DOI 10.1515/hc-2013-0141 — Heterocycl. Commun. 2013; 19(6): 375–386

**Review**

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# Polyoxin and nikkomycin analogs: recent design and synthesis of novel peptidyl nucleosides

**Abstract:** Polyoxins and nikkomycins are a class of naturally occurring peptidyl nucleoside antibiotics that show promise as potential antifungal agents due to their potent ability to inhibit chitin synthase, an enzyme responsible for fungal cell wall biosynthesis. Whole cell assays and *in vivo* studies have shown that these natural products have poor cellular uptake and are metabolically unstable, and there has been a concerted effort to improve their pharmokinetic properties by synthesizing analogs. These have either been designed as natural substrate analogs, transition state mimetics or mechanistic inhibitors. Recent synthetic efforts and the results of their biological studies are briefly described in this review, and the current trends in the design and construction of polyoxin and nikkomycin analogs are discussed.

**Keywords:** chitin synthase; chitin synthase inhibitors; nikkomycin; peptidyl nucleosides; polyoxin.

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## Introduction

The discovery of nucleoside antibiotics has presented synthetic chemists with a multitude of potential lead compounds in the development of novel antimicrobial agents [1, 2]. These unique secondary metabolites are important, in particular, due to the increasing observance of resistant strains of bacteria and fungi in immunocompromised patients such as those undergoing treatment for cancer, organ transplants and AIDS patients. Among the numerous types of nucleoside antibiotics found in nature, peptidyl nucleoside antibiotics have exhibited activity against a broad variety of microbes [2, 3]. One such example is a class of compounds named the polyoxins (**1**–**3**; Figure 1), which were isolated in the 1960s from *Streptomyces cacaoi* var. *asoensis* [4–8]. Following this discovery, the structurally related nikkomycins (**4**, **5**; Figure 1) were isolated from other *Streptomyces* strains [9–16].

Much of the interest in the polyoxins and nikkomycins has originated from their high selectivity and biological effects against various fungi and insects [8], which have been attributed to the competitive inhibition of chitin synthase. This enzyme is responsible for the synthesis of chitin, which consists of a β-1,4-linked polymer of *N*-acetylglucosamine (GlcNAc). Chitin and chitin-like substances are an essential cell wall component of fungi and protozoa, such as *Giardia*, and are responsible for imparting shape, strength and rigidity to the cell wall [17]. Additionally, chitin is a key component of the exoskeleton of arthropods and many molluscs [18–20]. Once the formation of chitin is disrupted in unicellular organisms, the cells are affected by osmotic sensitivity, abnormal morphology and growth arrest [21]. As chitin is absent in mammals, chitin synthase inhibitors such as the polyoxins and nikkomycins are considered an attractive option for antifungal treatment [22].

The chemical process initiated by chitin synthase can be thought of as a repetitive transfer of GlcNAc residues from the activated donor uridine diphosphate-*N*-acetylglucosamine (UDP-GlcNAc) to the growing chitin polysaccharide chain, with concomitant release of UDP (Scheme 1). The overall structural resemblance of polyoxins and nikkomycins to UDP-GlcNAc has been postulated as a possible reason for their high potency to bind to the catalytic site of chitin synthase [7].

Nikkomycin Z (**4**) is one of the most potent chitin synthase inhibitors and is currently the only chitin synthase inhibitor that has undergone clinical trials [23]. However, despite excellent *in vitro* results, clinical utility of these natural products is compromised by their poor cellular uptake and metabolic instability, resulting in a decrease in efficacy and high inhibitory concentrations [6, 24]. In addition, it has become apparent that there are isozymes of chitin synthase that have different roles in cell wall biosynthesis, for example, chitin synthases 1 and 2 in *Saccharomyces cerevisiae* are responsible for repair and cell division, respectively. Polyoxins and nikkomycins have

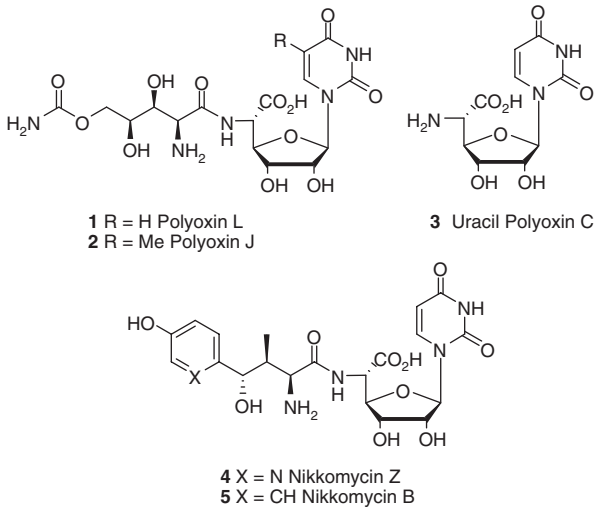



**Figure 1** Representative structures of the polyoxins and nikkomycins.

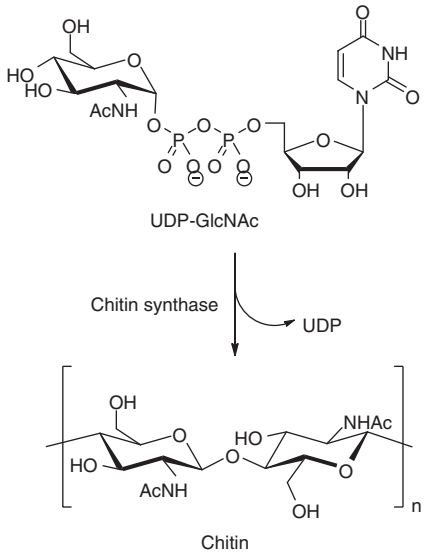
been shown to inhibit specific isozymes [25], and although selectivity is often a desired trait in a drug candidate, in this instance global inhibition of chitin synthase isoforms is preferred so that multiple pathways may be targeted and treatments can consequently be more effective.

## Design of polyoxin and nikkomycin analogs

Since the initial discovery of polyoxins and nikkomycins, a number of research groups have been working on the synthesis of analogs in order to improve their pharmacokinetic properties. These efforts have been highlighted in several review publications, particularly in the context of chitin synthase inhibition [8, 26–30]. Development of first generation polyoxin and nikkomycin analogs typically involved simple substitution or removal of specific functional groups to improve their bioavailability and elucidate a possible mode of action [31–38]. This provided important information about the structural features of the polyoxins and nikkomycins that are thought to contribute to their potent biological activity. For example, the 5′-carboxylate group was found to be essential and is believed to place a negative charge in a position analogous to that of the phosphate groups in the natural substrate UDP-GlcNAc [37].

These studies, combined with those on other classes of glycosyl donors, have also contributed to the understanding that transition state mimetics or bisubstrate analogs may be more effective as chitin synthase inhibitors than natural substrate analogs [39]. The aim of this method of drug design is to simulate aspects of the substrate in the transition state of the enzymatic reaction such as charge, shape and polarity. For example, one of the interactions at the active site of chitin synthase is believed to involve a six-membered ring complex with the pyrophosphate portion of UDP-GlcNAc and a divalent metallic species such as magnesium (Figure 2). Installing chelating functionalities that can emulate this transition state may enhance the activity of the molecule.

The two-active site mechanism has also been hypothesized as a potential mode of action and is based on the theory that the delivery of consecutive glycosyl units occurs in an alternating 180° pattern at adjacent active sites within the enzyme to generate the chitin polysaccharide chain (Figure 3, left) [40]. Recent results from the Finney group




**Scheme 1**

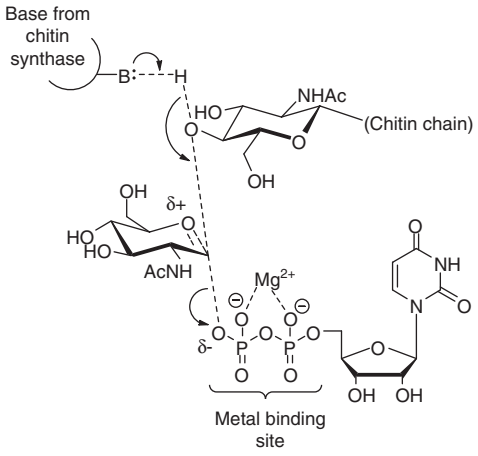



**Figure 2** Postulated transition state for chitin biosynthesis.

have demonstrated a weak but clear correlation between uridine dimers and chitin synthase inhibition, and will be discussed later in greater detail [41, 42]. It is possible that the efficacy of polyoxins and nikkomycins might be explained by their acting as bisubstrate inhibitors (Figure 3, right), effectively blocking two active sites of chitin synthase at the same time. Synthesis of analogs that possess terminal functional groups that can mimic the uracil base of uridine in UDP-GlcNAc, such as the carbamoyl group of polyoxins and the hydroxy-pyridyl moiety of nikkomycins, may allow access to more potent inhibitors.

In the recent literature, there have been a number of structural modifications made to the polyoxins and nikkomycins that can be grouped into several categories, including changes to the terminal amino acid, the nucleoside moiety and the bridging unit linking them together. These efforts have aimed to probe structure-activity relationships, to improve pharmokinetic properties and to target the transition state or bisubstrate mechanism. Synthetic efforts and biological results will be briefly described.

## Peptidyl side chain modifications

Matsuda and coworkers synthesized a series of novel nikkomycin analogs via peptide coupling of uracil polyoxin C (**3**) with various amino acids using standard dicyclohexylcarbodiimide (DCC) coupling conditions [43]. This series of compounds was designed to have a chemical structure that closely mimicked the structure of the natural products, yet had a variety of alkyl and aryl substituents at the terminal amino acid moiety (Scheme 2). Chitin synthase inhibitors from the first series of analogs (such as **6**) contained an *S*-arylmethyl-L-cysteine side chain. Additional analogs possessing hydrophobic aryl components such as phenyl, naphthyl and phenanthrenyl groups were subsequently synthesized and their inhibition of chitin synthase evaluated. Among them, the compound possessing a phenanthrene group at the terminal amino acid **7** was found to possess strong anti-chitin synthase activity comparable to that of nikkomycin Z (**4**) (0.31 μg/mL vs. 0.393 μg/mL). Importantly, this investigation demonstrated that two stereocenters could be removed from the overall nikkomycin structure while retaining the same level of inhibition.

Tsukuda and coworkers synthesized a combinatorial library of 450 nikkomycin analogs using the Ugi reaction on solid support (Scheme 3) [44]. The key step of this reaction was a multicomponent coupling of the known aldehyde **8**, a combination of 59 different carboxylic acids and 15 isocyanides and Rink amide resin. The final compounds were concomitantly cleaved from the Rink resin and deprotected in one step to give a variety of peptidyl nucleosides. The 450 crude products were then screened for inhibition of chitin synthases 1 and 2 of *Candida albicans*. It was found that 246 compounds exhibited more than 50% inhibition at 10 μg/mL concentrations. Similar to results from previous studies [25], compounds (**9**–**11**) that possessed chitin synthase 1 inhibition comparable to nikkomycin Z were found to be inactive against chitin synthase 2, with the exception of compound **9**.

Treatment of the uracil polyoxin C conjugate **12** with a variety of isoxazoles by Plant and colleagues generated a series of heterocyclic polyoxin analogs (Scheme 4) [45]. These compounds were also screened against a range of organisms including insect pests, fungal pathogens and weeds using *in vivo* high-throughput screening. However, no significant biological activity was observed, possibly due to poor cellular uptake or metabolic instability.

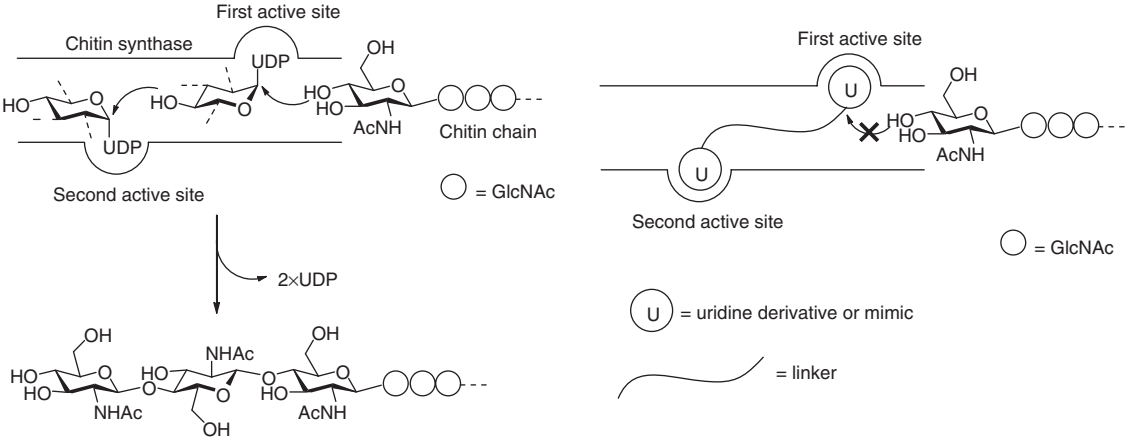
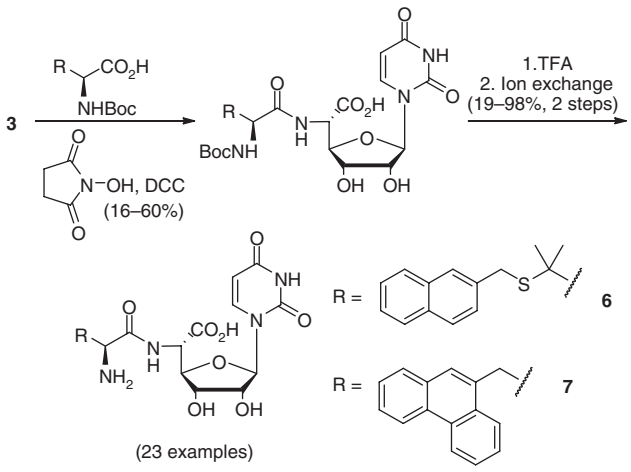
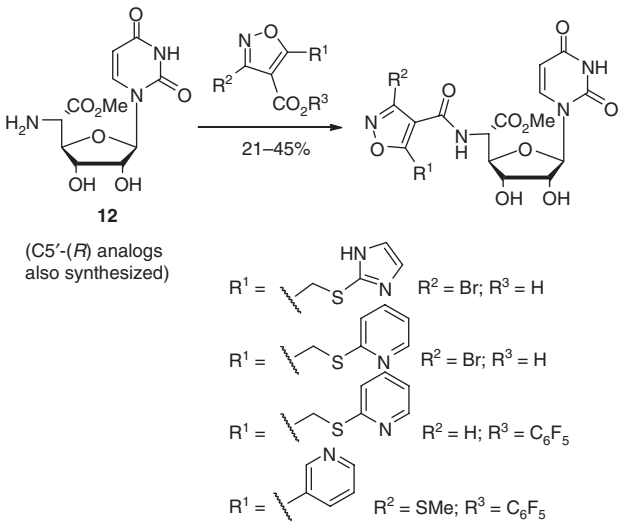



**Figure 3** Proposed mechanism of chitin biosynthesis (left) and bisubstrate inhibitors (right).




Scheme 2

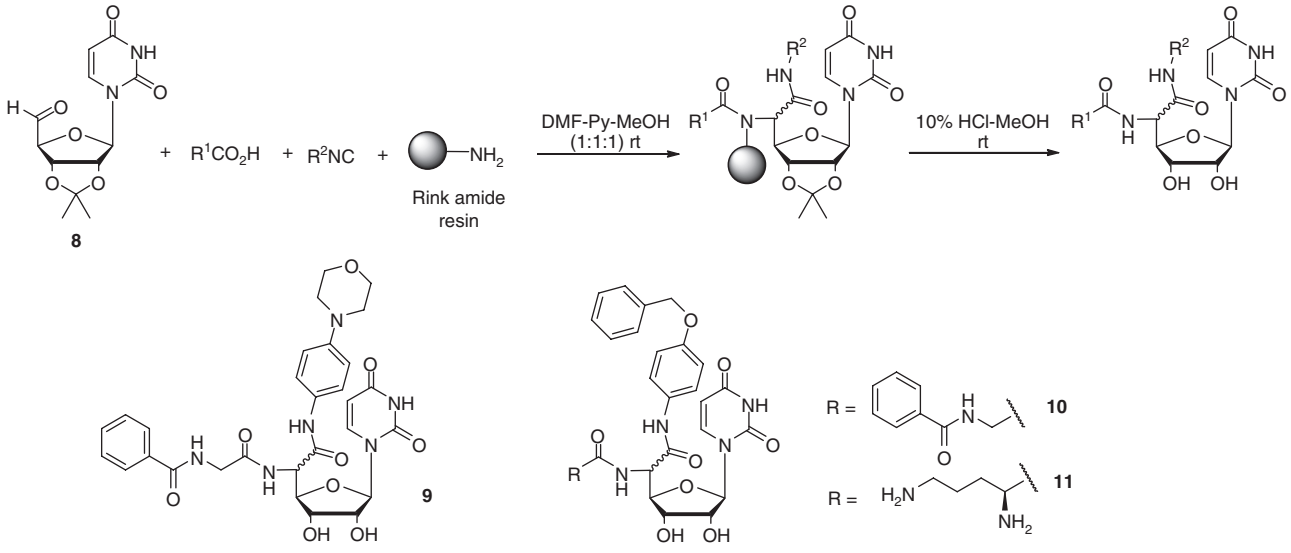



Scheme 4

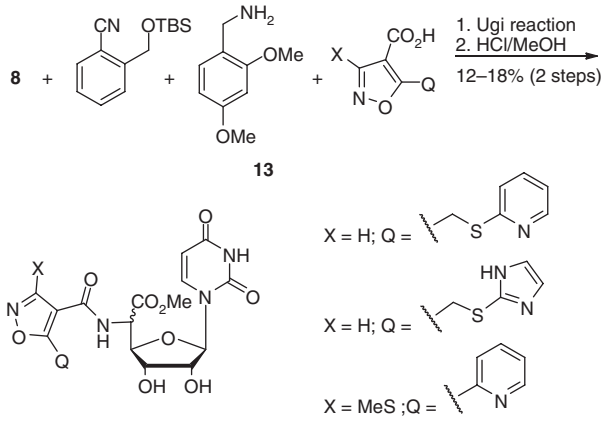
In addition, Plant et al. discovered that these compounds could also be synthesized via a highly chemoselective 4-component intramolecular Ugi reaction from aldehyde **8** and Lindermann's convertible nitrile **13** (Scheme 5) [46]. Although this particular study did not yield any compounds that displayed any significant biological activity, it provided a simple multicomponent sequence using readily available materials to rapidly produce compound libraries for further biological evaluation.

Guillerm and coworkers synthesized a variety of interesting nikkomycin analogs, aiming to mimic the metal-chelating transition state involving the pyrophosphate moiety of UDP-GlcNAc [47]. Several linkers such as malonic, tartaric and carbohydrate groups were chosen to replace the pyrophosphate moiety (Scheme 6). The key intermediates were synthesized and linked together via simple DCC-mediated couplings. Inhibition studies of these compounds were performed on a variety of fungal strains; however, only weak inhibition was observed. This indicated that binding affinity was not improved by replacing the pyrophosphate group of UDP-GlcNAc with these functional groups, several of which are susceptible to cleavage under metabolic conditions.

The Grigg laboratory developed an interesting 1,3-dipolar cycloaddition reaction of uracil polyoxin C (**3**) with mono- or dicarbonyl compounds in the presence of a dipolarophile to form heterocyclic polyoxin adducts

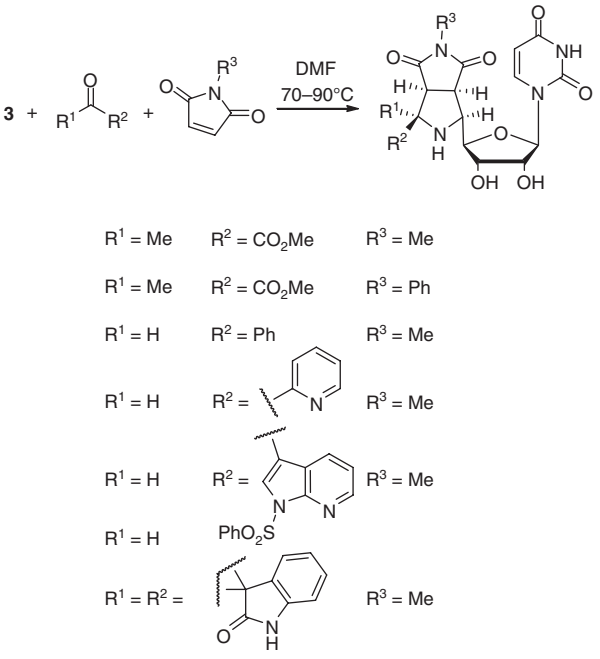



Scheme 3




**Scheme 5**

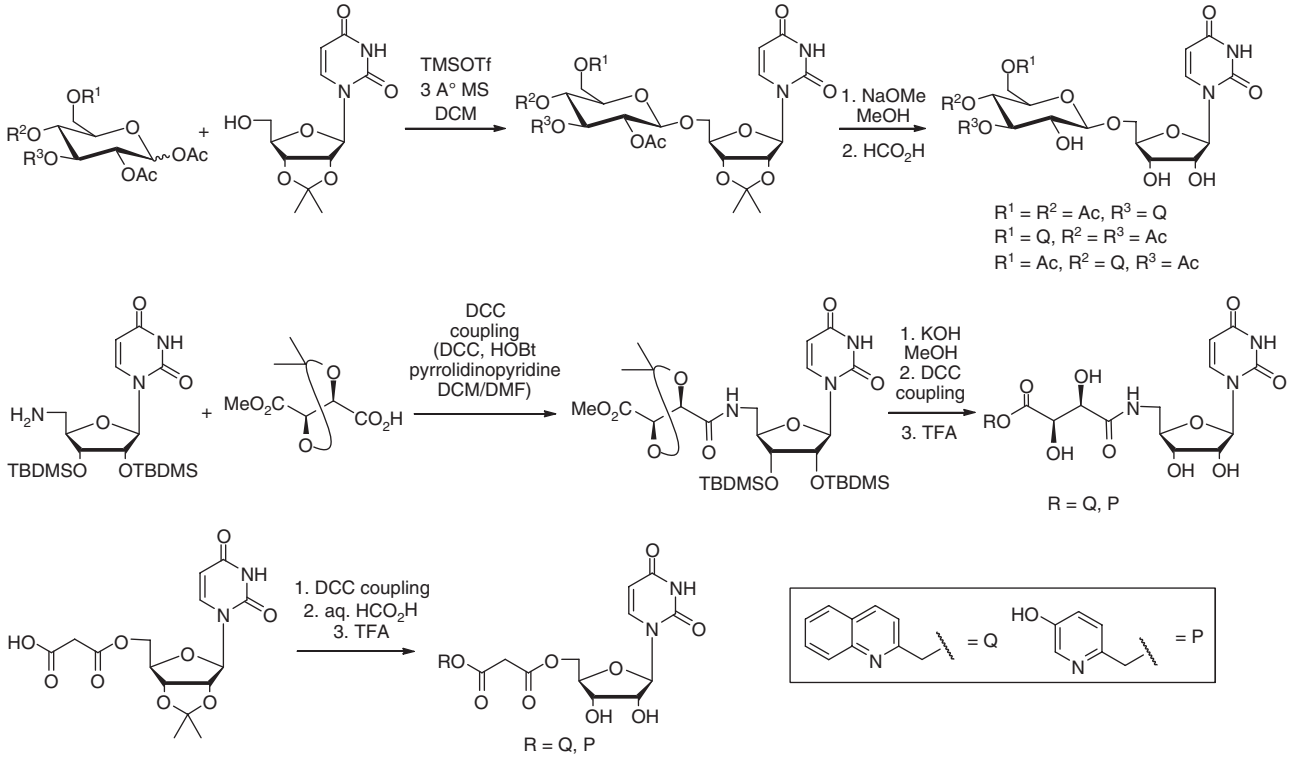
(Scheme 7) [48]. This synthetic transformation occurred in good yields with high diastereoselectivity; however, biological evaluation of these analogs did not uncover any potential lead compounds.



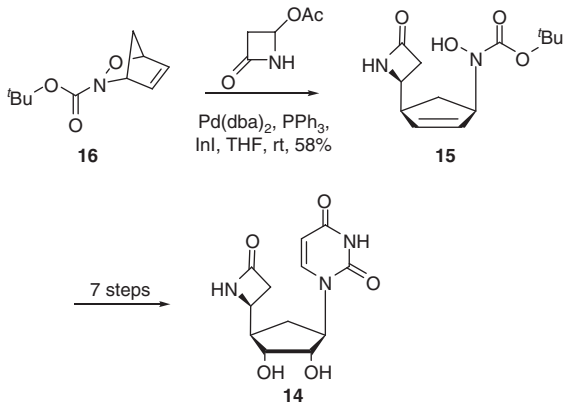

**Scheme 7**

## Nucleoside modifications

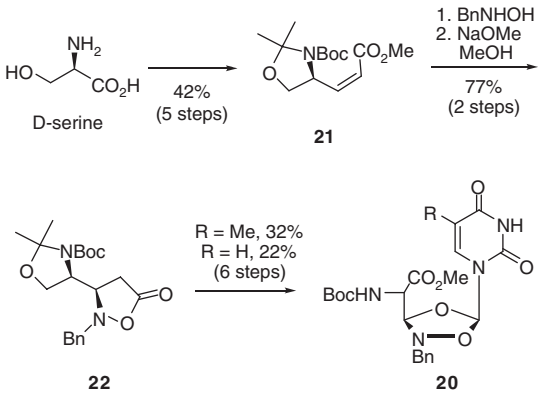
The central ribose core of the polyoxins and nikkomycins has been less extensively investigated compared to the peptidyl side chain; however, several groups have successfully synthesized carbocyclic analogs [49–53]. The most common approach involves the synthesis of carbocyclic polyoxin C, as this unit is present in both the polyoxin




**Scheme 6**

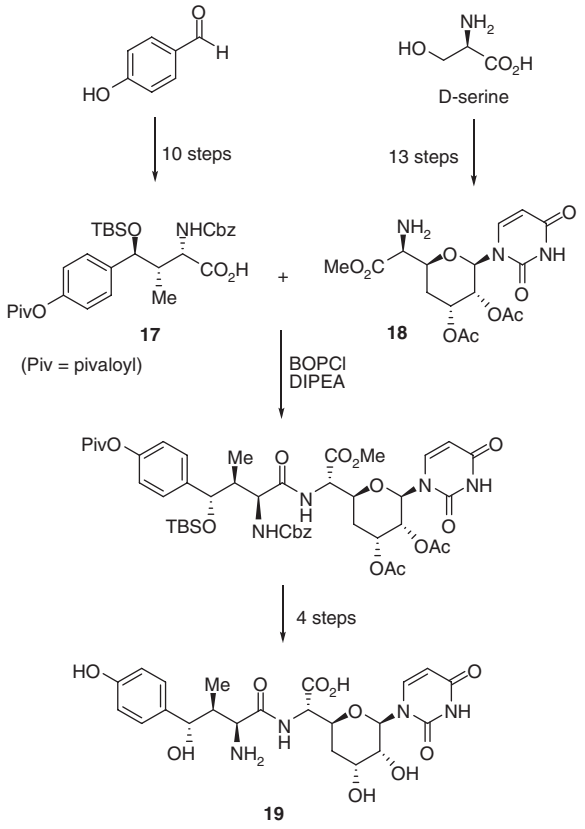



Scheme 8




Scheme 10

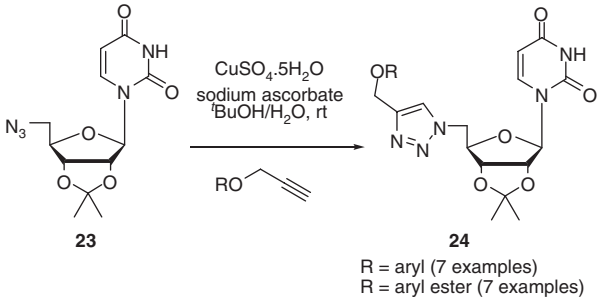
and nikkomycin structural framework. Recently, Miller and coworkers prepared a carbocyclic uracil polyoxin C analog **14** from an acylnitroso-derived hetero-Diels-Alder cycloadduct (**15**; Scheme 8) [54]. Palladium (0)/InI-mediated allylation of 4-acetoxy-2-azetidinone (**16**) was used to install the β-amino acid side chain at the C-5′ position of the carbocycle. A racemic mixture of the target compound **14** was generated, the biological activity of which has not yet been reported.



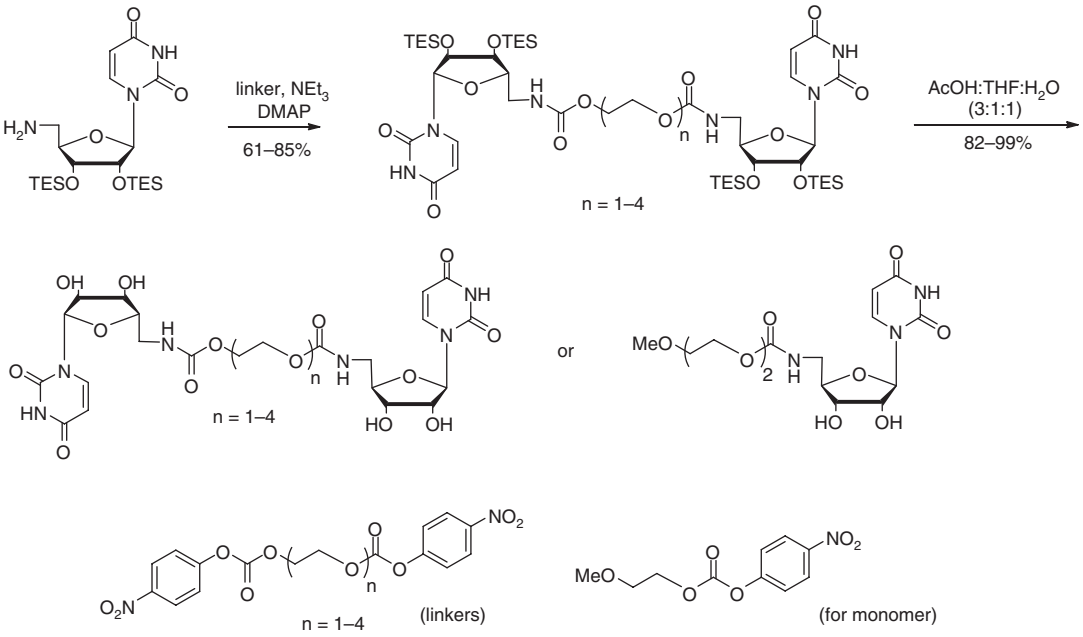

Scheme 9

Datta and coworkers also probed the structure-activity relationship of the ribose unit by developing a carbohydrate ring-expanded analog of nikkomycin B (**5**) [55]. This convergent synthetic route involved a stereocontrolled synthesis of the amino acid side chain fragment **17** and the pyranosyl nucleoside subunit **18** [56]. Peptide coupling of these two fragments completed the structural framework of the target compound **19** (Scheme 9). *In vitro* biological evaluation of **19** found that it displayed strong inhibitory activity against several human pathogenic fungal strains comparable to nikkomycin B (**5**) and was 10-fold more potent than the current antifungal treatment, amphotericin B.

Merino and coworkers, having earlier achieved the total synthesis of several polyoxins [57, 58], applied their experience with the asymmetric construction of heterocycles to synthesize analogs of uracil polyoxin C in which the furanose ring was replaced with an isoxazolidine ring (**20**; Scheme 10) [59]. After encountering issues with facial selectivity and several modifications to their synthetic approach, the key step of this synthesis involved Michael addition of *N*-benzylhydroxylamine to the *cis*-alkene **21**, generating the isoxazolidine ring **22** with a high degree of stereocontrol. Subsequent glycosylation with uracil or thymine provided the isoxazolidinyl nucleosides **20** with




Scheme 11




**Scheme 12**

the correct facial selectivity as the major product. Biological activity of these compounds has not yet been reported.

## Linker modifications

Replacing the peptide bond of the polyoxins and nikkomycins with a metabolically stable linker is expected to improve their biological profile. Chaudary and coworkers recently explored this hypothesis by utilizing Sharpless click chemistry to synthesize a series of 1,2,3-triazolyl-linked uridine derivatives (Scheme 11) [60]. Copper (I)-mediated 1,3-cycloaddition reactions were carried out using a selection of terminal alkynes and 5′-azidouridine (**23**) to generate a series of 1,4-disubstituted triazoles (**24**). These compounds were screened for biological activity against several fungal strains and displayed activity better than or comparable to nikkomycin Z (**4**) and a current treatment, fluconazole. These results were particularly interesting as the isopropylidene protecting groups were not removed prior to biological testing.

Following work investigating UDP-GlcNAc analogs as chitin synthase inhibitors [61–63], Finney and his group turned to designing a direct experimental proof of concept of the two active site mechanism using uridine dimers linked by various hydrocarbon linkers (Scheme 12) [41]. These compounds were synthesized via simple coupling of 5′-deoxy-5′-aminouridine units connected by ethylene glycol carbamate-based linkers. Their results demonstrated that the length of the linker affected the rate of inhibition, with the shorter chain dimers (n = 1 or 2) possessing a 10-fold higher inhibition that any of the longer chain-linked dimers or the monomer control. This observation was also supported by an additional chitin synthase inhibition study of a second generation of dimeric analogs possessing tartrate linkers (Figure 4)

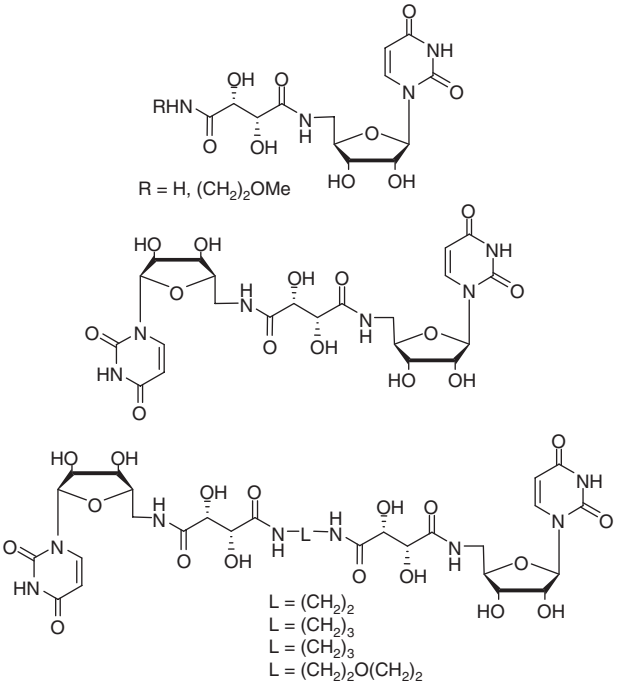
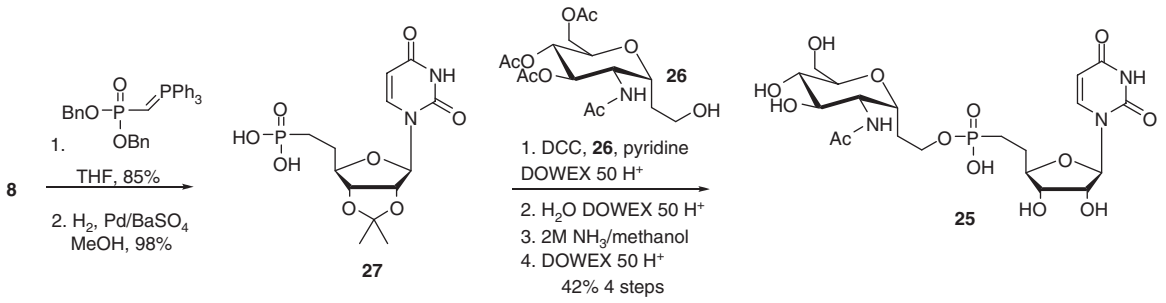



**Figure 4** Additional dimeric analogs.




**Scheme 13**

[42]. As the best percentile inhibition of the second generation dimer analogs was comparable to the most active analog in the first generation series, it was apparent that replacing the pyrophosphate group of UDP-GlcNAc with a tartrate group did not improve chitin synthase inhibition. However, these studies demonstrated the likelihood that chitin biosynthesis involves two catalytic sites in close proximity, and that the polyoxins and nikkomycins might be bisubstrate inhibitors.

Protozoan cyst walls are not as fully characterized as those in other organisms; however, it is known that some such as *Giardia*, *Entamoeba* and *Toxoplasma* contain chitin or a chitin-like polysaccharide, poly-*N*-acetylgalactosamine or poly(GalNAc) [64–68]. Recently reported enzymatic activity of cyst wall synthase in *Giardia* inspired us to investigate this as a novel drug target [66, 67]. The initial design of our polyoxin analogs, which we named phosphonoxins, incorporated a stable phosphonate linker between uridine and *N*-acetylglucosamine (GalNAc). Employing this functional group as a linker was anticipated to make the molecule more chemically and metabolically stable, and to improve cell membrane penetration [69, 70]. Our first inhibitor (**25**) was a substrate analog of UDP-GalNAc. This was synthesized from the known aldehyde **8** in six steps involving a DCC-mediated coupling of the GalNAc intermediate **26** to the nucleoside fragment **27** (Scheme 13). Phosphonoxin **25** was slightly more active against *Giardia* trophozoite culture than metronidazole [minimum inhibitory concentration (MIC) = 4.8 μM].

A second generation series of phosphonoxins was also synthesized (**28a–j**; Figure 5), containing an aza-sugar or aza-sugar analog in place of the GalNAc moiety of **25** [71]. These compounds were screened for biological activity, with the discovery of phosphonoxin **28i** as a potent inhibitor of *Giardia* trophozoite growth (MIC = 0.48 μM). This challenged existing therapeutics such as metronizadole and also showed remarkable inhibition of *Giardia* cyst formation (5.73% at 10 μM drug concentration). Phosphonoxin **28i** was synthesized in five steps from diethylvinylphosphonate (**29**; Scheme 14) [72]. DCC-mediated coupling of β-amino phosphonate **30** with isopropylidene-protected uridine followed by global deprotection gave the target phosphonoxin **28i**.

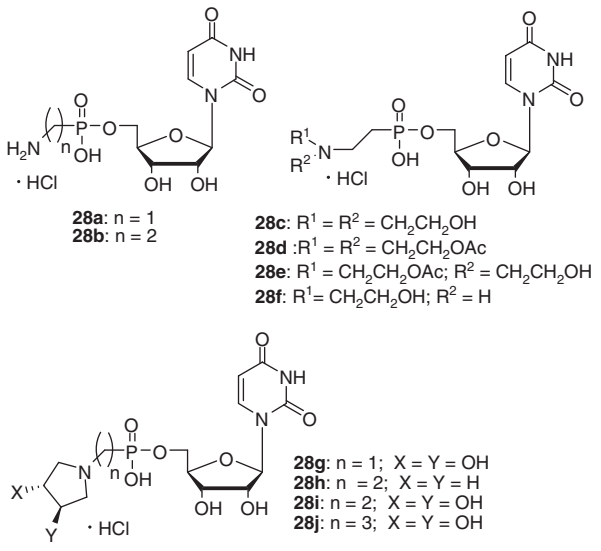
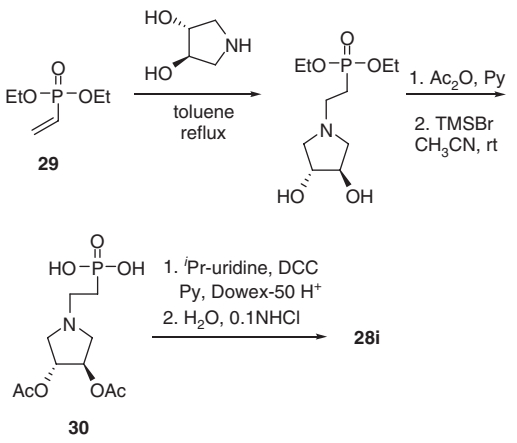
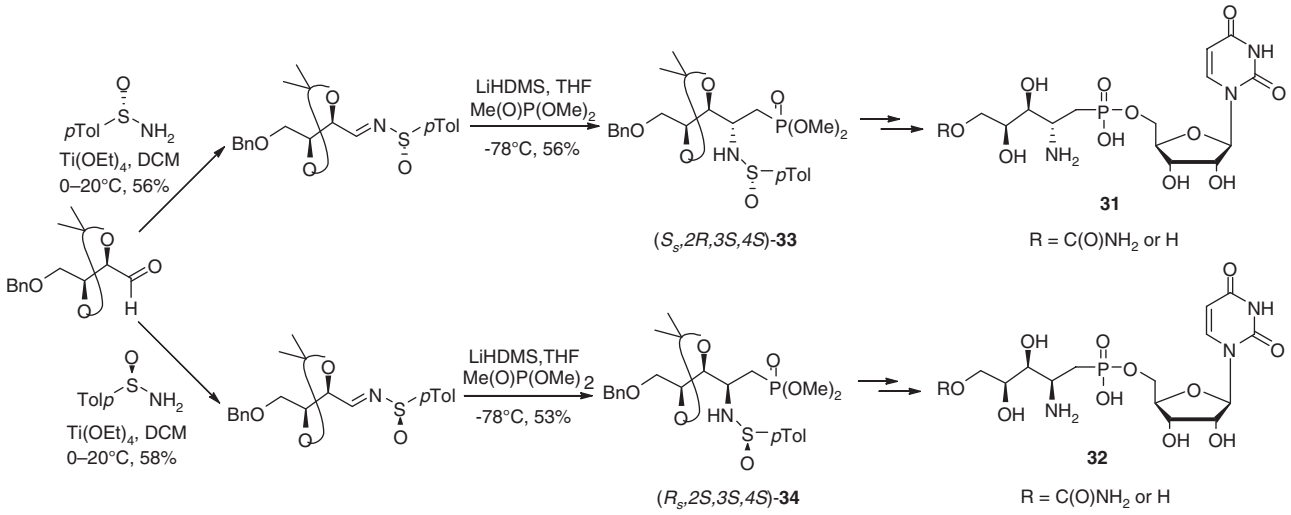



**Figure 5** Second generation phosphonoxins.



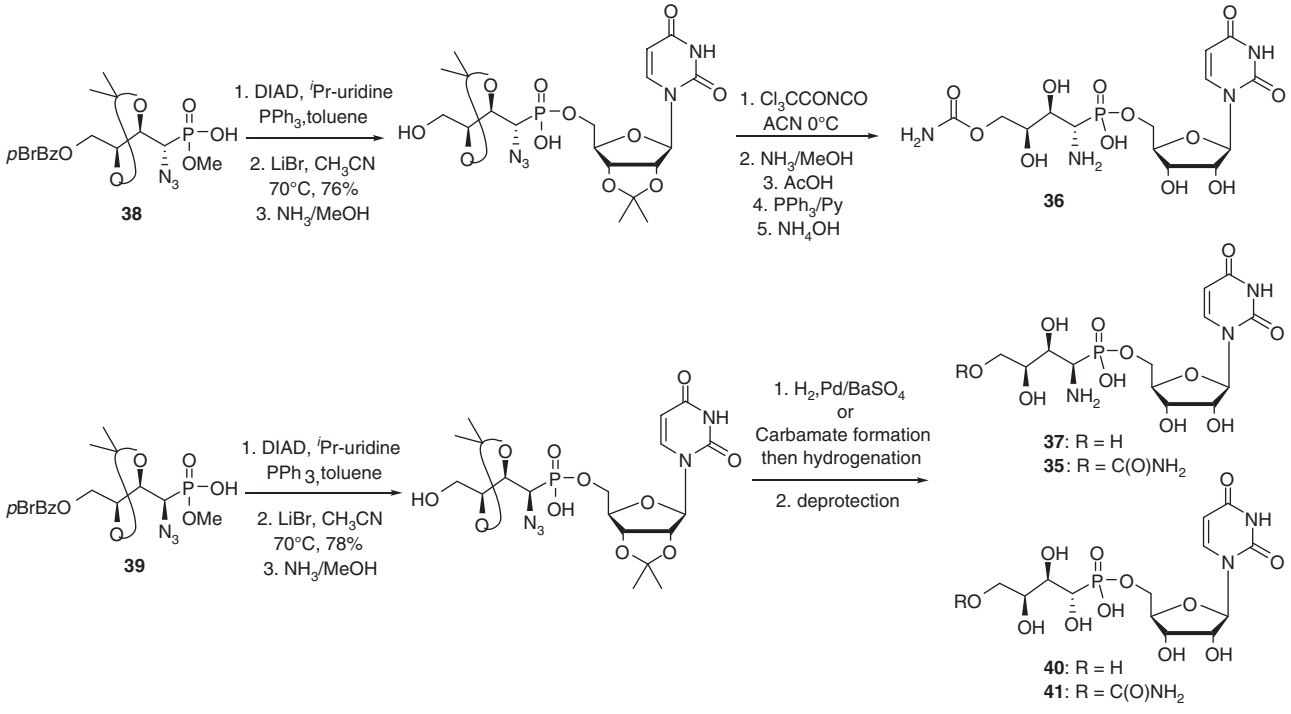

**Scheme 14**




**Scheme 15**

In addition to this work, a series of β-amino phosphonoxins, **31** and **32**, was prepared (Scheme 15) [73]. The key step of this synthesis was sulfinimine-mediated asymmetric formation of the aminophosphonates **33** and **34** as the major diastereoisomers. A double stereodifferentiation effect was not observed, and the diastereoselectivity is controlled by the absolute configuration of the sulfinyl group.

A variation of this asymmetric protocol was also employed in the synthesis of novel α-aminophosphonate analogs of the phosphonoxins (**35**–**37**; Scheme 16), which were designed to be more structurally similar to the polyoxins [74]. Mitsunobu coupling of the key fragments **38** and **39** with isopropylidene-protected uridine generated the phosphonoxin core. α-Hydroxyphosphonate analogs **40** and **41** were also obtained by taking advantage of an




**Scheme 16**

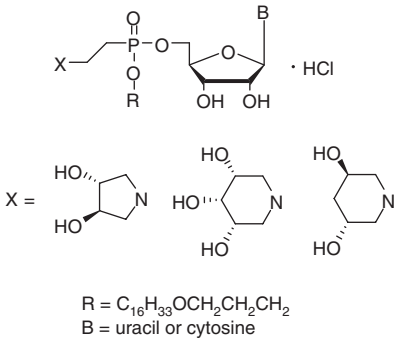



**Figure 6** Lipophosphoxin analogs.

unprecedented conversion of an azide to a hydroxyl group during hydrogenation. Phosphonoxin **41** was the most active compound in this series, with inhibition of *Giardia lamblia* trophozoite growth at a concentration of 2.3 μM. Chitin synthase inhibition of the phosphonoxin series is currently being evaluated.

Recently, Rejman and coworkers investigated the phosphonoxins further for antibacterial activity by improving their cellular uptake using a prodrug approach. The negative charge on the phosphonate moiety of several phosphonoxins was replaced by a lipophilic and metabolically cleavable hexadecyloxypropyl ester group and consequently named lipophosphonoxins (Figure 6) [75]. These compounds displayed promising activities against several Gram-positive bacterial species, including multiresistant strains. The MIC values of the best inhibitors were in the 1–12 μg/mL range, whereas their cytotoxic concentrations against human cell lines were significantly above this range.

## Conclusions

Recent examples of polyoxin and nikkomycin analogs in the literature have demonstrated a clear trend moving away from the synthesis of classical natural substrate-derived analogs towards the design of compounds that take the mechanistic implications of chitin biosynthesis into consideration. The scope of nucleoside chemistry has also been expanded by incorporating less traditional synthetic methods to generate novel structural motifs that improve the metabolic stability and biological profile of the polyoxins and nikkomycins. This has resulted in some impressive lead compounds and contributed to a greater knowledge of chitin biosynthesis. However, a more detailed understanding of this process is required before this class of compounds can eventually be translated into new, effective and nontoxic therapeutic agents.

Received August 29, 2013; accepted September 30, 2013

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