JOC The Journal of Organic Chemistry

Article

pubs.acs.org/joc

Synthesis of Isoxazolin-5-one Glucosides by a Cascade Reaction

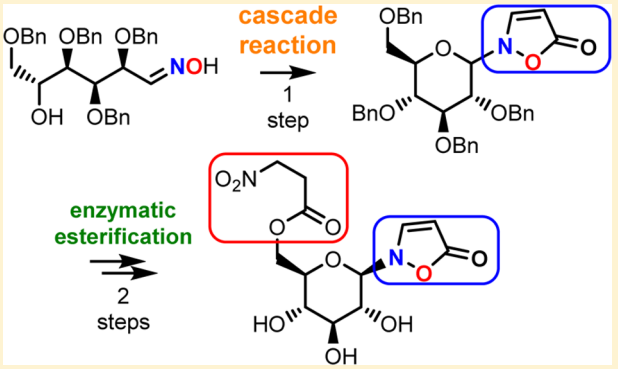
Tobias Becker,[†] Helmar Görls,[‡] Gerhard Pauls,[†] Roland Wedekind,[†] Marco Kai,[†] Stephan H. von Reuß,[†] and Wilhelm Boland*[,†]

[†]Max Planck Institute for Chemical Ecology, Beutenberg Campus, Hans-Knoell-Straße 8, D-07745 Jena, Germany

[‡]Institute for Inorganic and Analytical Chemistry, Humboldtstraße 8, D-07743 Jena, Germany

Supporting Information

ABSTRACT: A novel synthetic route was developed for the construction of isoxazolin-5-one glucosides using a cascade reaction. An X-ray crystal structure analysis of a isoxazolin-5-one glucoside confirmed the structure and stereochemistry of the heterocycle. The properties of the α- and β-anomers of the isoxazolin-5-one glucosides were compared. The first synthesis of 2-[6′-(3″-nitropropanoyl)-β-D-glucopyranosyl]-3-isoxazolin-5-one was realized by direct enzymatic esterification without need of further protective groups.



INTRODUCTION

Isoxazolin-5-one glucosides are natural compounds that were identified as metabolites in legumes (Fabaceae) as well as in certain species of leaf beetles (Chrysomelidae).[1−6] 2-(β-D-Glucopyranosyl)-3-isoxazolin-5-one (**1**) and its nitropropanoyl derivative **2** have been reported to be major components of the defensive secretions of adult leaf beetles.[1−3] Seedlings of *Lathyrus odoratus* show concentrations of compound **1** of up to 0.8% of their dry mass (figure 1).[4]

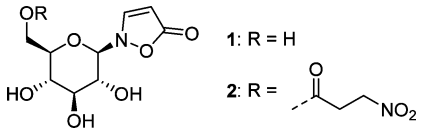


Figure 1. Isoxazolin-5-one glucosides.

A variety of different glucose derivatives of 3-nitropropanoic acid (3-NPA) have been found in several plant, insect, and fungi families.[7] It has been shown that the acid component 3-NPA, is an irreversible inhibitor of succinate dehydrogenase.[8] As a consequence the mitochondrial citric acid cycle is inhibited, causing neurodegenerative symptoms.[9] The esters of 3-NPA serve as pretoxins and storage molecules in different organisms and represent an important class of defensive compounds.[10−13] In order to provide standards for studies concerning the biosynthesis, quantification, and hydrolysis kinetics of **2**, the synthesis of compounds **1** and **2** is of interest. In the case of compound **1**, no efficient synthesis is known, and no synthesis of the 3-NPA derivative **2** has been reported.

The regioselective synthesis of pyranose esters via transesterification has been intensively studied.[14−18] Different strategies for the construction of isoxazolin-5-one moieties have been reported.[19−24] Van Rompuy et al. described a nucleophilic substitution of organohalides with the sodium salt of isoxazolin-5-one.[20−22] This approach requires complicated purification procedures and provides low yields because of the formation of many side products when applied to the synthesis of glucose derivatives.[22] Hence, Baldwin and co-workers developed an alternative method based on a 5-endo-dig cyclization reaction to synthesize amino acid derivatives of isoxazolin-5-one.[23−26]

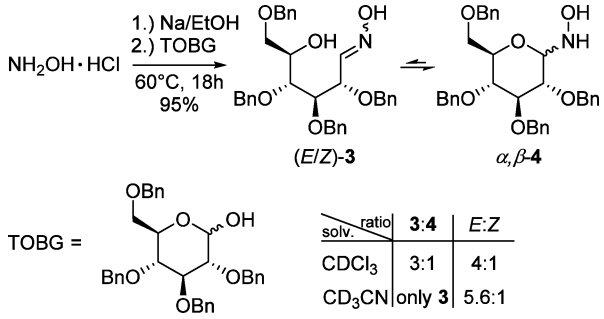
Herein we report a novel direct synthetic route for compound **1** based on a cascade reaction consisting of a 6-exo-trig ring closure followed by a 5-endo-dig reaction. The synthesis of 2-[6′-(3″-nitropropanoyl)-β-D-glucopyranosyl]-3-isoxazolin-5-one (**2**) was achieved by the regioselective transesterification of an activated ester of 3-NPA to compound **1**. Furthermore our novel synthetic strategy allows for the first time the synthesis of α-configured 3,4-unsaturated isoxazolin-5-one glucosides.

RESULTS AND DISCUSSION

We commenced our work with the synthesis of (*E*)- and (*Z*)-2,3,4,6-tetra-*O*-benzyl-D-glucose oxime (**3**) starting from commercially available 2,3,4,6-tetra-*O*-benzyl-D-glucopyranose (TOBG).[27] An excess of hydroxylamine formed in situ via deprotonation of its hydrochloride salt by sodium ethanolate solution in ethanol[28] afforded isomers (*E*/*Z*)-**3** in quantitative yield (Scheme 1).

Received: October 22, 2013
Published: November 22, 2013

Scheme 1. Solvent-Dependent Formation of 3 and 4

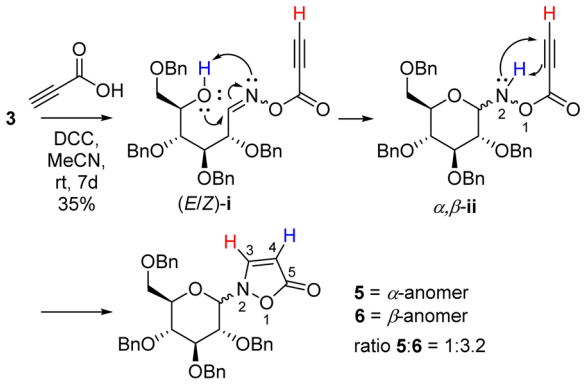


solv. \ ratio	3:4	E:Z
$CDCl_3$	3:1	4:1
CD_3CN	only 3	5.6:1

A solvent-dependent equilibrium between the aldoxime forms (*E*/*Z*)-**3** and the *N*-hydroxylamine pyranose isomers *α*,*β*-**4** was observed. ^{1}H NMR spectra in $CDCl_3$ showed a relative **3**/**4** ratio of 3:1, while in CD_3CN the cyclic isomers *α*,*β*-**4** were absent. Because of the complexity of the mixture, the *α*/*β*-ratio of isomers **4** was not determined.

We found (*E*/*Z*)-**3** to be transformed into isoxazolin-5-one derivatives **5** and **6** in a one-pot synthesis when propynoic acid was applied under Steglich conditions[29] in MeCN (Scheme 2).

Scheme 2. Proposed Mechanism and Reaction Pathway for the Cascade Reaction



The reaction is initiated by a chemoselective acylation of aldoxime **3** with propynoic acid to produce intermediates (*E*/*Z*)-**i** followed by two consecutive ring-closure steps to provide a mixture of **5** and **6**. The course of the reaction was followed by ^{1}H and ^{13}C NMR measurements at rt, which provided evidence for the formation of intermediates (*E*/*Z*)-**i** by a low-field shift of the oxime doublets from 7.41 ppm (H_E-1) and 6.87 ppm (H_Z-1) to 7.95 and 7.85 ppm, respectively. We observed that the H-1 oxime signals in **3** disappeared nearly quantitatively after the application of DCC/propynoic acid. Over a period of several days the integrals of the oxime doublets decreased while the intensities of the isoxazolin-5-one doublets increased. This observation can be explained by an intramolecular nucleophilic attack of the 5′-OH group on the 1′-carbon of the oxime moiety in (*E*/*Z*)-**i** (6-exo-trig reaction) to form the *α*-and *β*-anomers (*α*,*β*-**ii**). The isomers *α*,*β*-**ii** are transformed into the corresponding isoxazolin-5-one glucosides by a second nucleophilic attack of the nitrogen atom on the *β*-position of the propynoyl group (5-endo-dig reaction). For these sequential nucleophilic processes, the terms nucleophilic cascade reaction[30] or homodomino reaction[31] can be used. The integration of the heterocyclic protons (H-3 and H-4) in the ^{1}H NMR spectra indicated that the *α*- and *β*-anomers **5** and **6** are formed in a ratio of ca. 1:3. Compounds **5** and **6** were isolated by column chromatography, and their structures were confirmed by NMR, IR, and UV spectroscopy as well as HRMS. In addition, an X-ray crystal structure analysis of compound **6** was performed after crystallization from ethanol/ethyl acetate (Figure 2); this represents the first crystal

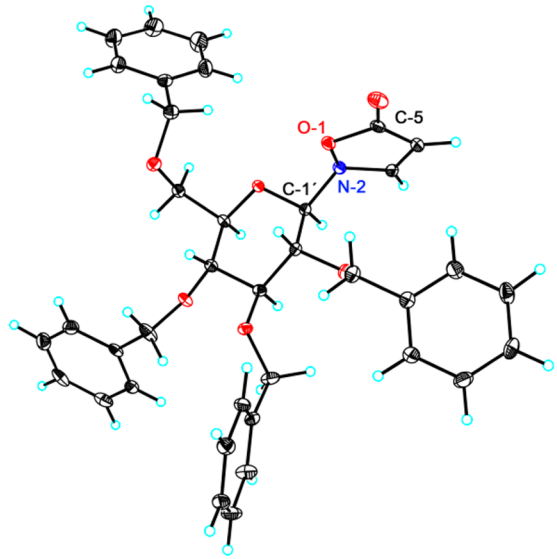


Figure 2. Ellipsoid plot of glucoside **6** (50% probability). Gray, carbon; red, oxygen; blue, nitrogen; green, hydrogen. Hydrogen atoms have been drawn with arbitrary units.

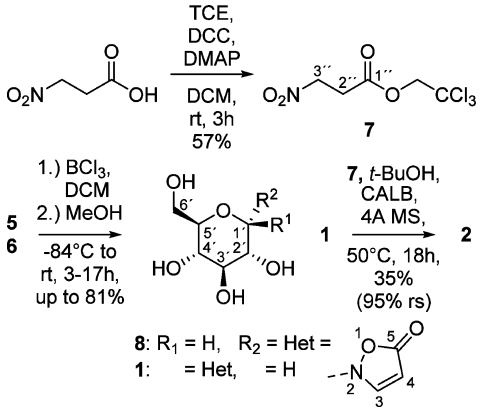
structure of an isoxazolin-5-one glycoside. The resulting bond lengths and angles confirmed the structure of the heterocycle as an isoxazolin-5-one instead of its isoxazolin-3-one isomer, as shown. This observation rules out a possible intramolecular propynoyl transfer from O-1 to N-2 in **ii**. Furthermore, the absolute configuration of compound **6** was confirmed.

The ^{1}H NMR spectra of compounds **5** and **6** show doublet signals with chemical shifts and coupling constants typical for isoxazolin-5-one moieties (8.3−8.1 and 5.3−5.1 ppm; $J_{3,4}$ = 3.7 Hz)[21,23,24] and anomeric protons of the glucose ring (*α*: 5.53 ppm, $J_{1',2'}$ = 5.7 Hz and *β*: 4.90 ppm, $J_{1',2'}$ = 9.1 Hz). The ^{13}C NMR spectra show signals at 154.8 (C_α-3), 155.2 (C_β-3), 89.3 (C_α-4), 92.8 (C_β-4), 85.9 (C_α-1′), and 89.2 ppm (C_β-1′). The glucosides **5** and **6** show UV absorptions around 205 nm (ε = 39 000 L mol^{-1} cm^{-1}) and 260 nm (ε = 12 100 L mol^{-1} cm^{-1}) corresponding to the benzyl and isoxazolin-5-one moieties. The IR spectra show two characteristic absorptions at 1750 cm^{-1} (C═O stretch) and 1550 cm^{-1} (C═C stretch of the isoxazolin-5-one double bond). The spectroscopic data are consistent with literature values.[21−24]

Deprotection of compounds **5** and **6** was achieved using boron trichloride at −84 °C in dichloromethane[32] to give 2-(*α*-D-glucopyranosyl)-3-isoxazolin-5-one (**8**) and 2-(*β*-D-glucopyranosyl)-3-isoxazolin-5-one (**1**) in high yields and purity (Scheme 3). The spectral data for compound **1** are identical with literature values.[3,22] The ^{1}H NMR spectrum of compound **8** shows doublets at 8.52 and 5.39 ppm ($J_{3,4}$ = 3.7 Hz) corresponding to the isoxazolin-5-one moiety, which can be discriminated from those of the *β*-anomer **1** (8.49 and 5.52 ppm, $J_{3,4}$ = 3.7 Hz). The anomeric proton in compound **8** (H_α-1′) shows a doublet at 5.73 ppm ($J_{1',2'}$ = 6.1 Hz), while the H_β-1′ doublet shows a signal at 5.14 ppm ($J_{1',2'}$ = 9.2 Hz). The

dx.doi.org/10.1021/jo4023155 | J. Org. Chem. 2013, 78, 12779−12783

Scheme 3. Reaction Conditions for the Synthesis of Compounds 1, 2, 7, and 8



corresponding ^{13}C NMR signals appear at 154.8 (C_α-3), 155.2 (C_β −3), 88.2 (C_α-4), 92.8 (C_β-4), 87.1 (C_α-1′), and 89.2 ppm (C_β-1′). NMR spectra showed that compounds **1** and **8** are stable in D_2O and CD_3OD at rt and neutral pH. The glucosides **1** and **8** exhibit strong UV absorption at 261 nm (ε = 10 800 L mol^{-1} cm^{-1}) as well as IR absorptions typical for isoxazolin-5-one moieties at 1720−1730 cm^{-1} (C═O stretch) and 1550 cm^{-1} (C═C stretch).

The regioselective acylation of glucosides can be achieved without the use of protective groups by enzymatic transesterification reactions in organic solvents.[14] 2,2,2-Trichloroethyl 3-nitropropanoate (**7**) was synthesized applying Steglich conditions[29] to commercially available 3-NPA and 2,2,2-trichloroethanol (TCE) and purified by chromatography. Esterification of **1** was realized using immobilized *Candida antarctica* lipase B (CALB) and **7** as an activated acyl transfer agent to obtain 2-[6′-(3″-nitropropanoyl)-β-D-glucopyranosyl]-3-isoxazolin-5-one (**2**) (Scheme 3). A regioselectivity of about 95% was determined. The nonconverted 2-(β-D-glucopyranosyl)-3-isoxazolin-5-one **1** could be recovered by chromatography. The spectral data of compound **2** were identical with the literature values.[3]

CONCLUSION

In conclusion, the first total synthesis of naturally occurring 2-[6′-(3″-nitropropanoyl)-β-D-glucopyranosyl]-3-isoxazolin-5-one (**2**) using an efficient four-step synthetic route is reported. A novel cascade reaction pathway was found to incorporate 3,4-unsubstituted isoxazolin-5-one moieties at the 1-position of glucose to afford 2-(β-D-glucopyranosyl)-3-isoxazolin-5-one (**1**). In addition, the first synthesis of α-configured isoxazolin-5-one glucosides (**5** and **8**) has been described. Finally, we have presented the first crystal structure of an isoxazoline-5-one glycoside.

EXPERIMENTAL SECTION

General Experimental Methods. Melting points were determined using a capillary melting point apparatus. Infrared spectra were measured with a IR spectrometer over the 700−4000 cm^{-1} range in transmission mode with a spectral resolution of 2 cm^{-1}. Optical rotations were measured at 589 nm and 22 °C. Ultraviolet spectra were recorded on a UV spectrophotometer over the range from 190 to 300 nm. NMR spectra were measured using a spectrometer operating at 500 MHz (^1H) and 125 MHz (^{13}C). Chemical shifts (δ) are quoted in parts per million (ppm) and are referenced to the signals of residual protonated solvents (CD_2HCN at 1.94 ppm and $CHCl_3$ at 7.26 ppm). Methanol was added as a reference for ^{13}C NMR spectra in D_2O. Assignment of peaks was carried out using 2D NMR experiments (COSY, HSQC, and HMBC). The multiplicities are given as follows: br, broad; s, singlet; d, doublet; t, triplet; dd, doublet of doublets; ddd, doublet of doublets of doublets; m, multiplet. High-resolution mass spectra were recorded on a UHR-qTOF and an APCI-OrbitrapXL mass spectrometer. The intensity data for the X-ray analysis were collected on a diffractometer using graphite-monochromatized Mo Kα radiation. Data were corrected for Lorentz and polarization effects but not for absorption effects.[33,34] The structures were solved by direct methods (SHELXS[35]) and refined by full-matrix least-squares techniques against F_o^2 (SHELXL-97[35]). HPLC-MS analyses were carried out using the APCI mode (vaporizer temperature 500 °C, capillary temperature 300 °C) connected to an HPLC system equipped with an RP18 column. Thin-layer chromatography was performed on TLC silica gel 60 F_{254} aluminum sheets. Compounds containing nitro groups were visualized using the modified Griess assay. Preparative column chromatography was carried out using silica gel (30−60 μm). All chemicals were purchased in the highest purity that was commercially available and used without further purification. All solvents except for diethyl ether were purchased in HPLC grade and used without further purification. Dichloromethane, *tert*-butyl alcohol, and acetonitrile were dried over activated molecular sieves (4 Å) under an argon atmosphere. Diethyl ether was distilled before use.

Synthesis of 2,3,4,6-Tetra-*O*-benzyl-2-(α-D-glucopyranosyl)-3-isoxazolin-5-one (5) and 2,3,4,6-Tetra-*O*-benzyl-2-(β-D-glucopyranosyl)-3-isoxazolin-5-one (6). To a solution of propynoic acid (1.12 g, 16 mmol, 1.1 equiv) in 1 mL of dry MeCN, a solution of oxime **3** (8.0 g, 14.4 mmol) in 7 mL and a solution of DCC (3.09 g, 15 mmol, 1.04 equiv) in 5.3 mL of dry MeCN were added dropwise over 20 min simultaneously at rt. After 7 days of stirring at rt, the mixture was concentrated under reduced pressure at 30 °C, and the residue was taken up in diethyl ether (10 mL). The colorless precipitate was filtered off, and the filtrate was concentrated under reduced pressure at 30 °C. The crude product was purified by column chromatography (DCM/MeCN 100:1 and $CHCl_3$/ethyl acetate 95:5). Methanol was added, and the solvents were removed under reduced pressure. This procedure was repeated five times to yield the title compound **6** as a colorless solid (2.5 g, 4.11 mmol, 28.5%). Crystals suitable for an X-ray crystal structure analysis were obtained via recrystallization from ethanol/ethyl acetate. $[\alpha]_D^{22}$ −16.6 (*c* 0.55, $CHCl_3$); R_f = 0.30 (ethyl acetate/$CHCl_3$ 5:95); ^1H NMR (500 MHz, CD_3CN) δ 8.16 (d, $J_{3,4}$ = 3.7 Hz, 1H, H-3), 7.36−7.20 (m, 20H, Ar−H), 5.33 (d, $J_{3,4}$ = 3.7 Hz, 1H, H-4), 4.90 (d, $J_{1',2'}$ = 9.1 Hz, 1H, H-1′), 4.87 (d, J = 11.2 Hz, 1H, OCH_2Ph), 4.84 (d, J = 11.2 Hz, 1H, OCH_2Ph), 4.78 (d, J = 10.9 Hz, 1H, OCH_2Ph), 4.76 (d, J = 11.0 Hz, 1H, OCH_2Ph), 4.63 (d, J = 11.0 Hz, 1H, OCH_2Ph), 4.57 (d, J = 10.9 Hz, 1H, OCH_2Ph), 4.52 (d, J = 12.1 Hz, 1H, OCH_2Ph), 4.48 (d, J = 12.1 Hz, 1H, OCH_2Ph), 3.90 (t, $J_{1',2'}$ = 9.1 Hz, 1H, H-2′), 3.76−3.72 (m, 1H, H-3′), 3.68−3.62 (m, 2H, H-6′), 3.61−3.56 (m, 2H, H-4′ and H-5′); ^{13}C NMR (125 MHz, CD_3CN) δ 171.6 (C-5), 155.2 (C-3), 139.6 (Ar-Cq), 139.3 (Ar-Cq), 139.3 (Ar-Cq), 139.0 (Ar-Cq), 129.4 (Ar-C), 129.4 (Ar-C), 129.3 (Ar-C), 129.3 (Ar-C), 129.2 (Ar-C), 129.2 (Ar-C), 129.1 (Ar-C), 129.0 (Ar-C), 128.9 (Ar-C), 128.8 (Ar-C), 128.8 (Ar-C), 128.8 (Ar-C), 128.7 (Ar-C), 128.6 (Ar-C), 128.6 (Ar-C), 128.5 (Ar-C), 92.8 (C-4), 89.2 (C-1′), 86.0 (C-3′), 78.9 (C-2′), 78.3 (C-4′ or C-5′), 77.7 (C-4′ or C-5′), 76.2 (OCH_2Ph), 75.6 (OCH_2Ph), 75.4 (OCH_2Ph), 73.7 (OCH_2Ph), 69.4 (C-6′); HRMS (APCI-Orbitrap) *m*/*z* calcd for $C_{37}H_{37}NO_7Na$ 630.2462 $[M + Na]^+$, found 630.2449; IR (thin film, cm^{-1}) 3063 (m), 3031 (m), 2961 (m), 2915 (m), 2869 (m), 1750 (s), 1556 (m), 1090 (s); UV (MeOH) λ_{max}/nm (ε/L mol^{-1} cm^{-1}) 205 (39340 ± 650), 261 (12140 ± 200); mp 97−99 °C. Compound **6** crystallized with two symmetrically independent molecules per asymmetric unit. The two molecules were identical except for the orientation of one benzyl group. The hydrogen atoms of the isoxazolin-5-one ring were located by difference Fourier synthesis and refined isotropically. All other hydrogen atoms were included at calculated positions with fixed thermal parameters. All non-hydrogen atoms were refined anisotropically.[35] XP was used for structure representations. Crystal data for **6**: $C_{37}H_{37}NO_7$, M_r = 607.703 g mol^{-1},

dx.doi.org/10.1021/jo4023155 | J. Org. Chem. 2013, 78, 12779−12783

colorless prism, size 0.10 mm × 0.10 mm × 0.09 mm, triclinic, space group *P*1, a = 10.6443(5) Å, b = 12.4845(6) Å, c = 12.8966(6) Å, α = 104.609(2)°, β = 96.556(2)°, γ = 104.520(2)°, V = 1576.38(13) Å^3, T = 23 °C, Z = 2, ρ_{calcd} = 1.280 g cm^{-3}, μ(Mo Kα) = 0.88 cm^{-1}, F(000) = 644, 70 532 reflections in h(−12/12), k(−14/14), l(−15/15) measured in the range 2.72° ≤ Θ ≤ 25.03°, completeness Θ_{max} = 99.9%, 11066 independent reflections, R_{int} = 0.0209, 10606 reflections with $F_o > 4\sigma(F_o)$, 1107 parameters, 3 restraints, R_1(obs) = 0.0253, wR_2(obs) = 0.0614, R_1(all) = 0.0273, wR_2(all) = 0.0626, GOF = 1.033, Flack parameter = 0.0(3), largest difference peak/hole 0.161/−0.155 e Å^{-3}. Crystallographic data (excluding structure factors) have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication CCDC-961971. Copies of the data can be obtained free of charge from the CCDC, 12 Union Road, Cambridge CB2 1EZ, U.K. (E-mail: deposit@ccdc.cam.ac.uk).

The α-anomer **5** was isolated as a colorless oil (605 mg, 0.99 mmol, 6.8%). $[\alpha]_D^{22}$ +78.5 (*c* 0.51, $CHCl_3$); R_f = 0.41 (ethyl acetate/$CHCl_3$ 5:95); ^{1}H NMR (500 MHz, CD_3CN) δ 8.26 (d, $J_{3,4}$ = 3.7 Hz, 1H, H-3), 7.36−7.21 (m, 20H, Ar−H), 5.53 (d, $J_{1',2'}$ = 5.7 Hz, 1H, H-1′), 5.17 (d, $J_{3,4}$ = 3.7 Hz, 1H, H-4), 4.88 (d, J = 11.1 Hz, 1H, OCH_2Ph), 4.80 (d, J = 11.1 Hz, 2H, OCH_2Ph) 4.65 (s, 2H, OCH_2Ph), 4.57 (d, J = 11.0 Hz, 1H, OCH_2Ph), 4.51 (d, J = 11.9 Hz, 1H, OCH_2Ph), 4.46 (d, J = 11.9 Hz, 1H, OCH_2Ph), 4.10 (t, $J_{3',4'}$ = 9.1 Hz, 1H, H-3′), 3.95 (dd, $J_{2',3'}$ = 9.6 Hz, $J_{1',2'}$ = 5.8 Hz, 1H, H-2′), 3.89−3.85 (m, 1H, H-5′), 3.68−3.62 (m, 2H, H-6′), 3.60 (dd, $J_{4',5'}$ = 10.2 Hz, $J_{3',4'}$ = 8.7 Hz, 1H, H-4′); ^{13}C NMR (125 MHz, CD_3CN) δ 171.7 (C-5), 154.8 (C-3), 139.7 (Ar-Cq), 139.4 (Ar-Cq), 139.3 (Ar-Cq), 138.7 (Ar-Cq), 129.4 (Ar-C), 129.3 (Ar-C), 129.2 (Ar-C), 129.1 (Ar-C), 128.9 (Ar-C), 128.9 (Ar-C), 128.8 (Ar-C), 128.6 (Ar-C), 128.5 (Ar-C), 89.3 (C-4), 85.9 (C-1′), 82.8 (C-3′), 79.7 (C-2′), 78.2 (C-4′), 75.9 (OCH_2Ph), 75.5 (OCH_2Ph), 75.4 (C-5′), 74.2 (OCH_2Ph), 73.8 (OCH_2Ph), 69.7 (C-6′); HRMS (APCI-Orbitrap) *m*/*z* calcd for $C_{37}H_{41}N_2O_7$ 625.2908 $[M + NH_4]^+$, found 625.2892; IR (thin film, cm^{-1}) 3087 (m), 3062 (m), 3030 (m), 2923 (m), 2867 (m), 1749 (s), 1552 (m), 1093 (s); UV (MeOH) λ_{max}/nm (ε/L mol^{-1} cm^{-1}) 204 (36740 ± 600), 264 (12110 ± 200).

Synthesis of 2,2,2-Trichloroethyl 3-Nitropropanoate (7). 3-Nitropropanoic acid (687 mg, 5.77 mmol), 2,2,2-trichloroethanol (3.45 g, 23.08 mmol, 4 equiv), and DMAP (63.4 mg, 0.52 mmol, 9 mol %) were dissolved in dry DCM (5.77 mL). The mixture was cooled to 0 °C, and DCC (1.308 g, 6.35 mmol, 1.1 equiv) was added all at once. After 10 min at 0 °C, the mixture was heated to rt and stirred for 3 h. After purification by flash column chromatography ($CHCl_3$) and removal of the solvent at 40 °C under reduced pressure, a colorless powder of **7** (834 mg, 3.33 mmol, 57.7%) was obtained. R_f = 0.78 ($CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.81 (s, 2H, CH_2CCl_3), 4.72 (t, $J_{2,3}$ = 6.1 Hz, 2H, CH_2NO_2), 3.16 (t, $J_{2,3}$ = 6.1 Hz, 2H, CH_2CO_2R); ^{13}C NMR (125 MHz, $CDCl_3$) δ 168.13, 94.46, 74.59, 69.35, 30.97; HRMS (APCI-Orbitrap) *m*/*z* calcd for $C_5H_7Cl_3NO_4$ 249.9435 $[M + H]^+$, found 249.9429; IR (thin film, cm^{-1}) 3012 (w), 2961 (m), 2926 (m), 1747 (s), 1549 (s), 1088 (s); mp 35−36 °C.

Synthesis of 2-(β-D-Glucopyranosyl)-3-isoxazolin-5-one (1). 2,3,4,6-Tetra-*O*-benzyl-2-(β-D-glucopyranosyl)-3-isoxazolin-5-one (**6**) (1.73 g, 2.85 mmol) was dissolved in dry DCM (150 mL) and cooled to −84 °C under an argon atmosphere. Boron trichloride (20 mL, 1 M, heptane, 7 equiv) was added dropwise. After 17 h at −84 to −79 °C, the mixture was quenched with methanol (20 mL) and warmed to rt. The solvents were removed at 25 °C under reduced pressure to yield a colorless oil. The residue was dissolved in methanol (5 mL), and silica (2 g) was added. The solvent was removed, and the residual colorless powder was applied to a silica gel column. After elution of the column (DCM/MeOH 5:1 to 2:1), the pure fractions were concentrated under high vacuum at rt to yield **1** as a colorless solid (522 mg, 2.11 mmol, 74.2%). $[\alpha]_D^{22}$ +13.5 (*c* 0.53, MeOH); R_f = 0.49 (DCM/MeOH 2:1); ^{1}H NMR (500 MHz, D_2O) δ 8.49 (d, $J_{3,4}$ = 3.7 Hz, 1H, H-3), 5.50 (d, $J_{3,4}$ = 3.7 Hz, 1H, H-4), 5.14 (d, $J_{1',2'}$ = 9.2 Hz, 1H, H-1′), 3.92−3.88 (m, 2H, H_A-6′ and H-2′), 3.73 (dd, J_1 = 6.9 Hz, J_2 = 5.6 Hz, 1H, H_B-6′), 3.64−3.57 (m, 2H, H-3′ and H-5′), 3.49 (t, $J_{3',4'}$ = 9.5 Hz, 1H, H-4′); ^{13}C NMR (125 MHz, D_2O) δ 174.8 (C-5), 154.8 (C-3), 91.1 (C-4), 88.8 (C-1′), 78.8 (C-5′), 76.7 (C-3′), 70.0 (C-2′), 69.5 (C-4′), 61.0 (C-6′); HRMS (ESI-TOF) *m*/*z* calcd for $C_9H_{13}NO_7Na$ 270.0584 $[M + Na]^+$, found 270.0575; IR (thin film, cm^{-1}) 3370 (br, s), 2932 (m), 1725 (s), 1544 (s), 1104 (s), 1040 (s); UV (MeOH) λ_{max}/nm (ε/L mol^{-1} cm^{-1}) 262 (10800 ± 200). The spectral data were in agreement with the literature values.[3,22]

Synthesis of 2-(α-D-Glucopyranosyl)-3-isoxazolin-5-one (8). 2,3,4,6-Tetra-*O*-benzyl-2-(α-D-glucopyranosyl)-3-isoxazolin-5-one (**5**) (100 mg, 0.165 mmol) was dissolved in dry DCM (8 mL) and cooled to −84 °C under an argon atmosphere. Boron trichloride (2 mL, 1 M, heptane, 10 equiv) was added dropwise. After 1.5 h at −84 to −79 °C, the mixture was warmed to rt and stirred for 1.5 h. The reaction was quenched with methanol (4 mL) at −75 °C and stirred for 30 min. The solvents were removed at 25 °C under reduced pressure to yield a colorless oil. The residue was dissolved in methanol (5 mL), and silica (500 mg) was added. The solvent was removed, and the residual colorless powder was applied to a silica gel column. After elution of the column (DCM/MeOH 5:1 to 2:1), the product fractions were concentrated under high vacuum at rt to yield **8** as a colorless oil (33.3 mg, 0.135 mmol, 81.8%). $[\alpha]_D^{22}$ +85.3 (*c* 1.5, MeOH); R_f = 0.49 (DCM/MeOH 2:1); ^{1}H NMR (500 MHz, D_2O) δ 8.52 (d, $J_{3,4}$ = 3.6 Hz, 1H, H-3), 5.73 (d, $J_{1',2'}$ = 6.1 Hz, 1H, H-1′), 5.39 (d, $J_{3,4}$ = 3.6 Hz, 1H, H-4), 4.10 (t, $J_{2',3'}$ = 9.6 Hz, 1H, H-3′), 4.02 (dd, $J_{2',3'}$ = 10.0 Hz, $J_{1',2'}$ = 6.2 Hz, 1H, H-2′), 3.87−3.80 (m, 2H, H-5′ and H_A-6′), 3.78−3.73 (m, 1H, H_B-6′) 3.53 (t, $J_{3',4'}$ = 9.5 Hz, 1H, H-4′); ^{13}C NMR (125 MHz, D_2O) δ 174.9 (C-5), 154.8 (C-3), 88.2 (C-4), 87.1 (C-1′), 77.1 (C-5′), 74.1 (C-3′), 70.6 (C-2′), 69.7 (C-4′), 61.1 (C-6′); HRMS (APCI-Orbitrap) *m*/*z* calcd for $C_9H_{14}NO_7$ 248.0765 $[M + H]^+$, found 248.0762; IR (thin film, cm^{-1}) 3382 (br, s), 2962 (m), 2923 (m), 1727 (s), 1552 (s), 1192 (s), 1063 (s); UV (MeOH) λ_{max}/nm (ε/L mol^{-1} cm^{-1}) 261 (10760 ± 200).

Synthesis of 2-[6′-(3″-Nitropropanoyl)-β-D-glucopyranosyl]-3-isoxazolin-5-one (2). A mixture of 2-(β-D-glucopyranosyl)-3-isoxazolin-5-one (**1**) (100 mg, 0.404 mmol), 2,2,2-trichloroethyl 3-nitropropanoate (**7**) (160.1 mg, 0.639 mmol), immobilized *C. antarctica* lipase B (150 mg), and 4 Å molecular sieves was suspended in dry *tert*-butyl alcohol (7 mL). The suspension was stirred at 50 °C under an argon atmosphere for 18 h. The enzyme was filtered off, and the filter cake was washed with *tert*-butyl alcohol (2 × 5 mL) at 40 °C and cold methanol (5 mL). The filtrate was concentrated under reduced pressure at 25 °C, and the residue was taken up in methanol. Silica was added, and the solvent was evaporated under reduced pressure at 25 °C to obtain a colorless powder. The dry powder was added to a silica column, and the product was purified by column chromatography (ethyl acetate/MeOH/DCM 10:1:1 to 2:1:0). The solvent was removed to yield **2** as a colorless solid (50 mg, 0.144 mmol, 35.6%). Nonconverted glucoside **1** could be recovered (31 mg, 0.125 mmol, 31%). $[\alpha]_D^{22}$ +30.1 (*c* 0.36, MeOH); R_f = 0.20 (ethyl acetate/MeOH/DCM 10:1:1); ^{1}H NMR (500 MHz, D_2O) δ 8.47 (d, $J_{3,4}$ = 3.7 Hz, 1H, H-3), 5.52 (d, $J_{3,4}$ = 3.7 Hz, 1H, H-4), 5.15 (d, $J_{1',2'}$ = 9.2 Hz, 1H, H-1′), 4.82 (t, $J_{2'',3''}$ = 5.8 Hz, 2H, H-3″), 4.51 (dd, $J_{A6',B6'}$ = 12.3 Hz, $J_{5',A6'}$ = 2.2 Hz, 1H, H_A-6′), 4.33 (dd, $J_{A6',B6'}$ = 12.4 Hz, $J_{5',B6'}$ = 5.2 Hz, 1H, H_B-6′), 3.91 (t, $J_{1',2'}$ = 9.2 Hz, 1H, H-2′), 3.78 (ddd, $J_{4',5'}$ = 10.0 Hz, $J_{5',B6'}$ = 5.2 Hz, $J_{5',A6'}$ = 2.2 Hz, 1H, H-5′), 3.62 (t, $J_{2',3'}$ = 9.3 Hz, 1H, H-3′), 3.50 (t, $J_{3',4'}$ = 9.5 Hz, 1H, H-4′), 3.13 (t, $J_{2'',3''}$ = 5.8 Hz, 2H, H-2″); ^{13}C NMR (125 MHz, D_2O) δ 174.7 (C-5), 172.4 (C-1″), 155.0 (C-3), 91.7 (C-4), 88.7 (C-1′), 76.5 (C-5′), 76.1 (C-3′), 70.7 (C-3″), 69.9 (C-2′), 69.5 (C-4′), 63.9 (C-6′), 31.6 (C-2″); HRMS (ESI-TOF) *m*/*z* calcd for $C_{12}H_{16}N_2O_{10}Na$ 371.06972 $[M + Na]^+$, found 371.06958; IR (thin film, cm^{-1}) 3374 (br, s), 2924 (m), 2887 (m), 1725 (s), 1549 (s), 1067 (br, s); UV (MeOH) λ_{max}/nm (ε/L mol^{-1} cm^{-1}) 201 (6250 ± 110), 261 (11040 ± 200). The spectral data were in agreement with the literature values.[3]

■ ASSOCIATED CONTENT

Supporting Information

Crystallographic data for compound **6** (CIF); NMR spectra for compounds **1**, **2**, and **5**−**8**; HRMS spectra of compounds **5**−**8**; HPLC-MS analyses of **1** and **8**; and ^{1}H NMR spectra of the

dx.doi.org/10.1021/jo4023155 | J. Org. Chem. 2013, 78, 12779−12783

formation of compounds **5** and **6**. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*E-mail: boland@ice.mpg.de.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We thank Dr. Maritta Kunert and Kerstin Ploss for the MS measurements and Mina Shaheed for the IR measurements.

REFERENCES

(1) Laurent, P.; Braekman, J.-C.; Daloze, D. *Top. Curr. Chem.* **2005**, *240*, 167−229.
(2) Pasteels, J. M.; Braekman, J. C.; Daloze, D.; Ottinger, R. *Tetrahedron* **1982**, *38*, 1891−1897.
(3) Rowell-Rahier, M.; Pasteels, J. M. *J. Chem. Ecol.* **1986**, *12*, 1189−1203.
(4) Ikegami, F.; Lambein, F.; Kuo, Y.-H.; Murakoshi, I. *Phytochemistry* **1984**, *23*, 1567−1569.
(5) Benn, M. H.; Majak, W.; Aplin, R. *Biochem. Syst. Ecol.* **1997**, *25*, 467−468.
(6) Sugeno, W.; Matsuda, K. *Appl. Entomol. Zool.* **2002**, *37*, 191−197.
(7) Anderson, R. C.; Majak, W.; Rassmussen, M. A.; Callaway, T. R.; Beier, R. C.; Nisbet, D. J.; Allison, M. J. *J. Agric. Food Chem.* **2005**, *53*, 2344−2350.
(8) Huang, L.-s.; Sun, G.; Cobessi, D.; Wang, A. C.; Shen, J. T.; Tung, E. Y.; Anderson, V. E.; Berry, E. A. *J. Biol. Chem.* **2006**, *281*, 5965−5972.
(9) Beal, M. F.; Brouillet, E.; Jenkins, B. G.; Ferrante, R. J.; Kowall, N. W.; Miller, J. M.; Storey, E.; Srivastava, R.; Rosen, B. R.; Hyman, B. T. *J. Neurosci.* **1993**, *13*, 4181−4192.
(10) Harlow, M. C.; Stermitz, F. R.; Thomas, R. D. *Phytochemistry* **1975**, *14*, 1421−1423.
(11) Garcez, W. S.; Garcez, F. R.; Barison, A. *Biochem. Syst. Ecol.* **2003**, *31*, 207−209.
(12) Baxter, R. L.; Abbot, E. M.; Greenwood, S. L.; McFarlane, I. J. *J. Chem. Soc., Chem. Commun.* **1985**, 564−566.
(13) Baxter, R. L.; Hanley, A. B.; Chan, H. W. S.; Greenwood, S. L.; Abbot, E. M.; McFarlane, I. J.; Milne, K. *J. Chem. Soc., Perkin Trans. 1* **1992**, 2495−2502.
(14) Therisod, M.; Klibanov, A. M. *J. Am. Chem. Soc.* **1987**, *109*, 3977−3981.
(15) Wong, C. H. *Science* **1989**, *244*, 1145−1152.
(16) Klibanov, A. M. *Acc. Chem. Res.* **1990**, *23*, 114−120.
(17) Boland, W.; Frössl, C.; Lorenz, M. *Synthesis* **1991**, 1049−1072.
(18) Woudenberg-van Oosterom, M.; Vitry, C.; Baas, J. M. A.; van Rantwijk, F.; Sheldon, R. A. *J. Carbohydr. Chem.* **1995**, *14*, 237−246.
(19) De Sarlo, F.; Dini, G.; Lacrimini, P. *J. Chem. Soc. C* **1971**, 86−89.
(20) Lambein, F.; Van Rompuy, L.; De Bruyn, A.; Van Parijs, R. *Arch. Int. Physiol. Biochem.* **1974**, *82*, 187.
(21) Van Rompuy, L.; Schamp, N.; De Kimpe, N.; Van Parijs, R. *J. Chem. Soc., Perkin Trans. 1* **1973**, 2503−2506.
(22) Van Rompuy, L.; Lambein, F.; De Gussem, R.; Van Parijs, R. *Biochem. Biophys. Res. Commun.* **1974**, *56*, 199−205.
(23) Baldwin, J. E.; Adlington, R. M.; Mellor, L. C. *Tetrahedron* **1994**, *50*, 5049−5066.
(24) Baldwin, J. E.; Adlington, R. M.; Birch, D. J. *Tetrahedron Lett.* **1985**, *26*, 5931−5934.
(25) Baldwin, J. E. *J. Chem. Soc., Chem. Commun.* **1976**, 734−736.
(26) Baldwin, J. E.; Thomas, R. C.; Kruse, L. I.; Silberman, L. *J. Org. Chem.* **1977**, *42*, 3846−3852.
(27) Zhang, F.; Vasella, A. *Carbohydr. Res.* **2007**, *342*, 2546−2556.
(28) Aebischer, B. M.; Hanssen, H. W.; Vasella, A. T.; Schweizer, W. B. *J. Chem. Soc., Perkin Trans. 1* **1982**, 2139−2147.
(29) Neises, B.; Steglich, W. *Angew. Chem., Int. Ed.* **1978**, *17*, 522−524.
(30) Nicolaou, K. C.; Edmonds, D. J.; Bulger, P. G. *Angew. Chem., Int. Ed.* **2006**, *45*, 7134−7186.
(31) Tietze, L. F. *Chem. Rev.* **1996**, *96*, 115−136.
(32) Williams, D. R.; Moore, J. L.; Yamada, M. *J. Org. Chem.* **1986**, *51*, 3916−3918.
(33) *COLLECT, data collection software*; Nonius BV: Delft, The Netherlands, 1998.
(34) Otwinowski, Z.; Minor, W. *Methods Enzymol.* **1997**, *276*, 307−326.
(35) Sheldrick, G. M. *Acta Crystallogr.* **2008**, *A64*, 112−122.