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Synthesis and Biological Evaluation of 6-[(1*R*)-1-hydroxyethyl]-2,4a(*R*),6(*S*),8a(*R*)-tetrahydropyrano-[3,2-b]-pyran-2-one and Structural Analogs of the Putative Structure of Diplopyrone

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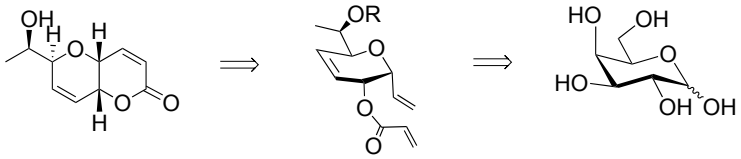


(-)-Diplopyrone *C*-Vinyl Glycoside D-Galactose

ABSTRACT

The phytotoxin diplopyrone is considered to be the main phytotoxin in a fungus that is responsible for cork oak decline. A carbohydrate-based synthesis of the enantiomer of the structure proposed for diplopyrone has been developed from a commercially available derivative of D-galactose. Key steps in the synthesis are a highly stereoselective pyranose chain-extension based on methyltitanium, preparation of a vinyl glycoside via Isobe *C*-alkynylation-rearrangment/reduction, and RCM-based pyranopyran construction. Crystallographic and NMR analysis confirms an earlier report that the structure originally proposed for diplopyrone may require revision. Structural analogs were prepared for biological evaluation, the most promising being a pyranopyran nitrile synthesized from tri-*O*-acetyl-D-galactal by Ferrier cyanoglycosidation, Wittig chain extension, and lactonization. Biological assays revealed potent

antibacterial activity for the nitrile analog against common bacterial pathogens *E. ictaluri* and *F. columnare* that cause enteric septicemia (ESC) and columnaris disease, respectively, in catfish. The IC50 value of 0.002 against *E. ictaluri* indicates approximately 100 times greater potency than the antibiotic florfenicol used commercially for this disease. Phytotoxic activity for all three target compounds against duckweed was also observed. The antibiotic and phytotoxic activities of the new pyranopyrans synthesized in this study demonstrate the potential of such compounds as antibiotics and herbicides.

INTRODUCTION

Diplopyrone is a phytotoxin isolated from the fungus *Diplodia mutila* and reported by Evidente and coworkers in 2003.[1,2] An endophytic fungus, meaning it can reside in the host plant for a period of time before causing damage,[3] *D. mutila* is thought to be responsible for cork oak decline in parts of southern Europe where the disease has caused large and negative economic and environmental impacts. Diplopyrone causes necrosis and wilting of cork oak (*Quercus suber*) cuttings and is considered the main phytotoxin responsible for the observed pathogenesis. Strains of this fungus also infect other oak species as well as cypress.[2] Recently, there have been studies of structure-activity relationships of fungal phytotoxins, in particular, the relationship of stereochemistry to biological activity.[4,5] These studies provide insight into the mechanisms of action of microbially-derived natural products and may also lead to the identification of new molecular targets for the development of environmentally benign herbicides.[6-8]

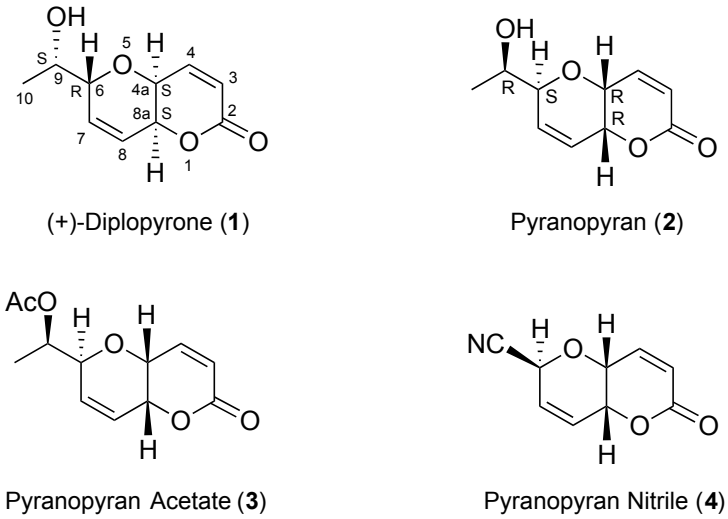


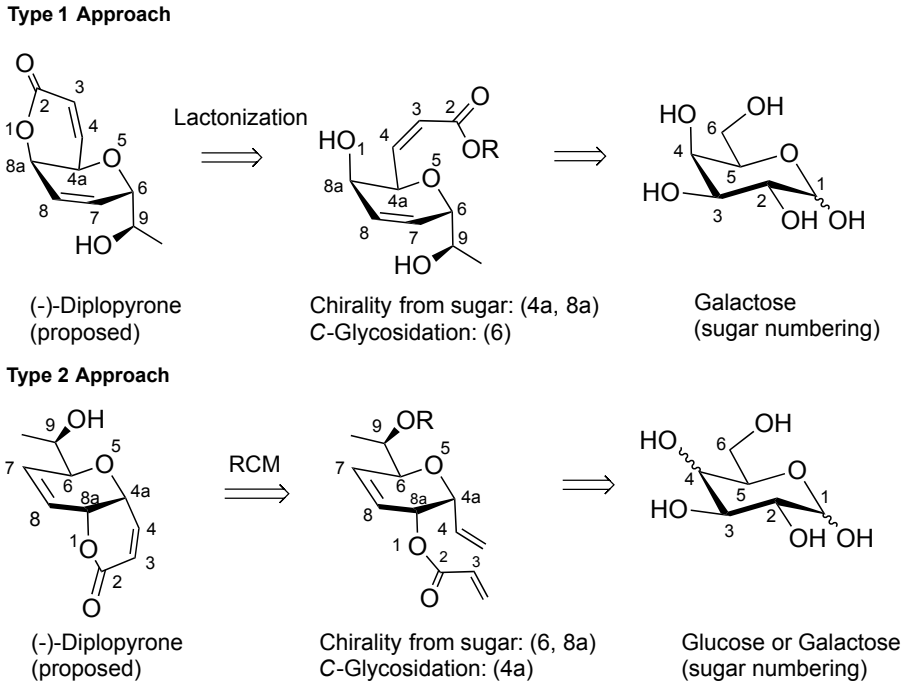
Figure 1. Putative (+)-Diplopyrone and Related Compounds in this Study

The proposed structure of diplopyrone contains an unusual cis-fused pyranopyran core and was assigned on the basis of spectroscopic data and theoretical calculations of its optical properties as 6-[(1*S*)-1-hydroxyethyl]-2,4a(*S*),6(*R*),8a(*S*)-tetrahydropyrano-[3,2-b]-pyran-2-one (**1**) (Figure1).[2] Pyranopyrans[9] such as those found in brevitoxin and other ladder ethers as well as in okadaic acid, thyrsiferol and other natural products are typically trans-fused, consistent with proposed biosynthetic origins by nucleophilic ring opening of polyepoxides.[10] The first synthesis of the putative structure of (+)-diplopyrone was achieved in 17 steps from *cis*-1,4-butenediol in 2017 by Mohaptra and coworkers.[11] Key steps in their synthesis are the Sharpless asymmetric epoxidation, Pd-catalyzed regioselective epoxide opening, tandem iodine-catalyzed allylation/cyclization for dihydropyran synthesis, and α-aminohydroxylation in the presence of L-proline to introduce the C-9 hydroxyl group stereoselectively (7:3 ratio). A comparison of NMR spectral data for their synthetic diplopyrone with that reported for the natural product revealed discrepancies in proton and carbon chemical shifts and led the authors to suggest that revisions to the proposed structure of diplopyrone are necessary. Natural material is scarce,[1,2] thereby increasing the need for compounds to be made available through synthesis to support structural verification and biological studies.

We have been seeking to develop an asymmetric, carbohydrate-based synthesis of enantiomeric (-)-diplopyrone and analogs for the past few years (Figure 1). There are two reasons for the choice of the enantiomer as the initial target. First, viewing diplopyrone as a "*C*-glycoside problem"[12] opens up multiple synthetic routes from common D sugars. Marine polyethers such as brevitoxin and the anticancer drug Halaven® have been synthesized by pathways that rely on a pyranopyran core structure accessible from carbohydrates via *C*-glycosides as intermediates.[9] Our design utilizes *C*-glycosides as precursors to the lactone ring of diplopyrone. The second reason, of a less practical nature but enticing nonetheless, stems from phytochemistry. It has been demonstrated that stereoisomers of phytotoxins may possess different biological acitivity.[4,5] For example, the dihydroxynaphthaleneone (-)-regiolone is produced by *Botrytis fabae,* which is a much more virulent pathogen than *Botrytis cinerea*. The latter produces the enantiomeric phytotoxin (+)-isosclerone.[13] Enantiomers of the phytotoxic lichen metabolite usnic acid possess different levels of activity, with (-)-usnic acid having more phytotoxic activity.[14] Sesquiterpenes (+)- and (-)-gossypol have divergent activities across a range of bioassays with the (-)-enantiomer showing more potency in most instances.[15] Will (-) diplopyrone exhibit necrotic effects in bioassays similar to the naturally occurring (+)-diplopyrone? Will it exhibit inverse agonism by blocking the action of (+)-diplopyrone, or antimicrobial activity against strains of *D. mutila* that infect other species? The compounds in this study will be used to determine structure-activity relationships for diplopyrone and to obtain biological data for assays against invasive plants, bacteria, and other pathogenic organisms.

Two carbohydrate-based routes to the enantiomer of the structure proposed for diplopyrone (pyranopyran **2**) are shown retrosynthetically in Scheme 1. Part of the appeal to a carbohydrate-based strategy for the enantioselective synthesis of diplopyrones lies in the pathways can be developed from simple starting materials. Six of the ten carbons of diplopyrone and two of its four chirality centers are derived from common carbohydrates in our approach. Viewing the target

from the standpoint of C-6 Wittig chain-extension[16] (Scheme 1, Type 1 approach) allows for formation of the pyranopyran core by intramolecular esterification of a chain-extended substrate, which is a "higher-carbon sugar."[17] In this approach, the chirality centers at C-4a and C-8a are derived from C-5 and C-4 of the carbohydrate starting material, D-galactose, and the hydroxyethyl side-chain is derived from a group that is introduced at the anomeric center by *C*-glycosidation. In an alternative approach, the pyranopyran core can potentially be formed from ring-closing metathesis (RCM) using a *C*-vinyl glycoside[18] (Scheme 1, Type 2 approach). In a Type 2 approach, the chirality centers at C-6 and C-8a as numbered in diplopyrone originate from C-5and C-2 of either D-glucose or D-galactose. *C*-glycosidation is required to introduce the vinyl group needed in the RCM precursor, while carbohydrate chain extension is required to create the hydroxyethyl side-chain. The approaches outlined in Scheme 1 present opportunities for the development of synthesis methodology in the areas of chain-extension and in *C*-vinyl glycoside synthesis, both of which are useful methods with well-known applications in synthetic carbohydrate chemistry.[19-24]

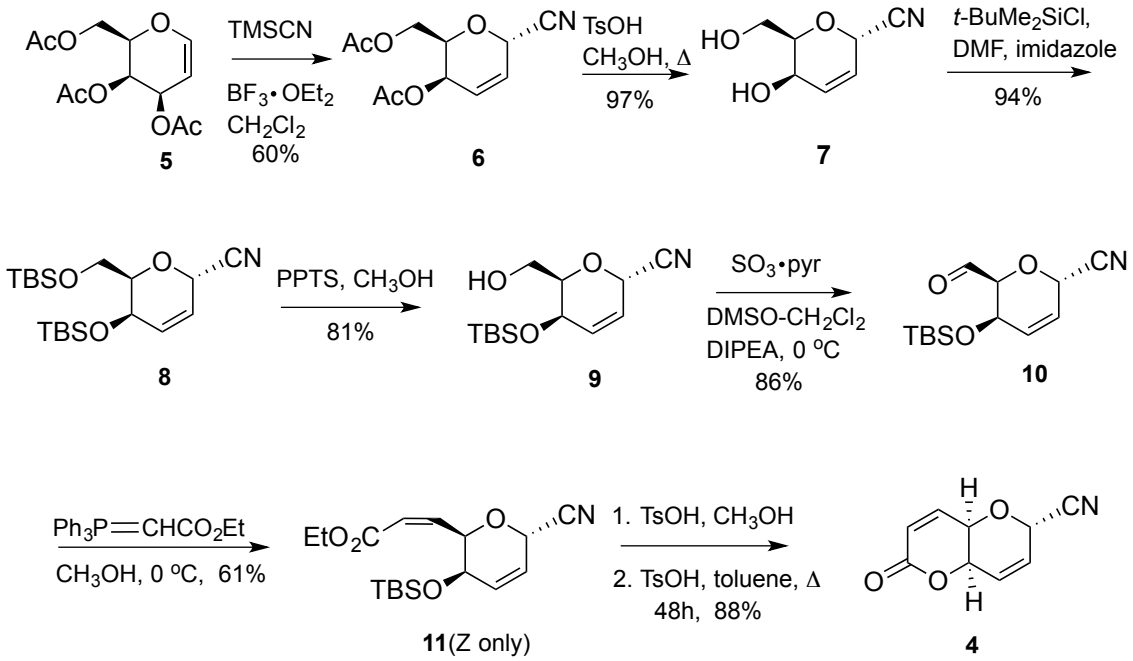


Scheme 1. *C*-Glycoside-based approaches to pyranopyran 2

In this paper we describe the first synthesis of enantiomeric (-)-diplopyrone, pyranopyran **2**, based on the structure originally proposed for the natural product. The synthesis was carried out in 13 steps from a commercially available derivative of D-galactose by the RCM (Type 2) approach. We also describe the syntheses of pyranopyran nitrile **4** in 8 steps from tri-*O*-acetyl-D-galactal using a Type 1 approach. Both syntheses are highly stereoselective and both are amenable for the preparation of additional analogs for use in biological studies. 'X-ray crystallographic analyses has been obtained for **3** and **4**. The crystallographic analyses coupled with NMR data provide further insight into the structure assignments for natural and synthetic diplopyrones in the literature. Biological assays were carried out at the USDA (Natural Products Utilization Research Unit). Compounds **2**, **3**, and **4** were tested for phytotoxic, fungicidal, and antibacterial activity, the latter against common bacterial pathogens in fish. It is of interest that compound **4** is significantly more active than a commercially used antibiotic used against *E. ictaluri*, a common pathogen in catfish. Results of these bioassays are presented herein.

RESULTS AND DISCUSSION

In our first approach to pyranopyran **2**, we chose a Type 1 strategy in which a nitrile group would be introduced by a Ferrier rearrangement to the anomeric center of D-galactal (Scheme 2). The nitrile group would then be converted to the required hydroxyethyl side-chain prior to chain extension and lactonization. Tri-*O*-acetyl-D-galactal **5** was treated with trimethyl-



Scheme 2. Synthesis of pyranopyran nitrile 4

silyl cyanide in the presense of boron trifluoride diethyl etherate to provide crystalline glycosyl nitrile **6** in 60% yield on a 20-gram scale.[25] Deacylation of **6** with *p*-toluenesulfonic acid in methanol gave diol **7** which was converted to di-*O*-TBS ether **8** in 91% overall yield. The nitrile was expected to be a suitable precursor for the hydroxyethyl side-chain by either of two sequences, one involving reduction to the corresponding aldehyde followed by methylation and the other consisting of prior methylation to give a methyl ketone which could be reduced. Our attempts at conversion of the nitrile to the hydroxyethyl side chain of pyranopyran **2** by both of these sequences were unsuccessful. Compound **8** and similar compounds were found to be prone to double bond migration, epimerization at the anomeric center in the presence of base, and vinylogous β-elimination. For example, addition of methyllithium to **8** followed by hydride reduction of the resulting ketone gave a hydroxyethyl derivative in 14% overall yield, along with products of elimination. In spite of these difficulties, it was possible to construct the pyranopyran core of diplopyrone from **8**, leaving the nitrile group intact. Selective deprotection of the 6-hydroxyl group of **8** was achieved using PPTS in methanol at room temperature. Migration of the

4-*O*-TBS group in **9** to the C-6 hydroxyl group was observed to occur upon standing at room temperature so it was necessary to carry out the next two steps in quick succession. Oxidation of the primary alcohol **9** by the Parikh-Doering method gave aldehyde **10** in 86% yield. Attempted Wittig reactions of **10** with methylenetriphenylphosphorane failed as did Peterson alkenation, again likely the result of the tendency for **10** to undergo vinylogous β-elimination of the 4-*O*-TBS group. Tebbe methylenation was also unsuccessful. The reaction of **10** with the stabilized Wittig reagent (carbethoxymethylidene)triphenylphosphorane under conditions described by Valverde and coworkers[26] gave ester **11** exclusively as the Z isomer. The cyclization of **11** to diplopyrone nitrile **4** proved more difficult than expected perhaps because the double bond in the pyranose ring places the C-4 hydroxyl and ester groups in poor proximity for ring closure. However, we were able to obtain **4** in 88% overall yield from **11** by a two-step procedure consisting of deprotection in methanol then heating in toluene, both in the presence of an acid catalyst. The overall yield of nitrile **4** from galactal derivative **5** is 20%. Nitrile **4** was crystalline and suitable for analysis by X-ray diffraction, which supports the assignment of its structure. The ORTEP diagram of **4** is included in the Supporting Information.

The pyranopyran nitrile **4** turned out to be an interesting analog, especially in view of its biological activity discussed later in this paper. Our lack of success in converting the nitrile group to a hydroxyethyl side-chain prompted us to explore an alternative (Type 2) strategy for the synthesis of pyranopyran **2**. In this approach, the hydroxyethyl group is introduced first, by chain extension at C-6 of a hexopyranoside. While many methods exist for the synthesis of higher-carbon sugars,[17] a challenge that emerges in the route to our target compounds is the control of stereochemistry at the off-ring, C-9 chirality center. Additions of organometallic reagents to dialdopyranosides **I** typically favor products **II** that result from chelation-control and which possess the incorrect stereochemistry for diplopyrone (Figure 2).[27] Felkin-Anh stereoselectivity

(**III**) is required for our synthesis. A second challenge in this approach is the need for an efficient synthesis of a *C*-vinyl glycoside precursor for the RCM-based route to the

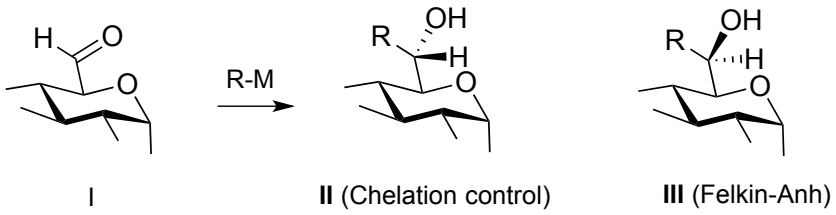
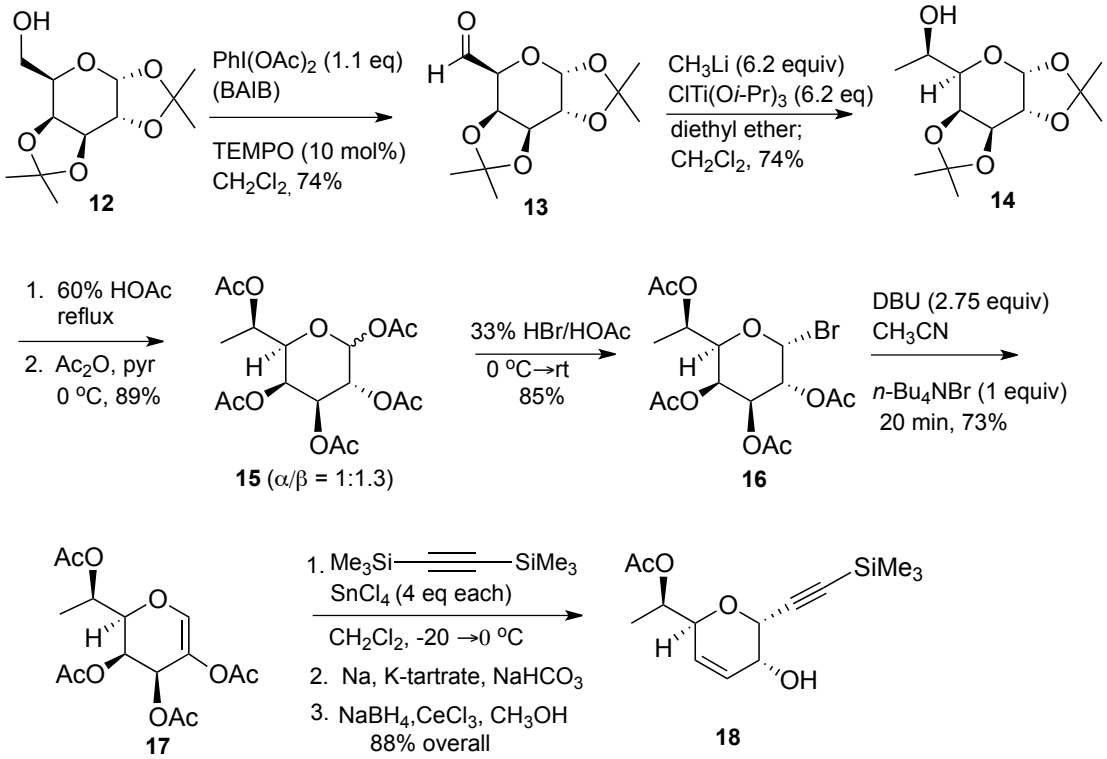


Figure 2. Chelation vs. Felkin –Anh Stereoselectivity in Dialdohexopyranosides

lactone (Scheme 1). *C*-Vinyl glycosides have been used as precursors to carbon-linked glycoconjugates, for example, *C*-galactosyl ceramides.[20] However, unlike their *C*-allyl glycoside counterparts,[28-30] simple, unsubstituted *C*-vinyl glycosides have proven to be more difficult to synthesize as illustrated in recent approaches to *C*-alkyl, *C*-aryl, and (substituted) *C*-vinyl glycosides, some of which are based on modern catalytic methods.[22-24] We were able to solve both the chain-extension and *C*-vinyl glycoside problems in our approach.

Commercially available 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose **12** was oxidized with catalytic TEMPO in the presence of bis(acetoxy)iodo-benzene (BAIB) on a 15-gram scale to give aldehyde **13** which was used immediately in the next step (Scheme 3).[31] This oxidation was also carried out using a polymer-bound reagent,[32] which gave improved yields on smaller (1-2 mmol) scales, but turned out to be less desirable for scaled-up preparations of **13**. The addition of organometallic reagents to aldehyde **13** has provided products that have found use as building blocks for trisaccharides used to study monoclonal antibodies,[33] enzyme inhibitors,[34] and higher-carbon sugars.[17] However, most of the addition reactions of organometallic reagents to **13** provide mixtures of diastereomers with the highest selectivity for **14** on the order of 2.5:1 to 3.0:1.[27] Our previous studies[35] of the addition of organometallic reagents to pentodialdo-1,4-furanoses revealed high levels of Felkin-Anh stereoselectivity when

methyl(triisopropoxy)titanium[36,37] was used instead of methyllithium or Grignard reagents. Methyl(triisopropoxy)titanium has received limited attention in natural product synthesis, there being only a few reported examples.[38-41] In need of a highly stereoselective route to **14**, we decided to examine the addition of methyl(triisopropoxy)titanium to **13** and were able to achieve a *d.r.* of 19:1 in favor of desired **14** on a 4-gram scale.[42] In the larger-scale preparation included in the experimental section of this paper, the minor isomer was estimated at 0.7%. The structure of **14** was assigned based on comparison of NMR data with that published by Lemieux.[33] Further confirmation of the structure of **14** was provided by x-ray analysis of the corresponding *p*-bromobenzoate, which shows that the configuration at the newly formed C-9 chirality center is *R*.[42] The yield and stereoselectivity of the methyltitanium-based route to chain-extension were critical to the overall success of our synthesis. Two of the four chirality centers of pyranopyran **2**, C-6 and C-9, are established in **14**, without the production of diastereomers.

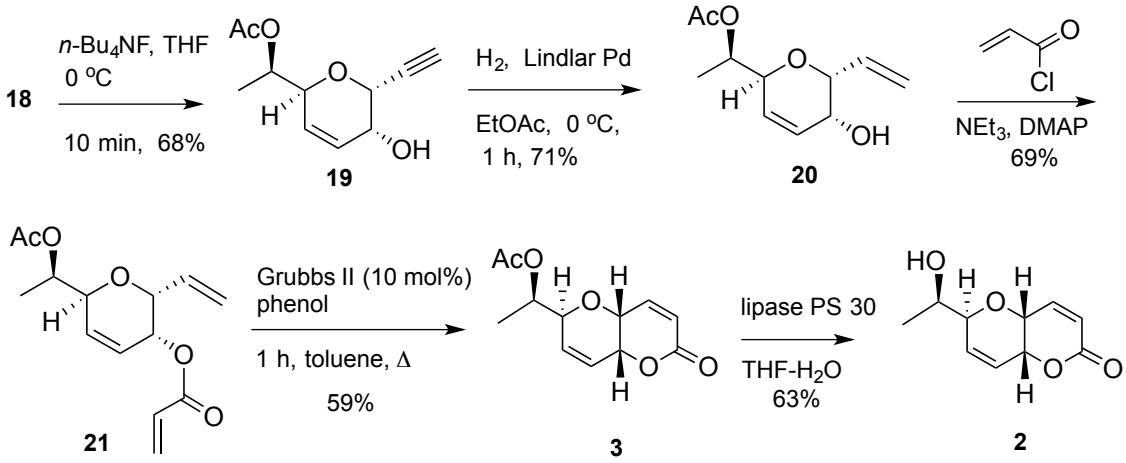


Scheme 3. Chain extension and *C*-glycosidation for pyranopyran synthesis

The second challenge in a Type 2 route to pyranopyran **2** was the synthesis for the *C*-vinyl glycoside needed for the lactone by RCM. Progress in the development of methods for the synthesis of *C*-alkynyl glycosides[43-45] suggested that a sequence consisting of *C*-alkynylation followed by Lindlar reduction would provide the vinyl glycoside needed as the RCM precursor. This approach was investigated by Franck and coworkers in their synthesis of *C*-galatosyl ceramides;[20] however, low overall yields *C*-vinyl glycoside from methyl galactopyranoside led the authors to pursue an alternative synthesis. We were encouraged by the recent work of Isobe in which the *C*-alkynylation of glycals with bis(trimethylsilyl)acetylene was carried out in good yield and high stereoselectivity.[43] As shown in Scheme 3, glycal **17** was prepared from **14** by a four-step sequence consisting of deisopropylidenation, acylation, and bromination to give glycosyl bromide **16**, which was converted to **17** by a modification of the procedure of Ferrier.[46,47] Substitution of DBU for diethylamine in the elimination step gave fewer side products. The *C*-alkynylation of **17** with bis(trimethylsilyl)acetylene, using conditions described by Isobe for glycals, followed by hydrolytic workup and sodium borohydride reduction as described gave exclusively alkynyl alcohol **18** in 88% overall yield. Four transformations occur in this two-step sequence: Ferrier rearrangement, enol acetate hydrolysis, β-elimination of the C-4 acetoxy group and ketone reduction. The C-3-C-4 double bond in **18** is in the correct location of diplopyrone and the configurations at C-1 and C-2 are introduced with complete stereoselectivity. At this point in the synthesis, all four of the chirality centers are established.

Final transformations culminating in a successful synthesis of the target pyranopyran **2** are shown in Scheme 4. Desilylation of alkyne **18** with TBAF gave alkynyl glycoside **19** in 68% yield. Selective reduction of the alkyne in **19** proved challenging with initial attempts with Lindlar's catalyst in methanol producing mixtures of *C*-vinyl and *C*-ethyl glycosides. Careful optimization of this reaction in terms of catalyst preparation (pre-treatment with quinoline under hydrogen), solvent (ethyl acetate), and temperature (0 °C) led to an efficient reduction of **19** to *C*-vinyl

glycoside **20** with only trace amount of over-reduced product. Acryloylation of **20** and RCM using Grubbs II catalyst in toluene gave pyranopyran acetate **3**. The addition of phenol to the RCM, as suggested by Forman for electron-deficient alkenes,[48] resulted in an improvement in yield (36% to 59%) and a significantly reduced reaction time (41 h to 1 h). Deacylation of **3** was expected to be challenging due to the presence of the unsaturated lactone that would preclude the use of sodium methoxide in methanol, which is known to undergo conjugate addition with carbohydrate enones.[49] Deacylation with methanol and *p*-toluenesulfonic gave (-)-diplopyrone that was contaminated with a side-product that appeared to be the result of conjugate addition of methanol to the unsaturated lactone. The ratio of the desired alcohol to the methanol adduct was estimated at 3:1. Lipases have been shown to catalyze selective deacylations in carbohydrate esters and we had some previous success in kinetic resolutions in our laboratory.[50] Several lipases were assayed against **3** and acceptable results were obtained with Amano PS-30 lipase which gave pyranopyran **2** in 63% yield (80% based on recovered **3**).



Scheme 4. Completion of the synthesis of pyranopyran 2

Not surprisingly, enzyme-catalyzed deacylation of **3** was slower than that reported for primary alcohols, but nonetheless was reproducible and less complicated by side-product formation than methanolysis. The route from commercially available **12** to pyranopyran **2** requires 13 steps, is highly stereoselective, and proceeds in 4% overall yield. Our route compares favorably with the

published route from butanediol (17 steps).[11] The final product **2** obtained in our synthesis was crystalline, while natural and synthetic diplopyrone **1** were reported as oils. Acetate **3** was also crystalline and suitable for x-ray analysis. The ORTEP diagram for **3** is shown in Figure 3.

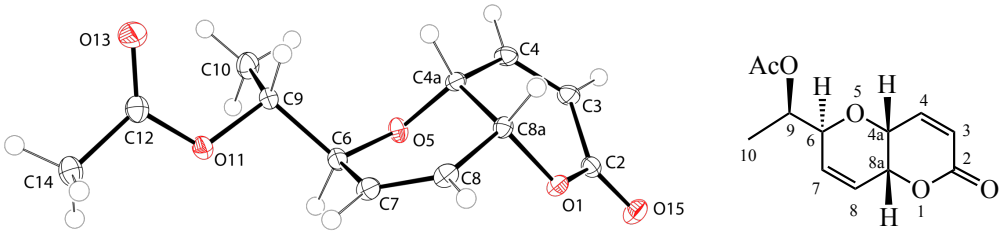


Figure 3. ORTEP diagram of 3

One of the goals of this study was to attempt to clarify the differences in analytical data, in particular NMR data, for diplopyrone isolated from the natural source with data obtained for material in the Mohaptra synthesis of "putative (+)-diplopyrone." Table 1 shows a comparison of NMR data of our synthetic pyranopyran **2** with the NMR data reported for natural, (+)-diplopyrone along with NMR data for material synthesized by Mohaptra.[11] The NMR data of **2** synthesized in this study match that reported for putative (+)-diplopyrone by Mohaptra and coworkers. The same discrepancies are observed when the data for **2** are compared to that reported for the natural product, most notably in proton chemical shifts for H-4a and H-8a, but also in other peak values. The structure was originally assigned on the basis of computational methods and NMR data.[1,2] While the structure is in need of revision, it is difficult to determine exactly where it is incorrect. The 2D 1H NOE (NOESY) data did not show an effect from H-4a to H-6, which would be consistent with a trans relationship of H-6 to both H-4a and H-8a.[1]

It is interesting to compare the conformations of compounds **3** and **4** obtained by x-ray analysis with the calculated conformation reported in the literature for (+)-diplopyrone **1**.[2] To facilitate this comparison, ORTEP structures of **3** and **4** can be inverted to show corresponding absolute stereochemistry (Figure 4). On the basis of molecular mechanics (MMII) calculations performed on the proposed structure of **1** two main conformers were predicted, in which the major

differences were in the conformation of the hydroxyethyl side chain and the ring to which it is attached. The major conformation predicted from these calculations is indicated as (a) in Figure 4, while the minor conformation is (b). Our crystallographic analyses of pyranopyran acetate **3** and nitrile **4** indicate solid state conformations for both that more closely match (b).

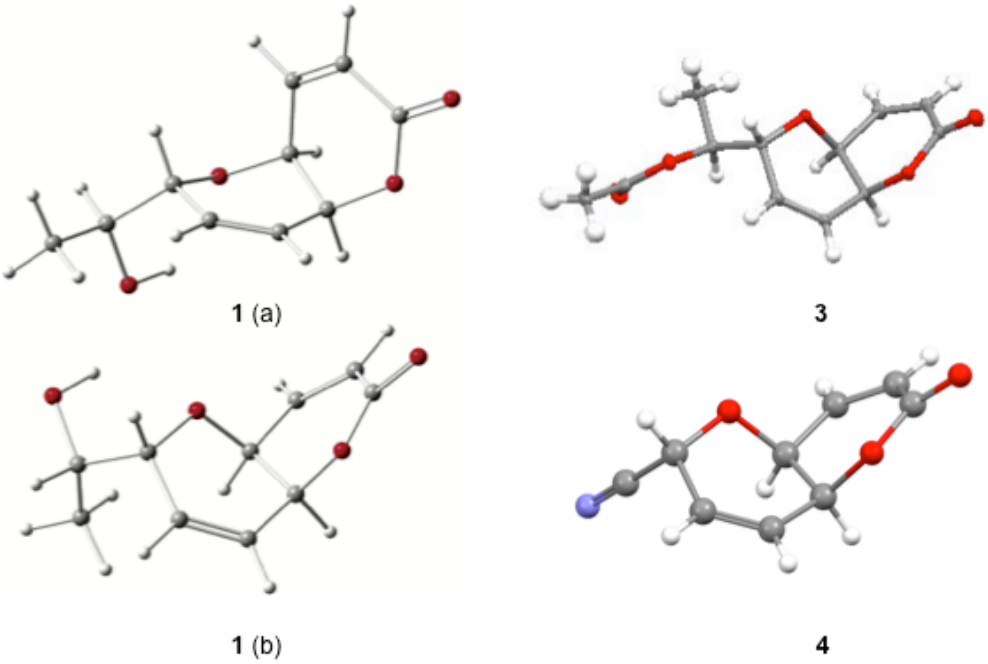


Figure 4. Comparison of conformations of pyranopyran acetate 3 and nitrile 4 from crystallographic data with calculated structures reported for diplopyrone 1

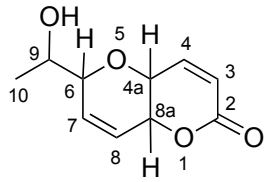
	^{1}H (natural) Ref. 2	^{1}H (synthetic) Ref. 11	^{1}H (synthetic) This work	^{13}C (natural) Ref. 2	^{13}C (synthetic) Ref. 11	^{13}C (synthetic) This work
2				**162.7**	**161.7**	**161.8**
3	**6.23 (d, *J* = 9.8 Hz)**	**6.07 (dd, *J* = 10.0, 1.6 Hz)**	**6.07 (dd, *J* = 10.0, 1.8 Hz)**	**124.8**	**123.3**	**123.2**
4	**6.88 (dd, *J* = 9.8, 5.8 Hz)**	**6.88 (ddd, *J* = 10.0, 3.1, 1.0 Hz)**	**6.88 (dd, J = 10.0, 2.8 Hz)**	**140.0**	**144.2**	**144.3**
4a	**4.09 (dd, *J* = 5.8, 2.8 Hz)**	**4.92 (ddd, *J* = 5.6, 3.1, 1.6 Hz)**	**4.91 (cm, 5.8, 2.8, 1.8 Hz)**	**64.9**	**64.9**	**64.8**
6	**4.16 (brs, *J* = 4.2, 3.5, 1.8 Hz)**	**4.08 (ddt, *J* = 4.2, 2.6, 2.6, 1.6 Hz)**	**4.07 (cm, *J* = 4.1, 3.0, 1.8 Hz)**	**78.9**	**74.8**	**74.8**
7	**6.18 (d, *J* = 10.4 Hz)**	**6.06 (td, *J* = 10.5, 1.6, 1.6 Hz)**	**6.01 (cm, 1H, *J* = 10.7, 1.8 Hz)**	**132.6**	**129.6**	**129.6**
8	**6.14 (ddd, *J* = 10.4, 4.6, 1.8 Hz)**	**6.04 (td, *J* = 10.5, 2.6, 2.6 Hz)**	**6.05 (cm, *J* = 2.0 Hz)**	**123.0**	**124.7**	**124.6**
8a	**4.65 (ddd, *J* = 4.6, 3.5, 2.8 Hz)**	**4.97 (m, *J* = 5.6, 2.6, 2.6, 1.6, 1.0 Hz)**	**4.97 (cm, *J* = 10.7, 5.8, 2.0 Hz)**	**69.7**	**70.0**	**70.0**
9	**3.92 (dq, *J* = 6.5, 4.2 Hz)**	**3.96 (dd, *J* = 6.4, 4.2 Hz)**	**3.96 (dq, 1H, *J* = 6.4, 4.1 Hz)**	**69.0**	**69.7**	**69.6**
10	**1.21 (d, *J* = 6.5 Hz)**	**1.25(d, *J* = 6.4 Hz)**	**1.24 (d, *J* = 6.5 Hz**	**17.8**	**18.7**	**18.7**

Table 1. Comparison of NMR data of proposed diplopyrone and synthetic products

BIOLOGICAL EVALUATION

Pyranopyran **2**, its acetyl derivative **3**, and nitrile analog **4** were evaluated for phytotoxic and antibacterial activity. Experimental procedures for the bioassays are included in the Supporting Information. Antibacterial activity was evaluated using common pathogenic bacteria in catfish. The largest segment of aquaculture in the United States of America (USA) is pond-raised catfish, with most production occurring in Mississippi, and USA pond-raised catfish are rated "Best Choice" by the Monterey Bay Aquarium Seafood Watch. The most common bacterial diseases in pond-raised catfish are enteric septicemia in catfish (ESC) and columnaris disease which are caused by *Edwardsiella ictaluri* and *Flavobacterium columnare,* respectively. These diseases can cost the catfish industry over US$100 million annually.[51] Catfish producers use numerous approaches to manage bacterial diseases in catfish ponds including the use of medicated feed containing the antibiotic florfenicol, live attenuated vaccines, and chemical therapeutants such as peroxides, copper sulfate ($CuSO_4 \cdot 5H_2O$), and potassium permanganate ($KMnO_4$). However, the use of chemicals in catfish ponds must be carefully monitored because these compounds have broad-spectrum toxicities toward non-target species, such as catfish and planktons. The discovery of efficacious natural or natural-based compounds that are toxic towards *E. ictaluri* and *F. columnare* would provide catfish producers with another beneficial management approach. During our initial screening to discover efficacious antibacterial compounds against *E. ictaluri* and *F. columnare*, we evaluated pyranopyran **2** and derivatives **3**, and **4** using a rapid bioassay (See Supporting Information).

Pyranopyran nitrile **4** was the most active of the three test compounds against *F. columnare* ALM-00-173, with a MIC of 17.7±0 mg/L, 24-h IC_{50} of 7.5±0.5 mg/L, and 24-h cellular viability IC_{50} of 16.8±0.9 mg/L (Table 2). The 24-h IC_{50} RDCF value of 14.0±0.9 indicates moderate activity of nitrile **4** against *F. columnare* ALM-00-173 when compared to the drug control florfenicol. Pyranopyran acetate **3** was only slightly active against *F. columnare* ALM-00-173

based on a MIC at the highest test concentration of 238 mg/L. Similar results were observed for evaluation of the three test compounds against *E. ictaluri* S02-1039, with **4** also as the most active compound based on a MIC of 0.002±0 mg/L and 24-h IC_{50} of 7.7±1.2 mg/L (Table 3). In comparison to the drug control, pyranopyran nitrile **4** had stronger activity than the drug control florfenicol against *E. ictaluri* S02-1039 based on the MIC RDCF value of 0.5±0 but was moderately active based on the 24-h IC_{50} RDCF value of 85.2±3.3. Pyranopyran **2** was moderately toxic against *E. ictaluri* S02-1039 based on a MIC of 19.6±0 mg/L, but it was less toxic than the nitrile with a 24-h IC_{50} of 46.1±1.0 mg/L. Because pyranopyran nitrile **4** had strong activity against *E. ictaluri* S02-1039, the minimum bactericidal concentration (MBC) was evaluated and determined to be >177.0 mg/L (the highest test concentration). These results indicate that pyranopyran nitrile **4** is more likely to be bacteriostatic against *E. ictaluri* S02-1039 rather than completely lethal at the active test concentrations.

The current study is the first to evaluate and report the antibacterial activities of pyranopyrans **2**, **3**, and **4**, against *F. columnare* and *E. ictaluri*, and, more specifically, the strong activity of **4** against *E. ictaluri* S02-1039 based on MIC results. Prior to efficacy studies (e.g., challenge tests), additional studies need to be performed such as the determination of fish toxicity (e.g., LC_{50}) for the nitrile. Following positive efficacy results, other studies would need to be performed such as the determination of pyranopyran nitrile **4** toxicity towards other non-target organisms in the environment. In addition, approval by the United States Food and Drug Administration would need to occur before aquaculturists could potentially use **4** as an alternative to currently approved therapeutants and antibiotics.

Compounds **2**, **3**, and **4** were also evaluated for phytotoxic activity using the aquatic plant *Lemna paucicostata* (L.) Hegelm. (duckweed). Only **4** was significantly phytotoxic, with an IC_{50} of less than 1 μM (Figure 5). At 33 μM some of the plants had no chlorophyll and were necrotic. At 100 μM most of the plants were devoid of chlorophyll and necrotic. The phytotoxicity of the

nitrile analog in the duckweed bioassay is similar to that of the commercial herbicides Isoproturon (IC_{50}= 0.35 μM), imazethapyr (IC_{50}= 0.93 μM), and pendimethalin (IC_{50}= 0.38 μM) in the same bioassay.[52] A figure showing the visual symptoms of the effects of **4** on the growth of *L. paucicostata* is included in the Supporting Information.

Table 2. Results of bioassay evaluations of diplopyrone derivatives for antibacterial activity against *Flavobacterium columnare* ALM-00-173. Numbers in parentheses are the standard error of the means.

Test material	MIC[a]	24-h IC_{50}[b]	MTT 24-h IC_{50}[c]	MIC[a] RDCF[d]	24-h IC_{50}[b] RDCF[d]
Florfenicol	0.36	0.54			
Pyranopyran (2)	196.0 (0)	76.4 (2.0)	78.4 (0)	544.4 (0)	141.6 (3.6)
Pyranopyran acetate (3)	238.0 (0)	>238.0	>238.0	661.1 (0)	>440.7
Pyranopyran nitrile (4)	17.7 (0)	7.5 (0.5)	16.8 (0.9)	49.2 (0)	14.0 (0.9)

[a]MIC = Minimum inhibitory concentration in mg/L. [b]24-h IC_{50} = 50% inhibition concentration in mg/L.

[c]24-h IC_{50} (mg/L) as measurement of cell viability using the tetrazolium bromide dye MTT.

[d]RDCF = Relative-to-drug-control florfenicol; values closer to 1.0 indicate higher antibacterial activity compared to florfenicol.

Table 3. Results of bioassay evaluations of diplopyrone derivatives for antibacterial activity against *Edwardsiella ictaluri* S02-1039. Numbers in parentheses are the standard error of the means.

Test material	MIC[a]	24-h IC_{50}[b]	MTT 24-h IC_{50}[c]	MIC[a] RDCF[d]	24-h IC_{50}[b] RDCF[d]
Florfenicol	0.36	0.14			
Pyranopyran (2)	19.6 (0)	46.1 (1.0)	22.1 (9.3)	54.4 (0)	329.0 (7.0)
Pyranopyran acetate (3)	238.0 (0)	165.4 (1.2)	90.4 (4.8)	661.1 (0)	1,181.5 (8.5)
Pyranopyran nitrile (4)	0.002 (0)	7.7 (1.2)	64.6 (4.4)	0.5 (0)	85.2 (3.3)

[a]MIC = Minimum inhibitory concentration in mg/L. [b]24-h IC_{50} = 50% inhibition concentration in mg/L.

[c]24-h IC_{50} (mg/L) as measurement of cell viability using the tetrazolium bromide dye MTT.

[d]RDCF = Relative-to-drug-control florfenicol; values closer to 1.0 indicate higher antibacterial activity compared to florfenicol.

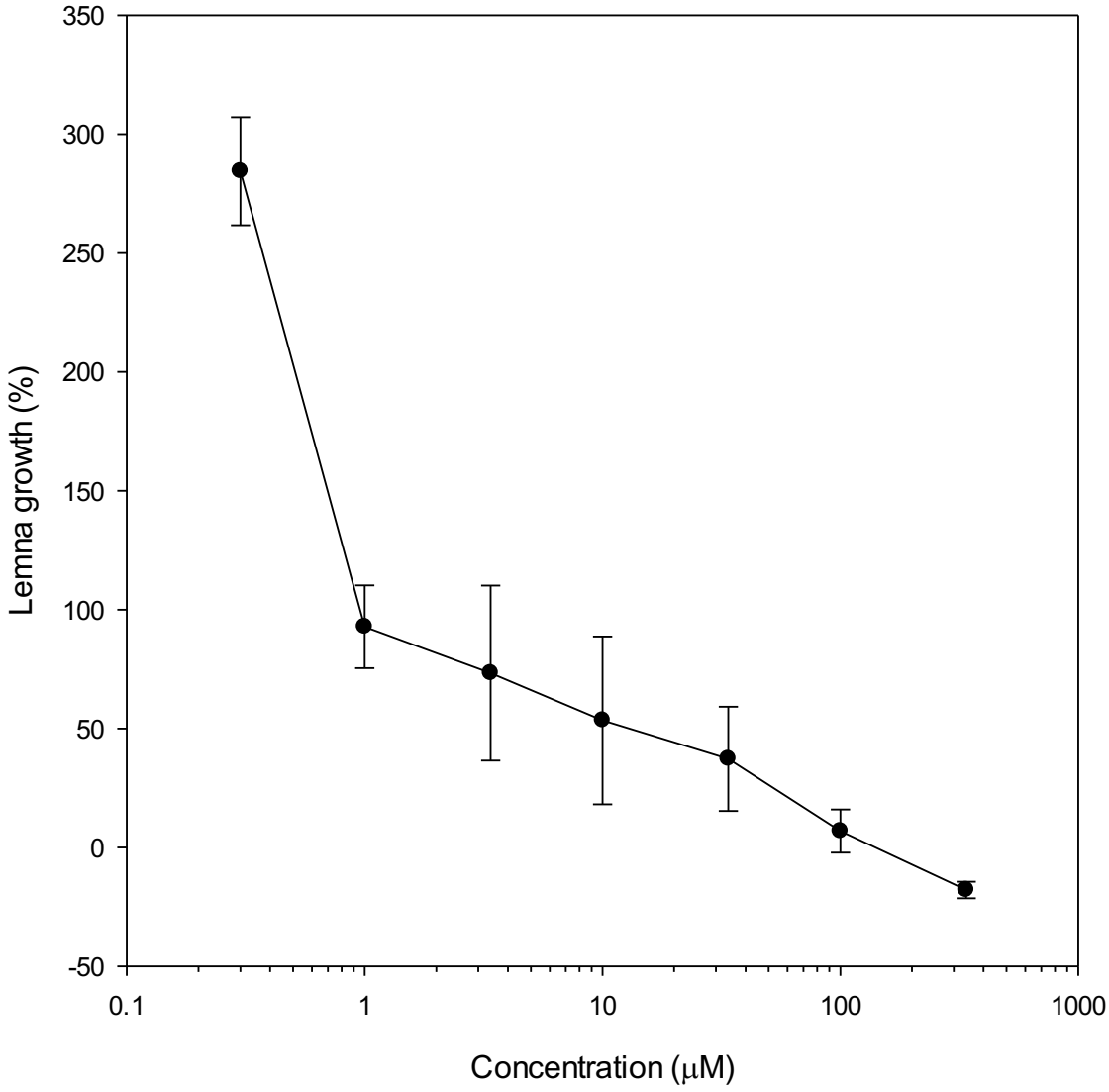


Figure 5. Dose/response curves for the nitrile pyranopyran (**4**) after 6 days of exposure. Error bars are ± 1 SE. The control (no test compound) is at 0.3 μM on the graph. The Y axis is the percent growth increase after exposure to the test compounds.

In a recent study, the antimicrobial activities of secondary metabolites, including diplopyrone, produced by the oak pathogenic fungus *Diplodia corticola* were isolated and determined to possess antifungal activities against plant pathogens.[53] Compounds **2**, **3**, and **4** were evaluated as fungicides using **i**solates of the fungal plant pathogens *Colletotrichum acutatum* Simmonds, *C. fragariae* Brooks, and *C. gloeosporioides*. Details of the fungicide bioassay are provided in the Supporting Information. None of the diplopyrone analogs were active as fungicides.

CONCLUSION

Highly stereoselective syntheses of the enantiomer of the structure originally proposed for the phytotoxin diplopyrone and structural analogs have been achieved using carbohydrate starting materials in two complementary approaches. Key steps in the synthesis of pyranopyran **2**, the enantiomer of putative diplopyrone, are a highly stereoselective pyranose chain-extension based on methyltitanium, preparation of a vinyl glycoside via Isobe *C*-alkynylation-rearrangment and chemoselctive reduction, and RCM-based pyranopyran construction. The synthesis of nitrile analog **4** is based on an alternative strategy using a Ferrier *C*-glycosidation to introduce the nitrile group and Wittig chain-extension, both of which are completely stereoselective. X-ray crystallographic analyses were obtained for the pyranopyran acetate **3** and the nitrile analog **4**. Our syntheses coupled with new crystallographic and NMR data provide further confirmation that the structure originally proposed for (+)-diplopyrone may require revision. Biological assays carried out at the USDA-ARS-Natural Products Utilization Research Unit revealed potent antibacterial activity for pyranopyran nitrile **4** against common bacterial pathogens in fish. Compound **4** is significantly more active in vitro than a commercially used antibiotic used against *E. ictaluri*, a common pathogen in catfish and it is also phytotoxic as determined by bioassay using *L. paucicostata* (duckweed). The antibiotic and phytotoxic activities of **2**, **3**, and **4** demonstrate the potential of these and similar pyranopyrans as antibiotics and herbicides. Significantly, the bioassay results show an intriguing structure-activity relationship for the C-6 position on the

pyranopyran scaffold that can be exploited using our synthetic methodology to generate additional analogs for biological testing. Both the routes to **2** and **3** (Type 1) and the route to **4** (Type 2) are amenable to modification to introduce other functionality at this key position. These studies and further biological evaluation are currently in progress.

EXPERIMENTAL SECTION

General Methods.

Melting points were recorded on a Thomas-Hoover apparatus and they are uncorrected. Thin-layer chromatography was carried out on aluminum foil-backed silica gel plates coated with a fluorescent indicator. Plates were developed with cerium molybdate stain. Flash chromatography was carried out using 230-400 mesh silica gel. Optical rotations were recorded on a Perkin-Elmer Model 341 polarimeter at 20 or 23 °C as indicated. HRMS analyses were conducted at the University of Illinois Mass Spectrometry Laboratory. NMR spectra were recorded on a Varian (Agilent) Mercury 300 Plus spectrometer in $CDCl_3$ for ^1H NMR at 300.0 MHz, tetramethysilane reference, δ = 0.0 ppm, and, ^{13}C NMR 75.4 MHz, $CDCl_3$ reference, δ = 76.9 ppm. Spectral assignments were confirmed using COSY and DQCOSY experiments. Data were collected on a Brüker-AXS Kappa APEX II CCD diffractometer with 0.71073 Å Mo-Ka radiation (See Supporting Information). Reagents and starting materials were purchased from Sigma-Aldrich with the exception of 5% Pd/$CaCO_3$ (Alfa Aesar), chlorotitanium triisopropoxide (Strem), and tri-O-acetyl-D-galactal (TCI).

2,3-Dideoxy-α-D-*threo*-hex-2-enopyranosyl cyanide (7). To a solution of diester **6** 365 mg (1.5 mmol) in methanol (15 mL) was added *p*-toluenesulfonic acid monohydrate (16 mg, 0.085 mmol). The mixture was stirred under reflux for 7 h and monitored by TLC (50% ethyl acetate/hexanes) until starting material was consumed ($R_f \sim 0$, 50% ethyl acetate/hexanes). The reaction mixture was concentrated to a small volume (2-3 mL) and cooled to -20 °C to give

crystalline diol **7** (120 mg, 52%). mp 134–137 °C; lit[54] mp 134– 135 °C; $[\alpha]_D^{20}$ -351.3 (*c* 0.97, EtOH); lit $[\alpha]_D^{54}$ -362.4 (*c* 1.0, EtOH). HRMS (ESI): *m/z* calcd for $C_7H_9NO_3$ $[M + Na]^+$: 178.0480. Found 178.0486. Larger scale deacylation of **6** (10.9 g, 45.8 mmol) was carried out by the same procedure in anhydrous methanol (140 mL) with *p*-toluenesulfonic acid monohydrate (430 mg, 2.3 mmol, 5 mol%) to afford crude diol **7** (6.91 g 97%) as an amorphous solid after concentration of the reaction mixture and drying. This product was suitable for use without further purification in the silylation step.

4,6-di-*O*-tert-butyldimethylsilyl-2,3-dideoxy-α-D-*threo*-hex-2-enopyranosyl cyanide (8). To a stirring solution of diol nitrile **7** (6.13 g, 39.5 mmol) in anhydrous DMF (15 mL) was added imidazole (13.5 g, 198 mmol, 5 eq) and *tert*-butyldimethylsilyl chloride (15.7 g, 104 mmol, 2.6 eq). The reaction was stirred at rt 12 h then quenched with sat'd aq NH_4Cl sol'n and extracted with ethyl acetate (3 x 30 mL). The combined organic extracts were washed with water (2 x 20 mL), brine (20 mL) then dried (Na_2SO_4) and concentrated to a syrup that was purified by flash chromatography with 5% ethyl acetate/hexanes to give 14.3 g (94%) of **8** as an oil that solidified. R_f = 0.79 (20% ethyl acetate/hexanes); mp 31 – 33 °C; $[\alpha]_D^{23}$ -181.2 (*c* 0.96, CH_2Cl_2); ^{1}H NMR ($CDCl_3$): δ 6.12 (ddd, 1H, $J_{2,3}$ = 10.0, $J_{3,4}$ = 5.4, $J_{1,3}$ = 2.0 Hz, H-3), 5.84 (dd, 1H, $J_{2,3}$ = 10.0, $J_{1,2}$ = 3.8 Hz, H-2), 5.08 (dd, 1H, $J_{1,2}$ = 3.8, $J_{1,3}$ = 2.1 Hz, H-1), 4.03 (dd, 1H, $J_{3,4}$ = 5.4, $J_{4,5}$ = 1.9 Hz, H-4), 3.86 – 3.70 (m, 3H, H-5,6,6'), 0.90, (*t*-BuMe_2Si), 0.89 (*t*-BuMe_2Si), 0.08 (*t*-BuMe_2Si), 0.07 (*t*-BuMe_2Si); ^{13}C{^{1}H} NMR($CDCl_3$): δ 130.4, 123.5, 115.9, 76.8, 62.7, 61.9, 61.2, 25.8, 25.7, 18.2, -4.1, -4.8, -5.2, -5.3. HRMS (CI-magnetic sector): m/z: $[M + H]^+$ Calcd for $C_{19}H_{38}NO_3Si_2$ 384.2390; Found 384.2389.

(2*S*,5*R*,6*S*)-5-(*tert*-butyldimethylsilyl)oxy)-6-formyl-5,6-dihydro-2*H*-pyran-2-carbonitrile (10). A mixture of di-*O*-TBS ether **8** (1.40 g, 3.65 mmol) and PPTS (185 mg, 0.74 mmol, 20 mol %) in anhydrous methanol (20 mL) was stirred at rt for 10 h, until TLC showed

consumption of the starting material. The reaction mixture was concentrated rapidly under reduced pressure using a high vacuum pump connected to a rotary evaporator *without heating* and the resulting oil diluted with dichloromethane (45 mL) and washed with cold water (15 mL), cold sat'd aq $NaHCO_3$ sol'n (10 mL), dried (Na_2SO_4) and concentrated to yield 800 mg (81%) alcohol **9** as an oil. R_f = 0.60 (25% ethyl acetate/hexanes); HRMS (CI): *m/z* calcd for $C_{13}H_{24}NO_3Si$ [M + H]$^+$: 270.1525. Found 270.1533. The crude product alcohol (630 mg, 2.34 mmol), was dissolved in anhydrous dichloromethane (5 mL) and to this solution was added anhydrous DMSO (2.0 mL, 2.2 g, 28 mmol, 12 eq), and diisopropylethylamine (2.5 mL, 1.9 g, 14 mmol, 6 eq). The mixture was cooled to 0 °C and SO_3•pyridine (1.86 g, 11.7 mmol, 5 eq) was added and the reaction stirred for 2 h. Water (30 mL) was added and the mixture was extracted with ethyl acetate (3 x 20 mL . The combined extracts were washed with water (4 x 10 mL), brine (10 mL), dried (Na_2SO_4), and concentrated under reduced pressure. Flash chromatography (16% ethyl acetate/hexanes) gave 540 mg (86%) of **10** as an oil.

R_f = 0.70 (25% ethyl acetate/hexanes); $[\alpha]_D^{20}$ -282 (*c* 1.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 9.61 (s, 1H, CHO), 6.15 (ddd, 1H, $J_{2,3}$ = 10.1, $J_{3,4}$ =5.1, $J_{1,3}$ = 2.1 Hz, H-3), 5.93 (ddd, 1H, $J_{2,3}$ = 10.1, $J_{1,2}$ = 3.7, $J_{2,4}$ = 0.7 Hz, H-2), 5.28 (dd, 1H, $J_{1,2}$ = 3.5, $J_{1,3}$ = 1.9 Hz, H-1), 4.45 (ddd, 1H, $J_{3,4}$ = 5.3, $J_{4,5}$ = 3.0, J = 0.6 Hz, H-4), 4.30 (d, 1H, $J_{4,5}$ = 2.9 Hz, H-5), 0.84 (*t*-BuMe$_2$Si), 0.08 (*t*-BuMe$_2$Si); ^{13}C{^{1}H} NMR ($CDCl_3$): δ 198.4, 129.4, 123.5, 115.1, 79.4, 62.3, 62.1, 25.5, 17.9, -4.2, -5.0. HRMS (ESI-TOF) m/z: [M + Na]$^+$ Calcd for $C_{13}H_{21}NO_3SiNa$ 290.1188; Found 290.1194.

Ethyl (*Z*)-3-((2*R*,3*R*,6*S*)-3-((*tert*-butyldimethylsilyl)oxy)-6-cyano-3,6-dihydro-2*H*-pyran-2-yl)acrylate (11). To a stirring solution of aldehyde **10** (402 mg, 1.5 mmol), in anhydrous methanol (25 mL), at 0 °C was added (carbethoxymethylene)-triphenylphosphorane (651 mg, 1.86 mmol, 1.5 eq) and the reaction was stirred 3 h. Concentration of the mixture and flash chromatography (5% ethyl acetate/hexanes) gave 310 mg (61%) of cis ester **11** as a colorless oil. R_f = 0.64 (10% ethyl acetate/hexanes); $[\alpha]_D^{20}$ -194 (*c* 1.8, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 6.22 (dd, 1H, $J_{6,7}$ = 11.9,

$J_{5,6}$ = 6.8 Hz, H-6), 6.13 (ddd, 1H, $J_{2,3}$ = 10.4, $J_{3,4}$ = 5.4, $J_{1,3}$ = 2.1 Hz, H-3), 5.93 (dd, 1H, $J_{6,7}$ = 11.8, $J_{5,7}$ = 1.4 Hz, H-7), 5.87 (ddd, 1H, $J_{2,3}$= 10.1, $J_{1,2}$ = 4.0, $J_{2,4}$ = 0.6 Hz,H-2), 5.36 (ddd, 1H, $J_{5,6}$ = 6.7, $J_{4,5}$ = 2.5, $J_{5,7}$ = 1.7 Hz, H-5), 5.12 (dd, 1H, $J_{1,2}$ = 3.9, $J_{1,3}$ = 2.1 Hz, H-1), 4.37 (dd, 1H, $J_{3,4}$= 5.4, $J_{4,5}$ = 2.5 Hz, H-4), 4.20 (q, 2H, J = 7.2 Hz), 1.30 (t, 3H, J = 7.2 Hz), 0.85 (*t*-BuMe$_2$Si), 0.018 (*t*-BuMe$_2$Si), 0.012 (*t*-BuMe$_2$Si); ^{13}C{^{1}H} NMR ($CDCl_3$): δ 165.3, 145.6, 130.6, 122.8, 120.6, 115.9, 73.9, 63.0, 62.2, 25.7, 18.1, 14.2, -4.34, -4.85.

HRMS (ESI-TOF) m/z: $[M + Na]^+$ Calcd for $C_{17}H_{27}NO_4SiNa$ 360.1607; Found 360.1615.

(4a*R*,6*S*,8a*R*)-6-Cyano-6,8a-dihydropyrano-[3,2-*b*]pyran-2(4a*H*)-one (Diplopyrone nitrile 4). Ester **11** (130 mg, 0.385 mol), and TsOH (11 mg, 15 mol%) were stirred under reflux in anhydrous methanol (10 mL) for 4.5 h. The mixture was then concentrated under reduced pressure and dried briefly under vacuum to remove trace methanol. Toluene (10 mL) was added and the mixture stirred overnight under reflux. The crude product was purified by flash chromatography (30% ethyl acetate/hexanes) to give 60 mg (88%, 34% overall from **6**) of crystalline **4**. R_f = 0.23 (30% ethyl acetate/hexanes); mp 162 – 164 °C; $[\alpha]_D^{20}$ -279° (*c* 1.0 $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 6.91 (dd, 1H, $J_{3,4}$ = 9.8, $J_{4,4a}$ = 5.8 Hz, H-4), 6.33 (m, 1H, H-8), 6.31 (dd, 1H, $J_{3,4}$ = 9.8, $J_{3,4a}$ = 0.6 Hz, H-3), 6.17 (ddd, 1H, $J_{7,8}$ = 10.1, $J_{6,7}$ = 4.0, $J_{6,8}$ = 0.8 Hz, H-7), 5.18 (ddd, 1H, $J_{6,7}$ = 4.1, $J_{6,8}$ = 2.0, J = 0.5 Hz, H-6), 4.74 (dd, 1H, $J_{8,8a}$ = 5.3, $J_{4a,8a}$ = 3.0 Hz, H-8a), 4.45 (ddd, 1H, $J_{4,4a}$ = 5.8, $J_{4a,8a}$ = 2.9, $J_{4a,8}$ = 0.5 Hz, H-4a); ^{13}C{1H} NMR ($CDCl_3$): δ 161.3, 138.3, 126.6, 125.6, 125.0, 114.9, 67.5, 62.7, 62.3. HRMS (ESI-TOF) m/z: M^+ Calcd for $C_9H_7NO_3$ 177.0426; Found 177.0431.

1,2;3,4-di-*O*-isopropylidene-α-D-galacto-1,6-dialdo-hexopyranose (13). A mixture of 1,2:3,4-diisopropylidene-α-D-galactopyranose **12** (14.99 g, 57.6 mmol) and TEMPO (900 mg, 5.76 mmol, 10 mol %) was stirred in dichloromethane (62 mL, anhydrous) at room temperature as diacetoxyiodobenzene (BAIB) (20.42 g, 63.4 mmol, 1.1 eq,) was added to the reaction mixture.

After several minutes of stirring, all the BAIB dissolved in the clear, bright orange reaction mixture. After stirring 4 h the reaction mixture was diluted with dichloromethane (35 mL), washed with sat. aq $Na_2S_2O_3$ (100 mL), and the aqueous layer was extracted with dichloromethane (4 X 50 mL). The combined organic layers were then washed with sat. aq $NaHCO_3$ sol'n (100 mL) followed by brine (100 mL), dried via sodium sulfate (anhydrous) and concentrated via rotary evaporation to yield a bright orange liquid that was purified by flash chromatography (40:60 ethyl acetate/hexanes) yielding 10.99 (74 %) of **13** as a bright yellow oil that was stored at -23 °C. R_f = 0.67 (1:1 ethyl acetate/hexanes); $[\alpha]_D^{20}$ -125.5 (*c* 0.98, $CHCl_3$); lit[55] $[\alpha]_D^{20}$ -131° (*c* 0.9, $CHCl_3$); lit[56] $[\alpha]_D^{21}$ -113° (*c* 3.4, $CHCl_3$). The ^{1}H NMR spectra of **13** matched that reported.[32]

7-Deoxy-1,2;3,4-di-O-isopropylidene-D-*glycero*-α-D-*galacto*-heptopyranose (14).

In a nitrogen glove box, chlorotitanium triisopropoxide (83 g, 318.1 mmol, 6.2 eq) was added to a Schlenk flask equipped with a teflon plug and a magnetic stir bar. The Schlenk flask was fitted with an addition funnel, sealed with a rubber septum, and removed from the glove box. The Schlenk flask was then connected to a Schlenk line, and the flask was placed under argon. Diethyl ether (220 mL, anhydrous) was added to the flask via a cannula transfer. The mixture was stirred at room temperature until all the solids dissolved, then the reaction mixture was cooled to -50 °C ($CO_{2(s)}$/acetone bath). Methyllithium (199 mL, 1.6 M in diethyl ether, 318.1 mmol, 6.2 eq) was added to the dropping funnel via cannula transfer. The methyllithium was then added to the reaction mixture at a rate so that the cooling bath temperature did not rise above -50 °C. Following this addition, the light-yellow reaction mixture was stirred for 1 h (the reaction warmed to -25 °C during this time) and a white precipitate formed. Under an argon counter flow, the addition funnel was replaced with a rubber septum. While maintaining the cooling bath temperature at -25 °C, the diethyl ether was removed *in vacuo* (using the Schlenk line, with a liquid nitrogen cooled trap to collect solvent). When the solvent was removed, the methyl(triisopropoxy)titanium thus formed was kept under argon.

Methyl(triisopropoxy)titanium was suspended in dichloromethane (220 mL, anhydrous), and the Schlenk flask was cooled to -78 °C (CO_2/acetone bath). A solution of 1,2:3,4-di-*O*-aldehyde **13** (13.24 g, 51.3 mmol) in dichloromethane (50 mL, anhydrous) was added dropwise via a syringe while maintaining a cooling bath temperature of -78 °C. The reaction mixture was allowed to slowly warm over 22 h under argon (a needle was affixed to an argon balloon and was inserted through the septum of the Schlenk flask in order to prevent pressure build up). At this point, the cooling bath reached 20 °C. The reaction mixture was quenched via the addition of water. The first 10 mL of water was added dropwise until vigorous effervescence subsided. Following this, an additional 240 mL of water was added to the reaction mixture followed by 300 mL of diethyl ether. The entire mixture (including solids formed) was transferred into an Erlenmeyer flask and stirred vigorously for 20 min. After filtration, the solids were washed with diethyl ether (200 mL), the filtrate was transferred to a separatory funnel, the organic and aqueous layers were separated, and the aqueous phase was extracted with diethyl ether (2 X 200 mL). The combined organic extracts were washed successively with H_2O (250 mL), brine (400 mL), dried via $MgSO_4$ (anhydrous) and concentrated to yield 10.36 g (78 %) of a bright yellow oil that was filtered through a pad of SiO_2, washed with diethyl ether (300 mL), and concentrated under reduced pressure to yield 10.34 g (74 %) of **14** as a pale yellow oil that was stored at -23 °C. R_f = 0.45 (1:1 ethyl acetate/hexanes) and used without further purification. The ^{1}H NMR spectrum matched that reported,[37] except for the presence of the minor β-L-glycero diastereomer that was seen previously (19:1 *dr*) but in this larger-scale preparation was estimated at approximately 0.7%.

1,2,3,4,6-Penta-*O*-acetyl-7-deoxy-D-*glycero*-D-*galacto*-heptopyranoside (15).

A mixture of 1,2;3,4-di-*O*-isopropylidene-7-deoxy-D-*glycero*-D-*galacto*-heptopyranoside **14** (10.30 g, 37.55 mmol) and acetic acid/water (80 mL, 60 % v/v) was heated and stirred under reflux. As the reaction progressed the reaction mixture became dark brown in color. After 2 h, TLC analysis (1:1 ethyl acetate/hexanes) showed the disappearance of starting material and the reaction

mixture was allowed to cool to room temperature. Once cooled the solvent was removed under reduced pressure (vacuum pump) at a temperature no greater than 30 °C. The resulting brown oil was taken up in ethanol (anhydrous), concentrated under reduced pressure, and dried under high vacuum. The crude material was dissolved in pyridine (150 mL, anhydrous) and cooled to 0 °C. Acetic anhydride (110 mL) was added via syringe. After five minutes of stirring at 0 °C, the reaction mixture was allowed to warm to room temperature and stirred 22 h. The reaction was quenched by the addition of 110 mL of H_2O and stirred for 10 minutes. Following this, the reaction mixture was taken up in 100 mL of dichloromethane and the organic and aqueous layers were separated. The aqueous layer was extracted with dichloromethane (4 x 100 mL). The combined organic layers were washed with sat'd aq $CuSO_4$ sol'n (4 x 500 mL), sat'd aq $NaHCO_3$ sol'n (250 mL), brine (250 mL), dried (Na_2SO_4), and concentrated under reduced pressure to yield 13.5 g (89 %) of **15** as a foam in a 1:1.3 mixture of α/β anomers that was stored at 0 °C. R_f = 0.46 (1:1 ethyl acetate/hexanes). The ^{1}H NMR spectrum of **15** matched that reported.[41] ^{13}C{^{1}H} NMR ($CDCl_3$): δ170.3, 170.3, 170.15, 170.00, 169.91, 169.74, 169.68, 169.37, 169.04, 168.89, 92.42, 92.42, 89.72, 77.23, 76.21, 73.12, 70.85, 67.75, 66.21, 65.90, 20.91, 20.87, 20.82, 20.67, 20.58, 17.5. HRMS (ESI-TOF) m/z: $[M + Na]^+$ Calcd for $C_{17}H_{24}O_{11}Na$ 427.1216; Found 427.1227.

2,3,4,6-Tetra-*O*-acetyl-7-deoxy-D-glycero-α-D-galacto-heptopyranoside bromide (16). 1,2,3,4,6-Penta-*O*-acetyl-7-deoxy-D-*glycero*-α/β-D-*galacto*-heptopyranoside **15** (13.45 g, 33.26 mmol) was cooled to 0 °C then dissolved in 33 % wt HBr solution in glacial acetic acid (75 mL). The dark brown reaction mixture was allowed to stir at 0 °C for 5 min. Following this the reaction mixture was allowed to warm to room temperature. After 3 h, TLC analysis (1:1 ethyl acetate/ hexanes) showed the disappearance of starting material, the reaction mixture was taken up in dichloromethane (250 mL) and washed with ice water (200 mL). The aqueous layer was extracted with dichloromethane (2 x 200 mL), the combined organic layers were washed with cold water until the washings were neutral (4 x 500 mL), dried (Na_2SO_4), and concentrated via reduced

pressure to 12.01 g (85 %) of **16** as a foam that was stored at -23 °C. R_f = 0.63 (1:1 ethyl acetate/hexanes); $[\alpha]_D^{20}$ +197.8 (*c* 1.4, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 6.66 (d, 1H, $J_{1,2}$ = 3.97 Hz, H-1), 5.54 (dd, 1H, $J_{3,4}$ = 3.29, $J_{5,4}$ = 1.23 Hz, H-4), 5.39 (dd, 1H, $J_{3,4}$ = 3.29, $J_{3,2}$ = 10.63 Hz, H -3), 5.02 (dq, 1H, $J_{6,7}$ = 6.24, $J_{6,5}$ = 9.38 Hz, H-6), 5.02 (dd, 1H, $J_{1,2}$ = 3.97,$J_{2,3}$ = 10.63, $J_{2,7}$ = 6.24 Hz, H-2), 4.12 (ddd, 1H, $J_{5,6}$ = 9.38 Hz, H-5), 2.11, 2.10, 2.00, 1.99 (4s, 12H, $OCOCH_3$), 1.26 (d, 3H, $J_{7,6}$ = 6.24, CH_3), $^{13}C\{^1H\}$ NMR ($CDCl_3$): δ 170.0, 169.7, 88.3, 75.1, 67.9, 67.8, 65.9, 65.7, 53.5, 20.8, 20.7, 20.5, 20.4, 17.3. HRMS (ESI-TOF) m/z: $[M + Na]^+$ Calcd for $C_{15}H_{21}O_9BrNa$ 447.0267; Found 447.0274.

2,3,4,6-Tetra-*O*-acetyl-7-deoxy-1,5-anhydro-D-*gluco*-hept-1-enitol (17). A mixture of glycosyl bromide **16** (11.87 g, 27.70 mmol) and tetra-*n*-butylammonium bromide (8.93, 27.7 mmol, 1 eq) was stirred in acetonitrile (6 mL, anhydrous) as DBU (11.15 mL, 76.2 mmol, 2.75 eq) was added via a syringe. Immediately following the addition of DBU the reaction became black. After 20 min TLC analysis (1:1 ethyl acetate/hexanes) showed the disappearance of starting material and the reaction mixture was diluted with 6 mL of dichloromethane and concentrated via rotary evaporation. The resulting syrup was taken up in dichloromethane (150 mL) and washed with 10 % HCl (150 mL), followed by saturated $NaHCO_3$ (150 mL), dried (Na_2SO_4), and concentrated via rotary evaporation to yield a dark oil that was purified by flash chromatography (1:1 ethyl acetate/hexanes) to yield 6.94 g (73 %) of **17** as a yellow oil that was stored at 0 °C. R_f = 0.50 (1:1 ethyl acetate/hexanes); $[\alpha]_D^{20}$ + 57.7 (*c* 1.3, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$): δ 6.63 (d, 1H, $J_{1,3}$ = 1.75 Hz, H-1), 5.89 (ddd, 1H, $J_{3,4}$ = 5.04, $J_{3,1}$ = 1.75 Hz, H-3), 5.53 (dd, 1H, $J_{4,3}$ = 5.04, $J_{4,5}$ = 0.95 Hz, H-4), 5.02 (dq, 1H, $J_{6,7}$ = 6.25, $J_{6,5}$ = 9.15 Hz, H-6), 3.99 (ddd, 1H, $J_{5,6}$ = 9.15 Hz, H-5), 2.11, 2.02, 2.01, (3s, 12H, $OCOCH_3$), 1.32 (d, 3H, $J_{7,6}$ = 6.25, CH_3); $^{13}C\{^1H\}$ NMR ($CDCl_3$): δ 170.3, 169.9, 169.7, 169.4, 138.8, 127.3, 77.6, 66.4, 64.0, 62.1, 20.9, 20.5, 20.4, 17.2. HRMS (ESI-TOF) m/z: $[M + Na]^+$ Calcd for $C_{15}H_{20}O_9Na$ 367.1005; Found 367.1005.

(*R*)-1-((2*S*,5*R*,6*R*)-5-Hydroxy-6-((trimethylsilyl)ethynyl)-5,6-dihydro-2*H*-pyran-2-yl)ethyl acetate (18). A homogeneous mixture of glycal **17** (1.9 g, 5.52 mmol) and bis(trimethylsilyl)acetylene (3.76 g, 22.1 mmol, 4 eq) in dichloromethane (25 mL, anhydrous) was degassed and place under argon. The reaction mixture was cooled to -20 °C (CO_2/acetone) and $SnCl_4$ (22.1 mL, 1 M in dichloromethane, 4 eq) was added via syringe. Following this addition, the reaction mixture was stored at 0 °C. After 70 h, TLC analysis showed the disappearance of starting material, and the reaction mixture was poured into a 1:1 mixture of sat'd aq $NaHCO_3$/Na, K tartrate sol'n (100 mL) and stirred for 30 min at 0 °C. After several minutes of stirring the reaction mixture became an orange/brown color. After 30 min, the reaction mixture was taken up in dichloromethane, and the organic and aqueous layers were separated. The aqueous layer was extracted with dichloromethane (4 x 100 mL) and the combined organic layers were washed with brine (200 mL), dried ($MgSO_4$), and concentrated via rotary evaporation to yield **18** as a light-yellow oil. To the crude product was added methanol (30 mL) and cerium(III)chloride heptahydrate (3.08 g, 8.28 mmol, 1.5 eq) to give an orange/brown homogeneous mixture that was cooled to 0 °C. Once cooled, $NaBH_4$ (0.313 g. 8.28 mmol, 1.5 eq) was added to the reaction mixture portion-wise over 5 minutes. After 1 h 40 min, TLC analysis showed the disappearance of starting material and the reaction mixture was quenched by the addition of 15 mL of ice cold H_2O. After 10 min of stirring, the methanol was removed via rotary evaporation at a temperature no greater than 35 °C. The resulting faint orange aqueous solution was filtered through cotton and extracted with dichloromethane (4 X 100 mL). The combined organic extracts were then washed with brine (200 mL), dried (Na_2SO_4), and concentrated under rotary evaporation to yield 1.37 g (88%) a yellow/orange liquid that crystallized overnight at 0 °C. R_f = 0.69 (1:1 ethyl acetate/hexanes); mp 44.5 °C – 46.0 °C; $[\alpha]_D^{20}$ -28.6 (*c* 1.4, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.82 (ddd, 1H, $J_{3,5}$ = 2.00, $J_{3,4}$ = 10.51, $J_{3,2}$ = 2.05, $J_{3,1}$ = 0.80, Hz, H-3), 5.74 (dt, 1H, $J_{4,5}$ = 1.71, $J_{4,3}$ = 10.51, $J_{4,2}$ = 1.64 Hz, H-4), 4.96 (dq, 1H, $J_{6,7}$ = 6.41, $J_{6,5}$ = 4.34 Hz, H-6), 4.90 (d, 1H, $J_{1,3}$ = 0.80, $J_{1,2}$ = 5.84

Hz, H-1), 4.25 (bs, 1H, $J_{2,3}$ = 2.05, $J_{2,1}$ = 5.84 Hz, H-2), 2.08 (s, 3H, $OCOCH_3$), 1.85 (bs, 1H, OH), 1.21 (d, 3H, $J_{7,6}$ = 6.41 Hz, CH_3), 0.18 (s, 9H, $SiMe_3$); $^{13}C\{^1H\}$ NMR ($CDCl_3$): δ 170.4, 129.4, 127.0, 99.2, 94.3, 72.0, 71.5, 67.9, 63.5, 21.3, 14.9, -0.2. HRMS (ESI-TOF) m/z: $[M + Na]^+$ Calcd for $C_{14}H_{22}O_4SiNa$ 305.1185; Found 305.1190.

(*R*)-1-((2*S*,5*R*,6*R*)-5-Hydroxy-6-ethynyl)-5,6-dihydro-2*H*-pyran-2-yl)ethyl acetate (19). To a stirring solution of alkynyl glycoside **18** (3.0 g, 10.6 mmol) in THF (10 mL) at 0 °C was added TBAF (3.18 mL, 1 M in THF, 0.3 eq) via syringe. After 15 min TLC analysis (1:1 ethyl acetate/hexanes) showed the disappearance of starting material. The THF was removed via rotary evaporation to yield a brown oil. This crude material was purified by flash chromatography (1:1 ethyl acetate/hexanes) to yield 1.52 g (68 %) of **19** as a faint yellow oil that was stored at – 23 °C. R_f = 0.41 (1:1 ethyl acetate/hexanes); $[\alpha]_D^{20}$ -47.8 (*c* 1.0, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$): δ 5.85 (ddd,1H, $J_{2,3}$ = 1.73, $J_{3,4}$ = 10.72, $J_{3,5}$ = 2.3 Hz, H-3), 5.77 (dt, 1H, $J_{2,4}$ = 1.73, $J_{4,5}$ = 1.73, $J_{4,3}$ = 10.72 Hz, H-3, H-4), 4.97 (dq, 1H, $J_{6,7}$ = 6.55, $J_{6,5}$ = 4.34 Hz, H-6), 4.93 (dd, 1H, $J_{1,3}$ = 0.78, $J_{1,2}$ = 5.8 Hz, H-1), 4.43 (cm, 1H, $J_{5,6}$ = 4.34, $J_{5,4}$ = 1.7, $J_{5,3}$ = 2.3, $J_{5,1}$ = 0.63 Hz, H-5), 4.43 (dddt, 1H, $J_{2,4}$ = 1.7, $J_{2,3}$ = 1.7, $J_{2,2\text{-OH}}$ = 11.0 $J_{2,1}$ = 5.8 Hz, H-2), 2.49 (d, 1H, $J_{CH,1}$ = 2.24 Hz, CH), 2.08 (s, 3H, $OCOCH_3$), 1.89 (d, $J_{OH,2}$ = 11.0 Hz, OH), 1.22 (d, 3H, $J_{7,6}$ = 6.55 Hz, CH_3); $^{13}C\{^1H\}$ NMR ($CDCl_3$): δ 170.5, 129.4, 127.0, 78.1, 76.5, 72.1, 71.4, 67.3, 63.6, 21.7, 14.8. HRMS (ESI): *m/z* calcd for $C_{11}H_{14}O_4Na$ $[M+ Na]^+$ = 233.0790. Found 233.0793

(*R*)-1-((2*S*,5*R*,6*R*)-5-Hydroxy-6-vinyl-5,6-dihydro-2*H*-pyran-2-yl)ethyl acetate (20). A heterogeneous mixture of 5% Pd/$CaCO_3$ (10 mg) and quinoline (23 μL, 0.19 mmol, 0.58 eq) was stirred in ethyl acetate (10 mL) at 0 °C as the reaction vessel was purged with hydrogen gas for 20 min. Alkyne **19** (70 mg, 0.33 mmol) was added to the reaction mixture in a solution of ethyl acetate (10 mL). The reaction mixture was purged with hydrogen and stirred 65 min, filtered through a pad of Celite, washed with ethyl acetate, and concentrated under reduced pressure to yield 50 mg (71 %) of vinyl glycoside **20** a colorless oil that was stored at 0 °C. R_f = 0.41 (1:1

ethyl acetate/hexanes); $[\alpha]_D^{20}$ -186.6 (*c* 0.98, $CHCl_3$), ^1H NMR ($CDCl_3$, 300 MHz): δ 6.10 (ddd, $J_{3,2}$ = 4.7, $J_{3,4}$ = 10.4, $J_{3,5}$ = 2.3 Hz, H-3), 5.97 (ddd, $J_{1',1}$ = 6.0, $J_{1',2'e}$ = 17.3, $J_{1',2'z}$ = 10.5 Hz, H-1'), 5.90 (ddd, $J_{4,3}$ = 10.4, $J_{4,5}$ = 3.0 Hz, H-4), 5.43 (dt, $J_{2e',1}$ = 17.3, $J_{2'e,2'z}$ = 1.6 Hz, H-2'e), 5.35 (dt, $J_{2'z,1'}$ = 10.5, $J_{2'z,2'e}$ = 1.6 Hz, H-2'z), 5.04 (pent, $J_{6,5}$ = 7.2, $J_{6,7}$ = 6.5 Hz, H-6), 4.33 (cm, $J_{1,2}$ = 2.9, $J_{1,1'}$ = 6.0, $J_{1,2'z}$ = 1.2, $J_{1,2'e}$ = 1.2 Hz, H-1), 4.18 (cm, $J_{5,3}$ = 2.3, $J_{5,4}$ = 3.0 Hz, H-5), 3.97 (bs, 1H, H-2), 2.06 (s, $OCOCH_3$), 1.70 (d, $J_{OH,2}$ = 11.51 Hz, OH), 1.30 (d, $J_{7,6}$ = 6.5 Hz, CH_3); $^{13}C\{^1H\}$ NMR($CDCl_3$): δ 170.4, 133.9, 128.6, 128.4, 118.7, 74.6, 73.8, 71.0, 63.8, 21.3, 16.8. HRMS (ESI-TOF) m/z: $[M + Na]^+$ Calcd for $C_{11}H_{16}O_4Na$ 235.0946; Found 235.0941.

(2*S*,3*R*,6*S*)-6-((*R*)-1-acetoxyethyl)2-vinyl-3,6-dihydro-2*H*-pyran-2-yl)ethyl acrylate (21). A pale yellow, homogeneous mixture of vinyl glycoside **20** (1.01 g, 4.76 mmol), acryloyl chloride (0.94 mL, 11.45 mmol, 2.4 eq), and triethylamine (2.5 mL, 19.1 mmol, 4 eq) was stirred in dichloromethane (10 mL) at 0 °C. After 10 min TLC analysis (1:1 ethyl acetate/hexanes) showed the disappearance of starting material. The reaction mixture was taken up in dichloromethane (40 mL) and washed with cold sat. aq $NaHCO_3$ sol'n (25 mL), dried (Na_2SO_4) and concentrated under reduced pressure to give a yellow solid that was purified by flash chromatography (30:70 ethyl acetate/hexanes) to yield 870 mg (69 %) of **21** as a colorless oil that was stored at -23 °C. R_f = 0.67 (1:1 ethyl acetate/hexanes); $[\alpha]_D^{20}$ -207.5 (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$, 300 MHz): δ 6.41 (dd, $J_{2'',1''}$ = 17.4, $J_{2'',2''z''}$ = 1.5 Hz, H-2''e), 6.39 (dt, $J_{2'e,1'}$ = 17.3 $J_{2'e,2'z}$ =ND, H- 2'e), 6.27 (dt, $J_{2'z,1}$ = 9.9, $J_{2'z,2'e}$ =ND, H-2'z), 6.11 (dd, $J_{1'',2''e}$ = 17.4, $J_{1'',2''z}$ =10.3 Hz, H-1''), 6.03 (cs, $J_{3,2}$ = ND, $J_{3,4}$ = ND, $J_{3,5}$ = ND, H-3), 6.03 (cs, $J_{4,3}$ = ND, $J_{4,5}$ = ~0, H-4), 5.89 (ddd, $J_{1',1}$ = 6.3, $J_{1,2'e}$ = ND, $J_{1',2'z}$ = ND, $J_{1',2'e}$ = 17.3, $J_{1',2'z}$ = 9.9 Hz, H-1'), 5.83 (dd, $J_{2'',1''}$ = 10.3, $J_{2''z,2''e}$ =1.5 Hz, H-2''z), 5.26 (cm, $J_{2,1}$ = 4.0, H-2), 5.03 (pent, $J_{6,5}$ = 6.5, $J_{6,7}$ = 6.4 Hz, H-6), 4.51 (ct, $J_{1,2}$ = 4.0, $J_{1,1'}$ = 6.3 Hz, $J_{1,2'z}$ = ND, $J_{1,2'e}$ = ND, H-1) 4.24 (d, $J_{5,4}$ = ~0, $J_{5,6}$ = 7.2 Hz, H-5), 2.06 (s, $OCOCH_3$), 1.30 (d, $J_{7,6}$ = 6.5 Hz, CH_3); $^{13}C\{^1H\}$ NMR ($CDCl_3$): δ 170.3, 165.6, 132.9, 131.4, 130.1, 128.0

124.7, 119.0, 73.2, 72.8, 71.1, 65.7, 21.3, 16.5. HRMS (ESI-TOF) m/z: $[M + Na]^+$ Calcd for $C_{14}H_{18}O_5Na$ 289.1052; Found 289.1052.

(4a*R*,6*S*,8a*R*)-6-((*R*)-1-Acetoxyethyl)-6,8a-dihydropyrano[3,2-b]pyran-2-(4a*H*)-one (3). A solution of **21** (86mg, 0.36 mmol) in anhydrous toluene (10 mL) was degassed under argon gas while stirring. Following this, Grubbs II (27 mg, 10 mol %) and phenol[51] (75 mg, 0.8 mmol, 2.5 eq) were added and the reaction mixture was degassed under argon once again and heated to 80 °C. After 1 h TLC analysis (1:1 ethyl acetate/hexanes) showed the disappearance of starting material. The reaction mixture was concentrated under reduced pressure to give a dark brown oil that was purified via flash chromatography (1:1 ethyl acetate/hexanes) to yield 45 mg (59%) of **3** as an oil that crystallized when stored at -23 °C. R_f = 0.33 (1:1 ethyl acetate/hexanes); mp 105 - 107.5 °C; $[\alpha]_D^{20}$ -98.0 (*c* 1.3, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 6.86 (dd, $J_{4,3}$ = 10.0, $J_{4,4a}$ = 3.5 Hz, H-4), 6.10 (dd, $J_{3,4}$ = 10.0, $J_{3,4a}$ = 1.6 Hz, H-3), 6.05 (cm, $J_{8,7}$ = 10.5, $J_{8,8a}$ = 2.5 H, H-8), 6.01 (cm, $J_{7,6}$ = 2.0, $J_{7,8}$ = 10.5 Hz, H-7), 5.02 (dq, $J_{9,6}$ = 5.5, $J_{9,10}$ = 6.4 Hz, H-9), 4.90 (cd, $J_{7,8}$ = 10.4, $J_{8a,8}$ = 2.5, $J_{8a,4a}$ = 5.2 Hz, H-8a), 4.75 (ct, $J_{4,4a}$ = 3.5, $J_{4a,3}$ = 1.6, $J_{4a,8a}$ = 5.2 Hz, H-4a), 2.08 (s, $OCOCH_3$), 1.28 (d, $J_{10,9}$ = 6.4 Hz, CH_3); $^{13}C\{^1H\}$ NMR ($CDCl_3$): δ 170.3, 161.8, 143.5, 129.9, 124.2, 123.6, 73.0, 71.1, 69.5, 64.1, 21.3, 15.8. HRMS (ESI-TOF): m/z: $[M + Na]^+$ Calcd for $C_{12}H_{15}O_5Na$ 239.0919; Found 239.0917.

(4a*R*,6*S*,8a*R*)-6-((*R*)-1-Hydroxyethyl)-6,8a-dihydropyrano[3,2-b]pyran-2-(4a*H*)-one (2). A mixture of (-)-diplopyrone acetate **3** (35 mg, 0.15 mmol) was stirred in THF as H_2O was added (five drops) followed by lipase PS 30 Amano (~3 eq wt). Not all the lipase dissolved in the reaction mixture. After nine days, the reaction mixture was concentrated under reduced pressure to yield a beige solid that was purified by column chromatography (1:1 ethyl acetate/hexanes followed by ethyl acetate) to yield 18.5 mg (63 %, 80% based on recovered **3**) of **2** as a a colorless oil that crystallized overnight at -23°C. R_f = 0.26 (9:1 ethyl acetate/hexanes); mp 73.2 - 74°C, $[\alpha]_D^{20}$ -79.2 (*c* 0.6, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 6.88 (dd, 1H, $J_{4,3}$ = 10.0, $J_{4,4a}$ = 2.8

Hz, H-4), 6.07 (dd, 1H, $J_{3,4}$ = 10.0, $J_{3,4a}$ = 1.8 Hz, H-3), 6.05 (cm, 1H, $J_{8,7}$ = ND, $J_{8,8a}$ = 2.0 Hz, H-8), 6.01 (cm, 1H, $J_{7,8}$ = 10.7, $J_{7,8a}$ = ND, $J_{7,6}$ = 1.8 Hz, H-7), 4.97 (cm, 1H, $J_{8,8a}$ = 2.0, $J_{8,7}$ = 10.7, $J_{8a,4a}$ = 5.8 Hz, H-8a), 4.91 (cm, 1H, $J_{4a,8a}$ = 5.8, $J_{4a,4}$ = 2.8, $J_{4a,3}$ = 1.8 Hz, H-4a), 4.07 (cm, 1H, $J_{6,9}$ = 4.1, $J_{6,8}$ = 3.0, $J_{6,8a}$ = ND, $J_{6,7}$ = 1.8 Hz, H-6), 3.96, (dq, 1H, $J_{9,10}$ = 6.5, $J_{9,6}$ = 4.1 Hz, H-9), 1.87 (bs, 1H, OH), 1.24 (d, 3H, $J_{10,9}$ = 6.5 Hz, CH_3), $^{13}C\{^1H\}$ NMR ($CDCl_3$): δ 161.8, 144.3, 129.6, 124.6, 123.2, 74.8, 70.0, 69.6, 64.8, 18.7. HRMS (ESI-TOF) m/z: $[M + H]^+$ Calc for $C_{10}H_{13}O_4$ 197.0814; Found 197.0812.

SUPPORTING INFORMATION

Experimental procedures for bioassays of compounds **2**, **3**, and **4**.
Figure showing visual effects of phytotoxic activity of **4** on *L. paucicostata.*
Experimental procedures for X-ray crystallographic analyses of **3** and **4**.
Thermal ellipsoid plots of **3** and **4**.
NMR spectra of all new compounds.

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