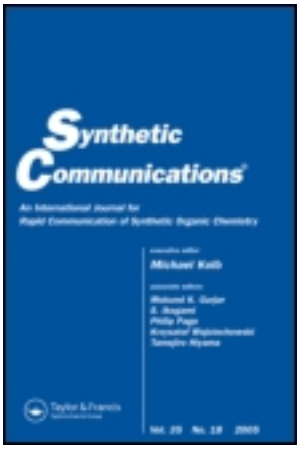

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Zearalenone Mimics: Synthesis of (E)-6-(1-Alkenyl)-substituted β-Resorcylic Acid Esters

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ZEARALENONE MIMICS: SYNTHESIS OF (E)-6-(1-ALKENYL)-SUBSTITUTED β-RESORCYLIC ACID ESTERS

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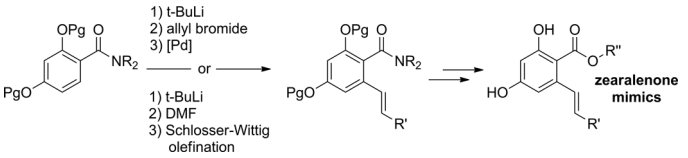
GRAPHICAL ABSTRACT



Abstract *Two versatile strategies for the synthesis of mimics of the* Fusarium *mycotoxin zearalenone (1) have been developed. Optimized preparation of (E)-6-(1-alkenyl) substituted β-resorcylic acid esters was realized via ortho-directed lithiation of variable substrates combined with allylation/isomerization or via formylation/Schlosser–Wittig olefination using different protective group patterns. Spontaneous decarboxylation of (E)-6-(1-alkenyl) substituted β-resorcylic acids indicated the influence of this substituent on the chemical behavior of these compounds. These mimics were already used for the development of optimized standard protocols for the synthesis of phase II metabolites of ZEN (glucosides, glucuronides), and further applications (i.e., sulfate conjugates) are still under investigation.*

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Keywords Mycotoxin; ortho-directed lithiation; β-resorcylic acid ester; Schlosser–Wittig olefination; zearalenone

INTRODUCTION

The rapid global re-emergence of Fusarium head blight disease of wheat and barley in the last 30 years along with contamination of grains with mycotoxins have spurred basic research on the fungal causal agent. Therefore *Fusarium graminearum* quickly has become one of the most intensively studied fungal plant pathogens.[1] One of the mycotoxins formed from this organism is the macrolide zearalenone (**1**) (Fig. 1). This toxin acts as an estrogenic substance and is responsible for many

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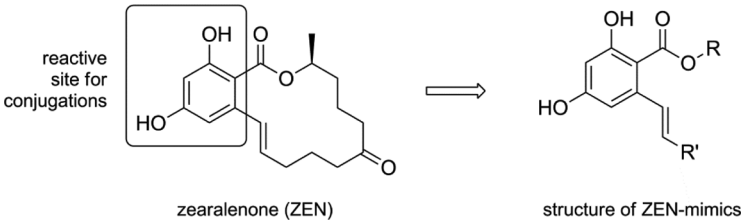


Figure 1. Structure of zearalenone and substituted β-resorcylic acid esters as mimics for conjugations at the reactive site of zearalenone.

breeding problems, especially infertility in swine.[2] Modern analytical methods made the detection of even small amounts of zearalenone (ZEN) possible and therefore safety values in food and feed were introduced.[3] One problem remaining is the occurrence of so- called masked mycotoxins, which are formed during phase II detoxification in plants, animals, and humans by conjugation of the toxin with hydrophilic moieties such as sugars, amino acids, or sulfate. These metabolites are usually not detected by using standard protocols and quantification is not possible because of lack of reference materials.[4] Glucuronides formed during xenobiotic metabolism are also considered to serve as possible biomarkers for daily uptake measurements.[5,6]

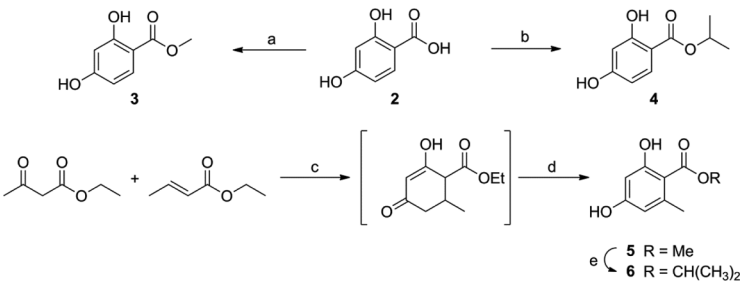
In the course of our research to synthesize zearalenone conjugates as reference materials for bioanalytical studies as well as potential biomarkers, we became very interested in the design and synthesis of (E)-6-(1-alkenyl)-substituted β-resorcylic acid esters as mimics for this mycotoxin (Fig. 1) in the development of synthetic strategies toward such conjugates. These mimics may also be used instead of expensively isolated and purified mycotoxin in metabolization or chemical reaction studies, for example, the decarboxylation of ZEN after hydrolysis of the lactone moiety as described first by Shipchandler[7] or recently by Utermark and coworkers.[8]

In this article we report different strategies for the synthesis of (E)-6-(1-alkenyl)-substituted β-resorcylic acid esters as mimics for zearalenone.

RESULTS AND DISCUSSION

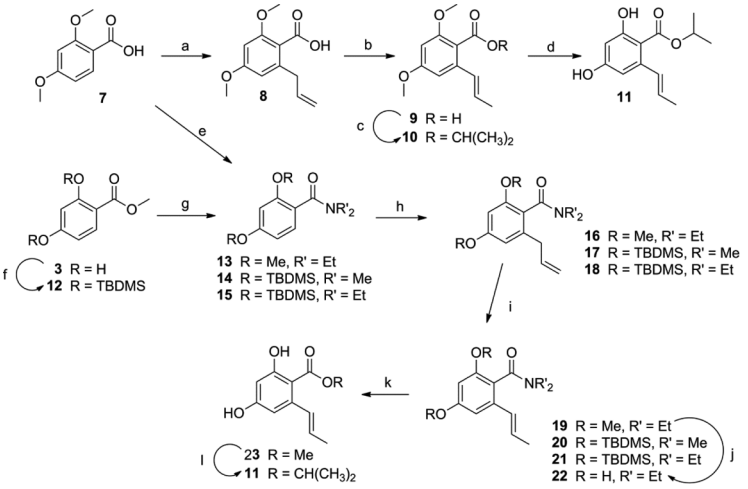
The site of conjugation of ZEN, therefore responsible for most metabolization reactions, is basically a β-resorcylic acid ester substituted in position 6.

Simple mimics were obtained by esterification of 2,4-dihydroxybenzoic acid (**2**), yielding the corresponding methyl ester (**3**)[9] and isopropyl ester (**4**). A second approach led us to 6-methyl substituted resorcylic acid esters. After condensation of ethyl acetoacetate and ethyl crotonate,[10] the formed intermediate was aromatized by bromination and elimination of hydrogen bromide following a known procedure[11] to afford the ethyl ester **5** in 56% overall yield (data identical with those reported in the literature.[12]) Basic transesterification using a method described by Kumar et al.[13] yielded 2,4-dihydroxy-6-methylbenzoic acid isopropyl ester (**6**) (Scheme 1).



Scheme 1. Reagents and conditions: (a) cat. H_2SO_4, MeOH, reflux, 96 h, 89%; (b) NaH, 2-bromopropane, DMF, 80 °C, 12 h, 74%; (c) NaOEt, EtOH, reflux, 2 h; (d) Ac_2O, Br_2, reflux, 2 h then HBr, H_2O, reflux, 2 h, 56% over two steps); and (e) Na, isopropanol, reflux, 24 h, 81%.

Because many 6-alkyl substituted resorcylic acids and 2,4,6-trihydroxybenzoic acid are stable compounds and often described in literature, we thought about the influence of an (E)-6-(1-alkenyl) substituent, such as that present in zearalenone, which undergoes spontaneous decarboxylation after ester hydrolysis. Excluding enzymatic reactions, we assume that the substituent in position 6 is crucial for the decarboxylation step and therefore important for the reactivity and the structure of ZEN mimics. Thus different strategies, all containing an ortho-directed lithiation as key step, were applied to prepare (E)-6-(1-alkenyl) substituted β-resorcylic acid esters (Scheme 2).

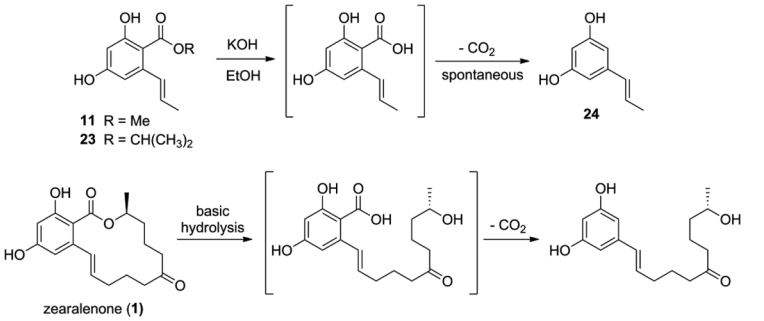


Scheme 2. Reagents and conditions: (a) TMEDA, s-BuLi, THF, 30 min, −90 °C then allyl bromide, 2 h, −80 °C, 44%; (b) $(CH_3CN)_2PdCl_2$, CH_2Cl_2, 24 h, room temperature (rt); (c) NaH, THF, 0 °C, 1 h then 2-bromopropane, 12 h, rt, 73% (over two steps); (d) BBr_3, CH_2Cl_2, 24 h, −15 °C, 59 %; (e) $SOCl_2$, 3 h, reflux then $HNEt_2$, 15 h, rt; (f) NaH, THF, 1 h, 0 °C then TBDMS-Cl, 1 h, rt, 94%; (g) $AlMe_3$, HNR_2, 50 min, rt then **12**, 20 h, reflux, 72% (**14**), 76% (**15**); (h) t-BuLi, THF, 1 h, −80 °C then CuBr • Me_2S, 30 min, −15 °C then allyl bromide, −80 °C to rt, 56% (**16**), 71% (**17**), 82% (**18**); (i) $(CH_3CN)_2PdCl_2$, EtOH, 24 h, rt, 89% (**19**), 83% (**20**), 88% (**21**); (j) BBr_3, CH_2Cl_2, 15 h, −80 °C to rt; (k) $Me_3O(BF_4)$, CH_2Cl_2, 15 h, rt then Na_2CO_3, H_2O, MeOH, 6 h, rt, 87% (from **20**), 86% (from **21**), 69% (over two steps from **19**); (l) Na, isopropanol, reflux, 24 h, 90%.

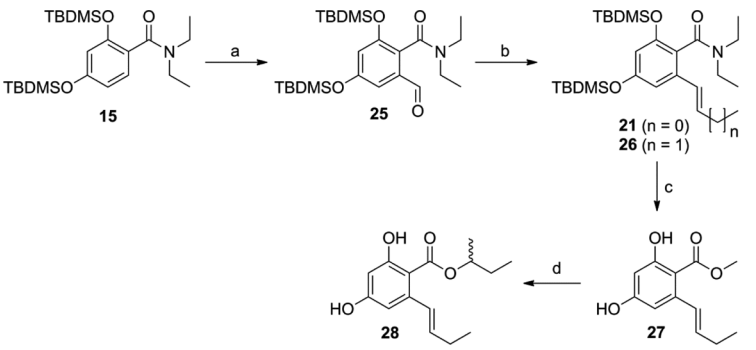
As first starting material for the lithiation, commercially available 2,4-dimethoxybenzoic acid (**7**) seemed to be applicable as it was used and described by Vintonyak et al.[14] Metalation with s-BuLi/tetramethylenediamine (TMEDA) and reaction with allyl bromide yielded only 44% of compound **8**. After changing different reaction parameters (temperature, reaction time, and number of equivalents of s-BuLi), the yield of this reaction was not improved and still far from values described in the original procedure (79% crude and 51% after subsequent methyl esterification). Stereoselective double-bond isomerization was achieved using catalytic amounts of bis(acetonitrile)palladium(II) dichloride in dichloromethane. The crude product **9** was converted to isopropyl ester **10** (73% over two steps) and methyl protective groups were then cleaved by reaction with BBr_3 to yield the first (E)-6-(1-alkenyl) substituted ZEN mimic **11**, which was characterized by ^1H NMR to confirm the E configuration of the conjugated double bond ($J_{CH=CH} = 15.5$ Hz).

To optimize the lithiation step, compound **7** was converted to N,N-diethyl-2,4-dimethoxybenzamide (**13**) following an already reported procedure,[15] and methyl ester **12**[16] was reacted with $AlMe_3/HNMe_2$ or $AlMe_3/HNEt_2$ to yield the tert-butyldimethylsilyl (TBDMS)–protected benzamides **14** and **15**,[16] respectively. After lithiation, transmetalation, and alkylation, yielding **16, 17**, and **18** in yields up to 82% (for **18**), the allyl substituent in position 6 was isomerized using again $(CH_3CN)_2PdCl_2$ as catalyst to afford **19, 20**, and **21** in good yields (83–89%). In this case, no conversion was observed using CH_2Cl_2 as solvent as used before for the synthesis of **9** and described in literature.[17,18] Thus different protic solvents were screened to accelerate proton transfer, and dry ethanol gave the best results. After demethylation of (E)-N,N-diethyl-6-(1-propenyl)benzamide (**19**) using BBr_3 to yield compound **22**, the corresponding methyl ester **23** was prepared by reaction with trimethyloxonium tetrafluoroborate (69% over two steps). Using these reaction conditions the TBDMS–protected benzamides **20** and **21** could be directly converted to **23** in good yields (>85%). Basic transesterification was achieved using sodium isopropoxide to yield **11** (90%).

After basic hydrolysis of compounds **11** and **23** we observed spontaneous decarboxylation to **24**, which was identified by comparison of spectral data with those reported in the literature.[19] As already mentioned, this reaction also occurs after ZEN hydrolysis (Scheme 3), which indicates the influence of an (E)-6-(1-alkenyl) substituent on the chemical behavior of β-resorcylic acid esters.



Scheme 3. Spontaneous decarboxylation after basic hydrolysis of (E)-6-(1-alkenyl) substituted β-resorcylic acid esters **11**, **23**, and zearalenone (**1**).



Scheme 4. Reagents and conditions: (a) t-BuLi, THF, 2 h, −80 °C then DMF, 2 h, −80 °C to 0 °C, 73%; (b) $EtPh_3P^+Br^-$ (for **21**) or $PrPh_3P^+Br^-$ (for **26**), LiBr, PhLi, THF, −75 °C to 20 °C then **25**, PhLi, 90 min, −75 °C to rt to −75 °C then HCl/Et_2O and KOtBu, 1 h, rt, 74% (**21**), 71% (**26**); (c) $Me_3O(BF_4)$, CH_2Cl_2, 15 h, rt then Na_2CO_3, H_2O, MeOH, 6 h, rt, 81% (**23**), 85% (**27**); (d) Na, 2-butanol, 24 h, reflux, 66%.

Another versatile strategy using a Schlosser–Wittig olefination[20] as key step was developed for the synthesis of (E)-6-(1-alkenyl)-substituted ZEN mimics (Scheme 4). Benzamide **15** was formylated by quenching with dimethylformamide after t-BuLi lithiation without transmetalation to yield compound **25** (73%). Stereoselective olefination [E/Z ratio of 8/1 as determined by gas chromatography–mass spectrometric (GC/MS] analysis) afforded the desired products **21** (in comparison to the allylation/isomerization approach described previosly) and **26** in 74% and 71% yields, respectively. After TBDMS deprotection and simultaneous conversion of the diethylamide to the corresponding methyl ester using trimethyloxonium tetrafluoroborate, basic transesterification of **27** was achieved using sodium in 2-butanol to yield the ZEN mimic **28** (29% overall yield starting from **15**).

In conclusion, different approaches to the synthesis of unsaturated substituted β-resorcylic acid esters as mimics for the mycotoxin zearalenone and other natural products were demonstrated and used to prove spontaneous decarboxylation of (E)-6-(1-alkenyl)-β-resorcylic acids.

Ortho-directed lithiation of the TBDMS-protected benzamide **15** using t-BuLi combined with either Pd-catalyzed double-bond isomerization after quenching with allyl bromide or formylation and subsequent Schlosser–Wittig olefination gave the best results for versatile introduction of (E)-6-(1-alkenyl) substituents to β-resorcylic acid derivatives. Because allylic halides and aldehydes are readily available as synthetic building blocks, these approaches may be useful for the preparation of many similar compounds as well as β-resorcylic acid lactones (RALs).

An optimized procedure for the chemical O-glucuronidation of these mimics and the first reported chemical synthesis of ZEN-14-β,D-glucuronide was published very recently.[21] Chemical sulfation of β-resorcylic acid esters and synthesis of zearalenone sulfates are currently under investigation using the herein reported ZEN mimics.

EXPERIMENTAL

All reactions were performed under an argon atmosphere. The progress of the reactions was monitored by thin-layer chromatography (TLC) over silica gel 60 F_{254}

(Merck). The chromatograms were visualized by irradiation with ultraviolet light or by heat staining with ceric ammonium molybdate in ethanol/sulfuric acid. Column chromatography was performed on silica gel 60 (Merck, 40–63 μm) using a Büchi Sepacore Flash system. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker DPX 200-MHz or Avance DRX 400-MHz spectrometer. Data were recorded and evaluated using Topspin 1.3 (Bruker Biospin). All chemical shifts are given in parts per million (ppm) relative to tetramethylsilane (TMS). The calibration was done using residual solvent signals. Multiplicities are abbreviated as s (singlet), d (doublet), t (triplet), q (quartet), and b (broad signal).

General Procedure for Lithiation and Allylation of Benzamides 13–15

Benzamide **13, 14**, or **15** (5 mmol) was dissolved in dry THF (20 ml), and t-BuLi (7.5 ml, 12.75 mmol, 1.7 M in pentane) was added dropwise at −80 °C and the yellow-orange solution was stirred for 1 h. After addition of CuBr • Me_2S (2.26 g, 11 mmol), the reaction mixture was slowly warmed to −15 °C and stirred for 30 min. Allyl bromide (3.02 g, 25 mmol) was then added dropwise at −80 °C, and after warming to room temperature, the reaction mixture was filtered through celite, diluted with EtOAc (50 ml), and washed with water (2 × 60 ml). The organic layer was dried over Na_2SO_4 and evaporated. The residue was purified by column chromatography (hexanes/EtOAc, 8:1 to 2:1) to afford the desired product.

General Procedure for Pd-Catalyzed Isomerization of 16–18

Benzamide **16**, **17**, or **18** (2.5 mmol) was dissolved in dry ethanol (10 ml) and $(CH_3CN)_2PdCl_2$ (65 mg, 0.25 mmol) was added. The reaction mixture was stirred for 24 h at room temperature, filtered through celite, and concentrated. The residue was purified by column chromatography (hexanes/EtOAc, 8:1 to 2:1) to afford the desired product.

General Procedure for Schlosser–Wittig Olefination

Phenyllithium solution (1.9 ml, 3.6 mmol, 1.9 M in dibutyl ether) was slowly added to a suspension of alkyl triphenylphosphonium bromide (3.6 mmol) and LiBr (624 mg, 7.2 mmol) in dry THF (40 ml) at −75 °C. After 30 min of vigorous stirring at room temperature, the reaction mixture was cooled to −75 °C to be treated with compound **25** (1.40 g, 3 mmol) dissolved in dry THF (10 ml). A few minutes later, complete decolorization occurred, again with phenyllithium solution (1.9 ml, 3.6 mmol, 1.9 M in dibutyl ether). After stirring each time 30 min at −75 °C, room temperature, and again −75 °C, hydrogen chloride (3.6 ml, 3.6 mmol, 1 M in Et_2O) was added. After addition of potassium *tert*-butoxide (448 mg, 4 mmol), the reaction mixture was stirred 1 h at room temperature before being poured into water (200 ml) and extracted with CH_2Cl_2 (3 × 200 ml). The combined organic layer was dried over Na_2SO_4 and evaporated. Purification by column chromatography (hexanes/EtOAc, 5:1 to 3:1) yielded the desired E-configured olefins.

The Supplemental Material, available online, contains experimental details and characterization data for compounds **4**, **10**, **11**, **14**, **16–23** and **25–28**. Furthermore,

^{1}H and ^{13}C NMR chemical shifts of ZEN mimics **4**, **11**, and **28** were compared with those of zearalenone (**1**).

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