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Abstract
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Disciplines
Chemistry | Environmental Chemistry | Inorganic Chemistry | Organic Chemistry | Other Chemistry | Polymer Chemistry

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Synthesis and Testing of Analogues of *Helminthosporium maydis* Race T Toxin

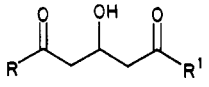
George. A. Kraus,* Michael Silveira, and S. J. Danko

Several analogues of *Helminthosporium maydis* race T toxin were prepared. The synthesis of the hydroxy diketones was accomplished by aldol condensations of 2-decanone with ketal aldehydes followed by deketalization. The dihydroxy ketone analogues were prepared by use of dihydroisoxazole intermediates. The analogues were tested and found to be less active than the parent toxin. The analogues still exhibited host specificity.

Helminthosporium maydis Nisikado (*Cochliobolus heterostrophus* Dreschler) is a fungus that is parasitic on corn, causing the southern leaf blight (Hesseltine et al., 1971). In the late 1960s, a new race, race T, appeared that was especially virulent on corn plants bearing the "Texas" or "cms-T" cytoplasmic gene for male sterility (Hooker et al., 1970). Corn plants with normal cytoplasm or with other cytoplasmic genes for male sterility are much less susceptible to the fungus. *H. maydis* race T produces a toxin that, when applied to corn plant leaves in the absence of the fungus, produces the most prominent symptom of the disease, chlorotic or necrotic streaks on the leaves (Turner and Martinson, 1972). Sensitivity to the toxin is always associated with Texas male sterility. Kono et al. (1980) have recently identified the structures of the major components of the toxin. These structures are depicted below. Of particular interest is the production of highly specific effects by relatively low molecular weight compounds. This is a useful system in which to explore the biochemical basis for specificity in host–parasite interactions. Since the reduction of the toxin with sodium borohydride does not result in the loss of host-specific activity, it is possible that simpler analogues may also exhibit useful activity. We report herein the synthesis and testing results of selected analogues of *H. maydis* race T toxin. Suzuki et al. (1982) have recently reported the synthesis of certain analogues of *H. maydis* race T toxin.

MATERIALS AND METHODS

Synthesis of Analogues. Several types of compounds were constructed as analogues of *H. maydis* race T toxin. Initially we sought to prepare the representative subunits **4** and **5**. There are many reliable and selective methods



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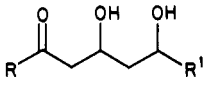

5

for the synthesis of simple β-hydroxy ketones. However, the combination of functional groups in **4** posed a problem in that the enolate anion of a methyl ketone would react with a β-ketoaldehyde to give products derived from an anion-exchange reaction rather than an aldol condensation. As illustrated in Scheme I, we managed to circumvent this problem by use of the ketal aldehyde **6** (Ban et al., 1972). The enolate of 2-decanone reacted with **6** to afford a high yield of hydroxy ketones **7** plus **8**. The hydrolysis of the ketal moiety required carefully defined conditions. The temperature and acid concentration had to be carefully adjusted in order to minimize side reactions. Chromatographic purification provided compounds **9** and **10** in 45% and 15% yields, respectively. Although ketone **10** was produced in low yeld, its testing will afford some insight into the effect of branching on activity. Analogue **11** was next synthesized by a similar route. Our strategy was to construct analogues by building outward from a central unit. We chose **12** because it incorporated both the pentamethylene spacer and the precursor to the central hydroxy diketone subunits present in **1** and **3**. The readily available diacid chloride of pimelic acid was treated with Meldrum's acid and pyridine followed by alcohol to produce a β-keto ester (Oikawa et al., 1978). The product was then subjected to ketalization, reduction, and oxidation by the method of Corey and Suggs (1975) to afford dialdehyde **12**. The C_{31} analogue **11** was synthesized by generating the enolate anion of 2-decanone, adding the dialdehyde, and then acidifying the reaction mixture. Chromatographic purification produced **11** (55%) and **14** (15%).

The dihydroxy ketone subunit was constructed from a dihydroisoxazole intermediate. The key reaction (depicted in Scheme II) was devised by Mukaiyama and Hoshino (1960). Recently several groups have applied this reaction to natural product synthesis. Analogue **15** was synthesized from 1-nitrohexane and 4-acetoxy-1-dodecene by using Mukaiyama's reaction conditions. Analogue **15** could be readily hydrolyzed to alcohol **16** which in turn could be oxidized to ketone **17**. The dihydroisoxazoles could be cleaved by using the method of Curran (1983) to afford **18**.

CO_2 Fixation Bioassay. Susceptible (Tms) and resistant (N) lines of W64A corn were grown in a controlled environment room at 25 °C under a 14-h photoperiod of fluoroescent light at 250 μEinstein m^{-2} s^{-1}. The assay solution (pH 6.4) consisted of 12.5 mM 3-morpholinopropanesulfonic acid, 20 mM KH_2PO_4, and 0.1 mM sodium pyruvate.

Fifteen leaf slices were placed in 475 μL of assay solution in 7-mL scintillation vials. Test compounds were added in 1–5 μL of dimethyl sulfoxide, and the vials were sealed with serum stoppers. Controls received an equivalent amount of dimethyl sulfoxide. Vials were incubated for 1 h in light (intensity 450 μEinsteins m^{-2} s^{-1}) at 29.5 °C. The vials were placed in darkness for 5 min before the addition of 25 μL of 60 mM $NaH^{14}CO_3$ (specific activity approximately 150 μCi $mmol^{-1}$).

After 15-min exposure to $NaH^{14}CO_3$, 0.25 mL of trichloroacetic acid–H_2O (1:1) was added, and the vials were either placed overnight in a hood or flushed with forced air for 30 min to allow diffusion of unfixed $^{14}CO_2$. Five milliliters of 3a70 scintillation fluid (Research Products International, Mount Prospect, IL) was added and radioactivity determined with a Packard Model 240 CL/D spectrometer. Counting efficiencies ranged from 55 to 65% as determined by the sample channels ratio. Control rates

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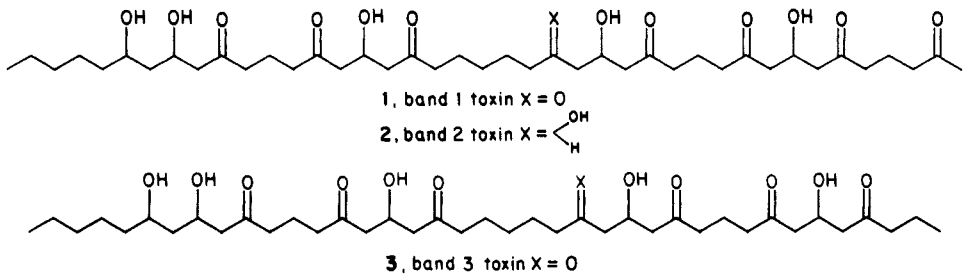
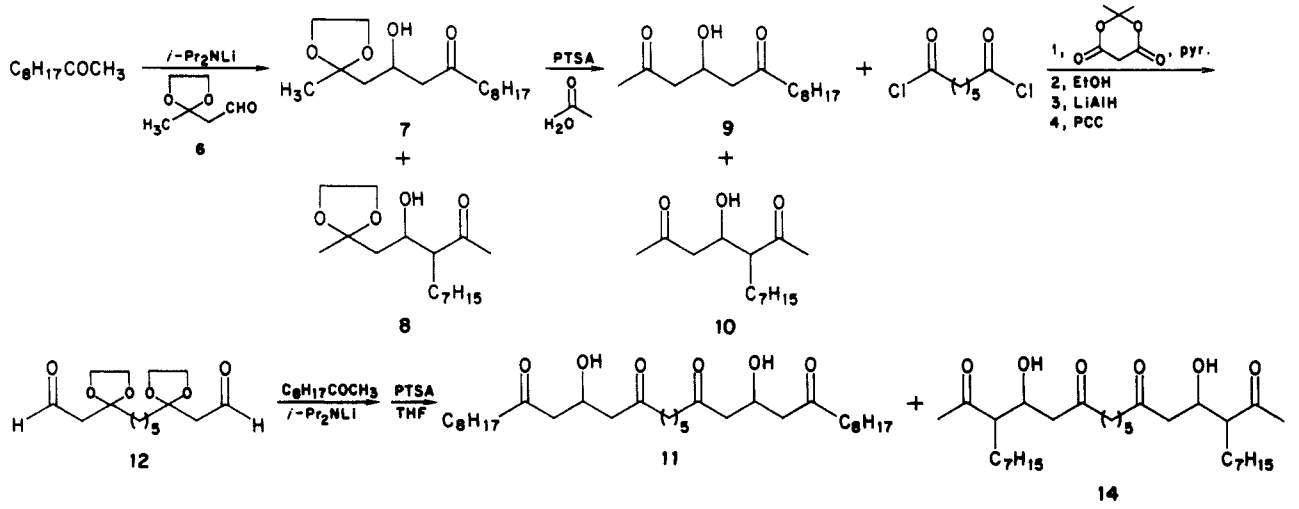
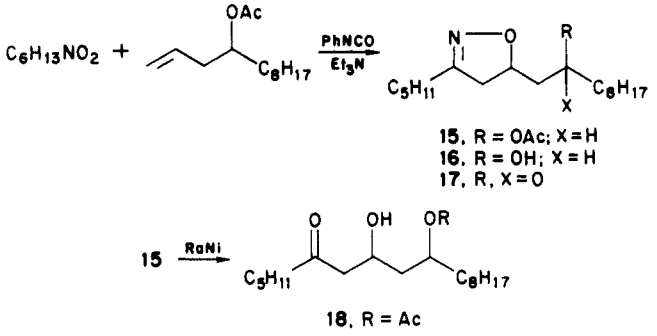


Scheme I



Scheme II



averaged 19–21 nmol of CO_2 (fixed slice)$^{-1}$ h^{-1}. All data are the average of three replicates per treatment, and each experiment was repeated at least twice.

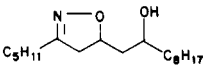
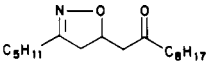
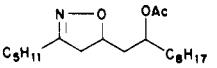
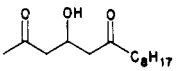
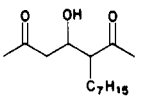
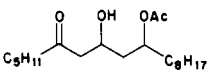
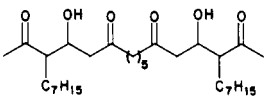
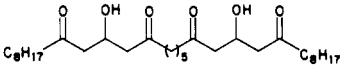
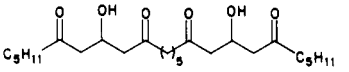
Experimental. All melting points were determined on a Fisher-Johns hot stage. ^{1}H NMR spectra were recorded on a Varian EM-360 spectrometer with Me_4Si as an internal standard. IR spectra were determined with a Beckmann IR-4250. Mass spectra were obtained with an AEI-MS902 instrument at 70 eV. Elemental analyses were performed by Galbraith Laboratories, Knoxville, TN.

4-Hydroxy-2,6-tetradecanedione 2-Ethylene Ketal (7) *and 4-Hydroxy-2,13-tetradecanedione 2-Ethylene Ketal* (8). To a solution of lithium diisopropylamide (LDA, 1.50 mmol) in 5 mL of tetrahydrofuran (THF) at −78 °C was added a solution of 2-decanone (0.234 g, 1.50 mmol) in 5 mL of THF over 1 min. The solution was stirred at −78 °C for 30 min. A solution of **6** (0.190 g, 1.46 mmol) in 5 mL of THF was then added, and the reaction was stirred for 30 min. Saturated ammonium chloride (5 mL) was then added, and the reaction was allowed to warm to ambient temperature. The reaction was diluted with ether–methylene chloride, and the organic layer was washed with brine, dried, and concentrated in vacuo. The crude product proved to be unstable and decomposed on standing. It was usually deketalized as soon as possible.

Deketalization to Produce 9 and 10. The crude product from the aldol reaction (0.260 g) was added to a solution of *p*-toluenesulfonic acid (0.020 g) in 10 mL of 20% aqueous acetone. The solution was stirred at ambient temperature for 5 h. The reaction was then diluted with methylene chloride and extracted with brine, dried over sodium sulfate, filtered, and concentrated in vacuo to afford 0.248 g of crude product. The product was chromatographed on silica gel to afford **9** (45%) and **10** (15%). **9**: NMR ($CDCl_3$) δ 0.75–1.81 (m, 15 H), 2.24 (s, 3 H), 2.25–2.81 (m, 6 H), 3.42–3.58 (m, 1 H), 4.32–4.68 (m, 1 H); IR (film) 3530, 1714 (br), 861 cm^{-1}; high-resolution mass spectrum for $C_{14}H_{26}O_3$ requires 242.18820, measured 242.18900. Anal. ($C_{14}H_{26}O_3$) C, H. **10**: NMR ($CDCl_3$) δ 0.68–1.75 (m, 15 H, 2.06 (s, 3 H), 2.10 (s, 3 H), 2.13–2.85 (m, 3 H), 3.12–3.40 (m, 1 H); IR (film) 3440, 1710 (br), 1359, 913 cm^{-1}; high-resolution mass spectrum $C_{14}H_{24}O_2$ ($M^+ - H_2O$) requires 224.17763, measured 224.17820. Anal. $C_{14}H_{26}O_3$) C, H.

1,3,9,11-Undecanetetraone Diethylene Ketal (12). A solution of the diacidchloride (7.80 g, 39.7 mmol), Meldrum's acid (12.66 g, 87.9 mmol), and pyridine (40 mL) in 100 mL of methylene chloride was stirred at ambient temperature for 4 h. The solution was diluted with ether–methylene chloride and extracted with 1 N HCl, brine, and then dried and concentrated in vacuo to afford 17.6 g of crude product. The crude product was dissolved in 300 mL of ethanol. One gram of PTSA was added, and the solution was heated to reflux for 3 h. The solution was cooled, concentrated, and then dissolved in methylene chloride. The solution was then extracted with brine, dried, and concentrated. Chromatography on silica gel using hexane–methylene chloride–acetone (9:3:1) afforded a 22% yield of the diketo diester. To 1.50 g (5.0 mmol) of the diketo diester in 50 mL of benzene was added 10 mL of ethylene glycol and 0.150 g of PTSA. The solution was heated to reflux with continuous removal of water for

Table I. Percent Inhibition of Dark CO_2 Fixation

		ng/mL		μg/mL				
entry	structure	10	100	1	10	50	100	500
1				0	15	25	27	47
2				−5	4	1	3	24
3				−15	−4	−1	0	11
4				1	1	8	10	48
5				5	11	14	27	52
6				3	−2	1	12	14
7		−3	6	20	34	49		56
8		−2	6	13	26	41		57
9	HM T toxin	15	42	52	51			
10[a]		14	26	50	52			

[a] Analogue prepared by Suzuki.

5 h. The solution was then cooled, diluted with methylene chloride, and extracted with brine. The organic layer was dried, filtered, and concentrated in vacuo. The crude product was reduced directly to the diol. To a stirred solution of 0.932 g (2.40 mmol) of the diester in 50 mL of anhydrous ether at 0 °C was added lithium aluminum hydride (0.190 g, 5.0 mmol). The suspension was stirred at 0 °C for 1.5 h. To the stirred suspension at 0 °C was cautiously added 5 mL of water followed by ether and 10% NaOH. The organic layer was washed with brine, dried, filtered, and concentrated. The diol was sufficiently pure to be taken on to the oxidation step. To a suspension of pyridinium chlorochromate (0.760 g, 3.0 mmol) and 0.040 g of sodium acetate in 8 mL of methylene chloride was added the diol (0.304 g, 1 mmol) in 5 mL of CH_2Cl_2. The suspension was stirred for 3 h. The reaction mixture was then diluted with ether and filtered through Fluorisil. The solution was washed with 1% NaOH and brine and then dried, filtered, and concentrated. The dialdehyde was obtained in 64% yield. **12**: NMR ($CDCl_3$) δ 0.80–1.92 (m, 10 H), 2.68 (d, J = 3 Hz, 4 H), 4.02 (s, 8 H), 9.80 (t, J = 3 Hz, 2 H); IR (film) 1720, 1138, 1050, 942 cm^{-1}. Anal. (for diester) ($C_{19}H_{32}O_8$) C, H.

11,21-Dihydroxy-9,13,19,23-nonatriacontatetranone (**11**) *and 3,15-Diheptyl-4,14-dihydroxy-2,6,12,16-heptadecatetraone* (**14**). To a −78 °C solution of lithium diisopropylamide (1.31 mmol) in 5 mL of THF was added a solution of 2-decanone (0.208 g, 1.33 mmol) in 5 mL of THF over 1 min. The solution was stirred for 30 min. at −78 °C. A solution of **12** (0.200 g, 0.67 mmol) in 5 mL of THF was then added. After 20 min 5 mL of ammonium chloride was added and the reaction mixture was allowed to warm to 0 °C. The mixture was diluted with methylene chloride. The organic layer was washed with brine, dried, filtered, and concentrated. The crude product (0.299 g) was immediately dissolved in 10% aqueous THF, 0.005 g of PTSA was added, and the solution was stirred for 24 h at ambient temperature. The solution was diluted with ether. The organic layer was washed with brine, dried, filtered, and concentrated. The crude material was purified by column chromatography to afford **11** (55%) and **14** (15%). **11**: NMR ($CDCl_3$) δ 0.64–1.92 (m, 36 H), 2.08–2.71 (m, 22 H), 3.41 (d, J = 3 Hz, 1 H), 3.98 (d, J = 3 Hz, 1 H); IR (film) 3420 (br), 1710, 910, 730 cm^{-1}, high-resolution mass spectrum for $C_{31}H_{54}O_5$ ($M^+ - H_2O$) requires 506.39714, measured 506.39648. Anal. ($C_{31}H_{56}O_6$) C, H. **14**: NMR ($CDCl_3$) δ 0.63–1.90 (m, 36 H), 2.08–2.71 (m, 16 H), 3.41 (d, J = 4 Hz, 1 H); IR (film) 3520, 1710, 1805 cm^{-1}; high-resolution mass spectrum for $C_{31}H_{54}O_5$ ($M^+ - H_2O$) requires 506.39714, measured 506.39698.

5-Amyl-3,4-dihydro-3-(2-acetoxydecyl)isoxazole (**15**). To a solution of 1-nitrohexane (0.650 g, 4.96 mmol), phenyl isocyanate (0.540 mL, 4.9 mmol), and 4-acetoxy-1-dodecene (2.30 g, 10.1 mmol) in 50 mL of benzene was added triethylamine (0.070 mL, 5.0 mmol). The solution was stirred for 48 h, diluted with 50 mL of hexane, and filtered. The crude product was chromatographed on silica gel by using ethyl acetate–hexane (3:7) to provide **15** (47% yield). **15**: NMR ($CDCl_3$) δ 0.68–1.83 (m, 24 H), 2.05 (s, 3 H), 2.10–3.22 (m, 4 H), 4.23–5.16 (m, 2 H); IR (film 3500, 1735, 1240, 1015 cm^{-1}.

5-Amyl-3,4-dihydro-3-(2-hydroxydecyl)isoxazole (**16**). To a solution of **15** (0.380 g, 1.00 mmol) in 5 mL of methanol was added anhydrous potassium carbonate (0.400 g, 2.90 mmol). The reaction was stirred at ambient temperature for 17 h and diluted with ether. The organic layer was washed with brine, dried, filtered, and concentrated in vacuo to afford pure **16** in 95% yield. **16**: NMR ($CDCl_3$) δ 0.67–1.86 (m, 24 H), 2.18–3.00 (m, 4 H), 3.60–3.89 (m, 1 H); IR (film) 3430, 1470, 865 cm^{-1}.

5-Amyl-3,4-dihydro-3-(2-oxodecyl)isoxazole (**17**). To a suspension of pyridinium chlorochromate (0.350 g, 1.40 mmol) and sodium acetate (0.020 g, 0.30 mmol) in 5 mL of methylene chloride was added **16** (0.350 g, 0.90 mmol)

Table II. Percent Inhibition of Dark CO_2 Fixation

entry	50 μg/mL	500 μg/mL	entry	50 μg/mL	500 μg/mL
1	1	16	5	1	32
2	8	10	6	3	12
3	12	27	7	12	31
4	5	38	8	11	26

in 5 mL of methylene chloride. The reaction mixture was stirred at ambient temperature for 3 h, diluted with ether, and filtered through Fluorisil. The ether layer was washed with 1% NaOH and brine. The organic layer was dried, filtered, and concentrated in vacuo to afford pure **17** in 61% yield. **17**: NMR ($CDCl_3$) δ 0.75–1.85 (m, 24 H), 2.12–3.15 (m, 9 H); IR (film) 1708, 1461, 1405, 1370 cm^{-1}; high-resolution mass spectrum for $C_{18}H_{33}O_2N$ requires 295.25114, measured 295.25163. Anal. ($C_{18}H_{33}O_2N$) C, H, N.

Dihydroisoxazole Cleavage To Provide Either 10-Acetoxy-8-hydroxy-6-octadecanone (**18**) *or 8,10-Dihydroxy-6-octadecanone* (**19**). To the requisite dihydroisoxazole (**15** or **16**) in 20% aqueous methanol (5 mL) was added 0.005 g of RaNi and 0.020 g of boric acid. The reaction was conducted under a hydrogen atmosphere for 3 h. After the mixture was diluted with ether, it was washed with brine, dried, filtered, and concentrated. The crude product was subjected to flash chromatography using 1:3 ethyl acetate–hexane to provide approximately a 60% yield of pure product. **18**: NMR ($CDCl_3$) δ 0.62–1.88 (m, 24 H), 2.01, 2.03 (s, 3H), 2.13–2.66 (m, 4 H), 3.33–3.62 (m, 1 H), 4.73–5.10 (m, 1 H); IR (film) 3500 (br), 1712 (br), 1360, 905 cm^{-1}; high-resolution mass spectrum for $C_{18}H_{34}O_2$ ($M^+ - CH_3CO_2H$) requires 282.25589, measured 282.25597. Anal. ($C_{20}H_{38}O_4$) C, H.

RESULTS

The array of analogues described above will enable one to study the effect of structural changes on activity. The effect of branching, heteroatom substitution, and chain length can be analyzed. The testing procedure used to compare the relative activity of our analogues was the inhibition of dark CO_2 fixation by susceptible corn. This test is a sensitive one (detection of ng/mL activity) and also an operationally convenient one. Our results are depicted in Table I.

The results in Table I indicate that even simple molecules with only one group of oxygen functions show toxic activity toward susceptible corn. Especially interesting is the fact that the nitrogen analogues (**15–17**) are as active as the corresponding ketones. The larger analogues with two groups of oxygen functions per molecule are more active than those with only one group. The only analogues that are not very toxic are the acetylated derivatives **15** and **18**. This observation is in agreement with the data of Payne et al. (1980), who reported that acetylated HmT toxin is less active than is native toxin. All active analogues are also host specific (Table II). While some of the analogues show slight effects on resistant corn, they are more toxic to susceptible corn and are only toxic to resistant corn at concentrations for which Payne et al. (1980) reported that native HmT toxin was toxic to resistant corn and to soybean.

However, none of the analogues described here are as active as native HmT toxin (entry 9 in Table I) or some of the analogues prepared independently by Suzuki et al. (1982). The activity of one of Suzuki's analogues (entry 10 in Table I) was directly compared with the activity of the analogues we prepared and was approximately 100 times as active as **11**, which is very similar in structure to Suzuki's analogue. Suzuki's analogue, however, has a shorter chain length, making it more polar than our analogues. Suzuki et al. (1983) have recently reported the synthesis of a C_{41} analogue of HmT toxin that was as active as the native toxin and have suggested that chain length may be important for maximum activity. Our results support this hypothesis and, in addition, suggest that a balance between polarity and lipophilicity may be important as well. Suitable modifications of the present analogues (e.g., by adding polar groups at the ends) may produce even more active analogues.

ACKNOWLEDGMENT

We thank Dr. Carl Tipton for preliminary testing of some analogues using the leaf streak test.

APPENDIX

Analyses.

compound	C, H, N found	C, H, N calcd
9	69.36, 10.98	69.38, 10.81
10	69.78, 11.13	69.38, 10.81
11	69.44, 10.57	69.71, 10.79
12[a]	58.87, 8.34	58.75, 8.30
17	73.22, 11.30, 4.81	73.17, 11.26, 4.74
18	70.27, 11.21	70.13, 11.18

[a] Diketal diester precursor to **12**.

Registry No. **6**, 18871-63-1; **7**, 91743-70-3; **8**, 91743-71-4; **9**, 91743-72-5; **10**, 91743-73-6; **11**, 91743-74-7; **12**, 91743-75-8; **14**, 91743-76-9; **15**, 91743-77-0; **16**, 91743-78-1; **17**, 91743-79-2; **18**, 91743-80-5; **19**, 91743-81-6; $ClC(O)(CH_2)_5C(O)Cl$, 142-79-0; Meldrum's acid, 2033-24-1; 2-decanone, 693-54-9; 3,9-dioxoundecane-1,11-dioic acid, 91743-82-7; diethyl 3,9-dioxoundecane-1,11-dioate, 91743-83-8; diethyl 3,9-dioxoundecane-1,11-dioate 3,9-diethylene ketal, 91743-84-9; 1,11-dihydroxyundecane-3,9-dione diethylene ketal, 91743-85-0; 1-nitrohexane, 646-14-0; 4-acetoxy-1-dodecene, 91743-86-1.

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