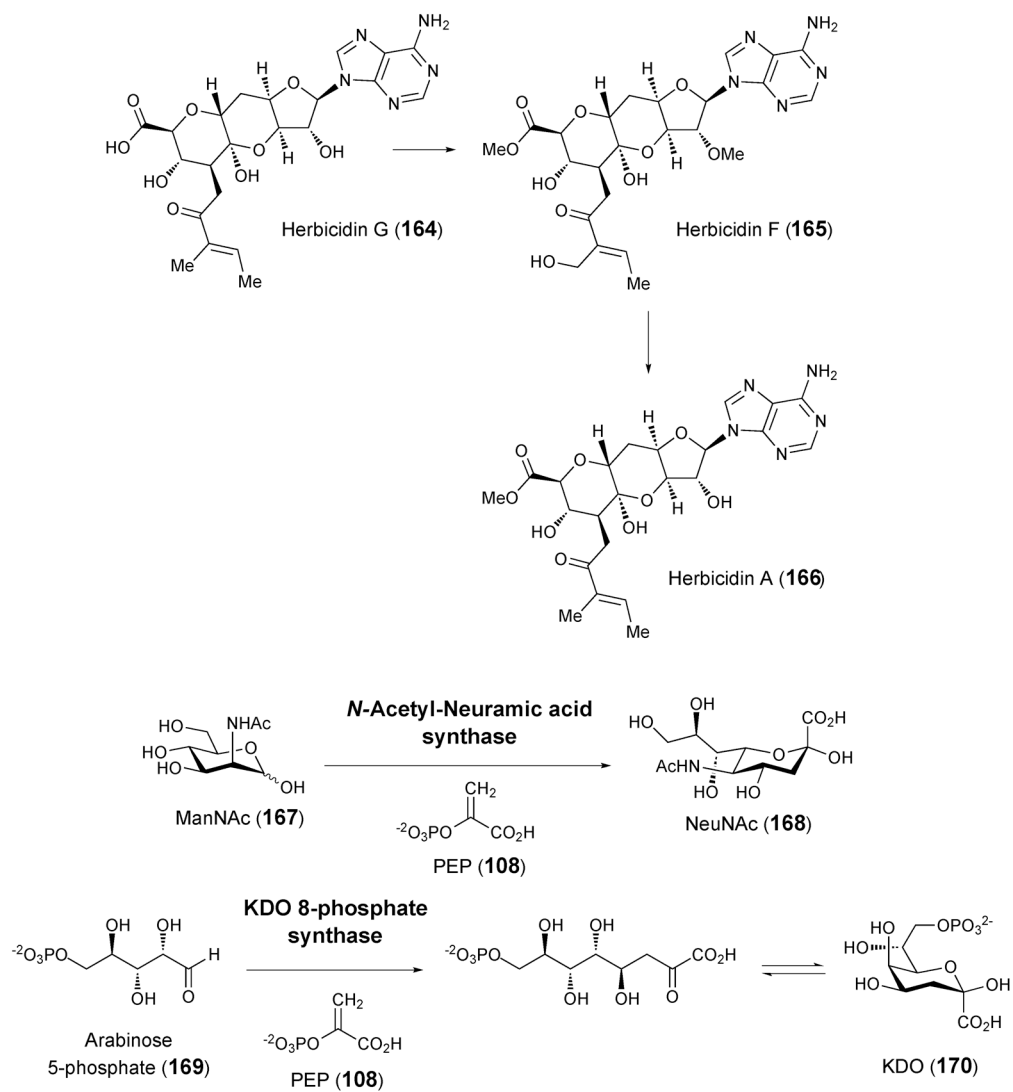
Proposed pathways for the biosynthesis of 4-thioheptouronic acid in albomycin



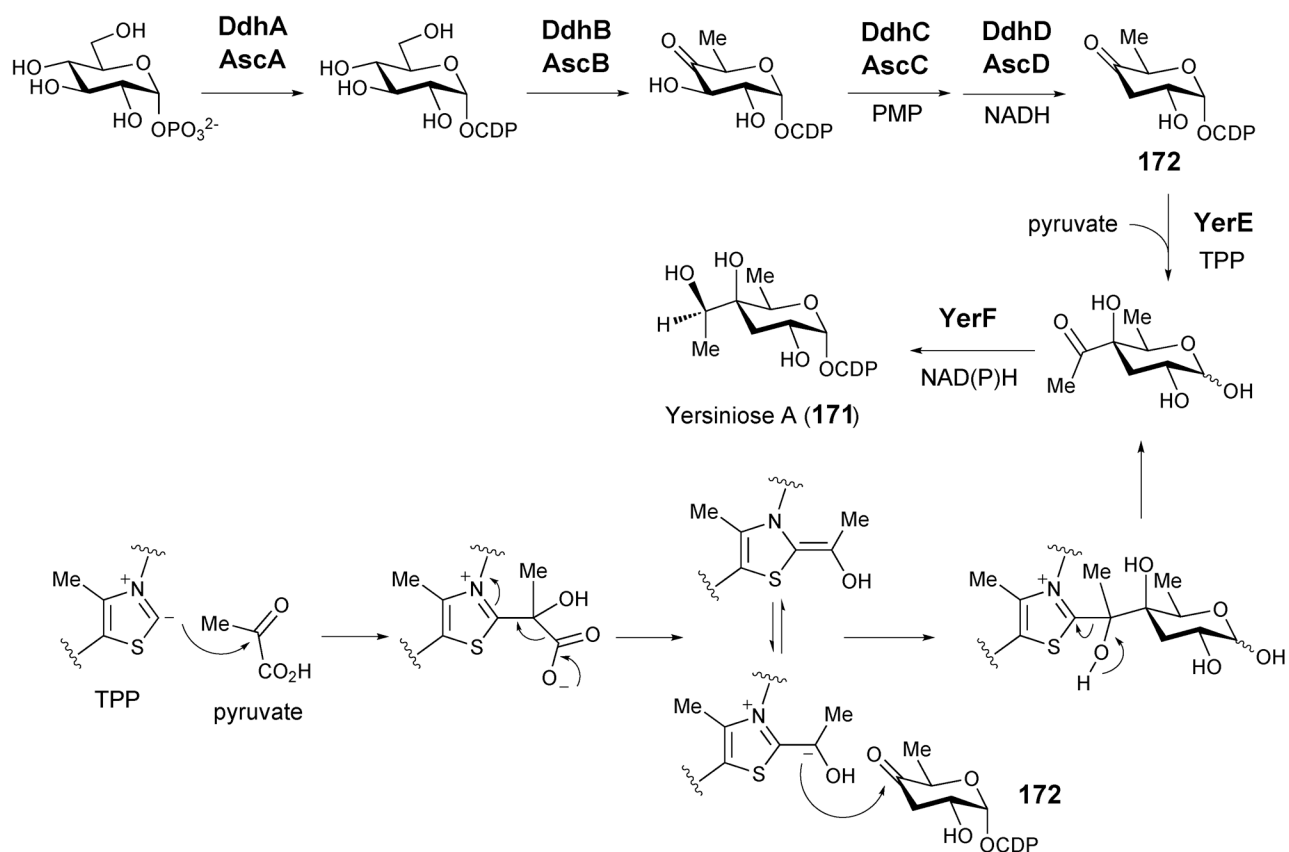


Scheme 21.

Proposed pathway for the biosynthesis of bicyclic octose moiety in apramycin



**Scheme 22.**  
Biosynthetic pathways for NeuNAc and KDO



Scheme 23.

Proposed pathway for the biosynthesis of yersiniose and mechanism of YerE