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Application of the Ugi Reaction for the One-Pot Synthesis of Uracil Polyoxin C Analogues

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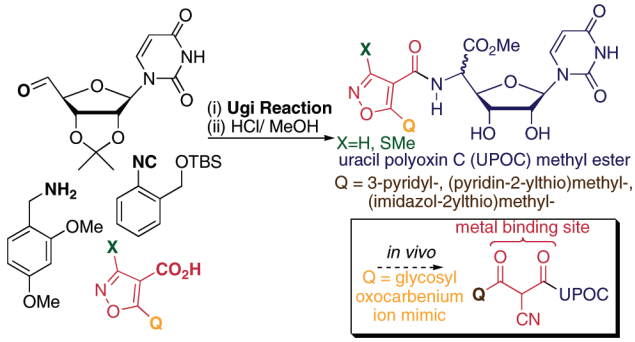
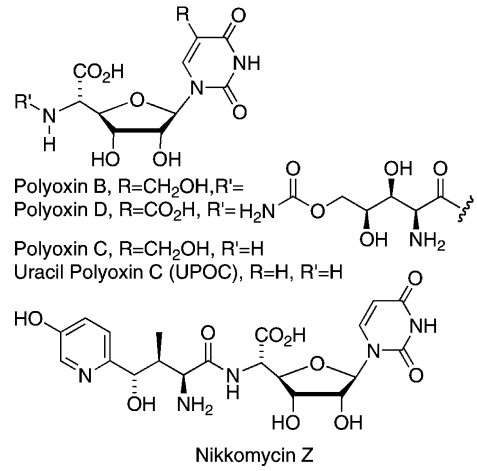


A simple, two-step synthesis of amide derivatives of uracil polyoxin C (UPOC) methyl ester using the Ugi reaction is described. The four components employed in the Ugi reaction are 2′,3′-isopropylidine-protected uridine-5′-aldehyde, 2,4-dimethoxybenzylamine, an isoxazolecarboxylic acid, and the convertible isonitrile *N*-(2-{[(*tert*-butyldimethylsilyl)oxy]methyl}phenyl)carbonitrile. Following the Ugi reaction, treatment with HCl in MeOH achieves deprotection of the isopropylidene group and the *N*-benzyl group and conversion of the isonitrile-derived amide (the Ugi product) into the corresponding methyl ester. The procedure is amenable to automated multiparallel synthesis of novel compounds related to the polyoxin and nikkomycin nucleoside−peptide antibiotics.

Polyoxins and nikkomycins (examples shown in Chart 1) are nucleoside−peptide antibiotics that display biological activity against fungal chitin synthase (CS) derived from *Candida albicans* and/or *Saccharomyces cerevisiae*.[1] Since chitin is absent from mammals and plants, CSs are attractive targets for inhibition in both fungi and insects. Literature-derived structure−activity relationships for the polyoxins, nikkomycins and their synthetic analogues suggest several important structural

CHART 1. Structures of Selected Polyoxins and Nikkomycins



requirements for biological activity. These include the pyrimidine base,[2,3] the C5′ carboxyl group and associated C5′-(*S*)-configuration[4−7] and the presence of a free NH on the amide backbone of the side chain.[8,9]

One of the simplest reported routes to uracil polyoxin C (UPOC) analogues involves a modified Strecker reaction between 2′,3′-isopropylidene-protected uridine-5′-aldehyde (**1**), an amino acid, and TMSCN in the presence a Lewis acid.[10] This affords UPOC precursors, 5′-α-amino nucleosides, in reasonable yield, but as a mixture of two diastereoisomers with ratios of between 6.5:1 and 4:1 in favor of the natural (C5′-*S*) configuration of UPOC. Stereoselective synthetic routes to polyoxin C and analogues thereof have also been described by a number of groups, including ourselves.[10−18]

In our own recent studies,[18] we reported a highly stereoselective, three-step synthesis of both natural (C5′-*S*) and unnatural (C5′-*R*) diastereoisomers of uracil polyoxin C methyl ester. A

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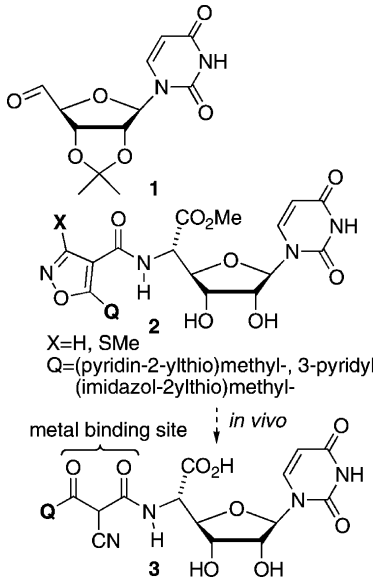

FIGURE 1. Structures of protected uridine aldehyde precursor (**1**) and polyoxin C derivatives designed as plant pro-pesticides.[18]

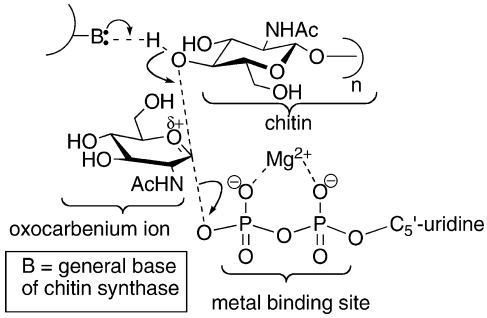

FIGURE 2. Putative transition state structure during chitin synthesis.

key step of this synthesis is the addition of TMSCN to an appropriate chiral sulfinimine derived from uridine aldehyde **1**. Further elaboration of UPOC methyl ester by conjugation to various isoxazole carboxylic acids gave compounds of general structure **2** (Figure 1), designed as potential pro-pesticidal agents directed against CS. In designing these compounds we sought to include features of both polyoxins/nikkomycins and of the putative transition state structure derived from the natural substrate UDP-GlcNAc during its enzymatic incorporation into chitin (Figure 2). The isoxazole unit was incorporated as a lipophilic, masked, metal-binding site, which we postulated would facilitate transmembrane permeability in plants and subsequently, upon cleavage of the heterocycle, form a 1,3-dicarbonyl moiety (structure **3**, Figure 1). This strategy has been shown to improve transmembrane permeability and root uptake of the herbicide isoxaflutole.[19] The analogues we have prepared to date do not display significant pesticidal activity, the reasons for which are currently under further investigation.

One goal of our initial studies was to produce compound libraries of structures based on **2** for high-throughput screening to identify new lead structures for novel crop protection agents. With this task in mind, we considered a short and effective means of generating compounds of general structure **2**. Retrosynthetic analysis of structure **2** suggested that the four-component Ugi reaction might be applicable (Figure 3), especially if a suitable convertible isonitrile could be used which could be easily transformed into the C5′-methyl ester (or other functionality likely to give rise to the corresponding carboxylic acid in vivo).

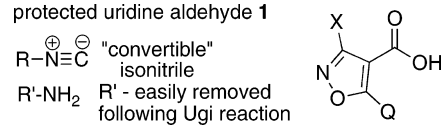

FIGURE 3. Components required for synthesis of UPOC analogues **2** via the Ugi reaction.

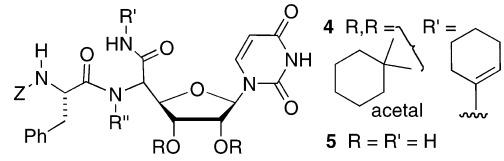

FIGURE 4. Polyoxin C analogues synthesized by the Ugi reaction.[8]

In a brief communication, Tsuchida et al. reported the synthesis of a protected C5′-carboxamide precursor to UPOC using the Ugi reaction[20] but provided no details of the conversion of this compound into UPOC. The Ugi reaction has also been employed in the solid-phase, combinatorial synthesis of nikkomycin analogues using a Rink amide resin.[21] However, since no convertible isonitriles were used, analogues with the important carboxyl group at the C5′-position were not synthesized. Boehm and Kingsbury have employed the Ugi reaction to synthesize polyoxin analogues containing *N*-methylated peptide bonds.[8] Thus, 2′,3′-protected uridine-5′-aldehyde, L-(carbobenzyloxy)phenylalanine (L-ZPhe), methylamine, and two different isonitriles were reacted in the presence of methanol to give Ugi products as a mixture of diastereoisomers (i.e., no stereocontrol at C5′). Treatment of the Ugi product **4** (derived using cyclohexenyl isonitrile) with acetic acid afforded the corresponding C5′-carboxamide **5** in 25% overall yield (Figure 4). Armstrong has shown that conversion of isonitriles with similar structures to **4** to the corresponding carboxylic acids requires a strong electron-donating *N*-acyl group[22] and often harsh acid conditions. Indeed, conversion of the carboxamide **5** to the C5′-carboxylic acid required treatment with nitrosyl sulfuric acid.[8] In the context of our own goals, the use of cyclohexenyl isonitrile was not considered to be ideal. First, the post-Ugi conversion to the carboxylic acid was expected to be difficult since our target compounds were not *N*-methylated, and in addition, we anticipated that this would be accompanied by hydrolysis of the secondary amide function of the polyoxin side chain. Futhermore, based on our own experience and that of others,[23] cyclohexenyl isonitrile is often difficult to prepare, is generally obtained in only moderate yield, and must be stored at −30 °C due to its low thermal stability.

Initially, to confirm that our target compounds could indeed be prepared by the Ugi reaction, we employed a simple (nonconvertible) isonitrile, together with the other three components shown in Scheme 1. Thus, the Ugi reaction with

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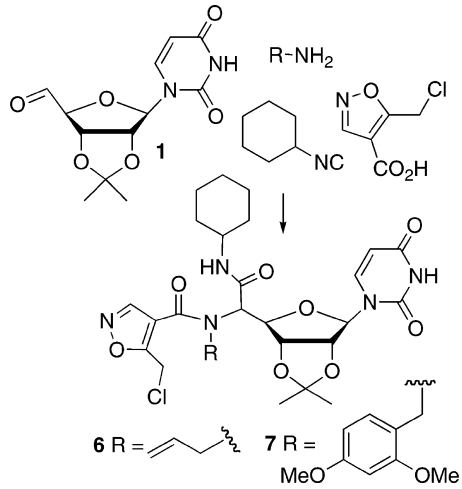
SCHEME 1. Ugi Reactions Using Cyclohexane Isonitrile

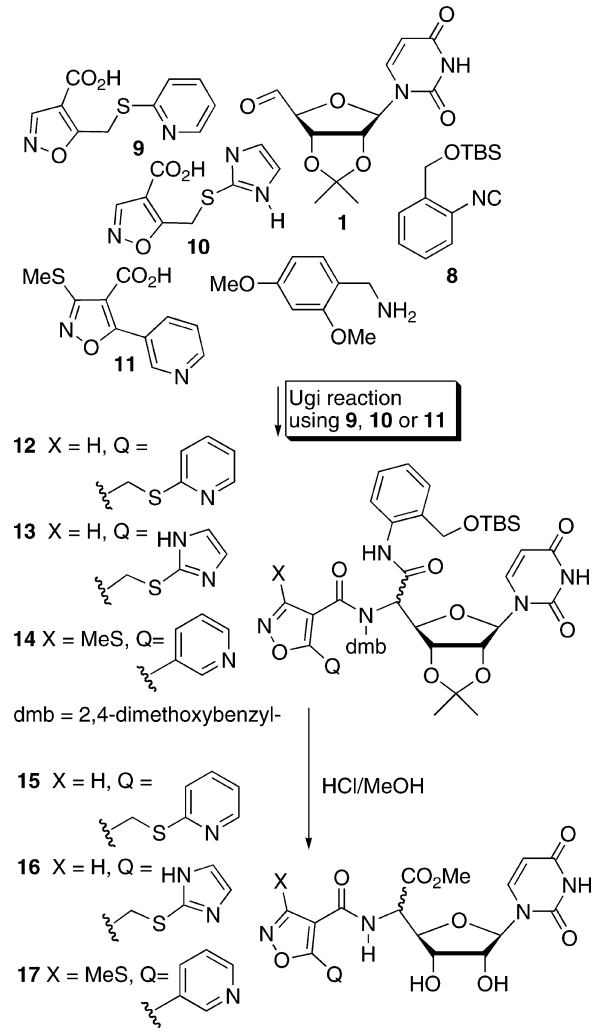


aldehyde **1**[24] in methanol gave compound **6** in 70% yield. To evaluate the viability of the Ugi reaction for synthesizing analogues using parallel synthesis and to generate a library of novel compounds based on structure **2**, we used a Zymark robot to perform the reaction. Thus, nucleoside **1**,[24] a variety of 20 different amines, 5-(chloromethyl)isoxazole-4-carboxylic acid,[18] and six different isonitriles were reacted for 24 h in methanol at room temperature to generate a 120-component library of Ugi products.

In order to obtain the desired C5′-NH derivatives of compound **6**, we investigated a variety of different conditions to remove the allyl protecting group. Unfortunately, all of these methods including Pd(0) and NDMBA,[25] sulfinic acid[26] or 2-mercaptobenzoic acid,[27] zirconium cyclopentadiene,[28] rhodium(I),[29] or Grubbs' first-generation carbene[30] proved unsuccessful. We attributed this to the possibility that the allyl group might be sterically shielded, thereby preventing the effective co-ordination of the respective metal complexes. Consequently, as an alternative, we investigated using 2,4-dimethoxybenzylamine as an ammonia equivalent in the analogous Ugi reaction, based on its utility during peptide synthesis and subsequent ease of debenzylation under acid conditions.[31] The Ugi product **7** was obtained in good yield (68%) as a 1:1 mixture of diastereoisomers. Treatment of compound **7** with HCl in methanol resulted in the successful cleavage of the 2,4-dimethoxybenzyl group and simultaneous removal of the sugar isopropylidene group.

With this result in hand, we next focused on the Ugi reaction using the convertible isonitrile **8** (Scheme 2) described by Linderman.[23] The synthesis of **8** is considerably easier than the synthesis of cyclohexenyl isonitrile. Furthermore, the formamide precursor to **8** is stable to long-term storage while in our hands the isonitrile itself also proved to be stable when stored at −18 °C.

SCHEME 2. Ugi Reactions Using Linderman's Convertible Isonitrile[23]



When Ugi reactions were performed with aldehyde **1**,[24] isonitrile **8**, dimethoxybenzylamine, and isoxazoles[18] **9**, **10**, or **11**, the desired Ugi products **12**−**14** were obtained with yields of 50%, 35%, and 45%, respectively (Scheme 2). In each case, the Ugi products are obtained as mixtures of diastereoisomers. Although for compounds **12** and **13** the two diastereoisomers were only partially resolved by silica TLC, for compound **14** it proved possible after repeated chromatography to obtain a faster and slower running component which we have characterized by NMR. This confirms that the individual diatereoismers are formed in the Ugi reaction in an approximately 1:1 ratio. Interestingly, the faster running diastereoisomer of **14** appears to consist of two conformational isomers as evidenced by NMR. Treatment of compounds **12**−**14** with HCl in methanol facilitated ketal cleavage, *N*-debenzylation, and conversion of the isonitrile-derived amides to the corresponding C5′-methyl ester derivatives of UPOC. The products **15**−**17** were obtained after flash chromatography in yields of 35%, 35%, and 31%, respectively, as mixtures of diastereoisomers.

Previously, we have shown that compounds **15** and **17** undergo isoxazole ring-opening under basic conditions and in the presence of Fe(II), respectively, to afford diketonitriles analogous to compound **2**.[18] The novel compound **16** could be converted under basic conditions (Et_3N in MeOH) to produce the diketonitrile **18** (Figure 5). Despite this, compound **16** did

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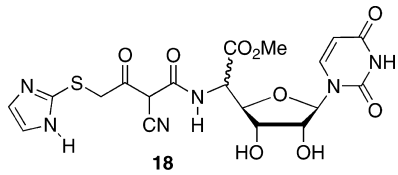

18

FIGURE 5. Structure of diketonitrile **18** derived from **16** under mildly basic conditions.

not display any significant biological activity using the assays that we have employed previously.[18]

In summary, we have shown that the four-component Ugi reaction may be employed for the synthesis of polyoxin/nikkomycin analogues from a readily available uridine 5′-aldehyde, dimethoxybenzylamine, Linderman's convertible isonitrile,[23] and novel isoxazolecarboxylic acids. Treatment of the Ugi products with HCl in methanol provides the polyoxin/nikkomycin analogues as their C5′-methyl esters in two steps overall and provides a simple means of producing compound libraries for biological evaluation.

Experimental Section

***N*-[(*tert*-Butyldimethylsilyloxymethyl)phenyl] 1,5-Dideoxy-1-(3,4-dihydro-2,4-dioxo-1-(2*H*)-pyrimidinyl)-5-{5-[(pyridin-2-ylthio)methyl]isoxazole-4-carbonyl(2,4-dimethoxybenzyl)-amino}-*β*-D-allofuranuronamide (α-L-Talofuranuronamide) (12).** Nucleoside **1**[24] (250 mg, 0.887 mmol), 2,4-dimethoxybenzylamine (148 mg, 0.887 mmol), and isoxazole **9**[18] (209 mg, 0.887 mmol) were dissolved in dry MeOH (10 mL). Isonitrile **7**[23] (219 mg, 0.887 mmol) was then added and the reaction stirred at room temperature under argon for 24 h. The mixture was then evaporated and the residue dissolved in EtOAc (50 mL) which was then washed sequentially with satd aq $NaHCO_3$ solution, water, and brine. The organic layer was dried ($MgSO_4$) and evaporated to a yellow solid. Purification by flash chromatography (silica, 1:1 hexane/EtOAc, then EtOAc) gave a beige-colored solid (405 mg, 0.44 mmol, 50%). HRMS (ESI): *m*/*z* calcd for $C_{45}H_{54}N_6O_{11}SSi{\cdot}Na$ $[M + Na]^+$ 937.3214, found 937.3238. ^1H NMR (500 MHz, $CDCl_3$): δ −0.01−0.07 (m, 6H), 0.86−0.89 (m, 9H), 1.20−1.29 (m, 3H), 1.53 (bs, 3H), 3.55−3.61 (m, 3H), 3.68−3.77 (m, 3H), 4.52−5.18 (m, 9H), 5.47−5.73 (m, 2H), 6.22−6.41 (m, 2H), 6.93−7.54 (m, 9H), 8.20−8.34 (m, 1H), 8.35−8.40 (m, 1H), 8.87−8.92 (m, 1H), 9.62 (bs, 1H), 9.72 (bs, 1H). ^{13}C NMR (126 MHz, CDCl3): δ −5.3, −5.2, 14.0, 18.2, 23.5, 23.6, 24.6, 24.9, 25.1, 25.8, 26.1, 26.9, 27.0, 55.0, 55.2, 60.2, 62.3, 62.7, 63.0, 80.7, 81.9, 82.0, 83.1, 83.7, 83.8, 85.7, 93.7, 95.3, 95.6, 98.5, 98.6, 102.1, 102.6, 102.8, 104.3, 104.4, 112.4, 112.5, 113.4, 114.9, 120.0, 120.1, 121.9, 122.0, 122.3, 122.6, 122.9, 124.4, 124.7, 124.8, 126.8, 127.3, 127.6, 127.7, 129.9, 130.1, 135.3, 136.1, 142.0, 142.3, 142.8, 148.4, 148.5, 148.8, 149.3, 149.4, 149.8, 150.5, 156.0, 156.1, 158.1, 160.9, 163.3, 163.8, 164.5, 165.1, 166.2, 166.7, 170.2.

Methyl 1,5-Dideoxy-1-(3,4-dihydro-2,4-dioxo-1-(2*H*)-pyrimidinyl)-5-{5-([pyridin-2-ylthio)methyl]isoxazole-4-carbonylamino}-*β*-D-allofuranuronate (α-L-Talofuranuronate) (15). Nucleoside **12** (200 mg, 0.22 mmol) was dissolved in MeOH (10 mL) and cooled to 0 °C. HCl was then bubbled through the solution for 1 h at 0 °C. The solution was then allowed to warm to room temperature and stirred for 5 h. Water (6 mL) was then added and the mixture stirred for a further 12 h and then evaporated. EtOAc (20 mL) and satd aq $NaHCO_3$ (20 mL) were added to the residue, the two layers were separated, and the aqueous layer was extracted with EtOAc (3 × 20 mL). The combined organic layers were dried ($MgSO_4$) and evaporated, and the crude product was purified by flash chromatography (silica, 0−20% MeOH in EtOAc) to give a beige-colored solid as a 1:1 mixture of diastereoisomers (40 mg, 0.08 mmol, 35%). HRMS (ESI), ^1H NMR, and ^{13}C NMR data were in agreement with those described previously.[18]

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Supporting Information Available: Experimental details for the syntheses of compounds **13**, **14**, and **16**−**18**. ^1H and ^{13}C NMR spectra for compounds **12**−**14**, **16**, and **18**. ^1H and ^{13}C NMR spectra and $^1H-^{13}C$-coupled and $^1H-^1H$-coupled spectra for fast and slow diastereoisomers of **14**. This material is available free of charge via the Internet at http://pubs.acs.org.

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