JOURNAL OF NATURAL PRODUCTS

Article

pubs.acs.org/jnp

Total Synthesis, Configuration Assignment, and Cytotoxic Activity Evaluation of Protulactone A

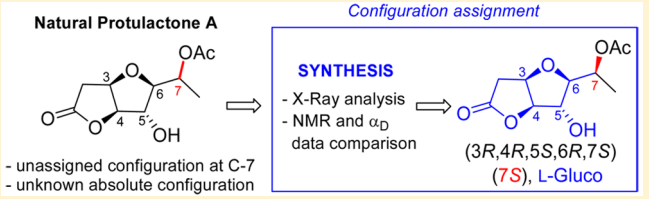
Martin Markovič,[†] Peter Koóš,[‡] Tomáš Čarný,[†] Saskia Sokoliová,[†] Nikola Boháčiková,[†] Ján Moncol',[§] and Tibor Gracza*,[†]

[†]Department of Organic Chemistry, Institute of Organic Chemistry, Catalysis and Petrochemistry, and [§]Department of Inorganic Chemistry, Institute of Inorganic Chemistry, Technology and Materials, Slovak University of Technology, Radlinského 9, SK-812 37 Bratislava, Slovakia

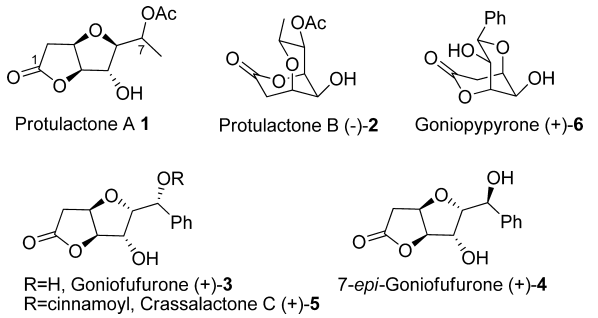
[‡]Georganics Ltd., Koreničova 1, SK-811 03 Bratislava, Slovakia

Supporting Information

ABSTRACT: The first total synthesis and absolute configuration assignment of protulactone A (**1**) has been achieved. Four stereoisomers, **1a**, *ent*-**1a**, **1b**, and *ent*-**1b**, of this natural polyketide were prepared by chiral pool synthesis starting from L- and D-arabinose, respectively. The absolute and relative configurations of all isomers were assigned by single-crystal X-ray analysis. Target compounds were screened for their *in vitro* cytotoxicity toward certain human tumor cells (NCI_{60} cancer cell line panel).



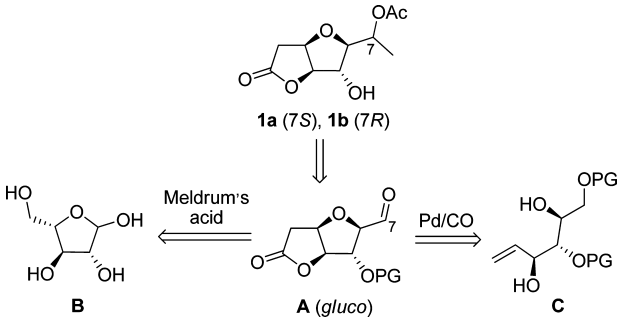
Protulactones A (**1**) and B ((−)-**2**) were isolated in 2010 from the marine-derived fungus *Aspergillus* sp. SF-5044.[1] The substructure unit of these two polyketide-derived fungal metabolites, a bicyclic lactone, is not a distinctive structure for the natural compounds isolated from fungi of the *Aspergillus* genus. However, similar compounds occur in plants of various families (e.g., *Goniothalamus giganteus* Hook f., Thomas (Annonaceae) and *Polyalthia crassa*).[2] The structures and relative configurations of protulactones A and B were determined by extensive NMR analysis. The absolute configuration of the rigid bicyclic skeleton of protulactone B ((−)-**2**) was assigned by Mosher's MTPA ester method. However, the relative configuration of the C-7 center and the absolute configuration of **1** have not been described.[1] The biological activities of these two natural compounds have not been evaluated presumably due to the limited supply from natural sources (only 3.7 mg of **1** was isolated from 2.0 g of the EtOAc extract). Naturally occurring and structurally similar lactones, goniofufurone ((+)-**3**), 7-*epi*-goniofufurone ((+)-**4**), goniopypyrone ((+)-**6**),[2a,b] and crassalactone C ((+)-**5**),[2c] have exhibited significant cytotoxic activity in tests with several human tumor cell lines.[2,3] The incomplete configuration assignment and promising biological activity of **1** led us to develop a total synthesis of protulactone A with the aim to establish its absolute structure and evaluate its antiproliferative activities against the NCI_{60} cancer cell line panel.



RESULTS AND DISCUSSION

Because the absolute and C-7 configuration of the isolated compound **1** are unknown, we have targeted four different stereoisomers. We herein report the synthesis of all of these isomers of **1** (**1a**, **1b**, *ent*-**1a**, and *ent*-**1b**) containing the bicyclic skeleton with a relative *gluco* configuration and different configurations at the C-7 center. The proposed retrosynthetic strategy of protulactone A (**1**) is shown in Scheme 1. In both selected routes, the methyl moiety is introduced at the end of

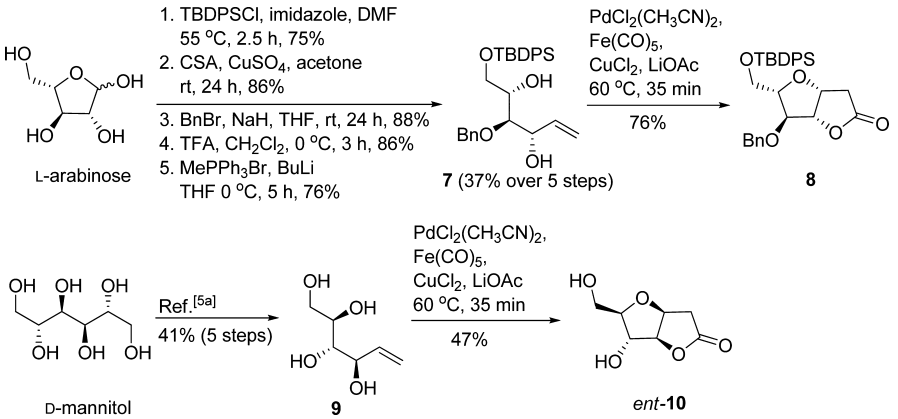
Scheme 1. Retrosynthetic Analysis of the Protulactone A



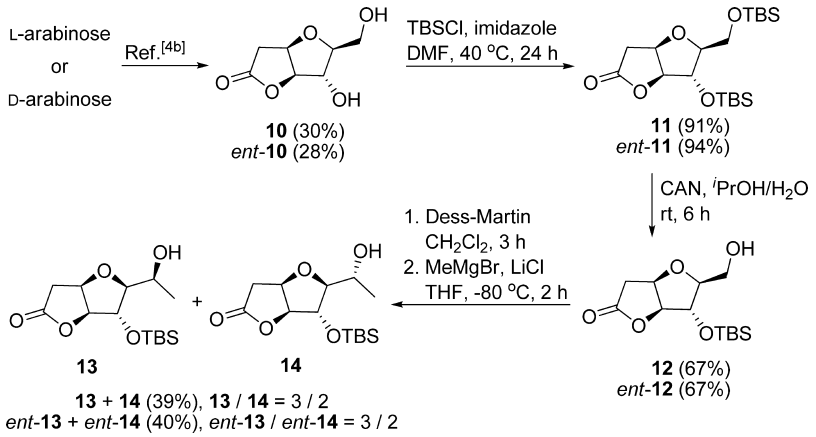
Received: March 10, 2017

DOI: 10.1021/acs.jnatprod.7b00212
J. Nat. Prod. XXXX, XXX, XXX−XXX

Scheme 2. Synthesis of Lactones 8 and *ent*-10



Scheme 3. Synthesis of Isomers 13, 14, *ent*-13, and *ent*-14



the synthesis via the Grignard addition on aldehyde **A**, providing both C-7 stereoisomers. The optically pure heptonolactone **A** having the required D- or L-*gluco* configuration can be obtained by a condensation reaction of arabinose **B** with Meldrum's acid[4] or using Pd-catalyzed carbonylative bicyclization of unsaturated tetraol[5a] **C**.

First, we applied a known oxycarbonylation strategy[6] for the stereoselective construction of the [3.3.0] bicyclic core of intermediary heptonolactone **A**. The key intermediates **C**, the known alkenol[5a] **9**, and partially protected tetraol[5b−e] **7** were obtained by standard carbohydrate chemistry procedures from D-mannitol and L-arabinose, respectively (Scheme 2).[5]

The crucial steps in this approach, homogeneous Pd-catalyzed carbonylation reactions of alkenes **7** and **9** using $Fe(CO)_5$ as an *in situ* donor of a stoichiometric amount of carbon monoxide, afforded corresponding optically pure lactones **8** (L-*gluco*, 76%) and *ent*-**10** (D-*gluco*, 47%).[7] In the second approach, the required [3.3.0] bicyclic structural unit was assembled by a single reaction of the corresponding aldose with Meldrum's acid in the presence of triethylamine.[4] Consequently, we prepared the known 3,6-anhydro-2-deoxy-L-*gluco*-heptono-1,4-lactone (**10**) and its enantiomer *ent*-**10** in one step in acceptable yields (30%/28%) using L- or D-arabinose.[4b] While both synthetic approaches provided lactone *ent*-**10** in similar (combined) yields, the second route has been chosen to prepare the key aldehyde **A** (Scheme 3).

The syntheses of both enantiomers were run in parallel and continued with the silyl protection of free hydroxy groups. Thus, bis-silylated lactones **11** and *ent*-**11** were isolated in excellent yields (91%/94%). Selective deprotection of the primary hydroxy group using cerium(IV) ammonium nitrate[8] in aqueous isopropyl alcohol provided primary alcohols **12**/*ent*-**12** (67%/67%). These alcohols were then subjected to the Dess−Martin oxidation followed by an addition of methylmagnesium bromide to the formed aldehyde. However, the addition reaction provided a poorly separable diastereomeric mixture of alcohols **13**/**14** and *ent*-**13**/*ent*-**14** in moderate yield. After several MPLC purifications, the analytically pure intermediates **13**, *ent*-**13**, **14**, and *ent*-**14** were isolated as white solids. Recrystallization of the individual materials from CH_2Cl_2/hexanes (1:10) furnished colorless needles suitable for single-crystal X-ray analysis. Accordingly, the absolute L-*gluco* configuration of the bicyclic skeleton and *S*-configuration of the newly formed stereocenter at C-7 in compound **13** were established (Figure 1). The alcohol **14** represents the C-7 epimer (7*R*). An X-ray study of the enantiomeric intermediates *ent*-**13** and *ent*-**14** confirmed the D-*gluco* configuration of the bicyclic moiety with the 7*S*/7*R*-configuration (Figure 1).[9]

Finally, crystalline lactones **13**, **14**, *ent*-**13**, and *ent*-**14** were then transformed in two steps into the final compounds. Thus, the acetylation of the free hydroxy group and buffered tetrabutylammonium fluoride (TBAF) deprotection of the silyl moiety provided **1a**/**1b** and *ent*-**1a**/*ent*-**1b** in good yields (Scheme 4).

Isolated stereoisomer **1a** was identified as the natural protulactone A, despite the fact that the value of specific rotation, $[\alpha]_D^{25}$ +35.6 (*c* 0.34, MeOH), of the synthetic sample **1a** differs from the literature value, $[\alpha]_D^{25}$ +6 (*c* 0.34, MeOH)

DOI: 10.1021/acs.jnatprod.7b00212
J. Nat. Prod. XXXX, XXX, XXX−XXX

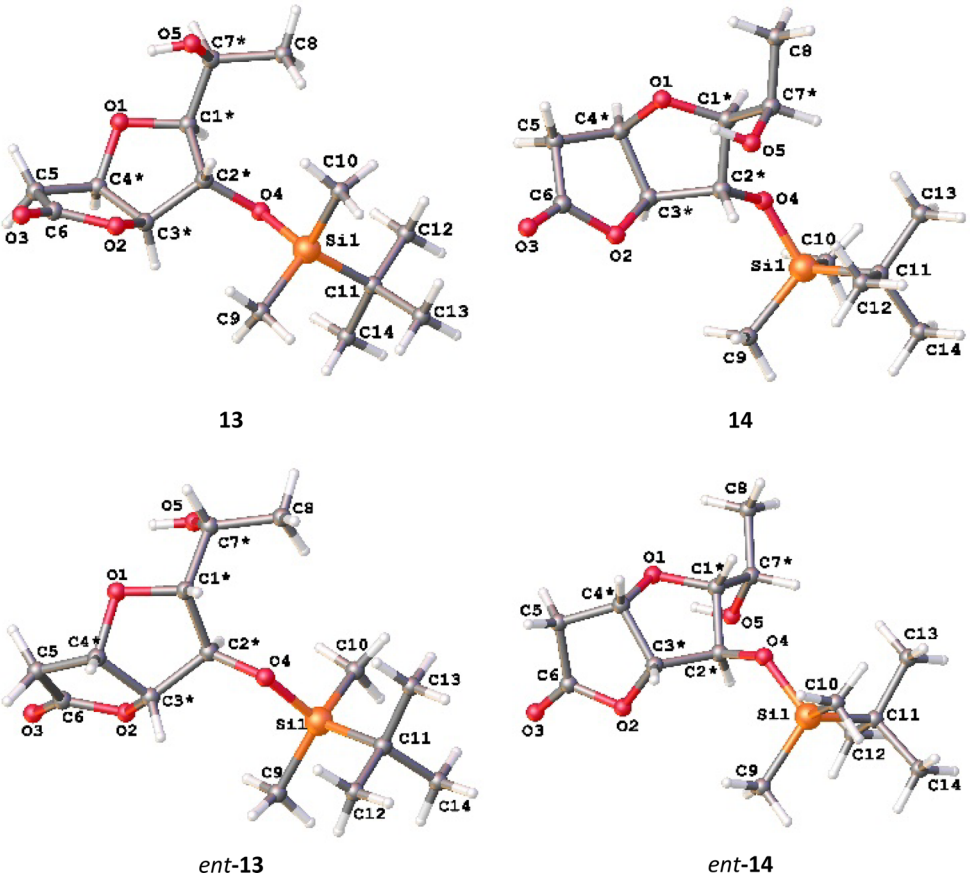
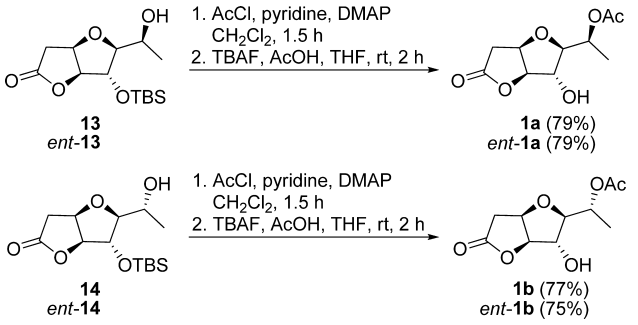
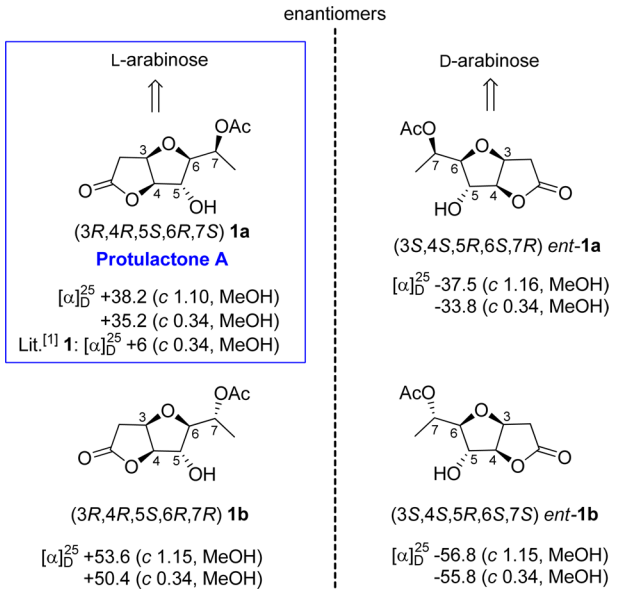


Figure 1. Ball-and-stick view of crystal structures **13**, **14**, *ent*-**13**, and *ent*-**14**[9]

Scheme 4. Synthesis of Protulactone A (1a) and Its Isomers 1b, *ent*-1a, and *ent*-1b



Scheme 5. Structures of Natural Protulactone A (1a), 7-Epimer 1b, and Unnatural Enantiomers *ent*-1a and *ent*-1b



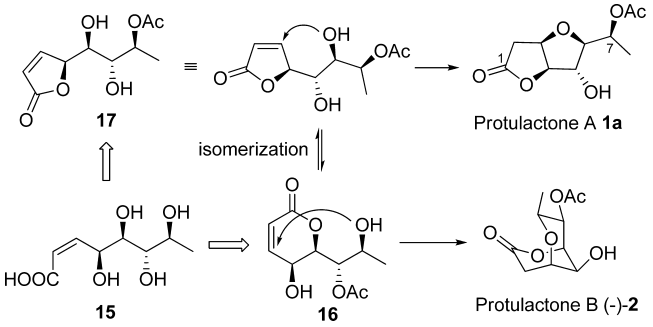
(Scheme 5). The ^{1}H and ^{13}C NMR spectroscopic data of our synthetic product **1a** matched well the reported values for the isolated natural compound.[1,10] The proton NMR spectrum of the second isomer **1b** does exhibit some differences in the chemical shifts. While the chemical shift of the H-5 proton (br d, 1*H*, J = 4.8 Hz) of the natural substance is 4.32 ppm,[1] the corresponding H-5 proton of the diastereomer **1b** is shifted to 4.15 ppm (d, 1H, J = 4.5 Hz).

Despite the inconsistency in the specific rotation data of isolated material **1** and our isomer **1a** (designated as natural protulactone A), the stereogenic centers of **1a** are consistent with the configuration of natural protulactone B (−)-**2**. According to the literature,[11] we have also outlined a possible biosynthetic pathway involving intermediates **16** and **17**, which can be transformed to the individual natural products by an intramolecular Michael addition (Scheme 6). As follows, lactones **16** and **17** can be derived from the same biosynthetic precursor **15**, pointing to the configuration uniformity of proposed protulactone A (**1a**).

Moreover, all prepared stereoisomers of **1** were then evaluated for their *in vitro* cytotoxicity against the NCI_{60} cancer cell line panel at the developmental therapeutics

DOI: 10.1021/acs.jnatprod.7b00212
J. Nat. Prod. XXXX, XXX, XXX−XXX

Scheme 6. Proposed Biosynthetic Pathway of Natural Protulactones A (1a) and B ((−)-2)



program, Division of Cancer Treatment and Diagnosis, National Cancer Institute. Interestingly, the screening of all prepared isomers of protulactone **1** at a single dose of 10 μM has shown low toxicity against the tested tumor cells (see one-dose mean graphs in the SI) in contrast to highly active structurally related lactones (goniofufurone (**3**), 7-*epi*-goniofufurone (**4**), and crassalactone C (**5**)).

In summary, we have developed a divergent synthesis of four stereoisomers of naturally occurring protulactone A from L- and D-arabinose. The absolute configurations of all prepared stereoisomers were established on the basis of the single-crystal X-ray analysis of the intermediates **13**, **14**, *ent*-**13**, and *ent*-**14**. The 3,6-anhydro-2,8-dideoxy-7-*O*-acetyl-L-*glycero*-L-*gluco*-octano-1,4-lactone ((+)-**1a**) (3*R*,4*R*,5*S*,6*R*,7*S*) was identified as the naturally occurring lactone **1** based on the comparison of the specific rotation and NMR spectroscopic data. The screening of the prepared compounds against the NCI_{60} cancer cell line panel has not shown significant cytotoxicity. However, these results bring new insights into the relationship between structure and biological activity of such bicyclic lactones. Finally, the presented approach is applicable in the synthesis of substantial amounts of new epimers and C-7 analogues of protulactone A (**1**), making it suitable for structure−activity relationship studies.

EXPERIMENTAL SECTION

General Experimental Procedures. Melting points were obtained using a Boecius apparatus and are uncorrected. Optical rotations were measured with a JASCO P-2000 polarimeter and are given in units of 10^{-1} deg·cm^2·g^{-1}. FTIR spectra were obtained on a Nicolet 5700 spectrometer (Thermo Electron) equipped with a Smart Orbit (diamond crystal ATR) accessory, using the reflectance technique (4000−400 cm^{-1}). ^{1}H and ^{13}C NMR spectra were recorded on either a 300 (75) MHz Unity Inova or a 600 (151) MHz VNMRS spectrometer from Varian. Standard chemical shifts are referenced to the corresponding solvent residual peak ($CDCl_3$: δ_H 7.26 ppm, δ_C 77.16 ppm; CD_3OD: δ_H 3.31 ppm, δ_C 49.00 ppm; DMSO-d_6: δ_H 2.50 ppm, δ_C 39.52 ppm) or tetramethylsilane as internal standard. High-resolution mass spectra (HRMS) were recorded on an OrbitrapVelos mass spectrometer (Thermo Scientific) with a heated electrospray ionization source. The mass spectrometer was operated with full scan (50−2000 amu) in positive or negative FT mode (at a resolution of 100 000). The analyte was dissolved in MeOH and infused via syringe pump at a rate of 5 mL/min. The heated capillary was maintained at 275 °C with a source heater temperature of 50 °C, and the sheath, auxiliary, and sweep gases were at 10, 5, and 0 units, respectively. Source voltage was set to 3.5 kV. Flash column liquid chromatography was performed on Kieselgel 60 silica gel (40−63 μm, 230−400 mesh), and analytical thin-layer chromatography (TLC) was performed on aluminum plates precoated with either 0.2 mm (DC-Alufolien, Merck) or 0.25 mm silica gel 60 F254 (ALUGRAM SIL G/UV254, Macherey-Nagel). The compounds were visualized by UV fluorescence and by dipping the plates in an aqueous H_2SO_4 solution of cerium sulfate/ammonium molybdate followed by charring with a heat gun.

Data collection and cell refinement of **13**, **14**, *ent*-**13**, and *ent*-**14** were made on a Stoe StadiVari diffractometer using a Pilatus 300 K HPAD detector and the microfocus source Xenocs FOX3D with Cu Kα. The structures were solved using SHELXT or SIR-2011 and refined by the full-matrix least-squares procedure with SHELXL. The structures were drawn using the OLEX2 package.[12] The absolute configurations of all enantiomers were determined. The Flack parameters were calculated by the classical Flack method (for **13** and *ent*-**14**) or Parsons method (for **14** and *ent*-**13**).[13]

Synthesis of 3-*O*-Benzyl-1-*O*-*tert*-butyldiphenylsilyl-L-*xylo*-hex-5-enitol (7). *5-O-tert-Butyldiphenylsilyl-L-arabinose.* L-Arabinose (8.00 g, 53.29 mmol, 1 equiv) was dissolved in DMF (120 mL) at 100 °C. After cooling the solution to 55 °C, imidazole (7.26 g, 106.57 mmol, 2 equiv) and *tert*-butyl(chloro)diphenylsilane (13.9 mL, 53.29 mmol, 1 equiv) were added, and the reaction mixture was stirred at the same temperature for 2.5 h. The reaction mixture was then concentrated to half of its volume, and EtOAc (800 mL) was added. The organic phase was then washed with deionized H_2O (4 × 200 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by MPLC (gradient EtOAc/hexanes, 0/100 to 70/30, then isocratic EtOAc/hexanes, 70/30), providing the desired product as a light yellow oil (75%, 15.51 g): TLC R_f 0.30 (EtOAc/hexanes, 70/30); $[\alpha]_D^{25}$ −17.7 (*c* 0.97, MeOH); IR (ATR) ν_{max} 502, 699, 1043, 1105, 1427, 2929, 3373 cm^{-1}; HRESIMS *m*/*z* 411.15996 [M + Na]$^+$ (calcd for $C_{21}H_{28}NaO_5Si$, 411.15982); NMR data were in good agreement with published data.[5b]

1,2-O-Isopropylidene-5-O-tert-butyldiphenylsilyl-β-L-arabinose. To a solution of 5-*O*-*tert*-butyldiphenylsilyl-L-arabinose (3.36 g, 8.64 mmol, 1 equiv) in dry acetone (34 mL) were added $CuSO_4$ (6.35 g, 39.8 mmol, 4.6 equiv) and camphorsulfonic acid (0.20 g, 0.87 mmol, 0.1 equiv), and the reaction mixture was stirred at room temperature (rt) overnight. Subsequently, the reaction mixture was neutralized using $NaHCO_3$ (1.45 g, 17.31 mmol, 2 equiv). The resulting mixture was then left to stir for 20 min and filtered through fiberglass filter paper. The filtrate was then concentrated under reduced pressure, and the residue was purified using MPLC (gradient EtOAc/hexanes, 0/100 to 25/75, then isocratic EtOAc/hexanes, 25/75), providing the desired product as a colorless oil (3.20 g, 86%): TLC R_f 0.25 (EtOAc/hexanes, 25/75); $[\alpha]_D^{25}$ −13.1 (*c* 1.05, MeOH); IR (ATR) ν_{max} 701, 1014, 1063, 2931, 3444 cm^{-1}; HRESIMS *m*/*z* 451.19121 [M + Na]$^+$ (calcd for $C_{24}H_{32}NaO_5Si$, 451.19112); NMR data were in good agreement with published data.[5b]

1,2-O-Isopropylidene-3-O-benzyl-5-O-tert-butyldiphenylsilyl-β-L-arabinose. To a solution of 1,2-*O*-isopropylidene-5-*O*-*tert*-butyldiphenylsilyl-β-L-arabinose (3.20 g 7.47 mmol, 1 equiv) in dry tetrahydrofuran (THF) (72 mL) under an argon atmosphere was added portionwise NaH (0.85 g, 22.41 mmol, 3 equiv, 63% dispersion in mineral oil). Subsequently, benzyl bromide (1.77 mL, 14.94 mmol, 2 equiv) was added, and the reaction mixture was stirred at rt for 12 h. The reaction was quenched with saturated NH_4Cl solution. The layers were then separated, and the aqueous layer was extracted using EtOAc (3 × 160 mL). Combined organic extracts were dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified using MPLC (gradient EtOAc/hexanes, 0/100 to 5/95, then isocratic EtOAc/hexanes, 5/95), providing 1,2-*O*-isopropylidene-3-*O*-benzyl-5-*O*-*tert*-butyldiphenylsilyl-β-L-arabinose as a colorless oil (3.41 g, 88%): TLC R_f 0.10 (EtOAc/hexanes, 5/95); $[\alpha]_D^{25}$ −13.4 (*c* 0.96, MeOH); IR (ATR) ν_{max} 504, 699, 1017, 1065, 1102, 2856, 2931 cm^{-1}; HRESIMS *m*/*z* 541.23814 [M + Na]$^+$ (calcd for $C_{31}H_{38}NaO_5Si$, 541.23807); NMR data were in good agreement with published data.[5c]

3-O-Benzyl-5-O-tert-butyldiphenylsilyl-L-arabinose. To a solution of 1,2-*O*-isopropylidene-3-*O*-benzyl-5-*O*-*tert*-butyldiphenylsilyl-β-L-arabinose (2.12 g, 4.09 mmol, 1 equiv) in CH_2Cl_2 (33 mL) at 0 °C was added trifluoroacetic acid (TFA) (4 mL, 44.96 mmol, 11 equiv, 90% w/w). The solution was stirred at 0 °C for 3 h followed by the addition of saturated $NaHCO_3$ solution until no evolution of CO_2 was

DOI: 10.1021/acs.jnatprod.7b00212
J. Nat. Prod. XXXX, XXX, XXX−XXX

observed. The resulting layers were separated, and the aqueous phase was extracted using CH_2Cl_2 (3 × 180 mL). Combined organic extracts were dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified using MPLC (gradient EtOAc/hexanes, 0/100 to 35/65, then isocratic EtOAc/hexanes, 35/65), providing 3-*O*-benzyl-5-*O*-*tert*-butyldiphenylsilyl-L-arabinose as a colorless oil (1.68 g, 86%): TLC R_f 0.22 (EtOAc/hexanes, 35/65); $[\alpha]_D^{25}$ −18.5 (*c* 1.07, MeOH); IR (ATR) ν_{max} 504, 699, 1047, 1105, 1427, 2856, 2929, 3400 cm^{-1}; HRESIMS *m*/*z* 501.20696 $[M + Na]^+$ (calcd for $C_{28}H_{34}NaO_5Si$, 501.20677); NMR data were in good agreement with published data.[5d]

3-*O*-Benzyl-1-*O*-*tert*-butyldiphenylsilyl-L-*xylo*-hex-5-enitol (7). To a suspension of $MePPh_3Br$ (2.99 g, 8.36 mmol, 4 equiv) in dry THF (50 mL) at 0 °C under an argon atmosphere was added dropwise *n*BuLi (3.43 mL, 8.57 mmol, 4.1 equiv of 2.5 M in hexane). The reaction mixture was left to stir at 0 °C for 5 min. Subsequently, the solution of 3-*O*-benzyl-5-*O*-*tert*-butyldiphenylsilyl-L-arabinose (1.00 g, 2.09 mmol, 1 equiv) in dry THF (10 mL) was added, and the reaction mixture was left to stir for another 2.5 h. The reaction was quenched with a saturated solution of NH_4Cl (15 mL). The resulting layers were separated, and the aqueous phase was extracted using EtOAc (3 × 100 mL). Combined organic extracts were dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by MPLC (gradient EtOAc/hexanes, 0/100 to 20/80, then isocratic EtOAc/hexanes, 20/80), providing the desired product **7** as a light yellow oil (0.75 g, 76%): TLC R_f 0.18 (EtOAc/hexanes, 20/80); $[\alpha]_D^{25}$ −13.4 (*c* 0.48, $CHCl_3$), −17.6 (*c*, 0.76 MeOH); IR (ATR) ν_{max} 504, 699, 738, 1070, 1105, 2929, 3417 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ_H 7.60−7.56 (m, 4H), 7.37−7.24 (m, 6H), 7.20−7.08 (m, 5H), 5.96 (ddd, *J* = 17.3, 10.6, 5.0 Hz, 1H), 5.30 (dt, *J* = 17.3, 1.7 Hz, 1H), 5.14 (dt, *J* = 10.6, 1.7 Hz, 1H), 4.53 (d, *J* = 11.2 Hz, 1H), 4.44 (d, *J* = 11.2 Hz, 1H), 4.36 (ddt, *J* = 5.0, 3.2, 1.7 Hz, 1H), 3.84 (ddd, *J* = 7.2, 5.7, 3.8 Hz, 1H), 3.76 (dd, *J* = 10.4, 3.8 Hz, 1H), 3.69 (dd, *J* = 10.4, 5.7 Hz, 1H), 3.51 (dd, *J* = 7.2, 3.2 Hz, 1H), 3.16 (br s, 2H), 0.99 (s, 9H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ_C 138.1, 137.8, 135.51, 135.47, 133.1, 132.9, 129.7, 128.2, 127.9, 127.7, 115.5, 80.0, 73.5, 71.7, 71.6, 64.9, 26.8, 19.2 ppm; HRESIMS *m*/*z* 499.22766 $[M + Na]^+$ (calcd for $C_{29}H_{36}NaO_4Si$, 499.22751).

3,6-Anhydro-2-deoxy-7-*O*-*tert*-butyldiphenylsilyl-L-*gluco*-heptono-1,4-lactone (8). To alkenol **7** (0.40 g, 0.84 mmol, 1 equiv), anhydrous LiOAc (0.22 g, 3.36 mmol, 4 equiv), and anhydrous $CuCl_2$ (0.45 g, 3.36 mmol, 4 equiv) in a 7 mL screw cap vial was added dry AcOH (3.36 mL, 0.25 M according to **7**). The reaction mixture was stirred for 15 min at rt followed by the addition of $PdCl_2(CH_3CN)_2$ (0.02 g, 0,08 mmol, 0,1 equiv) and $Fe(CO)_5$ (28.3 μL, 0.04 mmol, 0.25 equiv). ***Caution:*** *The vial must be immediately closed after the addition of* $Fe(CO)_5$ *to the* reaction *mixture!* The mixture was then stirred for 35 min at 60 °C and concentrated under reduced pressure. The crude product was absorbed onto silica gel and purified using MPLC (gradient EtOAc/Hex, 0/100 to 25/75, then isocratic EtOAc/hexanes, 25/75). The desired product **8** was obtained as a colorless oil (0.32 g) in 76% yield: TLC R_f 0.2 (EtOAc/hexanes, 25/75); $[\alpha]_D^{25}$ +9.02 (*c* 1.00, $CHCl_3$), +10.81 (*c* 1.02, MeOH); IR (ATR) ν_{max} 503, 699, 1047, 1105, 1143, 1786, 2929 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ_H 7.68−7.62 (m, 4H), 7.47−7.26 (m, 11H), 4.90 (dd, *J* = 4.4, 1.1 Hz, 1H), 4.79 (td, *J* = 4.4, 2.2 Hz, 1H), 4.65 (d, *J* = 11.7 Hz, 1H), 4.55 (d, *J* = 11.7 Hz, 1H), 4.25 (dd, *J* = 5.7, 1.1 Hz, 1H), 4.00 (dt, *J* = 5.7, 4.0 Hz, 1H), 3.79 (dd, *J* = 14.5, 4.0 Hz, 1H), 3.76 (dd, *J* = 14.5, 4.0 Hz, 1H), 2.77−2.64 (m, 2H), 1.04 (s, 9H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ_C 174.9, 137.1, 135.8, 135.7, 133.4, 133.2, 129.9, 128.7, 128.2, 128.0, 127.9, 88.0, 85.4, 83.3, 77.5, 72.7, 63.1, 36.1, 26.9, 19.4 ppm; HRESIMS *m*/*z* 525.20649 $[M + Na]^+$ (calcd for $C_{30}H_{34}NaO_5Si$, 525.20677).

3,6-Anhydro-2-deoxy-L-*gluco*-heptono-1,4-lactone (10). To a solution of L-arabinose (12.00 g, 79.97 mmol, 1 equiv) and Meldrum's acid (11.53 g, 79.97 mmol, 1 equiv) in anhydrous dimethylformamide (DMF) (42 mL) was added triethylamine (11.15 mL, 79.97 mmol, 1 equiv). The solution was left to stir at 40−50 °C under an argon atmosphere for 10 days. The mixture was then concentrated under reduced pressure and absorbed onto silica gel. The crude product was purified using MPLC (first separation: isocratic MeOH/CH_2Cl_2, 10/90, the second separation, gradient EtOAc/hexanes, 0/100, to EtOAc/hexanes, 100/0, then isocratic EtOAc/hexanes, 100/0), providing the desired compound **10** as a white crystalline solid (4.22 g, 30%): TLC R_f 0.18 (MeOH/CH_2Cl_2, 10/90); mp 50−52 °C; $[\alpha]_D^{25}$ +44.3 (*c* 1.03, MeOH); IR (ATR) ν_{max} 566, 655, 993, 1026, 1754, 2935, 3311 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ_H 5.60 (d, *J* = 5.0 Hz, 1H), 4.81 (t, *J* = 5.7 Hz, 1H), 4.75 (dd, *J* = 4.4, 1.0 Hz, 1H), 4.69 (t, *J* = 4.8 Hz,1H), 4.02 (t, *J* = 4.9 Hz, 1H), 3.68 (dd, *J* = 10.5, 4.8 Hz, 1H), 3.46 (ddd, *J* = 11.7, 5.5, 4.4 Hz, 1H), 3.38 (dd, *J* = 11.7, 5.8 Hz, 1H), 2.86 (dd, *J* = 18.2, 5.6 Hz, 1H), 2.44 (d, *J* = 18.1 Hz, 1H) ppm; ^{13}C NMR (75 MHz, CD_3OD) δ_C 175.6, 90.1, 87.2, 75.4, 61.2, 39.5, 36.0 ppm; HRESIMS *m*/*z* 175.06014 $[M + H]^+$ (calcd for $C_7H_{11}O_5$, 175.06010).

3,6-Anhydro-2-deoxy-5,7-di-*O*-*tert*-butyldimethylsilyl-L-*gluco*-heptono-1,4-lactone (11). To a solution of **10** (4.22 g, 24.25 mmol, 1 equiv) in anhydrous DMF (70 mL) were added imidazole (8.25 g, 121.27 mmol, 5 equiv) and *tert*-butyldimethylsilyl chloride (TBSCl) (12.79 g, 84.89 mmol, 3.5 equiv). The mixture was left to stir at 50 °C for 3 h and then diluted with EtOAc (450 mL). The organic phase was washed with deionized H_2O (3 × 150 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification of the residue using MPLC (gradient EtOAc/hexanes, 0/100, to EtOAc/hexanes, 10/90, then isocratic EtOAc/hexanes, 10/90) provided the desired product **11** as a colorless oil (8.84 g, 91%): TLC R_f 0.24 (EtOAc/hexanes, 10/90); $[\alpha]_D^{25}$ +17.2 (*c* 1.11, MeOH); IR (ATR) ν_{max} 776, 832, 1046, 1108, 1253, 1790, 2929 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ_H 4.79 (td, *J* = 4.5, 2.2 Hz, 1H), 4.70 (dd, *J* = 4.5, 1.3 Hz), 4.34 (dd, *J* = 5.1, 1.3 Hz, 1H), 3.80 (dt, *J* = 5.1, 4.3 Hz, 1H), 3.70 (dd, *J* = 9.8, 4.3 Hz, 1H), 3.66 (dd, *J* = 9.8, 4.3 Hz, 1H), 2.74−2.62 (m, 2H), 0.90 (s, 9H), 0.89 (s, 9H), 0.13 (s, 3H), 0.11 (s, 3H), 0.054 (s, 3H), 0.05 (s, 3H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ_C 175.2, 90.6, 87.6, 77.3, 76.9, 62.4, 36.2, 26.0, 25.8, 18.5, 18.1, −4.7, −4.9, −5.2, −5.3 ppm; HRESIMS *m*/*z* 403.23302 $[M + H]^+$ (calcd for $C_{19}H_{39}O_5Si_2$, 403.23305).

3,6-Anhydro-2-deoxy-5-*O*-*tert*-butyldimethylsilyl-L-*gluco*-heptono-1,4-lactone (12). To a solution of **11** (8.50 g, 21.11 mmol, 1 equiv) in isopropyl alcohol (210 mL) were added ceric ammonium nitrate (CAN) (11.57 g, 21.11 mmol, 1 equiv) and deionized H_2O (6 mL). The mixture was left to stir at rt for 6 h. Subsequently, the reaction mixture was diluted with EtOAc (1500 mL) and washed with saturated $NaHCO_3$ solution (400 mL) and deionized H_2O (3 × 250 mL). The separated organic phase was then dried over Na_2SO_4, filtered, and concentrated under reduced pressure, affording a crude solid product. The resulting white solid was then suspended in hot hexanes (75 mL), cooled to rt, and filtered, providing pure product **12** (4.08 g, 67%): TLC R_f 0.16 (EtOAc/hexanes, 35/65); mp 105.6−106.4 °C; $[\alpha]_D^{25}$ +30.3 (*c* 1.05, MeOH); IR (ATR) ν_{max} 779, 833, 1035, 1052, 1762, 2954, 3458 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ_H 4.81 (td, *J* = 4.5, 2.1 Hz, 1H), 4.74 (dd, *J* = 4.5, 1.4 Hz, 1H), 4.26 (dd, *J* = 5.8, 1.4 Hz, 1H), 3.87−3.80 (m, 2H), 3.66 (dd, *J* = 12.6, 5.9 Hz, 1H), 2.78−2.65 (m, 2H), 1.69 (t, *J* = 5.8 Hz, 1H), 0.90 (s, 9H), 0.14 (s, *J* = 7.6 Hz, 3H), 0.11 (s, *J* = 7.6 Hz, 3H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ_C 175.0, 90.8, 87.0, 77.3, 77.0, 61.9, 36.0, 25.8, 18.0, −4.7, −4.9 ppm; HRESIMS *m*/*z* 289.14659 $[M + H]^+$ (calcd for $C_{13}H_{25}O_5Si$, 289.14658).

3,6-Anhydro-2,8-dideoxy-5-*O*-*tert*-butyldimethylsilyl-L-*glycero*-L-*gluco*-octano-1,4-lactone (13) and 3,6-Anhydro-2,8-dideoxy-5-*O*-*tert*-butyldimethylsilyl-D-*glycero*-L-*gluco*-octano-1,4-lactone (14). To a solution of **12** (1.00 g, 3.47 mmol, 1 equiv) in CH_2Cl_2 (30 mL) were added Dess−Martin reagent (2.21 g, 5.20 mmol, 1.5 equiv) and solid $NaHCO_3$ (1.46 g, 17.34 mmol, 5 equiv). The resulting suspension was left to stir at rt for 3 h and then filtered through a column filled with 20 g of silica gel. The crude product was eluted with an EtOAc/hexanes mixture (30/70, 200 mL), and the filtrate concentrated to dryness under reduced pressure, providing a crude aldehyde.

The crude aldehyde and anhydrous LiCl (0.29 g, 6.93 mmol, 2 equiv) were then dissolved in anhydrous THF (14 mL), and a MeMgBr solution (1.27 mL, 3.81 mmol, 1.1 equiv, 3 M in Et_2O) was added dropwise (0.05 mL/min) at −78 °C under an argon

DOI: 10.1021/acs.jnatprod.7b00212
J. Nat. Prod. XXXX, XXX, XXX−XXX

atmosphere. The solution was left to stir for 2 h at −78 °C and then left to warm to rt over 10 min. The reaction was quenched by the addition of a saturated NH_4Cl solution (5 mL). The layers were separated, and the aqueous phase was extracted with EtOAc (2 × 25 mL). The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification of the residue using MPLC (isocratic MeOH/EtOAc/hexanes, 1/20/80) provided a mixture of diastereomers **13** and **14** as a white solid (0.41g, 39%, **13**/**14** = 3/2). A second MPLC purification (150 equiv of SiO_2, isocratic EtOAc/hexanes, 25/75) of this mixture provided pure products **13** and **14**.

Aldehyde: TLC R_f 0.25 (EtOAc/hexanes, 25/75); ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 9.53 (d, J = 1.5 Hz, 1H), 5.03 (t, J = 4.1 Hz, 1H), 4.67 (dd, J = 4.1, 0.9 Hz, 1H), 4.58 (dt, J = 1.5, 0.9 Hz, 1H), 4.30 (t, J = 1.5 Hz, 1H), 2.87 (d, J = 18.3 Hz, 1H), 2.74 (dd, J = 18.3, 4.1 Hz, 1H), 0.90 (s, 9H), 0.14 (s, 6H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ_C 199.5, 174.3, 92.0, 87.8, 79.4, 78.2, 36.3, 25.7, 18.1, −4.84 ppm.

Compound **13**: TLC R_f 0.32 (EtOAc/hexanes, 40/60); mp 61.7−63.5 °C; $[\alpha]_D^{25}$ +35.3 (c 1.00, $CHCl_3$); IR (ATR) ν_{max} 776, 835, 1114, 1772, 2860, 2929, 3483 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 4.78 (td, J = 4.2, 2.2 Hz, 1H), 4.69 (dd, J = 4.2, 0.8 Hz, 1H), 4.40 (dd, J = 5.3, 0.8 Hz, 1H), 3.93 (qd, J = 6.5, 4.3 Hz, 1H), 3.68 (dd, J = 5.3, 4.3 Hz, 1H), 2.76−2.62 (m, 2H), 1.20 (d, J = 6.5 Hz, 3H), 0.90 (s, 9H), 0.15 (s, 3H), 0.13 (s, 3H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ_C 175.1, 91.0, 90.6, 77.3, 76.3, 66.8, 36.1, 25.8, 18.7, 17.9, −4.7, −4.9 ppm; HRESIMS m/z 325.14413 $[M + Na]^+$ (calcd for $C_{14}H_{26}NaO_5Si$, 325.14417).

Compound **14**: TLC R_f 0.26 (EtOAc/hexanes, 40/60); mp 105.0−106.5 °C; $[\alpha]_D^{25}$ +23.2 (c 1.00, $CHCl_3$); IR (ATR) ν_{max} 783, 835, 1060, 1763, 2856, 2956, 3459 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 4.80 (td, J = 4.4, 2.2 Hz, 1H), 4.72 (dd, J = 4.4, 1.3 Hz, 1H), 4.24 (dd, J = 5.6, 1.3 Hz, 1H), 3.79 (qd, J = 6.5, 5.6 Hz, 1H), 3.60 (t, J = 5.6 Hz, 1H), 2.78−2.65 (m, 2H), 1.25 (d, J = 6.5 Hz, 3H), 0.90 (s, 9H), 0.15 (s, 3H), 0.12 (s, 3H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ_C 175.0, 90.9, 90.7, 77.7, 77.2, 67.4, 36.0, 25.8, 19.7, 18.0, −4.5, −4.9 ppm; HRESIMS m/z 325.14404 $[M + Na]^+$ (calcd for $C_{14}H_{26}NaO_5Si$, 325.14417).

3,6-Anhydro-2,8-dideoxy-L-*glycero*-L-*gluco*-octano-1,4-lactone (Protulactone A, 1a). To a solution of 4-dimethylaminopyridine (DMAP) (100 mg, 0.82 mmol, 2.2 equiv) in anhydrous CH_2Cl_2 (1 mL) was added AcCl (53 μL, 0.74 mmol, 2 equiv), and the mixture was left to stir for 15 min. Subsequently, the solution of **13** (112 mg, 0.37 mmol, 1 equiv) in anhydrous CH_2Cl_2 (2 mL) was added, and the reaction mixture was left to stir for 1.5 h followed by the addition of CH_2Cl_2 (25 mL). The resulting mixture was then washed with deionized H_2O (2 × 10 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure, providing a crude product.

Subsequently, the crude product was dissolved in THF (2.5 mL), and a solution of TBAF·3H_2O (175 mg, 0.56 mmol, 1.5 equiv) and AcOH (32 μL, 0.56 mmol, 1.5 equiv) in THF (2.5 mL) was added. The reaction mixture was left to stir for 2 h and then concentrated under reduced pressure to one-fifth of its volume. The crude product was purified using MPLC (gradient EtOAc/hexanes, 0/100, to EtOAc/hexanes, 100/0, then isocratic EtOAc/hexanes, 100/0), providing protulactone A (**1a**) as a colorless oil (67 mg, 79%): TLC R_f 0.17 (EtOAc/hexanes, 40/60); $[\alpha]_D^{25}$ +38.2 (c 1.1, MeOH), +35.6 (c 0.34, MeOH), +35.2 (c 1.05, $CHCl_3$); IR (ATR) ν_{max} 505, 552, 945, 960, 1728, 1780, 3446 cm^{-1}; ^{1}H NMR (300 MHz, CD_3OD) δ_H 5.07 (dq, J = 6.6, 4.0 Hz, 1H), 4.82−4.77 (m, 2H), 4.33 (d, J = 4.7 Hz, 1H), 3.80 (dd, J = 4.7, 4.0 Hz, 1H), 2.90−2.80 (m, 1H), 2.53 (dd, J = 18.1, 0.8 Hz, 1H), 2.00 (s, 3H), 1.25 (d, J = 6.6 Hz, 3H) ppm; ^{13}C NMR (75 MHz, CD_3OD) δ_C 177.5, 172.1, 92.2, 90.2, 79.2, 76.6, 70.6, 37.0, 21.0, 16.3 ppm; HRESIMS m/z 231.08639 $[M + H]^+$ (calcd for $C_{10}H_{15}O_6$, 231.08631).

3,6-Anhydro-2,8-dideoxy-D-*glycero*-L-*gluco*-octano-1,4-lactone (7-*epi*-protulactone A, 1b). To a solution of DMAP (71 mg, 0.58 mmol, 2.2 equiv) in anhydrous CH_2Cl_2 (1 mL) was added AcCl (38 μL, 0.53 mmol, 2 equiv), and the mixture was left to stir for 15 min. Subsequently, the solution of **14** (80 mg, 0.26 mmol, 1 equiv) in anhydrous CH_2Cl_2 (1 mL) was added, and the reaction mixture was left to stir for 1.5 h. The mixture was then diluted with CH_2Cl_2 (25 mL), washed with deionized H_2O (2 × 10 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure.

Subsequently, the crude product was dissolved in THF (1.5 mL), and a solution of TBAF·3H_2O (125 mg, 0.40 mmol, 1.5 equiv) and AcOH (26 μL, 0.40 mmol, 1.5 equiv) in THF (1.5 mL) was added. The resulting mixture was left to stir for 2 h and then concentrated under reduced pressure to one-fifth of its volume. The crude product was purified using MPLC (gradient EtOAc/hexanes, 0/100, to EtOAc/hexanes, 100/0, then isocratic EtOAc/hexanes, 100/0), providing **1b** as a colorless oil (47 mg, 77%): TLC R_f 0.19 (EtOAc/hexane, 40/60); $[\alpha]_D^{25}$ +53.6 (c 1.15, MeOH), +50.4 (c 0.34, MeOH), +65.3 (c 1.10, $CHCl_3$); IR (ATR) ν_{max} 553, 608, 945, 1041, 1731, 1782, 3451 cm^{-1}; ^{1}H NMR (300 MHz, CD_3OD) δ_H 5.04 (dq, J = 6.5, 5.0 Hz, 1H), 4.83−4.79 (m, 2H, 3-H), 4.15 (d, J = 4.5 Hz, 1H), 3.81 (t, J = 5.0 Hz, 1H), 2.85 (dd, J = 18.2, 4.8 Hz, 1H), 2.57 (dd, J = 18.2, 0.6 Hz, 1H), 2.02 (s, 3H), 1.26 (d, J = 6.5 Hz, 3H) ppm; ^{13}C NMR (75 MHz, CD_3OD) δ_C 177.6, 172.3, 92.0, 90.1, 79.2, 77.6, 71.0, 37.0, 21.0, 16.8 ppm; HRESIMS m/z 231.08636 $[M + H]^+$ (calcd for $C_{10}H_{15}O_6$, 231.08631).

3,6-Anhydro-2-deoxy-D-*gluco*-heptono-1,4-lactone (*ent*-10). *Procedure A.* To alkenol **9** (0.12 g, 0.81 mmol, 1 equiv), anhydrous LiOAc (0.27 g, 4.05 mmol, 5 equiv), and anhydrous $CuCl_2$ (0.54 g, 4.05 mmol, 5 equiv) in a 7 mL screw cap vial was added dry AcOH (3.24 mL, 0.25 M according to **7**). The reaction mixture was stirred for 15 min at rt followed by the addition of $PdCl_2(CH_3CN)_2$ (0.02 g, 0.08 mmol, 0.1 equiv) and $Fe(CO)_5$ (53 μL, 0.4 mmol, 0.5 equiv). ***Caution:*** *The vial must be immediately closed after the addition of $Fe(CO)_5$ to the reaction mixture!* The mixture was then stirred for 1 h at 60 °C and concentrated under reduced pressure. The crude product was absorbed onto silica gel and purified using MPLC (gradient EtOAc/hexanes, 0/100 to 100/0). The desired product *ent*-**10** was obtained as a white crystalline solid (0.66 g) in 47% yield.

Procedure B. To a solution of D-arabinose (15.00 g, 99.96 mmol, 1 equiv) and Meldrum's acid (14.41 g, 99.96 mmol, 1 equiv) in anhydrous DMF (53 mL) was added triethylamine (13.94 mL, 99.96 mmol, 1 equiv). The solution was left to stir at 40−50 °C under an argon atmosphere for 10 days. The mixture was then concentrated under reduced pressure and absorbed onto silica gel. The crude product was purified using MPLC (first separation: isocratic MeOH/CH_2Cl_2, 10/90, second separation, gradient EtOAc/hexanes, 0/100, to EtOAc/hexanes, 100/0, then isocratic EtOAc/hexanes, 100/0), providing the desired lactone *ent*-**10** as a white, crystalline solid (4.86 g, 28%): TLC R_f 0.27 (MeOH/CH_2Cl_2, 15/85); mp 50−52 °C; $[\alpha]_D^{25}$ −43.9 (c 1.03, MeOH); IR (ATR) ν_{max} 499, 1006, 1021, 1743, 2939, 3439, 3515 cm^{-1}; ^{1}H NMR (300 MHz, CD_3OD) δ_H 4.83−4.78 (m, 2H), 4.17 (d, J = 5.4 Hz, 1H), 3.81 (td, J = 5.6, 3.8 Hz, 1H), 3.70 (dd, J = 11.9, 3.8 Hz, 1H), 3.58 (dd, J = 11.9, 5.8 Hz, 1H), 2.85 (dd, J = 18.2, 5.1 Hz, 1H), 2.59 (d, J = 18.2 Hz, 1H) ppm; ^{13}C NMR (75 MHz, CD_3OD) δ_C 177.8, 92.2, 88.4, 78.9, 77.2, 62.8, 36.9 ppm; HRESIMS m/z 175.06022 $[M + H]^+$ (calcd for $C_7H_{11}O_5$, 175.06010); all spectroscopic data were in good agreement with published data.[5a]

3,6-Anhydro-2-deoxy-5,7-di-*O*-*tert*-butyldimethylsilyl-D-*gluco*-heptono-1,4-lactone (*ent*-11). To a solution of *ent*-**10** (4.19 g, 22.06 mmol, 1 equiv) in anhydrous DMF (72 mL) were added imidazole (8.19 g, 120.3 mmol, 5 equiv) and TBSCl (12.69 g, 84.21 mmol, 3.5 equiv). The mixture was left to stir at 50 °C for 3 h and then diluted with EtOAc (450 mL). The organic phase was then washed with deionized H_2O (3 × 150 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification of the residue using MPLC (gradient EtOAc/hexanes, 0/100, to EtOAc/hexanes, 10/90, then isocratic EtOAc/hexanes, 10/90) provided the desired product *ent*-**11** as a colorless oil (9.11 g, 94%): TLC R_f 0.5 (EtOAc/hexanes, 30/70); $[\alpha]_D^{25}$ −21.6 (c 3.30, MeOH); IR (ATR) ν_{max} 671, 833, 1046, 1108, 1790, 2858, 2929 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 4.77 (td, J = 4.5, 2.2 Hz, 1H), 4.69 (dd, J = 4.5, 1.3 Hz, 1H), 4.33 (dd, J = 5.1, 1.3 Hz, 1H), 3.80 (dt, J = 5.1, 4.3 Hz, 1H), 3.69 (dd, J = 12.0, 4.3 Hz, 1H), 3.65 (dd, J = 12.0, 5.1 Hz, 1H), 2.73−2.60 (m, 2H), 0.88 (s, 9H), 0.87 (s, 9H), 0.12 (s, 3H), 0.09 (s, 3H),

DOI: 10.1021/acs.jnatprod.7b00212
J. Nat. Prod. XXXX, XXX, XXX−XXX

0.04 (s, 3H), 0.036 (s, 3H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ_C 175.1, 90.6, 87.6, 77.2, 76.8, 62.4, 36.2, 26.0, 25.8, 18.5, 18.0, −4.7, −4.9, −5.2, −5.3 ppm; HRESIMS m/z 403.23354 $[M + H]^+$ (calcd for $C_{19}H_{39}O_5Si_2$, 403.23305).

3,6-Anhydro-2-deoxy-5-*O*-*tert*-butyldimethylsilyl-D-*gluco*-heptono-1,4-lactone (*ent*-12). To a solution of *ent*-**11** (9.11 g, 22.62 mmol, 1 equiv) in isopropyl alcohol (237 mL) was added CAN (12.40 g, 22.62 mmol, 1 equiv) and deionized H_2O (7 mL). The mixture was left to stir at rt for 6 h. Subsequently, the mixture was diluted with EtOAc (1500 mL) and washed with saturated $NaHCO_3$ solution (400 mL) and deionized H_2O (3 × 250 mL). The separated organic phase was then dried over Na_2SO_4, filtered, and concentrated under reduced pressure, affording a crude solid product. The resulting white solid was then suspended in hot hexanes (75 mL), cooled to rt, and filtered, providing pure product *ent*-**12** (4.40 g, 67%): TLC R_f 0.27 (EtOAc/hexanes, 35/65); mp 106.5−107.8 °C; $[\alpha]_D^{25}$ −29.3 (*c* 0.90, MeOH); IR (ATR) ν_{max} 434, 833, 1052, 1762, 2856, 2952, 3460 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 4.81 (td, *J* = 4.5, 2.1 Hz, 1H), 4.74 (dd, *J* = 4.5, 1.4 Hz, 1H), 4.26 (dd, *J* = 5.8, 1.4 Hz, 1H), 3.86−3.81 (m, 2H), 3.65 (dd, *J* = 12.6, 5.9 Hz, 1H), 2.78−2.65 (m, 2H), 0.90 (s, 9H), 0.14 (s, 3H), 0.11 (s, 3H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ_C 175.0, 90.8, 87.0, 77.4, 77.1, 62.0, 36.0, 25.8, 18.0, −4.7, −4.9 ppm; HRESIMS m/z 289.14663 $[M + H]^+$ (calcd for $C_{13}H_{25}O_5Si$, 289.14658).

3,6-Anhydro-2,8-dideoxy-5-*O*-*tert*-butyldimethylsilyl-D-*glycero*-D-*gluco*-octano-1,4-lactone (*ent*-13) and 3,6-Anhydro-2,8-dideoxy-5-*O*-*tert*-butyldimethylsilyl-L-*glycero*-D-*gluco*-octano-1,4-lactone (*ent*-14). To a solution of *ent*-**12** (1.00 g, 3.47 mmol, 1 equiv) in CH_2Cl_2 (30 mL) were added Dess−Martin reagent (2.21 g, 5.20 mmol, 1.5 equiv) and solid $NaHCO_3$ (1.46 g, 17.34 mmol, 5 equiv). The resulting suspension was left to stir at rt for 3 h and then filtered through a column filled with 20 g of silica gel. The crude product was eluted with an EtOAc/hexanes mixture (30/70, 200 mL) and the filtrate concentrated under reduced pressure, providing a crude aldehyde.

The crude aldehyde and andydrous LiCl (0.29 g, 6.93 mmol, 2 equiv) were then subsequently dissolved in anhydrous THF (14 mL), and MeMgBr solution (1.27 mL, 3.81 mmol, 1.1 equiv, 3 M in Et_2O) was added dropwise (0.05 mL/min) at −78 °C under an argon atmosphere. The solution was left to stir for 2 h at −78 °C and then left to warm to rt over 10 min. The reaction was quenched with saturated NH_4Cl solution (5 mL). The layers were separated, and the aqueous phase was extracted with EtOAc (2 × 25 mL). The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification of the residue using MPLC (isocratic MeOH/EtOAc/hexanes, 1/20/80) provided a mixture of diastereomers *ent*-**13** and *ent*-**14** as white solids (0.42g, 40%, *ent*-**13**/*ent*-**14** = 3/2). A second MPLC purification (150 equiv of SiO_2, isocratic EtOAc/hexanes, 25/75) of this mixture provided pure products *ent*-**13** and *ent*-**14**.

ent-Aldehyde: TLC R_f 0.25 (EtOAc/hexanes, 40/60); ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 9.55 (d, *J* = 1.5 Hz, 1H), 5.04 (t, *J* = 4.1 Hz, 1H), 4.68 (dd, *J* = 4.1, 0.9 Hz, 1H), 4.59 (ddd, *J* = 2.3, 1.5, 0.9 Hz, 1H), 4.30 (t, *J* = 1.5 Hz, 1H), 2.88 (d, *J* = 18.3 Hz, 1H), 2.75 (dd, *J* = 18.3, 4.1 Hz, 1H), 0.91 (s, 9H), 0.15 (s, 6H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ_C 199.6, 174.2, 92.1, 87.9, 79.5, 78.3, 36.3, 25.7, 18.1, −4.8, −4.84 ppm.

Compound ent-13: TLC R_f 0.18 (EtOAc/hexanes, 35/65); mp 61.2−63.0 °C; $[\alpha]_D^{25}$ −35.8 (*c* 1.00, $CHCl_3$); IR (ATR) ν_{max} 547, 834, 1048, 1774, 2858, 2929, 3467 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 4.78 (td, *J* = 4.2, 2.2 Hz, 1H, H-5), 4.69 (dd, *J* = 4.2, 0.8 Hz, 1H, H-1), 4.41 (dd, *J* = 5.3, 0.8 Hz, 1H), 3.94 (qd, *J* = 6.5, 4.3 Hz, 1H), 3.68 (dd, *J* = 5.3, 4.3 Hz, 1H), 2.76−2.62 (m, 2H), 1.20 (d, *J* = 6.5 Hz, 3H), 0.90 (s, 9H), 0.15 (s, 3H), 0.13 (s, 3H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ_C 175.0, 91.0, 90.6, 77.3, 76.3, 66.8, 36.1, 25.8, 18.7, 17.9, −4.5, −4.9 ppm; HRESIMS m/z 325.14409 $[M + Na]^+$ (calcd for $C_{14}H_{26}NaO_5Si$, 325.14417).

Compound ent-14: TLC R_f 0.13 (EtOAc/hexanes, 35/65); mp 104.2−106.3 °C; $[\alpha]_D^{25}$ −22.3 (c 1.00, $CHCl_3$); IR (ATR) ν_{max} 561, 835, 1041, 1765, 2856, 2927, 3462 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 4.79 (td, *J* = 4.4, 2.2 Hz, 1H), 4.71 (dd, *J* = 4.4, 1.3 Hz, 1H), 4.24 (dd, *J* = 5.6, 1.3 Hz, 1H), 3.78 (qd, *J* = 6.5, 5.6 Hz, 1H), 3.59 (t, *J* = 5.6 Hz, 1H), 2.77−2.65 (m, 2H), 1.24 (d, *J* = 6.5 Hz, 3H), 0.90 (s, 9H), 0.15 (s, 3H), 0.12 (s, 3H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ_C 175.0, 90.9, 90.7, 77.7, 77.2, 67.4, 36.0, 25.8, 19.8, 18.0, −4.5, −4.9 ppm; HRESIMS m/z 325.14400 $[M + Na]^+$ (calcd for $C_{14}H_{26}NaO_5Si$, 325.14417).

3,6-Anhydro-2,8-dideoxy-D-*glycero*-D-*gluco*-octano-1,4-lactone (*ent*-1a). To a solution of DMAP (135 mg, 1.11 mmol, 2.2 equiv) in anhydrous CH_2Cl_2 (2 mL) was added AcCl (72 μL, 1.01 mmol, 2 equiv), and the mixture was left to stir for 15 min. Subsequently, the solution of *ent*-**13** (152 mg, 0.50 mmol, 1 equiv) in anhydrous CH_2Cl_2 (2 mL) was added, and the resulting solution was left to stir for 1.5 h. The reaction mixture was then diluted with CH_2Cl_2 (25 mL), washed with deionized H_2O (2 × 15 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure.

Subsequently, the crude product was dissolved in THF (3.4 mL), and a solution of TBAF·$3H_2O$ (238 mg, 0.75 mmol, 1.5 equiv) and AcOH (43 μL, 0.75 mmol, 1.5 equiv) in THF (3.4 mL) was added. The resulting mixture was left to stir for 2 h and then concentrated under reduced pressure to one-fifth of its volume. The crude product was purified using MPLC (gradient EtOAc/hexanes, 0/100, to EtOAc/hexanes, 100/0, then isocratic EtOAc/hexanes, 100/0), providing *ent*-**1a** as a colorless oil (91 mg, 79% over 2 steps): TLC R_f 0.17 (EtOAc/hexanes, 40/60); $[\alpha]_D^{25}$ −37.5 (*c* 1.16, MeOH), −33.8 (c 0.34, MeOH), −35.2 (c 1.10, $CHCl_3$); IR (ATR) ν_{max} 552, 1040, 1240, 1729, 1780, 2937, 3437 cm^{-1}; ^{1}H NMR (300 MHz, CD_3OD) δ_H 5.07 (dq, *J* = 6.6, 4.0 Hz, 1H), 4.82−4.77 (m, 2H), 4.33 (d, *J* = 4.7 Hz, 1H), 3.80 (dd, *J* = 4.7, 4.0 Hz, 1H), 2.89−2.80 (m, 1H), 2.52 (dd, *J* = 18.1, 0.8 Hz, 1H), 2.00 (s, 3H), 1.25 (d, *J* = 6.6 Hz, 3H) ppm; ^{13}C NMR (75 MHz, CD_3OD) δ_C 177.5, 172.1, 92.2, 90.1, 79.1, 76.6, 70.6, 37.0, 21.0, 16.3 ppm; HRESIMS m/z 231.08639 $[M + H]^+$ (calcd for $C_{10}H_{15}O_6$, 231.08631).

3,6-Anhydro-2,8-dideoxy-L-*glycero*-D-*gluco*-octano-1,4-lactone (*ent*-1b). To a solution of DMAP (42 mg, 0.34 mmol, 2.2 equiv) in anhydrous CH_2Cl_2 (0.5 mL) was added AcCl (22 μL, 0.31 mmol, 2 equiv), and the mixture was left to stir for 15 min at rt. Subsequently, a solution of *ent*-**14** (47 mg, 0.16 mmol, 1 equiv) in anhydrous CH_2Cl_2 (1 mL) was added, and the resulting solution was left to stir for 1.5 h. The reaction mixture was then diluted with CH_2Cl_2 (15 mL), washed with deionized H_2O (2 × 5 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure.

Subsequently, the crude product was dissolved in THF (1 mL), and a solution of TBAF·$3H_2O$ (73 mg, 0.23 mmol, 1.5 equiv) and AcOH (13 μL, 0.23 mmol, 1.5 equiv) in THF (1 mL) was added. The resulting mixture was left to stir for 2 h and then concentrated under reduced pressure to one-fifth of its volume. The crude product was purified using MPLC (gradient EtOAc/hexanes, 0/100, to EtOAc/hexanes, 100/0, then isocratic EtOAc/hexanes, 100/0), providing *ent*-**1b** as a colorless oil (27 mg, 75% over 2 steps): TLC R_f 0.19 (EtOAc/hexanes, 40/60); $[\alpha]_D^{25}$ −56.8 (*c* 1.15, MeOH), −55.8 (*c* 0.34, MeOH) −65.5 (*c* 1.30, $CHCl_3$); IR (ATR) ν_{max} 463, 1039, 1239, 1728, 1779, 2939, 3437 cm^{-1}; ^{1}H NMR (300 MHz, CD_3OD) δ_H 5.04 (dq, *J* = 6.5, 5.0 Hz, 1H), 4.83−4.79 (m, 2H), 4.15 (d, *J* = 4.5 Hz, 1H), 3.81 (t, *J* = 5.0 Hz, 1H), 2.89−2.80 (m, 1H), 2.58 (dd, *J* = 18.2, 0.6 Hz, 1H), 2.02 (s, 3H), 1.26 (d, *J* = 6.5 Hz, 3H) ppm; ^{13}C NMR (75 MHz, CD_3OD) δ_C 177.6, 172.3, 92.0, 90.1, 79.2, 77.6, 71.0, 37.0, 21.0, 16.8 ppm; HRESIMS m/z 231.08635 $[M + H]^+$ (calcd for $C_{10}H_{15}O_6$, 231.08631).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.7b00212.

^{1}H and ^{13}C NMR spectra for all new compounds; one-dose mean graphs (PDF)

DOI: 10.1021/acs.jnatprod.7b00212
J. Nat. Prod. XXXX, XXX, XXX−XXX

crystallographic data (CIF) of compounds **13**, **14**, *ent*-**13**, and *ent*-**14** (CIF)

AUTHOR INFORMATION

Corresponding Author

*E-mail: tibor.gracza@stuba.sk. Tel: +421 2 59325160.

ORCID

Tibor Gracza: 0000-0002-7667-0663

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work was supported by Slovak Grant Agencies (VEGA No. 1/0488/14, APVV-14-0147, and ASFU, Bratislava, ITMS project no. 26240120025). This article was created also with the support of the MŠVVaŠ of the Slovak Republic within the Research and Development Operational Programme for the project "University Science Park of STU Bratislava" (ITMS project no. 26240220084) cofunded by the European Regional Development Fund.

REFERENCES

(1) Sohn, J. H.; Oh, H. *Bull. Korean Chem. Soc.* **2010**, *31*, 1695−1698.

(2) (a) Fang, X.-p.; Anderson, J. E.; Chang, C.-j.; Fanwick, P. E.; McLaughlin, J. L. *J. Chem. Soc., Perkin Trans. 1* **1990**, 1655−1661. (b) Fang, X.-p.; Anderson, J. E.; Chang, C.-j.; McLaughlin, J. L. *J. Nat. Prod.* **1991**, *54*, 1034−1043. (c) Tuchinda, P.; Munyoo, B.; Pohmakotr, M.; Thinapong, P.; Sophasan, S.; Santisuk, T.; Reutrakul, V. *J. Nat. Prod.* **2006**, *69*, 1728−1733.

(3) (a) Mereyala, H. B.; Joe, M. *Curr. Med. Chem.: Anti-Cancer Agents* **2001**, *1*, 293−300. (b) Popsavin, V.; Benedekovič, G.; Srečo, B.; Popsavin, M.; Francuz, J.; Kojič, V.; Bogdanovič, G. *Org. Lett.* **2007**, *9*, 4235−4238. (c) Popsavin, V.; Benedekovič, G.; Srečo, B.; Francuz, J.; Popsavin, M.; Kojič, V.; Bogdanovič, G.; Divjakovič, M. *Tetrahedron* **2009**, *65*, 10596−10607.

(4) (a) Mata, F. Z.; Martinez, M. B.; Galbis Perez, J. A. *Carbohydr. Res.* **1992**, *225*, 159−161. (b) Köll, P.; Wernicke, A.; Kovacs, J.; Lützen, A. *J. Carbohydr. Chem.* **2000**, *19*, 1019−1047.

(5) (a) Gracza, T.; Hasenöhrl, T.; Stahl, U.; Jäger, V. *Synthesis* **1991**, *1991*, 1108−1118. (b) Dahlman, O.; Garegg, P. J.; Mayer, H.; Schramek, S. *Acta Chem. Scand.* **1986**, *40*, 15−20. (c) Pakulski, Z.; Zamojksi, A. *Tetrahedron* **1995**, *51*, 871−908. (d) Varvogli, A.−A. C.; Karagiannis, I. N.; Koumbis, A. E. *Tetrahedron* **2009**, *65*, 1048−1058. (e) Li, Y.-L.; Wu, Y.-L. *Liebigs Annales* **1996**, *12*, 2079−2082.

(6) (a) Gracza, T. In *Stereoselective Synthesis of Drugs & Natural Products*; Andrushko, V.; Andrushko, N., Eds.; Wiley: Hoboken, NJ, USA, 2013; Chapter 15, pp 421−440. (b) Gracza, T.; Jäger, V. *Synlett* **1992**, *1992*, 191−193. (c) Gracza, T.; Jäger, V. *Synthesis* **1994**, *1994*, 1359−1368. (d) Babjak, M.; Kapitán, P.; Gracza, T. *Tetrahedron* **2005**, *61*, 2471−2479. (e) Kapitán, P.; Gracza, T. *ARKIVOC* **2008**, *viii*, 8−17. (f) Kapitán, P.; Gracza, T. *Tetrahedron: Asymmetry* **2008**, *19*, 38−44.

(7) Babjak, M.; Markovič, M.; Kandríková, B.; Gracza, T. *Synthesis* **2014**, *46*, 809−816.

(8) Hwu, J. R.; Jain, M. L.; Tsai, F.-Y.; Tsay, Sh.-Ch. *J. Org. Chem.* **2000**, *65*, 5077−5088.

(9) CCDC-1530640 (for **13**), CCDC-1530641 (for **14**), CCDC-1530642 (for *ent*-**13**), and CCDC-1530643 (for *ent*-**14**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

(10) ^{13}C shifts were all different by similar amounts (0.9−1.4 ppm), likely due to a systematic difference in referencing.

(11) Shing, T. K. M.; Tsui, H.-Ch; Zhou, Z.-H. *J. Org. Chem.* **1995**, *60*, 3121−3130.

(12) (a) Sheldrick, G. M. *Acta Crystallogr., Sect. A: Found. Adv.* **2015**, *A71*, 3−8. (b) Burla, M. C.; Caliandro, R.; Camalli, M.; Carrozzini, B.; Cascarano, G. L.; Giacovazzo, C.; Mallamo, M.; Mazzone, A.; Polidori, G.; Spagna, R. *J. Appl. Crystallogr.* **2012**, *45*, 351−356. (c) Sheldrick, G. M. *Acta Crystallogr.* **2015**, *C71*, 3−8. Dolomanov, O.; Bourhis, L. J.; Gildea, R. I.; Howard, J. A. K.; Puschmann, H. *J. Appl. Crystallogr.* **2009**, *42*, 339−341.

(13) (a) Flack, H. D. *Acta Crystallogr., Sect. A: Found. Crystallogr.* **1983**, *A39*, 876−881. (b) Parsons, S.; Flack, H. D.; Wagner, T. *Acta Crystallogr., Sect. B: Struct. Sci., Cryst. Eng. Mater.* **2013**, *B69*, 249−259.

DOI: 10.1021/acs.jnatprod.7b00212
J. Nat. Prod. XXXX, XXX, XXX−XXX