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# Stereoselective Synthesis of Novel Uracil Polyoxin C Conjugates as Substrate Analogues of Chitin Synthase

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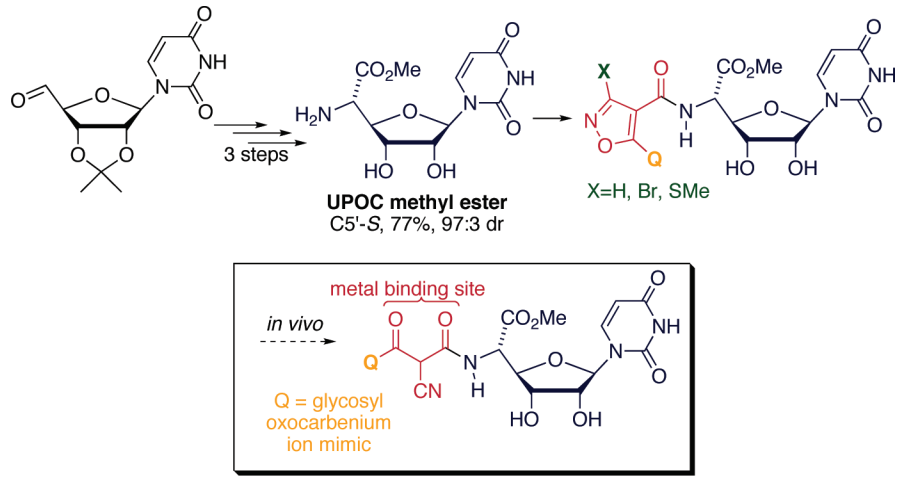


Stereoselective syntheses of both the natural (C5′-*S*) and unnatural (C5′-*R*) diastereoisomers of uracil polyoxin C methyl ester have been developed. The key stereocontrolled step involves nucleophilic addition of trimethylsilyl cyanide to the appropriate chiral sulfinimine derived from 2′,3′-protected 5′-formyluridine and (*S*)-(−)-*tert*-butanesulfinamide or (*R*)-(+)-*tert*-butanesulfinamide, respectively. A variety of substrate mimics designed to function as inhibitors of chitin synthase have been synthesized by conjugation of the methyl ester of uracil polyoxin C (UPOC) with activated isoxazole carboxylic acids. Amide bond formation was accomplished via coupling of the amino functionality of UPOC methyl ester with a free isoxazole acid using HBTU or alternatively an isoxazole pentafluorophenyl ester. The substrate mimics incorporate features of the nucleoside-peptide antibiotics, the polyoxins and the nikkomycins, as well as features of the transition state structure expected during polymerization of the natural chitin synthase substrate uridine diphosphoryl-*N*-acetylglucosamine (UDP-GlcNAc), namely, a metal-binding site and glycosyl oxocarbenium ion mimic.

## Introduction

Chitin is a homopolymer consisting of $\beta$-1,4-linked *N*-acetyl-D-glucosamine (GlcNAc) units. It is one of the most abundant natural polymers and is an essential structural component of the insect exoskeleton and the cell walls of fungi. The biosynthesis of chitin occurs by the polymerization of UDP-*N*-acetylglucosamine (UDP-GlcNAc) (Figure 1). Chitin synthases (CS) are attractive targets for the pharmaceutical and agrochemical industries due to their essential role in chitin biosynthesis in fungi and insects and due to their absence in mammals and plants.[1] Because CS are large integral membrane proteins, there has been limited progress toward the elucidation of detailed structural information that would be of benefit for the rational design of inhibitors. Furthermore the exact mechanistic details of chitin formation are still unresolved. Indeed the

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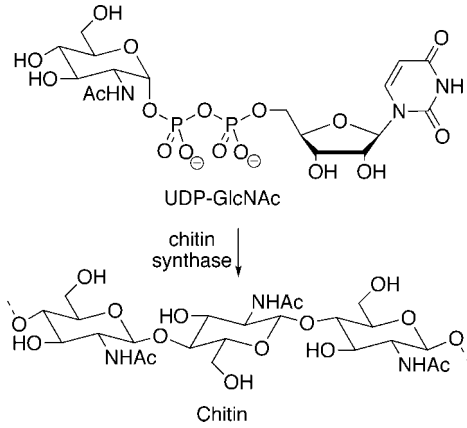
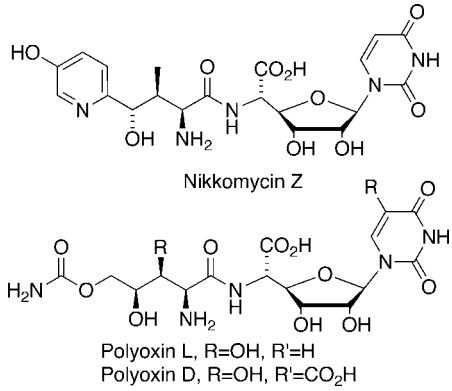



**FIGURE 1.** Synthesis of chitin by CS.

**CHART 1. Selected Nikkomycins and Polyoxins**




opposed orientations of the adjacent sugar units have prompted suggestions that CS might possess two active sites.[2]

Two major families of naturally occurring nucleoside-peptide antibiotics, the nikkomycins and the polyoxins (examples shown in Chart 1) possess some of the structural features of the natural substrate UDP-GlcNAc. Typically synthetic analogues of the nikkomycins or the polyoxins have been evaluated using fungal CS derived from *Candida albicans* and/or *Saccharomyces cerevisiae* and have highlighted several important structural requirements associated with biological activity. Features of particular note include the pyrimidine base,[3,4] the C5 carboxyl group and associated C5-(*S*)-configuration,[5–8] and the presence of a free NH on the amide backbone of the side chain.[9,10] Attempts to enhance the cellular uptake of analogues by incorporation of hydrophobic side chains have had some success,[11] while nonhydrolyzable analogues of UDP-Glc-NAc

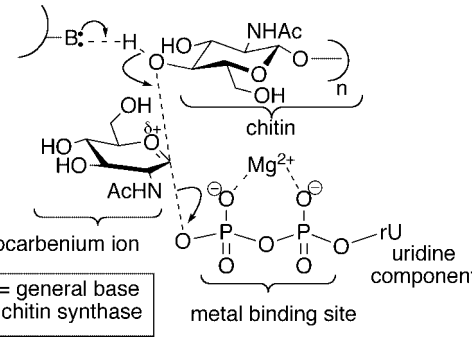



**FIGURE 2.** Postulated transition state for chitin synthesis.

have included its C1-methylenephosphonate[12] and related compounds in which the diphosphate moiety has been replaced by nonhydrolyzable linkages[13–15] such as tartrate and malonate. The Aggarwal group described the (+)-carbocyclic uracil polyoxin C,[16] for which the furanosyl oxygen of uracil polyoxin C is replaced by a methylene group. Such analogues display an enhanced stability of the glycosidic linkage and may be more amenable to cellular uptake. More recently, Miller and coworkers have provided an alternative synthesis for this analogue[17] and have also prepared its C5′-epimer.[18] Other analogues that have produced comparable activity to the nikkomycins and polyoxins as inhibitors of fungal CS have included an azasugar,[19] which might mimic the putative oxocarbenium ion transition state that ensues during glycosyl transfer. Recent studies from the Finney group have focused on designing dimeric analogues that assume a two-active-site mechanism for chitin synthase.[14,15,20] Although at present these latter studies have not provided unambiguous evidence for a two-active-site mechanism, it was demonstrated that dimeric analogues (that contain two uridyl moieties) displayed activity approximately 10-fold higher than that of corresponding monomeric analogues.

**Design of Novel CS Inhibitors as Potential Pesticidal Agents.** Our design of analogues that could function as inhibitors of CS in vivo focused on structural features of the nikkomycins, polyoxins, and the natural substrate UDP-Glc-NAc and also aimed to address the issues limiting in vivo activity. Specifically, we aimed to address problems of poor cellular uptake and bioavailability of such analogues while retaining the biologically important feature of the natural uracil polyoxin C. On the presumption of a transition state of the type shown in Figure 2, we envisaged three key features required of such compounds, namely, a uridyl component (preferably the polyoxin C nucleus), a metal-binding site, and a suitable surrogate of the oxocarbenium ion of the glycosyl unit.[21] Our strategy is unique in attempting to facilitate cellular uptake by synthesizing lipophilic

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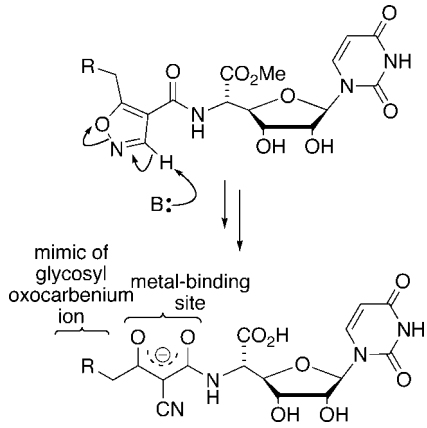


**FIGURE 3.** Target analogues for CS inhibition.

pro-pesticides[22–24] in which the polar metal binding site is masked within an isoxazole ring. Cleavage of the heterocycle reveals the 1,3-dicarbonyl functionality, which is capable of metal binding (Figure 3). This strategy has been shown to improve transmembrane permeability and root uptake of the herbicide isoxaflutole in planta.[25] Edmunds et al. have also reported that certain 3-alkylsulfinyl-4-nicotinoyl isoxazoles display herbicidal activity, presumably resulting from in vivo conversion to the corresponding diketonitriles.[26] Furthermore we sought to exploit the hypothesis that certain nicotinic acid derivatives are taken up into plants by active transport mechanisms.[27]

**Efficient Stereoselective Route to Polyoxin C.** Although several syntheses of uracil or thymine polyoxin C have been reported previously,[28–35] these either require a large number of synthetic steps or show poor stereoselectivity (Scheme 1). One of the shortest of these routes employed a modified Strecker reaction between 2′,3′-isopropylidene-protected uridine 5′-aldehyde, trimethylsilyl cyanide (TMSCN), and an amino acid in the presence of the Lewis acid $BF_3$ (route A, Scheme 1).[29] The 5′-α-aminonitrile nucleosides obtained were transformed to polyoxin C analogues following hydrolysis of the nitrile function. Depending on the amino acid used, diastereomeric ratios between 6:5 and 4:1 in favor of the natural polyoxin C (5′-(*S*)- configuration) were observed. In contrast, one of the more stereoselective routes to UPOC has been described by Barrett.[30] Thus, nucleophilic addition of potassium trimethylsilonate to the least hindered face of the *Z*-nitro alkene (Scheme 1, route B) gave the (*S*)-α-hydroxy thioester with a dr of greater than 15:1. However, a further six steps were required to produce uracil polyoxin C.

As the key step in an alternative synthesis (route C, Scheme 1), Evina and Guillerm[31] performed the asymmetric dihydroxylation of 5′-deoxy-5′-methylene uridine (derived from the corresponding 5′-aldehyde) using AD-mix-α with high diastereoselectivity (de 95%). The product was then converted to an O2,5′-cyclonucleoside which was subsequently treated with azide in HMPA. A further four steps afforded UPOC in 6% overall yield. Another approach[32] to the synthesis of UPOC has made use of α-L-talofuranosyluronic acid as the key intermediate. This in turn was obtained following the stereocontrolled nucleophilic addition of vinylmagnesium bromide to the *re* face of the protected ribose 5-aldehyde (obtained in 3 steps from D-ribose) as shown in Scheme 1 (route D). However, diastereoselectivity was limited, with optimized conditions giving a dr of only 3.7:1. Furthermore, the carbohydrate product requires five further synthetic steps for conversion into UPOC. More recently,[35] the Finney group has employed the same ribosyl aldehyde which undergoes the high yielding and stereospecific conversion to the acetylenic alcohol (route E, Scheme 1) upon reaction with a zinc acetylide in the presence of the chiral ligand (−)-*N*-methyl ephedrine. A further four steps were required to produce the azide (common to route D) as a precursor to UPOC.

Davis et al. have shown that nucleophilic addition of Grignard reagents to *p*-toluenesulfinylimino esters in the presence of a Lewis acid is highly stereoselective (dr 83:17 to 99:1) and following acid hydrolysis provides excellent yields of α-amino acids.[36] The mechanistic rationale for the control of the stereochemistry in these reactions is based on the selective coordination of the Lewis acid to the sulfinamide oxygen which shields one face of the imine toward nucleophilic attack. This methodology has also been applied to the stereoselective synthesis of polyoxamic acids in which HCN addition to the *p*-toluenesulfinylimines using ethylaluminium cyanoisopropoxide affords α-amino nitriles with a de of 91%.[37] More recently, examples of this chemistry have also been exploited by others,[38] and the addition of trimethylsilyl cyanide (TMSCN) to sulfinimines in the presence of Lewis bases has also been achieved with moderate-to-good stereoselectivity.[39,40] A solvent-controlled asymmetric Strecker reaction has recently been described for the synthesis α-trifluoromethylated amino acids using TMSCN addition to *N-tert*-butylsulfinylimines[41] In this case, the addition of TMSCN to sulfinylimines was investigated in various solvents, with hexane giving the most successful results (dr up to 99:1). In the nucleoside area, Jung et al.[42] have exploited the stereoselective addition of MeMgBr to a *N-tert*-

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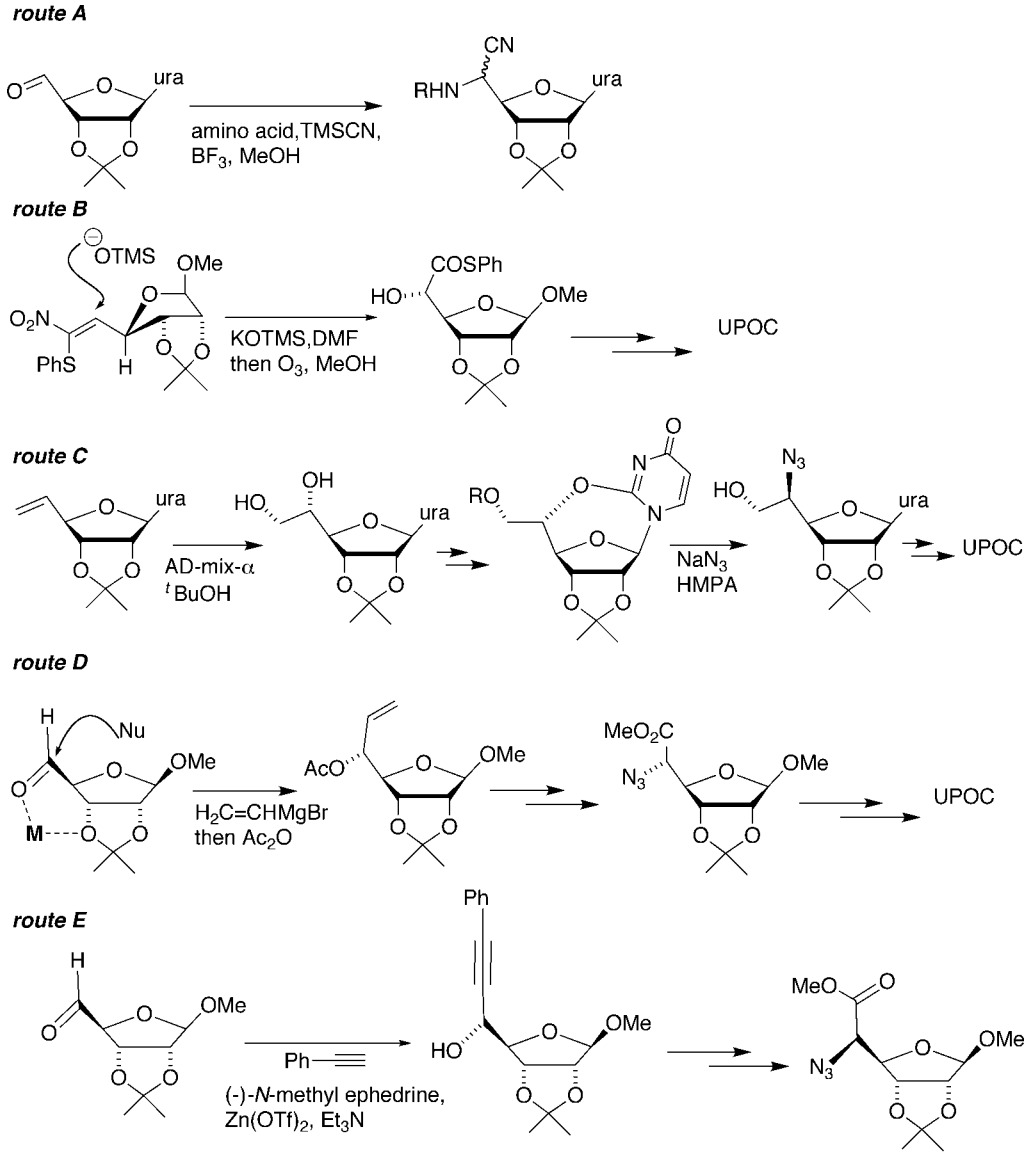
**SCHEME 1. Selected Literature Routes to Uracil Polyoxin C (UPOC)**




butylsulfinylimine derivative of 2′-deoxyuridine-5′-aldehyde to prepare the corresponding 5′-amino-5′-(*S*)-methyl uridine analogue.

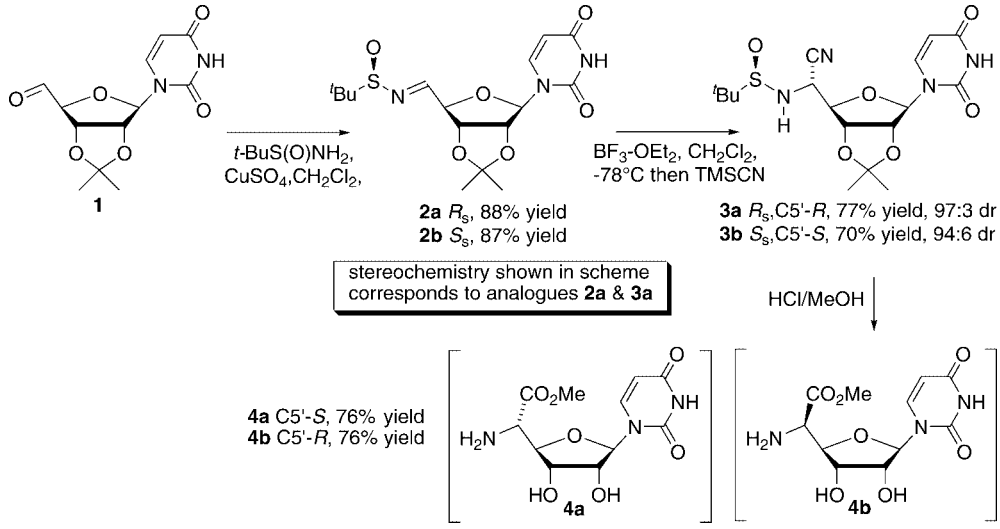
Herein we report an efficient and highly stereoselective synthesis of both diastereoisomers of the methyl ester of UPOC in which the key stereoselective step involves the Lewis acid catalyzed addition of cyanide ion to an *N*-*tert*-butylsulfinylimine derivative of uridine. The further elaboration of the methyl ester of UPOC to potential CS inhibitors and the biological evaluation of these compounds in vivo is also reported.

## Results and Discussion

**Efficient Stereoselective Route to Polyoxin C.** The reaction of the aldehyde **1**[43] with (*R*)-(+)-*tert*-butanesulfinamide in dry dichloromethane containing anhydrous copper sulfate afforded the sulfinimine nucleoside **2a**, isolated exclusively as its *E*-isomer in 88% yield (Scheme 2). Nucleoside **2a** was then dissolved in dichloromethane and $BF_3$-etherate added at −78 °C. After 30 min TMSCN was added dropwise, and the solution then allowed to warm to room temperature. The desired nucleoside **3a** with the $R_S$,*R* configuration was formed with a dr of 97:3 as determined following analysis by $^1$H NMR and was isolated in 77% yield following silica chromatography. The same route using the enantiomeric (*S*)-(−)-*tert*-butanesulfinamide **2b** was also successfully applied to the synthesis of nucleoside **3b** with the $S_S$,*S* configuration which was obtained in 70% yield and formed with a dr of 94:6. Treatment of the α-amino nitriles **3a** or **3b** with HCl in methanol produced the corresponding UPOC methyl esters with concomitant removal of the isopropylidene protecting groups and the Ellman's auxiliary. Due to their polar nature, compounds **4a** and **4b** were not easily purified but instead were isolated as crude products (following neutralization and ether wash) that were used directly in the conjugation reactions described below. The synthesis of both the natural (C5′-(*S*)) and unnatural diastereoisomers of the UPOC methyl ester allowed the assignment of C5-stereochemistry based on literature data[28] for UPOC (i.e., the free carboxylic acid derivative). Hence the signals for H6, H5, and H1′ all appear downfield in the $^1$H NMR of the natural diastereoisomer.

(43) More, J. D.; Finney, N. S. *Org. Lett.* **2002**, *4*, 3001–3003.

**SCHEME 2. Stereoselective Syntheses of Methyl Esters of (*R*) and (*S*)-Polyoxin C**




The observed stereocontrol during the addition of cyanide to the sulfinimines is consistent with the model proposed by Davis et al.[36] in which the sulfinyl oxygen is coordinated to the Lewis acid, while we assume that there is minimum induction from the stereogenic centers of the ribose. This interaction with the sulfinimine (for the $R_s$ isomer) directs the addition of cyanide from the least hindered Re face of the imine to give the Cram product (Figure 4). Interestingly, the sulfinimine **5**, (Figure 4) when reacted with TMSCN in the presence of boron trifluoride, afforded the corresponding α-amino nitrile **6** (Figure 4) with little or no stereocontrol (Figure 4). Thus, two diastereomeric products were obtained, which were separated by silica column chromatography, the faster- and slower-eluting products (**6a** and **6b**, respectively) being isolated in a ratio of 2:3. Although the bulkier protecting groups on the sugar and the presence of the benzyl group on the pyrimidine of **5** may be significant, we are uncertain of the exact reason for the loss of stereocontrol during this reaction.

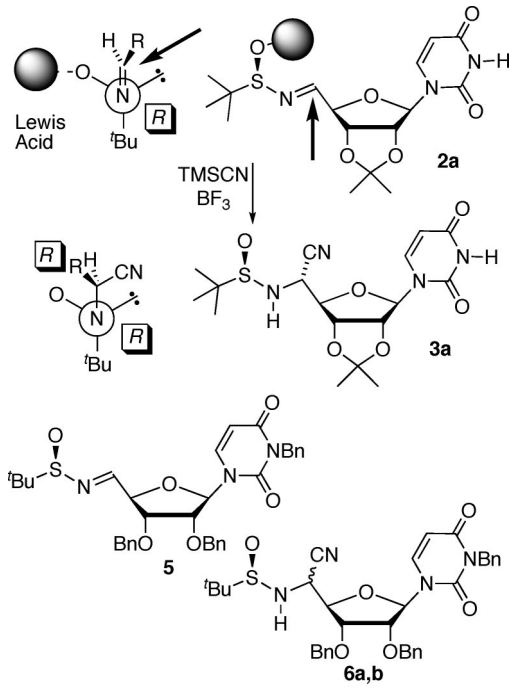



**FIGURE 4.** Proposed origin of stereocontrol during cyanide addition to sulfinimines.

**Synthesis of Isoxazole Analogues for Conjugation to UPOC Methyl Ester.** The structures in which we were interested required the syntheses of isoxazole-4-carboxylic acid analogues bearing a 3-H, Br, or MeS substituent and a tethered basic group at C5. These were synthesized using either 1,3-dipolar cycloaddition chemistry or reaction of a three-carbon unit with hydroxylamine (Scheme 3). Thus treatment of the alkyne **7** with oxime **8** as described by Moore et al.[44] afforded the desired isoxazole **9** in 77% yield. Deprotection of **9** gave the corresponding alcohol **10** which was then mesylated to afford **11**. Displacement of the mesylate of **11** by 2-mercaptopyridine or 2-mercaptoimidazole gave **12** and **13**, respectively, both in excellent yield. These were subsequently debenzylated using $BCl_3$ to give the corresponding carboxylic acids **14** and **15**.

Isoxazoles bearing a 3-H or 3-SMe substituent were prepared by reaction of a three-carbon unit with hydroxylamine. Thus compound **16**, obtained in turn from methyl 4-chloroacetoacetate and triethylorthoformate, was reacted with hydroxylamine to give the isoxazole **17** in 75% yield as a single regioisomer. Our attempts to introduce substituents via $S_N2$ displacement of chloride from **17** using nucleophiles such as azole-derived anions or a variety of amines were all unsuccessful, instead leading to base-mediated ring opening of the isoxazole (as shown in Figure 3). Indeed the only types of nucleophile that resulted in displacement of chloride rather than ring opening were thiols. Thus we successfully prepared isoxazole **18** in 74% yield using 2-mercaptopyridine as the nucleophile. Acid hydrolysis of **18** gave the carboxylic acid **19**. When we repeated the reaction with 2-mercaptoimidazole, acid hydrolysis of the product to afford the carboxylic acid resulted in a zwitterion that proved difficult to purify. Consequently we first converted the isoxazole **17** to the corresponding carboxylic acid **20** under acidic conditions and then displaced the chloride with 2-mercaptoimidazole. The product **21** precipitated from the solution and required no further purification. The reaction of hydroxylamine with the ketene dithioacetal **22**, derived from methyl nicotinylacetate, gave the MeS-substituted isoxazole **23** in 81% yield,

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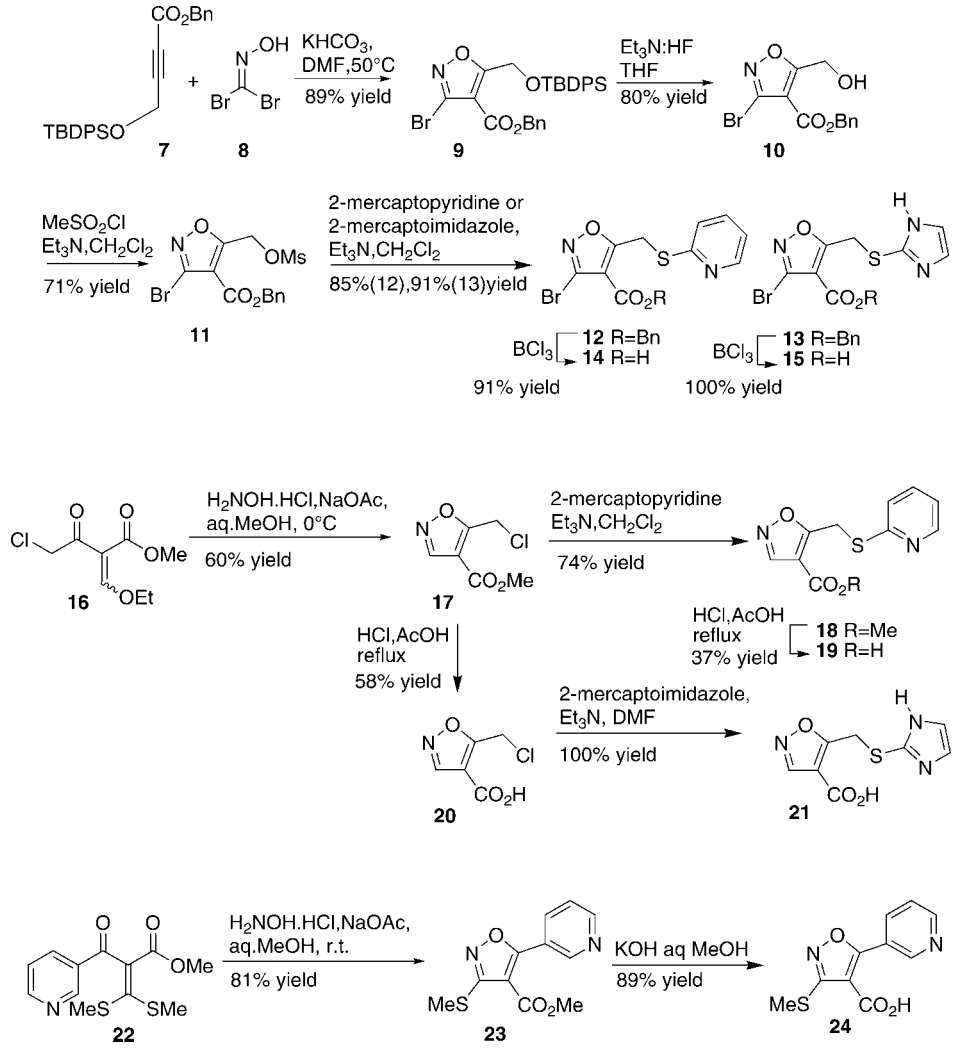
**SCHEME 3. Syntheses of Isoxazole Carboxylic Acids**



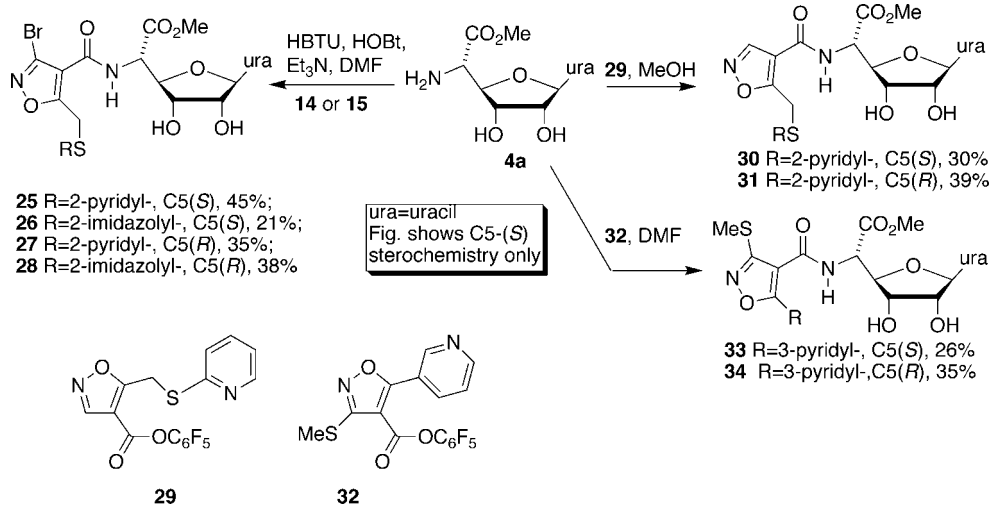

again as the only regioisomer. Basic hydrolysis of **23** gave the carboxylic acid **24**.

**Synthesis of UPOC−Isoxazole Conjugates: Conjugation of Isoxazole Analogues to UPOC Methyl Ester.** Initially we attempted to react the UPOC methyl ester **4a** with isoxazole **19** following its activation as the *N*-hydroxysuccinimidyl (NHS) ester using DCC. Although the NHS ester formation occurred, this coincided with isoxazole ring opening as shown by MS analysis and the absence of the imidazole 3-H ring proton in the NMR spectrum. As an alternative conjugation method, we investigated the use of *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HBTU). Thus, the 3-bromoisoxazole derivatives **14** and **15** both reacted with UPOC methyl ester (*S* and *R* diastereoisomers) in the presence of HBTU, hydroxybenzotriazole, and triethylamine in DMF. Yields of the target nucleosides **25**−**28** (Scheme 4) were between 21 and 45% (for two steps from **3a/3b** via **4a/4b**). However, when the same reaction was attempted using the 3-H isoxazole **19**, no reaction was observed.

The propensity for ring opening of **19** in the presence of base led us to consider alternative strategies for activation of the carboxylic acid. Thus, we successfully prepared the pentafluorophenyl (PfP) ester **29** in 66% yield following reaction of **19** with pentafluorophenol and EDC in DMF. In this case only very limited ring opening of the isoxazole had occurred, presumably due to the short reaction time (30 min) and low p$K_a$ of the pentafluorophenol. Attempts to react **29** with UPOC methyl ester initially failed using DMF as solvent. A study of the stability of **29** revealed that ring opening was essentially complete after simply stirring in anhydrous DMF or DMSO (in the absence of any base) for 24 h. However, the compound was stable in dioxane, acetone, acetonitrile, and methanol. Indeed both (*S*)- and (*R*)-diastereoisomers of UPOC methyl ester (**4a** and **4b**, respectively) could be successfully acylated with **29** in methanol to give the products **30** and **31** in 30% and 39% yield (two steps from **3a** or **3b**), respectively. Attempts to prepare the PfP ester of **15** were unsuccessful leading to a number of products such as those from ring opening and as evidenced by MS, the formation of an acyl imidazolium ion derived from acylation of the imidazole ring by the pentafluorophenyl ester. In contrast, preparation of the pentafluorophenyl ester **32** was possible, which in turn allowed the synthesis of the analogues **33** and **34** containing the 3-MeS isoxazole.

**Ring-Opening Reactions and Biological Screening of UPOC−Isoxazole Conjugates.** An essential design characteristic of the synthesized UPOC-isoxazole conjugates was the assumption that isoxazole ring opening to the corresponding diketonitriles would take place in planta, mediated either by mildly basic conditions (R = H), by enzyme catalysis (R = SMe), or alternatively, via single electron transfer processes

**SCHEME 4. UPOC−Isoxazole Conjugates (Yields from 3a or 3b Shown)**




(R = Br). To provide reference standards the putative pro-pesticides **25**, **30**, and **33** were ring-opened by applying known methodologies to afford the corresponding diketonitriles shown in Figure 5.

As expected, compound **30** containing the 3-H isoxazole underwent ring opening under mildly basic conditions (triethylamine in methanol) to produce the cyano-1,3-diketone **35** in 75% yield.[45] In contrast, under basic conditions (pH 10 buffer) the analogues **33** and **26** underwent hydrolysis of their respective amide bonds faster than any ring-opening reactions. However the corresponding diketonitriles derived from these conjugates, namely, **35** and **36**, respectively, could be formed upon treatment with iron(II) chloride, a single-electron transfer process.[46,47] However the strong chelation of Fe(III) by both of these analogues made purification and hence $^1$H NMR unfeasible, and these compounds were characterized by MS only.

Physicochemical studies[48,49] revealed that, as expected, compound **30** displayed a relatively short half-life (of less than 1 h) for conversion into **35** at pH 7 and 40 °C (data not shown). Compounds **25** (R = Br) and **33** (R = SMe) were stable under these conditions. Pesticides are often applied as spray solutions to crop plants and in order to gain a feel for the metabolic stability of our novel pro-pesticides in an agronomically relevant biological system we examined selected compounds in a maize cell culture assay.[50] In this assay, compounds are incubated in maize cell culture and growth medium and at predetermined times acetonitrile is added, the sample frozen and then thawed, and the supernatant analyzed by HPLC and MS. In the current study we analyzed samples for unchanged **30** and **33** and their ring-opened products **35** and **36**, respectively. Surprisingly we observed negligible metabolism over a 24 h period (for compounds **30** and **33** values of 16% and 13% respectively were determined). HPLC and MS analysis indicated that ring opening of the isoxazole rings had not taken place. We also evaluated the compounds **25**−**28**, **30**, **31**, **33**, and **34** against a range of commercial target and model organisms including insect pests, fungal pathogens, and weeds using in vivo high-throughput screening.[51] Fungal species were evaluated in mycelial growth tests in artificial media or in leaf disk assays for obligate pathogens. The test compounds were applied prior to inoculation or incorporated into the media. Insect species were evaluated on artificial diet or cotton leaf disk assays, treated with test compounds, depending on species and developmental stage. Herbicide effects were evaluated using monocotyledonous and dicotyledonous model species grown on artificial media amended with the test compounds. All tests were completed in 96-well microtiter plates and incubated under controlled conditions for 4−10 days, at which time inhibitory or lethal effects were assessed visually and scored. We did not detect any significant levels of biological activity for any of the compounds reported herein. Since the biological evaluation of these compounds was performed in whole-cell or whole-organism assays, the lack of activity may be due to a variety of possible reasons not directly related to the abilities of these compounds to act as CS inhibitors or otherwise. Thus, additional factors that might preclude their biological activity potentially include poor cellular uptake per se. Alternatively it is feasible that following cellular uptake these compounds may not undergo ring opening to the dicarbonyl species and/or may be deactivated, for example, following enzymatic hydrolysis of the amide bond. Future strategies that might be employed include tuning the conditions for which the isoxazole moiety undergoes ring opening and increasing meta-

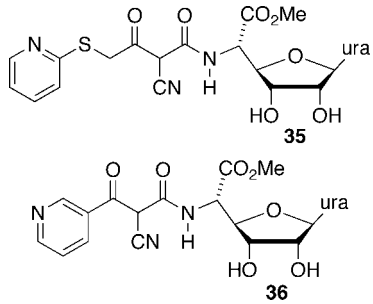



**FIGURE 5.** Diketonitrile derivatives derived from ring-opening reactions of UPOC−isoxazole conjugates.

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bolic stability using strategies that have been persued by other groups as outlined above. In the former case, for example the incorporation of various lipophilic ester functional groups at the 3-position of the isoxazole might be envisaged since these may undergo better cell uptake and following enzymatic hydrolysis to the carboxylic acid would undergo subsequent decarboxylation and ring opening.[53]

## Conclusions

In summary, we have developed an efficient and highly diatereoselective synthetic route that allows the preparation of the either the *R* or *S* diastereoisomer of UPOC methyl ester. We have developed methods for the conjugation of UPOC methyl ester (*R* or *S*) to a variety of isoxazole carboxylic acids which incorporate a basic side chain. These conjugates can undergo ring opening under basic conditions or in the presence of divalent metal ions to reveal a metal-binding site composed of a diketonitrile function. As such, the compounds possess structural features of both the transition state of the natural substrate during chitin biosynthesis and of the natural polyoxin C inhibitors of chitin synthase. Unfortunately, these novel compounds lack any appreciable in vivo pesticidal activity. Studies are ongoing to evaluate the intrinsic activity against CS and to better understand the factors influencing bioavailability.

## Experimental Methods

**($R_S$)-2-Methyl-2-propanesulfinic Acid 2′,3′-*O*-Isopropyluridine-5′-methyleneamide (2a).** Nucleoside **1**[43] (9.05 g, 32.10 mmol), (*R*)-(+)-*tert*-butanesulfinamide (4.28 g, 35.31 mmol) and anhydrous $CuSO_4$ (10.25 g, 64.10 mmol) were dissolved in dry $CH_2Cl_2$ (100 mL) and stirred at room temperature for 24 h. The suspension was filtered using filter aid and the filtrate was evaporated to a foam. Flash chromatography (1:1 hexane/EtOAc) gave a white solid (10.82 g, 28.10 mmol, 88%). HRMS (ESI) *m*/*z* calcd for $C_{16}H_{24}N_3O_6S$ $[M + H]^+$ 386.1386, found 386.1382. $^1$H NMR (250 MHz, DMSO-$d_6$): $\delta$ 1.13 (s, 9H), 1.31 (s, 3H), 1.49 (s, 3H), 4.88 (dd, $J$ = 3.2 Hz, 1H), 5.03 (dd, $J$ = 6.1, 3.2 Hz), 5.21 (d, $J$ = 6.4 Hz, 1H), 5.61 (dd, $J$ = 7.9, 1.8 Hz, 1H), 5.85 (s, 1H), 7.81 (d, $J$ = 7.9 Hz, 1H), 7.87 (d, $J$ = 3.4 Hz, 1H), 11.44 (s, 1H). $^{13}$C NMR (60 MHz, DMSO-$d_6$): $\delta$ 22.1, 25.0, 26.7, 56.6, 84.1, 84.1, 89.7, 95.6, 101.5, 112.9, 144.4, 150.4, 163.4, 168.3.

**($R_S$,*R*)-2-Methyl-2-propanesulfinic Acid 2′,3′-*O*-Isopropyluridine-5′-cyanomethylamide (3a).** Nucleoside **2a** (3.34 g, 8.68 mmol) was dissolved in dry $CH_2Cl_2$ (100 mL) under argon. $BF_3OEt_2$ (1.65 mL, 13.02 mmol) was added and the solution was cooled to −78 °C and stirred for 10 min. TMSCN (2.30 mL, 17.35 mmol) was then added and the reaction was stirred at −78 °C for 2 h. The reaction was then allowed to warm to room temperature and was stirred for 3 h. Brine (100 mL) was added and the reaction was stirred for 5 min. The organic layer was then washed with brine, dried ($MgSO_4$), and evaporated. Flash chromatography (50% EtOAc, 50% hexane) gave a white foam (2.76 g, 6.70 mmol, 77%). HRMS (ESI) *m*/*z* calcd for $C_{17}H_{25}N_4O_6S$ $[M + H]^+$ 413.1495, found 413.1497. $^1$H NMR (250 MHz, DMSO-$d_6$): $\delta$ 1.15 (s, 9H), 1.28 (s, 3H), 1.47 (s, 3H), 4.24 (dd, $J$ = 9.5, 2.4 Hz, 1H), 4.77 (t, $J$ = 9.2 Hz, 1H), 4.92 (dd, $J$ = 6.1, 2.7 Hz, 1H), 5.20 (d, $J$ = 6.4 Hz, 1H), 5.68 (d, $J$ = 7.9 Hz, 1H), 5.80 (s, 1H), 6.65 (d, $J$ = 8.9 Hz, 1H), 7.84 (d, $J$ = 7.9 Hz, 1H), 11.56 (s, 1H). $^{13}$C NMR (60 MHz, DMSO-$d_6$): $\delta$ 20.2, 22.7, 24.6, 47.9, 55.6, 81.1, 82.7, 86.1, 96.2, 100.4, 112.7, 116.1, 143.5, 149.5, 163.4.

**(*S*)-Uracil Polyoxin C Methyl Ester (4a).** Nucleoside **3a** (5.10 g, 12.38 mmol) was dissolved in dry MeOH (100 mL) and cooled to 0 °C. HCl gas was then slowly bubbled through the solution for 1 h. The resulting dark red solution was then left to stir at room temperature for 48 h. The reaction was then cooled to 0 °C, water (10 mL) was added slowly, and the mixture was then evaporated to give a red solid. This was dissolved in water and neutralized with 0.1 M aqueous NaOH. The solution was then extracted with ether and the aqueous layer evaporated to give a yellow solid that was used without further purification (5.24 g, 76% pure). HRMS (ESI) *m*/*z* calcd for $C_{11}H_{16}N_3O_7$ $[M + H]^+$ 302.0988, found 302.0996. $^1$H NMR (250 MHz, DMSO-$d_6$): $\delta$ 3.63 (s, 3H), 3.67−3.70 (m, 1H), 3.95 (dd, $J$ = 5.5 Hz, 2.7 Hz, 1H), 4.04−4.15 (m, 2H, H2′), 5.67 (d, $J$ = 7.9 Hz, 1H), 5.78 (d, $J$ = 6.4 Hz, 1H), 7.91 (d, $J$ = 7.9 Hz, 1H). $^{13}$C NMR (60 MHz, $D_2O$): $\delta$ 53.5, 54.3, 69.2, 72.4, 82.2, 92.0, 102.6, 143.3, 151.5, 166.2, 170.5.

**Benzyl 4-*tert*-Butyl(diphenyl)hydroxysilane-but-2-ynoate (7).** *tert*-Butyl(diphenyl)(prop-2-ynyloxy)silane[52] (19.00 g, 64.60 mmol) was dissolved in dry THF (100 mL) and the solution was cooled to −78 °C under $N_2$. Next, 2.0 M *n*BuLi in hexane (38.60 mL, 96.90 mmol) was added dropwise over 20 min. The reaction was then allowed to warm to room temperature, causing a color change from colorless to brown. The reaction was then cooled again to −78 °C and benzyl chloroformate (13.70 mL, 96.90 mmol) in dry THF (50 mL) was added dropwise to give a yellow solution. This was stirred at −78 °C for 5 h and then allowed to warm to room temperature overnight. The reaction was then quenched with saturated aqueous $NH_4Cl$ solution (100 mL) and extracted with ether, and the combined organic layers were dried ($MgSO_4$) and evaporated to a yellow oil. Flash chromatography (0−10% EtOAc in hexane) gave a colorless oil (25.07 g, 58.57 mmol, 91%). HRMS (ESI) *m*/*z* calcd for $C_{27}H_{28}O_3NaSi$ $[M + Na]^+$ 451.1705, found 451.1685. $^1$H NMR (250 MHz, DMSO-$d_6$): $\delta$ 0.98 (s, 9H), 4.54 (s, 2H), 5.19 (s, 2H), 7.37−7.64 (m, 15H). $^{13}$C NMR (60 MHz, $CDCl_3$): $\delta$ 19.2, 26.7, 52.3, 67.6, 76.6, 86.1, 127.5, 127.9, 128.4, 128.4, 128.5, 128.6, 128.7, 128.8, 130.0, 132.4, 134.9, 135.6, 153.1.

**Benzyl 3-Bromo-5-(*tert*-butyl(diphenyl)methylhydroxysilane)isoxazole-4-carboxylate (9).** Alkyne **7** (4.90 g, 11.45 mmol) and hydroxycarbonimidic dibromide **8**[44] (2.30 g, 11.45 mmol) were dissolved in dry DME (50 mL). $KHCO_3$ (2.30 g, 22.90 mmol) was then added and the resulting suspension was stirred at 50 °C overnight under $N_2$. The mixture was then cooled and filtered and the filtrate was evaporated. Flash chromatography (49:1 hexane/EtOAc) gave a colorless oil (5.62 g, 10.24 mmol, 89%). HRMS (ESI) *m*/*z* calcd for $C_{28}H_{29}NO_4SiBr$ $[M + H]^+$ 550.1049, found 550.1022. $^1$H NMR (250 MHz, DMSO-$d_6$): $\delta$ 0.97 (s, 9H), 5.11 (s, 2H), 5.16 (s, 2H), 7.27−7.64 (m, 15H). $^{13}$C NMR (60 MHz, $CDCl_3$): $\delta$ 19.3, 26.7, 58.3, 67.0, 109.3, 127.8, 127.9, 128.3, 128.6, 128.7, 128.8, 129.9, 130.1, 132.3, 134.8, 135.5, 135.6, 135.7, 140.5, 159.4, 176.7.

**Benzyl 3-Bromo-5-(hydroxymethyl)isoxazole-4-carboxylate (10).** Isoxazole **9** (5.00 g, 9.11 mmol) was dissolved in dry THF under $N_2$. Triethylamine trihydrofluoride (1.47 g, 45.53 mmol) was then added and the solution was stirred for 24 h at room temperature and then evaporated. The residue was dissolved in EtOAc (50 mL) and washed with saturated aqueous $NaHCO_3$ solution, water and brine. The organic layer was then dried ($MgSO_4$) and evaporated to a yellow oil. Flash chromatography (hexane to 1:1 hexane/EtOAc) gave a colorless oil (2.27 g, 7.28 mmol, 80%). HRMS (ESI) *m*/*z* calcd for $C_{12}H_{11}NO_4Br$ $[M + H]^+$ 311.9871, found 311.9874. $^1$H NMR (250 MHz, DMSO-$d_6$): $\delta$ 4.85 (s, 2H), 5.33 (s, 2H), 7.30−7.49 (m, 5H). $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ 55.5, 66.6, 108.6, 128.1, 128.4, 128.5, 128.6, 128.6, 135.8, 140.7, 159.2, 178.7.

**Benzyl 3-Bromo-5-[(methylsulfonyl)oxymethyl]isoxazole-4-carboxylate (11).** Isoxazole **10** (2.00 g, 8.01 mmol) was dissolved in dry $CH_2Cl_2$ (10 mL) under Ar and dry triethylamine (1.62 mL, 16.02 mmol) was added followed by methanesulfonyl chloride (1.83 g, 16.02 mmol). The reaction was stirred at room temperature for

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24 h, diluted with EtOAc, and then washed with saturated aqueous $NaHCO_3$ solution, water and brine. The organic layer was dried ($MgSO_4$) and then evaporated and the residue was purified by flash chromatography (hexane to 1:1 hexane/EtOAc) to give a white solid (2.21 g, 5.67 mmol, 71%). HRMS (ESI) *m/z* calcd for $C_{13}H_{12}NO_6NaSBr$ $[M + Na]^+$ 411.9466, found 411.9470. $^1H$ NMR (250 MHz, DMSO-$d_6$): $\delta$ 2.40 (s, 3H), 5.13 (s, 2H), 5.37 (s, 2H), 7.35−7.51 (m, 5H). $^{13}C$ NMR (60 MHz, $CDCl_3$): $\delta$ 33.3, 67.6, 68.4, 107.6, 128.5, 128.8, 129.1, 134.6, 140.9, 159.0, 173.1.

**Benzyl 3-Bromo-5-[(pyridine-2-ylthio)methyl]isoxazole-4-carboxylate (12).** Isoxazole **11** (1.00 g, 2.56 mmol) and 2-mercaptopyridine (342 mg, 3.08 mmol) were dissolved in dry $CH_2Cl_2$ (10 mL) under argon. Triethylamine (0.42 mL, 3.08 mmol) was then added and the solution was stirred for 24 h at room temperature and then evaporated. The residue dissolved in EtOAc was washed with saturated aqueous $NaHCO_3$ solution, water and brine. The organic layer was then dried ($MgSO_4$) and evaporated and the residue was purified by flash chromatography (hexane to 3:1 hexane/EtOAc) to give a white solid (886 mg, 2.18 mmol, 85%). HRMS (ESI) *m/z* calcd for $C_{17}H_{14}N_2O_3SBr$ $[M + H]^+$ 404.9909, found 404.9899. $^1H$ NMR (250 MHz, DMSO-$d_6$): $\delta$ 4.89 (s, 2H), 5.37 (s, 2H), 7.15 (ddd, $J$ = 7.3 Hz, 4.9 Hz, 0.9 Hz, 1H), 7.33−7.50 (m, 6H), 7.67 (td, $J$ = 7.3 Hz, 1.8 Hz, 1H), 8.37 (ddd, $J$ = 4.9 Hz, 1.8 Hz, 0.9 Hz, 1H). $^{13}C$ NMR (60 MHz, $CDCl_3$): $\delta$ 24.7, 67.2, 110.3, 120.3, 122.2, 128.5, 128.6, 128.7, 135.0, 136.4, 140.1, 149.5, 155.7, 159.8, 176.3.

**Benzyl 3-Bromo-5-[(1*H*-imidazol-2-ylthio)methyl]isoxazole-4-carboxylate (13).** Isoxazole **11** (950 mg, 2.43 mmol) and 2-mercaptoimidazole (282 mg, 2.82 mmol) were dissolved in dry $CH_2Cl_2$ (10 mL) under Ar. Triethylamine (0.39 mL, 2.82 mmol) was then added and the mixture was stirred for 24 h at room temperature. More 2-mercaptoimidazole (122 mg, 1.22 mmol) and triethylamine (0.17 mL, 1.22 mmol) were then added and the reaction was stirred for a further 24 h. The reaction was then worked up as for **12**. Flash chromatography (hexane to 3:1 hexane/EtOAc) gave a white solid (887 mg, 2.25 mmol, 92%). HRMS (ESI) *m/z* calcd for $C_{15}H_{13}N_3O_3SBr$ $[M + H]^+$ 393.9861, found 393.9853. $^1H$ NMR (250 MHz, DMSO-$d_6$): $\delta$ 4.53 (s, 2H), 5.23 (s, 2H), 6.96 (bs, 1H), 7.18 (bs, 1H), 7.32−7.47 (m, 5H), 12.45 (bs, 1H). $^{13}C$ NMR (60 MHz, $CDCl_3$): $\delta$ 30.4, 67.3, 110.0, 125.1, 128.5, 128.7, 128.7, 128.9, 134.8, 136.2, 140.7, 159.4, 175.6.

**3-Bromo-5-[(pyridine-2-ylthio)methyl]isoxazole-4-carboxylic Acid (14).** Isoxazole **12** (730 mg, 1.80 mmol) was dissolved in dry $CH_2Cl_2$ (6 mL) and $BCl_3$ (3.90 mL, 1 M solution in heptane) was added slowly over 5 min. The reaction was then stirred for 1 h at room temperature, then EtOAc was added and the product was extracted into saturated aqueous $NaHCO_3$ solution. The aqueous layer was then acidified (pH 2) and extracted with EtOAc. The organics were then dried ($MgSO_4$) and evaporated to give a white solid (519 mg, 1.66 mmol, 91%). HRMS (ESI) *m/z* calcd for $C_{10}H_8N_2O_3SBr$ $[M + H]^+$ 314.9439, found 314.9426. $^1H$ NMR (250 MHz, DMSO-$d_6$): $\delta$ 4.91 (s, 2H, $C\underline{H}_2S$), 7.17 (dd, $J$ = 7.3 Hz, 4.9 Hz, 1H), 7.39 (dd, $J$ = 7.9 Hz, 0.9 Hz, 1H), 7.69 (td, $J$ = 7.3 Hz, 1.8 Hz, 1H), 8.43 (dt, $J$ = 4.9 Hz, 0.9 Hz, 1H), 13.84 (bs, 1H). $^{13}C$ NMR (100 MHz, DMSO-$d_6$): $\delta$ 24.2, 110.5, 120.7, 122.0, 137.1, 141.2, 149.5, 155.5, 160.9, 175.9.

**3-Bromo-5-[(1*H*-imidazol-2-ylthio)methyl]isoxazole-4-carboxylic Acid (15).** Treatment of isoxazole **13** (750 mg, 1.90 mmol) in $CH_2Cl_2$ (6 mL) with $BCl_3$ (4.00 mL, 1 M solution in heptane) as described for **14**, followed by flash chromatography (EtOAc to 1:1 EtOAc/methanol) gave a white solid (579 mg, 1.90 mmol, quant). HRMS (ESI) *m/z* calcd for $C_8H_7N_3O_3SBr$ $[M + H]^+$ 303.9389, found 303.9389. $^1H$ NMR (250 MHz, DMSO-$d_6$): $\delta$ 4.66 (s, 2H), 7.02 (s, 2H). $^{13}C$ NMR (100 MHz, DMSO-$d_6$): $\delta$ 24.7, 114.4, 124.0, 137.2, 141.4, 164.9, 171.9.

**Methyl-2-(chloroacetyl)-3-ethoxyacrylate (16).** Methyl-4-chloroacetoacetate (30.60 mL, 265.70 mmol), triethylorthoformate (88.40 mL, 531.40 mmol) and acetic anhydride (25.00 mL, 265.70 mmol) were refluxed together for 24 h. Volatile components were removed under vacuum to leave an orange solid (54.50 g, 265.00 mmol, quant) as a mixture of *E* and *Z* isomers. This was used without further purification. HRMS (ESI) *m/z* calcd for $C_8H_{11}O_4NaCl$ $[M + Na]^+$ 229.0244, found 229.0250. $^1H$ NMR (250 MHz, DMSO-$d_6$): $\delta$ 1.04−1.30 (m, 3H), 3.63−3.71 (m, 3H), 4.08−4.37 (m, 2H), 4.62−4.68 (m, 2H), 7.99−8.01 (m, 1H). $^{13}C$ NMR (60 MHz, DMSO-$d_6$): $\delta$ 14.1, 15.1, 47.0, 50.0, 51.6, 51.7, 72.5, 72.9, 109.0, 112.0, 164.5, 165.0, 167.1, 167.4, 188.1, 188.2.

**Methyl-5-(chloromethyl)isoxazole-4-carboxylate (17).** To compound **16** (25.00 g, 121.00 mmol) in MeOH (100 mL) at 0 °C was added a solution of hydroxylamine hydrochloride (12.60 g, 182.00 mmol) and sodium acetate trihydrate (24.90 g, 183.00 mmol) in water (100 mL). After stirring for 4 h, the mixture was extracted with $CH_2Cl_2$ and the organic layer was dried ($MgSO_4$) and evaporated. Purification by flash chromatography (67% hexane, 33% EtOAc) gave a colorless oil (19.30 g, 110.00 mmol, 60%). HRMS (EI) *m/z* calcd for $C_6H_6NO_3Cl$ $[M + H]^+$ 175.0036, found 175.0043. $^1H$ NMR (250 MHz, DMSO-$d_6$): $\delta$ 3.83 (s, 3H), 5.13 (s, 2H), 9.03 (s, 1H). $^{13}C$ NMR (60 MHz, $CDCl_3$): $\delta$ 32.5, 52.3, 110.8, 150.2, 160.9, 170.5.

**Methyl-5-[(pyridine-2-ylthio)methyl]isoxazole-4-carboxylate (18).** 2-Mercaptopyridine (3.33 g, 30.00 mmol) was dissolved in dry DMF (100 mL) and triethylamine (4.20 mL, 30.00 mmol) was added. This solution was stirred for 10 min and then cooled to −40 °C and compound **17** (5.00 g, 28.57 mmol) dissolved in dry DMF (20 mL) was slowly added. Stirring then continued at −40 °C for 3 h and then the mixture was allowed to warm to room temperature over 12 h. The mixture was then worked up as described for **12** and gave a yellow oil (5.3 g, 21.14 mmol, 74%). This was used without further purification. HRMS (EI) *m/z* calcd for $C_{11}H_{10}N_2O_3S$ $[M + H]^+$ 250.0412, found 250.0424. $^1H$ NMR (250 MHz, DMSO-$d_6$): $\delta$ 3.82 (s, 3H), 4.91 (s, 2H), 7.18 (t, $J$ = 6.4 Hz, 1H), 7.38 (d, $J$ = 7.9 Hz, 1H), 7.56−7.72 (m, 1H), 8.44 (d, $J$ = 4.9 Hz, 1H), 8.93 (s, 1H). $^{13}C$ NMR (100 MHz, DMSO-$d_6$): $\delta$ 23.3, 52.1, 109.5, 120.6, 122.0, 137.1, 149.5, 150.6, 155.6, 161.1, 173.5.

**5-[(Pyridine-2-ylthio)methyl]isoxazole-4-carboxylic Acid (19).** Compound **18** (5.80 g, 23.20 mmol) was dissolved in glacial acetic acid (50 mL), concentrated HCl was added (25 mL) and the mixture was stirred at reflux for 6 h. The reaction was poured onto ice and neutralized to pH 8 with $Na_2CO_3$. The solution was then extracted with EtOAc and then acidified with concentrated HCl. The product was then extracted into EtOAc, dried ($MgSO_4$), and evaporated to give a white solid (2.02 g, 8.56 mmol, 37%). HRMS (EI) *m/z* calcd for $C_{10}H_8N_2O_3S$ $[M + H]^+$ 236.0256, found 236.0256. $^1H$ NMR (250 MHz, DMSO-$d_6$): $\delta$ 4.90 (s, 2H), 7.18 (t, $J$ = 6.0 Hz, 1H), 7.38 (d, $J$ = 8.2 Hz, 1H), 7.69 (d, $J$ = 7.8 Hz, 1H), 8.45 (d, $J$ = 4.9 Hz), 8.85 (s, 1H), 13.43 (s, 1H). $^{13}C$ NMR (60 MHz, DMSO-$d_6$): $\delta$ 23.3, 110.5, 120.6, 121.9, 137.0, 149.5, 150.9, 155.8, 162.2, 173.0.

**5-(Chloromethyl)Isoxazole-4-carboxylic Acid (20).** Compound **17** (13.00 g, 74.3 mmol) was reacted as described for the preparation of **19** using glacial acetic acid (30 mL) and concentrated HCl (15 mL) to afford a white solid (6.96 g, 43.22 mmol, 58%). HRMS (ESI) *m/z* calcd for $C_5H_4NO_3Cl$ $[M + H]^+$ 160.9879, found 160.9883. $^1H$ NMR (250 MHz, DMSO-$d_6$): $\delta$ 5.12 (s, 2H), 8.97 (s, 1H), 13.60 (br s, 1H). $^{13}C$ NMR (60 MHz, $CDCl_3$): $\delta$ 32.4, 110.3, 150.4, 166.1, 171.9.

**5-[(Imidazol-2-ylthio)methyl]isoxazole-4-carboxylic Acid (21).** Isoxazole **20** (3.00 g, 18.63 mmol) was dissolved in dry DMF (5 mL), then triethylamine (2.60 mL) was added and the solution was stirred for 5 min. The mixture was then added to a separate flask containing 2-mercaptoimidazole (1.87 g, 18.63 mmol) dissolved in dry DMF (5 mL). The reaction was then stirred for 24 h at room temperature under Ar. The white precipitate that formed was removed by filtration, washed with ice-cold MeOH and dried to leave a white powder. No further purification was necessary. HRMS (ESI) *m/z* calcd for $C_8H_6N_3O_3S$ $[M - H]^-$ 224.0130, found 224.0121. $^1H$ NMR (250 MHz, DMSO-$d_6$): $\delta$ 4.57 (s, 2H), 7.08

(s, 2H), 8.80 (s, 1H). $^{13}$C NMR (60 MHz, DMSO-$d_6$): $\delta$ 27.9, 110.7, 124.4, 136.1, 150.9, 162.0, 172.3.

**Methyl 3,3-Bis(methylthio)-2-(pyridin-3-ylcarbonyl)acrylate (22).** Methyl nicotinoylacetate (3.00 g, 16.76 mmol) and potassium carbonate (2.80 g, 16.76 mmol) were dissolved in dry DMF (30 mL) and stirred for 2 h at room temperature to give a white suspension. Carbon disulfide (3.82 g, 50.28 mmol) was then added to give an orange precipitate. After 2 h, iodomethane (4.76 g, 33.52 mmol) was added and stirring was continued for a further 12 h. The solution was then poured onto ice, extracted with EtOAc, dried ($MgSO_4$), and evaporated. Flash chromatography (1:1 hexane/EtOAc) gave an orange solid (3.55 g, 12.53 mmol, 75%). HRMS (ESI) *m/z* calcd for $C_{12}H_{14}NO_3S_2$ $[M + H]^+$ 284.0415, found 284.0416. $^1$H NMR (250 MHz, DMSO-$d_6$): $\delta$ 2.23 (bs, 3H), 2.53 (bs, 3H), 3.60 (s, 3H), 7.59 (dd, $J$ = 7.9 Hz, 4.9 Hz, 1H), 8.20 (dt, $J$ = 7.9 Hz, 2.0 Hz, 1H), 8.82(dd, $J$ = 4.9 Hz, 1.8 Hz, 1H), 8.98 (d, $J$ = 2.4 Hz, 1H). $^{13}$C NMR (60 MHz, $CDCl_3$): $\delta$ 17.4, 19.5, 52.2, 123.7, 130.6, 132.5, 136.1, 150.6, 153.5, 159.2, 163.3, 190.2.

**Methyl 3-(Methythio)-5-(pyridin-3-yl)isoxazole-4-carboxylate (23).** To compound **22** (3.50 g, 12.37 mmol) in MeOH (20 mL) was added a solution of hydroxylamine hydrochloride (4.30 g, 61.84 mmol) and sodium acetate trihydrate (8.45 g, 61.84 mmol) in water (20 mL). Further MeOH was then added after a precipitate appeared. The yellow solution was stirred at room temperature for 24 h, then extracted with $CH_2Cl_2$, dried ($MgSO_4$), and evaporated. Flash chromatography (1:1 hexane/EtOAc) gave a yellow solid (2.50 g, 10.02 mmol, 81%). HRMS (ESI) *m/z* calcd for $C_{11}H_{11}N_2O_3S$ $[M + H]^+$ 251.0365, found 251.0372. $^1$H NMR (250 MHz, DMSO-$d_6$): $\delta$ 2.56 (s, 3H), 3.76 (s, 3H), 7.62 (dd, $J$ = 7.9 Hz, 4.9 Hz, 1H), 8.28 (dt, $J$ = 7.9 Hz, 2.0 Hz, 1H), 8.79 (dd, $J$ = 4.9 Hz, 1.5 Hz, 1H), 9.02 (d, $J$ = 2.1, 1H). $^{13}$C NMR (60 MHz, MeOH-$d_4$): $\delta$ 13.8, 52.5, 109.9, 125.0, 138.5, 150.3, 152.6, 162.5, 164.0, 171.7.

**3-(Methylthio)-5-(pyridin-3-yl)isoxazole-4-carboxylic Acid (24).** Compound **23** (2.50 g, 10.00 mmol) was dissolved in MeOH (37.50 mL) and a 15% (w/v) KOH solution (12.50 mL) added slowly. The reaction was stirred for 30 min at room temperature and then water (50 mL) was added. The basic solution was taken to pH 10 with dilute aqueous HCl and then extracted with EtOAc to remove unreacted starting material. The aqueous layer was then acidifed with concentrated HCl and extracted with EtOAc, and the combined organic layers were dried ($MgSO_4$) and evaporated to afford a beige-colored solid (2.10 g, 8.90 mmol, 89%). HRMS (ESI) *m/z* calcd for $C_{10}H_9N_2O_3S$ $[M + H]^+$ 237.0334, found 237.0345. $^1$H NMR (250 MHz, DMSO-$d_6$): $\delta$ 2.54 (s, 3H), 7.61 (dd, $J$ = 7.9 Hz, 4.9 Hz, 1H), 8.28 (dt, $J$ = 7.9 Hz, 2.0 Hz), 8.77 (dd, $J$ = 4.9 Hz, 1.5 Hz, 1H), 9.03 (d, $J$ = 2.1 Hz, 1H), 13.51 (bs, 1H). $^{13}$C NMR (60 MHz, DMSO-$d_6$): $\delta$ 13.1, 109.3, 122.8, 123.4, 136.8, 149.4, 151.8, 161.8, 162.4, 170.2.

**Methyl-1,5-dideoxy-1-(3,4-dihydro-2,4-dioxo-1-($^2$H)-pyrimidinyl)-5-{3-bromo-5-[(pyridin-2-ylthio)methyl]isoxazole-4-carbonylamino}-$\beta$-D-allofuranuronate (25).** UPOC methyl ester **4a** (351 mg, 1.17 mmol), isoxazole **14** (400 mg, 1.28 mmol), HBTU (486 mg, 1.28 mmol) and HOBt·$H_2O$ (196 mg, 1.28 mmol) were dissolved in dry DMF (5 mL) under argon. Triethylamine (0.36 mL, 2.58 mmol) was then added and the reaction was stirred for 24 h at room temperature Solvent was evaporated and the slurry was partitioned between EtOAc and water. The aqueous layer was further extracted with EtOAc and the combined organic layers were dried ($MgSO_4$) and evaporated to give a yellow foam. Flash chromatography (EtOAc to 20:1 EtOAc/MeOH) gave an off-white foam (315 mg, 0.54 mmol, 45% (from compound **3a**)). HRMS (ESI) *m/z* calcd for $C_{21}H_{21}BrN_5O_9S$ $[M + H]^+$ 598.0243, found 598.0231. $^1$H NMR (250 MHz, DMSO-$d_6$): $\delta$ 3.65 (s, 3H), 4.08 (m, 1H), 4.20−4.27 (m, 2H), 4.74 (s, 2H), 4.95 (dd, $J$ = 8.2 Hz, 6.7 Hz, 1H), 5.41−5.43 (m, 1H), 5.53−5.55 (m, 1H), 5.72 (dd, $J$ = 7.9 Hz, 2.1 Hz, 1H), 5.78 (d, $J$ = 5.5 Hz, 1H), 7.13 (dd, $J$ = 6.4 Hz, 5.2 Hz, 1H), 7.35 (d, $J$ = 8.2 Hz, 1H), 7.55−7.72 (m, 2H, Py), 8.39 (d, $J$ = 4.9 Hz, 1H), 9.38 (d, $J$ = 8.2 Hz, 1H), 11.41 (bs, 1H). $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ 24.1, 52.6, 54.5, 70.8, 72.0, 83.3, 88.8, 102.7, 114.9, 120.8, 122.3, 137.6, 140.5, 142.2, 149.9, 151.1, 155.9, 159.4, 163.4, 170.0, 171.4.

**Methyl-1,5-dideoxy-1-(3,4-dihydro-2,4-dioxo-1-($^2$H)-pyrimidinyl)-5-{3-bromo-5-[(imidazol-2-ylthio)methyl]isoxazole-4-carbonylamino}-$\beta$-D-allofuranuronate (26).** UPOC methyl ester **4a** (216 mg, 0.72 mmol), isoxazole **15** (240 mg, 0.79 mmol), HBTU (299 mg, 0.79 mmol) and HOBt·$H_2O$ (121 mg, 0.79 mmol) were dissolved in dry DMF (4 mL) under Ar. Triethylamine (0.22 mL, 1.58 mmol) was then added and the reaction was stirred for 24 h at room temperature Work-up and purification as for **25** gave a beige-colored foam (87 mg, 0.15 mmol, 21% (from compound **3a**)). HRMS (ESI) *m/z* calcd for $C_{19}H_{20}BrN_6O_9S$ $[M + H]^+$ 587.0196, found 587.0179. $^1$H NMR (250 MHz, DMSO-$d_6$): $\delta$ 3.67 (s, 3H), 4.06−4.22 (m, 3H), 4.68 (s, 2H), 4.88−4.94 (m, 1H), 5.44 (bs, 1H), 5.55 (d, $J$ = 5.5 Hz, 1H), 5.71 (d, $J$ = 7.9 Hz, 1H), 5.76 (d, $J$ = 5.5 Hz, 1H), 6.96 (bs, 1H), 7.18 (bs, 1H), 7.60 (d, $J$ = 8.2 Hz, 1H), 10.76 (d, $J$ = 8.5 Hz, 1H), 11.41 (bs, 1H), 12.42 (bs, 1H). $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ 22.6, 52.6, 54.4, 71.0, 72.0, 83.0, 88.7, 102.6, 114.9, 119.5, 124.8, 138.1, 140.8, 141.4, 151.0, 158.9, 163.3, 170.2, 170.2.

**Pentafluorophenyl 5-[(Pyridine-2-ylthio)methyl]isoxazole-4-carboxylate (29).** Isoxazole **19** (2.00 g, 8.47 mmol) and EDC (1.63 g, 8.47 mmol) were dissolved in dry DMF (15 mL) under Ar. A solution of pentafluorophenol (1.72 g, 9.32 mmol) in dry DMF (5 mL) was then added slowly and the mixture was stirred at room temperature for 30 min and then evaporated. The residue was dissolved in acetone (100 mL), 0.01 M pH 6 phosphate buffer (50 mL) was added and the mixture was extracted into ether. The combined organic layers were washed with water and brine, dried ($MgSO_4$), and evaporated. Flash chromatography (3:1 hexane/EtOAc) afforded a white solid (2.36 g, 5.87 mmol, 69%). HRMS (ESI) *m/z* calcd for $C_{16}H_8N_2O_3F_5S$ $[M + H]^+$ 403.0176, found 403.0172. $^1$H NMR (250 MHz, DMSO-$d_6$): $\delta$ 4.88 (s, 2H, $C\underline{H}_2S$), 6.99 (dd, $J$ = 7.3 Hz, 4.9 Hz, 1H), 7.17 (d, $J$ = 7.9 Hz, 1H), 7.48 (td, $J$ = 7.6 Hz, 1.8 Hz, 1H), 8.35 (d, $J$ = 4.9 Hz, 1H), 8.58 (s, 1H). $^{13}$C NMR (60 MHz, $CDCl_3$): $\delta$ 24.5, 107.4, 119.0, 120.8, 122.8, 136.0, 136.9, 139.2, 140.0, 141.7, 143.2, 149.3, 150.0, 155.5, 156.7, 176.4. $^{19}$F NMR (250 MHz, acetone-$d_6$): $\delta$ −172.1 to −171.9 (m, 1F), −166.2 to −166.0 (t, 1F), −163.4 to −163.2 (t, 1F), −158.5 to −158.3 (t, 1F), −153.3 to −153.2 (d, 1F).

**Methyl-1,5-dideoxy-1-(3,4-dihydro-2,4-dioxo-1-($^2$H)-pyrimidinyl)-5-{5-[(pyridin-2-ylthio)methyl]isoxazole-4-carbonylamino}-$\beta$-D-allofuranuronate (30).** UPOC methyl ester **4a** (1.00 g, 3.32 mmol) and isoxazole **29** (1.50 g, 3.72 mmol) were dissolved in dry MeOH (10 mL) and stirred at room temp under Ar. After 48 h the solvent was removed under vacuum and the residue was dissolved in water (50 mL), which was extracted with EtOAc. The combined organic layers were then dried ($MgSO_4$) and evaporated. Purification by flash chromatography (EtOAc to 4:1 EtOAc/MeOH) gave an off-white solid (500 mg, 0.96 mmol, 30% (from compound **3a**)). HRMS (ESI) *m/z* calcd for $C_{21}H_{22}N_5O_9S$ $[M + H]^+$ 520.1138, found 520.1160. $^1$H NMR (250 MHz, DMSO-$d_6$): $\delta$ 3.67 (s, 3H), 4.08−4.23 (m, 4H), 4.89 (s, 2H), 5.39 (d, $J$ = 4.9 Hz, 1H), 5.54 (d, $J$ = 5.2 Hz, 1H), 5.68 (d, $J$ = 8.2 Hz, 1H), 5.77 (d, $J$ = 5.8 Hz, 1H), 7.13−7.18 (m, 1H), 7.36 (d, $J$ = 7.9 Hz, 1H), 7.57 (d, $J$ = 7.9 Hz, 1H), 7.68 (m, 1H), 8.43−8.46 (m, 1H), 9.05 (s, 1H), 9.10−9.13 (d, $J$ = 7.9 Hz, 1H), 11.43 (bs, 1H). $^{13}$C NMR (60 MHz, MeOH-$d_4$): $\delta$ 25.0, 53.2, 55.5, 71.6, 74.1, 84.4, 93.8, 103.2, 113.8, 121.5, 123.3, 138.2, 143.6, 150.3, 150.6, 152.3, 158.1, 163.1, 165.9, 171.1, 172.5.

**Pentafluorophenyl 3-(Methylthio)-5-pyridin-3-ylisoxazole-4-carboxylate (32).** Compound **24** (1.50 g, 6.36 mmol) was dissolved in dry DMF under argon (20 mL) and EDC (1.34 g, 7.00 mmol) added. A solution of pentafluorophenol (1.29 g, 7.00 mmol) in dry DMF (2 mL) was then added, the mixture was stirred overnight at room temperature then poured onto acetone (50 mL), and 0.01 M pH 6 phosphate buffer (50 mL) was added. The solution was then extracted with EtOAc and the organic layers were combined, dried

($MgSO_4$), and evaporated. Flash chromatography (1:3 hexane/EtOAc) gave a white solid (2.34 g, 5.84 mmol, 91%). HRMS (ESI) *m/z* calcd for $C_{16}H_8N_2O_3F_5S$ $[M + H]^+$ 403.0176, found 403.0159. $^1H$ NMR (250 MHz, $CDCl_3$): $\delta$ 2.60 (s, 3H), 7.53 (dd, $J = 7.9$ Hz, 4.9 Hz, 1H), 8.29 (ddd, $J = 7.9$ Hz, 2.1 Hz, 1.5 Hz, 1H), 8.72 (dd, $J = 5.2$ Hz, 1.5 Hz, 1H), 9.07 (d, $J = 2.1$ Hz, 1H). $^{13}C$ NMR (60 MHz, $CDCl_3$): $\delta$ 13.8, 106.7, 123.4, 124.2, 132.6, 136.3, 138.0, 139.2, 140.0, 143.3, 148.4, 151.1, 156.7, 163.1, 172.3.

**Methyl-1,5-dideoxy-1-(3,4-dihydro-2,4-dioxo-1-(2*H*)-pyrimidinyl)-5-[5-(pyridin-3-yl)isoxazole-4-carbonylamino]-$\beta$-D-allofuranuronate (33).** UPOC methyl ester **4a** (647 mg, 2.15 mmol) and isoxazole **32** (1.30 g, 3.23 mmol) were dissolved in dry DMF (5 mL) and stirred at room temperature under Ar. After 48 h the solvent was removed, and the residue was dissolved in water (30 mL) and extracted with EtOAc. The combined organic layers were dried ($MgSO_4$) and evaporated. Flash chromatography (EtOAc to 4:1 EtOAc/MeOH) gave a cream-colored solid (286 mg, 0.55 mmol, 26% from compound **3a**). HRMS (ESI) *m/z* calcd for $C_{21}H_{21}N_5O_9NaS$ $[M + Na]^+$ 542.0958, found 542.0933. $^1H$ NMR (250 MHz, DMSO-$d_6$): $\delta$ 2.57 (s, 3H), 3.66 (s, 3H), 3.96−4.00 (m, 1H), 4.07−4.20 (m, 2H), 4.85 (t, $J = 7.0$ Hz, 1H), 5.32 (d, $J = 5.5$ Hz, 1H), 5.54 (d, $J = 5.5$ Hz, 1H), 5.64 (d, $J = 7.9$ Hz, 1H), 5.75 (d, $J = 5.8$ Hz, 1H), 7.55−7.59 (m, 2H), 8.13−8.17 (m, 1H), 8.71 (d, $J = 4.0$ Hz, 1H), 8.92 (bs, 1H), 9.23 (d, $J = 7.6$ Hz, 1H), 11.39 (bs, 1H). $^{13}C$ NMR (125 MHz, DMSO-$d_6$): $\delta$ 13.3, 52.1, 54.0, 70.1, 71.5, 82.2, 88.4, 102.2, 112.1, 122.5, 124.0, 134.7, 141.2, 147.6, 150.7, 151.6, 160.4, 160.4, 162.9, 164.8, 169.0.

**Methyl-1,5-dideoxy-1-(3,4-dihydro-2,4-dioxo-1-(2*H*)-pyrimidinyl)-5-[2-cyano-3-oxo-4-(pyridine-2-ylthio)butanoylamino]-$\beta$-D-allofuranuronate (35).** Nucleoside **33** (150 mg, 0.29 mmol) was dissolved in MeOH (2 mL) and triethylamine (0.12 mL, 0.87 mmol) was added. The reaction was stirred for 24 h at room temperature under Ar. The solution was then diluted with water (2 mL) and acidified to pH 4. Solvents were then removed under vacuum and the remaining yellow solid was purified by flash chromatography (EtOAc to 3:1 EtOAc/MeOH) to give a white solid (111 mg, 0.21 mmol, 74%). HRMS (ESI) *m/z* calcd for $C_{21}H_{21}N_5O_9S.Na$ $[M + Na]^+$ 542.0958, found 542.0938. $^1H$ NMR (250 MHz, DMSO-$d_6$): $\delta$ 3.60 (s, 3H), 3.93−4.14 (m, 5H), 4.71 (dd, $J = 7.9$ Hz, 5.8 Hz, 1H), 5.34 (d, $J = 4.3$ Hz, 1H), 5.48 (d, $J = 5.7$ Hz, 1H), 5.57 (d, $J = 7.9$ Hz, 1H), 5.78 (d, $J = 6.7$ Hz, 1H), 7.07 (dd, $J = 6.5$ Hz, 4.9 Hz, 1H), 7.32 (d, $J = 7.9$ Hz, 1H), 7.51 (d, $J = 8.2$ Hz, 1H), 7.61 (t, $J = 7.3$ Hz, 1H), 8.39 (d, $J = 4.3$ Hz, 1H), 9.86 (d, $J = 8.2$ Hz, 1H), 11.35 (bs, 1H). $^{13}C$ NMR (60 MHz, MeOD-$d_4$): $\delta$ 37.54, 51.5, C5′, 69.7, 73.0, 73.2, 84.1, 88.3, 101.8, 102.1, 119.5, 121.5, 136.5, 140.6, 148.8, 151.0, 159.0, 164.4, 169.5, 170.3, 186.9.

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**Supporting Information Available:** Experimental details for compounds **2b**, **3b**, **4b**, **5**, **6a**, **6b**, **27**, **28**, **31**, **34**; $^1H$ and $^{13}C$ NMR spectra for all novel compounds described in the Experimental Section. This material is available free of charge via the Internet at http://pubs.acs.org.

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