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Trioxane Dimers Have Potent Antimalarial, Antiproliferative and Antitumor Activities In Vitro

Gary H. Posner,*[a] Poonsakdi Ploypradith,[a] Whitney Hapangama,[a] Dasong Wang,[a] Jared N. Cumming,[a] Patrick Dolan,[b] Thomas W. Kensler,[b] Donna Klinedinst,[c] Theresa A. Shapiro,[c] Qun Yi Zheng,[d] Christopher K. Murray,*[d] Lynn G. Pilkington,[d] Lalith R. Jayasinghe,[d] Jeff F. Bray[d] and Randy Daughenbaugh[d]

[a]*Department of Chemistry, School of Arts and Sciences, The Johns Hopkins University, Baltimore, MD 21218, U.S.A.*
[b]*Division of Toxicological Sciences and The Environmental Health Sciences Center, Department of Environmental Health Sciences, School of Hygiene and Public Health, The Johns Hopkins University, Baltimore, MD 21205, U.S.A.*
[c]*Department of Medicine, School of Medicine, The Johns Hopkins University, Baltimore, MD 21205, U.S.A.*
[d]*Synthetic Chemistry Research and Development Group, Hauser Chemical Research Co., Inc., 5555 Airport Boulevard, Boulder, CO 80301, U.S.A.*

Abstract—A series of tetracyclic and tricyclic trioxane dimers has been prepared with ether and ester tethers of varying length and flexibility. Several of these trioxane dimers have been found to have potent and potentially therapeutically valuable antimalarial, antiproliferative, and antitumor activities in vitro. © 1997 Elsevier Science Ltd.

As part of our research program, we have been searching for bioactive compounds based on natural product leads. Based on antimalarial 1,2,4-trioxanes like the clinically useful Chinese drug qinghaosu (artemisinin, **1**) and its derivative arteether (**2**),[1–3] we have prepared trioxane dimers **3–13**. These dimers incorporate the tetracyclic trioxane skeleton of artemisinin (dimers **3**) or a simplified tricyclic trioxane skeleton (dimers **4–13**)[4–6] as well as linker groups representing ether and ester functionalities of various length and flexibility. Of the simplified trioxane dimers, only ether dimer **4** (IC_{50} = 1.9 nM) and phosphate dimer **11b** (IC_{50} = 0.96 nM) are considerably more antimalarially potent than artemisinin (IC_{50} = 10 nM) in vitro against chloroquine-sensitive *Plasmodium falciparum* (NF54) parasites.[6] Some other previously made trioxane dimers have been found to possess high antimalarial activities[7–10] and moderate antitumor activities.[11] Pursuing the recent discovery by Hauser that trioxane dimer **3b** has strong in vitro growth-inhibitory activity, we now report that several of these dimers **3–13** have high antiproliferative activities in normal murine keratinocytes and high antitumor activities in various cancer cell lines.

Antiproliferative activities, measured in vitro using murine keratinocytes as described previously,[12] are shown in Figure 1 for four highly active dimers. Note that some of these trioxane dimers, even at physiologically relevant 10–100 nM concentrations, are as antiproliferative as calcitriol (1α,25-dihydroxyvitamin D_3)[12] that is the hormonally active form of vitamin D and that is used clinically as a drug to treat psoriasis, a skin disorder characterized by uncontrolled proliferation of cells. Of the dihydroartemisinin polyethyleneglycol dimers **3a–3c**, the one with β-stereochemistry at both of the lactol acetal positions (i.e. **3b**) is very active. Likewise, the β,β-dimers **3d** and **3e** are highly antiproliferative. Of the simplified trioxane ether dimers **4–6**, the C_4-unsubstituted dimer **4** and the C_4-β-methyl[13] substituted dimer **5** are the most antiproliferative. Of the aromatic carboxylate ester dimers **7**, both the *meta*-phthalate dimer **7b** and the terephthalate dimer **7c**[14] have high antiproliferative activity. *meta*-Phthalate dimer **7b** has the highly desirable practical characteristic of being a crystalline solid that is stable for prolonged periods at room temperature. Of the aliphatic carboxylate ester dimers **8**, the glutarate diester is the most active. Neither the phosphate dimer **11** nor the phosphonate dimer **12** have significant antiproliferative activity. C_4-β-Hydroxymethyl terephthalate dimer **13** has significant antiproliferative activity. The high activities of glutarate diester **8b** and *meta*-phthalate diester **7b**, each with a 3-carbon linker group, mirror the

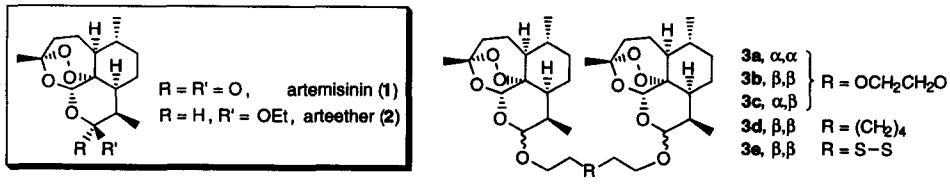
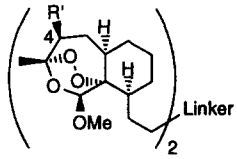
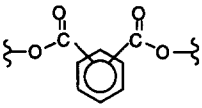
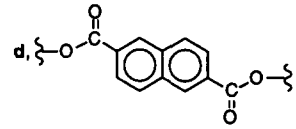
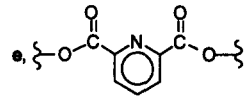
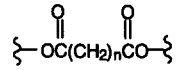
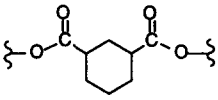
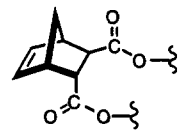
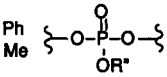
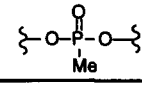
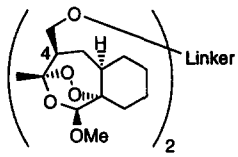
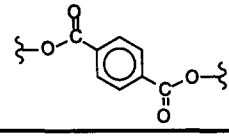


Table 1.



Compound	R'	Linker
4	H	
5	Me	
6	$PhCH_2$	
7	H	a, *ortho* b, *meta* c, *para*  d,  e, 
8	H	a, n = 2 b, n = 3 c, n = 4 
9	H	a, 1,3-*cis* b, 1,3-*trans* 
10	H	
11	H	a, R" = Ph b, R" = Me 
12	H	



Compound	Linker
13	

high activity of a glutarate diester of dihydroartemisinin;[15,16] also a pentamidine analogue having a 3-carbon linker has been found to have very strong DNA-binding affinity.[17]

Antitumor activities, measured in vitro as described previously[18] using a panel of 60 cancer cell lines in the National Cancer Institute's (NCI's) Developmental and Therapeutics Program, are shown in Figure 2 for four of the most potent dimers. In examining total growth inhibition (TGI), bars extending to the right of the centerline indicate cells more sensitive than average to a particular dimer, whereas bars to the left indicate less sensitive cells. All of these four dimers are selectively cytotoxic to leukemia cells. *meta*-Phthalate dimer **7b** is exquisitely specific in killing only leukemia cells and one kind of colon cancer cells but not any of the 53 other cell lines tested. The recent finding that our trioxane dimer **3b** and ether dimer **4** show no acute toxicity in vivo even at a maximum tolerated dose of 400 mg/kg in the NCI's mouse assay is very important for possible anticancer therapeutic use of these dimers. Other nonperoxidic dimers[19,20] and bis-electrophiles[21] have been reported recently to have potent biological activities and desirable pharmacological properties.[22]

Synthesis of these various trioxane dimers **3–13** was achieved easily in one chemical operation starting with the corresponding trioxane alcohol as shown schematically in equation 1. Acidic conditions were used in equation 1 for assembly of the lactol acetal dimers **3**, whereas basic conditions were used for construction of the dimers **4–13**.

$$\text{2 Trioxane—OH + X—Linker—X} \rightarrow \text{Trioxane—O—Linker—O—Trioxane} \qquad (1)$$

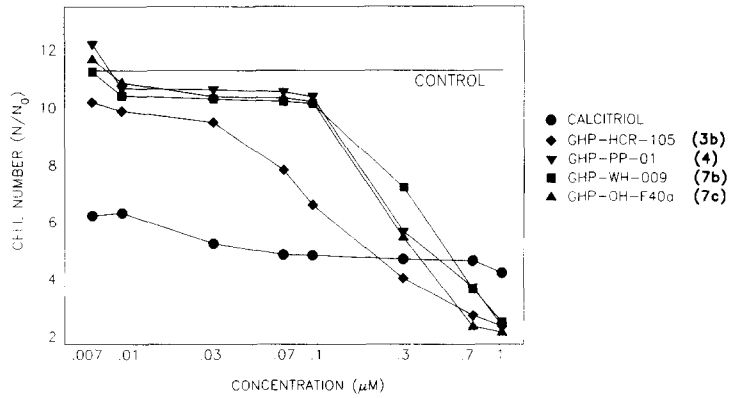


Figure 1. Dose response effects of dimers on keratinocyte proliferation (96 h).

Many bis-electrophiles[21] are powerful alkylating agents, often capable of damaging critical biomolecules such as proteins and DNA. It is tempting, therefore, to speculate that the potent antimalarial, antiproliferative, and antitumor activities of some of the trioxane dimers **3–13** may be due to their bis-alkylating ability once they are activated. The detailed mechanisms of their activation and of their cell growth-regulating capacities, as well as further in vivo biological evaluations, are under study.

Experimental

General

Unless otherwise noted, reactions were run in flame-dried round-bottomed flasks under an atmosphere of ultra high purity (UHP) argon. Diethyl ether (ether) and tetrahydrofuran (THF) were distilled from sodium benzophenone ketyl prior to use. Methylene chloride (CH_2Cl_2) was distilled from calcium hydride prior to use. All other compounds were purchased from Aldrich Chemical Company and used without further purification. Analytical thin-layer chromatography (TLC) was conducted with Silica Gel 60 F_{254} plates (250 μm thickness, Merck). Column chromatography was performed using short path silica gel (particle size <230 mesh), flash silica gel (particle size 400–230 mesh), or Florisil® (200 mesh). Yields are not optimized. High-performance liquid chromatography (HPLC) was carried out with a Rainin HPLX system equipped with two 25 mL/min preparative pump heads using Rainin Dynamax 10 mm$_\phi$ × 250 mm (semi-preparative) columns packed with 60 Å silica gel (8 μm pore size), either as bare silica or as C18-bonded silica. Melting points were measured using a Mel-Temp metal-block apparatus and are uncorrected. Nuclear magnetic resonance (NMR) spectra were obtained either on a Varian XL-400 spectrometer, operating at 400 MHz for ^1H and 100 MHz for ^{13}C, or on a Varian XL-500 spectrometer, operating at 500 MHz for ^1H and 125 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 (b). 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). Combustion analyses were conducted by Atlantic Microlab (Norcross, GA).

Synthesis of dihydroartemisinin triethylene glycol dimers 3a–c. To a solution of dihydroartemisinin (593 mg, 2.11 mmol) in toluene (60 mL) at room temperature was added triethylene glycol (0.144 mL, 1.06 mmol) followed by $BF_3{\cdot}Et_2O$ (0.064 mL, 0.53 mmol). The reaction was stirred at room temperature for 3 h. The mixture was then diluted with CH_2Cl_2 and was washed twice with water. The organic portions were collected, dried over magnesium sulfate, and concentrated. The crude product was purified by column chromatography (flash, 20–50% ethyl acetate/hexane) to produce dimers **3** (30 mg α,α dimer **3a**, 228 mg β,β dimer **3b**, 93 mg α,β dimer **3c**, 0.51 mmol total, 49%). **3a** (α,α dimer): ^1H NMR (400 MHz, $CDCl_3$) δ 5.32 (s, 2H), 4.50 (d, J = 9.2 Hz, 2H), 4.05 (m, 2H), 3.56 (m, 10H), 2.40 (m, 4H), 1.98 (m, 2H), 1.44 (s, 6H), 1.84–1.19 (m, 14H), 0.91 (d, J = 6.2 Hz, 6H), 0.88 (d, J = 7.3 Hz, 6H); IR (neat) 2962, 2925, 2854, 1716, 1456, 1261, 1099, 1024 cm^{-1}; LRMS (Electrospray) *m/e* found 700.5 (M + NH_4^+). **3b** (β,β dimer): ^1H NMR (400 MHz, $CDCl_3$) δ 5.36 (s, 2H), 4.76 (d, J = 3.3 Hz, 2H), 3.88 (m, 2H), 3.53–3.67 (m, 10H), 2.55 (m, 2H), 2.30 (dt, J = 13.6, 4.0 Hz, 2H), 1.98 (m, 2H), 1.84–1.39 (m, 14H), 1.38 (s, 6H), 1.28 (m, 2H), 1.19 (m, 2H), 0.91 (d, J = 6.2 Hz, 6H), 0.86 (d, J = 7.3 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 104.0, 102.1, 87.9, 81.1, 70.6, 67.4, 52.6, 44.5, 37.5, 36.5, 34.7, 30.9, 26.2, 24.8, 24.5, 20.4, 13.0; IR (neat) 2939, 2920, 2872, 1728, 1375, 1193, 1029 cm^{-1}; LRMS (Electrospray) *m/e* found 700.4 (M + NH_4^+); HRMS (FAB) *m/e* calcd for $C_{36}H_{59}O_{12}$ (M + H^+) 683.4007, found 683.3984. **3c** (α,β dimer): ^1H NMR (400 MHz, $CDCl_3$) δ 5.42 (s, 1H), 5.32 (s, 1H), 4.81 (d, J = 3.3 Hz, 1H), 4.50 (d, J = 9.2 Hz, 1H), 4.05 (m, 1H), 3.90 (m, 1H), 3.56 (m, 10H), 2.60 (m, 1H), 2.38 (m, 1H), 1.98 (m, 2H), 1.84–1.19 (m, 18H), 1.42 (s, 6H), 0.89 (d, J = 6.2 Hz, 6H), 0.86 (d, J = 7.3 Hz, 6H); IR (neat) 2940, 2925, 1731, 1462, 1377, 1261, 1157, 1030 cm^{-1}; LRMS (Electrospray) *m/e* found 700.5 (M + NH_4^+).

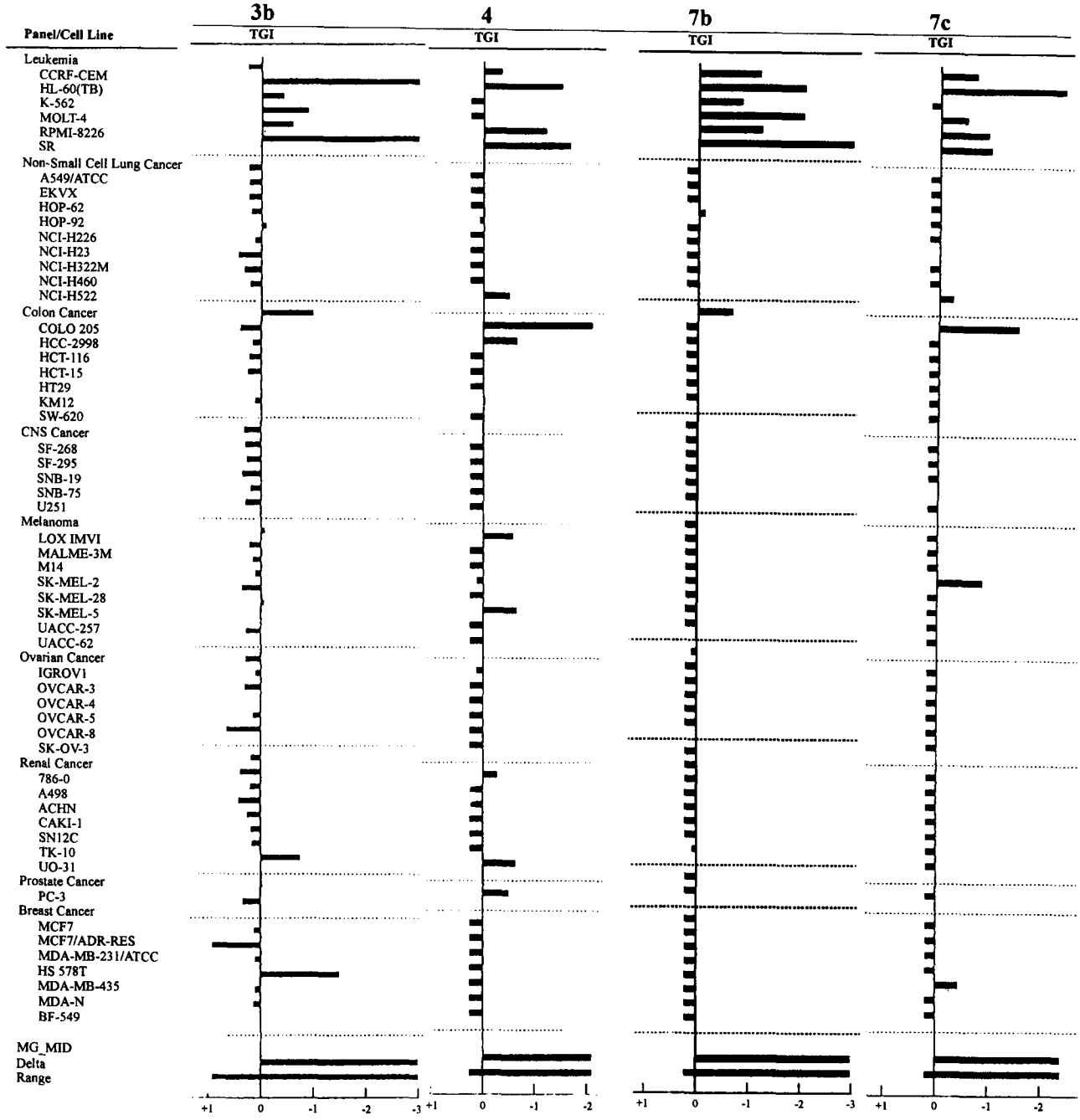


Figure 2. NCI 60 cell line assay.

Synthesis of dihydroartemisinin octamethylene dimer 3d. 1,8-octanediol (2.3 g, 15 mmol), $BF_3{\cdot}Et_2O$ (0.094 mL, 0.77 mmol) and molecular sieves were added to a solution of dihydroartemisinin (273 mg, 0.960 mmol) in toluene (18 mL). The reaction was stirred at 0 °C for 8 h and then slowly quenched with H_2O (20 mL). This mixture was diluted with CH_2Cl_2, the layers were separated, and the aqueous phase was extracted with CH_2Cl_2 (20 mL × 2). The organic portions were combined, dried over Na_2SO_4, and concentrated. This crude material was purified by column chromatography (flash, 20–50% EtOAc/hexanes) to give a monomeric dihydroartemisinin–octanol adduct (141 mg, 0.342 mmol, 36%).

A portion of this monomer (30 mg, 0.073 mmol) was dissolved in toluene (2 mL) and treated sequentially with dihydroartemisinin (21 mg, 0.073 mmol) and $BF_3{\cdot}Et_2O$ (0.072 mL, 0.58 mmol). The reaction was stirred for 7 h at 0 °C and then quenched with H_2O (3 mL). The resulting mixture was diluted with CH_2Cl_2, the layers were separated, and the aqueous portion was further extracted with CH_2Cl_2 (10 mL × 2). The organic washes were combined, dried over Na_2SO_4, and concentrated. The crude product was purified by column chromatography (flash, 20–33% EtOAc/hexanes) to give the desired dimer (16 mg, 0.024 mmol, 66%). ^1H NMR (400 MHz, $CDCl_3$) δ 5.38 (s, 2H), 4.79 (d, 2H), 3.80 (2H), 3.35 (m, 2H), 2.60 (m, 2H), 2.35 (m,

2H), 1.45 (s, 6H), 2.10–1.20 (m, 36H), 0.95 (d, 6H), 0.85 (d, 6H); LRMS (Electrospray) *m/e* found 701.0 (M + Na^+), 696.0 (M + NH_4^+).

Synthesis of dihydroartemisinin diethylene disulfide dimer 3e. Mercaptoethanol (0.59 mL, 8.3 mmol), molecular sieves, and $BF_3{\cdot}Et_2O$ (0.051 mL, 0.42 mmol) were added to a solution of dihydroartemisinin (148 mg, 0.520 mmol) in toluene (10 mL) at 5 °C. The reaction was stirred for 5.5 h at 5 °C, then diluted with water (5 mL) and CH_2Cl_2 (4 mL). The layers were separated, and the organic phase was washed with water, dried over Na_2SO_4, and concentrated. The crude material was purified by column chromatography (flash, CH_2Cl_2 then 10–40% EtOAc/CH_2Cl_2) to produce a monomeric dihydroartemisinin–thiol (68 mg, 0.20 mmol, 38%).

To a portion of this monomer (30 mg, 0.087 mmol) in MeOH (0.6 mL) at 0 °C was added Et_3N (0.036 mL, 0.26 mmol) followed by I_2 (11 mg, 0.043 mmol) as a solution in MeOH (0.25 mL). The reaction was warmed to room temperature and stirred for 1.5 h. The solvent was then removed, and the resulting yellow-brown oil was purified by column chromatography (flash, CH_2Cl_2 then 10% EtOAc/CH_2Cl_2) to give the desired disulfide (21 mg, 0.031 mmol, 71%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.45 (s, 2H), 4.81 (d, 2H), 4.04 (m, 2H), 3.68 (m, 2H), 2.90 (m, 4H), 2.60 (m, 2H), 2.35 (m, 2H), 2.10–1.20 (m, 24H), 1.55 (s, 6H), 0.95 (d, 6H), 0.89 (d, 6H); LRMS (Electrospray) *m/e* found 704.0 (M + Na^+), 709.0 (M + NH_4^+).

General procedure 1: synthesis of trioxane ether dimers 4–6

Freshly opened trifluoromethanesulfonic anhydride (2 equiv) was added, via syringe, to a solution of 2,6-di-*tert*-butyl-4-methylpyridine (2 equiv) in CH_2Cl_2 (20 mL/mmol starting alcohol) at 0 °C. A solution of starting trioxane alcohol (1 equiv) in CH_2Cl_2 (10 mL/mmol starting alcohol) at 0 °C was then added via cannula. The reaction was stirred at 0 °C until TLC analysis indicated consumption of alcohol (approximately 3 h). The reaction was quenched with satd aqueous sodium bicarbonate at 0 °C and diluted with methylene chloride. Two layers were separated and the aqueous phase was extracted with methylene chloride. The combined organic layers were washed with brine, dried over magnesium sulfate, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography (flash gel or Florisil®, EtOAc/hexanes) to provide the desired dimer.

Synthesis of trioxane ether dimer 4. Using general procedure 1, dimer **4** (12 mg, 0.023 mmol, 64%) was prepared and then further purified by HPLC (silica, 15% EtOAc/hexanes, 3 mL/min, 254 nm, R_t = 8.1 min) to afford a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.16 (s, 2H), 3.53–3.42 (m, 4H), 3.50 (s, 6H), 2.36–2.28 (m, 2H), 2.25–2.19 (m, 2H), 2.01 (ddd, J = 14.4, 4.4, 2.8 Hz, 2H), 1.85–1.48 (m, 16H), 1.38 (s, 6H), 1.34–1.20 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 105.1, 100.3, 85.3, 69.7, 69.5, 56.8, 48.6, 48.5, 42.3, 42.2, 37.5, 31.1, 29.92, 29.87, 29.6, 29.5, 27.2, 26.0, 25.27, 25.26; IR ($CHCl_3$) 3018, 2932, 2862, 1451, 1443, 1408, 1376, 1266, 1224, 1218, 1210, 1136, 1121, 1101, 1078, 1007 cm^{-1}; LRMS (CI, NH_3, rel intensity) 544 (M + 18, 13), 484 (10), 424 (10), 255 (19), 223 (11), 196 (14), 195 (100), 137 (7); HRMS (CI) *m/z* calcd for $C_{28}H_{50}O_9N$ (M + NH_4^+) 544.3486, found 544.3489.

Synthesis of trioxane ether dimer 5. Using general procedure 1, dimer **5** (9.5 mg, 0.018 mmol, 65%) was obtained and further purified by HPLC (silica, 10% EtOAc/hexanes, 3 mL/min, 254 nm, R_t = 10.3 min) to give a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.16 (s, 2H), 3.48 (s, 6H), 3.47 (m, 4H), 2.48–2.38 (m, 2H), 2.26–2.16 (m, 2H), 1.78–1.46 (m, 16H), 1.35–1.17 (m, 6H), 1.28 (s, 6H), 0.97 (d, J = 7.2 Hz, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 107.4, 100.34, 100.32, 84.7, 69.7, 69.5, 56.9, 47.5, 42.11, 42.05, 39.9, 37.4, 30.9, 29.88, 29.85, 29.6, 29.5, 25.28, 25.26, 23.3, 19.2; IR ($CHCl_3$) 3000, 2931, 2859, 1465, 1373, 1408, 1376, 1218, 1213, 1138, 1122, 1100, 1009, 998, 947 cm^{-1}; LRMS (CI, NH_3, rel intensity) 572 (M + NH_4^+, 11), 452 (9), 269 (17), 237 (16), 209 (100), 137 (12); HRMS (CI) *m/z* calcd for $C_{30}H_{54}O_9N$ (M + NH_4^+) 554.3799, found 554.3808.

Synthesis of trioxane ether dimer 6. Using general procedure 1, dimer **6** (4.0 mg, 0.0057 mmol, 19%) was prepared and then further purified by HPLC (silica, 10% EtOAc/hexanes, 3 mL/min, 254 nm, R_t = 9.0 min) to produce a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30–7.26 (m, 4H), 7.23–7.20 (m, 6H), 5.20 (s, 2H), 3.51–3.42 (m, 4H), 3.04 (dd, J = 13.2, 4.0 Hz, 2H), 2.66 (m, 2H), 2.30 (t, J = 12.8, 2H) 2.20 (m, 2H), 1.80–1.10 (m, 18H), 1.42 (s, 6H), 0.88 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 140.7, 129.0, 128.3, 125.9, 107.3, 100.6, 90.7, 84.8, 69.7, 69.5, 57.0, 47.3, 46.4, 42.00, 41.95, 39.0, 33.2, 30.8, 29.88, 29.86, 29.59, 29.56, 25.2, 23.6; IR ($CHCl_3$) 3001, 2931, 2860, 1670, 1465, 1409, 1374, 1215, 1137, 1122, 1008, 972, 948 cm^{-1}; LRMS (CI, NH_3, rel intensity) 724 (M + NH_4^+, 10), 664 (33), 604 (31), 345 (12), 285 (100); HRMS (CI) *m/z* calcd for $C_{42}H_{62}O_9N$ (M + NH_4^+) 724.4425, found 724.4433.

General procedure 2: synthesis of bis-ester dimers (7–10)

4-(dimethylamino)pyridine and CH_2Cl_2 were added to a flask charged with vacuum-dried trioxane alcohol (2 equiv). This mixture was cooled to 0 °C, and a bis-acid chloride (2 equiv), either commercially available or synthetic (made from the reaction of the corresponding commercially available dicarboxylic acid with thionyl chloride) was added via a syringe. The reaction was stirred overnight, and the crude mixture was then directly adsorbed onto flash gel and loaded onto a column. Chromatography (ethyl acetate/hexanes) gave the desired dimer.

Synthesis of trioxane bis-ester dimer 7a. Using general procedure 2, trioxane dimer **7a** (15 mg, 0.022 mmol,

40%) was prepared and then further purified by HPLC (silica, 20% EtOAc/hexanes, 2 mL/min, 254 nm, R_t = 16.5 min) to give a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.73–7.67 (m, 2H), 7.53–7.48 (m, 2H), 5.15 (s, 2H), 4.43–4.37 (m, 2H), 4.35–4.29 (m, 2H), 3.48 (s, 6H), 2.40–2.22 (m, 4H), 2.05–1.94 (m, 2H), 1.88–1.61 (m, 6H), 1.61–1.41 (m, 6H), 1.41–1.30 (m, 6H), 1.30–1.15 (m, 6H), 0.93–0.77 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.5, 132.2, 130.9, 128.8, 105.2, 100.1, 85.1, 64.6, 56.7, 48.5, 42.2, 37.5, 31.0, 29.3, 29.0, 27.1, 25.9, 25.2; IR ($CDCl_3$) 2955, 2931, 2849, 2249, 1710, 1467, 1431, 1373, 1296–1261, 1143–1120, 1073, 1008, 926–891, 761–697, 644 cm^{-1}; HRMS calcd for $C_{36}H_{54}O_{12}N$ (M + NH_4^+) 692.3646, found 692.3663.

Synthesis of trioxane bis-ester dimer 7b. Using general procedure 2, trioxane dimer **7b** (14 mg, 0.021 mmol, 37%) was prepared and then further purified by HPLC (silica, 20% EtOAc/hexanes, 2 mL/min, 254 nm, R_t = 11.7 min) to afford a white crystalline solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.62 (t, J = 1.4 Hz, 1H), 8.15 (dd, J = 7.8, 1.8 Hz, 2H), 7.46 (t, J = 7.8 Hz, 1H), 5.15 (d, J = 0.8 Hz, 2H), 4.41–4.28 (m, 4H), 3.46 (s, 6H), 2.46–2.07 (m, 4H), 2.05–1.84 (m, 2H), 1.85–1.58 (m, 10H), 1.58–1.39 (m, 6H), 1.32 (s, 6H), 1.27–0.94 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.8, 133.7, 130.8, 130.2, 128.5, 105.3, 100.1, 85.2, 64.5, 56.7, 48.6, 42.4, 37.5, 31.0, 29.6, 29.3, 27.1, 25.9, 25.2; IR ($CDCl_3$) 2928, 2857, 1718, 1243, 1210, 1136, 1008, 780, 772, 762, 756, 752, 746, 735, 668 cm^{-1}; HRMS calcd for $C_{36}H_{54}O_{12}N$ (M + NH_4^+) 692.3646, found 692.3656.

Synthesis of trioxane bis-ester dimer 7d. Using general procedure 2, trioxane dimer **7d** (16 mg, 0.022 mmol, 30%) was prepared and then recrystallized to produce a white crystalline solid: mp 178–180 °C; R_f = 0.7, 20% EtOAc/hexanes; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.63 (s, 2H), 8.12 (dd, J = 8.4, 1.6 Hz, 2H), 8.01 (d, J = 8.4 Hz, 2H), 5.23 (s, 2H), 4.58–4.34 (m, 4H), 3.50 (s, 6H), 2.59–2.40 (m, 2H), 2.35 (dt, J = 14.2, 3.6 Hz, 2H), 2.11–1.96 (m, 2H), 1.93–1.74 (m, 6H), 1.74–1.66 (m, 4H), 1.66–1.48 (m, 8H), 1.40 (s, 6H), 1.32–1.15 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.3, 134.5, 130.6, 129.6, 129.5, 126.0, 105.3, 100.1, 85.2, 64.4, 56.7, 48.6, 42.3, 37.5, 31.0, 29.5, 29.3, 27.1, 26.0, 25.2; IR ($CDCl_3$) 2919, 2849, 2355, 2249 1708, 1602, 1461, 1443, 1402, 1378, 1337, 1280, 1262, 1210, 1183, 1135, 1118, 1096, 1077, 1007, 961, 951 cm^{-1}; HRMS calcd for $C_{40}H_{56}O_{12}N$ (M + NH_4^+) 742.3803, found 742.3814.

Synthesis of trioxane bis-ester dimer 7e. Using general procedure 2, trioxane dimer **7e** (10 mg, 0.015 mmol, 27%) was prepared and then further purified by HPLC (silica, 20% EtOAc/hexanes, 2 mL/min, 254 nm, R_t = 10.3 min) to give a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.27 (d, J = 8.0 Hz, 2H), 7.99 (t, J = 8.0 Hz, 1H), 5.21 (s, 2H), 4.51–4.47 (m, 4H), 3.51 (s, 6H), 2.51–2.37 (m, 1H), 2.37–2.23 (m, 2H), 2.08–1.93 (m, 1H), 1.93–1.70 (m, 6H), 1.70–1.46 (m, 12H), 1.37 (s, 6H), 1.29–1.16 (s, 4H), 0.95–0.73 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.5, 148.6, 138.1, 127.8, 105.2, 100.1, 85.2, 65.5, 56.7, 48.5, 42.5, 37.5, 31.0, 29.7, 29.3, 27.1, 25.9, 25.2; IR ($CDCl_3$) 3683, 2989, 2919, 2861, 2364, 2334, 1737, 1719, 1602, 1584, 1449, 1320, 1284, 1243, 1220, 1142, 1121, 759, 663, 452 cm^{-1}.

Synthesis of trioxane bis-ester dimer 8a. Using general procedure 2, trioxane dimer **8a** (8.0 mg, 0.013 mmol, 14%) was prepared and further purified by HPLC (silica, 30% EtOAc/hexanes, 2 mL/min, 254 nm, R_t = 6.6 min) to afford a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.12 (d, J = 0.8 Hz, 2H), 4.20–4.07 (m, 4H), 3.48 (s, 6H), 2.60 (s, 4H), 2.59–2.43 (m, 12H), 2.43–2.30 (m, 6H), 2.29–2.09 (m, 12H), 1.95–1.69 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.3, 105.2, 100.1, 85.2, 63.7, 56.7, 48.5, 42.2, 37.5, 31.0, 29.4, 29.2, 29.1, 27.1, 25.9, 25.2; IR ($CDCl_3$) 3013, 2978, 2919, 2861, 1725, 1461, 1237, 1208, 1055, 1002, 897, 769, 767, 754, 743, 738, 734, 728, 672 cm^{-1}; HRMS calcd for $C_{32}H_{54}O_{12}N$ (M + NH_4^+) 644.3646, found 644.3649.

Synthesis of trioxane bis-ester dimer 8b. Using general procedure 2, trioxane dimer **8b** (7.2 mg, 0.011 mmol, 12%) was prepared and then further purified by HPLC (silica, 30% EtOAc/hexanes, 2 mL/min, 254 nm, R_t = 7.0 min) to produce a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.12 (d, J = 1.2 Hz, 2H), 4.19–4.04 (m, 4H), 3.48 (s, 6H), 2.34 (t, J = 7.4 Hz, 4H), 2.32–2.24 (m, 2H), 2.24–2.14 (m, 2H), 2.08–1.99 (m, 8H), 1.98 (ddd, J = 14.4, 4.6, 2.6 Hz, 2H), 1.92 (t, J = 7.4 Hz, 2H), 1.86–1.73 (m, 2H), 1.73–1.62 (m, 4H), 1.62–1.44 (m, 8H), 1.35 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.9, 105.2, 100.1, 85.1, 63.4, 56.7, 48.5, 42.2, 37.5, 33.4, 31.0, 29.4, 29.2, 27.1, 25.9, 25.2, 20.1; IR ($CDCl_3$) 3683, 3030, 2997, 2931, 2859, 1727, 1601, 1214, 1138, 1002, 756, 673 cm^{-1}; HRMS calcd for $C_{33}H_{56}O_{12}N$ (M + NH_4^+) 658.3803, found 658.3795.

Synthesis of trioxane bis-ester dimer 8c. Using general procedure 2, trioxane dimer **8c** (14 mg, 0.021 mmol, 14%) was prepared as a colorless oil: R_f = 0.6, 50% EtOAc/hexanes; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.12 (s, 2H), 4.24–4.12 (m, 4H), 3.48 (s, 6H), 3.13 (dt, J = 9.0, 2.4 Hz, 4H), 2.39–2.19 (m, 9H), 2.19–2.04 (m, 2H), 2.04–1.92 (m, 2H), 1.92–1.73 (m, 4H), 1.73–1.43 (m, 12H), 1.36 (s, 6H), 1.29–1.12 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.3, 126.8, 117.1, 105.2, 100.1, 85.2, 64.3, 56.7, 54.8, 48.5, 42.1, 38.1, 37.5, 31.0, 29.3, 27.4, 25.9, 25.2, 21.0; IR ($CDCl_3$) 3015, 2933, 2861, 1729, 1210, 1134, 1003, 780, 774, 770, 755, 752, 747, 741, 734, 728 cm^{-1}; HRMS calcd for $C_{34}H_{58}O_{12}N$ (M + NH_4^+) 672.3959, found 672.3948.

Synthesis of trioxane bis-ester dimers 9a and 9b. Using general procedure 2, a mixture of trioxane dimers **9a** and **9b** was prepared as a colorless oil. The isomers were separated by HPLC (silica, 20% EtOAc/hexanes, 2 mL/min, 254 nm, R_t (**9a**) = 14.8 min, (**9b**) 15.3 min). **9a**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.14 (s, 2H), 4.27–3.98 (m, 4H), 3.50 (s, 6H), 2.77–2.61 (m, 2H), 2.40–2.25 (m, 4H), 2.25–2.19 (m, 2H), 2.19–2.11 (m, 2H), 2.07–1.91 (m, 4H), 1.91–1.74 (m, 4H), 1.73–1.63 (m, 6H), 1.63–1.47 (m, 14H), 1.37 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.2, 105.2, 100.1, 85.1, 63.3, 56.7, 48.5, 42.6,

42.2, 37.5, 31.0, 29.3, 29.1, 28.3, 27.9, 27.1, 25.9, 25.2, 24.8; IR ($CDCl_3$) 2931, 2861, 1716, 1454, 1374, 1359, 1264, 1204, 1137, 1120, 1007, 922, 916, 910, 902, 894, 757, 752, 747, 742, 737, 732, 726, 723 cm^{-1}; LRMS (CI, rel intensity) 698 (M + NH_4^+, 8), 638 (51), 578 (100), 529 (16), 195 (70), 151 (13). **9b**: ^1H NMR (400 MHz, $CDCl_3$) δ 5.14 (d, J = 1.2 Hz, 2H), 4.28–4.40 (m, 4H), 3.49 (s, 6H), 2.74–2.57 (m, 2H), 2.44–2.25 (m, 4H), 2.25–2.13 (m, 4H), 2.09–1.92 (m, 4H), 1.77–1.63 (m, 6H), 1.63–1.46 (m, 8H), 1.37 (s, 6H), 1.30–1.12 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.1, 105.2, 100.1, 85.1, 63.3, 60.3, 56.7, 48.5, 42.6, 42.2, 37.5, 31.0, 29.3, 29.1, 28.3, 27.1, 25.9, 25.2, 24.8; IR ($CDCl_3$) 2938, 2861, 2259, 2247, 1722, 1464, 1449, 1377, 1261, 1207, 1136, 1121, 1009, 921, 915, 909, 904, 900, 896, 758, 750, 742, 733, 723, 718, 655, 477 cm^{-1}.

Synthesis of trioxane bis-ester dimer 10. Using general procedure 2, trioxane dimer **10** was prepared as a colorless oil: ^1H NMR (400 MHz, $CDCl_3$) δ 6.30–6.27 (m, 1H), 6.11–6.06 (m, 1H), 5.16–5.13 (m, 2H), 4.34–3.99 (m, 6H), 3.50 (d, J = 2.4 Hz, 6H), 3.39–3.38 (m, 2H), 3.30–3.21 (m, 1H), 3.16–3.09 (m, 1H), 2.43–2.18 (m, 2H), 2.09–1.93 (m, 2H), 1.92–1.42 (m, 8H), 1.38 (s, 6H), 1.35–1.16 (m, 6H); IR ($CDCl_3$) 2931, 2861, 2249, 1725, 1461, 1373, 1265, 1248, 1210, 1185, 1114, 1045, 1008, 904, 733, 656, 651, 646 cm^{-1}; LRMS (CI, rel intensity) 708 (M + NH_4^+, 8), 648 (55), 588 (100), 539 (15), 195 (46).

General procedure 3: synthesis of bis-trioxane phosphate esters 11

Lithium hexamethyldisilazide (LHMDS, 1.0 M in THF, 2 equiv) was added, via syringe, to a solution of trioxane alcohol (2 equiv) in THF at 0 °C. The resulting mixture was stirred for 10 min at 0 °C and the appropriate dichlorophosphate (1 equiv) was added. The reaction was kept at 0 °C for 2 h and was then warmed to room temperature and stirred for 40 min. The mixture was then cooled back to 0 °C, quenched with water, and the organic layer was extracted three times with ether. The combined organic portions were washed with satd aqueous sodium chloride, and dried over magnesium sulfate. Column chromatography (EtOAc/hexanes) produced the desired dimer.

Synthesis of bis-trioxane phosphate ester 11a. Using general procedure 3, trioxane dimer **11a** (12 mg, 0.017 mmol, 52%) was prepared and then recrystallized (15% EtOAc/hexanes) to afford a white solid: mp 162–164 °C; R_f = 0.3, 20% EtOAc/hexanes; ^1H NMR (400 MHz, $CDCl_3$) δ 7.38–7.23 (m, 2H), 7.23–7.13 (m, 2H), 7.13–7.02 (m, 1H), 5.04 (s, 2H), 4.42–4.04 (m, 4H), 3.41 (d, J = 1.6 Hz, 6H), 2.54–2.12 (m, 4H), 2.12–1.88 (m, 4H), 1.88–1.43 (m, 12H), 1.41–1.28 (m, 8H), 1.31 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 129.7, 124.9, 120.1, 105.2, 100.1, 85.1, 67.4, 67.3, 56.7, 48.5, 41.7, 37.5, 30.9, 29.4, 29.3, 27.1, 25.9, 25.1; IR ($CDCl_3$) 4200, 3683, 3613, 3025, 2931, 2390 1519, 1425, 1213, 1020, 926–885, 790–703, 667, 644 cm^{-1}.

Synthesis of bis-trioxane phosphate ester 11b. Using general procedure 3, trioxane dimer **11b** (32 mg, 0.052 mmol, 69%) was prepared and further purified by HPLC (silica, 10% i-PrOH/hexanes, 2 mL/min, 254 nm, R_t = 16.0 min) to give a colorless oil: ^1H NMR (400 MHz, $CDCl_3$) δ 5.11 (s, 2H), 4.12–4.07 (m, 4H), 3.74 (m, 3H), 3.48 (s, 6H), 2.39–2.20 (m, 4H), 2.07–1.90 (m, 2H), 1.90–1.72 (m, 2H), 1.72–1.65 (m, 4H), 1.65–1.44 (m, 12H), 1.35 (s, 6H), 1.28–1.16 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 105.2, 100.1, 85.1, 66.6, 56.7, 54.3, 48.5, 41.7, 37.5, 31.0, 29.4, 29.3, 27.1, 25.9, 25.1; IR ($CDCl_3$) 2249, 1461, 1378, 1214, 1096, 1008, 908, 779, 714, 644 cm^{-1}; LRMS (CI) 638 (M + NH_4^+, 4), 621 (M + H^+, 1), 561 (5), 307 (22), 195 (100).

Synthesis of bis-trioxane phosphonate ester 12. LHMDS (0.14 mL, 1.0 M in THF) was added dropwise via syringe to a solution of trioxane alcohol (30 mg, 0.11 mmol) in THF (2.5 mL) at 0 °C. The resulting mixture was stirred for 10 min at −78 °C, and then methylphosphonic acid dichloride (5 μL, 0.06 mmol) was added. The reaction was kept at −78 °C, then warmed to room temperature and stirred for 40 min. The resulting mixture was cooled back to 0 °C, quenched with water, and the organic layer was extracted three times with ether. The combined organic portions were washed with satd aqueous sodium chloride, dried over magnesium sulfate, and concentrated. Column chromatography (flash, 15% EtOAc/hexanes) produced the product as an oil (20 mg, 0.033 mmol, 60%) that was further purified by HPLC (silica, 20% EtOAc/hexanes, 2 mL/min, 254 nm, R_t = 9.9 min): ^1H NMR (400 MHz, $CDCl_3$) δ 5.14 (d, J = 1.2 Hz, 2H), 3.73–3.54 (m, 4H), 3.49 (s, 6H), 2.31 (dt, J = 13.6, 3.5 Hz, 2H), 2.15 (dq, J = 9.6, 2.0 Hz, 2H), 2.00 (dq, J = 14.4, 2.4 Hz, 2H), 1.91–1.74 (m, 4H), 1.74–1.64 (m, 6H), 1.64–1.61 (m, 2H), 1.57 (s, 6H), 1.37 (s, 3H), 1.34–1.33 (m, 8H), 0.95–0.78 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 105.2, 100.1, 85.1, 66.59, 66.53, 56.7, 48.5, 41.6, 37.5, 31.0, 29.3, 27.1, 25.9, 25.1; IR ($CDCl_3$) 3695, 3013, 2955, 2931, 2865, 2360, 2339, 1220, 1216, 1210, 790, 784, 779, 772, 768, 761, 754, 750, 740, 736, 732 cm^{-1}.

Synthesis of C_4-β-hydroxymethyl trioxane terephthalate dimer 13. A 10-mL three-necked round-bottomed flask was fitted with an inlet line from an argon gas tank, an outlet line, and a septum. This flask was charged with paraformaldehyde (753 mg, 25.1 mmol), and the outlet line was connected through a glass tube to a 100 mL three-necked round-bottomed flask, also fitted with an outlet line to a bubbler and a septum. A solution of Z-methoxyethylidene-2-(2′-cyanoethyl)cyclohexanone[23] (900 mg, 5.02 mmol) in THF (39 mL) at −78 °C was added, via cannula, to a freshly prepared solution of Li(i-PrN)$_2$ (5.52 mmol) in THF/hexane (7.1 mL/3.9 mL) at −78 °C in the 100 mL flask. After stirring at −78 °C for 5 min, the reaction mixture was warmed to room temperature and stirred for 20 min. This yellow/brown enolate solution was cooled to −78 °C while the paraformaldehyde was heated to 160 °C. The resulting gaseous formaldehyde was blown over the vigorously stirred enolate solution with argon overpressure. After

the addition, the mixture was stirred at −78 °C for 15 min, warmed to room temperature over 2 h, and stirred at room temperature for 6 h. The reaction was quenched by the dropwise addition of H_2O (1 mL). The resulting mixture was diluted with H_2O (50 mL) and ether (50 mL). The organic phase was separated, and the aqueous phase was extracted with ether (50 mL × 2). The organic portions were combined, washed with satd aq NaCl, dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. This crude product was purified by column chromatography (flash, 5–20% EtOAc/hexane) to give the desired product, a 1:1 diastereomeric mixture (931 mg, 4.45 mmol, 89%), as a pale-yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.87 (d, J = 1.6 Hz, 1H), 5.86 (d, J = 1.6 Hz), 3.83 (m, 2H), 3.76 (m, 2H), 3.54 (s, 3H), 3.53 (s, 1H), 3.09 (m, 1H), 2.96 (m, 1H), 2.67 (m, 2H), 2.24 (m, 2H), 2.08–1.94 (m, 2H), 1.88 (m, 4H), 1.78–1.64 (m, 4H), 1.63–1.52 (m, 8H), 1.46 (m, 2H), 1.30–1.18 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 141.2, 141.1, 121.9, 121.5, 117.3, 117.2, 63.1, 61.8, 59.3, 59.2, 33.6, 32.4, 31.8, 31.1, 31.0, 30.3, 29.9, 28.03, 27.96, 26.4, 26.2, 21.7, 21.3; IR ($CHCl_3$) 3617, 3470, 3020, 2933, 2858, 2242, 1675, 1449, 1239, 1127 cm^{-1}; LRMS (EI, rel intensity) 209 (M^+, 11), 125 (100), 93 (17), 84 (5), 45 (11); HRMS (EI) m/z calcd for $C_{12}H_{19}NO_2$ (M^+) 209.1416, found 209.1419.

To a solution of the above α-hydroxymethyl nitrile (280 mg, 1.34 mmol) in CH_2Cl_2 (13 mL) at 0 °C was added via syringe 2,6-lutidine (234 μL, 2.01 mmol). This mixture was stirred for 5 min at 0 °C. At that time, t-$BuMe_2SiOTf$ (400 μL, 1.74 mmol) was added via syringe, and the solution was stirred for an additional 30 min at 0 °C. The reaction was quenched by the addition of H_2O (3 mL). The resulting mixture was diluted with H_2O (20 mL) and ether (20 mL). The organic phase was separated, and the aqueous phase was extracted with ether (20 mL × 2). The organic portions were combined, washed with satd aq NaCl, dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. This crude product was purified by column chromatography (short path, 1–10% EtOAc/hexane) to give the desired product, a 1:1 mixture of diastereomers (397 mg, 1.22 mmol, 91%), as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.86 (d, J = 1.6 Hz, 1H), 5.84 (d, J = 2.0 Hz, 1H), 3.81–3.66 (m, 4H), 3.54 (s, 3H), 3.51 (s, 3H), 3.10 (m, 1H), 2.92 (m, 1H), 2.59 (m, 2H), 2.02–1.81 (m, 7H), 1.78–1.65 (m, 4H), 1.60 (m, 3H), 1.53 (m, 5H), 1.45 (m, 1H), 1.29–1.17 (m, 2H), 0.91 (s, 9H), 0.90 (s, 9H), 0.09 (s, 6H), 0.08 (d, J = 2.0 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 141.28, 141.27, 141.2, 141.1, 122.1, 121.4, 117.3, 117.34, 117.32, 63.6, 62.1, 59.2, 33.5, 32.5, 31.9, 31.3, 31.11, 31.06, 30.4, 29.9, 28.2, 28.2, 26.4, 26.3, 25.8, 21.8, 21.5, 18.24, 18.22, −5.4, −5.5; IR ($CHCl_3$) 3017, 2931, 2858, 2243, 1677, 1463, 1258, 1128, 839 cm^{-1}; LRMS (EI, rel intensity) 323 (M^+, 2), 266 (100), 234 (26), 160 (17), 125 (23), 89 (33), 73 (19); HRMS (EI) m/z calcd for $C_{18}H_{33}NO_2Si$ (M^+) 323.2281, found 323.2280.

Over a period of 5 min, MeLi (1.4 M in ether, 2.2 mL, 3.1 mmol) was added via syringe to a solution of the above t-butyldimethylsilyl ether (330 mg, 1.02 mmol) in ether (7.8 mL) at −78 C. The resulting mixture was stirred at −78 °C for 5 min then warmed to room temperature and stirred for 3 h. At that time, the reaction was cooled to 0 °C and quenched with the dropwise addition of H_2O (1 mL). The resulting mixture was diluted with H_2O (20 mL) and ether (20 mL). The organic phase was separated, and the aqueous phase was extracted with ether (20 mL × 2). The organic portions were combined, washed with satd aq NaCl, dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. This crude product was purified by column chromatography (short path, 1–20% EtOAc/hexane) to give the desired product, a 1:1 diastereomeric mixture (260 mg, 0.76 mmol, 75%), as a yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.78 (d, J = 2.0 Hz, 1H), 5.73 (d, J = 2.4 Hz, 1H), 3.75–3.52 (m, 4H), 3.49 (s, 3H), 3.47 (s, 3H), 2.81 (m, 1H), 2.72 (m, 2H), 2.63 (m, 1H), 2.18 (s, 3H), 2.14 (s, 3H), 1.98–1.83 (m, 3H), 1.80–1.68 (m, 4H), 1.57 (m, 3H), 1.46 (m, 7H), 1.25 (ddd, J = 14.0, 6.8, 4.8 Hz, 1H), 1.15 (m, 1H), 0.844 (s, 9H), 0.840 (s, 9H), 0.00 (d, J = 4.0 Hz, 6H), −0.05 (d, J = 4.0 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 213.1, 212.4, 140.4, 140.1, 119.05, 118.95, 65.7, 64.5, 59.1, 58.9, 53.2, 52.7, 32.1, 32.00, 31.96, 31.2, 30.8, 30.7, 29.6, 29.3, 28.44, 28.37, 26.4, 26.3, 25.8, 21.41, 21.38, 18.2, −5.59, −5.63; IR ($CHCl_3$) 3009, 2931, 2857, 1707, 1679, 1463, 1361, 1257, 1127, 788; LRMS (EI, rel intensity) 283 (M^+−t-Bu, 1), 251 (11), 223 (13), 138 (100), 75 (13); HRMS (EI) m/z calcd for $C_{19}H_{36}O_3Si$ (M^+) 283.1729, found 283.1728.

A 125-mL sulfonation (three-necked) flask was fitted with a gas inlet line, an outlet line with a stopcock, and a septum. To this flask was added solid methylene blue (ca. 5 mg) followed by a solution of the above ketone (200 mg, 0.585) in CH_2Cl_2 (60 mL). The resulting solution was cooled to −78 °C while UHP oxygen was passed through a drying column and bubbled (ca. 3 mL/s) through the solution. The reaction mixture was then irradiated with UV light (medium pressure Hg lamp) with continuous O_2 bubbling just until TLC analysis showed >95% consumption of starting material (ca. 1 h). After irradiation, an argon source was introduced through the septum, the outlet stopcock was closed, and the gas inlet line was replaced with a stopper. A −78 °C solution of t-$BuMe_2SiOTf$ (148 μL, 0.644 mmol) in CH_2Cl_2 (1.5 mL) was then added by cannula to this reaction mixture, which was still at −78 °C. The resulting solution was stirred for 8 h at −78 °C. At that time, the reaction was quenched by the addition, over 2 min, of Et_3N (268 μL, 1.93 mmol) via syringe. The mixture was allowed to warm to room temperature slowly over 10 h and was then concentrated under reduced pressure to a total volume of ca. 1 mL. The resulting syrup was purified by column chromatography (Florisil®, 1–10% EtOAc/hexanes) to give the desired product, a 1:1 mixture of diastereomers (ca. 140 mg, 0.375 mmol, 64%), as a yellow oil.

A solution of Bu_4NF (monohydrate, 62 mg, 0.24 mmol) in THF (0.60 mL) at 0 °C was added via cannula to a

solution of these trioxane silyl ethers (44 mg, 0.12 mmol) in THF (0.60 mL) at 0 °C. This mixture immediately turned to a yellow/brown color. The solution was stirred at 0 °C for 6 h. The reaction was then quenched with H_2O (1 mL). The resulting mixture was diluted with H_2O (5 mL) and ether (5 mL). The organic phase was separated, and the aqueous phase was extracted with ether (5 mL × 2). The organic portions were combined, washed with satd aq NaCl, dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. This crude product was purified by column chromatography (Florisil®, 1–20% EtOAc/hexane) to give the desired product, a 1:1 diastereomeric mixture (20 mg, 0.078 mmol, 66%), as a colorless oil. The $C_{4\beta}$-hydroxymethyl trioxane was separated from its $C_{4\alpha}$ diastereomer by HPLC (silica, 5% *i*-PrOH/hexanes, 3 mL/min, 230 nm, R_t = 16.2 min) to afford a white solid: mp 101–102 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.95 (d, J = 1.2 Hz, 1H), 3.70 (d ABq, J_d = 5.2 Hz, $\Delta\nu_{AB}$ = 28.4 Hz, J_{AB} = 10.8 Hz, 2H), 3.51 (s, 3H), 2.46 (m, 1H), 1.86 (m, 2H), 1.74–1.62 (m, 8H), 1.46 (s, 3H), 1.43 (br s, 1H), 1.28–1.15 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 106.0, 104.5, 82.7, 64.8, 57.2, 47.7, 45.8, 35.1, 31.0, 30.8, 24.9, 23.6, 22.8; IR ($CHCl_3$) 3623, 3011, 2936, 2861, 1446, 1144, 1224, 1021 cm^{-1}; LRMS (CI, rel intensity) 276 (M + NH_4^+, 64), 244 (24), 227 (100), 209 (45), 181 (47), 138 (8); HRMS (CI) *m/z* calcd for $C_{13}H_{22}O_5$ (M + NH_4^+) 276.1811, found 276.1815.

A one-dram vial was charged with the above $C_{4\beta}$-hydroxymethyl trioxane (8.3 mg, 0.032 mmol) and dissolved with CH_2Cl_2 (0.35 mL). Et_3N (ca. 5 μL, 0.04 mmol), terephthaloyl chloride (3.3 mg, 0.016 mmol), and DMAP (3.9 mg, 0.032 mmol) were added sequentially, via syringe, to this solution at room temperature. The mixture was stirred for 5 h at room temperature, quenched with H_2O (2 mL) and diluted with ether (3 mL). The phases were separated and the aqueous phase extracted with ether (2 × 3 mL). The organic portions were combined, washed with satd aq NaCl, dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. This crude product was purified by column chromatography (Florisil®, 1–10% EtOAc/hexane) to give the desired dimer product **13** (9.8 mg, 0.015 mmol, 94%) as a pale yellow oil. This sample was further purified by HPLC (silica, 20% CH_2Cl_2/hexanes, 3 mL/min, 240 nm, R_t = 7.1 min) to give an oily solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.13 (s, 4H), 4.99 (d, J = 1.2 Hz, 2H), 4.37 (d ABq, J_d = 5.2 Hz, $\Delta\nu_{AB}$ = 64.8 Hz, J_{AB} = 11.2 Hz, 4H), 3.54 (s, 3H), 2.77 (m, 2H), 1.95 (br q, J = 12 Hz, 2H), 1.86 (m, 2H), 1.79–1.62 (m, 14H), 1.48 (s, 6H), 1.29–1.15 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.5, 133.9, 129.6, 105.6, 104.2, 82.7, 67.0, 56.9, 45.8, 45.2, 35.2, 31.4, 30.7, 24.9, 23.6, 23.2; IR ($CHCl_3$) 3031, 2934, 2863, 1719, 1446, 1272, 1122, 1010 cm^{-1}; LRMS (CI, rel intensity) 664 (M + NH_4^+, 1), 604 (8), 544 (17), 364 (13), 198 (30), 181 (100); HRMS (CI) *m/z* calcd for $C_{34}H_{46}O_{12}$ (M + NH_4^+) 664.3333, found 664.3339.

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