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Synthesis of Pyranopyrans Related to Diplopyrone and Evaluation as Antibacterials and Herbicides

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ABSTRACT: Stereoselective syntheses of new pyranopyrans that are related to the natural product diplopyrone, which is a phytotoxin implicated in cork oak decline, have been achieved from carbohydrate starting materials in two approaches that are based on *C*-glycosides as key intermediates. A *C*-alkynyl glycoside prepared by Ferrier rearrangement was used as the precursor to a new pyranopyran alkyne that showed potent antibacterial activity against the common bacterial pathogen *Edwardsiella ictaluri* that causes enteric septicemia in catfish. The *C*-alkynyl glycoside also showed herbicidal activity. New bioassay data for the pyranopyran nitrile (4a*R*,6*S*,8a*R*)-6-cyano-6,8a-dihydropyrano-[3,2-*b*]pyran-2(4a*H*)-one, the most potent of the pyranopyrans synthesized to date, were obtained in greenhouse studies that revealed additional herbicidal activity. Other new analogues that were synthesized included desmethylpyranopyrans that were prepared by Isobe *C*-alkynylation-rearrangement/reduction and RCM-based pyranopyran construction. The antibiotic and phytotoxic activities of the new pyranopyrans synthesized in this study highlight the importance of substituents on the nonlactone ring and demonstrate the potential of such compounds as antibiotics and herbicides.

KEYWORDS: *pyranopyrans, diplopyrone, phytotoxin, cork oak, C-alkynyl glycoside, enteric septicemia*

INTRODUCTION

Fungal phytotoxins provide a diverse pool of natural products with which to explore the development of novel antibacterial agents and herbicides based on core secondary metabolite structures. Natural phytotoxins and natural product-based compounds may prove valuable as leads from which to develop more effective and environmentally benign agents.[1−3] For several years, our laboratory has been exploring the synthesis and biological evaluation of compounds that are based on bicyclic pyranopyrans,[4] which is a core structure found in numerous natural products, for example, complex marine polyethers such as brevitoxins and halichondrins, okadaic acid, thyrsiferol, and dactomelynes.[5,6] These compounds contain one or more bicyclic pyranopyran units, as illustrated by the examples included in Figure 1. Owing to their biological activities, there has been much interest in developing synthetic routes to pyranopyran-containing natural products and their derivatives, and therapeutic agents have been developed, for example, eribulin mesylate.[5,6]

Diplopyrone **1** is a pyranopyran phytotoxin isolated from the fungus *Diplodia mutila* and reported by Evidente and co-workers in 2003.[7] This fungus is thought to be the causative agent in cork oak decline in parts of southern Europe where the disease has negative environmental and economic effects. Originally assigned as 9*S*,6*R*,4a*S*,8a*S*,[8] the absolute configuration of natural diplopyrone was reinvestigated and reassigned as 9*R*,6*S*,4a*S*,8a*S* on the basis of optical spectroscopy and computational methods.[9] Our laboratory has pursued the synthesis and biological properties of structural analogues of diplopyrone based on pyranopyran structure **1** (Figure 2).[4] The reason for the choice of this template stems from the design of our synthesis that uses simple D-sugars as starting materials and the potential flexibility for access to a variety of pyranopyrans that contain different functional groups at the C-6 position that appears to be important to the biological activity of this class of compounds.

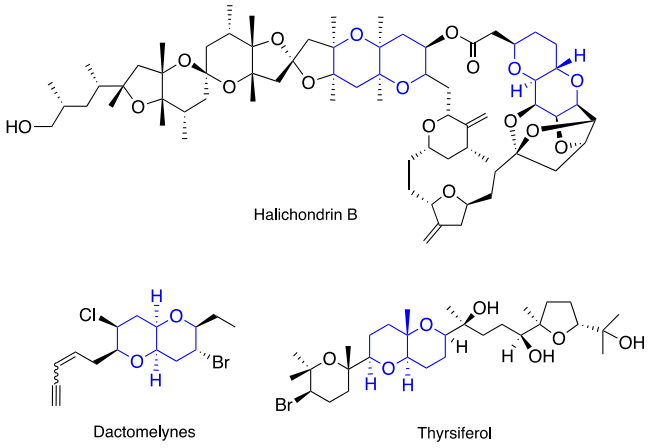

Figure 1. Structures of pyranopyran-containing natural products.

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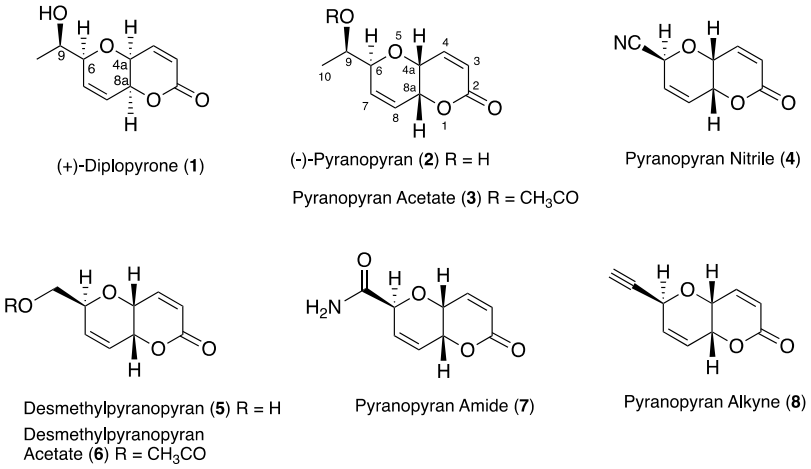

Figure 2. Pyranopyrans based on the structure of diplopyrone.

We have recently completed and reported the synthesis of compounds **2**–**4** from carbohydrate starting materials and evaluated them in bioassays for antibacterial, herbicidal, and antifungal activity in collaboration with the USDA-ARS Natural Products Utilization Research Unit.[4]

These tests revealed that one of the structural analogues, pyranopyran nitrile **4**, has antibacterial activity against common bacterial pathogens *Edwardsiella ictaluri* and *Flavobacterium columnare* that cause enteric septicemia (ESC) and columnaris disease, respectively, in catfish.[10] Pyranopyran nitrile **4** also possessed phytotoxic activity as evidenced by an assay using *Lemna paucicostata* (L.) Helgelm (duckweed). Compounds **2** and **3** possessed antibacterial and phytotoxic activity but were less potent than the nitrile. Although these results are promising, questions emerged during the course of our earlier study which need to be addressed in relation to our goal of developing novel antibacterials and herbicides based on a core natural product structure. Would our carbohydrate-based synthesis be adaptable to the preparation of structural analogues that contain diverse functionality? Can additional biologically active compounds be identified from our approach? Will an expanded set of pyranopyran analogues help us to gain further insight into the apparent structure–activity relationship involving substituents on the pyranopyran scaffold? In the work described herein, we report that we have successfully synthesized additional analogues through modification of our original synthetic approach. Two of these analogues possessed activity in antibacterial and phytotoxicity assays. Most significant toward the latter question and our overall goal is the preparation of pyranopyran alkyne **8**, which exhibits the most potent antibacterial and phytotoxic activity after the nitrile **4**. The activity of **8**, relative to other analogues, provides further evidence of the importance of the functional group at the C-6 position.

MATERIALS AND METHODS

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 °C as indicated. HRMS analyses were conducted at the University of Illinois Mass Spectrometry Laboratory. NMR spectra were recorded on Varian (Agilent) Mercury 300 Plus and JEOL 400 and 500 MHz spectrometers in $CDCl_3$ unless otherwise noted. For ^1H NMR at 300.0 MHz, tetramethysilane reference δ = 0.0 ppm, and for ^{13}C NMR at 75.4 MHz, $CDCl_3$ reference δ = 76.9 ppm. Spectral assignments were confirmed using correlation spectroscopy (COSY) and double quantum coherence COSY experiments. Reagents and starting materials were purchased from Sigma-Aldrich with the exception of 5% Pd/$CaCO_3$ (Alfa Aesar) and tri-*O*-acetyl-D-galactal (CarboSynth).

((2*R*,3*R*)-3-Acetoxy-6-((trimethylsilyl)ethynyl)-3,6-dihydro-2*H*-pyran-2-yl)methyl Acetate (10). Using the procedure of Yamada,[11] a solution of commercially available 3,4,6-tri-*O*-acetyl-D-galactal (3,4,6-tri-*O*-acetyl-1,5-anhydro-2-deoxy-D-*lyxo*-hex-1-enitol) **9** (0.3030 g, 1.1130 mmol) and bis(trimethylsilyl)acetylene (0.3793 g, 2.2260 mmol, 2.0 equiv) in anhydrous dichloromethane (10 mL) was stirred in a three-neck round-bottomed flask that was fitted with a stopper, a two-way adaptor, and a septum. The reaction solution was cooled to −20 °C (CO_2 (s)/*i*-PrOH), degassed, and placed under argon, after which $SnCl_4$ (2.23 mL, 2.23 mmol, 1 M solution in CH_2Cl_2, 2 equiv) was added dropwise via a syringe over 20 min. After the addition of $SnCl_4$, the reaction mixture darkened and was allowed to warm to 0 °C. The reaction was monitored by thin-layer chromatography (TLC; 30% ethyl acetate/hexanes) until the starting material was consumed, usually in 1 h, after which the reaction solution was poured into a mixture of ice and saturated Rochelle's salt and allowed to stir vigorously for a period of 45 min to 1 h. The biphasic solution was extracted with dichloromethane (2 × 25 mL), washed with saturated $NaHCO_3$ (25 mL) and brine (25 mL), dried (Na_2SO_4), and concentrated to yield a yellow oil that was purified by flash chromatography to afford 0.2289 g (66%) of **10** as a clear, colorless oil. R_f = 0.45 (30% ethyl acetate/hexanes); $[\alpha]_D^{20}$ −283° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz): δ 5.96 (dd, 1H, $J_{2,3}$ = 10.1 Hz, $J_{1,2}$ = 3.6 Hz, H-2), 5.90 (ddd, 1H, $J_{2,3}$ = 10.1 Hz, $J_{3,4}$ = 5.1 Hz, $J_{1,3}$ = 1.8 Hz, H-3), 4.98 (dd, 1H, $J_{3,4}$ = 5.1 Hz, $J_{4,5}$ = 2.3 Hz, H-4), 4.92 (dd, 1H, $J_{1,2}$ = 3.6 Hz, $J_{1,3}$ = 1.8 Hz, H-1), 4.25 (ddd, 1H, $J_{5,6'}$ = 7.3 Hz, $J_{5,6}$ = 4.9 Hz, $J_{4,5}$ = 2.4 Hz, H-5), 4.19 (dd, 1H, $J_{6,6'}$ = 11.4 Hz, $J_{5,6}$ = 4.9 Hz, H-6), 4.05 (dd, 1H, $J_{6,6'}$ = 11.4 Hz, $J_{5,6'}$ = 7.3 Hz, H-6′), 1.98 (s, 3H, OAc), 1.97 (s, 3H, OAc), 0.07 (s, 9H, $SiMe_3$); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 170.5, 170.2, 131.8, 122.3, 100.2, 91.9, 69.6, 64.3, 63.2, 62.8, 53.8, 29.2, 20.7, 20.6, 14.1, −0.3. The NMR data for **10** matched that reported.[11]

(2*R*,3*R*)-6-Ethynyl-2-(hydroxymethyl)-3,6-dihydro-2*H*-pyran-3-ol (11). A solution of **10** (0.2344 g, 0.7551 mmol) in anhydrous methanol (10 mL) was stirred in a round-bottomed flask as anhydrous K_2CO_3 (0.4175 g, 3.0204 mmol, 4 equiv) was added. Upon addition, the solution darkened to a yellow color, and the resulting heterogenous mixture was stirred at room temperature. The reaction was monitored by TLC (2:1 ethyl acetate/hexanes), with consumption of the starting material typically occurring in 1.5–2 h,

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after which the solution was acidified to a pH of 7 using Dowex 50 × 8 resin as indicated by pH paper (1−12). The heterogenous mixture was filtered, and the filtrate was concentrated in vacuo to yield an orange solid that was purified by flash chromatography (3:1 ethyl acetate/hexanes) to yield 0.1024 g (88%) of **11** as a white waxy solid that was dried by azeotropic distillation with pentane (5 × 10 mL) and further dried under vacuum overnight. mp 103−105 °C; lit[11] mp 103−105 °C; $[\alpha]_D^{20}$ −541° (*c* 0.25, $CHCl_3$); lit[11] $[\alpha]_D^{27}$ −500 (*c* 0.23, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 6.08 (ddd, 1H, $J_{2,3}$ = 9.9 Hz, $J_{3,4}$ = 5.4 Hz, $J_{1,3}$ = 1.9 Hz, H-3), 5.96 (dd, 1H, $J_{2,3}$ = 10.0 Hz, $J_{1,2}$ = 3.8 Hz, H-2), 5.01 (dd, 1H, $J_{1,2}$ = 4.0 Hz, $J_{1,3}$ = 2.1 Hz, H-1), 4.08 (ddd, 1H, $J_{5,6'}$ = 6.6 Hz, $J_{5,6}$ = 4.7 Hz, $J_{4,5}$ = 2.2 Hz, H-5), 4.1−3.8 (bm, 3H, H-4,6,6′), 2.51 (d, 1H, $J_{1,alkynyl}$ = 2.3 Hz, H-alkynyl), 2.22−2.12 (br s, 1H), 2.00−1.92 (bd, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 128.8, 125.9, 76.4, 76.0, 75.6, 73.8, 72.4, 62.9, 61.8, 61.8, 0.0. The NMR data for **11** matched that reported.[11]

***tert*-Butyl(((2*R*,3*R*)-3-((*tert*-butyldimethylsilyl)oxy)-6-ethynyl-3,6-dihydro-2*H*-pyran-2-yl)methoxy)dimethylsilane (12).** Method A: A solution of diol **11** (0.5000 g, 3.2432 mmol) in anhydrous DMF (20 mL) was stirred at room temperature as imidazole (1.100 g, 16.2160 mmol, 5.0 equiv) was added, followed by the addition of *tert*-butyldimethylsilyl chloride (1.2700 g, 8.4323 mmol, 2.6 equiv). The resulting cloudy yellow solution was allowed to stir at room temperature. Reaction progress was monitored by TLC (10% ethyl acetate/hexanes), with the starting material typically consumed after 20−25 h, after which the reaction solution was quenched with saturated NH_4Cl and extracted with ethyl acetate (4 × 10 mL). The combined extracts were washed with water (2 × 10 mL) and brine (10 mL), dried (Na_2SO_4), and concentrated in vacuo to yield a yellow oil which was purified by flash chromatography (10% ethyl acetate/hexanes) to yield 0.4500 g (36%) of **12** as a pale yellow oil. Method B: To a clear, colorless stirring suspension of diol **11** (15.3 mg, 0.0992 mmol) in anhydrous dichloromethane (1 mL) was added TBSOTf (68.4 μL, 3 equiv, 0.2977 mmol), followed by the addition of 2,6-lutidine (46.2 μL, 4 equiv, 0.3970 mmol) via a micropipette. Upon the addition of TBSOTf, the reaction solution immediately darkened to a light brown color and became homogenous. The resulting clear, light brown solution was allowed to stir at room temperature with reaction progress being monitored by TLC (10% ethyl acetate/hexanes). Consumption of the starting material typically occurred after 20 min, after which the reaction solution was transferred to a separatory funnel, diluted with 20 mL of dichloromethane, washed with saturated $NaHCO_3$ (3 × 5 mL), water (5 mL), and brine (5 mL), dried (Na_2SO_4), and concentrated in vacuo to yield a yellow oil that was purified by flash chromatography (10% ethyl acetate/hexanes) to yield 35.4 mg (93%) of **12**. $[\alpha]_D^{20}$ −196° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 5.93 (ddd, 1H, $J_{2,3}$ = 10.0 Hz, $J_{3,4}$ = 5.0 Hz, $J_{1,3}$ = 1.8 Hz, H-3), 5.87 (dd, 1H, $J_{2,3}$ = 10.0 Hz, $J_{1,2}$ = 3.5 Hz, H-2), 4.97 (dt, 1H, $J_{1,2}$ = 3.8 Hz, $J_{1,3}$ = 2.0 Hz, H-1), 4.00 (dd, 1H, $J_{3,4}$ = 5.0 Hz, $J_{4,5}$ = 2.3 Hz, H-4), 3.93 (td, 1H, $J_{5,6}$ = 6.2 Hz, $J_{4,5}$ = 2.4 Hz, H-5), 3.84 (dd, 1H, $J_{6,6'}$ = 10.6 Hz, $J_{5,6}$ = 6.1 Hz, H-6), 3.73 (dd, 1H, $J_{6,6'}$ = 10.5 HJz, $J_{5,6'}$ = 6.3 Hz, H-6′), 2.45 (d, 1H, $J_{1,alkynyl}$ = 2.3 Hz, H-alkynyl), 0.90 (s, 9H, ^{t}BuMe$_2$Si), 0.89 (s, 9H, ^{t}BuMe$_2$Si), 0.09 (s, 6H, ^{t}BuMe$_2$Si), 0.08 (s, 6H, ^{t}BuMe$_2$Si); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 128.9, 127.5, 80.6, 75.0, 74.1, 63.6, 62.5, 26.1, 18.5, 18.4, 1.2, −3.9, −4.5, −5.0, −5.1.

HRMS (ESI-TOF) *m*/*z*: $[M + H]^+$ calcd for $C_{20}H_{39}O_3Si_2$, 383.2438; found, 383.2420.

((2*R*,3*R*)-3-((*tert*-Butyldimethylsilyl)oxy)-6-ethynyl-3,6-dihydro-2*H*-pyran-2-yl)methanol (13). A solution of di-*O*-TBS ether **12** (0.43 g, 1.236 mmol) in anhydrous methanol (20 mL) was stirred in a round bottom flask as pyridinium *p*-toluenesulfonate (0.0565 g, 0.2247 mmol, 20.0 mol %) was added. The resulting solution was stirred at room temperature. Reaction progress was monitored by TLC (30% ethyl acetate/hexanes), with the majority of the starting material typically being consumed after 20−24 h, with only minimal diol formation as shown by TLC, after which the solvent was removed under high vacuum, attached to a rotary evaporator whose water bath was maintained at 0 °C. The resulting colorless oil was immediately diluted with cold dichloromethane (40 mL), washed with 0 °C water (15 mL) and 0 °C saturated $NaHCO_3$ (10 mL), dried (Na_2SO_4), and concentrated to yield 0.2215 g (73%) of **13** as a clear oil that was immediately used without further purification, with an aliquot being removed for optical rotation data and HRMS analysis. $[\alpha]_D^{20}$ −244° (*c* 1.0, $CHCl_3$). NMR data were not obtained because of the instability of **13**.

HRMS (ESI-TOF) *m*/*z*: $[M + H]^+$ calcd for $C_{14}H_{25}O_3Si$, 269.1573; found, 269.1572.

(2*S*,3*R*)-3-((*tert*-Butyldimethylsilyl)oxy)-6-ethynyl-3,6-dihydro-2*H*-pyran-2-carbaldehyde (14). A solution of primary alcohol **13** (0.1315 g, 0.4899 mmol) in anhydrous dichloromethane (20 mL) was stirred at 0 °C as DIPEA (0.3799 g, 0.51 mL, 2.9393 mmol, 6 equiv), DMSO (0.4592 g, 0.42 mL, 5.8786 mmol, 12 equiv), and SO_3·pyridine (0.3899 g, 2.4494 mmol, 5 equiv) were added. The resulting clear, slightly brown solution was stirred at 0 °C with reaction progress being monitored by TLC (30% ethyl acetate/hexanes) with consumption of the starting material typically occurring in 2−3 h. After the reaction was complete, as shown by TLC, the reaction solution was quenched with cold water (30 mL) and extracted with cold ethyl acetate (3 × 20 mL), and the combined extracts were washed with cold water (4 × 15 mL) and cold brine (10 mL), dried (Na_2SO_4), and concentrated to yield 0.0806 g (62%) of **14** as a red-brown oil. $[\alpha]_D^{20}$ −241° (*c* 1.0, $CHCl_3$), ^{1}H NMR ($CDCl_3$, 300 MHz): δ 9.63 (s, 1H, CHO), 5.92 (s, 1H, H-3), 5.91 (s, 1H, H-2) 5.12 (t, 1H, *J* = 2.0 Hz, H-1), 4.38−4.32 (m, 2H, H-4,5), 2.51 (d, 1H, *J* = 2.3 Hz, H-alkynyl), 0.83 (s, 9H, ^{t}BuMe$_2$Si), 0.06 (s, 3H, ^{t}BuMe$_2$Si), 0.04 (s, 3H, ^{t}BuMe$_2$Si); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 201.0, 129.0, 126.3, 79.3, 78.6, 75.4, 63.4, 63.3, 25.8, 18.2, −4.0, −4.8.

HRMS (ESI-TOF) *m*/*z*: $[M + H]^+$ calcd for $C_{14}H_{23}O_3Si$, 267.1416; found, 267.1418.

Ethyl (*Z*)-3-((2*R*,3*R*,6*S*)-3-((*tert*-Butyldimethylsilyl)oxy)-6-ethynyl-3,6-dihydro-2*H*-pyran-2-yl)acrylate (15). To a clear, slightly yellow solution of aldehyde **14** (0.0606 g, 0.2275 mmol) in anhydrous methanol (5 mL) was added (carbethoxymethylene)-triphenylphosphorane (0.1189 g, 0.3412 mmol, 1.5 equiv). The resulting homogenous solution was stored at −10 °C overnight with reaction progress being monitored by TLC (10% ethyl acetate/hexanes) with the starting material typically being consumed in 30−35 h, after which the solvent was removed and the crude product was immediately purified by flash chromatography (10% ethyl acetate/hexanes) to yield 0.0422 g (55%) of **15** a clear oil. $[\alpha]_D^{20}$ −233.2° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 6.27 (dd, 1H, $J_{6,7}$ = 11.9 Hz, $J_{5,6}$ = 7.0 Hz, H-6), 5.96−5.83 (m, 3H, H-2, 3, 7), 5.46 (ddd, 1H, $J_{5,6}$ = 7.0 Hz, $J_{5,4}$ = 2.7 Hz, $J_{5,7}$ = 1.5 Hz, H-5), 4.98 (dd, 1H, $J_{1,2}$ = 3.0 Hz, $J_{1,alkynyl}$ = 2.4, Hz, H-1), 4.27 (dd, 1H, $J_{3,4}$ = 4.5 Hz, $J_{4,5}$ = 2.6 Hz, H-4), 4.17 (qd, 2H, *J* = 10.5 Hz, *J* = 7.1 Hz, CH_2), 2.46 (d, 1H, $J_{1,alkynyl}$ = 2.3 Hz, H-alkynyl), 1.29−1.26 (t, 3H, *J* = 7.1 Hz, CH_3), 0.83 (s, 9H, ^{t}BuMe$_2$Si), −0.01 (s, 3H, ^{t}BuMe$_2$Si), −0.03 (s, 3H, ^{t}BuMe$_2$Si); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 165.9, 147.9, 128.3, 127.4, 120.1, 80.2, 74.5, 72.0, 63.9, 63.1, 60.34 25.9, 18.3, 14.2, −4.2, −4.7.

HRMS (ESI-TOF) *m*/*z*: $[M + H]^+$ calcd for $C_{18}H_{29}O_4Si$, 337.1835; found, 337.2834.

(4a*R*,6*S*,8a*R*)-6-Ethynyl-6,8a-dihydropyrano[3,2-*b*]pyran-2-(4a*H*)-one (8). A solution of ester **15** (0.0370 g, 0.1100 mmol) in anhydrous methanol (2 mL) was heated to reflux, after which *p*-toluene sulfonic acid (0.0032 g, 0.0165 mmol, 15.0 mol %) was added. The resulting colorless solution was stirred in a round-bottomed flask with reaction progress being monitored by TLC (30% ethyl acetate/hexanes). The starting material was typically consumed after 5−6 h, after which methanol was removed using a rotary evaporator, and toluene was added to the reaction vessel. The resultant clear, colorless solution was allowed to stir overnight at reflux with the starting material typically being consumed after an additional 20 h (∼26 h in total). The reaction solution was concentrated in vacuo to yield a brown, crystalline solid that was purified by flash chromatography (30% ethyl acetate/hexanes) to yield 0.0104 g (53%) of **7** as a white crystalline solid. mp 153−154 °C; $[\alpha]_D^{20}$ 338° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 6.88 (dd, $J_{3,4}$ = 9.8 Hz, $J_{4,4a}$ = 5.8 Hz, H-4), 6.23 (d, 1H, $J_{3,4}$ = 9.8 Hz, H-3),

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6.15 (dd, 1H, $J_{7,8}$ = 10.1 Hz, $J_{6,7}$ = 3.9 Hz, H-7), 6.06 (ddd, 1H, $J_{7,8}$ = 10.1 Hz, $J_{8,8a}$ = 5.1 Hz, $J_{6,8}$ = 1.8 Hz, H-8), 5.03 (dt, 1H, $J_{6,7}$ = 4.2 Hz, $J_{6,8}$ = 2.1 Hz, H-6), 4.63 (dd, 1H, $J_{8,8a}$ = 5.1 Hz, $J_{4a,8a}$ = 3.1 Hz, H-8a), 4.52 (dd, 1H, $J_{4,4a}$ = 5.8 Hz, $J_{4a,8a}$ = 3.1 Hz, H-4a), 2.58 (d, 1H, J = 2.3 Hz, H-alkynyl); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 162.5, 140.2, 132.0, 125.1, 121.7, 78.8, 75.9, 68.7, 63.6, 61.2.

HRMS m/z: $[M + Na]^+$ calcd for $C_{10}H_8O_3Na$, 199.0371; found, 199.0364.

2,3,4,6-Tetra-*O*-acetyl-1-deoxy-D-*arabino*-hex-1-enopyranose (16). Glycal **16** was prepared following the published procedure[12] from commercially available 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (20.0 g, 48.6 mmol). Concentration of the crude product gave a red syrup to which a minimum amount of hot isopropanol was added. The solution was cooled to rt and then stored at 0 °C overnight. The white crystalline product was collected. Two more crops of crystals were isolated from the mother liquor, and the combined crystal harvests were dried under high vacuum to afford 12.5 g (77%) of glycal. R_f = 0.4 (40% ethyl acetate 60% hexanes); mp 60−63 °C; lit mp 61, 65−66 °C; $[\alpha]_D^{23}$ −18° (c 1.0, ethanol); lit $[\alpha]_D^{23}$ −20° (c 1.8, ethanol); ^{1}H NMR ($CDCl_3$): δ 6.62 (s, 1H), 5.84 (d, 1H, J = 4.7 Hz), 5.48 (dd, 1H, J = 5.0 Hz, 2.0 Hz), 4.42−4.19 (m, 3H), 2.13 (s, 3H), 2.11 (s, 3H), 2.08 (s, 3H), 2.04 (s, 3H); ^{13}C NMR ($CDCl_3$): δ 170.2, 169.9, 169.3, 169.3, 139.2, 127.4, 74.1, 67.5, 66.3, 60.9, 20.5, 20.5, 20.3.

(2*S*,5*R*,6*R*)-5-Hydroxy-6-(trimethylsilyl)ethynyl-5,6-dihydro-2*H*-pyran-2-yl)methyl Acetate (17). TMS-alkynyl glycoside **17** was prepared from glycal **16** by the method of Isobe.[13] From 9.0 g (27.2 mmol) of glycal, 4.20 g (57%) of **17** was obtained as an oil, after purification by flash chromatography. R_f = 0.4 (40% ethyl acetate/hexanes); $[\alpha]_D^{23}$ −100.2° (c 1.8, $CHCl_3$); lit $[\alpha]_D^{23}$ −99.2° (c 1.2, $CHCl_3$). The NMR data for **17** matched those reported.[13]

(2*S*,5*R*,6*R*)-6-Ethynyl-5-hydroxy-5,6-dihydro-2*H*-pyran-2-yl-methyl Acetate (18). A solution of TMS-alkynyl glycoside **17** (0.72 g, 2.69 mmol) in anhydrous THF (10 mL) was stirred at 0 °C as TBAF (0.806 mL, 1 M solution in THF, 30 mol %) was added via a micropipette. Upon addition, the solution immediately darkened. The resulting homogenous yellow solution was allowed to stir at 0 °C with reaction progress being monitored by TLC (1:1 ethyl acetate/hexanes) with consumption of the starting material typically occurring in 15−20 min, after which the reaction solution was concentrated and immediately purified by flash chromatography (1:1 ethyl acetate/hexanes). Desilylated **18** (420 mg, 87%) was obtained as a white solid. R_f = 0.18 (40% ethyl acetate/hexanes); mp 73−76 °C; $[\alpha]_D^{23}$ −52.4° (c 1.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 5.88 (dd, 1H, J = 10.5 Hz, 2.3 Hz, H-3), 5.75 (dd, J = 10.5 Hz, 2.0, Hz H-4), 4.90 (dd, 1H, J = 5.2 Hz, 2.3 Hz, H-1), 4.58 (octet, 1H, H-5), 4.32 (m, 1H, J = 10.3 Hz, 5.2 Hz, 2.3 Hz, 2.0 Hz, H-2), 4.14 (d, 2H, J = 5.0 Hz, H-6,6′), 2.52 (d, 1H, J = 2.3 Hz), 2.09 (s, 3H), 2.00 (d, 1H, J = 10.3, OH); ^{13}C NMR ($CDCl_3$): δ 170.8, 129.5, 127.0, 78.2, 76.4, 69.2, 67.1, 65.0, 63.5, 20.7.

HRMS (ESI): calcd for $C_{10}H_{12}O_4Na$ $[M + Na]^+$, 219.0633; found, 219.0631.

(2*S*,5*R*,6*R*)-6-Ethenyl-5-hydroxy-5,6-dihydro-2*H*-pyran-2-yl-methyl Acetate (19). A 250 mL round-bottom flask fitted with a two-way adaptor and balloon was charged with ethyl acetate (40 mL), quinoline (222.1 μL, 1.8 mmol, 0.58 equiv), and Lindlar's catalyst (50.0 mg, 10 wt %) and cooled in an ice bath. The mixture was purged three times with hydrogen and then stirred for 20 min at 0 °C. A solution of alkyne **18** (400 mg, 2.6 mmol) in ethyl acetate (30 mL) was added, and the flask was again purged three times and then stirred at 0 °C. The reaction was monitored by TLC until the starting material was consumed, typically in 1−1.5 h. The mixture was filtered through a pad of Celite which was washed with ethyl acetate, and the filtrate concentrated to give an oil that was purified by flash chromatography (40% ethyl acetate/hexanes). **19** (405 mg; 97%) was obtained as an oil. The crude product contained a small amount of *C*-ethyl glycoside that could not be separated. R_f = 0.12 (40% ethyl acetate/hexanes); $[\alpha]_D^{23}$ −112.3° (c 0.9, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 6.10 (ddd, 1H, J = 10.1 Hz, 5.1 Hz, 2.1 Hz, H-3), 5.92 (ddd, 1H, J = 17.4 Hz, 10.6 Hz, 5.98 Hz, HC═CH_2), 5.76 (dd, 1H, J = 10.1 Hz, 3.1 Hz, H-4), 5.36 (dd, 1H, J = 17.4 Hz, 1.5 Hz, HC═CH_2), 5.27 (dd, 1H, J = 10.6 Hz, 1.5 Hz, HC═CH_2), 4.44 (m, 1H, J = 8.4 Hz, 3.7 Hz, 3.1 Hz, 2.1 Hz, H-5), 4.33−4.24 (m, 2H, J = 11.9 Hz, 8.4 Hz, 5.9 Hz, 1.5 Hz, H-1, H-6), 3.96 (dd, 1H, J = 11.9 Hz, 3.7 Hz, H-6′), 3.85 (m, 1H, H-2), 2.02 (s, 3H, CH_3), 1.94 (br s, 1H, OH); ^{13}C NMR ($CDCl_3$): δ 170.8, 134.2, 129.7, 129.3, 128.2, 128.0, 118.3, 73.8, 73.7, 71.5, 71.1, 63.7, 63.3, 62.9, 62.6, 20.9.

HRMS (ESI): calcd for $C_{10}H_{14}O_4Na$ $[M + Na]^+$, 221.0790; found, 219.0790.

(2*R*,3*R*,6*S*)-6-(Acetyloxy)methyl]-2-ethenyl-3,6-dihydro-2*H*-pyran-3-yl Prop-2-enoate (20). To a stirring solution of vinyl glycoside **19** (167 mg, 0.84 mmol), acryloyl chloride (128 μL, 1.3 mmol), and triethylamine (155 μL, 0.21 mmol 2.5 equiv) in anhydrous dichloromethane (5 mL) at 5 °C was added a solution of DMAP (5 mg) in 500 μL of dichloromethane. The reaction was monitored by TLC (30% ethyl acetate/hexanes) and completed in 20 min. The mixture was then diluted with dichloromethane (25 mL) and washed once with satd aq $NaHCO_3$ soln, dried (Na_2SO_4), and concentrated to an oil that was purified by flash chromatography using 30% ethyl acetate/hexanes. Acryloyl derivative **20** (185 mg, 88%) was obtained. R_f = 0.46 (40% ethyl acetate/hexanes); $[\alpha]_D^{23}$ −292° (c 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 6.78 (dd, 1H, J = 10.4 Hz, 1.5 Hz, HC═CH_2), 6.36, (dd, 1H, J = 17.3 Hz, 1.5 Hz, HC═CH_2), 6.06, (ddd, 1H, J = 17.3 Hz, 10.4 Hz, HC═CH_2), 6.04 (ddd, 1H, J = 10.6 Hz, 4.6 Hz, 2.3 Hz, H-3), 5.91 (ddd, 1H, J = 10.3 Hz, 2,8 Hz, 1.0 Hz, H-4), 5.81 (ddd, 1H, J = 17.5 Hz, 10.7 Hz, 5.6 Hz, HC═CH_2), 5.33 (dt, 1H, J = 17.4 Hz, 1.3, Hz, HC═CH_2), 5.20 (dt, 1H, J = 10.7 Hz, 1.3 Hz, HC═CH_2), 5.13 (m, J = 4.6 Hz, 3.0 Hz, 1.0 Hz, H-2), 4.47 (m, 2H, J = 7.6 Hz, 5.6 Hz, 4.0 Hz, 3.0 Hz, 2.8 Hz, 2.3 Hz, 1.3 Hz, H-1, H-5), 4.30 (dd, 1H, J = 10.9 Hz, 7.6 Hz, H-6), 3.98, (dd, H, J = 10.9 Hz, 4.0 Hz, H-6′), 2.02 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$): δ 170.6, 165.6, 133.1, 131.2, 130.1, 128.1, 125.2, 118.4, 72.0, 70.6, 65.5, 63.5, 20.7.

HRMS (ESI): calcd for $C_{13}H_{16}O_5Na$ $[M + Na]^+$, 275.0895; found, 275.0891.

(2*S*,4a*R*,8a*R*)-6-Oxo-2,4a,6,8a-tetrahydropyrano[3,2-*b*]-pyran-2-yl]methyl Acetate (6). A flame-dried 50 mL three-neck round bottom flask equipped with a reflux condenser and septum was flushed with argon and charged with 7.0 mL of anhydrous toluene which had previously been degassed and stored under argon and the Grubbs II catalyst (22.3 mg, 10 mmol %). The reaction vessel was then degassed three times and heated in an oil bath to 80 °C. Purified acryloyl ester **20** (90 mg, 0.35 mmol) was dissolved in 0.5 mL of toluene and added via a syringe. The temperature of the oil bath was increased to initiate reflux, and the reaction was stirred for 48 h. After that, the resulting black solution was concentrated and subjected to flash chromatography (50% ethyl acetate/hexanes) to afford **6** (64 mg, 81%). R_f = 0.48 (1:1 acetone/hexanes); $[\alpha]_D^{23}$ −97° (c 0.88, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 6.86 (dd, 1H, J = 9.9 Hz, 4.7 Hz, H-4), 6.16 (ddd, 1H, J = 10.4 Hz, 4.0, Hz 2.0 Hz, H-8), 6.16 (dd, 1H, J = 9.9 Hz, 1.0 Hz, H-3), 6.04 (ddd, 1H, J = 10.4 Hz, 2.4 Hz, 1.0 Hz, H-7), 4.78 (t, 1H, J = 4.0 Hz, 1.0 Hz, H-8a), 4.60 (t, 1H, J = 4.7 Hz, 4.0 Hz, 1.0 Hz, H-4a), 4.50 (m, 1H, J = 8.2 Hz, 2.7 Hz, 2.4 Hz, 2.0 Hz, H-6), 4.45 (dd, 1H, J = 10.9 Hz, 8.2 Hz, H-9a), 4.02 (dd, 1H, J = 10.9 Hz, 2.7 Hz, H-9b); ^{13}C NMR ($CDCl_3$): δ 170.6, 162.1, 142.0, 130.3, 124.1, 124.0, 71.1, 69.0, 63.1, 61.9, 20.7.

HRMS (ESI): calcd for $C_{11}H_{12}O_5Na$ $[M + Na]^+$, 225.0763; found, 225.0760.

(4a*R*,6*S*,8a*R*)-6-(Hydroxymethyl)-6,8a-dihydropyrano[3,2-*b*]pyran-2(4a*H*)-one (5). A solution of **6** (44.0 mg, 0.24 mmol) in THF (2 mL) containing a drop of water was added to a stirring suspension of lipase PS 30 (44.0 mg), and the mixture was stirred for 2 days. TLC (50% acetone/hexane) showed complete consumption of the starting material and the formation of a more polar component. The crude reaction mixture was filtered to remove solids, and the filtrate was concentrated to a residue that was purified by flash chromatography (50% acetone/hexanes) to give **5** (33.0 mg, 92%) as a clear oil. R_f = 0.25 (50% acetone/hexanes); $[\alpha]_D^{23}$ −66° (c 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 6.89 (dd, 1H, J = 10.1 Hz, 4.1 Hz, H-4), 6.13 (dd, 1H, J = 10.0 Hz, 1.5 Hz, H-3), 6.07 (ddd, 1H, J = 10.5 Hz, 3.6 Hz, 2.1 Hz, H-8), 5.99 (ddd, 1H, J = 10.5 Hz, 2.3 Hz, 1.1 Hz,

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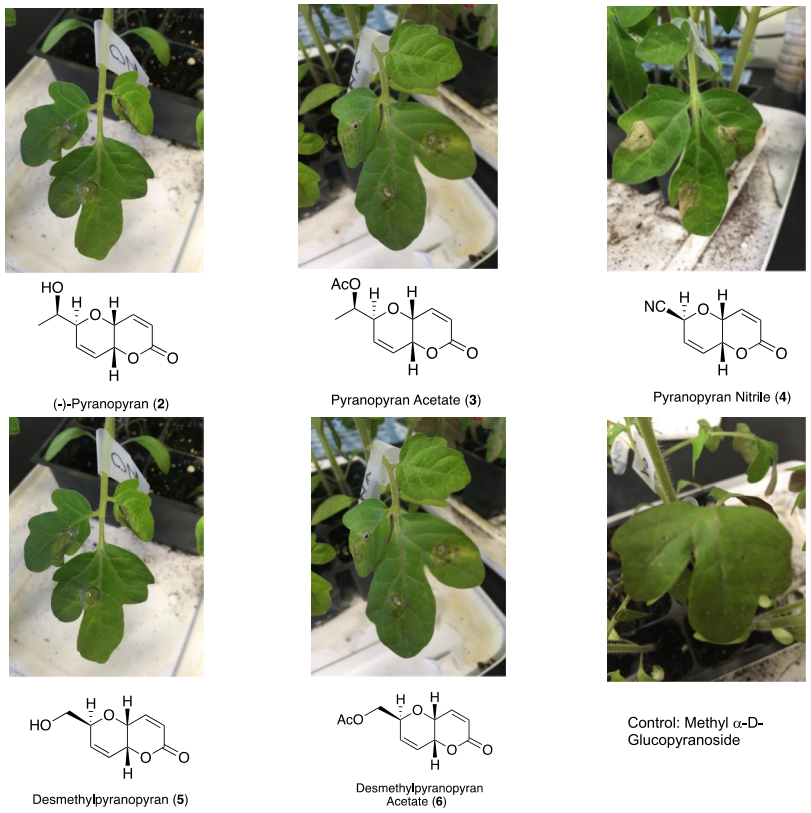

Figure 3. Leaf puncture assay for pyranopyrans **2**, **3**, **4**, **5**, and **6** in tomato plants (*L. esculentum*). Photographs of leaves are for 3 day exposure at 1.0 mg/mL.

H-7), 4.86 (m, 1H, H-8a), 4.73 (td, 1H, J = 4.9 Hz, 1.5 Hz, H-4a), 4.34 (m, 1H, H-6), 3.72 (m, 2H, H-9a,9b), 1.88 (m, 1H, OH); ^{13}C NMR ($CDCl_3$): δ 162.0, 143.0, 130.5, 124.2, 123.8, 72.9, 69.6, 63.6, 63.1.

HRMS (ESI): calcd for $C_9H_{10}O_4Na$ $[M + Na]^+$, 205.0477; found, 225.0480.

(2*S*,4a*R*,8a*R*)-6-Oxo-2,4a,6,8a-tetrahydropyrano[3,2-b]-pyran-2-carboxamide (7). Compound 7 was prepared by modification of the procedure of Kiss and Hell.[14] The solvent was changed from methanol to *tert*-butanol. A mixture of nitrile **4** (0.040 g, 0.226 mmol), the Cu^{II}-4 Å catalyst (0.022 g), acetaldoxime (0.040 g, 0.678 mmol), and *tert*-butanol (2 mL) was stirred at 70 °C for 4 h. The mixture was filtered through a pad of Celite and concentrated to a yellow-brown solid that was purified by cartridge chromatography on a Waters vacuum manifold system using 5% methanol/chloroform as the eluant (flash chromatography was also successful using 10% methanol/chloroform). Concentration of fractions left a white solid; yield, 0.0227 g (51.5%).R_f 0.2 (10% methanol/chloroform); mp 160–164 °C; $[\alpha]_D$ −268 (c 0.8, methanol; IR (ATR) ν: 3425, 3325, 3219, 1710, 1670, 1618 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.10 (dd, 1H, $J_{3,4}$ = 10.1 Hz, $J_{4,4a}$ = 5.4 Hz, H-4), 6.40 (ddd, 1H, $J_{7,8}$ = 10.2 Hz, $J_{6,7}$ = 3.6 Hz, $J_{7,8a}$ = 1.2 Hz, H-7), 6.16 (d, 1H, $J_{3,4}$ = 10.5 Hz, H-3), 6.10 (m, 1H, H-8), 4.86 (br s, 2H, NH_2), 4.80 (m, 2H, H-6, H-8a), 4.61 (ddd, 1H, $J_{4a,4}$ = $J_{4a,8a}$ = 4.5 Hz, $J_{4a,8}$ = 1.2 Hz, H-4a); ^{13}C NMR ($CDCl_3$): δ 174.1, 164.8, 143.7, 131.8, 124.5, 123.3, 74.0, 70.2, 64.4.

HRMS (ESI-TIF) m/z: calcd for $C_9H_{10}NO_4$, 196.0610; found, 196.0607.

Leaf Puncture Assay. Tomato leaves (*Lycopersicon esculentum*) from seedlings (~1 month old) were micropunctured with a needle and then exposed to 20 μL droplets of various test compounds dissolved in sterile water–methanol (1:1). The leaves, depicted in Figure 3, were exposed for 3 days to a concentration of 1.0 mg/mL of each of the test compounds and the control. The assay is similar to the assay described by Evidente.[15]

Antibacterial Bioassays of Pyranopyran Analogues. *Microorganisms and Preparation of the Culture Material for Antibacterial Bioassay.* The isolate of *F. columnare* [isolate ALM-00-173 (genomovar II)] was obtained from Dr. Covadonga Arias (School of Fisheries, Aquaculture and Aquatic Sciences, Auburn University, Auburn, Alabama). Cultures of *F. columnare* ALM-00-173 were maintained separately on modified Shieh (MS) agar plates (pH 7.2–7.4) to maintain purity.[16] Colonies of *F. columnare* ALM-00-173 were used to prepare the assay culture material by growing in 75 mL of MS broth for 24 h at 29 ± 1 °C on a rotary shaker at 150 rpm (model C24KC; New Brunswick Scientific, Edison, New Jersey). After 16 h of incubation, a 0.5 McFarland standard of the *F. columnare* ALM-00-173 culture material was prepared by transferring cells from the broth culture to fresh MS broth.[17]

The isolate of *E. ictaluri* (isolate S02-1039) was obtained from Mr. Tim Santucci (formerly with the College of Veterinary Medicine, Mississippi State University, Stoneville, Mississippi). Cultures of *E. ictaluri* S02-1039 were maintained on 3.8% Mueller–Hinton (MH) agar plates (pH 7.3) (Becton, Dickinson and Company, Sparks, Maryland) to maintain purity. Colonies of *E. ictaluri* S02-1039 were used to prepare the assay culture material by aseptically transferring bacterial cells from colonies to 45 mL of 3.8% MH broth to form a bacterial cell density of the 0.5 McFarland standard.

Antibacterial Bioassay. The pure test compounds **4**–**7** were dissolved in technical grade 100% methanol. The four test compounds were evaluated for antibacterial activity against *F. columnare* ALM-00-173 and *E. ictaluri* S02-1039 using a rapid 96-well microplate bioassay and following previous procedures (Schrader and Harries 2006). Florfenicol was included as a positive drug control. Control wells (no test material added) were also included in each assay. Final test concentrations of compounds and florfenicol were 0.01, 0.1, 1.0, 10.0, 100.0, and 1000.0 μM. Three replications were used for each dilution of each test compound and drug control. Final results were converted to units of mg/L to permit comparison with previous studies.

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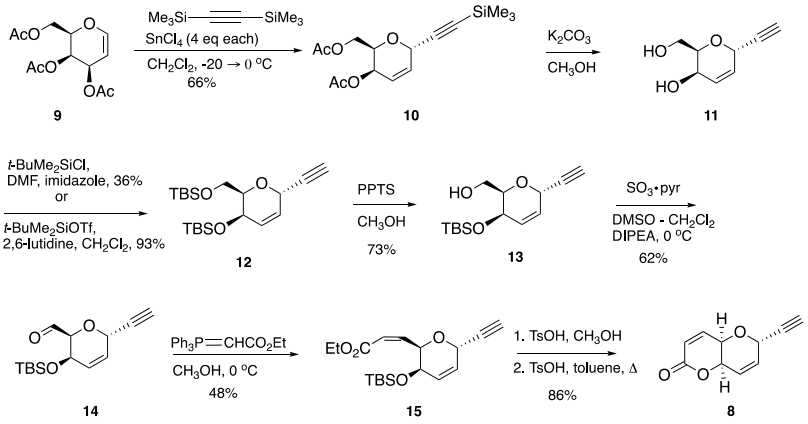
Scheme 1. Synthesis of Pyranopyran Alkyne 8



The 24 h 50% inhibition concentration (IC_{50}) and minimum inhibitory concentration (MIC) were determined using sterile 96-well (flat bottom) polystyrene microplates (Corning Costar Corp., Acton, Massachusetts). Initially, dissolved test compounds or florfenicol were transferred using a micropipette separately into individual microplate wells (10 μL/well), and the solvent was allowed to completely evaporate before 0.5 MacFarland bacterial culture was added to the microplate wells (200 μL/well). Microplates were incubated at 29 ± 1 °C (VWR model 2005 incubator; Sheldon Manufacturing, Inc., Cornelius, Oregon). A microplate photometer (SpectraCount, Packard Instrument Company, Meriden, Connecticut) was used to measure the absorbance (630 nm) of the microplate wells at time 0 and 24 h. Two replicates of each microplate bioassay were made. Cell viability of *F. columnare* ALM-00-173 and *E. ictaluri* S02-1039 was determined at the completion of the growth bioassay for each test compound using the yellow dye 3(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) and previously outlined procedures.[17]

The means and standard errors of absorbance measurements were calculated, graphed, and compared to controls to determine the MIC, 24 h IC_{50}, and MTT 24 h IC_{50} for each test compound. The 24 h IC_{50} and MIC results for each test compound were divided by the respective 24 h IC_{50} and MIC results obtained for the positive control florfenicol to determine the relative-to-drug-control florfenicol (RDCF) values.

Primary Bioassay for Herbicidal Activity. One filter paper disk (Whatman, Grade 1, 1.5 cm) was placed into each well of a 24-well plate (Corning Incorporated, CoStar 3524). The solution (200 μL, containing 180 μL of water and 20 μL of appropriate dilution of the sample) was transferred into each well. The plate also contained two types of control wells: 1. only with 200 μL of water and 2. with 180 μL of water and 20 μL of the solvent. For monocot screen, 20 mg of creeping bentgrass (*Agrostis stolonifera*) seeds and for dicot, five seeds of lettuce (*Lactuca sativa*) were added into each well. In the experiment, each condition was triplicate. The seeds were surface-sterilized in 2.5% bleach for 10 min and washed with deionized, sterile water. All steps were carried out under sterile conditions under laminar flow hood. Plates were sealed with parafilm and placed in a growth chamber at 26 °C and 120.1 μmol s^{-1} m^{-1} average light intensity. After 7 days of incubation, a growth level of seedlings was ranked. The phytotoxicity level was assessed based on the germination rate and ranked by the scale from 0 to 5 (0 = no effect and 5 = complete inhibition of germination).

Secondary Bioassay—*Lemna pausicostata*. The assay was carried out in nonpyrogenic polystyrene six-well plates (CoStar 3506, Corning Incorporated). Each well contained 4950 μL of Hoagland's media and 50 μL of water or the solvent or the compound solution. Three wells contained solution with the same concentration. Two three-frond *L. pausicostata* plants of the same age (4−5 day old) and approximate size were transferred into each well. All six-well plates were placed for 7 days in the Percival growth chamber at 26 °C and 120.1 μmol s^{-1} m^{-2} average light intensity. The area of leaf growth was measured at day 0 and 7 using the Scanalyzer PL (LemnaTec). The results of the treatments were analyzed with R studio (version 3.4.1.) with the drc package. IC_{50} values were calculated using four-parameter logistic functions.

Greenhouse Studies. In a greenhouse experiment, the phytotoxic activity of pyranopyran nitrile was tested on four plant species velvetleaf (*Abutilon theophrasti*) (10 days old), field bindweed (*Convolvulus arvensis*) (14 days old), crabgrass (*Digitaria sanguinalis*) (14 days old), and barnyard grass (14 days old). The plants grew in pots (10 cm/10 cm/10 cm) in soil Metro-Mix (Sungro). The application of the test compound was performed with Generation III Spray Booth (Bellspray Inc.). The track sprayer released the test solution with a pressure of 207 kPa psi and moved the spray boom with a speed of 1.48 km/h over the plants which gave 360 L of applied solution per hectare. The applied rate for the active ingredient was 1 kg per hectare. The final concentration in the solution of acetone was 1% and Tween 20 was 0.1%.

RESULTS AND DISCUSSION

In view of the potent antibacterial activity of pyranopyran nitrile **4**, we decided to pursue the synthesis of the corresponding pyranopyran alkyne **8**, similar in size but much different in the polarity of the side chain. Compound **8** was synthesized as illustrated in Scheme 1, by modification of the route we developed previously for the nitrile **4**, in which a silyl alkyne was substituted for trimethylsilyl cyanide in the Ferrier reaction. The *C*-alkynylation of tri-*O*-acetyl-D-galactal **9** with bis(trimethylsilyl)acetylene, as described by Yamada,[11] gave the alkynyl glycoside **10** exclusively as the α-anomer. Deprotection of both hydroxy groups and the alkyne was achieved in a single step by treatment of **10** with potassium carbonate in methanol.[11]

Conversion of **11** to the di-*O*-TBS ether **12** using TBDMSCl/imidazole was less efficient than expected; however, the yield could be improved to 93% using TBDMSOTf as the silylating agent and 2,6-lutidine as the base. The sequence leading from di-*O*-TBS ether **12** to the Wittig product **15** was carried out without the isolation of intermediate 4-*O*-TBS ether **13**, as our previous studies had shown that similar systems were prone to silyl group migration from the 4-OH to the 6-OH. Oxidation of **13** to aldehyde **14** was carried out with the sulfur trioxide−pyridine complex (Parikh−Doering method).[18] Side products from overoxidation were not observed with this method. The reaction of aldehyde **14** with the stabilized ylide under conditions described by Valverde gave **15** as the *Z*-isomer exclusively.[19] Desilylation of **15** followed by cyclization gave **8** in 86% yield.

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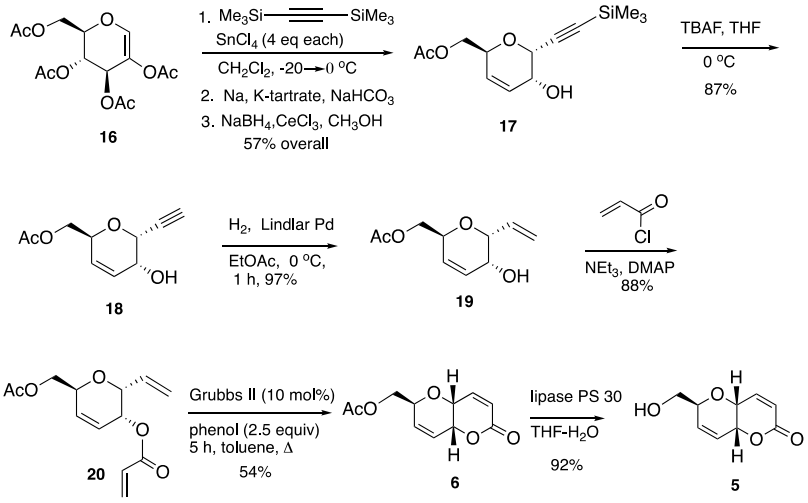
Scheme 2. Synthesis of Desmethylpyranopyrans 4 and 5

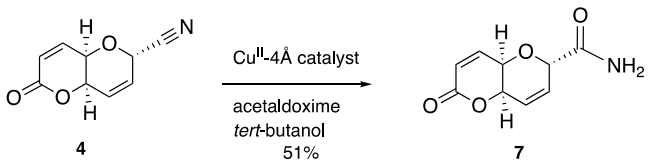


The synthesis of **8** was completely stereoselective. Two of its three chirality centers were provided by the starting carbohydrate, the third was established by the α-selective *C*-alkynylation, and the Wittig reaction gave only the *Z*-isomer.

Desmethylpyranopyran analogues **5** and **6** were synthesized by a highly stereoselective sequence that is similar to the route we developed for the synthesis of pyranopyrans **2** and **3** with modifications in certain key steps and a different choice of the carbohydrate starting material.[4] The starting glycal in our route to **5** and **6** was 2,3,4,6-tetra-*O*-acetyl-1-deoxy-D-*arabino*-hex-1-enopyranose (**16**) which is readily available from acetobromoglucose or acetobromogalactose by modifications of the reported preparation.[13] The *C*-alkynylation of **16** with bis(trimethylsilyl)acetylene as described by Isobe,[14] followed by hydrolytic workup and sodium borohydride reduction as described, gave exclusively alkynyl alcohol **17** in 57% overall yield with the desired stereochemistry at C-2 (Scheme 2). Four transformations occur in this three-step sequence: Ferrier rearrangement, enol acetate hydrolysis, β-elimination of the C-4 acetoxy group, and ketone reduction. Selective deprotection with TBAF gave alkyne **18**. Selective reduction of the alkyne in **18** to the *C*-vinyl glycoside **19** proved more difficult than expected. In our initial attempts with Lindlar's catalyst in methanol, we obtained mixtures of *C*-vinyl and *C*-ethyl glycosides. We found that overreduction could be minimized with a procedure consisting of pretreatment of the catalyst (Pb-poisoned) with quinoline, changing the solvent from methanol to ethyl acetate and lowering the temperature to 0 °C. Efficient reduction of *C*-alkynyl to *C*-vinyl glycoside **19** occurred under these conditions. The sequence from starting glycal **16** to **19** proceeds in 48% overall yield. Acryloylation of **19** was straightforward, setting up the key lactone ring closure by RCM, which occurred with the Grubbs II catalyst in toluene at 80 °C to give **6**. The reaction time was longer than expected, perhaps because one of the alkenes is electron deficient. A shorter reaction time was realized by including phenol in the RCM, as described by Forman.[20] The authors suggest that the rate enhancement that results from the addition of phenol to alkene metathesis is the result of stabilization of the active form of the catalyst and activation of the carbene−ruthenium complex. Carbohydrate deacylation is typically carried out with sodium methoxide in methanol; however, sugar enones are known to react rapidly with methoxide,[21] and we were concerned that side reactions might occur with **6** under these conditions. Based on our previous synthesis of amicetose by lipase-catalyzed transesterification,[22] we subjected **6** to Amano PS-30 lipase in wet THF and observed clean deprotection to give desmethylpyranopyran **5** in 92% yield.

The syntheses illustrated in Schemes 1 and 2 are amenable for the preparation of structural analogues with different substitutions at the crucial C-6 position on the pyranopyran scaffold. As an example, we were able to carry out chemoselective hydration of the nitrile functional group in **4** to obtain pyranopyran amide **7**. Hydrations of nitriles to amides are often carried out under acidic or basic conditions that might affect the dense functionality in **4**, so we sought a catalytic method that would proceed under neutral conditions. Kiss and Hell reported the use of a copper (II) catalyst supported on molecular sieves in the presence of acetaldoxime for this conversion.[14] We substituted *tert*-butanol for methanol as the solvent in their procedure in order to avoid possible conjugate addition of methanol to the unsaturated lactone. Crystalline pyranopyran amide **7** was prepared by this method (Scheme 3). X-ray crystallographic analysis of **7** revealed that it

Scheme 3. Synthesis of Pyranopyran Amide 6



adopts a similar conformation in both rings to the nitrile but with intermolecular hydrogen bonds between amide groups on adjacent molecules in the crystal lattice.[23]

Early indications that these novel pyranopyrans may possess herbicidal activity were observed by a leaf puncture assay using tomato leaves as described by Evidente and co-workers in their study of afritoxinones A and B obtained from *Diplodia africana*.[15] The data, shown in Figure 3, for compounds **2**, **3**, **4**, **5**, and **6** show necrotic lesions that are produced after exposure for 3 days to these compounds at a concentration of 1 mg/mL. All five compounds were phytotoxic in this assay, although not to the same extent. These results encouraged us to undertake a broader biological evaluation of the

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pyranopyrans. Bioassay results for compounds **2**, **3**, and **4** as antibacterials against catfish pathogens in aquaculture and against *L. paucicostata* (L.) Helgelm (duckweed) were reported in our previous study.[4] In this previous study, only nitrile **4** was significantly phytotoxic in the duckweek bioassay. Compound **4** was the most promising compound in the antibacterial assays against catfish pathogens. A minimum inhibitory concentration (MIC) of 0.002 mg/L against *E. ictaluri* indicated approximately 100 times greater potency than that of the antibiotic florfenicol used commercially for this disease. Further results of the biological testing of nitrile **4** for phytotoxic activity in bioassays including a greenhouse study and test results for the new analogues **5**, **6**, **7**, and **8** in both phytotoxicity and antibacterial assays are described below.

Micro-bioassays for phytotoxicity are needed when the amount of test compound is limited. The phytotoxicity bioassays that we used in this study have been routinely used by the USDA NPURU laboratory, for example, in Favaretto et al.[24] Phytotoxic activity of compounds **4**–**8** against monocots represented by bentgrass (*A. stolonifera*) and dicots by lettuce (*L. sativa*) was evaluated in a primary ranking bioassay (Table 1). Bentgrass and lettuce are frequently used by numerous laboratories in preliminary tests of herbicidal activity of chemicals. Fast germination of both species (in 2–3 days) enables us to complete these tests within 7 days. Moreover, as described in the Materials and Methods section, we use only 200 μL of the solution containing the compound per replicate. Such a minimalization enables the fast and efficient analysis of phytotoxic properties of tested compounds. Additionally, simultaneous testing of bentgrass and lettuce lets a very rapid identification of compounds with selective properties against monocots or dicots. In this pre-emergence test, seeds are placed in 1000 μM solution of the compound and incubated in a growth chamber for 7 days. Two compounds demonstrated significant phytotoxic potential against lettuce: pyranopyran nitrile **4** and pyranopyran alkyne **8** were ranked **4** and **3**, respectively. All tested pyranopyrans had a range of activity against monocot species. Interestingly, **4** is less active than **8** as a pre-emergence agent against the monocot bentgrass, while based on IC_{50} values of post-emergence bioassay on the monocot duckweed, their herbicidal effect is reversed. As reported previously, neither (−)-diplopyrone **2** nor its acetate derivative **3** was active in the phytotoxicity assay against duckweed. In this study, a similar lack of activity was observed for the corresponding desmethyl compounds **5** and **6**. However, the alkyne analogue **8** showed potent activity in this assay. The calculated IC_{50} for the alkyne analogue reached 38.1 μM, and it is the most phytotoxic compound after the nitrile.

Table 1. Evaluation of Phytotoxic Activity of Pyranopyprans in Primary and Secondary Bioassays[a]

		ranking		IC_{50} ± SE
#	sample ID	lettuce	bentgrass	duckweed (μM)
4	pyranopyran nitrile	4	1	<3 [ref 4]
7	pyranopyran amide	0	0	>1000
8	pyranopyran alkyne	3	4	38.1
6	desmethyldiplopyrone acetate	0	1	>1000
5	desmethyldiplopyrone	0	2	>1000

[a]The primary bioassay tests the pre-emergence effect of treatment on lettuce and bentgrass and is based on the scale from 0 to 5 (0 = no effect and 5 = complete inhibition of germination). The half maximal inhibitory concentration (IC_{50}) values from the growth change rate of duckweed fronds.

Good results from our micro-bioassays are generally followed up by greenhouse assessments of effects on potted plants.[24,25] The pyranopyran nitrile **4** was evaluated in a greenhouse study with four plant species velvetleaf (*A. theophrasti*), field bindweed (*C. arvensis*), crabgrass (*D. sanguinalis*), and barnyardgrass (*Echinochloa crusgalli*). Leaf tissue of both monocot species crabgrass and barnyardgrass developed signs of injuries as soon as 24 h after application (1 DAT). Results for crabgrass are shown in Figure 4; similar results for barnyardgrass are not shown. Later, these light gray spots turned into necrotic brown lesions (4 DAT) (Figure 4). Leaves of dicot species showed little or no signs of damage. Nevertheless, the treated pool grew new leaves that are healthy and in size comparable to the control group (data not shown). The necrosis spots are the result of direct contact of plant tissue with droplets of sprayed solution. Both this evidence and the lack of translocation signs suggest that pyranopyran nitrile acts as a contact herbicide.

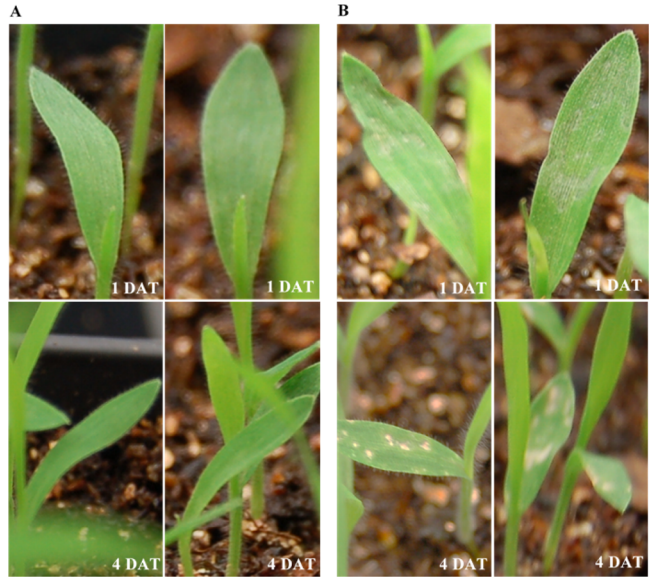

Figure 4. Postemergence effect of pyranopyran nitrile **4** on crabgrass **1** and **4** DAT. Pictures present examples of treated seedlings (A) and control (B) pyranopyran nitrile—1 kg a. l. ha^{-1}. The crabgrass seedlings exposed to **4** developed necrosis.

Summarizing phytotoxic properties of tested pyranopyran analogues: only compounds **4** and **8** showed significant phytotoxic activity, while **5**–**7** were not phytotoxic. Both pyranopyran nitrile (**4**) and pyranopyran alkyne (**8**) efficiently inhibit growth of *L. paucicostata,* while **8** demonstrates more universal pre- and postemergence potential against monocot species.

Among the four diplopyrone analogues evaluated for antibacterial activity in this study, pyranopyran alkyne **8** was the most active against *E. ictaluri* S02-1039 based on MIC results of 1.8 mg/L (Table 2). Compound **8** had the best MIC RDCF value of 4.9, also indicating the strongest activity of the four analogues when compared to the drug control florfenicol. Although compound **4**, as reported in a previous study,[4] had a lower MIC (0.002 mg/L) than **8**, the 24 h IC_{50} (7.7 mg/L) of **4** was greater than the MIC by more than 3 orders of

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Table 2. Results of Bioassay Evaluations of Diplopyrone Derivatives for Antibacterial Activity Against *E. ictaluri* S02-1039[a]

test material	MIC[b]	24 h IC_{50}[c]	MTT 24 h IC_{50}[d]	MIC[a] RDCF[e]	24 h IC_{50}[b] RDCF[e]
florfenicol	0.36	0.18			
5	18.2 (0)	48.2 (2.7)	14.7 (9.0)	50.6 (0)	358.5 (33.5)
6	22.4 (0)	39.2 (1.1)	35.8 (4.5)	62.2 (0)	290.5 (2.5)
7	19.5 (0)	20.5 (2.9)	16.6 (8.8)	54.2 (0)	135.8 (10.5)
8	1.8 (0)	22.0 (2.6)	6.2 (0.2)	4.9 (0)	181.2 (42.9)

[a]Numbers in parentheses are the standard error of the means. [b]MIC = Minimum inhibitory concentration in mg/L. [c]24 h IC_{50} = 50% inhibition concentration in mg/L. [d]24 h IC_{50} (mg/L) as measurement of cell viability using the tetrazolium bromide dye MTT. [e]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 *Flavobacterium columnare* ALM-00-173[a]

test material	MIC[b]	24 h IC_{50}[c]	MTT 24 h IC_{50}[d]	MIC[b] RDCF[e]	24 h IC_{50}[c] RDCF[e]
florfenicol	0.36	0.69			
5	182.0 (0)	76.4 (3.6)	79.2 (2.7)	505.6 (0)	109.1 (2.1)
6	224.0 (0)	>224.0	>224.0	622.2 (0)	>329.4
7	107.3 (87.8)	54.6 (2.0)	75.1 (1.0)	297.9 (243.8)	38.5 (31.7)
8	176.0 (0)	43.1 (7.9)	59.0 (2.6)	488.9 (0)	63.0 (8.0)

[a]Numbers in parentheses are the standard error of the means. [b]MIC = Minimum inhibitory concentration in mg/L. [c]24 h IC_{50} = 50% inhibition concentration in mg/L. [d]24 h IC_{50} (mg/L) as measurement of cell viability using the tetrazolium bromide dye MTT. [e]RDCF = Relative-to-drug-control florfenicol; values closer to 1.0 indicate higher antibacterial activity compared to florfenicol.

magnitude, which overall indicated significant sensitivity of *E. ictaluri* S02-1039 to **4**, while higher amounts were required for 50% inhibition. Compound **8** had an IC_{50} of 22.0 mg/L which was approximately 1 order of magnitude less active than **4**.

None of the four diplopyrone analogues were highly active against *F. columnare* ALM-00-173 based on MIC results (Table 3). Compound **7** had the most activity against *F. columnare* ALM-00-173, with an MIC of 107.3 mg/L. Compound **8** had the lowest 24 h IC_{50} results of 43.1 mg/L. These results are similar to the lack of strong activity by the other diplopyrone compounds reported in the first study.[4]

Challenge studies using channel catfish would determine the efficacy of compounds **4** and **8** in the potential management of ESC. Because these two compounds were not very toxic against *F. columnare*, they do not appear to possess broad-spectrum toxicity against Gram-negative bacteria in general. Bioassay evaluation of natural and natural product-based compounds is the first step in the discovery of novel compounds that could be used to manage common diseases of cultured catfish. These discoveries involve novel compounds, and the exact modes of antibacterial actions against specific bacteria are unknown. Additional research may lead to the synthesis of more active compounds as structure−activity relationships are determined.

The synthesis of the four new pyranopyrans based on the core structure of diplopyrone has been achieved using carbohydrate starting materials and *C*-glycosides as key intermediates.

The synthesis of pyranopyrans **5**, **6**, and **7** was carried out in seven or eight steps from carbohydrates; no stereoisomers are produced at any stage. The most promising of these new compounds in terms of its biological activity is pyranopyran alkyne **8**, which was synthesized in eight steps from tri-*O*-acetyl-D-galactal. Compound **8** possesses potent antibacterial activity against the bacterial pathogen *E. ictaluri* that causes enteric septicemia (ESC) in catfish and herbicidal activity in bioassay with monocots *A. stolonifera* (bentgrass), *L. sativa* (lettuce), and *L. paucicostata* (L.) Helgelm (duckweed). Further tests of herbicidal activity in greenhouse studies with pyranopyran nitrile **4** showed potential against four plant species. Both the nitrile **4** and the alkyne **8** were active against monocots. The biological activities of pyranopyrans **4** and **8** demonstrate the potential of these compounds as antibacterials and herbicides. The bioassay results of **4** and **8** show an intriguing structure−activity relationship for the C-6 position on the pyranopyran scaffold. Amide **7** was synthesized to provide a compound that would have greater polarity and aqueous solubility, yet it proved to be much less active than either **4** or **8**. An intriguing aspect of pyranopyran **8** is the possibility of exploiting the alkyne functional group in azide−alkyne cycloadditions to obtain triazole analogues.[26] Bioassay results suggest an interplay between the pyranopyran ring system and the C-6 substituent. Further studies are required to speculate on the specific role of substituents at the crucial C-6 position, and additional analogues would be useful in this regard. Additionally, studies of the mechanisms of action and off-target effects of these compounds will need to be conducted to determine their safety and potential applications.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jafc.0c02564.

NMR spectra for compounds **5**, **6**, **8**, **10**, **11**, **12**, **14**, **15**, **17**, **18**, **19**, and **20** (PDF)

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Notes

The authors declare no competing financial interest.

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