First Synthesis of (−)-Altenuene-D₃ Suitable as Internal Standard for Isotope Dilution Mass Spectrometry

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Abstract: Metabolites from Alternaria fungi exhibit a variety of biological properties such as phytotoxic, cytotoxic, or antimicrobial activity. Optimization of a literature procedure culminated in an efficient total synthesis of (−)-altenuene as well as a stable isotope-labeled derivative suitable for implementation in a LC-MS/MS method for mycotoxin analysis.

Keywords: altenuene; Alternaria mycotoxins; food safety; isotope-labeled; SIDA-LC-MS/MS; Suzuki coupling

1. Introduction

1.1. Alternaria Mycotoxins

Invading crops at the pre- and post-harvest stage, certain filamentous fungi can contaminate food and feedstuff by producing a variety of toxic secondary metabolites, which are referred to as mycotoxins [1]. Derived from the extremely wide-spread genus, Alternaria, the commonly named Alternaria toxins [2,3] are frequently found in agricultural crops, such as grains, fruits, and vegetables, as well as soil, wall papers, and textures, and have thus been implicated in several animal and human health disorders. The European Food Safety Authority (EFSA) assessed the risks for public health related to the presence of Alternaria toxins in food and feed. Although the toxicological data on various Alternaria toxins was limited, it recommended the supervision of those toxins in foods and feeds [4]. Considering the need for possible future regulation, a suitable LC-MS/MS standard method for the determination of the most relevant Alternaria metabolites is required (Figure 1).

Figure 1. Relevant Alternaria mycotoxins.
With (-)-altenuene (ALT, 1a) [5] being the most acutely toxic in mice (LD50 > 50 mg/kg) [4], the development of new analytical methods for its detection and quantification has become of great importance for human and animal health risk assessment. Due to its high selectivity, sensitivity, and multi-analyte suitability, LC-MS/MS has become the method of choice for trace analysis of mid polar and polar organic contaminants in food and feed. As a major step forward in the improvement of accuracy (trueness and precision), isotope labeled compounds (mostly 13C-, 2D- or 15N-labeling) are widely used as internal standards to quantify the target analytes by LC-MS/MS. This so-called stable isotope dilution assay (SIDA, SIDA-LC-MS/MS) is considered the primary ratio method, representing a high level of metrology. Thus, the development of isotope labeled standards for mycotoxins and their implementation in LC-MS/MS methods have received much attention over recent years [6].

Moreover, 1a has recently been reported to exhibit interesting cytotoxic activity against HCT116 cell lines with an IC50 value of 3.13 μM. This makes it a potential lead compound for the development of new anti-tumor drug candidates [7], which further supported our interest in working towards an efficient and reliable access to ALT (1a) and ALT-D3 (1b) by total synthesis.

1.2. Retrosynthetic Analysis

Based on the only total synthesis of 1a reported to date [8], we herein wish to present the first preparation of a deuterated (-)-altenuene derivative (ALT-D3, 1b) following an improved procedure. To prevent any ‘cross talk’ between the native and the labeled analyte we aimed to synthesize ALT-D3 (1b) by coupling the deuterated boronate 4b and halogenated allylic alcohol 5b, which should permit a facile isotope incorporation using commercially available reagents in an analogous fashion as described for the native compound 1a (Scheme 1) [8].

![Scheme 1. Retrosynthetic analysis of (-)-altenuene (1a) and (-)-altenuene-D3 (1b).](image)

2. Results and Discussion

2.1. Synthesis of Deuterated Boronate 4b

The synthesis of the deuterated boronic acid derivative 4b was efficiently achieved in analogy to a literature procedure [9] but starting rather with a regioselective alkylation of the 4-hydroxy group using commercial iodomethane-D3 (99.5atom% D) as the deuterium source (Scheme 2). The slightly lower yield compared to the Mitsunobu reaction employed by Podlech et al. is compensated by the simplicity and the low cost of the reagents used in this protocol.

![Scheme 2. Synthesis of boronate 4b. Reagents and conditions: a TF2O, pyridine, 0 °C – r.t. (82%), b PinBH, NEt3, Pd(PPh3)4, dioxane, 80 °C (73%).](image)
2.2. Synthesis of Bromo Alcohol 5b

A major drawback in the original synthesis of 1b is the unfavorable diastereoselectivity of ~1:6 in the Grignard reaction affording the required tertiary alcohol 5a (Scheme 1) [8]. In order to tackle this issue, we first decided to optimize the key carbonyl addition reaction. With the labile iodo substrate 10a limiting the number of applicable reagents, we envisioned the usage of the much more stable bromo enone derivative 10b, which should allow us to investigate other organometallic reagents. Thus, bromination of the known enone 9 obtained in 4 steps from inexpensive D-(−)-quinic acid (8) according to the literature, gave bromo enone 10b with an excellent overall yield (Scheme 3).

Scheme 3. Synthesis of the bromo enone 10b; for the synthesis of compound 9 see [10].

We then focused on the optimization of the inefficient methylation of halo enones 10a and 10b (Table 1). Applying the original reaction conditions (Table 1, Entry 1) to the bromo enone 10b provided a ~1:4 mixture of 5b and the undesired isomer epi-5b (Table 1, Entry 2). A further refinement to a ~1:2 ratio could be achieved by the addition of stoichiometrical amounts of CeCl3 (Table 1, Entry 3). Interestingly, changing from the methyl Grignard reagent to the more reactive methyllithium, the diastereoselectivity changed completely with the desired carbinol 5b now being the major isomer (d.r. ~ 1:4:1, Table 1, Entry 6). While the addition of CeCl3 proved to be disadvantageous in this case (Table 1, Entry 7), lowering the reaction temperature to −78 °C resulted in the best diastereomeric ratio obtained with ~1:7:1 in favor of 5b (Table 1, Entry 8) but came at the cost of incomplete conversion of the starting material 10b. Applying the same conditions to iodo enone 10a only nonspecific decomposition was observed as expected (Table 1, Entry 9), emphasizing the initially proposed enhanced chemical stability of the bromo derivative 10b.

Table 1. Screening of methylation conditions of halo enones 10a and 10b.

<table>
<thead>
<tr>
<th>Entry</th>
<th>X</th>
<th>M</th>
<th>Conditions [a]</th>
<th>d.r. 5b/epi-5b [b]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>I</td>
<td>Mgl</td>
<td>−40 °C / THF</td>
<td>1:6</td>
</tr>
<tr>
<td>2</td>
<td>Br</td>
<td>Mgl</td>
<td>−40 °C / THF</td>
<td>1:4</td>
</tr>
<tr>
<td>3</td>
<td>Br</td>
<td>Mgl</td>
<td>−40 °C / CeCl3</td>
<td>1:2</td>
</tr>
<tr>
<td>4</td>
<td>Br</td>
<td>MgBr</td>
<td>r.t. / Fe-Josiphos</td>
<td>only 1,4-addition</td>
</tr>
<tr>
<td>5</td>
<td>Br</td>
<td>MgBr</td>
<td>−78 °C / Fe-Josiphos CuBr-SMe2 / THF</td>
<td>1,4-addition + traces of 5b</td>
</tr>
<tr>
<td>6</td>
<td>Br</td>
<td>Li</td>
<td>−40 °C / THF</td>
<td>1:4:1</td>
</tr>
<tr>
<td>7</td>
<td>Br</td>
<td>Li</td>
<td>−40 °C / CeCl3</td>
<td>1:1</td>
</tr>
<tr>
<td>8</td>
<td>Br</td>
<td>Li</td>
<td>−78 °C / THF</td>
<td>1:1</td>
</tr>
<tr>
<td>9</td>
<td>I</td>
<td>Li</td>
<td>−78 °C / THF</td>
<td>decomposition</td>
</tr>
<tr>
<td>10</td>
<td>Br</td>
<td>AlMe2</td>
<td>0 °C / THF</td>
<td>−1:3</td>
</tr>
<tr>
<td>11</td>
<td>Br</td>
<td>AlMe2</td>
<td>0 °C / [Rh(cod)Cl2] / BINAP (rac.) / THF / n-heptane</td>
<td>only 1,4-addition</td>
</tr>
<tr>
<td>12</td>
<td>Br</td>
<td>DABAL</td>
<td>0 °C / [Rh(cod)Cl2] / BINAP (rac.) / THF / n-heptane</td>
<td>no reaction</td>
</tr>
<tr>
<td>13</td>
<td>Br</td>
<td>ZnMe2</td>
<td>r.t. / THF</td>
<td>no reaction</td>
</tr>
<tr>
<td>14</td>
<td>Br</td>
<td>ZnMe2</td>
<td>r.t. / Ti(iPrO)4 / toluene</td>
<td>traces</td>
</tr>
</tbody>
</table>

[a] 0.1 mmol scale. [b] determined by GC-MS analysis of the crude mixture; retention times: tR (5a) = 17.20 min, tR (5b) = 17.05 min (separation conditions are given in Section 3, Materials and Methods).
Any attempts to further improve the diastereoselectivity of the 1,2-addition by employing either chiral catalytic systems (e.g., BINAP + Josiphos, which is actually known to promote 1,4-addition [11]) and/or alternative methyl donating reagents (e.g., AlMe₃, DABAL, ZnMe₂) [12–14] resulted in the formation of either the 1,4-addition product or only traces of the desired alcohol 5b (Table 1, Entries 4,5,10–14).

On a preparative more useful scale, the newly established conditions (Table 1, Entry 8) delivered the requisite bromo alcohol 5b with an essentially improved yield of 38% after chromatographic separation of both isomers by MPLC. With substantial amounts of the labeled boronate 4b and the crucial alcohol 5b in hand we started assembling the pieces.

2.3. Suzuki Coupling of Bromo Alcohol 5b and Boronate 4b

Alcohol 5b and boronate 4b were subsequently subjected to the reported Suzuki cross coupling conditions (Pd(OAc)₂, S-Phos, Cs₂CO₃, dioxane/H₂O, 80 °C) [8], approved for the arylation of iodo alcohol 5a. Unfortunately, this test almost exclusively resulted in homodimerization of the boronic ester 4b. After thoroughly scouring the literature for alternative catalytic systems, which would permit the desired hetero coupling to proceed, we were delighted to find that a simple alteration of catalyst and base (Pd(dppf)Cl₂, NEt₃, THF/H₂O, 70 °C) [15] facilitated a smooth conversion, providing the advanced intermediate 11b with 81% isolated yield (Scheme 4).

Interestingly, only traces of concomitant lactonization product 12b were observed illustrating the remarkable mildness of this adjusted protocol. Cyclization using K₂CO₃ in methanol and cleavage of the bisketal protecting group by refluxing in aqueous AcOH finally gave rise to 1b in 81% yield after two steps and purification by preparative HPLC (purity > 99.9% and > 99atom% D). Lastly, submitting alcohol 5b and the unlabeled boronate 4a (synthesized analogously using iodomethane [9]) to the same reaction sequence delivered ALT (1a) with identical yields.

2.4. Implementation of the ALT-D₃ Standard (1b) in a LC-MS/MS Method

There are some recent LC-MS/MS methods available for the quantification of Alternaria toxins including ALT (1a) in food and feed based on positive or negative ionization mode as well using acidic or alkaline LC conditions [16–18]. Due to better performance data, ESI(−) mode under alkaline LC conditions was used to set up/optimize the MS/MS and LC parameters of the synthesized ALT-D₃ (1b) (Table 2). HPLC: Agilent 1200 with autosampler; column: Eurospher 100-5 C18 P, particle size 5 µm, 250 × 4 mm (Knauer, Berlin, Germany); Inj.-vol: 10 µL; oven temp.: 30 °C; flow: 0.5 mL/min; eluent A: water with 5 mM ammonium acetate and ammonium hydroxide (pH 8.7), eluent B: Methanol with 5 mM ammonium acetate. 0–5 min 90% A, 5–22 min 0% A, 22–30 min 90% A. MS/MS: AB-Sciex QTrap4000; turbo ion spray, single reaction monitoring (SRM; negative polarity); TEM: 500 °C, CUR: 50 a.u., CAD: 12 a.u., IS: −2000 V; DP: −60 V; CE: −40 V; CXP: −10 V.
Table 2. Recorded mass transitions for native (1a) and isotope labeled ALT (1b).

<table>
<thead>
<tr>
<th>Substance</th>
<th>Q1 Mass (Da)</th>
<th>Q3 Mass (Da)</th>
<th>Q3 Mass (Da)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT (1a)</td>
<td>291.0</td>
<td>203.0</td>
<td>248.0</td>
</tr>
<tr>
<td>ALT-D₃ (1b)</td>
<td>294.0</td>
<td>203.0</td>
<td>248.0</td>
</tr>
</tbody>
</table>

ALT-D₃ (1b) does not show any signals for the mass transition of native ALT (1a) and vice versa with identical retention times (tᵣ (1a/1b) = 16.67 min, Figure 2). This is important for ideal compensation of ionization effects (mostly matrix suppression effects) and the use of ALT-D₃ (1b) as internal standard. Moreover, the presented SIDA-LC-MS/MS method does not only allow the analysis of ALT (1a) and ALT-D₃ (1b) but is also applicable to other relevant Alternaria toxins, e.g., alternariol (2a), alternariol monomethyl ether (2b), tentoxin or tenuazonic acid (3).

Figure 2. HPLC-MS/MS runs of (a) synthesized ALT-D₃ (1b) and (b) native ALT (1a) standards. Displayed are the selected ion chromatograms (SRM(−)-mode) of the quantifier mass transitions for ALT-D₃ (red) and ALT (blue).
3. Materials and Methods

Commercial chemicals and solvents were used as received without any further purification. Triethylamine was dried over KOH, distilled in vacuo, and stored under an atmosphere of nitrogen. All reactions were carried out under an inert gas atmosphere using dry grade reagents and solvents unless stated otherwise. Reactions were monitored by thin-layer chromatography on Merck TLC Silica gel 60 F254 sheets with UV-visualization (254 nm and 336 nm) or KMnO4 staining. The diastereomeric ratios of the compounds 5 and epi-5 were determined using a hp Series II 5860 GC device (SGE Analytical Science column, 25 m × 0.22 μm, BP × 5 × 0.25 μm, HP Inc., Palo Alto, CA, USA; Trajan Scientific, Ridgewood, Victoria, Australia) connected to a hp 5971 Series mass selective detector. Melting points were determined with the MP 90 melting point device by Mettler Toledo (Columbus, OH, USA). MPLC purification was performed with a Shimadzu MPLC system (Shimadzu Corp., Kyoto, Japan). The conditions and devices used for LC-MS/MS analysis of compounds 1a and 1b are stated in Section 2.4. ESI-high resolution mass spectra were recorded with a Bruker Daltonik microTOF coupled with a LC Packings Ultimate HPLC system (Bruker Corp., Billerica, MA, USA; Dionex/LC Packings, Sunnyvale, CA, USA). NMR spectra were either recorded on a Varian Mercury Plus 300 (300.8 MHz), Varian Mercury Plus 400 (399.95 MHz), or a Bruker Avance III HD (400.13 MHz) spectrometer (Varian/Agilent Technologies Inc., Santa Clara, CA, USA; Bruker Corp., Billerica, MA, USA). All signals were referenced to the respective solvent signals reported in the literature [19]. All coupling constants J refer to hydrogen–hydrogen interactions unless stated otherwise. The 1H and 13C NMR spectra of all new compounds as well as UV/Vis- and IR-spectra of the native and labeled natural products can be found in the Supplementary Materials.

3.1. Synthesis of (2S,3S,4aR,8aR)-7-bromo-2,3,4a,5-tetrahydro-2,3-dimethoxy-2,3-dimethylbenzo[b][1,4]dioxin-6(8aH)-one (10b)

Enone 9 (2.91 g, 12.0 mmol, 1.00 equiv) was dissolved in 32 mL DCM in a 250 mL round-bottom flask and cooled to 0 °C. A solution of 632 μL (1.96 g, 12.3 mmol, 1.02 equiv) bromine in 32 mL DCM was added slowly over 1 h with a dripping funnel and the mixture was stirred for another 30 min at the same temperature. After that, 2.85 mL (1.96 g, 20.4 mmol, 1.70 equiv) NEOF was added and the resulting blue solution was warmed to room temperature while stirring. After 1 h GC-MS analysis indicated the complete consumption of the starting material and the reaction was quenched with NaHCO3 (100 mL) and the phases were separated. The aqueous phase was extracted with DCM (3 × 75 mL), and the combined organic phases were washed with brine (50 mL), dried over Na2SO4, and the solvent was evaporated. The crude product was purified by chromatography using a CH/EE-gradient (15 → 25% EE) yielding 3.3 g (86%) of the title compound 10b as a white solid. Rf (CH/EE, 4:1) = 0.38. MP = 202 °C (decomposition). HR-MS: Calc. for [M + Na]+ = 343.0152, found: 343.0161. 1H-NMR (300 MHz, CDCl3): δ = 1.32 (s, 3 H), 1.36 (s, 3 H), 2.59 (dd, J = 13.5, 16.4 Hz, 1 H), 2.94 (dd, J = 4.8 Hz, 16.4 Hz, 1 H), 3.26 (s, 3 H), 3.31 (s, 3 H), 4.07 (ddd, J = 4.8, 9.1, 13.5 Hz, 1 H), 4.48 (ddd, J = 0.3, 2.0, 9.1 Hz, 1 H), 7.30 (dd, J = 0.3, 2.0 Hz, 1 H). 13C-NMR (75 MHz, CDCl3): δ = 17.7, 17.8, 41.3, 48.4, 48.5, 67.6, 70.4, 100.0, 101.1, 124.4, 148.9, 188.8.

3.2. Synthesis of (2S,3S,4aR,6R,8aR)-7-Bromo-2,3,4a,5,6,8a-hexahydro-2,3-dimethoxy-2,3,6-trimethylbenzo[b] [1,4]dioxin-6(8aH)-one (5b)

To a solution of bromo enone 10b (1.0 g, 3.1 mmol, 1.0 equiv) in 62 mL dry THF (0.05 M) at −78 °C was added methyllithium (1.6 M in Et2O, 3.0 mL, 4.8 mmol 1.5 equiv) and the reaction mixture was slowly warmed to r.t. over 1 h. Saturated NH4Cl-solution (40 mL) was added and the aqueous phase was extracted with MTB (3 × 50 mL). The combined organic phases were dried (Na2SO4) and the solvent was evaporated. Purification of the crude product by normal-phase chromatography (YMC-Gel, 6 nm S-15 μm) using MTB/heptane (3:7) and evaporation of the solvents yielded 374 mg (38%) of alcohol 5b as a light yellow solid. Rf (n-Hep/MTBE, 7:3) = 0.18. GC-MS: [M − OMe]+ = 305/307, tR = 17.05 min. MP = 73.9 °C. HRMS (ESI): Calc. [M + Na]+ = 359.0475, found: 359.0465. [α]D22(C = 0.1, CHCl3) = +9.
A degassed solution of alcohol \(5b\) (250 mg, 0.74 mmol, 1.00 equiv), boronate \(4b\) (312 mg, 0.92 mmol, 1.50 equiv), freshly distilled NEt3 (2.19 mL, 15.7 mmol, 21.2 equiv) in 8.2 mL (0.09 M)
THF/H$_2$O 9:1 (v/v). Pd(dppf)Cl$_2$-CH$_2$Cl$_2$ (48 mg, 60 µmol, 8.0 mol%) was added, and the reaction mixture was stirred for 2 h at 70 °C before the solvents were evaporated. The residue was redissolved in a small amount of DCM and directly purified by FC using EtOAc/cyclohexane 1:2 (v/v). Evaporation of the solvents furnished 279 mg (81%) of the deuterated coupling product 11b as a white solid. $R_f$ (CH/EE, 2:1) = 0.17. MP = 165–185 °C (decomposition). HRMS (ESI): Calc. [M + Na]$^+$ = 490.2127, found: 490.2121. $[\alpha]_{D}^{22}$ (c = 0.1, MeOH) = +1.5. $^1$H-NMR (300 MHz, DMSO-D$_6$): $\delta$ (ppm) = 1.20 (s, 6 H), 1.66 (s, 6 H), 1.69 (s, 1 H), 1.84 (m, 1 H), 3.15 (s, 3 H), 3.19 (s, 3 H), 3.95–4.09 (m, 2 H), 5.04 (s, 2 H), 5.20 (s, 1 H), 5.69 (d, $J$ = 2.5 Hz, 1 H), 6.77–6.70 (m, 1 H). $^{13}$C-NMR (75 MHz, DMSO-D$_6$): $\delta$ = 17.6, 17.8, 24.5, 25.8, 27.2, 42.3, 47.3, 47.4, 65.2, 69.7, 71.0, 99.1, 99.8, 100.8, 104.9, 105.6, 112.6, 112.6, 124.6, 142.1, 144.7, 157.9, 159.0, 162.9.

3.7. Synthesis of (6aR,7aR,9S,10S,11aR)-4-Hydroxy-2,9,10-trimethoxy-7a,9,10-trimethyl-6a,7,7a,9,10,11a-hexahydro-5H-benzo[c]1,4]dioxino[2,3-g]-chromen-5-one-D$_3$ (12b)

To a solution of the coupling product 11b (240 mg, 0.51 mmol) in MeOH (10 mL), K$_2$CO$_3$ (78 mg, 0.6 mmol, 1.1 equiv) was added, and the reaction mixture was stirred for 1 h at r.t. before the solvent was evaporated. Saturated NH$_4$Cl solution (15 mL) was added, and the aqueous phase was extracted with EtOAc (3 × 20 mL). The combined organic phases were dried (Na$_2$SO$_4$), and the solvent was evaporated. Chromatography with n-hexane/ethyl acetate 8:1 (v/v) provided 217 mg (100%) of the protected ALT derivative 12b as a white solid. $R_f$ (n-Hex/EE, 8:1) = 0.05. MP = 158–160 °C. HRMS (ESI): Calc. [M + Na]$^+$ = 432.1708, found: 432.1705. $[\alpha]_{D}^{22}$ (c = 0.1, CHCl$_3$) = +6. $^1$H-NMR (400 MHz, CDC$_3$): $\delta$ = 1.33 (s, 3 H), 1.34 (s, 3 H), 1.48 (s, 3 H), 1.90 (dd, $J$ = 12.9, 14.5 Hz, 1 H), 2.49 (dd, $J$ = 4.5, 14.5 Hz, 1 H), 3.26 (s, 3 H), 3.32 (s, 3 H), 3.87 (ddd, $J$ = 4.5, 8.9, 13.2 Hz, 1 H), 4.25 (dd, $J$ = 1.1, 8.9 Hz, 1 H), 6.14 (d, $J$ = 1.7, 1 H), 6.42 (d, $J$ = 2.4 Hz, 1 H), 6.49 (d, $J$ = 2.4 Hz, 1 H). $^{13}$C-NMR (101 MHz, CDC$_3$): $\delta$ = 17.90, 17.9, 28.4, 38.9, 48.28, 48.33, 66.2, 69.6, 81.5, 100.0, 100.5, 100.6, 100.9, 103.0, 128.8, 133.9, 139.0, 164.2, 166.3, 169.0.

3.8. Synthesis of (2R,3R,4aR)-2,3,4,4a-Tetrahydro-2,3,7-trihydroxy-9-methoxy-4a-methylbenzo[c]chromen-6-one-D$_3$ (−(−)-altenuene-D$_3$, 1b)

The bisketal protected natural product 12b (150 mg, 0.37 mmol) was heated to 100 °C while stirring in 5.2 mL of a 4:1 mixture of AcOH and H$_2$O for 2 h after which the solvents were evaporated. Residual acetic acid was removed by successive addition and evaporation of DCM (two or three times). Then the crude product was purified by RP chromatography on a C$_{18}$ stationary phase with H$_2$O/MeOH 4:6, delivering 87 mg (81%) of the deuterium labeled natural product 1b as a white solid. $R_f$ (DCM/MeOH, 20:1) = 0.18. MP = 117–120 °C. LC-MS (neg): [M – H]$^-$ = 294, $t_R$ = 16.67 min. HRMS (ESI): Calc. [M + Na]$^+$ = 318.1027, found: 318.1031. $[\alpha]_{D}^{22}$ (c = 0.03, MeOH) = −9. $^1$H-NMR (400 MHz, DMSO-D$_6$): $\delta$ = 1.47 (s, 3 H), 1.95 (dd, $J$ = 7.3, 14.0 Hz, 1 H), 2.26 (dd, $J$ = 3.5, 14.0 Hz, 1 H), 3.64–3.76 (m, 1 H), 3.91–4.00 (m, 1 H), 5.13 (d, $J$ = 3.8 Hz, 1 H), 5.29 (d, $J$ = 6.2 Hz, 1 H), 6.30 (d, $J$ = 3.3 Hz, 1 H), 6.50 (d, $J$ = 2.4 Hz, 1 H), 6.74 (d, $J$ = 2.4 Hz, 1 H), 11.29 (s, 1 H). $^{13}$C-NMR (101 MHz, DMSO-D$_6$): $\delta$ = 22.5, 38.5, 68.8, 69.5, 81.0, 100.0, 100.9, 102.4, 131.8, 139.2, 163.0, 165.8, 168.2.

3.9. Synthesis of (2R,3R,4aR)-2,3,4,4a-tetrahydro-2,3,7-trihydroxy-9-methoxy-4a-methylbenzo[c]chromen-6-one-(−(−)-altenuene, 1a)

The native mycotoxin 1a was synthesized analogously with 374 mg (1.16 mmol, 1.00 equiv) allylic alcohol 5b and 580 mg (1.74 mmol, 1.50 equiv) boronate 4a, yielding 222 mg (81%) (−)-altenuene (1a) as a white solid. $R_f$ (DCM/MeOH, 20:1) = 0.18. MP = 117–120 °C. LC-MS (neg): [M – H]$^-$ = 291, $t_R$ = 16.67 min. HRMS (ESI): Calc. [M + Na]$^+$ = 315.0839, found: 315.0836. $^1$H-NMR (400 MHz, DMSO-D$_6$): $\delta$ (ppm) = 1.47 (s, 3 H), 1.95 (dd, $J$ = 7.5, 14.1 Hz, 1 H), 2.26 (dd, $J$ = 3.5, 14.0 Hz, 1 H), 3.70 (dd, $J$ = 3.8, 7.6 Hz, 1 H), 3.86 (s, 3 H), 3.95 (dt, $J$ = 4.4, 6.2 Hz, 1 H), 5.13 (d, $J$ = 3.8 Hz, 1 H), 5.29 (d, $J$ = 6.1 Hz, 1 H), 6.30 (d, $J$ = 3.3 Hz, 1 H), 6.50 (d, $J$ = 2.3 Hz, 1 H), 6.75 (d, $J$ = 2.4 Hz, 1 H),
11.29 (s, 1 H). 13C-NMR (101 MHz, DMSO-D6): δ (ppm) = 27.4, 38.6, 55.9, 68.8, 69.5, 81.1, 100.0, 100.9, 102.3, 131.0, 131.8, 139.2, 163.0, 165.8, 168.2.

4. Conclusions

In summary, the successful optimization of a reported ALT (1a) synthesis provides efficient access to a novel deuterated derivative 1b. Starting from commercially available D-(−)-quinic acid (8) as an inexpensive chiral pool compound, the D3-labeled natural product 1b was obtained with an overall yield of 17% after nine steps. The newly synthesized ALT-D3 (1b) proved to be suitable as an internal standard for SIDA LC-MS/MS and thus ensures the availability of appropriate methods for the reliable screening of foods and feeds. Moreover, the optimized procedure may facilitate the development of new anti-tumor drug candidates with (−)-altenuene (1a) being a possible lead compound.

Supplementary Materials: The following are available online at http://www.mdpi.com/1420-3049/24/4/4563/s1: 1H- and 13C-NMR-Spectra of all new compounds as well as UV/Vis- and IR-spectra of the native and labeled natural products.

Author Contributions: J.G. designed the project. M.A.S. and J.G. decided which methodology to use. M.A.S. and J.G. carried out the syntheses. T.S. did the HPLC-MS/MS optimization and analysis. M.A.S., J.G. and M.K. wrote the original draft.

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Abbreviations

ALT: (−)-altenuene; BINAP: (2,2′-bis(diphenylphosphino)-1,1′-binaphthyl) ligand; CH: cyclohexane; DABAL: bis(trimethylaluminum)1,4-diazabicyclo[2.2.2]octane adduct; DCM: dichloromethane; d.r.: diastereomeric ratio; EE: ethyl acetate; ESI: electrospray ionization; HPLC: high pressure liquid chromatography; LC-MS: liquid-chromatography-tandem mass spectrometry; LD50: lethal dose, 50%; MPLC: medium pressure liquid chromatography; MTBE: methyl tert-butyl ether; SIDA: stable isotope dilution assay; SRM: selected reaction monitoring; THF: tetrahydrofuran.

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Sample Availability: ALT and ALT-D3 are available at HPC Standards (www.hpc-standards.com).