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Expedited Articles

Antimalarial, Antiproliferative, and Antitumor Activities of Artemisinin-Derived, Chemically Robust, Trioxane Dimers

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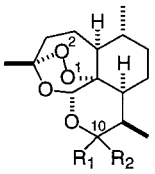
Nine C-10 non-acetal derivatives of the natural trioxane artemisinin (**1**) were prepared as dimers using some novel chemistry. As designed, each dimer was stable chemically. C-10 Olefinic dimers **7** and C-10 saturated dimers **8**−**13** all showed good to excellent antimalarial and antiproliferative activities in vitro. Dimers **8**, **10**, and **12** were especially potent and selective at inhibiting growth of some human cancer cell lines in the NCI in vitro 60-cell line assay.

Introduction

Some dimeric chemical structures have especially high biological activities. Examples include bismustard cross-linked lexitropsins,[2] dimeric steroid−pyrazine marine alkaloids,[3] and DNA-cross-linking dimeric benzodiazepines[4,5] and bis(enediynes).[6] Some 1,2,4-trioxane dimers have high antimalarial, antiproliferative, and antitumor activities in vitro, but often they are hydrolytically unstable.[7−10] Most efforts to prepare chemically more robust semisynthetic derivatives of the natural antimalarial 1,2,4-trioxane artemisinin (**1**) have involved replacing the C-10 acetal functionality in ester and ether derivatives **2**−**5** by less hydrolytically prone functional groups.[11−18] Recent success along these lines has led to a series of orally active, hydrolytically stable antimalarial trioxanes in the artemisinin family.[19] Herein, we report synthesis and preliminary biological evaluation of nine C-10 non-acetal artemisinin-derived trioxane dimers, some of which are not only potent antimalarials but also potent antiproliferative and antitumor agents.



1	$R_1 = R_2 = O$	artemisinin
2	$R_1 = H$, $R_2 = OMe$	artemether
3	$R_1 = H$, $R_2 = OEt$	arteether
4	$R_1 = H$, $R_2 = OOCCH_2CH_2CO_2Na$	sodium artesunate
5	$R_1 = H$, $R_2 = OCH_2PhCO_2Na$	sodium artelinate

Synthesis and Antimalarial Activities

C-10 Olefinic non-acetal dimers **7a**−**c** were prepared in high overall yield and in a novel way via a bis-Wittig coupling reaction with the recently reported artemisinin-derived α,β-unsaturated aldehyde **6** (Scheme 1).[20] The coupling reaction was optimized to nearly quantitative yield by generating the bis-phosphonium ylide reagent via in situ deprotonation of the corresponding bis-phosphonium bromide salt in the presence of aldehyde **6** using in situ generated lithium ethoxide as base.[21] The three geometric isomers **7a**−**c** were separated from one another chromatographically and were assigned trans or cis stereochemistry by ^{1}H NMR

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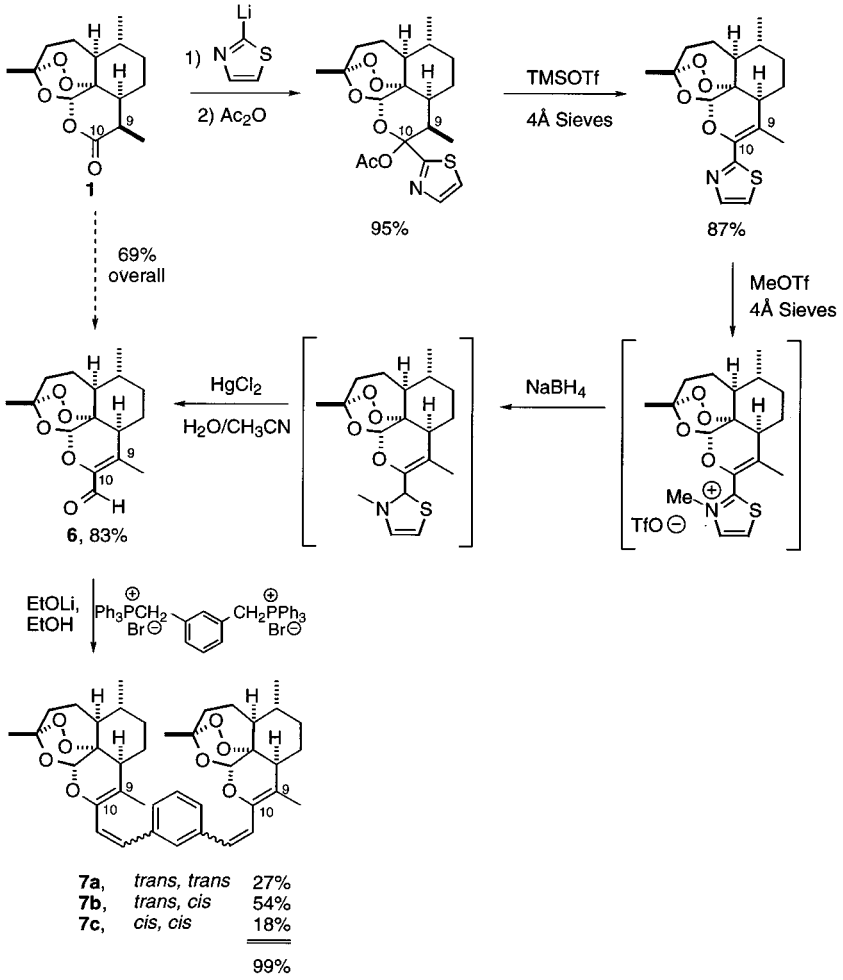
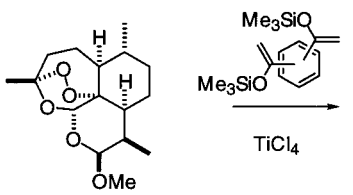
Scheme 1



spectroscopy (see Experimental Section). The corresponding *p*-xylylene dimers were prepared from aldehyde **6** in a similar fashion, but they were found to be unstable, decomposing during several hours in $CDCl_3$.

Using our standard assay,[22] the antimalarial potencies of *m*-xylylene dimers **7** in vitro against chloroquine-sensitive *Plasmodium falciparum* (NF54) parasites were found to be as follows: **7a**, $IC_{50} = 77$ nM; **7b**, $IC_{50} = 35$ nM; **7c**, $IC_{50} = 18$ nM; in comparison, the IC_{50} value for artemisinin is 9.7 nM. C-10 Saturated non-acetal dimers **8**−**13** were prepared from either artemether (**2**) via novel titanium-promoted condensations (eq 1) or



p-dimer **8**, 26%
m-dimer **9**, 33% (1)

from the recently described artemisinin-derived C-10 fluoride **14** via Friedel−Crafts or aluminum acetylide condensations (Scheme 2).[18] Although benzoylmethylene dimers **8** and **9** and acetylenic dimers **12** and **13** are stereochemically β-linked to C-10 of the artemisinin skeleton, aryl dimers **10** and **11** are α-linked; the basis for this difference in stereochemistry of attachment is

Scheme 2

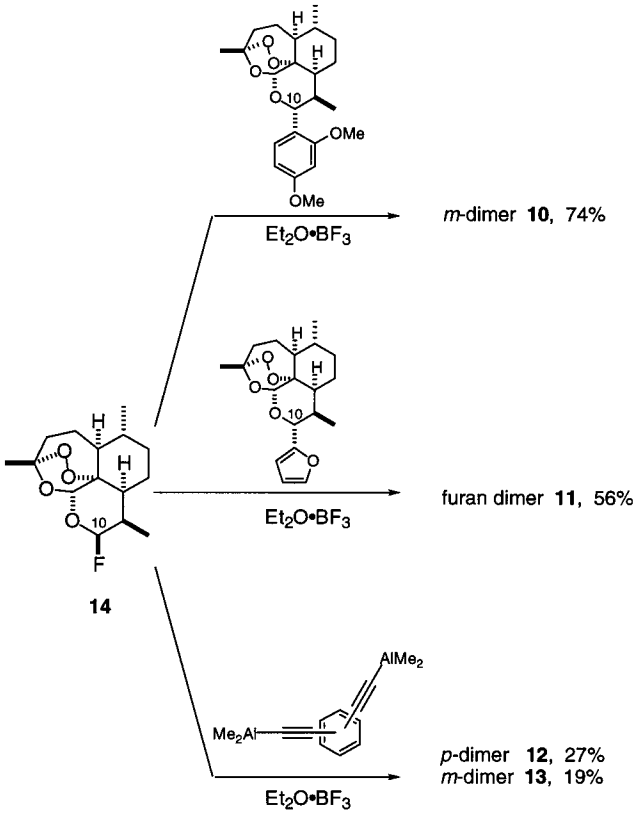


Table 1. C-10 Deoxoartemisinin Dimers **8**−**13**

Dimer	LINKER	Antimalarial IC_{50} (nM)[a]
8	10β −CH$_2$ C(=O) C$_6$H$_4$ C(=O) CH$_2$− 10β	1.9
9	10β −CH$_2$ C(=O) C$_6$H$_4$ C(=O) CH$_2$− 10β	1.9
10	10α C$_6$H$_2$(MeO)$_2$ 10α	1.3
11	10α C$_4$H$_2$O 10α	3.2
12	10β C$_6$H$_4$ 10β	30
13	10β C$_6$H$_4$ 10β	36
Artemisinin (**1**)		9.7 ± 1.8

[a] Antimalarial activity was determined against the chloroquine-sensitive NF54 strain of *P. falciparum* as reported previously.[22] The standard deviation for each set of quadruplicates was an average of 11% (≤ 57%) of the mean. R^2 values for the fitted curves were ≥ 0.992. Artemisinin activity is mean ± standard deviation of concurrent control (n = 6).

not fully understood. Unlike the bis-acetylene dimers **12** and **13**, benzoylmethylene dimers **8** and **9**, aryl dimer **10**, and furan dimer **11** are considerably more potent antimalarial agents (IC_{50} = 1.3−3.2 nM) than natural artemisinin (IC_{50} = 9.7 nM) (Table 1).

Antiproliferative and Antitumor Activities

Antiproliferative activities, measured in vitro using murine keratinocytes as described previously,[23] are shown in Figure 1 for C-10 olefinic *m*-xylylene dimers **7** and in Figure 2 for some of the C-10 saturated dimers **8**−**13**. It is noteworthy that most of these trioxane dimers are considerably more effective at 1 μM concentration than calcitriol (1α,25-dihydroxyvitamin D_3), the hormonally active form of vitamin D_3 that is used clinically as a topical drug to treat psoriasis,[24] a skin disorder characterized by uncontrolled proliferation of cells. However, these trioxane dimers are less effective than calcitriol at nanomolar concentrations.

Growth inhibitory activities at nanomolar to micromolar concentrations, measured in vitro as described previously using a diverse panel of 60 human cancer cell lines in the National Cancer Institute (NCI's) Developmental and Therapeutic Program,[25] indicate that all of our dimers are particularly inhibitory to leukemia cells and some of these dimers are notably active in a few other (e.g. colon 205) cancer cell lines. Furthermore, the NCI COMPARE program[25] evaluated the cell sensitivity profile of our dimers versus the 60-cell line sensitivity profile for all compounds in the NCI database and found that most of our dimers are similar in profile to platinum compounds; such compounds are DNA intrastrand cross-linking agents that inhibit cell replication.[26] Whether this molecular mechanism is the biological basis of action of our dimers, however, remains to be established by appropriate additional experiments in the future.

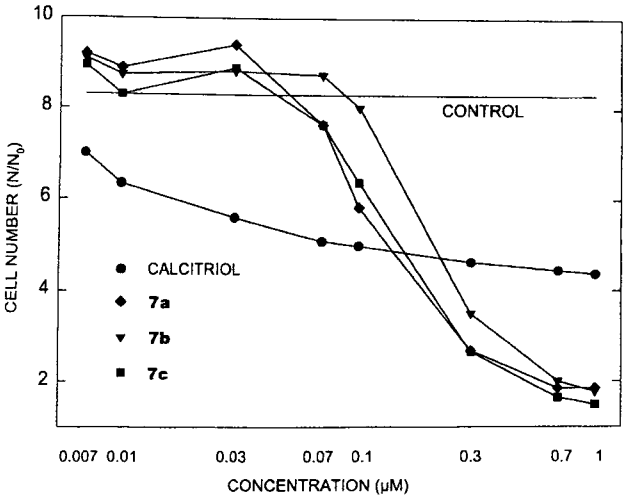


Figure 1. Dose−response effects of analogues on keratinocyte proliferation (96 h).

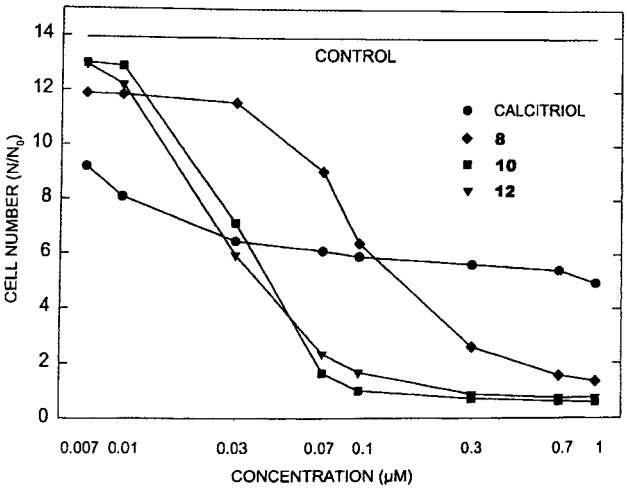


Figure 2. Dose−response effects of analogues on keratinocyte proliferation (96 h).

Preliminary in vivo antitumor evaluation of dimer **8** was performed using the NCI mouse hollow fiber assay. This mouse model involves implanting intraperitoneally (ip) and also subcutaneously (sc) polyvinylidene hollow fibers containing various human cancer cell lines and then administering the dimer via the ip route. The effect of a dimer on diminishing the viable cancer cell mass compared to that of controls was examined. Trioxane dimer **8** substantially diminished cancer cell mass and was as effective at the remote sc implant site as at the ip implant site, suggesting that dimer **8** is potent and stable in this in vivo assay.

In conclusion, the C-10 non-acetal trioxane dimers reported here represent a new series of chemically robust, biologically potent compounds having potential for diverse therapeutic uses. Further study of these new

chemical entities will reveal their mechanism(s) of action and their broad efficacy/safety profile.

Experimental Section

General. Unless otherwise noted: Reactions were run in flame-dried round-bottomed flasks under an atmosphere of ultrahigh purity (UHP) argon. Diethyl ether (ether) and tetrahydrofuran (THF) were distilled from sodium benzophenone ketyl prior to use. Dichloromethane (CH_2Cl_2) and triethylamine (TEA) were distilled from calcium hydride prior to use. All other compounds were purchased from Aldrich Chemical Co. Column chromatography was performed using short path silica gel (particle size < 230 mesh), flash silica gel (particle size 400−230 mesh), or Florisil gel (200 mesh). Analytical thin-layer chromatography (TLC) was performed with silica gel 60 F_{254} plates (250 μm thickness; Merck). Yields are not optimized. Nuclear magnetic resonance (NMR) spectra were obtained using a Varian XL-400 spectrometer, operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C, or a Bruker, operating at 300 MHz for ^{1}H and 75 MHz for ^{13}C. Chemical shifts are reported in parts per million (ppm, δ) downfield from tetramethylsilane. Splitting patterns are described as singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), and broad (br). Infrared (IR) spectra were obtained using a Perkin-Elmer 1600 FT-IR spectrometer. Resonances are reported in wavenumbers (cm^{-1}). Low-resolution (LRMS) and high-resolution (HRMS) mass spectra were obtained on a VG Instruments 70-S spectrometer run at 70 eV for electronic ionization (EI) and run with ammonia (NH_3) as a carrier for chemical ionization (CI). High-performance liquid chromatography (HPLC) was performed using a Rainin HPLX gradient system equipped with two 25 mL/min preparative pump heads using Rainin Dynamax 10-mm × 250-mm (semipreparative) columns packed with 60 Å silica gel (8-mm pore size) as bare silica. Melting points were measured using a Mel-Temp metal-block apparatus and are uncorrected. Combustion analyses were conducted by Atlantic Microlab (Norcross, GA).

General Procedure 1: Synthesis of TMS Enol Ether of Aryl Methyl Ketones. To a solution of aryl methyl ketone (1.0 equiv) in ether (2 mL/mmol of ketone) at 0 °C was added Et_3N (1.1 equiv) via syringe. To this mixture at 0 °C was slowly added trimethylsilyl trifluoromethanesulfonate (TMSOTf; 1.1 equiv) via gastight syringe. The resulting mixture was stirred at 0 °C for 15 min, warmed to room temperature, and stirred for 2 h. Two phases were separated and the ethereal layer was concentrated under reduced pressure.

***m*-Benzene Dimers 7.** *m*-Xylylene bis(triphenylphosphonium bromide) (0.13 g, 0.17 mmol) and aldehyde **6**[20] (0.10 g, 0.34 mmol) in EtOH (1.0 mL) at 0 °C were treated with lithium bis(trimethylsilyl)amide (1.0 M in THF, 0.68 mL, 4.0 equiv). The reaction was stirred for 10 min at 0 °C, then for 10 min at room temperature. The reaction was concentrated, then diluted with ether, washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude product was chromatographed on a flash silica gel column with 10% EtOAc/hexanes as eluent to give the desired product **7** as a mixture of all three possible *Z*/*E* isomers as an oil (0.11 g, 0.17 mmol, 99%), *ZE*:*EE*:*ZZ* = 6:3:2 (as determined by ^{1}H NMR). The three isomers were separated by HPLC (silica semipreparative column, 10% EtOAc/hexanes at 3 mL/min): *ZZ* (t_R = 11 min), *EE*/*EZ* (mixture; t_R = 13 min). Silica semipreparative column, 80% CH_2Cl_2/hexanes at 3 mL/min, gave separation of the *EE*/*EZ*: mixture; *EE* t_R = 9.0 min, *EZ* t_R = 19.0 min; **7a** $[\alpha]^{23}_D = 0$ (c = 0.48, EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.53 (br s, 1 H), 7.22 (m, 3 H), 6.99 (d, J = 15.6 Hz, 2 H), 6.87 (d, J = 15.6 Hz, 2 H), 5.72 (s, 2 H), 2.46−2.36 (m, 2 H), 2.08−1.80 (m, 6 H), 1.87 (s, 6 H), 1.72−1.40 (m, 8 H), 1.46 (s, 6 H), 1.32−1.02 (m, 6 H), 0.99 (d, J = 5.6 Hz, 6 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 142.0, 137.9, 128.5, 127.6, 125.4, 125.1, 119.6, 108.5, 104.4, 90.1, 78.7, 51.0, 47.0, 37.6, 36.2, 34.2, 29.4, 25.8, 24.5, 20.2, 16.1; HRMS (CI, NH_3) *m*/*z* calcd for $C_{40}H_{50}O_8$ (M^+) 658.3506, found 658.3515; **7b** $[\alpha]^{23}_D$ = +77 (c = 0.49, EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.49 (br s, 1 H), 7.44 (m, 1 H), 7.29 (m, 1 H), 7.22 (t, J = 7.6 Hz, 1 H), 6.96 (d, J = 15.6 Hz, 1 H), 6.85 (d, J = 15.6 Hz, 1 H), 6.50 (d, J = 12.0 Hz, 1 H), 6.05 (d, J = 12.0 Hz, 1 H), 5.70 (s, 1 H), 5.65 (s, 1 H), 2.46−2.35 (m, 2 H), 2.08−1.80 (m, 8 H), 1.86 (s, 3 H), 1.74−1.38 (m, 6 H), 1.44 (s, 3 H), 1.41 (s, 3 H), 1.40 (s, 3 H), 1.30−1.02 (m, 6 H), 0.99 (d, J = 5.6 Hz, 3 H), 0.97 (d, J = 6.0 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 142.0, 141.3, 137.5, 137.2, 132.3, 128.2, 128.0, 127.8, 127.2, 125.4, 122.8, 119.3, 108.2, 105.5, 104.4, 104.3, 90.0, 89.9, 78.704, 78.695, 51.1, 51.0, 46.9, 45.9, 37.6, 36.3, 36.2, 34.2, 34.1, 29.4, 29.2, 25.8, 24.5, 20.5, 20.2, 16.5, 16.1; HRMS (CI, NH_3) *m*/*z* calcd for $C_{40}H_{50}O_8$ (M^+) 658.3506, found 658.3508; **7c** $[\alpha]^{23}_D$ = +228 (c = 0.49, EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.50 (br s, 1 H), 7.43 (d, J = 7.6 Hz, 1 H), 7.39 (d, J = 7.6 Hz, 1 H), 7.18 (t, J = 8.0 Hz, 1 H), 6.48 (d, J = 12.0 Hz, 2 H), 5.99 (d, J = 12.0 Hz, 2 H), 5.66 (s, 2 H), 2.46−2.36 (m, 2 H), 2.04 (m, 2 H), 1.92 (m, 4 H), 1.70−1.38 (m, 10 H), 1.41 (s, 6 H), 1.36 (s, 6 H), 1.28−1.02 (m, 4 H), 0.98 (d, J = 5.6 Hz, 6 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 141.4, 136.8, 132.7, 129.8, 128.0, 127.6, 122.5, 105.0, 104.4, 89.9, 78.7, 51.2, 45.9, 37.6, 36.4, 34.1, 29.2, 25.9, 24.5, 20.3, 16.5; HRMS (CI, NH_3) *m*/*z* calcd for $C_{40}H_{50}O_8$ (M^+) 658.3506, found 658.3515.

1,4-Diacetylbenzene Bis-TMS Enol Ether. 1,4-Diacetylbenzene (1.6 g, 10 mmol) was treated according to the general procedure 1. The crude was purified by Kügelrohr distillation to give the desired product (1.8 g, 5.9 mmol, 59%). The mixture was used for the next step without further purification: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.56 (s, 4 H), 4.95 (d, J = 2.0 Hz, 2 H), 4.45 (d, J = 2.0 Hz, 2 H), 0.29 (s, 18 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.3, 137.2, 124.9, 91.3, 0.11; IR (neat) 2960, 1687, 1608, 1314, 1253, 1114, 1011 cm^{-1}.

10-*p*-Acetylphenylartemisinin Dimer 8. With the use of a glovebag under argon, neat $TiCl_4$ (0.12 mL, 1.1 mmol) was added to CH_2Cl_2 (1 mL) at room temperature. To this pale yellow mixture at −78 °C was added a −78 °C solution of β-artemether (**2**) (0.30 g, 1.0 mmol) in CH_2Cl_2 (1.5 mL) via cannula. The resulting mixture was stirred at −78 °C for 5 min. To this mixture at −78 °C was slowly added a −78 °C solution of 1,4-diacetylbenzene bis-TMS enol ether (0.21 g, 0.55 mmol) in CH_2Cl_2 (2.5 mL). The reaction mixture was stirred at −78 °C for 1 h. The mixture was quenched with H_2O (5 mL) and diluted with $CHCl_3$ (5 mL). Two phases were separated and the aqueous phase was extracted with $CHCl_3$ (5 mL × 2). The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude mixture was purified by column chromatography (Florisil, 1%→30% EtOAc/hexanes) to give the product (0.09 g, 0.13 mmol, 26%). Further purification by HPLC (silica, 25% EtOAc/hexanes, 3.0 mL/min, 254 nm, t_R = 18.3 min) provided the desired *p*-diacetyl dimer **8** as a white solid: mp = 87−88 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02 (s, 4 H), 5.33 (s, 2 H), 5.09 (ddd unresolved, 2 H), 3.20 (dABq, J_d = 6.6 Hz, J_{AB} = 16.0 Hz, $\Delta\nu_{AB}$ = 84.3 Hz, 4 H), 2.79 (m, 2 H), 2.31 (ddd, J = 14.4, 13.2, 4.0 Hz, 2 H), 2.00 (ddd, J = 14.6, 4.8, 3.2 Hz, 2 H), 1.93 (m, 2 H), 1.83 (ddd, J = 13.6, 8.0, 4.0 Hz, 2 H), 1.71 (m, 4 H), 1.47−1.23 (m, 10 H), 1.31 (s, 6 H), 0.98 (d, J = 6.0 Hz, 6 H), 0.90 (d, J = 7.6 Hz, 6 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 197.6, 140.0, 128.4, 102.9, 89.6, 80.8, 70.0, 52.0, 44.0, 40.5, 37.5, 36.5, 34.4, 29.9, 25.8, 24.8, 20.1, 13.0; IR ($CHCl_3$) 2956, 2930, 2877, 1686, 1453, 1378, 1359, 1265, 1092, 1053, 1011 cm^{-1}; HRMS (CI, NH_3) *m*/*z* calcd for $C_{40}H_{58}NO_{10}$ ($M + NH_4^+$) 712.4061, found 712.4054.

1,3-Diacetylbenzene Bis-TMS Enol Ether. 1,3-Diacetylbenzene (1.6 g, 10 mmol) was treated according to the general procedure 1. The crude was purified by Kügelrohr distillation to give the desired product (1.8 g, 5.9 mmol, 59%). The mixture was used for the next step without further purification: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83 (m, 1 H), 7.52 (d, J = 2.0 Hz, 1 H), 7.50 (d, J = 2.0 Hz, 1 H), 7.27 (apparent t, J = 7.8 Hz, 1 H), 4.93 (d, J = 1.6 Hz, 2 H), 4.44 (d, J = 1.6 Hz, 2 H), 0.27 (s, 18 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.5, 137.3, 127.8, 125.1, 122.1, 91.2, 0.13; IR (neat) 2962, 1689, 1607, 1312, 1253, 1114, 1011 cm^{-1}.

10-*m*-Acetylphenylartemisinin Dimer 9. To a solution of β-artemether (**2**) (0.12 g, 0.4 mmol) in CH_2Cl_2 (1.2 mL) at

−78 °C was added $TiCl_4$ (1.0 M in CH_2Cl_2, 0.44 mL, 0.44 mmol) via gastight syringe. The resulting mixture was stirred at −78 °C for 5 min. To the mixture was added a −78 °C solution of 1,3-diacetylbenzene bis-TMS enol ether (0.070 g, 0.22 mmol) in CH_2Cl_2 (1.2 mL) via cannula at −78 °C. The reaction mixture was stirred at −78 °C for 1 h. The mixture was quenched with H_2O and diluted with $CHCl_3$. Two phases were separated and the aqueous phase was extracted twice with $CHCl_3$. The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude mixture was purified by column chromatography (Florisil, 1%→30% EtOAc/hexanes) to give the product (0.045 g, 0.065 mmol, 33%). Further purification by HPLC (silica, 25% EtOAc/hexanes, 3.0 mL/min, 254 nm, t_R = 19.7 min) provided the desired *m*-diacetyl dimer **9** as a white solid: mp = 78−79 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.51 (m, 1 H), 8.14 (m, 2 H), 7.57 (m, 1 H), 5.34 (s, 2 H), 5.08 (ddd unresolved, 2 H), 3.22 (dABq, J_d = 6.4 Hz, J_{AB} = 16.4 Hz, $\Delta\nu_{AB}$ = 93.4 Hz, 4 H), 2.81 (m, 2 H), 2.31 (ddd, J = 14.4, 13.2, 4.0 Hz, 2 H), 1.99 (ddd, J = 14.6, 4.8, 3.2 Hz, 2 H), 1.92 (m, 2 H), 1.84 (ddd, J = 13.6, 8.0, 4.0 Hz, 2 H), 1.71 (m, 4 H), 1.47−1.22 (m, 10 H), 1.30 (s, 6 H), 0.97 (d, J = 6.0 Hz, 6 H), 0.91 (d, J = 8.0 Hz, 6 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 197.3, 137.2, 132.4, 128.9, 103.0, 89.4, 80.8, 70.2, 52.0, 44.1, 40.0, 37.5, 36.5, 34.4, 29.9, 25.8, 24.8, 20.1, 13.1; IR ($CHCl_3$) 3007, 2956, 2929, 2877, 1688, 1598, 1452, 1433, 1378, 1359, 1180, 1124, 1092, 1053, 1012 cm^{-1}; LRMS (CI, NH_3, rel intensity) 712 (M + NH_4^+, 1), 620 (28), 446 (37), 400 (97), 383 (100), 369 (28), 365 (95), 284 (30), 270 (28), 222 (14), 183 (17); HRMS (CI, NH_3) *m*/*z* calcd for $C_{40}H_{58}NO_{10}$ (M + NH_4^+) 712.4061, found 712.4069.

Dimethoxyphenyl Dimer 10. 12α-(2′,4′-Dimethoxyphenyl)-10-deoxoartemisinin (0.065 g, 0.16 mmol) and 10β-fluoro-10-deoxoartemisinin (**14**; 0.023 g, 0.08 mmol) were dissolved in dry dichloromethane (0.8 mL) and the solution was cooled to −78 °C. Boron trifluoride diethyl etherate (14 mL, 0.11 mmol) was added and the reaction was stirred at −78 °C for 1 h. The solution was warmed to −40 °C over 1 h. After being stirred at this temperature for an additional 4 h, the reaction was quenched with saturated aqueous sodium bicarbonate (1 mL). The organic phase was separated, and the aqueous phase was extracted with dichloromethane (2 × 10 mL). The organic portion was combined, washed with water, dried with magnesium sulfate, and concentrated under reduced pressure. Purification by column chromatography on silica gel gave the product (0.04 g, 0.059 mmol, 74%). Further purification by HPLC (silica, 30% EtOAc/hexanes, 3.0 mL/min, 254 nm, t_R = 8.8 min) provided the desired dimer **10** as a white solid: mp = 168.0−169.2 °C; $[\alpha]_D^{25}$ = +148.3 (c = 0.89, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.91 (br s, 1 H), 6.31 (s, 1 H), 5.39 (s, 2 H), 4.91 (d, J = 9.9 Hz, 2 H), 3.76 (s, 6 H), 2.56 (m, 2 H), 2.33 (m, 2 H), 1.99 (m, 2 H), 1.89−0.88 (m, 18 H), 1.43 (s, 6 H), 0.95 (d, J = 6.2 Hz, 6 H), 0.58 (d, J = 7.0 Hz, 6 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 157.1, 128.8, 122.8, 104.4, 94.2, 92.4, 80.6, 70.0, 56.1, 52.5, 46.8, 37.8, 36.9, 34.8, 26.4, 25.3, 22.0, 20.8, 13.9; IR (KBr) 2934, 2868, 1608, 1509, 1457, 1377, 1294, 1201, 1068, 883, 847 cm^{-1}. Anal. Calcd for $C_{18}H_{54}O_{10}$: C, 68.03; H, 8.11. Found: C, 67.88; H, 8.11.

Furan Dimer 11. 10α-(2′-Furyl)-10-deoxoartemisinin (0.022 g, 0.066 mmol) and 10β-fluoro-10-deoxoartemisinin (**14**; 0.019 g, 0.066 mmol) were dissolved in dry dichloromethane (1 mL) and the solution was cooled to −78 °C. Boron trifluoride diethyl etherate (0.011 g, 10 mL, 0.079 mmol) was added and the reaction was warmed to −50 °C and kept at −50 °C for 4 h. Saturated aqueous sodium bicarbonate (1 mL) was added. The solution was extracted with dichloromethane (3 × 2 mL). The combined organic solution was dried with magnesium sulfate, concentrated under vacuum, and chromatographed on Florisil to give the product (0.022 g, 0.037 mmol, 56%). Further purification by HPLC (silica, 1% *i*-PrOH/CH_2Cl_2, 3.0 mL/min, 236 nm, R_t = 5.0 min) provided the furan dimer **11** as a white foam: $[\alpha]_D^{25}$ = +56.8 (c = 1.20, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.33 (s, 2 H), 5.35 (s, 2 H), 4.46 (d, J = 10.8 Hz, 2 H), 2.80−2.70 (m, 2 H), 2.37 (ddd, J = 14.4, 13.6, 4.0 Hz, 2 H), 2.01 (ddd, J = 14.4, 4.8, 2.8 Hz, 2 H), 1.88 (m, 2 H), 1.73 (m, 4 H), 1.40 (s, 6 H), 1.60−1.00 (m, 12 H), 0.96 (d, J = 6.0 Hz, 6 H), 0.66 (d, J = 7.2 Hz, 6 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 152.9, 108.6, 104.1, 92.1, 80.4, 71.2, 52.0, 45.9, 37.3, 36.3, 34.1, 32.0, 26.0, 24.7, 21.3, 20.3, 13.8; IR ($CHCl_3$) 2926, 2873, 1452, 1377, 1197, 1127, 1042, 926, 879, 754 cm^{-1}; HRMS (CI, NH_3) *m*/*z* calcd for $C_{34}H_{52}NO_9$ (M + NH_4^+) 618.3642, found 618.3650.

1,4-Diethynylbenzene. To a solution of lithium diisopropylamide (LDA; Aldrich; 1.5 M, 8.7 mL, 13 mmol) in THF (55 mL) at −78 °C was added a solution of 1,4-diacetylbenzene (1.0 g, 6.2 mmol) in THF (10 mL) via syringe. The resulting mixture was stirred at −78 °C for 1 h. To this mixture was added diethyl chlorophosphate (2.0 mL, 14 mmol) via syringe. The resulting dark brown mixture was stirred at −78 °C for 5 min, slowly warmed to room temperature, and stirred for 15 min. This orange mixture was cooled to −78 °C and added into a −78 °C solution of LDA (Aldrich; 1.5 M, 19 mL, 28 mmol) in THF (55 mL) via cannula. The resulting dark blue-green mixture was stirred at −78 °C for 40 min, slowly warmed to room temperature, and stirred for 3.5 h. At this time, TLC analysis indicated complete consumption of starting material diketone. The reaction was quenched with a HCl solution (1 M, 3 mL) and diluted with ether (20 mL). Two layers were separated and the aqueous phase was extracted with ether (30 mL × 2). The combined organic layers were washed with a HCl solution (1 M, 10 mL) and a saturated $NaHCO_3$ solution (10 mL), dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography (flash, 1% EtOAc/hexanes) to furnish the desired product (0.60 g, 4.76 mmol, 77%) as a white solid: mp = 90.0−91.5 °C (lit. mp 96.5 °C); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 (s, 4 H), 3.17 (s, 2 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 132.0, 122.5, 83.0, 79.1. These spectroscopic data matched those reported previously.[27−29]

10-*p*-Diethynylphenylartemisinin Dimer 12. To a solution of 1,4-diethynylbenzene (0.12 g, 0.97 mmol) in ether (1 mL) at 0 °C was added *n*-butyllithium (1.6 M, 1.3 mL, 2.0 mmol). The resulting mixture was stirred at 0 °C for 45 min. To this mixture at 0 °C was slowly added dimethylaluminum chloride (1.0 M, 2.0 mL, 2.0 mmol) via syringe and the resulting suspension was stirred at 0 °C for 2 h. To the reaction mixture at −78 °C was added boron trifluoride diethyl etherate (0.26 mL, 2.0 mmol) and a solution of β-fluoroartemisinin (**14**) (0.58 g, 2.0 mmol) in CH_2Cl_2 (20 mL). The resulting reaction mixture was stirred at −78 °C for 20 min, slowly warmed to −50 °C, and stirred for 3 h. The reaction was quenched with H_2O (5 mL) and diluted with $CHCl_3$ (10 mL). Two layers were separated and the aqueous phase was extracted with $CHCl_3$ (20 mL × 2). The combined organic layers were washed with brine (20 mL), dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography (Florisil, 10%→20% EtOAc/hexanes) to furnish the product (0.18 g, 0.27 mmol, 27%). Further purification by HPLC (silica, 15% EtOAc/hexanes, 3.0 mL/min, 254 nm, t_R = 14.1 min) provided the desired *p*-diethynyl dimer **12** as a white solid: mp = 164.5−166.0 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34 (s, 4 H), 5.62 (s, 2 H), 4.96 (d, J = 5.6 Hz, 2 H), 2.85 (m, 2 H), 2.38 (ddd, J = 14.4, 13.6, 4.0 Hz, 2 H), 2.19 (apparent dq, J_d = 3.3 Hz, J_q = 13.6 Hz, 2 H), 2.06 (m, 2 H), 1.92−1.78 (m, 6 H), 1.70−1.51 (m, 6 H), 1.45 (s, 6 H), 1.42−1.25 (m, 4 H), 1.04 (d, J = 7.6 Hz, 6 H), 0.96 (d, J = 6.4 Hz, 6 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 131.4, 122.7, 104.3, 89.6, 88.9, 88.3, 80.9, 67.8, 52.7, 45.4, 37.4, 36.3, 34.6, 30.3, 26.1, 24.6, 23.0, 20.3, 13.9; IR ($CHCl_3$) 2999, 2956, 2928, 2875, 2854, 1508, 1452, 1379, 1369, 1042 cm^{-1}; FAB-MS 659 (M + H^+), 658 (M^+), 307, 209, 182, 169, 165, 154, 136, 120, 107, 89, 77, 65, 43; HRMS (CI, NH_3) *m*/*z* calcd for $C_{40}H_{51}O_8$ (M + H^+) 659.3584, found 659.3594.

10-*m*-Diethynylphenylartemisinin Dimer 13. To a solution of 1,3-diethynylbenzene (TCI; 0.078 g, 0.62 mmol) in ether (0.6 mL) at 0 °C was added *n*-butyllithium (1.6 M, 0.75 mL, 1.2 mmol). The resulting mixture was stirred at 0 °C for 45 min. To this mixture at 0 °C was slowly added dimethylaluminum chloride (1.0 M, 1.2 mL, 1.2 mmol) via syringe and the resulting suspension was stirred at 0 °C for 2 h. To the reaction

mixture at −78 °C was added boron trifluoride diethyl etherate (0.17 mL, 1.3 mmol) and a solution of β-fluoroartemisinin (**14**) (0.38 g, 1.3 mmol) in CH_2Cl_2 (12 mL). The resulting reaction mixture was stirred at −78 °C for 20 min, slowly warmed to −50 °C, and stirred for 3 h. The reaction was quenched with H_2O (5 mL) and diluted with $CHCl_3$ (10 mL). Two layers were separated and the aqueous phase was extracted with $CHCl_3$ (20 mL × 2). The combined organic layers were washed with brine (20 mL), dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography (Florisil, 10%→20% EtOAc/hexanes) to furnish the product (0.08 g, 0.12 mmol, 19%). Further purification by HPLC (silica, 15% EtOAc/hexanes, 3.0 mL/min, 254 nm, $t_R = 14.2$ min) provided the desired *m*-diethynyl dimer **13** as a white foam: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.42−7.24 (m, 4 H), 5.62 (s, 2 H), 4.96 (d, $J = 5.6$ Hz, 2 H), 2.85 (m, 2 H), 2.38 (ddd, $J = 14.4$, 13.6, 4.0 Hz, 2 H), 2.19 (apparent dq, $J_d = 3.3$ Hz, $J_q = 13.6$ Hz, 2 H), 2.05 (m, 2 H), 1.92−1.78 (m, 6 H), 1.70−1.51 (m, 6 H), 1.45 (s, 6 H), 1.42−1.25 (m, 4 H), 1.04 (d, $J = 7.3$ Hz, 6 H), 0.96 (d, $J = 6.2$ Hz, 6 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 134.2, 131.4, 128.5, 123.0, 104.3, 89.6, 87.8, 87.7, 80.9, 67.8, 52.7, 45.4, 37.4, 36.3, 34.6, 30.3, 26.1, 24.6, 23.0, 20.3, 13.9; IR ($CHCl_3$) 2925, 2869, 1458, 1372, 1189, 1137, 1050 cm^{-1}.

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Supporting Information Available: Figures 3 and 4 as bar graphs indicating total growth inhibition (TGI) for dimers **7**, **8**, **10**, and **12** in the NCI 60-human cell line in vitro assay. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Current address: Johnson & Johnson, Spring House, PA. (b) Current address: MethylGene, Inc., Montreal, Quebec. (c) Recipient of a 1998 Pfizer Undergraduate Summer Fellowship.
(2) Chen, Y.-H.; Liu, J.-X.; Lown, J. W. Design, Synthesis and Evaluation of Novel Bismustard Cross-linked Lexitropsins. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 2223−2228.
(3) Drögemüller, M.; Flessner, T.; Jautelat, R.; Scholz, U.; Winterfeldt, E. Synthesis of Cephalostatin Analogues by Symmetrical and Non-Symmetrical Routes. *Eur. J. Org. Chem.* **1998**, 2811−2831.
(4) Reddy, B. S. P.; Damayanthi, Y.; Lown, J. W. Design and Efficient Synthesis of Novel DNA Interstrand Cross-Linking Agents: C2−Linked Pyrrolo[2,1-*c*][1,4]benzodiazepine Dimers. *Synlett* **1999**, *7*, 1112−1114.
(5) Gregson, S. J.; Howard, P. W.; Jenkins, T. C.; Kelland, L. R.; Thurston, D. E. Synthesis of a Novel C2/C2′-*exo* Unsaturated Pyrrolobenzodiazepine Cross-linking Agent With Remarkable DNA Binding Affinity and Cytotoxicity. *J. Chem. Soc., Chem. Commun.* **1999**, 797−798.
(6) Borman, S. Higher Yield Synthetic Route to Cyclic Enediynes Developed. *Chem. Eng. News* **1995**, 28−30.
(7) Beekman, A. C.; Barenstsen, A. R. W.; Woerdenbag, H. J.; Van Uden, W.; Pras, N. Stereochemistry-Dependent Cytotoxicity of Some Artemisinin Derivatives. *J. Nat. Prod.* **1997**, *60*, 325−330.
(8) Galal, A. M.; Ahmad, M. S.; El-Feraly, F. S. Preparation and Characterization of a New Artemisinin-Derived Dimer. *J. Nat. Prod.* **1996**, *59*, 917−920.
(9) Venugopalan, B.; Bapat, C. P.; Karnik, P. J.; Catterjee, D. K.; Iyer, N.; Lepcha, D. Antimalarial Activity of Novel Ring-Contracted Artemisinin Derivatives. *J. Med. Chem.* **1995**, *38*, 1922−1927.
(10) Posner, G. H.; Ploypradith, P.; Hapangama, W.; Wang, D.; Cumming, J. N.; Dolan, P.; Kensler, T. W.; Klinedinst, D.; Shapiro, T. A.; Zheng, Q. Y.; Murray, C. K.; Pilkington, L. G.; Jayasinghe, L. R.; Bray, J. F.; Daughenbaugh, R. Trioxane Dimers Have Potent Antimalarial, Antiproliferative, and Antitumor Activities In Vitro. *Bioorg. Med. Chem.* **1997**, *5*, 1257−1265.
(11) Aboubdellah, A.; Bégué, J.-P.; Bonnet-Delpon, D.; Gontier, J.-C.; Nga, T. T.; Thac, T. D. Synthesis and in vivo Antimalarial Activity of 12α-Trifluoromethyl-hydroartemisinin. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2717−2720.
(12) Pu, Y. M.; Ziffer, H. Synthesis and Antimalarial Activities of 12β-Allyldeoxoartemisinin. *J. Med. Chem.* **1995**, *38*, 613−616.
(13) Vroman, J. A.; Khan, I. A.; Avery, M. A. Copper(I)-Catalyzed Conjugate Addition of Grignard Reagents to Acrylic Acids: Homologation of Artemisinic Acid and Subsequent Conversion to 9-Substituted Artemisinin Analogues. *Tetrahedron Lett.* **1997**, *38*, 6173−6176.
(14) Avery, M. A.; Alvim-Gaston, M.; Woolfrey, J. R. Synthesis and Structure−Activity Relationships of Peroxidic Antimalarials Based on Artemisinin. *Adv. Med. Chem.* **1999**, *4*, 125−217.
(15) Jung, M.; Lee, S. Synthesis and Cytotoxicity of Novel Artemisinin Analogues. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 1091−1094.
(16) Jung, M.; Lee, S. Stability of Acetal and Nonacetal Type Analogues of Artemisinin in Simulated Stomach Acid. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 1003−1006.
(17) Haynes, R. K.; Vonwiller, S. C. Efficient Preparation of Novel Qinghaosu (Artemisinin) Derivatives. *Synlett* **1992**, 481−483.
(18) Woo, S. H.; Parker, M. H.; Ploypradith, P.; Northrop, J.; Posner, G. H. Direct Conversion of Anomeric OH → F → R in the Artemisinin Family of Antimalarial Trioxanes. *Tetrahedron Lett.* **1998**, *39*, 1533−1536.
(19) Posner, G. H.; Parker, M. H.; Northrop, J.; Elias, J. S.; Ploypradith, P.; Xie, S.; Shapiro, T. A. Orally Active, Hydrolytically Stable, Semi-synthetic, Antimalarial Trioxanes in the Artemisinin Family. *J. Med. Chem.* **1999**, *42*, 300−304.
(20) O'Dowd, H.; Ploypradith, P.; Xie, S.; Shapiro, T. A.; Posner, G. H. Antimalarial Artemisinin Analogues. Synthesis via Chemoselective C−C Bond Formation and Preliminary Biological Evaluation. *Tetrahedron* **1999**, *55*, 3625−3636.
(21) Campbell, T. W.; McDonald, R. N. Synthesis of Hydrocarbon Derivatives by the Wittig Synthesis. I. Distyrylbenzenes. *J. Org. Chem.* **1959**, *24*, 1246−1251.
(22) For experimental details of the testing protocol, including references to development of the assay, see: Posner, G. H.; González, L.; Cumming, J. N.; Klinedinst, D.; Shapiro, T. A. Synthesis and Antimalarial Activity of Heteroatom-Containing Bicyclic Endoperoxides. *Tetrahedron* **1997**, *53*, 37−50.
(23) Posner, G. H.; Nelson, T. D.; Guyton, K. Z.; Kensler, T. W. New Vitamin D_3 Derivatives Having Unexpected Antiproliferative Activity: 1-Hydroxymethyl-25-hydroxyvitamin D_3 Homologues. *J. Med. Chem.* **1992**, *35*, 3280−3287.
(24) Holick, M. F., Ed. *Vitamin D*; Humana Press: Totowa, NJ, 1999.
(25) Boyd, M. R.; Paull, K. D. Some Practical Considerations and Applications of the National Cancer Institute In Vitro Anticancer Drug Discovery Screen. *Drug Dev. Res.* **1995**, *34*, 91−109.
(26) Zipp, A. P.; Zipp, S. G. $Pt(NH_3)_2Cl_2$ and Cancer. *J. Chem. Educ.* **1977**, *54*, 739−741.
(27) Negishi, E.; King, A. O.; Klima, W. L. Conversion of Methyl Ketones into Terminal Acetylenes and (*E*)-Trisubstituted Olefins of Terpenoid Origin *J. Org. Chem.* **1980**, *45*, 2526−2528.
(28) Hay, A. S. Preparation of *m*- and *p*-Diethynylbenzenes. *J. Org. Chem.* **1960**, *25*, 637.
(29) Royles, B. J. L.; Smith, D. M. J. The 'Inverse Electron-demand' Diels−Alder Reaction in Polymer Synthesis. Part 1. A Convenient Synthetic Route to Diethynyl Aromatic Compounds. *J. Chem. Soc., Perkin Trans 1* **1994**, 355−358.

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