Malaria-Infected Mice Are Cured by a Single Oral Dose of New Dimeric Trioxane Sulfones Which Are Also Selectively and Powerfully Cytotoxic to Cancer Cells

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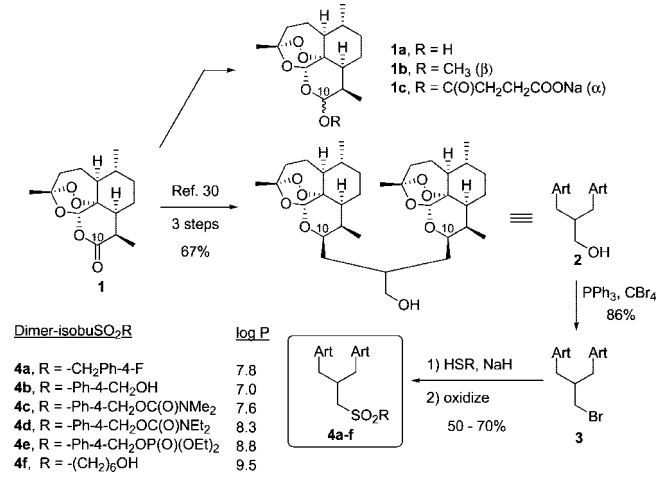
A new series of 6 dimeric trioxane sulfones has been prepared from the natural trioxane artemisinin in five or six chemical steps. One of these thermally and hydrolytically stable new chemical entities (**4c**) completely cured malaria-infected mice via a single oral dose of 144 mg/kg. At a much lower single oral dose of only 54 mg/kg combined with 13 mg/kg of mefloquine hydrochloride, this trioxane dimer **4c** as well as its parent trioxane dimer **4b** also completely cured malaria-infected mice. Both dimers **4c** and **4b** were potently and selectively cytotoxic toward five cancer cell lines.

Introduction

Even in 2009, one child dies of malaria approximately every 30 seconds.[1−3] No vaccine is yet available to prevent malaria infection in humans.[4] Chemotherapy of malaria-infected patients has been used broadly with great success. Now, unfortunately, there is widespread malaria parasite resistance to many of the standard amine antimalarial drugs like chloroquine.[5] During the past few decades,[6−13] a new, nonamine class of antimalarial 1,2,4-trioxanes has emerged from traditional Chinese herbal remedies. Natural 1,2,4-trioxane artemisinin (**1**) and its semisynthetic daughter trioxanes dihydroartemisinin (**1a**), artemether (**1b**), and water-soluble sodium artesunate (**1c**) are the fastest-acting antimalarial drugs known.[6−13] They are now often combined, as recommended by the World Health Organization (WHO), with standard antimalarial amines like lumefantrine or amodiaquine; such artemisinin combination therapy (ACT[a]), the current chemotherapeutic method of choice where malaria is endemic, requires a repeated dosing regimen.[14−17] For example, a 1:6 fixed combination of the trioxane artemether and lumefantrine is curative in a three-day six-dose regimen.[18] A single dose cure would involve better patient compliance and lower cost. Toward this challenging and urgent goal, we have reported a series of trioxane dimers able to cure malaria-infected mice after only a single subcutaneous dose[19] as well as a related series of trioxane dimers curative after three oral doses.[20−22] Such trioxane dimers are significantly more efficacious in curing malaria-infected mice than twice the dose of the corresponding trioxane monomers, as well as being significantly more cytotoxic than the corresponding monomeric trioxanes toward cancer cells.[20−22] Here, as proof of principle, we describe a new series of dimeric trioxane sulfones **4** (Scheme 1), two of which are curative after only a single oral dose to malaria-infected mice.

Scheme 1



Some of these new dimeric trioxane sulfones **4** also have selective and potent anticancer activity.[23] Increasingly widespread evidence indicates that human cancer cells, richer than normal cells in iron-transport transferrin receptors,[24,25] selectively activate trioxanes to produce various cytotoxic intermediates; this process is similar to that in the triggering of trioxanes by heme iron in malaria-infected human erythrocytes.[8] The anticancer properties of trioxanes have been reviewed.[26−29] Here we disclose that some of the new dimeric trioxane sulfones **4** powerfully inhibit the growth (submicromolar IC_{50} values) of various cancer cells in vitro without strongly affecting noncancerous fibroblasts.

Results and Discussion

Chemistry. As outlined in Scheme 1, trioxane dimer primary alcohol **2** was prepared in three steps from natural artemisinin,

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[a] Abbreviations: ACT, artemisinin combination therapy; *m*CPBA, *meta*-chloroperbenzoic acid; DMSO, dimethyl sulfoxide; MTS, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium; SRB, sulforhodamine B; DMF, dimethyl formamide; NaH, sodium hydride; EtOAc, ethyl acetate; LAH, lithium aluminum hydride; DMDO, dimethyldioxirane.

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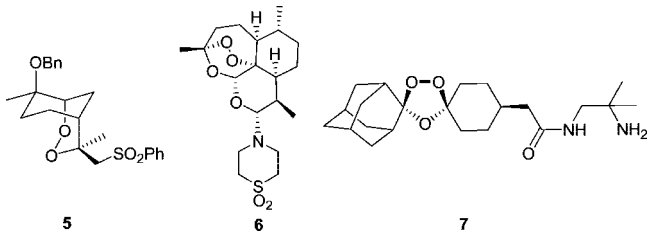
Table 1. Antimalarial Efficacy Using a Single Oral Dose of Dimeric Trioxane Sulfones **4** in *P. berghei*-Infected Mice

dimeric trioxane sulfone	average survival (days) after infection; trioxane dose (mg/kg)			% suppression of parasitemia (on day 3 post infection)		
	1 × 54	1 × 72	1 × 144	1 × 54	1 × 72	1 × 144
4a	14.6	–	–	97.1	–	–
4b	20.4 (11, 12, 19, 30, 30)[a]	25.0 (12, 25, 27, 30, 30)[a]	30.0 (2 sick, 3 cured)	99.8	99.6	>99.9
4c	22.8 (11, 24, 26, 26, 27)[a]	23.0 (11, 14, 30, 30, 30)[a]	**30.0 (all cured)**	99.5	98.6	99.3
4d	16.4	–	–	99.2	–	–
4e	14.8	–	–	92.4	–	–
4f	11.0	–	–	2.0	–	–
vehicle (no drug)	–	6.4 (6, 6, 6, 7, 7)[a]	–	–	–	–
1b	11 (8, 10, 11, 13, 13)[a]	9.8 (9, 9, 10, 10, 11)[a]	12.4 (12, 12, 12, 13, 13)[a]	99.4	98.7	99.8
1c	–	7.6[b]	–	–	–	–
7	–	10.7[b]	–	–	99.95[b]	–

[a] Actual mouse survival until day. [b] Data from Supporting Information in ref 39 using a single oral dose of 30 mg/kg. A dash in the table means not tested.

as described previously.[30] Primary bromide **3** was easily prepared in good yield by treating alcohol **2** with CBr_4 and Ph_3P.[31] Displacement of the bromide anion by a mercaptide anion, followed by sulfide→sulfone oxidation with *meta*-chloroperbenzoic acid (*m*CPBA) proceeded also in good yields without disruption of the crucial peroxide pharmacophore. This is an especially noteworthy result because most peroxides are cleaved by mercaptide anions. Jung and co-workers reported the first example of mercaptide displacement of a trioxane primary bromide leading to a dimeric trioxane sulfone.[22] We prepared dimeric trioxane sulfones **4a**−**f** from natural artemisinin in 5−6 chemical steps and good overall yields. The calculated octanol/water partition coefficient (log P) of these sulfone dimers ranged from 7.0 to 9.5. Scale up to multigram synthesis and even to kilogram manufacture of sulfones **4** is not expected to be a problem. Some endoperoxide sulfones like synthetic bicyclic sulfone **5**[32] and semisynthetic sulfone **6**[33] among others[34−37] have excellent antimalarial activities. The extraordinary antimalarial efficacy of dimeric trioxane sulfone benzylic alcohol **4b** as well as the chemical versatility of its primary hydroxyl group prompted us to prepare the corresponding carbamates **4c** and **4d** and phosphate **4e** as potential prodrugs. It was expected that, in vivo, esterase enzymes would convert carbamates **4c** and **4d** and phosphate **4e** back into their parent alcohol **4b**. Benzylic alcohol **4b** and dimethyl carbamate **4c** are stable in the solid state at 60 °C for ≥24 h; carbamate **4c** is stable in the solid state at 60 °C even for one week. Both dimers **4b** and **4c** are stable for at least 12 h at room temperature in 80/20 DMSO/water at pH 7.4.



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Biology. Each trioxane dimer **4a**−**4f** (7.2 mg) was dissolved in 0.11 mL of 7:3 Tween 80:ethanol and then diluted with 1.10 mL of water for oral administration to 5-week old C57BL/6J male mice (from the Jackson Laboratory) weighing about 22 g that were infected intraperitoneally on day 0 with the *Plasmodium berghei*, ANKA strain (2×10^7 parasitized erythrocytes).[38] Each of five mice in a group was treated orally with a single dose of 0.20 mL (0.20 mL/1.21 mL × 7.2 mg = 1.2 mg) of diluted compound solution, corresponding to a dose of 54 mg/kg, 24 h postinfection. In separate experiments, a single oral dose of 72 mg/kg and a single oral dose of 144 mg/kg were also used. Blood parasitemia levels as well as monitoring the duration of animal survival compared to survival time of animals receiving no drug are both widely accepted as a measure of a drug's efficacy in antimalarial drug development. Three days after infection, an average of 16.2% blood parasitemia was observed in the control (no drug) group. Animals receiving no drug die typically 6−8 days postinfection. A widely accepted yardstick of cure (i.e., 100% efficacy) is survival of animals to day 30 postinfection, with no detectable malaria parasites in the animal's blood at that time. Average survival results are summarized in Table 1. The clinically used monomeric trioxane drugs **1b** and **1c** and the synthetic trioxolane peroxide drug development candidate OZ277 maleate (**7**) are included as standards.

It is clear from the data in Table 1 that all of the dimeric trioxane sulfones **4a**−**4f** prolonged average survival time at least as effectively as trioxolane **7**, which is in phase II clinical trials.[39] It is also apparent from the data in Table 1 that the dimeric trioxane sulfones **4b** and **4c**, at a single oral dose of 54 mg/kg, were the most efficacious among sulfones **4a**−**4f** at prolonging survival. Therefore, sulfones **4b** and **4c** were tested further using a higher single oral dose of 72 mg/kg and separately using an even higher single oral dose of 144 mg/kg. As shown in Table 1, dimeric sulfones **4b** and **4c** at a single oral dose of 72 mg/kg prolonged average survival to days 23−25. At a higher oral dose, if 144 mg/kg, the benzyl alcohol sulfone **4b** prolonged average survival to day 30, but two of the five mice had 7−9% parasitemia at day 30 and appeared sick. In contrast, carbamate sulfone **4c** at a single oral dose of 144 mg/kg completely cured all of the malaria-infected mice with no adverse effects. Using the same experimental protocol but combining benzylic alcohol dimer **4b** (40 mg/kg) with mefloquine hydrochloride (13 mg/kg) in a single oral dose caused a 99.9% suppression of parasitemia on day 3 post infection and raised average survival to 28 days; a slightly higher single oral dose of dimer **4b** (54 mg/kg) along with mefloquine hydrochloride (13 mg/kg) caused >99.9% parasitemia suppression on day 3 post infection and completely cured the mice. Similar curative results were obtained by combining sulfone carbamate dimer **4c** (54 mg/kg) with mefloquine (13 mg/kg). In a control experiment, a single oral dose of mefloquine hydrochloride (13 mg/kg) alone prolonged average survival to only day 11. Neither overt toxicity nor behavioral change attributable to trioxane drug administration was observed in any of the malaria-infected animals cured by carbamate **4c** or cured by the dimer plus mefloquine hydrochloride combination. The standard monomeric trioxane antimalarial drugs **1b** and **1c** and the trioxolane drug candidate

Table 2. Anticancer Activity of Dimeric Trioxane Sulfones **4b** and **4c** in Human Cancer Cell Lines

cancer cell line	IC_{50} values (μM)[a] dimer **4b**	dimer **4c**
U-937-lymphoma	0.28 ± 0.15	0.28 ± 0.13
HL-60-leukemia	0.13 ± 0.09	0.06 ± 0.02
SK-MEL-5-melanoma	0.14 ± 0.09	0.06 ± 0.03
UACC-62-melanoma	0.03 ± 0.01	0.04 ± 0.02
HeLa-cervical	1.1 ± 0.95	0.11 ± 0.06
control: WT-MEF noncancerous mouse embryonic fibroblasts	>50	>50
Hs888Lu noncancerous human lung fibroblasts	>50	>50

[a] Average of at least three independent experiments with standard error of the mean. Compound toxicity was determined by the MTS assay (for U-937 and HL-60) or the SRB assay (for all other cell lines) after 72 h of cell treatment with the trioxane dimer as described in the experimental section. The IC_{50} value is the concentration, at which 50% of the cells are no longer viable (MTS assay), or where 50% of the cell biomass has been reduced (for the SRB assay).

7, although able to lower parasitemia levels considerably by day 3 post infection, were not efficacious in prolonging the average survival time beyond day 11.

Table 2 summarizes the in vitro cytotoxic activity of dimeric trioxane sufones **4b** and **4c** in five different cancer cell lines. The IC_{50} values are shown as an average of at least three experiments with standard error of the mean using a standard MTS assay or sulforhodamine B (SRB) assay. Clearly, both dimers **4b** and **4c** are strongly cytotoxic, often comparing well with the anticancer potency of doxorubicin, a clinically used anticancer drug.[40] Doxorubicin, however, has serious side effects in humans.[41] In this study, we have found that doxorubicin is toxic toward the WT-MEF and Hs888Lu noncancerous immortalized fibroblast cell lines (with IC_{50} values of 3.4 ± 1.3 μM and 1.4 ± 0.7 μM, respectively). Neither dimer **4b** or **4c** significantly affected these noncancerous fibroblast cell lines (see Table 2). This selective cytotoxicity of both dimers **4b** and **4c** is of special importance for potential anticancer drug development of this class of dimeric trioxane sulfones.

In conclusion, at only a single oral dose of 144 mg/kg, dimeric trioxane sulfone carbamate **4c** completely cured malaria-infected mice. Combining a much lower amount (54 mg/kg) of dimer alchol **4b** or of dimer carbamate **4c** with a small amount of mefloquine hydrochloride (13 mg/kg) in a single oral dose also completely cured the malaria-infected mice. Both dimers **4b** and **4c** have powerful and selective anticancer activities. This desirable combination[42] of high antimalarial activity and high anticancer activity encourages further lead optimization and preclinical drug development of trioxane dimer sulfones like **4b** and **4c**.

Experimental Section

Synthesis of Dimer Primary Bromide 3. Dimeric trioxane primary alcohol[30] **2** (76 mg, 0.13 mmol) was dissolved in CH_2Cl_2 (4 mL) under argon. Triphenylphosphine (49 mg, 0.19 mmol) and carbon tetrabromide (62 mg, 0.19 mmol) were added to the solution at room temperature and allowed to stir overnight. After 18 h, the reaction was quenched with distilled water (2 mL). The aqueous layer was extracted with CH_2Cl_2 (2 × 3 mL) and washed with brine (3 mL). The organic layer was dried with $MgSO_4$, filtered, and concentrated in vacuo. The crude product was purified by flash silica gel column chromatography (0 → 20% EtOAc/hexanes) to yield dimeric trioxane bromide **3** as an amorphous white solid (72 mg, 0.11 mmol, 86%). $[\alpha]_D^{21.9}$ +70.8° (c = 0.56, $CHCl_3$). IR (thin film) 2951, 2875, 1451, 1377, 1252, 1206, 1119, 1055, 1007, 942, 879, 735 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 5.37 (s, 1H), 5.30 (s, 1H), 4.41−4.37 (m, 1H), 4.24−4.20 (m, 1H), 3.91−3.88 (dd, J_1 = 10 Hz, J_2 = 5.0 Hz, 1H), 3.82−3.78 (dd, J_1 = 10 Hz, J_2 = 5.0 Hz, 1H), 2.73−2.68 (q, 1H), 2.62−2.57 (q, 1H), 2.36−2.27 (m, 2H), 2.19−2.17 (m, 1H), 2.04 (m, 1H), 2.00 (m, 1H), 1.91−1.23 (m, 27H including singlets for 3H each at 1.41 and 1.39), 0.99−0.95 (m, 7H), 0.88−0.86 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 103.3, 102.9, 89.6, 89.0, 81.2, 81.2, 74.7, 70.6, 52.5, 52.1, 44.6, 44.1, 40.7, 37.5, 37.4, 36.7, 36.6, 36.2, 34.5, 34.4, 32.1, 31.9, 30.6, 30.5, 26.1, 26.1, 24.9, 24.9, 24.8, 24.7, 20.3, 20.1, 13.3, 12.7. HRMS (ESI) m/z calcd for $C_{34}H_{53}BrO_8Na$ $(M + Na)^+$ 691.2816, found 691.2797.

Synthesis of Dimeric Fluorobenzylic Sulfone 4a. Bis-trioxane bromide **3** (10 mg, 0.015 mmol) was dissolved in DMF (0.5 mL) under argon. To the solution was added 4-fluorobenzyl mercaptan (2 μL, 0.018 mmol) and NaH (0.5 mg, 0.018 mmol) consecutively and the reaction was heated at 90 °C for 1 h. (Note: the reaction went from colorless to pink almost instantly upon heating. The pink color faded to a very light yellow over the course of the hour.) The reaction was allowed to cool and was diluted with EtOAc (1 mL). The organic layer was then washed with ice-cold distilled water (1 mL) and brine (1 mL). The organic layer was dried with $MgSO_4$, filtered, and concentrated in vacuo. The crude product was dissolved in CH_2Cl_2 (0.5 mL) and *m*CPBA (4 mg, 0.020 mmol) was added. The reaction was stirred at room temperature for 5 h and then washed with saturated sodium bisulfite solution (1 mL) and saturated sodium bicarbonate solution (1 mL) sequentially. The organic layer was dried with $MgSO_4$, filtered, and concentrated in vacuo. The crude product was purified by flash silica gel column chromatography (0 → 20% EtOAc/hexanes) to yield ASR-isobu-SO_2−CH_2Ph-4-F **9** (6.5 mg, 0.010 mmol, 66%) as an amorphous white solid. $[\alpha]_D^{21.3}$ +67.7° (c = 0.35, $CHCl_3$). IR (thin film) 2938, 2876, 1509, 1456, 1377, 1309, 1226, 1118, 1099, 1056, 1007, 941, 878, 843 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 7.48−7.44 (m, 2H), 7.08−7.04 (m, 2H), 5.48 (s, 1H), 5.31 (s, 1H), 4.40−4.38 (m, 1H), 4.35 (s, 2H), 4.15−4.13 (m, 1H), 3.54−3.50 (dd, J_1 = 14.3 Hz, J_2 = 10.1 Hz, 1H), 3.04−2.98 (dd, J_1 = 14.4 Hz, J_2 = 6.4 Hz, 1H), 2.78−2.72 (m, 1H), 2.65−2.56 (m, 2H), 2.39−2.28 (m, 3H), 2.06−1.95 (m, 2H), 1.92−1.88 (m, 3H), 1.81−1.73 (m, 2H), 1.69−1.19 (m, 21H including singlets for 3H each at 1.42 and 1.37), 0.96−0.93 (dd, J_1 = 6.1 Hz, J_2 = 3.0 Hz, 7H), 0.86 (s, 3H), 0.84 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 133.0, 132.9, 123.7, 123.7, 115.9, 115.7, 103.4, 103.0, 89.5, 88.7, 81.3, 81.2, 77.2, 74.1, 70.5, 58.6, 54.6, 52.6, 52.1, 44.6, 44.2, 37.5, 37.2, 36.6, 34.6, 34.4, 31.5, 31.4, 30.4, 30.3, 30.1, 26.3, 26.1, 24.8, 24.8, 24.7, 24.7, 20.3, 20.1, 13.4, 12.8. HRMS (FAB) m/z calcd for $C_{41}H_{60}FO_{10}S$ $(M + H)^+$ 763.3891, found 763.3850.

Synthesis of Dimeric Sulfone Benzylic Alcohol 4b. Bis-trioxane bromide **3** (39 mg, 0.058 mmol) was dissolved in acetonitrile (3 mL) under argon at room temperature. To the solution was added 4-hydroxymethyl thiophenol (16 mg, 0.116 mmol), easily prepared by LAH reduction of the commercially available parent carboxylic acid, and NaH (3.0 mg, 0.116 mmol) consecutively, and the reaction was stirred at room temperature overnight. The reaction was quenched with sat. aq sodium bicarbonate (2 mL) and extracted with EtOAc (2 mL). The organic layer was then washed with ice cold distilled water (2 × 2 mL) and brine (2 mL). The organic layer was dried with $MgSO_4$, filtered, and concentrated in vacuo. The crude product was dissolved in dichloromethane (3 mL). *m*CPBA (10 mg, 0.057 mmol) was added at room temperature and stirred for 2 h. The reaction was quenched with saturated aqueous sodium bisulfite (2 mL), and the organic layer was extracted with sat. aq sodium bicarbonate (2 mL). The crude product was purified by flash silica gel column chromatography (20 → 30% EtOAc/hexanes) to yield dimeric sulfone benzylic alcohol **4b** (25 mg, 0.033 mmol, 57%) as an amorphous white solid; mp = 109−112 °C; $[\alpha]_D^{21.3}$ +55° (c = 0.55, $CHCl_3$). IR (thin film) 3511, 2948, 2848, 1454, 1408, 1306, 1197, 1142, 1094, 1051, 1008, 936, 880, 837, 763 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 7.97−7.95 (d, 2 7.49−7.47 (d, 2H), 5.44 (s, 1H), 5.32 (s, 1H), 4.76 (s, 2H), 4.11−4.06 (m, 1H), 3.63−3.58 (dd, J_1 = 14 Hz, J_2 = 8.0 Hz, 1H), 3.34−3.29 (dd, J_1 = 14 Hz, J_2 = 8.0 Hz, 1H), 2.71−2.64 (m, 1H), 2.57−2.49 (m, 1H), 2.51−2.44 (m, 1H), 2.37−2.25 (m, 2H), 2.20−1.16 (m, 29H, including singlets at 1.42 and 1.34 for 3H each), 0.95−0.91

(m, 8H), 0.83−0.80 (m, 7H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 146.8, 139.2, 128.3, 127.0, 103.4, 102.9, 89.5, 88.8, 81.3, 81.2, 73.9, 71.0, 62.2, 60.4, 58.9, 52.5, 52.1, 44.6, 44.1, 37.4, 36.6, 36.6, 34.5, 34.4, 31.2, 31.1, 30.7, 30.4, 26.2, 26.1, 24.8, 24.8, 21.1, 20.3, 20.1, 14.2, 13.3, 12.7. HRMS (FAB) *m/z* calcd for $C_{41}H_{60}O_{11}SNa$ $(M + Na)^+$ 783.3749, found 783.3707.

Synthesis of Dimeric Sulfone Benzylic Dimethyl Carbamate 4c. Dimeric sulfone benzylic alcohol **4b** (20 mg, 0.026 mmol) was dissolved in DMF (1 mL) under argon at room temperature. To the solution was added dimethylcarbamyl chloride (5 μL, 0.052 mmol) and NaH (1.2 mg, 0.052 mmol) consecutively, and the reaction was stirred at room temperature for 2 h. The reaction was quenched with sat. aq sodium bicarbonate (1 mL) and extracted with dichloromethane (2 × 2 mL). The organic layer was dried with $MgSO_4$, filtered, and concentrated in vacuo. The crude product was purified by flash silica gel column chromatography (40% EtOAc/hexanes) to yield dimeric sulfone benzylic dimethyl carbamate **4c** (16 mg, 0.019 mmol, 73%) as an amorphous white solid. $[\alpha]_D^{23.4}$ +73.7° (c = 0.12, $CHCl_3$). IR (thin film) 2939, 2876, 1711, 1495, 1454, 1394, 1377, 1306, 1181, 1145, 1089, 1054, 1007, 878, 830, 767, 669 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02−8.00 (d, J = 8.0 Hz, 2H), 7.50−7.48 (d, J = 8.0 Hz, 2H), 5.48 (s, 1H), 5.34 (s, 1H), 5.18 (s, 2H), 4.45−4.43 (m, 1H), 4.14−4.10 (m, 1H), 3.63−3.58 (dd, J_1 = 14.4 Hz, J_2 = 7.2 Hz, 1H), 3.38−3.32 (dd, J_1 = 14.4 Hz, J_2 = 7.2 Hz, 1H), 2.95 (s, 6H), 2.71−2.65 (m, 1H), 2.58−2.50 (m, 2H), 2.39−2.26 (m, 2H), 2.24−2.15 (m, 1H), 2.09−1.97 (m, 2H), 1.95−1.84 (m, 3H), 1.82−1.16 (m, 22H including singlets for 3H each at 1.43 and 1.35), 0.95−0.03 (m, 8H), 0.84−0.82 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.7, 142.7, 139.9, 128.4, 127.9, 103.3, 102.9, 89.5, 88.8, 81.3, 81.2, 77.2, 74.0, 71.0, 66.0, 58.9, 56.6, 52.5, 52.1, 44.6, 44.1, 37.4, 37.3, 36.7, 36.6, 34.6, 34.4, 31.2, 31.1, 30.7, 30.4, 26.2, 26.1, 24.8, 24.8, 24.7, 24.7, 20.3, 20.1, 13.3, 12.7. HRMS (ESI) *m/z* calcd for $C_{44}H_{65}NO_{12}SNa$ $(M + Na)^+$ 854.4120, found 854.4100.

Synthesis of Dimeric Sulfone Benzylic Diethyl Carbamate 4d. Dimeric sulfone benzylic alcohol **4b** (18 mg, 0.024 mmol) was dissolved in DMF (1 mL) under argon at room temperature. To the solution was added diethylcarbamyl chloride (4 μL, 0.026 mmol) and NaH (0.62 mg, 0.026 mmol) consecutively, and the reaction was stirred at room temperature for 2 h. The reaction was quenched with sat. aq sodium bicarbonate (2 mL) and extracted with dichloromethane (2 × 2 mL). The organic layer was dried with $MgSO_4$, filtered, and concentrated in vacuo. The crude product was purified by flash silica gel column chromatography (30% EtOAc/hexanes) to yield dimeric sulfone benzylic diethyl carbamate **4d** (15.5 mg, 0.018 mmol, 76%) as an amorphous white solid. $[\alpha]_D^{23.6}$ +41.6° (c = 0.12, $CHCl_3$). IR (thin film) 2924, 2853, 1703, 1457, 1377, 1313, 1274, 1168, 1146, 1089, 1053, 1007, 878, 825, 766 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03−8.01 (d, J = 8 Hz, 2H), 7.49−7.47 (d, J = 8 Hz, 2H), 5.48 (s, 1H), 5.34 (s, 1H), 5.19 (s, 2H), 4.47−4.44 (m, 1H), 4.16−4.12 (dd, J = 4.4 Hz, 1H), 3.65−3.60 (dd, J_1 = 9.6 Hz, J_2 = 7.4 Hz, 1H), 3.38−3.33 (dd, J_1 = 9.6 Hz, J_2 = 7.4 Hz, 1H), 3.35−3.31 (m, 4H), 2.71−2.67 (m, 1H), 2.58−2.53 (m, 2H), 2.37−2.27 (m, 2H), 2.22−2.17 (m, 1H), 2.05−1.99 (m, 2H), 1.92−1.82 (m, 2H), 1.79−1.73 (m, 4H), 1.65−1.18 (m, 19H including singlets for 3H each at 1.43 and 1.34), 1.15−1.12 (t, J = 6.8 Hz, 6H), 0.96−0.94 (d, J = 6.4 Hz, 8H), 0.84−0.82 (d, J = 7.2 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.9, 142.9, 140.0, 128.4, 127.8, 103.3, 102.9, 89.5, 88.8, 81.3, 81.2, 73.9, 71.0, 65.7, 58.8, 52.6, 52.2, 44.6, 44.1, 37.4, 37.3, 36.7, 36.6, 34.6, 34.4, 31.3, 31.2, 31.2, 30.7, 30.4, 29.7, 26.2, 26.1, 24.8, 24.8, 24.7, 24.7, 20.2, 20.1, 13.2, 12.7. HRMS (ESI) *m/z* calcd for $C_{46}H_{69}NO_{12}SNa$ $(M + Na)^+$ 882.4433, found 882.4419.

Synthesis of Dimeric Sulfone Benzylic Diethyl Phosphate 4e. Dimeric sulfone benzylic alcohol **4b** (20 mg, 0.026 mmol) was dissolved in anhydrous dichloromethane (2 mL) under argon at room temperature. To the solution was added diethyl chlorophosphate (19 μL, 0.13 mmol) and pyridine (11 μL, 0.13 mmol) consecutively, and the reaction was stirred at room temperature for 18 h. The reaction was quenched with sat. aq sodium bicarbonate (2 mL) and extracted with dichloromethane (2 × 2 mL). The organic layer was dried with $MgSO_4$, filtered, and concentrated in vacuo. The crude product was purified by flash silica gel column chromatography (50% EtOAc/hexanes) to yield dimeric sulfone benzylic diethyl phosphate **4e** (8.2 mg, 0.009 mmol, 37%) as an amorphous white solid. $[\alpha]_D^{24.1}$ +27.1° (c = 0.06, $CHCl_3$). IR (thin film) 2925, 2854, 1699, 1653, 1558, 1541, 1521, 1507, 1457, 1375, 1270, 1036 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.06−8.03 (d, J = 8.0 Hz, 2H), 7.54−7.52 (d, J = 8.0 Hz, 2H), 5.49 (s, 1H), 5.35 (s, 1H), 5.14−5.12 (d, J = 8.0 Hz, 2H), 4.48−4.43 (m, 1H), 4.16−4.08 (m, 5H), 3.66−3.61 (dd, J_1 = 14 Hz, J_2 = 6.8 Hz, 1H), 3.39−3.34 (dd, J_1 = 14 Hz, J_2 = 6.8 Hz, 1H), 2.70−2.66 (m, 1H), 2,58−2.53 (m, 2H), 2.38−2.28 (m, 2H), 2.23−2.16 (m, 1H), 2.06−2.00 (m, 2H), 1.93−1.90 (m, 2H), 1.79−1.21 (m, 29H including singlets for 3H each at 1.44 and 1.35), 0.96−0.95 (m, 8H), 0.85−0.82 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 140.5, 138.0, 128.6, 127.7, 103.3, 102.9, 89.6, 88.9, 81.3, 81.2, 74.0, 70.9, 67.8, 67.7, 64.1, 64.1, 58.8, 57.2, 57.1, 52.6, 52.2, 44.6, 44.1, 37.5, 37.4, 36.7, 36.7, 34.4, 31.3, 30.7, 30.4, 29.7, 26.2, 26.1, 24.8, 24.8, 20.2, 20.1, 16.2, 16.1, 14.2, 13.2, 12.6. HRMS (ESI) *m/z* calcd for $C_{45}H_{69}O_{14}PSNa$ $(M + Na)^+$ 919.4038, found 919.3999.

Synthesis of Dimeric Sulfone Hexanol 4f. Dimeric trioxane bromide **3** (20 mg, 0.030 mmol) was dissolved in anhydrous acetonitrile (2 mL) under argon at room temperature. To the solution was added 6-mercapto-1-hexanol (9 μL, 0.066 mmol) and sodium hydride (NaH, 2.0 mg, 0.066 mmol) consecutively, and the reaction was stirred at room temperature for 2 h. The reaction was quenched with sat. aq sodium bicarbonate (2 mL) and extracted with dichloromethane (2 × 2 mL). The organic layer was dried with $MgSO_4$, filtered, and concentrated in vacuo. The crude product was dissolved in freshly made dimethyldioxirane solution in acetone (DMDO, 2 mL) at 0 °C and stirred for 1 h. The reaction was concentrated the crude product was purified by flash silica gel column chromatography (50% EtOAc/hexanes) to yield dimeric sulfone hexanol **4f** (12 mg, 0.015 mmol, 51%) as an amorphous white solid. $[\alpha]_D^{22.5}$ +58.1° (c = 0.15, $CHCl_3$). IR (thin film) 3520 (br), 2925, 2854, 1456, 1377, 1296, 1121, 1055, 1007, 878, 845 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.41 (s, 1H), 5.34 (s, 1H), 4.46−4.42 (m, 1H), 4.20−4.16 (m, 1H), 3.65−3.62 (t, J = 6.4 Hz, 2H), 3.49−3.42 (dd, J_1 = 14.2 Hz, J_2 = 7.2 Hz, 1H), 3.10−3.05 (m, 3H), 2.74−2.69 (m, 1H), 2.60−2.54 (m, 2H), 2.35−2.27 (m, 3H), 2.03−1.21 (m, 34H including singlets for 3H each at 1.40 and 1.25), 0.96−0.94 (m, 8H), 0.87−0.85 (m, 8H)). ^{13}C NMR (100 MHz, $CDCl_3$) δ 103.3, 102.9, 89.7, 88.8, 81.3, 81.2, 73.7, 70.3, 62.7, 55.7, 53.0, 52.6, 52.1, 44.5, 44.1, 37.5, 37.3, 36.7, 36.6, 34.6, 34.4, 32.3, 31.9, 31.6, 30.6, 30.3, 29.8, 29.7, 28.2, 26.2, 26.1, 25.1, 24.8, 24.8, 24.7, 24.7, 21.6, 20.3, 20.1, 13.2, 12.7. HRMS (ESI) *m/z* calcd for $C_{40}H_{66}O_{11}SNa$ $(M + Na)^+$ 777.4218, found 777.4211.

Cell Culture Conditions and Determination of IC_{50} Values. HL-60, U-937, SK-MEL-5, UACC-62, and HeLa cell lines were cultured in RPMI 1640 media supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin. WT-MEF and Hs888Lu cell lines were cultured in DMEM media supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin. All cell lines were obtained from the American Type Culture Collection (Manassas, VA), with the exception of the HL-60 line, which was a generous gift from Professor Huimin Zhao (University of Illinois), and were grown at 37 °C with CO_2/air (5:95). 99 μL of cells in media were plated in a 96-well plate. Adherent cell lines were incubated at least 5 h to allow cells to attach to the plate. A volume of 1 μL of a range of compound concentrations was added in to the cells, and the plates were incubated for 72 h. Cell viability in HL-60 and U-937 suspension cell lines were evaluated using the CellTiter 96 AQ_{ueous} nonradioactive cell proliferation assay (Promega, Inc.). The 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium (MTS)/ phenazine methosulfate (PMS) solution was added to the cells, and absorbance at 490 nm was measured in a Spectra Max Plus 384 plate reader (Molecular Devices, Sunnyvale, CA) following dye development. Biomass was quantitated in SK-MEL-5, UACC-62, HeLa, WT-MEF, and Hs888Lu cells using the sulforhodamine B assay (SRB).[43] Briefly, the cells were fixed with 10% trichloroacetic acid

overnight at 4 °C, washed with water, and 100 μL of 0.057% w/v sulforhodamine in 1% acetic acid was added to each well for 30 min. After rinsing the plates in 1% acetic acid, 200 μL of 10 mM tris base (pH > 10) was added to each well for 30 min and absorbance was read at 510 nm. Logistical dose response curves and IC_{50} values were generated from the MTS and SRB data using TableCurve 2D 5.01 (SYSTAT Software, Inc., Richmond, CA).

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Supporting Information Available: ^{1}H, ^{13}C NMR spectra for all of the new trioxane dimers. This material is available free of charge via the Internet at http://pubs.acs.org.

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