Gentiobiosylation of β-Resorcylic Acid Esters and Lactones: First Synthesis and Characterization of Zearalenone-14-β,D-Gentiobioside

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Abstract: The development of an optimized protocol for the gentiobiosylation of β-resorcylic acid esters and lactones (β-RAL) is presented. Different gentiobiosyl donors were prepared and used for regioselective and diastereoselective glycosylation affording a reliable synthetic strategy towards this class of natural product glycosides. The improved procedure was finally used for the preparation of the masked *Fusarium* mycotoxin zearalenone-14-β,D-gentiobioside.

Key words: carbohydrates, glycosides, glycosylation, phenols, natural products

Zearalenone (ZEN, **1**, Figure 1) is a mycotoxin produced by several plant pathogenic *Fusarium* species, including *Fusarium graminearum*, *Fusarium culmorum*, and *Fusarium cerealis*. This mycotoxin is common in maize, but also barley, oats, wheat, and rice are susceptible to contamination with ZEN.[1] Fusarium species are probably the most prevalent toxin-producing fungi of the northern temperate regions and are commonly found in cereals grown worldwide.[2] ZEN and its metabolites possess estrogenic activity in mammals, including pigs, cattle, and sheep.[3] Problems of the reproductive tract as well as impaired fertility and abnormal fetal development in farm animals can be caused by ZEN.[4] Furthermore, this mycotoxin can interfere with various enzymes involved in steroid metabolism, which was recently investigated.[5] Therefore ZEN is of significant importance from an agricultural, economic, and health perspective.[6]

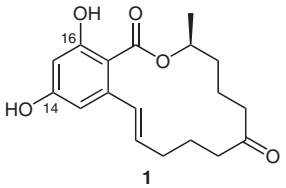

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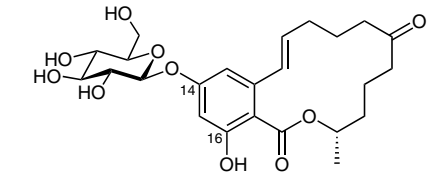
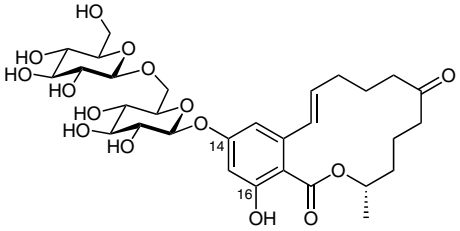
Figure 1 Structure of zearalenone (ZEN, **1**)

Conjugated mycotoxins, especially glycosides, can emerge after metabolization by living plants. The occurrence of ZEN-14-β,D-glucoside (**2**, Figure 2) in wheat was shown by Schneweis et al.[7] Furthermore ZEN-14-β,D-gentiobioside (formerly described as ZEN-14-diglucoside) was shown to be one of the major (late) phase II metabolites of **1** formed in the model plant *Arabidopsis thaliana* (Figure 2).[8] Awareness of such altered forms, often called masked mycotoxins, is increasing, but reliable analytical methods, standards as well as structural, occurrence, and toxicity data are still scarce.[9]



zearalenone-14-β,D-glucoside (**2**)



zearalenone-14-β,D-gentiobioside (**3**)

Figure 2 Structures of glycosylated ZEN metabolites (masked mycotoxins)

Gentiobiosylation has been shown to be an important metabolic pathway and several biologically active and relevant compounds have been described.[10] Additionally, further mycotoxin gentiobiosides have been identified as natural contaminants of corn and are also reported to be present in beer samples.[11]

In the course of ongoing research in the emerging field of masked and conjugated mycotoxins we focused on the synthesis of ZEN-14-β,D-gentiobioside (**3**) for structure confirmation and an accurate differentiation between **3** and the isomeric metabolite ZEN-14,16-di(β,D-glucoside).[12]

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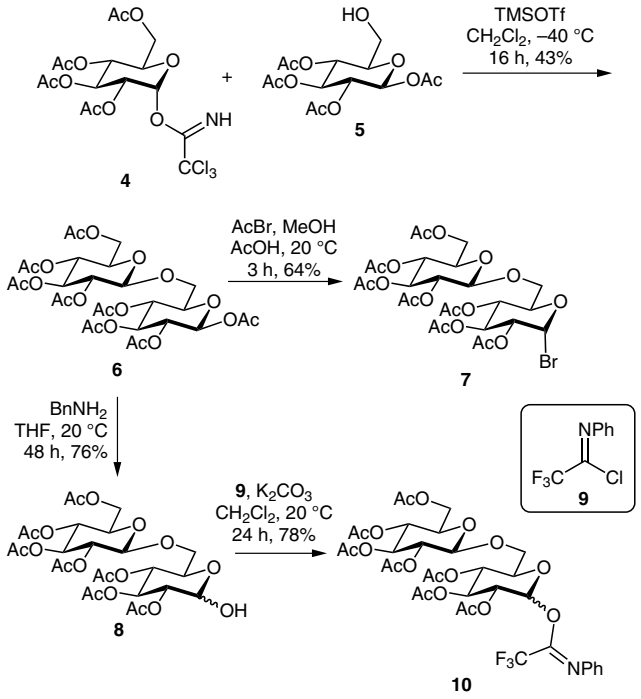
The first synthesis of a ZEN-glycoside, ZEN-14-β,D-glucoside (**2**) was achieved applying a Königs–Knorr procedure under phase-transfer conditions,[13] but the analogous reaction to produce ZEN-14-β,D-glucuronide was described to be unsuccessful under a variety of coupling conditions.[14] We were able to develop a fast and reproducible procedure for the chemical synthesis of ZEN-14-β,D-glucuronide, which was published very recently.[15]

Gentiobiosylation of phenols is often achieved by stepwise glucosylation and application of protective-group strategies.[16] Although similar approaches were developed for direct phenol glycosylation using a disaccharidic donor,[17] to the best of our knowledge there is no procedure reported for direct gentiobiosylation of phenols. Shimoda and coworkers applied cultured cells of *Eucalyptus perriniana* as biocatalysts for the synthesis of a mixture of glucosides and gentiobiosides of the isoflavonoids genistein and glycitein.[18]

Considering already described procedures for glucosylation of β-resorcylic acid esters and lactones (β-RAL), different disaccharidic donors were prepared for direct β-gentiobiosylation. Since acetyl protective groups were shown to be applicable for diastereoselective glycosylation of β-RAL, β-gentiobiose octaacetate (**6**) was selected as key intermediate. A reliable procedure[19] for β-glucosylation of 1,2,3,4-tetra-*O*-acetyl-β,D-glucoside (**5**)[20] using the trichloroacetimidate donor **4**[21] was applied to obtain larger amounts of **6**.[22] Gentiobiosyl bromide **7** was prepared according to Hunsen et al.,[23] and anomeric deprotection of **6** by reaction with benzyl amine[24] yielded the OH-sugar **8**,[25] which was subsequently reacted with *N*-phenyl-2,2,2-trifluoroacetimidoyl chloride (**9**)[26] to obtain the acetimidate gentiobiosyl donor **10**[27] (Scheme 1).



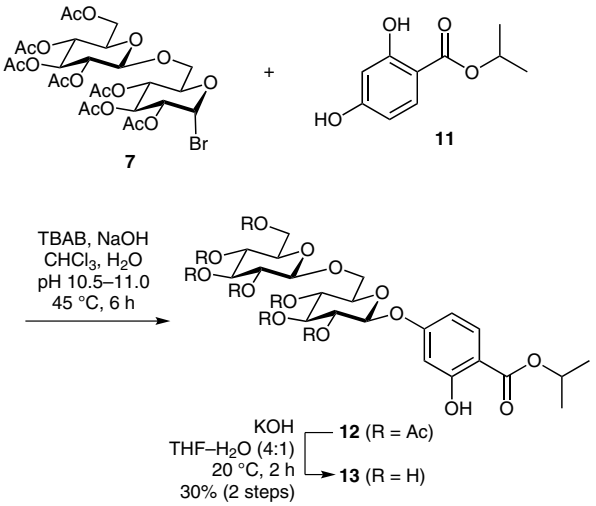
Scheme 1 Synthesis of gentiobiosyl donors **7** and **10**

2,4-Dihydroxybenzoic acid isopropyl ester (**11**)[28] was used as ZEN mimic to investigate the gentiobiosylation of resorcylic acid esters applying **7** and **10** as glycosyl donors under different reaction conditions. Selected data of reaction optimization and screening is shown in Table 1. Königs–Knorr glycosylation applying silver(I) salts for activation[29] was observed to result in nearly quantitative conversion after 48 hours. The highest yield (as determined by ^{1}H NMR after standard addition to the crude product mixture) was obtained when using Ag_2CO_3 for activation of the gentiobiosyl donor **7**.

Lewis acid mediated glycosylation[30] applying the acetimidate donor **10** gave only poor conversion or even complete rearrangement of **10** into the corresponding *N*-glycosyl acetamide (as indicated by NMR spectroscopy).

Phase-transfer glycosylation (Table 1, entry 7) was carried out applying an optimized procedure[31] according to the described approach for ZEN glucosylation.[13] The obtained results in terms of conversion and yield were quite comparable to that observed applying classic Königs–Knorr conditions.

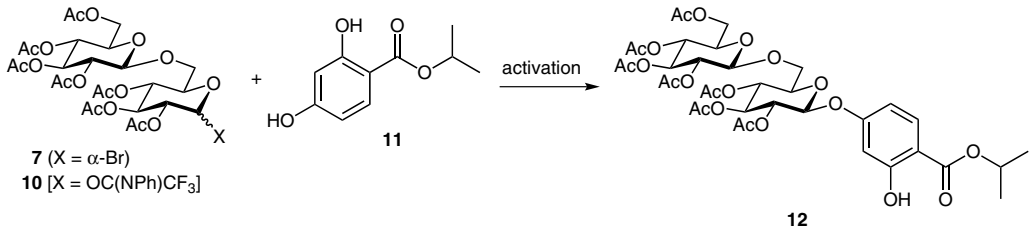
In all cases **12** was obtained within a complex product mixture mainly containing carbohydrate byproducts, but best results were achieved using phase-transfer glycosylation. Most of the impurities were removed by silica gel filtration, and the crude product was used without further purification in the deprotection step. Basic hydrolysis was achieved by reaction with an excess of KOH in THF–H_2O (4:1),[32] and the deacetylated product **13**[33] was finally isolated by reversed-phase (C18) column chromatography (Scheme 2).



Scheme 2 Synthesis of gentiobioside **13**

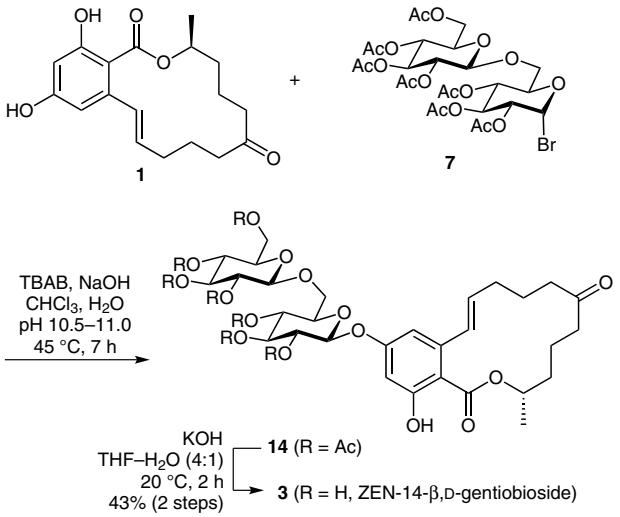
Finally, we were able to prepare ZEN-14-β,D-gentiobioside (**3**)[34] starting from ZEN (**1**) in an overall yield of 43% following general procedures for glycosylation and subsequent basic hydrolysis of the acetylated intermediate **14** according to the synthesis of the mimic gentiobioside **13** (Scheme 3).

Table 1 Gentiobiosylation of ZEN Mimic **11** Using Glycosyl Donors **7** and **10**



Entry	Donor (equiv)	Activation (equiv)	Solvent	Conversion of **11** (%)[a]	Yield (%)[b]
1	**7** (2.5)	Ag_2O (1.5)	MeCN	100	54
2	**7** (2.5)	Ag_2O (1.5)	CH_2Cl_2	90	52
3	**7** (2.5)	Ag_2CO_3 (1.5)	CH_2Cl_2	95	83
4	**10** (1.5)	TMSOTf (0.1)	CH_2Cl_2	13	9
5	**10** (2.5)	TMSOTf (0.02)	1,4-dioxane–toluene	0[c]	0[c]
6	**10** (1.5)	$BF_3{\cdot}OEt_2$ (0.1)	CH_2Cl_2	24	18
7	**7** (2.8)	TBAB (1.0), NaOH	$CHCl_3$–H_2O	92	52

[a] As determined by ^{1}H NMR.
[b] Nonisolated yield, determined by ^{1}H NMR spectroscopy (after standard addition of **11**).
[c] Rearrangement of the acetimidate **10** to the corresponding *N*-glycosyl acetamide was observed, as indicated by NMR spectroscopy.



Scheme 3 Gentiobiosylation of **1** and preparation of the target compound ZEN-14-β,D-gentiobioside (**3**)

In summary we were able to develop a reliable procedure for the gentiobiosylation of β-RAL, which in general should be applicable for many phenolic compounds. The first synthesis of a mycotoxin gentiobioside was accomplished yielding **3** in reasonable amounts for further studies.

Acknowledgment

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Supporting Information for this article is available online at http://www.thieme-connect.com/ejournals/toc/synlett. General experimental details and full characterization data (MS and NMR spectra) of **3** are included.

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(19) **Procedure for the Lewis Acid Mediated Synthesis of β-Gentiobiose Octaacetate (6)**
1,2,3,4-Tetra-*O*-acetyl-β,D-glucose (**4**, 3.8 g, 11 mmol) and trichloroacetimidoyl donor **5** (5.8 g, 11.8 mmol) were dissolved in dry CH_2Cl_2 (100 mL). MS 3 Å (10 g) was added, and the mixture was stirred at r.t. under argon for 1 h. After cooling to –40 °C, TMSOTf (0.2 mL, 1.1 mmol) was added, and the reaction mixture was stirred at –40 °C for 16 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 obtain **6** (3.1 g, 43%) as a white solid. Analytical data matched those reported in the literature.[22]

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(24) **Procedure for Anomeric Deprotection of 6**
To a solution of **6** (1.31 g, 1.9 mmol) in dry THF (45 mL) was added benzylamine (0.23 mL, 2.1 mmol), and the reaction mixture was stirred at r.t. for 48 h. The solvent was removed under reduced pressure, and the residue was dissolved in CH_2Cl_2 (100 mL), washed with 1 M HCl (2 × 100 mL) and H_2O (100 mL). The organic layer was dried over Na_2SO_4 and concentrated. Column chromatography (hexanes–EtOAc, 1:1 to 1:3) afforded the desired product **8** (0.91 g, 76%) as a white solid. Analytical data matched those reported in the literature.[25]

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(27) **Procedure for the Synthesis of Gentiobiosyl Acetimidate Donor 10**
To a solution of gentiobiose heptaacetate (**8**, 0.8 g, 1.3 mmol) in dry CH_2Cl_2 (20 mL) was added K_2CO_3 (0.36 g, 2.6 mmol) and *N*-phenyl-2,2,2-trifluoroacetimidoyl chloride (**9**, 0.4 mL, 2.6 mmol). The reaction mixture was stirred at r.t. and under argon for 24 h. The solvent was removed on a rotary evaporator, and the residue was purified by column chromatography (hexanes–EtOAc, 3:1) to yield **10** (0.82 g, 78%) as a white solid.
Analytical Data for 10
^{1}H NMR (200 MHz, $CDCl_3$): δ = 7.39–7.22 (m, 2 H), 7.18–7.06 (m, 1 H), 6.86 (d, *J* = 7.6 Hz, 2 H), 5.77 (br s, 1 H), 5.25–5.15 (m, 2 H), 5.11 (d, *J* = 10.3 Hz, 1 H), 5.05–4.92 (m, 3 H), 4.56 (d, *J* = 7.8 Hz, 1 H), 4.25 (dd, *J* = 12.3, 4.7 Hz, 1 H), 4.12 (dd, *J* = 12.3, 2.3 Hz, 1 H), 3.90 (d, *J* = 9.4 Hz, 1 H), 3.76–3.56 (m, 3 H), 2.08 (s, 3 H), 2.06 (s, 3 H), 2.03 (s, 3 H), 2.02 (s, 3 H), 2.01 (s, 3 H), 2.00 (s, 3 H), 1.95 (s, 3 H). ^{13}C NMR (50 MHz, $CDCl_3$): δ = 170.8 (s, 1 C), 170.3 (s, 1 C), 170.27 (s, 1 C), 169.6 (s, 1 C), 169.52 (s, 1 C), 169.49 (s, 1 C), 169.1 (s, 1 C), 142.9 (s, 1 C), 128.8 (d, 2 C), 124.6 (d, 1 C), 119.2 (d, 2 C), 100.5 (d, 1 C), 94.3 (d, 1 C), 74.1 (d, 1 C), 72.6 (d, 1 C), 72.4 (d, 1 C), 71.9 (d, 1 C), 70.8 (d, 1 C), 70.2 (d, 1 C), 68.3 (d, 1 C), 68.2 (d, 1 C), 67.3 (t, 1 C), 61.7 (t, 1 C), 20.9 (q, 1 C), 20.7 (q, 4 C), 20.6 (q, 1 C), 20.5 (q, 1 C). HRMS (ESI$^+$): *m/z* calcd for $C_{34}H_{40}F_3NNaO_{18}^+$ $[M + Na]^+$: 830.2090; found: 830.2099.

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(29) **General Procedure A: Silver(I)-Activated Königs–Knorr Glycosylation Using Gentiobiosyl Bromide 7**
To a solution of the glycosyl acceptor (1 equiv) and gentiobiosyl bromide **7** (2.5 equiv) in CH_2Cl_2 or MeCN (5 mL/mmol) MS 3 Å (0.1 g/mL) was added, and the reaction mixture was stirred at r.t. under argon for 1 h. After addition of silver(I) salt (1.5 equiv) stirring was continued in the dark for an additional period of 48 h. Filtration through Celite and concentration under reduced pressure afforded the crude product mixture.

(30) **General Procedure B: Lewis Acid Mediated Glycosylation Using Acetimidate 10**
To a solution of the glycosyl acceptor (1 equiv) and gentiobiosyl bromide **7** (1.5–2.5 equiv) in CH_2Cl_2 or dioxane–toluene (5 mL/mmol) MS 3 Å (0.1 g/mL) was added, and the reaction mixture was stirred at r.t. under argon for 1 h. After cooling to –10 °C, TMSOTf or $BF_3{\cdot}OEt_2$ (0.02–0.1 equiv) was added, and stirring was continued at –10 °C for an additional period of 16 h. The reaction mixture was filtered through Celite, washed with sat. aq $NaHCO_3$, dried over Na_2SO_4, and concentrated under reduced pressure to afford the crude product mixture.

(31) **General Procedure C: Phase-Transfer Glycosylation Using Gentiobiosyl Bromide 7**
Glycosyl acceptor (1 equiv), **7** (2.8 equiv), and TBAB (1 equiv) were dissolved in $CHCl_3$ (80 mL/mmol). Borate buffer (80 mL/mmol) was added, and the reaction mixture was warmed to 45 °C. The pH was kept between 10.5 and 11.0 by dropwise addition 0.1 M aq NaOH. After 6 h the organic layer was separated, dried over Na_2SO_4, and concentrated to afford the crude product mixture.

(32) **General Procedure D: Basic Hydrolysis of Crude Acetyl-Protected Gentiobiosides**
Crude acetyl-protected gentiobioside (**12** or **14**) was filtered over silica gel (hexanes–EtOAc = 3:1 to 1:2) to remove most of the carbohydrate impurities. Appropriate fractions were pooled and concentrated. The residue was dissolved in THF–H_2O (4:1, 50 mL/mmol), KOH (10 equiv) was added, and the reaction mixture was stirred for 2 h at r.t. After addition of 0.1 N HCl (pH 6.8), the solution was diluted with H_2O and immediately extracted with EtOAc. The combined organic layer was dried over Na_2SO_4 and concentrated. RP-C18 column chromatography (MeCN–H_2O gradient elution, used for **13**) or preparative RP-C18-HPLC (MeCN–H_2O gradient elution, used for **3**) yielded the desired gentiobioside.

(33) **Synthesis of Gentiobioside 13**
Starting from ZEN mimic **11** (11.7 mg, 0.06 mmol) and following general procedures C and D, gentiobioside **13** was obtained as a white solid (9.3 mg, 30%).
Analytical Data for 13
^{1}H NMR (400 MHz, MeOD): δ = 7.77 (d, *J* = 8.8 Hz, 1 H), 6.71 (d, *J* = 1.8 Hz, 1 H), 6.64 (dd, *J* = 8.8, 1.8 Hz, 1 H), 5.26 (sept, *J* = 6.2 Hz, 1 H), 4.98 (d, *J* = 6.9 Hz, 1 H), 4.36 (d, *J* = 7.5 Hz, 1 H), 4.17 (d, *J* = 11.3 Hz, 1 H), 3.91–3.83 (m, 1 H), 3.88–3.78 (m, 2 H), 3.81–3.74 (m, 1 H), 3.67 (dd, *J* = 11.3, 5.4 Hz, 1 H), 3.50–3.44 (m, 2 H), 3.44–3.38 (m, 1 H), 3.36–3.30 (m, 1 H), 3.28–3.20 (m, 2 H), 1.38 (d, *J* = 6.2 Hz, 6 H). ^{13}C NMR (100 MHz, MeOD): δ = 170.7 (s, 1 C), 164.6 (s, 1 C), 164.4 (s, 1 C), 132.4 (d, 1 C), 109.6 (d, 1 C), 108.4 (s, 1 C), 104.94 (d, 1 C), 104.88 (d, 1 C), 101.32 (d, 1 C), 78.0 (d, 1 C), 77.9 (d, 1 C), 77.8 (d, 1 C), 77.3 (d, 1 C), 75.1 (d, 1 C), 74.7 (d, 1 C), 71.6 (d, 1 C), 71.4 (d, 1 C), 70.2 (d, 1 C), 70.1 (t, 1 C), 62.7 (t, 1 C), 22.1 (q, 2 C). HRMS (ESI$^-$): *m/z* calcd for $C_{22}H_{31}O_{14}^-$ [M – H]$^-$: 519.1719; found: 519.1707.

(34) **Synthesis of ZEN-14-β,D-gentiobioside (3)**
Starting from ZEN (**1**, 33.4 mg, 0.105 mmol) and following general procedures C and D, ZEN-14-β,D-gentiobioside (**3**) was obtained as a white solid (29 mg, 43%).
Analytical Data for 3
^{1}H NMR (400 MHz, DMSO-d_6): δ = 10.43 (br s, 1 H), 6.65 (d, *J* = 1.5 Hz, 1 H), 6.53 (d, *J* = 1.5 Hz, 1 H), 6.40 (d, *J* = 15.4 Hz, 1 H), 6.01 (dt, *J* = 15.4, 7.1, 1 H), 5.36 (d, *J* = 4.3 Hz, 1 H), 5.18 (d, *J* = 3.2 Hz, 1 H), 5.11–5.04 (m, 1 H), 4.98–4.86 (m, 2 H), 4.90 (d, *J* = 7.8 Hz, 1 H), 4.53 (br s, 1 H), 4.19 (d, *J* = 7.8 Hz, 1 H), 3.98 (d, *J* = 10.2 Hz, 1 H), 3.66 (d, *J* = 12.1 Hz, 1 H), 3.63–3.55 (m, 2 H), 3.47–3.41 (m, 1 H), 3.30–3.18 (m, 3 H), 3.16–3.09 (m, 1 H), 3.08–3.03 (m, 2 H), 2.98 (t, *J* = 8.1 Hz, 1 H), 2.39–2.32 (m, 1 H), 2.32–2.26 (m, 2 H), 2.25–2.16 (m, 1 H), 2.07–1.95 (m, 1 H), 1.83–1.71 (m, 1 H), 1.70–1.59 (m, 3 H), 1.58–1.45 (m, 2 H), 1.27 (d, *J* = 6.1 Hz, 6 H). ^{13}C NMR (100 MHz, DMSO-d_6): δ = 211.2 (s, 1 C), 168.7 (s, 1 C), 160.0 (s, 1 C), 158.5 (s, 1 C), 138.4 (s, 1 C), 133.3 (d, 1 C), 129.8 (d, 1 C), 112.8 (s, 1 C), 105.2 (d, 1 C), 103.9 (d, 1 C), 103.1 (d, 1 C), 100.6 (d, 1 C), 77.3 (d, 1 C), 77.1 (d, 1 C), 76.8 (d, 1 C), 74.0 (d, 1 C), 73.6 (d, 1 C), 71.9 (d, 1 C), 70.4 (d, 1 C), 69.9 (d, 1 C), 68.9 (t, 1 C), 61.4 (t, 1 C), 43.5 (t, 1 C), 37.1 (t, 1 C), 34.8 (t, 1 C), 31.4 (t, 1 C), 21.4 (t, 2 C), 20.3 (q, 2 C). HRMS (ESI$^-$): *m/z* calcd for $C_{30}H_{41}O_{15}^-$ [M – H]$^-$: 641.2451; found: 641.2455.

