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FULL PAPER

Synthesis of Isotope Labeled Deoxynivalenol-15-*O*-Glycosides

Julia Weber,[a] Philipp Fruhmann,[a,b] Christian Hametner,[a] Alois Schiessl,[c] Georg Häubl,[c] Johannes Fröhlich[a] and Hannes Mikula*[a]

Abstract: A versatile and efficient protocol for Schmidt glycosylation of 3-acetyldeoxynivalenol (3-ADON) and subsequent deprotection has been developed to gain access to deoxynivalenol-15-*O*-glycosides in reasonable amounts for bioanalysis and further investigations. Applying this protocol in combination with [$^{13}C_6$]glycosyl donors we were able to prepare isotope labeled deoxynivalenol-15-*O*-glycosides, which are pivotal to enable accurate quantification of masked mycotoxins by LC-MS.

Introduction

Deoxynivalenol (DON **1**, Fig. 1a) is a trichothecene mycotoxin produced by various *Fusarium* species, including *Fusarium graminearum* and *Fusarium culmorum*. These species colonize mainly wheat, barley, corn and oat in temperate regions of Europe.[1] Infection of the crop by Fusarium fungi is favored during prolonged cool, moist growing and harvest season.[2] DON is the most commonly detected trichothecene with the highest concentration in cereal-based food. It is a potent protein synthesis inhibitor, and has an acute toxicity resulting in vomiting, nausea, abdominal pain and diarrhea. Chronic exposure to low doses of DON can cause anorexia, growth retardation, immune dysregulation, impaired reproduction and development.[3,4] To decrease the exposure to DON for humans and animals, the European Union set maximum levels, guidance values and started monitoring programs. In 2002 the Scientific Committee for Food (SCF) defined a provisional tolerable daily intake for DON and its acetylated derivatives of 1 µg/kg body weight.[2]

As Fusarium mycotoxins are plant pathogens, they are prone to metabolization by plants.[5] Infected plants are capable of biochemically modifying DON during the xenobiotic metabolism. In phase I of this detoxification process, xenobiotics are oxidized or hydrolyzed, while in phase II conjugates are formed by glycosylation, sulfation, addition of glutathione, etc. The most prominent conjugate of DON is DON-3-*O*-ß,D-glucoside (Fig. 1b). Until recently, this metabolite was the only DON conjugate proven to occur in naturally infected cereals (e.g. wheat).[5] Schmeitzl *et al.* have recently reported on the formation of DON-15-*O*-ß,D-glucoside (**2**, Fig. 1b) in wheat.[6] These altered forms, often called masked mycotoxins, escape routine detection but can release the parent toxin during food processing or digestion.[7] Therefore, the presence of such metabolites can cause an underestimation of the potential toxicity of a particular sample and thus represent a risk for food and feed safety.[8]

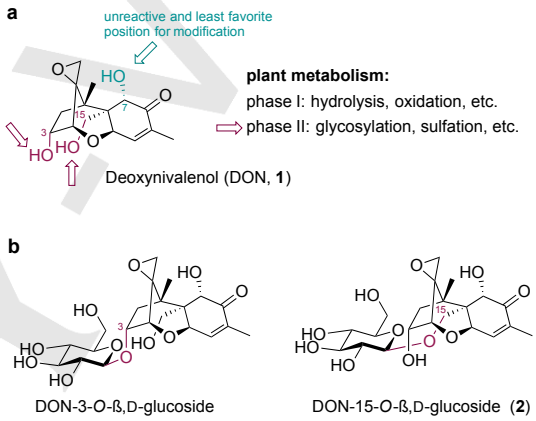

Figure 1. (a) Chemical structure of deoxynivalenol (DON, **1**) and reaction sites for metabolic modification; (b) naturally occurring DON-glucosides

Awareness of these DON-metabolites is increasing, but reference compounds for structure elucidation, analytics and toxicity testing are still scarce.[8,9] For example, **2** could only be tentatively identified by Schmeitzl *et al.* via mass spectrometry analysis and thus, a method for accurate quantification could not be achieved.[6] For the development of improved and accurate analytical methods, and toxicological risk assessment sufficient quantities of these compounds are required in high purity. Furthermore, isotope labeled conjugates are crucial for accurate quantification of mycotoxin metabolites by LC-MS.[6,10]

Previously described glycosylation reactions of DON were carried out under Königs-Knorr conditions ($CdCO_3$ in toluene under reflux) with 38% yield.[11] Recent advances have been made in the preparation of DON-3-*O*-ß,D-glucoside via an enzymatic approach. Michlmayr *et al*. reported on a DON-conjugating UDP-glucosyltransferase from rice that produces DON-3-*O*-ß,D-glucoside in 69% under optimized conditions.[12] The synthesis of ^{13}C-labeled DON-3-*O*-ß,D-[$^{13}C_6$]glucoside was accomplished via Königs-Knorr glycosylation (Ag_2CO_3 in dichloromethane) by Habler *et al.* very recently. However, the authors reported several drawbacks of their method: (i) an excess of isotope labeled glucosyl donor, (ii) a very long reaction time (5 days), and (iii) failed upscaling of the reaction.[10]

Herein we present the development of an efficient general procedure for the preparation of DON-15-*O*-glycosides and the

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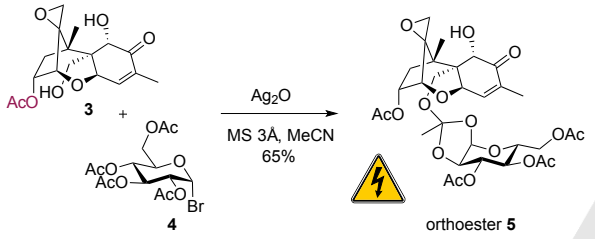
synthesis of isotope labeled analogs applying a $^{13}C_6$-labeled N-phenyltrifluoroacetimidoyl glucosyl donor.

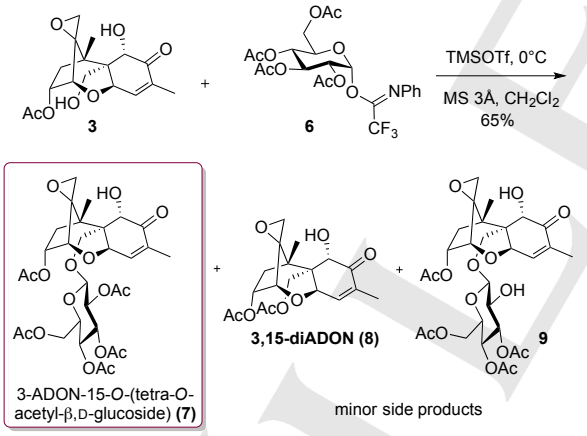
Results and Discussion

For glycosylation at position 15, 3-acetyldeoxynivalenol (3-ADON, **3**)[13,14] was used as starting material as the second free OH group at position 7 is known to be biochemically and chemically inert.[5,15,16] Considering previously described procedures for the glycosylation of DON, we tested Königs-Knorr conditions (acetobromo-α,D-glucose (**4**), Ag_2O, ACN) to synthesize DON-15-*O*-β,D-glucoside, but only observed exclusive formation of the undesired orthoester **5** (Scheme 1).

Applying Schmidt glucosylation using N-phenyltrifluoro-acetimidoyl donor **6**[17] at 0 °C in a second approach, successfully yielded the acetyl-protected 3-ADON-glucoside **7** in good yield (67%) with only two minor side products **8** and **9** (Scheme 2).

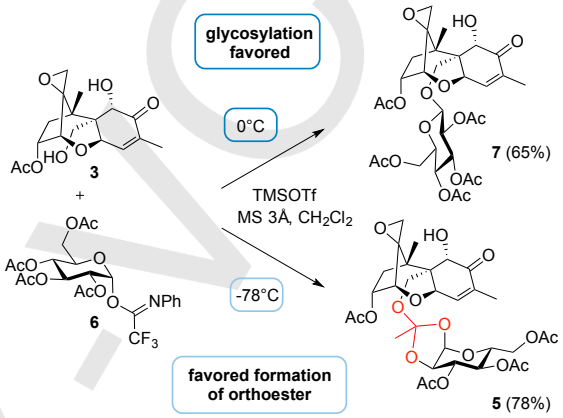


Scheme 1. Königs-Knorr glucosylation of 3-ADON leading to undesired orthoester formation.



Scheme 2. Schmidt glucosylation of 3-ADON.

To avoid undesired formation of 3,15-diacetyl-DON (3,15-diADON, **8**), the reaction temperature was lowered to -78°C fully preventing acyl transfer. However, no desired glucoside was formed. Instead, orthoester **5** was isolated in excellent yield (Scheme 3). As rarely described in the literature,[18] temperature-dependent orthoester- vs. glucoside-formation was observed, also confirming the results of DFT calculations done by Berces *et al.* showing that acyl transfer is a related but separate reaction from orthoester formation. To prevent acyl transfer without leading to orthoester formation the steric bulk of the acyl residue at O-2 needs to be increased.[19] Thus, to improve the glycosylation reaction, a more complex glycosyl donor would have to be synthesized. As the aim of this study was to develop a simple procedure (starting from commercially available peracetylated glucose), we did not consider any further modification of the glycosyl donor. In principal, orthoesters can often be converted under acidic conditions to form the glucoside, but in that case acyl transfer again emerges as undesired side reaction.[20,21]



Scheme 3. Temperature-dependent formation of orthoester vs. glycosylation.

Removal of acyl groups is usually done by saponification (basic hydrolysis) or base-catalyzed transesterification (Zemplén conditions).[22–25] Using sodium methoxide in methanol for deacetylation of **7** led to the formation of a mixture of desired DON-15-*O*-β,D-glucoside (**2**) and isoDON-15-*O*-β,D-glucoside (**10**) in a ratio of 1:0.6, which was confirmed by NMR.[26] Preparative separation of the two isomers by column chromatography did not work in our hands, but the isomers can be analytically distinguished based on different UV absorption maxima (**2**: 228 nm, **10**: 280 nm; Figure 2).

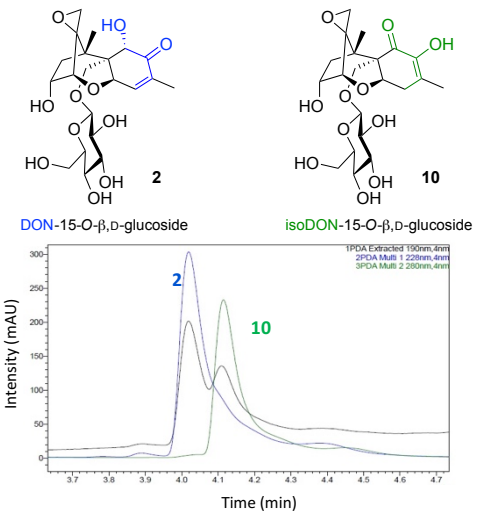
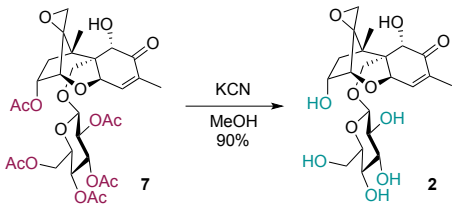
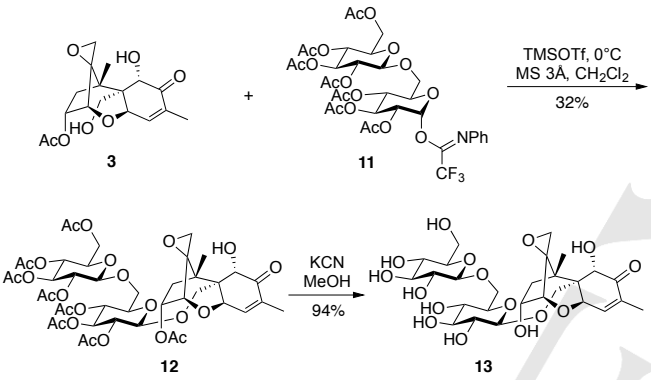


Figure 2. HPLC analysis of an inseparable mixture of DON-15-*O*-β,D-glucoside (**2**) and isoDON-15-*O*-β,D-glucoside (**10**).

We tested several base-catalyzed and saponification methods such as K_2CO_3 in MeOH,[27] KOH in THF/H_2O,[23] and $Mg(OMe)_2$ in MeOH[28] all leading to formation of an inseparable mixture of **2** and **10**. Finally, selective deprotection of **7** was successfully carried out applying a very mild procedure using potassium cyanide in MeOH[29] affording DON-15-*O*-β,D-glucoside (**2**) in excellent yield (Scheme 4).



Scheme 4. Deprotection of **7** affording DON-15-*O*-β,D-glucoside (**2**).



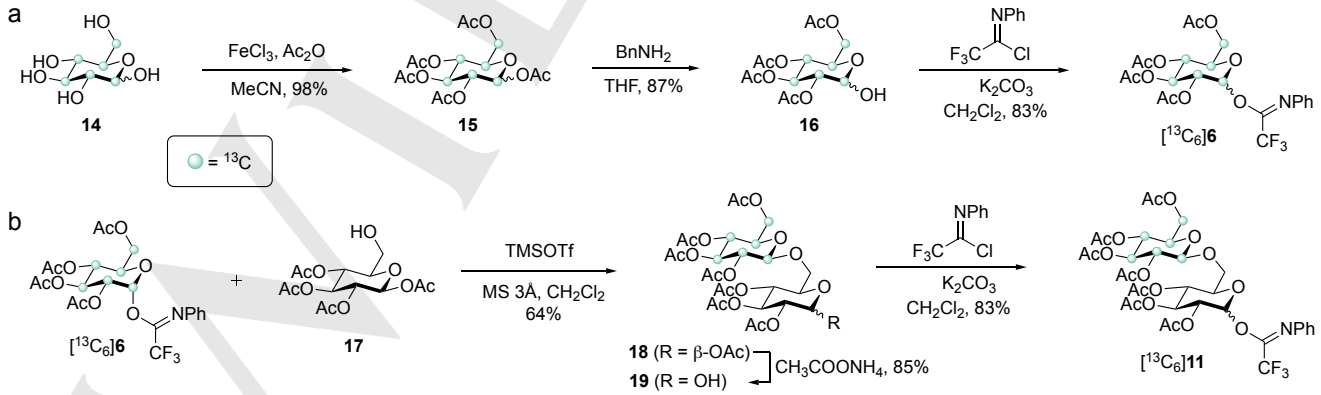
Scheme 5. Synthesis of DON-15-*O*-β-gentiobioside (**13**).

Having developed an optimized procedure, we tested its application for the synthesis of other DON-15-*O*-glycosides. Gentiobiosyl donor **11**[30] was reacted with **3** followed by deprotection of the intermediate **12** to obtain DON-15-β-gentiobioside (**13**) (Scheme 5). Even though a lower yield of 32% was achieved in the glycosylation step (compared to 65% for the synthesis of **7**) due to a more complex product mixture, and thus more difficult separation, the final product was obtained in an overall yield of 30%.

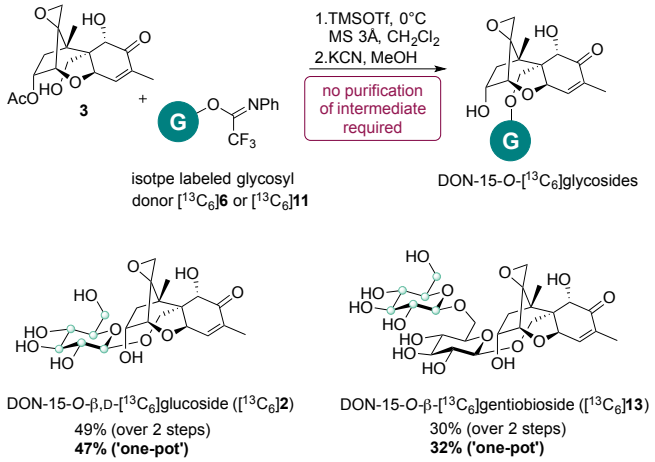
As the availability of isotope labeled compounds as internal standards is essential for the development of robust and accurate analytical methods, we have prepared ^{13}C-labeled glycosyl donors to enable the preparation of isotope labeled DON-15-*O*-glycosides. In comparison to using [$^{13}C_{15}$]DON[31] the application of [$^{13}C_6$]glycosyl donors makes the synthesis of isotope labeled DON-glycosides significantly easier and cheaper. The resulting partially isotope labeled DON-15-*O*-[$^{13}C_6$]glycoside can be used as a standard for reliable quantification in LC-MS analysis.

The $^{13}C_6$-labeled glucosyl donor [$^{13}C_6$]**6** was synthesized starting from [$^{13}C_6$]glucose (**14**) by acetylation with Ac_2O and a catalytic amount of $FeCl_3$ under ultrasonic irradiation to afford **15**.[32] Anomeric deprotection with benzyl amine[33] yielded the OH-sugar **16** that was subsequently reacted with N-phenyltrifluoro-acetimidoyl chloride[34] to obtain the [$^{13}C_6$]glucosyl donor [$^{13}C_6$]**6** in high yield (Scheme 6a). [$^{13}C_6$]Gentiobiosyl donor [$^{13}C_6$]**11** was synthesized by preparing [$^{13}C_6$]gentiobiose octaacetate (**18**) via Schmidt glycosylation of 1,2,3,4-tetra-*O*-acetyl-β,D-glucose (**17**) with [$^{13}C_6$]glucosyl donor [$^{13}C_6$]**6**. Anomeric deprotection of **17** with ammonium acetate[35] to obtain OH-sugar **19** followed by introduction of a N-phenyltrifluoroacetimidoyl leaving group afforded the $^{13}C_6$-labeled gentiobiosyl donor [$^{13}C_6$]**11** in good overall yield (Scheme 6b).

Following our developed procedure, Schmidt glycosylation of **3** using the $^{13}C_6$-labeled glycosyl donors [$^{13}C_6$]**6** and [$^{13}C_6$]**11,** and subsequent deprotection afforded DON-15-*O*-β,D-[$^{13}C_6$]glucoside ([$^{13}C_6$]**2**) and DON-15-*O*-β-[$^{13}C_6$]gentiobioside ([$^{13}C_6$]**13**), respectively. Furthermore, to avoid laborious purification of the intermediate acetyl-protected 3-ADON-glycosides, a 'one-pot' procedure was developed. Applying this fast and efficient



Scheme 6. Synthesis of isotope labeled glycosyl donors [$^{13}C_6$]**6** and [$^{13}C_6$]**11** for Schmidt glycosylation starting from [$^{13}C_6$]glucose (**14**).



Scheme 7. Synthesis of isotope labeled DON-15-*O*-[$^{13}C_6$]glycosides.

procedure, we were able to obtain both products ([$^{13}C_6$]**2** and [$^{13}C_6$]**13**) in sufficient quantities (up to 40 mg per batch; only limited by the amount of **3**) in less than 8 h (including final purification by preparative HPLC) (Scheme 7).

Conclusions

In summary, we have developed an efficient method for the synthesis of DON-15-*O*-glycosides by Schmidt-glycosylation of 3-ADON (**3**) followed by deprotection under mild reaction conditions avoiding the formation of undesired byproducts. This procedure was successfully applied for the preparation of isotope labeled [$^{13}C_6$]glycosides. The method was further optimized and simplified by the development of a 'one-pot' protocol avoiding troublesome and time-consuming purification of the intermediate protected glycosides. We are convinced that this method will find application towards the synthesis of further (isotope labeled) DON-15-*O*-glycosides in reasonable amounts for bioanalysis and ongoing investigations in the field of masked and conjugated mycotoxins. Starting from 15-*O*-acetyl-DON instead of 3-ADON (**3**) this method might furthermore be useful for the synthesis of DON-3-O-glycosides. Even though the glycosylation step might require further optimization (considering the lower reactivity of the secondary 3-OH in contrast to the primary 15-OH) the optimized reaction conditions for deprotection avoiding the formation of isoDON derivatives can be used for the synthesis of any DON conjugate.

Experimental Section

All reactions were performed under an argon atmosphere. 3-Acetyldeoxynivalenol (3-ADON, **3**) was obtained from Romer Labs (Tulln, Austria) and all other chemicals were purchased from ABCR (Germany), Sigma-Aldrich (Germany) or Carbosynth (UK). Anhydrous solvents (dichloromethane, tetrahydrofuran, methanol and diethyl ether) were dried using a PureSolv system (inert technology, Amesbury, MA, USA). Molecular sieves (3 Å) were activated under vacuum at 200°C before use. The progress of the reactions was monitored by thin layer chromatography (TLC) over silica gel 60 F_{254} (visualization either by using UV light or by heat staining using ceric ammonium molybdate in ethanol/sulfuric acid). LC-ESI-MS/MS was performed on an HCT ion trap mass spectrometer (Bruker, Germany) in full scan mode. Chromatographic separation was done on a 1200 series HPLC system (Agilent Technologies, Germany) using a Luna RP-C18 column (3.0 x 150 mm, 3 µm particle size, Phenomenex, Germany). Preparative column chromatography was performed on silica gel 60 (Merck, 40-63 µm) using a Sepacore™ Flash System or a Grace Reveleris Prep Purification System (Büchi, Switzerland). Preparative HPLC separation was done on a Grace Reveleris Prep system (Büchi, Switzerland) using a Luna Prep C18(2), 10 µm, 250x10 mm column (Phenomenex, Germany). NMR spectra were recorded on an Avance IIIHD 600Mhz spectrometer equipped with a Prodigy BBO cryo probe (Bruker, Germany) or on an Avance DRX-400 MHz spectrometer (Bruker, Germany) at 20°C. Data was recorded and evaluated using TOPSPIN 3.5 (Bruker Biospin, Germany). All chemical shifts are given in ppm relative to tetramethylsilane. The calibration was done using residual solvent signals.

General procedure A: Preparation of *O*-glycosyl N-phenyl trifluoro-acetimidates

To a solution of 1-hydroxy sugar (1 equiv) in CH_2Cl_2 (9 mL/mmol) was added K_2CO_3 (2 eq.) followed by *N*-phenyl-2,2,2-trifluoroacetimidoyl chloride (2 eq.). The reaction mixture was stirred at room temperature for 24 h, filtered and concentrated to afford the crude product. Purification by flash chromatography (gradient elution, hexanes-EtOAc) gave the corresponding trifluoroacetimidate.

General procedure B: glycosylation of 3-ADON (3)

To a solution of 3-ADON (**3**) (1 equiv) and glycosyl donor (1.5 equiv) in dry CH_2Cl_2 (8 mL/mmol) was added molecular sieve (3 Å, 0.1 g/mL), and the reaction mixture was stirred at rt for 30 min. After cooling the reaction mixture to 0 °C, TMSOTf (0.1 equiv) was added. The reaction mixture was stirred at 0 °C for 2 h, and quenched by the addition of Et_3N (0.15 equiv). The reaction mixture was filtered through Celite and concentrated. The crude product was purified by flash chromatography (MeOH in DCM, gradient elution) and preparative HPLC (RP-C18, MeCN in H_2O, gradient elution).

General procedure C: Deprotection of acetyl-protected 3-ADON-15-*O*-glycosides

To a solution of acetyl-protected glycoside (1 equiv.) in MeOH (14 mL/µmol) was added KCN (0.5 equiv.) at 0 °C. The reaction mixture was slowly warmed to room temperature and stirred for 2-4 h. Water (1 mL) was added and MeOH was evaporated under reduced pressure. Purification by preparative HPLC (RP-C18, MeCN in H_2O, gradient elution) afforded the desired glycoside.

General procedure D ('One-pot')

To a solution of 3-ADON (**3**) (1 equiv) and glycosyl donor (1.5 equiv) in dry CH_2Cl_2 (8 mL/mmol) was added molecular sieve (3 Å, 0.1 g/ mL), and the reaction mixture was stirred at rt for 30 min. After cooling the reaction mixture to 0 °C, TMSOTf (0.1 equiv) was added. The reaction mixture was stirred at 0 °C for 2 h, and quenched by the addition of Et_3N (0.15 equiv). The reaction mixture was filtered through Celite and concentrated. The crude product mixture was dissolved in MeOH (14 mL/µmol) and KCN (0.5 equiv.) was added at 0 °C. The reaction mixture was slowly warmed

to room temperature and stirred for 2-4 h. Water (1 mL) was added and MeOH was evaporated. Preparative HPLC (RP-C18, MeCN in H_2O, gradient elution) afforded the desired glycoside.

3-ADON-15-*O*-(tetra-*O*-acetyl-β,D-glucoside) (7)

General procedure B; starting from 3-ADON (**3**) (40 mg, 0.12 mmol) and donor **6**[17] (92 mg, 0.18 mmol) **7** was obtained as a white solid (54 mg, 68%); ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.54 (dq, J = 5.8, 1.5 Hz, 1H), 5.20 (dt, J = 11.3, 4.5 Hz, 1H), 5.12 (t, J = 9.4 Hz, 1H), 5.04 (t, J = 9.6 Hz, 1H), 4.84 (dd, J = 9.4, 7.8 Hz, 1H), 4.78 (d, J = 2.0 Hz, 1H), 4.72 (d, J = 5.4 Hz, 1H), 4.29 (d, J = 8.2 Hz, 1H), 4.27 (dd, J = 12.4, 5.0 Hz, 1H), 4.11 (dd, J = 12.3, 2.5 Hz, 1H), 4.03 (d, J = 10.2 Hz, 1H), 3.88 (d, J = 4.3 Hz, 1H), 3.67 (d, J = 1.9 Hz, 1H), 3.66 – 3.60 (m, 1H), 3.48 (d, J = 10.1 Hz, 1H), 3.15 (d, J = 4.5 Hz, 1H), 3.10 (d, J = 4.3 Hz, 1H), 2.42 (d, J = 15.2, 4.3 Hz, 1H), 2.17 (s, 3H), 2.11 (dd, J = 15.0, 11.2 Hz, 1H), 2.09 (s, 3H), 2.08 (s, 3H), 2.01 (s, 3H), 1.98 (s, 3H), 1.85 (s, 3H), 1.09 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 199.5 (s, 1C), 170.7 (s, 1C), 170.6 (s, 1C), 170.3 (s, 1C), 169.5 (s, 1C), 169.4 (s, 1C), 138.7 (d, 1C), 135.8 (s, 1C), 100.6 (d, 1C), 79.2 (d, 1C), 73.9 (d, 1C), 72.9 (d, 1C), 72.1 (d, 1C), 71.2 (d, 1C), 71.1 (d, 1C), 69.7 (t, 1C), 68.5 (d, 1C), 68.1 (d, 1C), 65.1 (s, 1C), 62.0 (t, 1C), 51.5 (s, 1C), 47.6 (t, 1C), 45.9 (s, 1C), 40.6 (t, 1C), 21.1 (q, 1C), 20.9 (q, 1C), 20.8 (q, 1C), 20.7 (q, 2C), 15.3 (q, 1C), 13.8 (q, 1C); HRMS calcd for $C_{31}H_{40}O_{16}^+$ $[M+Na]^+$ 691.2209, found 691.2206.

DON-15-*O*-β,D-glucoside (2)

General procedure C; starting from **7** (27.8 mg, 42 µmol) **2** was obtained as a white solid white solid (18.7 mg, 98%); ^{1}H NMR (600 MHz, MeOD): δ 6.50 (dq, J = 5.9, 1.5 Hz, 1H), 4.98 (d, J = 6.2 Hz, 1H), 4.85 (s, 1H), 4.37 (dt, J = 11.2, 4.4 Hz, 1H), 4.18 (d, J = 10.5 Hz, 1H), 4.03 (d, J = 7.9 Hz, 1H), 3.84 (dd, J = 11.9, 1.9 Hz, 1H), 3.63 (dd, J = 11.7, 5.6 Hz, 1H), 3.57 (d, J = 10.6 Hz, 1H), 3.53 (d, J = 4.7 Hz, 1H), 3.30 – 3.27 (m, 1H), 3.24 – 3.18 (m, 2H), 3.10 (d, J = 4.4 Hz, 1H), 3.07 (d, J = 4.4 Hz, 1H), 3.04 (dd, J = 9.2, 7.8 Hz, 1H), 2.44 (dd, J = 14.8, 4.3 Hz, 1H), 1.96 (dd, J = 14.7, 11.2 Hz, 1H), 1.83 (s, 3H), 1.11 (s, 3H); ^{13}C NMR (150 MHz, MeOD): δ 202.5 (s, 1C), 140.3 (d, 1C), 136.9 (s, 1C), 104.8 (d, 1C), 82.2 (d, 1C), 78.0 (d, 1C), 77.8 (d, 1C), 75.7 (d, 1C), 75.2 (d, 1C), 71.5 (d, 2C), 69.8 (t, 1C), 69.7 (d, 1C), 66.7 (s, 1C), 62.8 (t, 1C), 53.3 (s, 1C), 48.1 (t, 1C), 47.3 (s, 1C), 44.5 (t, 1C), 15.3 (q, 1C), 14.8 (q, 1C); HRMS calcd for $C_{21}H_{30}O_{11}^+$ $[M+Na]^+$ 481.1681, found 481.1685.

3-ADON-15-(hepta-*O*-acetyl-β-gentiobioside) (12)

General procedure B; starting from 3-ADON (**3**) (50 mg, 148 µmol) and donor **11**[30] (180 mg, 223 µmol) **12** was obtained as a white solid (46 mg, 32 %); ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.55 (dq, J = 6.0, 1.4 Hz, 1H), 5.25-5.19 (m, 1H), 5.16 (t, J = 9.4 Hz, 1H), 5.12 (t, J = 9.4 Hz, 1H), 5.06 (t, J = 9.6 Hz, 1H), 4.96 (dd, J = 9.3; 8.2 Hz, 1H), 4.90-4.82 (m, 3H), 4.76 (d, J = 5.9 Hz, 1H), 4.63 (d, J = 8.2 Hz, 1H), 4.28-4.22 (m, 2H), 4.19 (d, J = 10.1 Hz, 1H), 4.11 (dd, J = 12.5, 2.4 Hz, 1H), 3.94 (d, J = 1.96 Hz, 1H), 3.89 (d, J = 4.2 Hz, 1H), 3.79 (dd, J = 11.7, 1.9 Hz, 1H), 3.68 (dd, J = 11.9, 7.2 Hz, 1H), 3.64-3.57 (m, 2H), 3.34 (d, J = 9.8 Hz, 1H), 3.20 (d, J = 4.3 Hz, 1H), 3.11 (d, J = 4.0 Hz, 1H), 2.60 (dd, J = 15.3, 4.3 Hz, 1H), 2.17 (s, 3H), 2.09 (s, 3H), 2.08 (s, 3H), 2.02 (s, 3H), 2.01 (s, 6H), 2.00 (s, 3H), 1.98 (s, 3H), 1.85 (s, 3H), 1.61 (s, 1H), 1.09 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 199.6 (s, 1C), 170.7 (s, 1C), 170.5 (s, 1C), 170.4 (s, 1C), 170.3 (s, 1C), 169.6 (s, 1C), 169.5 (s, 1C), 169.4 (s, 1C), 169.2 (s, 1C), 138.3 (d, 1C), 136.0 (s, 1C), 100.8 (d, 1C), 100.6 (d, 1C), 79.4 (d, 1C), 74.4 (d, 1C), 74.3 (d, 1C), 73.1 (d, 1C), 72.7 (d, 1C), 72.1 (d, 1C), 71.5 (d, 1C), 71.3 (d, 1C), 71.2 (d, 1C), 69.4 (d, 1C), 68.9 (d, 1C), 68.4 (d, 1C), 68.3 (t, 1C), 67.5 (t, 1C), 65.3 (s, 1C), 61.9 (t, 1C), 51.5 (s, 1C), 47.7 (t, 1C), 45.9 (s, 1C), 40.7 (t, 1C), 21.1 (q, 1C), 20.9 (q, 2C), 20.8 (q, 1C), 20.7 (q, 4C), 15.3 (q, 1C), 13.8 (q, 1C); HRMS calcd for $C_{43}H_{56}O_{24}^+$ $[M+Na]^+$ 979.3054, found 979.3060.

DON-15-*O*-β-gentiobioside (13)

General procedure C; starting from **12** (37.7 mg, 39.4 µmol) **13** was obtained as a white solid (23 mg, 94 %); ^{1}H NMR (600 MHz, MeOD): δ 6.62 (dq, J = 6.0, 1.6 Hz, 1H), 4.98 (d, J = 4.98 Hz, 1H), 4.84 (s, 1H), 4.39-4.34 (m, 2H), 4.15 (d, J = 10.6 Hz, 1H), 4.12 (dd, J = 11.6, 1.9 Hz, 1H), 4.03 (d, J = 7.9 Hz, 1H), 3.86 (dd, J = 11.9, 2.2 Hz, 1H), 3.74 (dd, J = 11.9, 6.3 Hz, 1H), 3.66 (dd, J = 11.8, 5.5 Hz, 1H), 3.57 (d, J = 10.6 Hz, 1H), 3.53 (d, J = 4.4 Hz, 1H), 3.42-3.38 (m, 1H), 3.37 (t, J = 8.9, 1.9 Hz, 1H), 3.32-3.23 (m, 4H), 3.20 (dd, J = 9.0, 8.0 Hz, 1H), 3.10 (d, J = 4.4 Hz, 1H), 3.07-3.03 (m, 2H), 2.45 (dd, J = 14.7, 4.4 Hz, 1H), 1.96 (dd, J = 14.6, 11.2 Hz, 1H), 1.83 (s, 3H), 1.12 (s, 3H); ^{13}C NMR (150 MHz, MeOD): δ 202.4 (s, 1C), 140.2 (d, 1C), 136.8 (s, 1C), 104.9 (d, 1C), 104.5 (d, 1C), 82.2 (d, 1C), 78.0 (d, 1C), 77.9 (d, 1C), 77.7 (d, 1C), 77.2 (d, 1C), 75.7 (d, 1C), 75.2 (d, 1C), 75.0 (d, 1C), 71.6 (d, 1C), 71.5 (d, 1C), 71.4 (d, 1C), 69.8 (t, 1C), 69.7 (d, 1C), 69.7 (t, 1C), 66.8 (s, 1C), 62.7 (t, 1C), 53.3 (s, 1C), 48.1 (t, 1C), 47.3 (s, 1C), 44.5 (t, 1C), 15.5 (q, 1C), 14.9 (q, 1C); HRMS calcd for $C_{27}H_{40}O_{16}^+$ $[M+Na]^+$ 643.2209, found 643.2221.

[$^{13}C_6$]Glucose pentaacetate (15)

To a suspension of [$^{13}C_6$]glucose (**14**) (1 g, 5.37 mmol, 1 eq.) in acetonitrile (3 mL) cooled to 0 °C was added $FeCl_3$ (87 mg, 0.54 mmol, 0.1 eq.) followed by dropwise addition of acetic anhydride (2.8 mL, 29.6 mmol, 5.5 eq.). The reaction mixture was warmed to room temperature and treated with ultrasonic irradiation for 45 min. The reaction mixture was diluted with CH_2Cl_2 (10 mL) and washed with water. The organic layer was dried over Na_2SO_4 and concentrated. The crude product was filtered through a pad of silica gel eluting with hexanes/EtOAc (1:1) to afford **15** (2.1 g, 98%) as a white solid. Analytical data matched those reported in the literature.[36]

2,3,4,6-Tetra-*O*-acetyl-D-[$^{13}C_6$]glucopyranose (16)

To a solution of glucose pentaacetate **(15)** (2.1 g, 5.3 mmol, 1 eq.) in dry THF (40 mL) was added benzylamine (625 mg, 5.8 mmol, 1.1 eq.). The reaction mixture was stirred at room temperature for 24 h, concentrated and dissolved in CH_2Cl_2 (40 mL). The solution was washed with 1 N HCl (25 mL) and water (25 mL). The organic layer was dried over Na_2SO_4 and concentrated. Flash chromatography (hexanes/EtOAc 1:1, 90 g silica gel) afforded the title compound **16** (1.64 g, 87 %) as a yellowish oil, which slowly crystallized during storage. Analytical data matched those reported in the literature.[36]

2,3,4,6-Tetra-*O*-acetyl-α,D-[$^{13}C_6$]glucopyranosyl-1-(*N*-phenyl)-2,2,2-trifluoroacetimidate ([$^{13}C_6$]6)

General procedure A; starting from **16** (1.54 g, 4.4 mmol) [$^{13}C_6$]**6** was obtained as a colorless oil (1.9 g, 83 %) that slowly crystallized during storage in the freezer; ^{13}C-decoupled ^{1}H-NMR matched those reported in the literature[17]; HRMS calcd for $C_{16}(^{13}C)_6H_{24}NO_{10}$ $[M+Na]^+$ 548.1446, found 548.1462.

3-ADON-15-*O*-(tetra-*O*-acetyl-β,D-[$^{13}C_6$]glucoside) ([$^{13}C_6$]7)

General procedure B; starting from 3-ADON (**3**) (80 mg, 0.24 mmol) and donor [$^{13}C_6$]**6** (189 mg, 0.36 mmol) [$^{13}C_6$]**7** was obtained as a white solid (85 mg, 53 %); ^{13}C-decoupled ^{1}H NMR data matched those of compound **7**; ^{13}C NMR (150 MHz, $CDCl_3$): δ 199.5 (s, 1C), 170.7 (s, 1C), 170.6 (s, 1C), 170.3 (s, 1C), 169.5 (s, 1C), 169.4 (s, 1C), 138.7 (d, 1C), 135.8 (s, 1C), 100.9-100.1 (m, 1C), 79.2 (d, 1C), 73.9 (d, 1C), 73.5-70.5 (m, 4C), 69.7 (t, 1C), 68.8-67.8 (m, 2C), 65.1 (s, 1C), 62.4-61.5 (m, 1C), 51.5 (q, 1C), 47.6 (t, 1C), 45.9 (s, 1C), 40.6 (t, 1C), 21.1 (q, 1C), 20.9 (q, 1C), 20.8 (q, 1C), 20.7 (q, 2C), 15.3 (q, 1C), 13.8 (q, 1C); HRMS calcd for $C_{25}(^{13}C)_6H_{40}O_{16}$ $[M+Na]^+$ 697.2410, found 697.2430.

DON-15-*O*-β,D-[$^{13}C_6$]glucoside ([$^{13}C_6$]2)

General procedure C; starting from [$^{13}C_6$]**7** (62 mg, 92 µmol) [$^{13}C_6$]**2** was obtained as a white solid (40 mg, 93 %); ^{13}C-decoupled ^{1}H NMR data matched those of compound **2**; ^{13}C NMR (150 MHz, MeOD): δ 202.5 (s, 1C), 140.3 (d, 1C), 136.9 (s, 1C), 104.0-102.9 (m, 1C), 82.2 (d, 1C), 77.3-75.7 (m, 2C), 75.7 (d, 1C), 75.6-74.7 (m, 1C), 71.9-71.1 (m, 2C), 69.8 (t, 1C), 69.7 (d, 1C), 66.7 (s, 1C), 63.1-62.4 (m, 1C), 53.3 (s, 1C), 48.1 (t, 1C), 47.3 (s, 1C), 44.5 (t, 1C), 15.3 (q, 1C), 14.8 (q, 1C); HRMS calcd for $C_{15}(^{13}C)_6H_{30}O_{11}^+$ $[M+Na]^+$ 487.1882, found 487.1884.

[$^{13}C_6$]Gentiobiose octaacetate (18)

1,2,3,4-Tetra-O-acetyl-β,D-glucose (**17**) (80 mg, 0.23 mmol, 1 eq.) and [$^{13}C_6$]glucosyl donor [$^{13}C_6$]**6** (144 mg, 0.27 mmol, 1.2 eq) were dissolved in dry CH_2Cl_2 (3 mL). MS 3Å (300 mg) was added and the mixture was stirred at rt under argon for 1 h. After cooling to -60 °C, TMSOTf (68 mg, 0.03 mmol, 0.15 eq) was added, the reaction mixture was slowly warmed to rt and stirred for 1 h. The reaction was quenched by addition of Et_3N, filtered through Celite and concentrated. The crude product was purified by column chromatography (hexanes/EtOAc, 5:1 to 1:1) to afford **18** (100 mg, 64 %) as a white solid. ^{13}C-decoupled ^{1}H-NMR matched those reported in the literature[37]; HRMS calcd for $C_{22}(^{13}C)_6H_{38}O_{19}^+$ $[M+Na]^+$ 707.2101, found 707.2117.

[$^{13}C_6$]Gentiobiose heptaacetate (19)

To a solution of [$^{13}C_6$]gentiobiose octaacetate (**18**) (138 mg, 0.20 mmol, 1 eq.) in dry DMF (1 mL) was added ammonium acetate (31 mg, 0,40 mmol, 2 eq.). The reaction mixture was stirred for 16 h at rt, concentrated and purified by column chromatography to afford **19** (109 mg, 85 %) as a white solid. ^{13}C-decoupled ^{1}H-NMR matched those reported in the literature[38]; HRMS calcd for $C_{20}(^{13}C)_6H_{36}O_{18}^+$ $[M+Na]^+$ 665.1996, found 665.2007.

1-*N*-Phenyl-2,2,2-trifluoroacetimidoyl-[$^{13}C_6$]gentiobiose heptaacetate ([$^{13}C_6$]11)

General procedure A; starting from **19** (67 mg, 0.10 mmol) [$^{13}C_6$]**11** was obtained as a colourless viscous syrup (70 mg, 83 %), which crystallized in the freezer; ^{13}C-decoupled ^{1}H-NMR matched those of non-labeled **11** as reported in the literature[30]; HRMS calcd for $C_{28}(^{13}C)_6H_{40}F_3NO_{18}^+$ $[M+Na]^+$ 836.2291, found 836.2283.

DON-15-*O*-β-[$^{13}C_6$]gentiobioside ([$^{13}C_6$]13)

General procedure D ('one-pot'); starting from 3-ADON (**3**) (38 mg, 113 µmol) and donor [$^{13}C_6$]**11** (138 mg, 170 µmol) [$^{13}C_6$]**13** was obtained as a white solid (23 mg, 32 %); ^{13}C-decoupled ^{1}H NMR data matched those of non-labeled **13**; ^{13}C NMR (150 MHz, MeOD): δ 202.4 (s, 1C), 140.2 (d, 1C), 136.8 (s, 1C), 105.3-104.5 (m, 1C), 104.5 (d, 1C), 82.2 (d, 1C), 78.5-77.5 (m, 3C), 77.2 (d, 1C), 75.7 (d, 1C), 75.5-74.7 (m, 2C), 72.1-70.9 (m, 3C), 69.8 (t, 1C), 69.7 (d, 1C), 69.7 (t, 1C), 66.8 (s, 1C), 63.23-61.9 (m, 1C), 53.3 (s, 1C), 48.1 (t, 1C), 47.3 (s, 1C), 44.5 (t, 1C), 15.5 (q, 1C), 14.9 (q, 1C); HRMS calcd for $C_{21}(^{13}C)_6H_{40}O_{16}^+$ $[M+H]^+$ 627.2590, found 627.2591.

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Keywords: glycosylation • orthoester • trichothecenes • masked mycotoxin • isotope labeling

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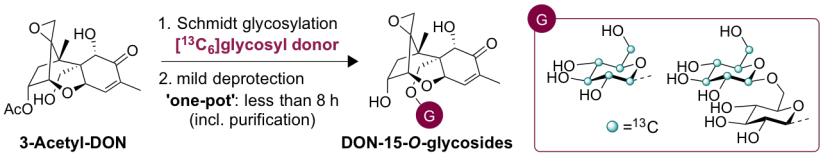

Glycosylation

*Julia Weber, Philipp Fruhmann, Christian Hametner, Alois Schiessl, Georg Häubl, Johannes Fröhlich, and Hannes Mikula**

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Synthesis of isotope labeled deoxynivalenol-15-*O*-glycosides

An efficient and fast procedure for the synthesis of deoxynivalenol-15-*O*-glycosides was developed enabling access to isotope labeled compounds. Applying this method DON-15-*O*-β,D-[$^{13}C_6$]glucoside and DON-15-*O*-β-[$^{13}C_6$]gentiobioside were prepared in reasonable amounts for characterization and as reference materials for further investigations.