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Total Synthesis of (*S*)-(+)-Citreofuran by Ring Closing Alkyne Metathesis

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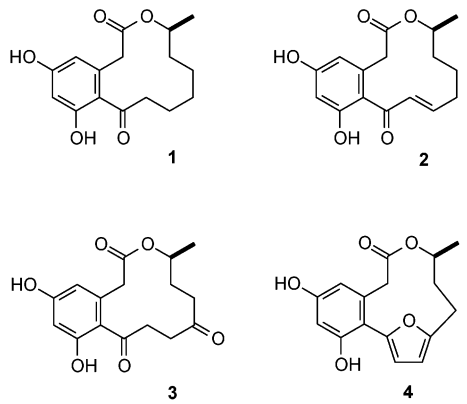
Received November 11, 2002

A concise total synthesis of citreofuran **4** is described, a structurally unique octaketide derivative belonging to the curvularin family. Key steps involve the elaboration of orsellinic acid methyl ester **5** to acid **14**, which converts, on attempted formation of the corresponding acid chloride, to the 3-alkoxyisocoumarin derivative **20**. This heterocycle can be used as an activated ester to give ketone **21** on treatment with 3-pentynylmagnesium bromide in the presence of TMSCl as the activating agent. Ring- closing alkyne metathesis (RCAM) of diyne **21** catalyzed by $(tBuO)_3W{\equiv}CCMe_3$ affords the strained cycloalkyne **22**. Treatment with acid renders its triple bond susceptible to nucleophilic attack by the adjacent carbonyl group, thus leading to a transannular cycloaromatization with formation of the intact skeleton of citreofuran. An X-ray crystallographic study reveals conformational details about this natural product. Finally, it is shown that **4** as well as its protected precursor **23** are able to cleave double-stranded DNA under oxidative conditions.

Introduction

Curvularin **1** and related polyketide metabolites such as **2** and **3** isolated from various *Curvularia*, *Penicillium*, *Alternaria*, and *Cochliobolus* species are known to elicit diverse biological effects ranging from phytotoxicity to antibacterial and antifungal activity.[1] Most noticeable, however, is their ability to arrest cell division at low concentrations by disrupting microtubulin assembly via a mechanism similar to that of colchicin or the combretastatins.[2−4] This ability is likely to originate from the conformation of their macrocyclic ring, which mimics the *M*-helicity of the colchicin skeleton,[5] a stereochemical feature known to be important for tubulin binding of these agents.[3] Consequently, the curvularins have been the subject of many preparative[6] and biosynthetic studies.[7] Moreover, it has been found that some of the producing strains lend themselves to biotechnological modification by the cell fusion technique. The resulting hybrid strains not only produce a host of novel curvularin type compounds with different oxidation states along the backbone, but also turned out to be surprisingly rich sources of secondary metabolites of totally different and rather diverse structures.[8]



Citreofuran **4** is a new member of the curvularin family produced by the hydrid strain *Penicillium citreo-viride*

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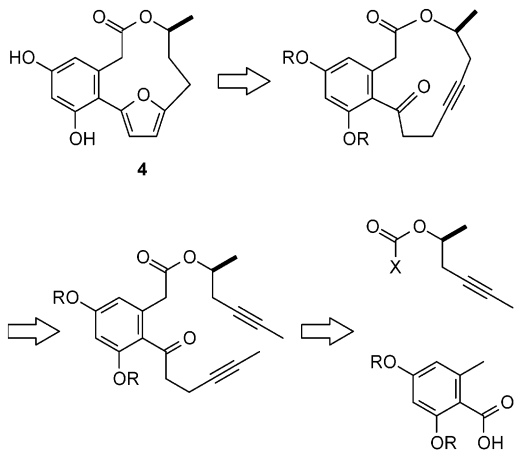
10.1021/jo026686q CCC: $25.00
Published on Web 01/23/2003

SCHEME 1. Retrosynthetic Analysis of Citreofuran



ME 0005.[9] While its biosynthesis has been studied in detail and its activity has been claimed to be promising, the only previous total synthesis of this particular metabolite suffered from the poor yield (15%) obtained in the intramolecular Friedel−Crafts acylation reaction forming the macrocyclic skeleton.[10] Therefore we targeted **4** by an entirely different route as part of an ongoing project dealing with bioactive furan,[11] resorcinol,[12] and orsellinic acid derivatives.[13,14] Described below is a concise total synthesis of this natural product together with a preliminary evaluation of its previously unknown DNA-damaging capacity.

Results and Discussion

Strategy and Retrosynthetic Analysis. Rather than taking recourse to established retrosynthetic logic that suggests strategic disconnections at the biaryl axis (cross coupling) and the ester bond (macrolactonization), we were prompted to use citreofuran as a testing ground for some of the methodology recently developed in our laboratory (Scheme 1).

Specifically, it was envisaged to encode the furan ring of **4** as a macrocyclic yne-one derivative,[15] which should derive from ring-closing alkyne metathesis (RCAM) of a suitable diyne precursor.[16] Although RCAM has already borne scrutiny in target-oriented synthesis, it was invariably applied so far in combination with Lindlar- or Birch-type reductions as post metathesis transformations.[17−23] Therefore the projected synthesis of citreofuran bears a chance to illustrate that RCAM has a significantly broader scope than just the preparation of stereodefined olefins and may prove relevant for heterocycle synthesis as well.

The required diyne precursor itself appears to be readily available from rather simple building blocks, notably from well-accessible orsellinic acid. Proper choice of the protecting groups during the assembly stages should result in a convenient and high-yielding access to this key synthon.

Total Synthesis. Our synthesis of **4** starts from orsellinic acid methyl ester **5**, which is readily available on a multigram scale by base-induced cyclodimerization of methyl acetoacetate. Alkylation of the phenolic −OH groups[24] followed by saponification of the methyl ester affords acid **6** in excellent overall yield.

Dianions derived from *o*-methylbenzoic acid by deprotonation with strong bases can be trapped at the benzylic position with various electrophiles including methyl chloroformate.[25] Therefore several attempts were made to adopt this methodology to the present series. Unfortunately, however, only complex mixtures were obtained upon quenching the dilithio derivative formed from **6** and LDA (≥3 equiv) at −78 °C with 2-hexyl chloroformate **7** as the model compound. A closer inspection showed the diacylation product **9** and unreacted starting material **6** to be the major components. This distribution is best explained by the fact that the product of the first acylation, i.e., compound **8**, is more acidic than the starting material, therefore being preferentially deprotonated by the residual dilithio derivative, and can hardly

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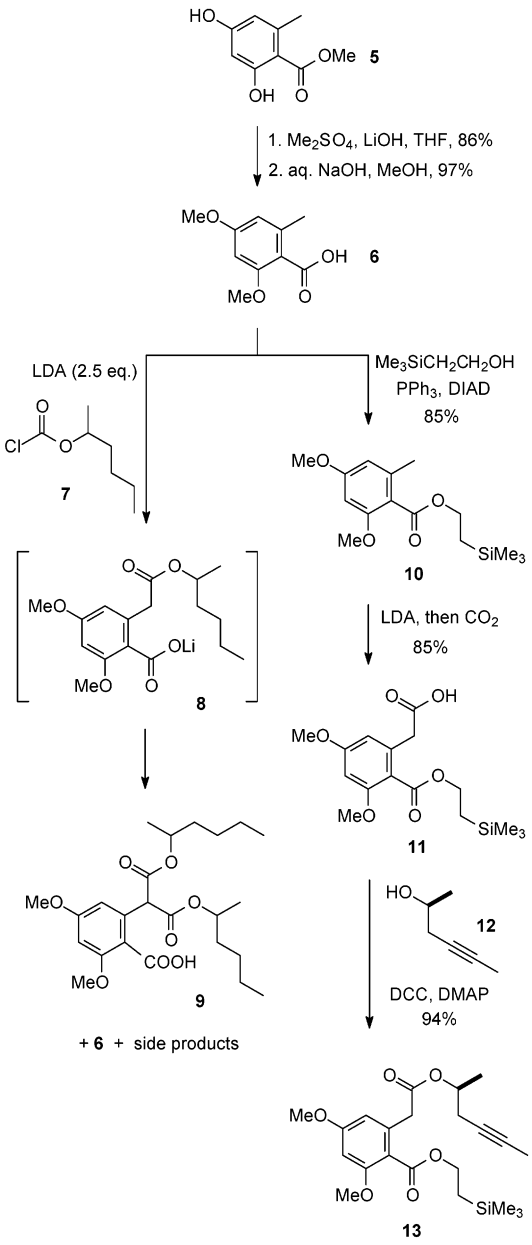
SCHEME 2

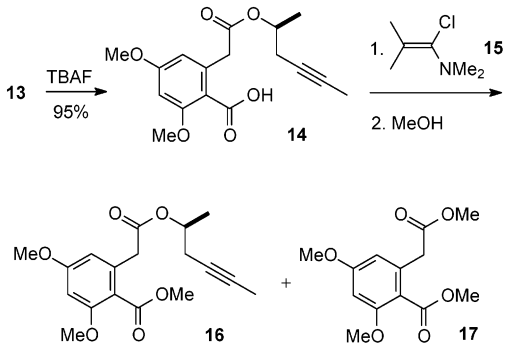


survive under the reaction conditions (Scheme 2). On the basis of this analysis, we did not pursue the envisaged C-acylation route any further despite the seemingly encouraging literature precedence.[25a,b,26]

A much more convenient preparation of the desired alkyne metathesis precursor commences with a Mitsunobu-type esterification of acid **6** and 2-trimethylsilyl ethanol to give compound **10**, which can be deprotonated at the benzylic site with LDA at low temperature; quenching of the reaction mixture with CO_2 furnishes acid **11** in 85% yield after acidic workup. Subsequent esterification with the enantiomerically pure alcohol **12**, derived from a ring-opening reaction of (*S*)-propene oxide with propynyllithium in THF/DMPU, gives compound **13** in high yield (Scheme 2). Its treatment with TBAF in

(26) It has previously been reported that the C-silylation (stannylation) of orsellinic acid esters can also lead to substantial amounts of disilylated (stannylated) products, cf. ref 25b.

SCHEME 3



THF results in selective saponification of the β-trimethylsilylethyl ester[6b] and sets the stage for the introduction of the second alkyne group.

Although many procedures are known for the conversion of carboxylic acid derivatives to the corresponding ketones,[27] this step initially turned out to be delicate. Attempted acyl-Negishi coupling reactions[28] of the putative acid chloride derived from **14** and the chloroenamine reagent **15**[29] with 3-pentynylzinc iodide invariably led to rather complex mixtures. Quenching of the transient acid chloride with MeOH also leads to erratic results, giving rise to a mixture of compound **16** and the unexpected dimethylester **17** in somewhat variable yields and ratios (Scheme 3). This outcome might be explained by a neighboring group participation of the residual ester in **14**, thus leading to an equilibrium between the desired acid chloride **18** and the cyclic chloroacetal derivative **19** (or the corresponding oxocarbenium cation derived thereof)[30] (Scheme 4) which compete with one another for the added nucleophile. This interpretation is corroborated by the fact that treatment of the reaction mixture with NEt_3 leads to the clean and virtually quantitative formation of alkoxyisocoumarin **20** as judged by NMR, although the isolated product rapidly degrades upon storage.

It was anticipated that this isocoumarin behaves as an activated ester able to replace the capricious acid chloride in the envisaged ketone formation step. Because of its instability, however, it seemed necessary to avoid isolation of this intermediate but to react the crude product with an appropriate nucleophile.[31] Although initial attempts to attack the isocoumarin ring of **20** with 3-pentynylmagnesium bromide[32] were unsuccessful, a smooth conversion to the desired diyne **21** takes place if

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(30) The available information at this point does not allow us to draw an unambiguous conclusion as to the actual constitution of this intermediate.

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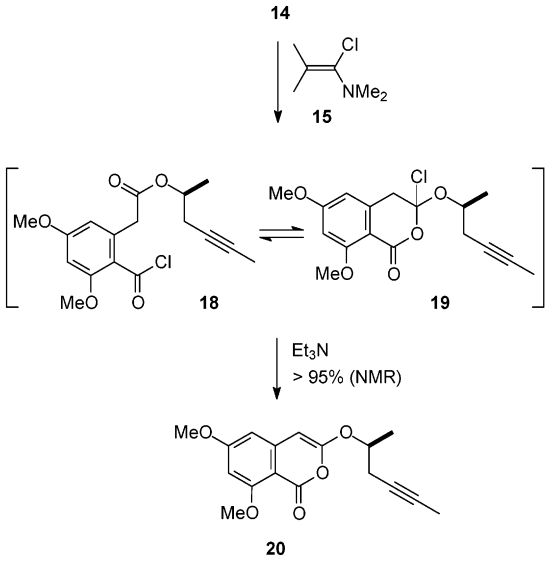
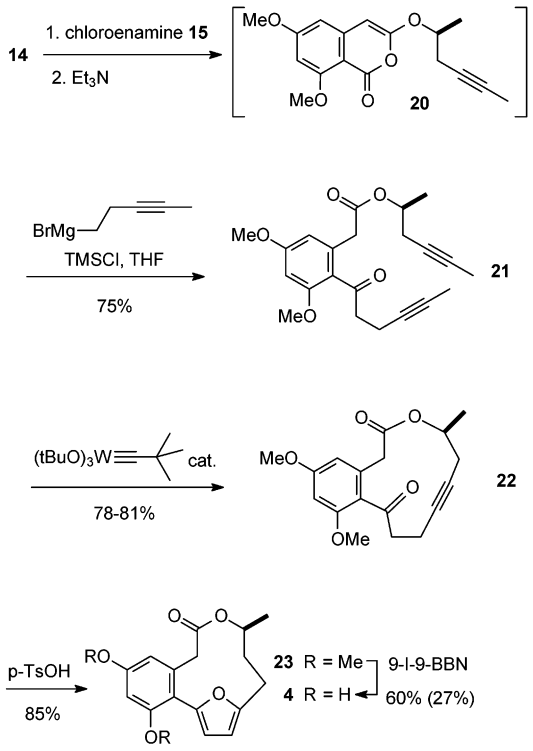
(32) It is important to keep the temperature below 40 °C during the formation of this Grignard reagent to minimize undesirable side reactions.

SCHEME 4



SCHEME 5



the reaction is carried out at low temperature in the presence of TMSCl as a mild activating agent.[33] Under these conditions, product **21** is obtained by a three-step/one-pot operation in 75% yield starting from acid **14** (Scheme 5).

Gratifyingly, the RCAM reaction proceeds in excellent yield on exposure of diyne **21** to Schrock's tungsten alkylidyne complex $(tBuO)_3W{\equiv}CCMe_3$[34] in toluene at 85 °C. It was noticed, however, that the substrate must be devoid of any trace impurities to avoid catalyst poisoning.[35] The fact that the yield is highly dependent on the chosen dilution is deemed to reflect the strain in the benzo-annellated oxacyclododecyne ring of compound **22**, with best results being obtained at $c = 0.0085$ M (78−81% isolated yield).

The subsequent formation of the furan ring from the γ-alkynyl ketone substructure in **22** essentially follows literature procedures describing similar transformations.[15] Thereby, the use of *p*-toluenesulfonic acid (1 equiv) in toluene at 85 °C turned out to be optimal in terms of yield and reaction rates. In contrast to the ease of this cyclization reaction, however, the final deprotection of compound **23** thus formed was far from trivial. Specifically, the use of BBr_3 entailed considerable decomposition, although it was possible to identify the desired citreofuran in the NMR spectrum of the crude mixture. Therefore, we reasoned that 9-iodo-9-BBN, a more moderate congener that has previously proven highly successful in the deprotection of different resorcinol derivatives,[12a] might lead to a more favorable outcome. This is indeed the case. Thus, treatment of **23** with 9-iodo-9-BBN in CH_2Cl_2 at −10 °C effects the consecutive cleavage of the two methyl ether groups, with side reactions coming into play only on prolonged stirring.[36] Although the crude mixture is quite clean, it turned out to be very difficult to remove traces of different boron impurities due to their very similar retention times. Therefore, analytically pure samples of citreofuran **4** had to be prepared by preparative HPLC (27%). Its physical and spectroscopic properties are in excellent agreement with those reported in the literature.[9]

Structural Aspects. As mentioned in the Introduction, the ability of curvularin derivatives to bind to tubulin and hence to disrupt the assembly of the microtubules during mitosis has been ascribed to the conformational peculiarities of their macrocyclic ring. Therefore, it seemed appropriate to study the structure of citreofuran in more detail and to compare it with the structure of the other members of this family.

Single crystals of **4** suitable for X-ray analysis have been grown from CH_2Cl_2. As can be seen from the structure depicted in Figure 1, the macrocycle of **4** adopts

(33) This experiment shows that the TMSCl-promoted addition of RMgX to the activated ester is faster than the interception of the Grignard reagent as R-$SiMe_3$. While the use of TMSCl as activating agent in 1,2-additions of RMgX to carbonyl compounds seems to be largely unexplored, its accelerating effect on 1,4-additions under "Kharasch conditions" (RMgX + Cu(I) cat.) or with preformed organocuprates as the reagents is well precedented. See the following for leading references and literature cited therein: (a) Corey, E. J.; Boaz, N. W. *Tetrahedron Lett.* **1985**, *26*, 6015−6018. (b) Nakamura, E.; Matsuzawa, S.; Horiguchi, Y.; Kuwajima, I. *Tetrahedron Lett.* **1986**, *27*, 4029−4032. (c) Linderman, R. J.; Godfrey, A. *Tetrahedron Lett.* **1986**, *27*, 4553−4556. (d) Alexakis, A.; Berlan, J.; Besace, Y. *Tetrahedron Lett.* **1986**, *27*, 1047−1050. (e) Lipshutz, B. H.; Aue, D. H.; James, B. *Tetrahedron Lett.* **1996**, *37*, 8471−8474. (f) Reetz, M. T.; Kindler, A. *J. Organomet. Chem.* **1995**, *502*, C5−C7. (g) Yamazaki, T.; Umetani, H.; Kitazume, T. *Tetrahedron Lett.* **1997**, *38*, 6705−6708. (h) Bertz, S. H.; Miao, G.; Rossiter, B. E.; Snyder, J. P. *J. Am. Chem. Soc.* **1995**, *117*, 11023−11024.

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(35) The catalyst is known to be poisoned by amines or thioethers, cf. ref 16c. Therefore it is particularly important that the substrate is devoid of any trace of Et_3N used in the previous step.

(36) Due to the formation of unidentified byproducts toward the end of the reaction, the mixture was quenched before the deprotection was complete. This affords a mixture of the monoprotected and the fully deprotected compounds in an ca. 1:6.4 ratio.

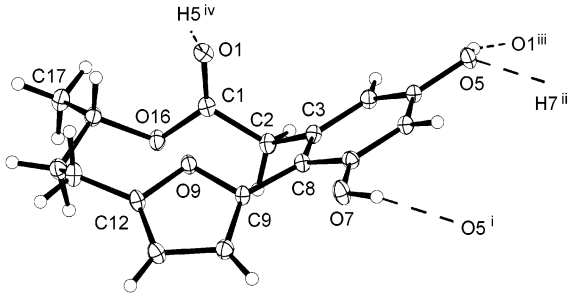

FIGURE 1. Molecular structure of citreofuran **4** obtained from single-crystal structure determination. Anisotropic displacement parameters are shown at the 50% probability level. Dashed bonds indicate hydrogen bonds to atoms of neighboring molecules. $O5^{i}\cdots H7 = O5\cdots H7^{ii}$ 1.963(1) Å, $O1^{iii}\cdots H5 = O1\cdots H5^{iv}$ 1.877(1) Å (iv = $1 - x$, $y - {}^{1}/_{2}$, $1 - z$).

a double-chair conformation. The benzo ring is found in the equatorial position while the furan is rotated in such a manner as to point the ring oxygen toward the inner perimeter of the macrocycle directly opposite the carbonyl oxygen, which is axially oriented. The distance between the furan ring oxygen atom and the carbonyl carbon atom is only 2.551(2) Å (which is at least 0.7 Å shorter than the sum of the VDW radii). The dihedral angle between the benzo ring and the furan is 41.28(6)°. The hydrogen bonding of **4** consists of a cooperative hydrogen bond network based on molecules related to each other via the 2_1-screw axis. A chain running parallel to the *b*-axis at [0, *y*, $^{1}/_{2}$] is formed by the hydroxyl groups $O5^{i}\cdots H7-C7-C6-C5-O5\cdots H7^{ii}$ (i = $-x$, $y - {}^{1}/_{2}$, $1 - z$; ii = $-x$, $y + {}^{1}/_{2}$, $1 - z$) and is supplemented by an additional hydrogen bond between $O1^{iii}$ and H5 (iii = $1 - x$, $y + {}^{1}/_{2}$, $1 - z$).

As is evident from Figure 2, the ring conformations of citreofuran **4** and curvularin **1** are remarkably similar. The rms based on all ring atoms excluding C9 to C13 is 0.21 Å. In contrast, dehydrocurvularin **2** adopts a completely different conformation of the macrocycle,[5] which is characterized by the anticlinal arrangement of C12 and C15 and the boatlike conformation of that half of the macrocycle annellated to the benzo ring (Figure 2, bottom).

Assessment of the DNA-Cleaving Properties. It is well-established in the literature that phenols are regioselectively oxygenated by O_2 in the presence of copper−amine complexes as catalysts. The resulting catechols are further oxidized to *o*-quinones and derivatives thereof by a mechanism that leads to the concomitant formation of H_2O_2.[37] Its subsequent cleavage by the metal cation then produces diffusible oxygen radicals which entail massive DNA damage. The catalytic action of copper in this overall process is remarkably specific, as this metal cation can hardly be replaced by other ones known to effect the catalytic decomposition of H_2O_2.

It has previously been shown that many natural products bearing hydroxylated aromatic or heteroaromatic rings, though rather diverse in structure, are able to effect DNA cleavage under oxidative conditions by this

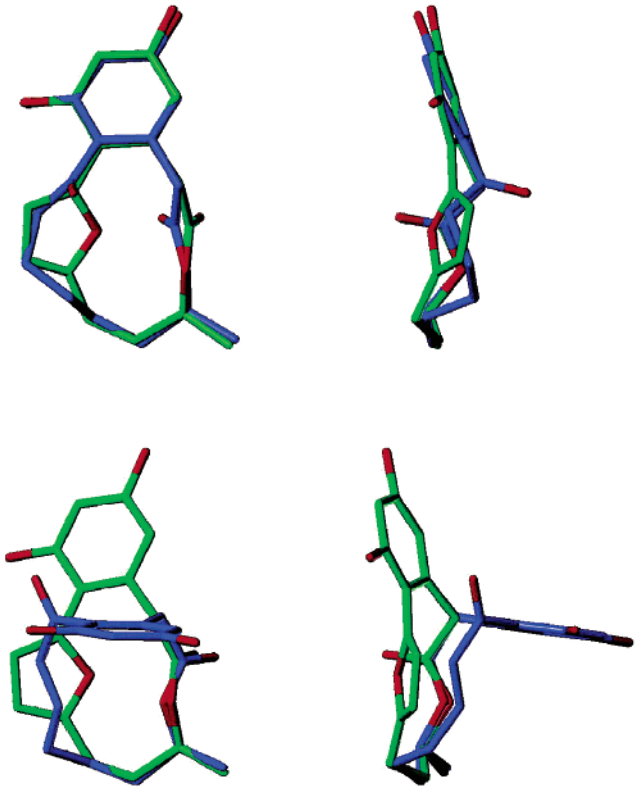

FIGURE 2. Top: Superposition of the X-ray structures of **4** (green) and **1** (blue)[5b] shown in an orthographic projection. Bottom: Superposition of the X-ray structures of **4** (green) and **2** (blue)[5a] based on atoms C13 to O16 plus C1 and the methyl carbon atom. All hydrogen atoms have been omitted for clarity.

specific mode of action.[12b,38,39] Therefore, we expected citreofuran to exert similar functions due to its resorcinol substructure.

In fact, **4** readily relaxes the supercoiled plasmid DNA of the bacteriophage ΦX174 (form **I**) to the nicked form **II** and even to the linear form **III** in the presence of Cu(OAc)$_2$ and *n*-BuNH$_2$ (Figure 3). Surprisingly, however, this ability to cause single- and even double-strand cleavage *is not directly linked to the presence of the free −OH groups in the resorcinol ring* as evident by comparison with lane 5 of the agarose gel, which shows that the di-*O*-methyl derivative **23** leads to similar effects. This observation is in striking contrast to the behavior of other resorcinol derivatives which are completely inert as long as their hydroxyl groups are protected as the corresponding methyl ethers.[12b,38] Therefore, a second mode of action must be operative, at least in part, which is likely related to the presence of the furan moiety. It has previously been shown that certain heteroaromatic systems engender DNA cleavage in the presence of Cu(II) by a mechanism that is triggered by oxidation to the corresponding radical cations.[40,41] Because the rather electron-rich biaryl system in **23** is susceptible to such oxidation, it is thought to be responsible for the DNA-

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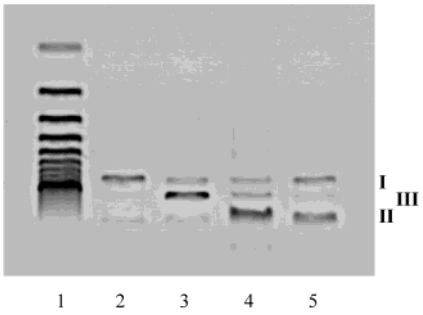

FIGURE 3. Result of an agarose gel electrophoresis showing the extent of DNA cleavage produced by compounds **4** and **23** (200 μM) in the presence of $Cu(OAc)_2$ and *n*-$BuNH_2$ after an incubation time of 1.5 h at 37 °C. Lane 1: DNA marker (500 base pairs molecular weight difference). Lane 2: DNA alone. Lane 3: DNA enriched in linear form **III** (partial cleavage of parent DNA by restriction endonuclease *Xho* I). Lane 4: DNA + compound **4** + Cu^{II} + *n*-$BuNH_2$. Lane 5: DNA + compound **23** + Cu^{II} + *n*-$BuNH_2$.

cleaving properties of this molecule. A more detailed investigation of this and related aspects together with other biological evaluations of citreofuran are subject to further studies in this laboratory.

Experimental Section

General. All reactions were carried out under Ar. The solvents were purified by distillation over the drying agents indicated and were transferred under Ar: THF, Et_2O, DME (Mg-anthracene), CH_2Cl_2 (P_4O_{10}), MeCN, Et_3N, pyridine, DMF (CaH_2), MeOH (Mg), hexane, toluene (Na/K). Flash chromatography: Merck silica gel 60 (230−400 mesh). NMR: chemical shifts (δ) are given in ppm relative to TMS, coupling constants (J) in Hz. Melting points are uncorrected. All commercially available compounds were used as received.

Methyl 2,4-Dihydroxy-6-methylbenzoate (5). Methyl acetoacetate (22.4 mL, 207 mmol) was slowly added to a stirred suspension of NaH (6.50 g, 271 mmol) in THF (100 mL) at 0 °C. After the evolution of gas had ceased, the reaction mixture was cooled to −78 °C and n-BuLi (1.6 M in hexane, 108 mL, 173 mmol) was added dropwise. The mixture was allowed to warm to ambient temperature overnight and was then refluxed for 24 h. The resulting dark red suspension was treated at 0 °C with aq HCl (2 M) until a pH of ca. 2 was reached and stirring was continued at ambient temperature for 2 h. A standard extractive workup with EtOAc followed by flash chromatography of the crude product (hexane/EtOAc, 10/1 → 4/1) provided ester **5** as a colorless solid (12.73 g, 67%). Mp 134−136 °C (lit.[42] 136−138 °C). ^{1}H NMR (400 MHz, acetone-d_6): δ 2.45 (s, 3H), 3.91 (s, 3H), 6.22−6.29 (m, 2H), 9.03 (br s, OH), 11.6 (br s, OH). ^{13}C NMR (100 MHz, acetone-d_6): δ 24.4, 52.3, 101.8, 112.4, 114.5, 163.4, 166.5, 173.2. MS: *m*/*z* (rel intensity): 182 ([M^+], 45), 151 (24), 150 (100), 122 (39), 94 (11). IR (film): 3369, 3312, 3044, 2984, 2958, 1640, 1582, 1503, 1446, 1391, 1380, 1313, 1267, 1201, 1160, 1112, 1062, 1033, 995, 953, 854, 838, 800, 753, 703, 642, 624, 576, 524 cm^{-1}.

2,4-Dimethoxy-6-methylbenzoic Acid (6). A solution of ester **5** (5.086 g, 27.95 mmol) in THF (60 mL) was treated with $LiOH·H_2O$ (3.696 g, 88.1 mmol) at ambient temperature for 10 min. Dimethyl sulfate (7.8 mL, 82.5 mmol) was added and the mixture was stirred for 3 h at 50 °C. The reaction was quenched with water, the aqueous phase was extracted with EtOAc, and the combined organic layers were successively washed with aq ammonia solution (10% w/w) and brine, dried (Na_2SO_4), and evaporated. Flash chromatography of the crude product (hexane/EtOAc, 4:1) afforded 2,4-dimethoxy-6-methylbenzoic acid methyl ester as a colorless solid (5.058 g, 86%) that shows the following spectroscopic properties: ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.28 (s, 3H), 3.785 (s, 3H), 3.79 (s, 3H), 3.87 (s, 3H), 6.31 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.0, 52.1, 55.5, 56.0, 96.4, 106.9, 116.6, 138.4, 158.4, 161.5, 168.8. A solution of this compound (0.667 g, 3.17 mmol) in aq NaOH (4 M, 12 mL) and MeOH (15 mL) was heated for 24 h at 60 °C. After cooling to ambient temperature, the mixture was acidified with aq HCl (2 M) until a pH of ca. 2 was reached. A standard extractive workup afforded acid **6**, which was used in the next step without further purification (0.601 g, 97%). Its analytical and spectroscopic data are in agreement with those previously reported.[43] ^{1}H NMR (300 MHz, acetone-d_6): δ 2.3 (s, 3H), 3.81 (s, 3H), 3.82 (s, 3H), 6.42 (d, 1H, J = 2.2 Hz), 6.46 (d, 1H, J = 2.2 Hz). ^{13}C NMR (75 MHz, acetone-d_6): δ 20.2, 55.7, 56.3, 96.9, 108.0, 117.8, 139.0, 159.2, 162.4, 168.7.

2,4-Dimethoxy-6-methylbenzoic Acid 2-Trimethylsilanylethyl Ester (10). To a solution of carboxylic acid **6** (0.798 g, 4.07 mmol), PPh_3 (2.736 g, 10.43 mmol), and trimethylsilylethanol (0.9 mL, 6.28 mmol) in THF (22 mL) at 0 °C was added diisopropylazodicarboxylate (DIAD, 1.85 mL, 9.41 mmol) and the resulting mixture was stirred for 3 h at 0 °C and then for 40 min at ambient temperature. The solution was partitioned between Et_2O and sat aq $NaHCO_3$, the aqueous phase was extracted with Et_2O, and the combined organic extracts were dried over Na_2SO_4, filtered, and concentrated. Purification of the crude product by flash chromatography afforded ester **15** (1.027 g, 85% yield) as a colorless solid. Mp 37−39 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.06 (s, 9H), 1.08−1.13 (m, 2H), 2.29 (s, 3H), 3.78 (s, 3H), 3.79 (s, 3H), 4.36−4.40 (m, 2H), 6.30 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ −1.4, 17.5, 20.0, 55.4, 55.9, 63.3, 96.3, 106.7, 117.1, 138.0, 158.2, 161.3, 168.6. MS, *m*/*z* (rel intensity): 296 ([M^+], 21), 253 (25), 196 (11), 179 (100), 178 (24), 152 (4), 136 (5), 121 (3), 93 (2), 73 (19). IR (KBr): 3026, 3024, 2956, 2896, 2839, 1717, 1606, 1585, 1467, 1458, 1267, 839 cm^{-1}.

2-Carboxymethyl-4,6-dimethoxybenzoic Acid 2-trimethylsilanylethyl Ester (11). n-BuLi (1.6 M in hexane, 8.2 mL, 13.12 mmol) was added to a solution of diisopropylamine (2 mL, 14.27 mmol) in THF (20 mL) at 0 °C. After 15 min, the resulting solution of LDA was cooled to −78 °C and a solution of compound **10** (2.014 g, 6.90 mmol) in THF (30 mL) was added dropwise. The reaction mixture was stirred for 25 min at that temperature prior to the addition of dry ice, which leads to an immediate disappearance of the deep red color. The reaction mixture was stirred at −78 °C for 15 min before it was hydrolyzed with aq HCl (2 M). After reaching ambient temperature, it was acidified until a pH of 2 was reached and extracted with EtOAc. The organic extract was washed with brine, dried over Na_2SO_4, filtrated, and evaporated. Flash chromatography of the residue (hexane/EtOAc/CH_3COOH, 1:1:0.01) afforded acid **11** (1.985 g, 85%) as a colorless solid. Mp 62−63 °C. ^{1}H NMR (300 MHz, acetone-d_6): 0.08 (s, 9H), 1.10 (t, 2H, J = 8.5 Hz), 3.66 (s, 2H), 3.81 (s, 3H), 3.83 (s, 3H), 4.32 (t, 2H, J = 8.5 Hz), 6.54 (s, 2H). ^{13}C NMR (75 MHz, acetone-d_6): δ −1.4, 17.8, 39.4, 55.8, 56.3, 63.4, 98.1, 108.8, 117.9, 136.1, 159.6, 162.4, 168.0, 171.8. MS, *m*/*z* (rel intensity): 340 ([M^+], 47), 297 (44), 269 (31), 253 (37), 223 (77), 205 (38), 194 (64), 178 (84), 149 (14), 73 (100). IR (KBr): 3087, 3001, 2951, 2897, 2843, 1718, 1707, 1606, 1491, 1425, 1241, 1165, 835 cm^{-1}. HRMS ($C_{16}H_{24}O_6Si$): calcd 340.1342, found 340.1343.

(*S*)-Hex-4-yn-2-ol (12). DMPU (15 mL) was added to a suspension of propynyllithium (1.614 g, 39.4 mmol) in THF (10 mL). After the mixture was stirred for 30 min at −20 °C,

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a solution of (*S*)-methyloxirane in DMPU (5 mL) was slowly added and the resulting mixture was stirred overnight at −20 °C before it was allowed to reach ambient temperature. After being stirred for another 6 h, the reaction mixture was quenched with sat aq NH_4Cl, the aqueous layer was extracted with Et_2O, and the combined organic phases were dried over Na_2SO_4, filtrated, and evaporated. Purification of the residue by filtration through a pad of silica (pentane/Et_2O, 4:1) afforded alcohol **12** as a colorless syrup (0.726 g, 52%). Its spectral and analytical data are in agreement with those reported in the literature.[44] $[\alpha]_D^{20}$ +19.2° (*c* 1.19, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.18 (d, 3H, J = 6.2 Hz), 1.75 (t, 3H, J = 2.6 Hz), 2.04 (s, 1H), 2.15−2.34 (m, 2H, CH_2), 3.81−3.88 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 3.6, 22.3, 29.5, 66.7, 75.5, 78.5. MS, *m*/*z* (rel intensity): 98 (0.16), 83 (15), 79 (7), 54 (100), 51 (11), 45 (62), 43 (25), 39 (36), 27 (22). IR (KBr): 3362, 2972, 2921, 2217, 1713, 1375, 1116, 1085 cm^{-1}.

2,4-Dimethoxy-6-(1-methylpent-3-ynyloxycarbonylmethyl)benzoic Acid 2-Trimethylsilanylethyl Ester (13). To a stirred solution of acid **11** (1.033 g, 3.13 mmol), alcohol **12** (0.409 g, 4.17 mmol), and DMAP (0.318 g, 2.6 mmol) in CH_2Cl_2 (30 mL) was added DCC (0.715 g, 3.5 mmol) and the resulting mixture was stirred overnight. The white precipitate was filtered off through a short pad of silica and the filtrate was partitioned between H_2O and EtOAc. The combined organic phases were dried over Na_2SO_4, filtrated, and evaporated, and the residue was purified by flash chromatography (hexane/EtOAc, 4:1) to furnish ester **13** as a colorless oil (1.238 g, 94%). $[\alpha]_D^{20}$ −13.6° (*c* 1.145, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.06 (s, 9H), 1.07−1.14 (m, 2H), 1.30 (d, 3H, J = 6.3 Hz), 1.76 (t, 3H, J = 2.6 Hz), 2.30−2.44 (m, 2H), 3.65 (s, 2H), 3.80 (s, 3H), 3.81 (s, 3H), 4.32−4.39 (m, 2H), 4.88−5.01 (m, 1H), 6.39 (d, 1H, J = 2.25 Hz), 6.41 (d, 1H, J = 2.25 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ −1.4, 3.6, 17.5, 19.2, 25.9, 39.7, 55.5, 56.1, 63.4, 70.0, 74.5, 77.9, 98.0, 107.3, 116.9, 134.9, 158.9, 161.6, 167.6, 170.3. MS, *m*/*z* (rel intensity): 420 ([M$^+$], 58), 303 (20), 295 (57), 267 (22), 223 (100), 205 (59), 194 (48), 178 (35), 81 (27), 73 (95), 53 (14). IR (KBr): 2953, 2901, 2841, 1734, 1606, 1588, 1458, 1426, 1272, 1162, 839 cm^{-1}. HRMS ($C_{22}H_{32}O_6Si$): calcd 420.1968, found 420.1966.

2,4-Dimethoxy-6-(1-methylpent-3-ynyloxycarbonylmethyl)benzoic Acid (14). To a solution of ester **13** (1.109 g, 2.64 mmol) in THF (40 mL) was added TBAF (1 M in THF, 4.6 mL, 4.6 mmol). The resulting mixture was stirred overnight before it was hydrolyzed with saturated aqueous NH_4Cl and repeatedly extracted with EtOAc. The combined organic phases were dried over Na_2SO_4, filtered, and evaporated, and the residue was purified by flash chromatography (hexane/EtOAc/HOAc, 1:1:0.01) to give acid **14** as a colorless solid (0.806 g, 95%). Mp 84−85 °C. $[\alpha]_D^{20}$ −23.8° (*c* 1.02, $CHCl_3$). ^{1}H NMR (400 MHz, acetone-d_6): δ 1.26 (d, 3H, J = 6.3 Hz), 1.73 (t, 3H, J = 2.6 Hz), 2.26−2.46 (m, 2H), 3.76 (s, 2H), 3.85 (s, 3H), 3.89 (s, 3H), 4.85−4.90 (m, 1H), 6.54 (d, 1H, J = 2.3 Hz), 6.60 (d, 1H, J = 2.3 Hz). ^{13}C NMR (75 MHz, acetone-d_6): δ 3.3, 19.4, 26.3, 40.5, 56.0, 56.7, 70.3, 75.4, 78.3, 98.2, 109.8, 116.6, 137.6, 160.1, 162.8, 167.9, 170.7. MS, *m*/*z* (rel intensity): 320 ([M$^+$], 25), 240 (13), 223 (100), 195 (58), 178 (21), 79 (13), 53 (11). IR (KBr): 3100, 3068, 2983, 2940, 2920, 2852, 2056, 1727, 1676, 1604, 1574, 1462, 1433, 1277, 1172 cm^{-1}. HRMS ($C_{17}H_{20}O_6$): calcd 320.1260, found 320.1258.

2-Hex-4-ynoyl-3,5-dimethoxyphenylacetic Acid 1-Methylpent-3-ynyl Ester (21). To a solution of acid **14** (83.1 mg, 0.26 mmol) in THF (3 mL) was added chloroenamine **15** (43 μL, 0.32 mmol)[29] and the resulting mixture was stirred for 2.5 h prior to the additon of Et_3N (73 μL, 0.52 mmol). After being stirred for another 10 min, the reaction mixture was quenched with water, the aqueous layer was repeatedly extracted with EtOAc, the combined organic phases were dried over Na_2SO_4, filtered, and evaporated, and the residue was rapidly passed through a short pad of silica (hexane/EtOAc, 1:1) to give isocoumarin **20**, which was immediately used in the next step. Characteristic data for compound **20**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.43 (d, 3H, J = 6.3 Hz), 1.77 (t, 3H, J = 2.6 Hz), 2.39−2.616 (m, 2H), 3.86 (s, 3H), 3.93 (s, 3H), 4.68−4.75 (m, 1H), 5.50 (s, 1H), 6.25 (d, 1H, J = 2.1 Hz), 6.27 (d, 1H, J = 2.1 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 3.6, 13.3, 26.2, 55.7, 56.3, 74.1, 74.6, 78.5, 82.8, 96.5, 99.0, 100.7, 145.2, 157.7, 158.0, 163.7, 165.7. To a solution of isocoumarin **20** prepared as described above in THF (5 mL) was successively added a solution of pent-3-ynylmagnesium bromide (0.7 mL, 0.54 M in THF)[32,45] and TMSCl (33 μL, 0.26 mmol), and the resulting mixture was stirred for 30 min at −78 °C before it was allowed to warm to ambient temperature. After being stirred for 3 h, the reaction mixture was hydrolyzed at 0 °C with sat aq NH_4Cl. A standard extractive workup followed by flash chromatography (hexane/EtOAc, 4:1) provided ketone **21** as a colorless solid (72.5 mg, 75%). Mp 67−68 °C. $[\alpha]_D^{20}$ −18.4 ° (*c* 1.005, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.29 (d, 3H, J = 4.8 Hz), 1.76 (t, 3H, J = 2.3 Hz), 2.34−2.50 (m, 4H), 3.03−3.09 (m, 2H), 3.63 (s, 2H), 3.81 (s, 3H), 3.82 (s, 3H), 4.90−4.97 (m, 1H), 6.37 (d, 1H, J = 2.2 Hz), 6.39 (d, 1H, J = 2.2 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 3.6, 3.65, 14.0, 19.2, 26.0, 39.2, 43.9, 55.6, 55.8, 70.0, 74.6, 75.7, 77.9, 78.6, 97.6, 108.1, 123.7, 135.0, 159.1, 161.7, 170.8, 204.7. MS, *m*/*z* (rel intensity): 370 ([M$^+$], 5), 290 (32), 273 (33), 231 (11), 223 (100), 203 (10), 197 (6), 195 (42), 81 (16), 79 (13), 53 (12). IR (KBr): 3098, 2980, 2919, 2843, 1728, 1660, 1602, 1577, 1457, 1426, 1167 cm^{-1}.

Cycloalkyne 22. To a solution of ketone **21** (93.5 mg, 0.253 mmol) in toluene (20 mL) was added a solution of $(tBuO)_3W{\equiv}CCMe_3$ (11.9 mg, 0.025 mmol)[34] in toluene (10 mL) and the mixture was stirred at 85 °C for 1 h. For workup, the solvent was evaporated and the residue was purified by flash chromatography (hexane/EtOAc, 3:1) to give product **22** as a colorless solid (62.7 mg, 78%). Mp 163−166 °C. $[\alpha]_D^{20}$ +83.5° (*c* 1.02, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.21 (d, 3H, J = 6.35 Hz), 2.10−2.36 (m, 3H), 2.70−2.78 (m, 1H), 3.05−3.27 (m, 2H), 3.29 (d, 1H, J = 17.6 Hz), 3.78 (s, 3H), 3.81 (s, 3H), 4.30 (d, 1H, J = 17.6 Hz), 5.33 (m, 1H), 6.33 (d, 2H, J = 2.1 Hz), 6.40 (d, 2H, J = 2.1 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 13.3, 20.2, 27.6, 38.8, 42.3, 55.5, 55.7, 68.9, 76.4, 82.2, 97.4, 109.1, 124.6, 134.4, 158.2, 161.3, 171.4, 205.0. IR (KBr): 3015, 2962, 2929, 2840, 1738, 1693, 1603, 1586, 1458, 1425, 1409, 1167, 1157, 837 cm^{-1}. MS, *m*/*z* (rel intensity): 316 ([M$^+$], 71), 288 (25), 272 (37), 257 (51), 244 (24), 223 (63), 205 (23), 195 (100), 178 (30), 135 (16), 92 (13), 79 (84), 77 (44). HRMS ($C_{18}H_{20}O_5$): calcd 316.13107, found 316.13112.

Di-*O*-methylcitreofuran (23). To a solution of compound **22** (63.5 mg, 0.201 mmol) in toluene (5 mL) was added *p*-toluenesulfonic acid (47.1 mg, 0.248 mmol) and the reaction mixture was stirred at 85 °C for 5.5 h. Quenching of the reaction at 0 °C with aq sat $NaHCO_3$ followed by a standard extractive workup and flash chromatography of the crude product (hexane/EtOAc, 4:1) afforded compound **23** as a colorless solid (54.0 mg, 85%). Mp 138−140 °C. $[\alpha]_D^{20}$ +94.4° (*c* 0.465, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.26 (d, 3H, J = 6.5 Hz), 1.75−1.87 (m, 1H), 2.01−2.09 (m, 1H), 2.66−2.87 (m, 2H), 3.19−3.31 (m, 2H), 3.76 (s, 3H), 3.85 (s, 3H), 5.20−5.27 (m, 1H), 6.10 (d, 1H, J = 3 Hz), 6.27 (d, 1H, J = 3 Hz), 6.46 (d, 1H, J = 2.25 Hz), 6.54 (d, 1H, J = 2.25 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 21.2, 25.7, 36.1, 42.2, 55.6, 56.0, 72.4, 97.8, 106.8, 109.0, 110.3, 113.7, 138.9, 147.2, 155.0, 158.7, 161.0, 171.7. MS, *m*/*z* (rel intensity): 316 ([M$^+$], 100), 229 (8), 202 (17), 187 (12), 115 (8). IR (KBr): 3007, 2980, 2909, 2840, 1739, 1617, 1601, 1578, 1479, 1460, 1438, 1200, 1157, 788 cm^{-1}. HRMS ($C_{18}H_{20}O_5$): calcd 316.13107, found 316.13098.

Citreofuran (4). A solution of 9-iodo-9-BBN (57.8 mg, 0.233 mmol) in CH_2Cl_2 (0.2 mL) was added to a solution of compound

(44) Adam, W.; Saha-Möller, C. R.; Schmid, K. S. *J. Org. Chem.* **2001**, *66*, 7365.

(45) The concentration of the Grignard solution was determined as described in: Bergbreiter, D. E.; Pendergrass, E. *J. Org. Chem.* **1981**, *46*, 219.

23 (19.0 mg, 0.060 mmol) in CH_2Cl_2 (1.25 mL) at −10 °C and the resulting mixture was stirred for 4 h at that temperature. During this time, the course of the reaction was followed by HPLC/MS, which indicated consecutive cleavage of the methyl ether groups. After 4 h the formation of byproducts becomes significant; therefore the reaction was quenched with sat aq $Na_2S_2O_3$ (4 mL) and aq sat $NaHCO_3$ (1.5 mL), the aqueous layer was repeatedly extracted with CH_2Cl_2, and the combined organic layers were consecutively washed with aq sat $NaHCO_3$ and brine, dried over Na_2SO_4, and evaporated to give crude citreofuran (10.4 mg, 60%). An analytically pure sample was obtained by preparative HPLC (Nucleosil-100-7-C18/A column, 125 mm, ∅ 20 mm; MeOH:H_2O 60:40; 6.8 MPa, 10.0 mL/min) (4.5 mg, 27%). Pale brown solid, Mp 202−205 °C (lit.[9] brownish needles, mp 203−205 °C). $[\alpha]_D^{27}$ +104.9 (*c* 0.185, EtOH) [lit.[9] $[\alpha]_D^{27}$ +112 (*c* 0.18, EtOH)]. ^{1}H NMR (600 MHz, MeOH-d_4): δ 1.23 (d, 3H, J = 6.5 Hz), 1.77 (m, 1H), 2.04 (m, 1H), 2.66 (ddd, 1H, J = 2.9, 11.4, 14.7 Hz), 2.84 (ddd, 1H, J = 2.8, 6.1, 15.3 Hz), 3.10 (d, 1H, J = 14.3 Hz), 3.20 (d, 1H, J = 14.6 Hz), 5.16 (m, 1H), 6.09 (d, 1H, J = 3.0 Hz), 6.21 (d, 1H, J = 3.0 Hz), 6.30 (d, 1H, J = 2.3 Hz), 6.33 (d, 1H, J = 2.3 Hz). ^{13}C NMR (150 MHz, MeOH-d_4): δ 21.2, 26.6, 37.3, 42.6, 74.0, 102.6, 107.6, 111.1, 112.0, 112.3, 139.8, 148.8, 156.2, 157.5, 159.7, 174.2. MS, *m*/*z* (rel intensity): 289 (18), 288 ([M^+], 100), 273 (10), 205 (14), 174 (27). IR (KBr): 3419, 2976, 2932, 1707, 1627, 1590, 1560, 1459, 1322, 1261, 1243, 1160, 1123, 1051, 1026, 1011, 845, 784 cm^{-1}.

Acknowledgment. Generous financial support by the Deutsche Forschungsgemeinschaft (Leibniz award to A.F.) and the Fonds der Chemischen Industrie is gratefully acknowledged. We thank Mrs. Helga Krause for performing the gel electrophoresis experiment and Mr. A. Deege and his crew for performing the HPLC separation.

Supporting Information Available: NMR spectra of all new compounds and crystal structure data for product **4** including atomic positions, bond length, and bond and dihedral angles, as well as hydrogen bonds. This material is available free of charge via the Internet at http://pubs.acs.org.

JO026686Q