Articles

Structure–Activity Relationships of Lactone Ring-Opened Analogs of the Antimalarial 1,2,4-Trioxane Artemisinin

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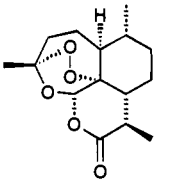
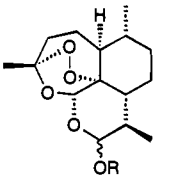
1,2,4-Trioxane benzylic ethers **8a–e** were prepared as simplified, tricyclic versions of the clinically used tetracyclic antimalarial drug artemisinin (**1**). Five additional artemisinin analogs (**9–11**) were prepared. Neither water solubility (analogs **8e** and **11b**) nor chelating ability (analogs **9** and **10**), however, produced trioxanes of especially high *in vitro* antimalarial activity. Trioxane fluorobenzyl ether **8b** is the most active in this series (more active than artemisinin) against *Plasmodium falciparum* parasites *in vitro*, with substantial activity also in mice infected with *Plasmodium berghei* parasites and with 10 times higher activity than artemisinin (**1**) in killing immature *P. falciparum* gametocytes.

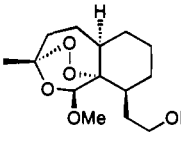
Multidrug resistance of malaria parasites to standard antimalarial drugs such as the alkaloids quinine and chloroquine is rapidly increasing.[1] Based on Chinese folk medicine, a new, nonalkaloidal, sesquiterpene lactone 1,2,4-trioxane has been isolated and identified;[2] natural endoperoxide artemisinin (qinghaosu, **1**) and its semisynthetic dihydro derivatives **2–5** have already cured well over one million Chinese malaria patients,[3] and clinical trials of arteether (**4**) are now being sponsored by the World Health Organization.[4] Also, clinical studies using suppositories containing water-soluble sodium artesunate (**5**) have produced impressive malaria cure rates.[5,6] Rational design and laboratory synthesis of structurally simpler analogs of tetracyclic artemisinin have led to various 1,2,4-trioxanes, some of which have excellent antimalarial activities.[7] For example, tricyclic trioxane primary alcohol **6**, prepared in only six steps from commercial cyclohexanone, was converted in one subsequent step into a series of 20 different ester and ether derivatives.[8] Preclinical *in vivo* evaluation of benzyl ether derivative **7** in *Aotus* monkeys infected with multidrug-resistant (MDR) *Plasmodium falciparum* malaria parasites revealed trioxane **7** to be curative at a dose of 48 mg/kg, without recrudescence after 6 months; this result matched that obtained with clinically used arteether (**4**) as a control.[9] On the basis of these promising findings, we have designed and synthesized a series of benzylic ethers, **8**, as potential next-generation antimalarials. Herein we record preparation and preliminary antimalarial testing of these benzylic ether tricyclic trioxanes **8** and some related trioxane analogs (**9–11**) as well as some generalizations based on qualitative structure–activity relationship (SAR) considerations.



1 (Artemisinin)



2, R = H (Dihydroartemisinin)
3, R = Me (Artemether)
4, R = Et (Arteether)
5, R = $COCH_2CH_2COONa$ (Sodium Artesunate)



6, R = H
7, R = CH_2Ph
8, R = CH_2Ar

Results and Discussions

The choice of the benzylic group was governed by the commercial availability of various benzylic halides for alkylation of (Williamson coupling[10] with) trioxane primary alcohol **6** (eq 1) and especially by the desire to incorporate a lipophilic group (**8a**), a fluorine-containing aromatic group (**8b**), and a nitrogen-containing heteroaromatic group (**8c,d**) that could be quaternized to provide a water-soluble analog (**8e**). Incorporation of a lipophilic group has been noted previously to promote antimalarial activity,[2,8] possibly due to improved transport in a living organism (human or parasite). Incorporation of a fluorine atom was based generally on the conventional wisdom that a fluorine substituent, similar in size to a hydrogen atom but much more polar, often

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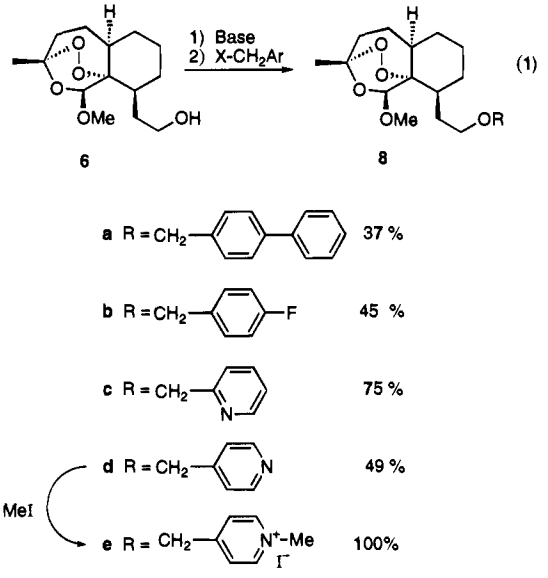
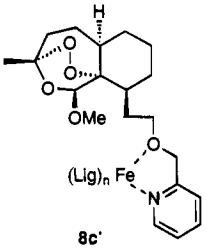
Table 1. Antimalarial Activities of Benzylic Ether Trioxanes

Trioxane R		IC_{50} (ng/ml) D-6 Clone	IC_{50} (ng/ml) W-2 Clone
-CH$_2$-Ph	(**7**)	8	3
-CH$_2$-Ph-Ph	(**8a**)	6	6
-CH$_2$-Ph-F	(**8b**)	3	4.5
-CH$_2$-(2-pyridyl)	(**8c**)	18	7
-CH$_2$-(4-pyridyl)	(**8d**)	10	7.5
-CH$_2$-(4-N$^+$-Me pyridyl) I$^-$	(**8e**)	--	85
Artemisinin		8	8

increases biological potency without increasing toxicity;[11] specifically, some fluorine-containing trioxanes have recently been shown to be very promising antimalarials.[12] Finally, water solubility of a new antimalarial drug candidate is desirable so that it can be administered intravenously for rapid action especially against severe cerebral malaria.[13] Indeed, quaternary ammonum salt **8e** is water-soluble.



Preliminary *in vitro* antimalarial evaluation of these *O*-benzylic trioxanes (Table 1) allows several qualitative SAR generalizations, as follows: (1) except for water-soluble pyridinium salt **8e**, all of these trioxane benzylic ethers compare favorably in antimalarial activity with artemisinin; (2) the additional lipophilicity provided by the biphenyl group in trioxane **8a** does not improve antimalarial activity relative to the phenyl system of parent trioxane benzyl ether **7**; (3) the position of the nitrogen atom in pyridyl derivatives **8c,d** does not change their antimalarial activity in a substantial way, even though the 2-pyridyl system of trioxane **8c** could, in principle, coordinate in a bidendate fashion[14] more effectively with a Lewis acidic iron species (see **8c'**) than



could 4-pyridyl **8d** and thus could position the reducing iron entity near the peroxide linkage of the trioxane ring system, thereby triggering free radical formation;[15,16] and (4) *p*-fluorobenzyl trioxane **8b**, the most active in this series of second-generation benzyl ether analogs, is considerably more active than artemisinin in the W-2 clone and especially in the African Sierra Leone D-6 clone of malarial parasites that is resistant to mefloquine but sensitive to chloroquine, thereby making fluorobenzyl trioxane **8b** a possible drug candidate for further *in vivo* evaluation as an antimalarial blood schizontocide for malaria chemotherapy.

p-Fluorobenzyl trioxane **8b** stands out among these tricyclic artemisinin analogs also as a potent gametocytocidal agent.[17] This *in vitro* stage-specific effect of trioxane **8b** against young *P. falciparum* gametocytes, as determined according to the previously described protocol,[17] is about 10 times higher than that observed with trioxanes **7** and **8a,c** as well as about 10 times higher than that observed with artemisinin (1), itself a potent gametocytocidal agent[17](Figure 1). Thus, fluorobenzyl trioxane **8b** may turn out to be useful also as a chemopreventive agent for interrupting transmission of malaria from humans to mosquitoes.

p-Fluorobenzyl trioxane **8b** also showed significant *in vivo* antimalarial activity when administered to mice subcutaneously (but not orally) as a single daily dose for 4 days against the drug-sensitive N strain of *Plasmodium berghei* malaria parasites according to the protocol described previously.[18] Results allowed determination of ED_{50} and ED_{90} (mg/kg) values as follows: trioxane **8b**, 7.6 and 13.5; arteether (**3**), 0.3 and 0.5; and sodium artesunate (**5**), 1.5 and 4.9. Thus, trioxane **8b** has significant antimalarial activity against a second species of *Plasmodium* malaria parasites.

Two additional series of artemisinin analogs were studied. First, carboxylate esters **9a,b** and sulfonate ester **10** were selected because they possess one or more potentially chelating nitrogen atoms[14] for sequestering the iron needed by the parasite for its survival and/or for localizing a reducing iron species near the peroxide linkage of the trioxane ring system; none of these analogs, however, showed *in vitro* antimalarial activity against the W-2 clone of *P. falciparum* of more than $^1/_6$ that of artemisinin. Second, as members of a new subgroup of our tricyclic trioxanes, tertiary amine analog **11a** had about $^1/_3$ the *in vitro* antimalarial activity of artemisinin in both the W-2 and D-6 clones, whereas its water-soluble quaternary ammonum salt **11b** was inactive.

In conclusion, a series of trioxane benzylic ethers has been designed and synthesized as structurally simplified, tricyclic versions of the clinically used antimalarial tetracyclic trioxane artemisinin. Among these, trioxane *p*-fluorobenzyl ether **8b** stands out as the most active *in vitro* against *P. falciparum*, with considerable activity

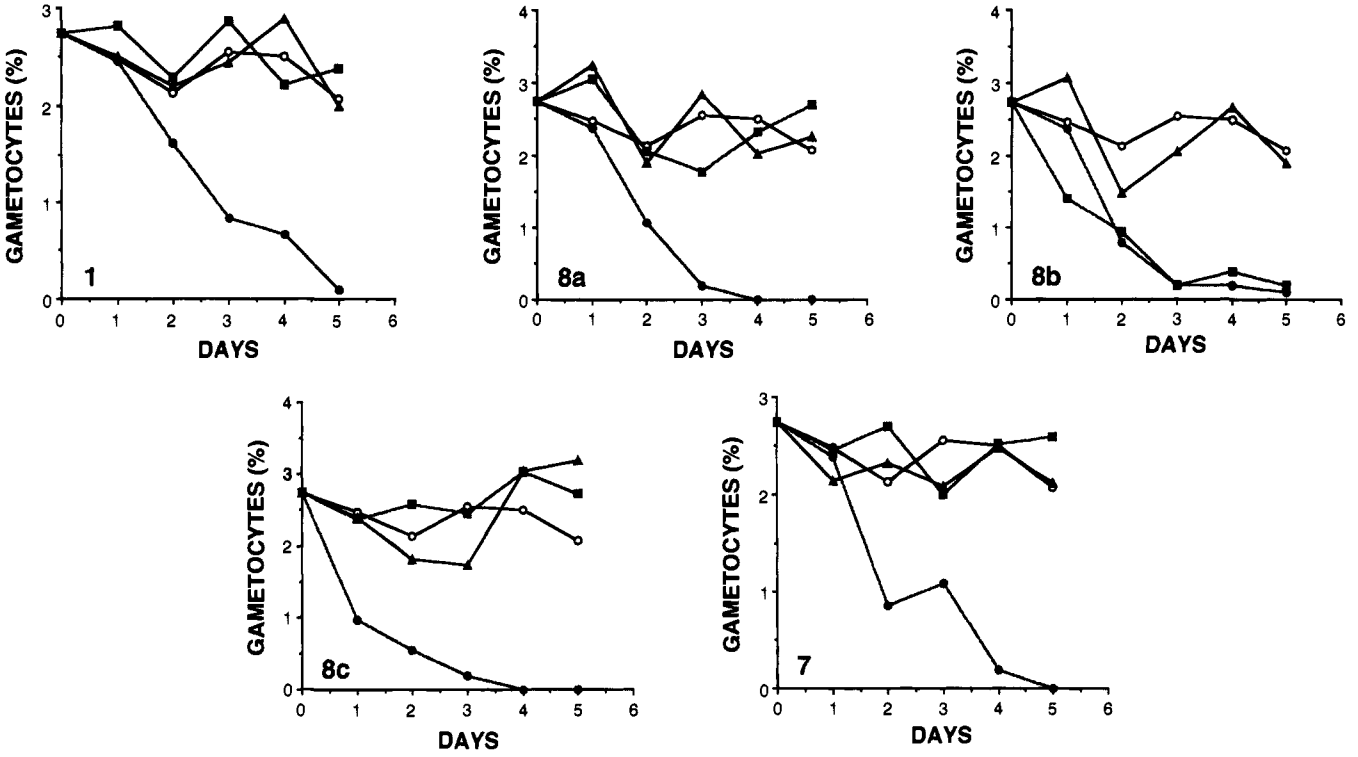
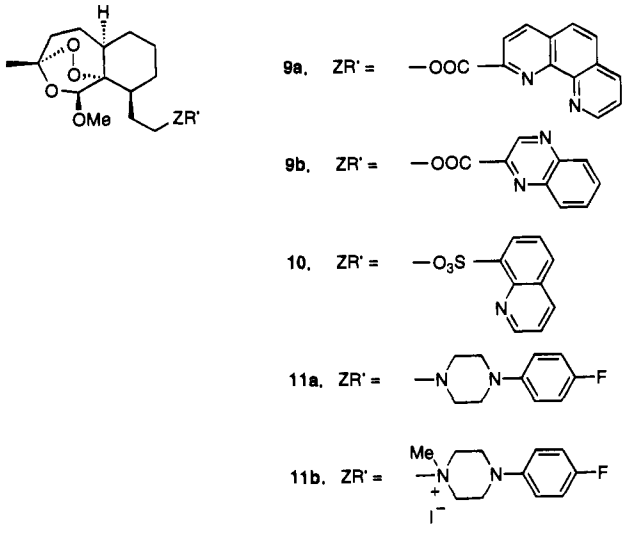


Figure 1. Effect of trioxanes on young gametocytes (day 9 in culture) of *P. falciparum* (NF54). Parasites were treated with DMF solvent control (open circles), 0.1 (closed circles), 0.01 (closed squares), and 0.001 (closed triangles) μM concentration of each drug. The experimental details are exactly as in ref 17.



also in mice infected with *P. berghei* and with 10 times higher activity than artemisinin in killing immature *P. falciparum* gametocytes. Several potentially chelating derivatives (i.e., 2-pyridyl system **8c**, heteroaromatic carboxylates **9**, and quinolenesulfonate **10**) and two water-soluble quaternary ammonum salts (i.e., **8e** and **11b**) are not especially active antimalarials *in vitro*. These qualitative SAR results may help design of better trioxane drugs for chemoprevention and chemotherapy of malaria.

Experimental Section

General. Melting points were determined with a Thomas-Hoover capillary melting point apparatus and are uncorrected. Infrared spectra (IR) were recorded on a Perkin-Elmer 1600 FT-IR spectrometer. Varian XL 300 and 400 spectrometers were employed for ^1H and ^{13}C NMR spectra with tetramethylsilane or chloroform as an internal reference. Resonances, in δ units downfield from internal Me_4Si, are noted as singlet (s), doublet (d), triplet (t), or multiplet (m). Mass spectra were recorded at 70 eV electron energy with a VG analytical 70-S mass spectrometer. Silica gel 60 (70–230 mesh; Merck) and Florisil (200 mesh; Aldrich) were used for column chromatography. Analytical thin-layer chromatography was performed by using Merck 60 F254 silica gel (precoated sheets, 0.2 mm thick). Dichloromethane was freshly distilled from calcium hydride, and diethyl ether and tetrahydrofuran were distilled from sodium benzophenone ketyl prior to use. All other compounds, unless noted, were purchased from Aldrich Chemical Co. Yields are not optimized. Purity of products was judged to be >95% on the basis of their chromatographic homogeneity.

Trioxane 8a. Trioxane alcohol **6** (56.2 mg, 0.21 mmol) was stirred in 3 mL of dry *N,N*-dimethylformamide at 0 °C under argon. Potassium hydride (70 mg of 40% KH, 0.68 mmol, in mineral oil) was added. After stirring for 10 min, *p*-phenylbenzyl bromide[19] (254.3 mg, 1.04 mmol) was added. After 13 h at room temperature, the reaction was quenched at −78 °C with 3 mL water, and the mixture warmed to room temperature. The mixture was washed with water and then brine, dried with $MgSO_4$, filtered, and evaporated under reduced pressure. The residue was purified by column chromatography to give trioxane **8a** (33.6 mg, 37%) as a yellow oil: FT-IR ($CHCl_3$, cm^{-1}) 3019, 2930, 2860, 1600, 1488; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.58 (m, 5H), 7.45 (m, 3H), 7.35 (m, 1H), 5.16 (d, J = 1.2 Hz, 1H), 4.55 (ABq, J_{ab} = 12.0 Hz, $\Delta\nu$ = 22.8 Hz, 2H), 3.57 (m, 2H), 3.49 (s, 3H), 2.31 (m, 2H), 2.05 (m, 1H), 2.00–1.40 (m, 10H), 1.38 (s, 3H), 1.26 (m, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 141.01, 140.45, 137.66, 128.72, 128.14, 127.19, 127.12, 127.10, 105.17, 100.34, 85.38, 72.39, 69.28, 56.72, 48.58, 42.19, 37.52, 31.10, 29.92, 29.54, 27.17, 25.97, 25.26; LRMS (EI, rel intensity) 411 (30), 371 (24), 167 (21), 159 (39), 149 (43), 91 (28); HRMS (CI, NH_3) calcd for $C_{27}H_{38}NO_5$ (M + 18) 456.2750, found 456.2759.

Trioxane 8b. Trioxane alcohol **6** (25.6 mg, 0.09 mmol) was stirred in 4 mL of dry *N,N*-dimethylformamide at 0 °C under argon. The solution was cannulated onto potassium hydride (60 mg of 40% KH, 0.58 mmol, in mineral oil, washed with hexane). After 10 min *p*-fluorobenzyl bromide (60 μL, 0.48 mmol) was added via syringe. The reaction was quenched 55

min later with 6 mL water and the mixture extracted three times with ether, washed with brine, dried with $MgSO_4$, filtered, and evaporated under reduced pressure. The crude product was separated using column chromatography to afford trioxane **8b** (16.2 mg, 45%) as a colorless oil: FT-IR ($CHCl_3$, cm^{-1}) 3003, 2931, 2862, 1604, 1510; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.29 (m, 2H), 7.01 (m, 2H), 5.15 (d, J = 1.2 Hz, 1H), 4.46 (ABq, J_{ab} = 11.6 Hz, $\Delta\nu$ = 19.2 Hz, 2H), 3.53 (t, J = 10.8 Hz, 2H), 3.48 (s, 3H), 2.30 (m, 2H), 2.01 (m, 1H), 1.85–1.45 (m, 8H), 1.38 (s, 3H), 1.35–1.15 (m, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 162.23 (d, J = 243.5 Hz), 134.31 (d, J = 3.8 Hz), 129.38 (d, J = 8.4 Hz), 115.14 (d, J = 21.2 Hz), 105.14, 100.27, 85.33, 71.92, 69.17, 48.56, 42.11, 37.50, 31.08, 29.86, 29.58, 29.51, 27.15, 25.96, 25.23; LRMS (CI, NH_3, rel intensity) 398 (M + 18, 17), 255 (18), 239 (29), 207 (40), 195 (70), 137 (100), 109 (35); HRMS (CI, NH_3) calcd for $C_{21}H_{33}O_5NF$ (M + 18) 398.2343, found 398.2340.

Trioxane 8c. 2-Picolyl chloride hydrochloride (16.2 mg, 0.06 mmol) was added to potassium hydride (60 mg of 40% KH, 0.58 mmol, in mineral oil, washed with hexane). Trioxane alcohol **6** (5.4 mg, 0.02 mmol) was added via syringe in 2.5 mL of *N,N*-dimethylformamide. After stirring at 0 °C for 20 min, the reaction was quenched with 3 mL water and the mixture washed with brine, dried with $MgSO_4$, filtered, and evaporated under reduced pressure. Column chromatography (50% ethyl acetate/hexane) produced trioxane **8c** (5.4 mg, 75%) as a yellow oil: FT-IR ($CHCl_3$, cm^{-1}) 3008, 2928, 2856, 1595, 1478; ^1H NMR ($CDCl_3$, 300 MHz) δ 8.54 (d, J = 4.7 Hz, 1H), 7.69 (dt, J = 1.6, 7.7 Hz, 1H), 7.44 (d, J = 7.8 Hz, 1H), 7.17 (m, 1H), 5.16 (d, J = 0.8 Hz, 1H), 4.63 (ABq, J_{ab} = 13.5 Hz, $\Delta\nu$ = 17.1 Hz, 2H), 3.62 (m, 2H), 3.49 (s, 3H), 2.31 (m, 2H), 2.00 (m, 1H), 1.80–1.40 (m, 8H), 1.37 (s, 3H), 1.35–1.05 (m, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 158.66, 148.85, 136.47, 122.11, 121.21, 105.00, 100.14, 85.17, 73.52, 69.86, 56.56, 48.44, 42.06, 37.37, 30.95, 29.82, 29.38, 27.02, 25.82, 25.11; LRMS (CI, NH_3, rel intensity) 364 (M + 1, 29), 305 (20), 304 (100), 260 (16), 151 (18), 93 (17); HRMS (CI, NH_3) calcd for $C_{20}H_{30}NO_5$ (M + 1) 364.2124, found 364.2129.

Trioxane 8d. 4-Picolyl chloride hydrochloride (52.0 mg, 0.32 mmol) was dissolved in a mixture of saturated sodium bicarbonate solution (5 mL) and ether (10 mL) in a separatory funnel. The resulting mixture was vigorously shaken to allow clean two layers to be formed. The ether layer was separated, washed with saturated sodium chloride solution (5 mL), dried over anhydrous magnesium sulfate, filtered, and evaporated to give pure 4-picolyl chloride. The chloride was dissolved in dry DMF (2 mL) and cannulated into a 5 mL flask containing trioxane alcohol **6** (12.2 mg, 0.04 mmol). To the mixture at 0 °C was added sodium hydride (60% in mineral oil, 14 mg, 0.35 mmol). The final reaction mixture was cooled to 0 °C, treated with water (10 mL), and extracted twice with ether (20 mL × 2). The combined ether solution was washed with saturated sodium chloride solution (10 mL), dried over anhydrous $MgSO_4$, filtered, and evaporated under reduced pressure. The residue was separated on column chromatography to give trioxane **8d** (7.9 mg, 49%): R_f = 0.3 (50:50 hexane:ethyl acetate); FT-IR (neat, cm^{-1}) 2929, 1562, 1467, 1377, 1210; ^1H NMR (400 MHz, $CDCl_3$) δ 8.58 (d, J = 5.8 Hz, 2H), 7.37 (d, J = 5.8 Hz, 2H), 5.16 (d, J = 0.8 Hz, 1H), 4.59 (d, J = 14.0 Hz, 1H), 4.54 (d, J = 14.0 Hz, 1H), 3.60 (dd, J = 7.60, 5.62 Hz, 2H), 3.50 (s, 3H), 2.34 (dd, J = 14.4, 3.6 Hz, 1H), 2.29 (ddd, J = 9.6, 8.0, 6.0 Hz, 1H), 2.02 (ddd, J = 14.4, 3.6, 2.8 Hz, 1H), 1.90–1.51 (m, 8H), 1.38 (s, 3H), 1.43–1.18 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 150.07, 148.10, 148.00, 122.19, 105.23, 100.18, 85.34, 70.70, 69.89, 56.69, 48.58, 42.00, 37.49, 31.04, 29.89, 29.69, 29.56, 27.11, 25.95, 25.23; HRMS *m/e* calcd for $C_{20}H_{30}NO_5$ (M + 1) 364.2124, found 364.2126.

Trioxane Salt 8e. A 0.5 dram vial was weighed and charged with a $CDCl_3$ solution of the 1,2,4-trioxane **8d**. Evaporation of the above solution under a vacuum pump for 3 h gave the pure amine **8d** in 0.8 mg. The vial was charged with a small stir bar and dry THF (0.3 mL). To the above THF solution (0.8 mg, 0.002 mmol) was injected iodomethane (10 mL, 0.16 mmol) via a gas-tight syringe. After being stirred for 4 h, the reaction mixture was concentrated to give trioxane salt **8e** as an orange oil quantitatively: ^1H NMR (400 MHz, $CDCl_3$) δ 9.07 (d, 2H), 8.02 (d, J = 6.8 Hz, 2H), 5.16 (br s, 1H), 4.80 (br s, 2H), 4.66 (s, 3H), 3.75 (m, 2H), 3.51 (m, 3H), 2.36–2.26 (m, J = 2 Hz), 2.05–1.95 (m, 1H), 1.88–1.83 (m, 2H), 1.79–1.52 (m, 9H), 1.39 (s, 3H), 1.34–1.20 (m, 3H).

Trioxane Carboxylate Ester 9a. To a dichloromethane (3.0 mL) solution of 1,10-phenanthroline-2-carbonyl chloride (70 mg, 0.29 mmol) was added triethylamine (150 μL, 1.1 mol) at 0 °C under argon atmosphere. After being stirred for 10 min, the resulting solution was treated with 1 mL of a dichloromethane solution of the trioxane alcohol **6** (24.5 mg, 0.09 mmol). The resulting solution was stirred for 3 h at room temperature and concentrated under reduced pressure. The residue was separated on column chromatography to give the corresponding carboxylate derivative (15.6 mg, 36%) as a waxy solid. Recrystallization from ether gave a white solid: mp 110–113 °C; R_f = 0.4 (methanol:CH_2Cl_2 = 1:10); FT-IR (neat, cm^{-1}) 1718, 1618, 1560; ^1H NMR (400 MHz, $CDCl_3$) δ 9.34 (dd, J = 4.0, 1.0 Hz, 1H), 8.44 (d, J = 10 Hz, 1H), 8.41 (d, J = 10.8 Hz, 1H), 8.37 (dd, J = 8.0, 1.2 Hz, 1H), 7.93 (d, J = 8.8 Hz, 1H), 7.89 (d, J = 8.8 Hz, 1H), 7.74 (dd, J = 8.2, 4.4 Hz, 1H), 5.26 (s, 1H), 4.59 (t, J = 7.8 Hz, 2H), 3.54 (s, 3H), 2.50 (m, 1H), 2.33 (m, J = 14.4, 13.2, 3.6 Hz, 1H), 2.05–1.99 (m, 1H), 1.88–1.52 (m, 7H), 1.44–1.38 (m, 1H), 1.38 (s, 3H), 1.31–1.22 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.46, 150.62, 148.17, 145.83, 145.42, 137.10, 136.34, 130.16, 128.90, 128.65, 126.04, 123.44, 123.42, 105.21, 100.18, 85.23, 65.70, 56.83, 48.56, 42.48, 37.49, 31.02, 29.79, 29.41, 27.13, 25.93, 25.20; HRMS (CI) calcd for $C_{27}H_{31}N_2O_6$ (M + 1) 479.2182, found 479.2187.

Trioxane Carboxylate Ester 9b. A dichloromethane (1.0 mL) solution of the trioxane alcohol **6** (18.4 mg, 0.07 mmol) and triethylamine (150 μL, 1.1 mol) was cooled to 0 °C and treated with 2-quinoxaloyl chloride (34.1 mg, 0.18 mmol) under argon atmosphere. The resulting solution was stirred for 2 h at room temperature and concentrated under reduced pressure. The residue was separated on column chromatography to give the corresponding carboxylate derivative **9b** (16.1 mg, 57%): R_f = 0.7 (ethyl acetate:hexane = 1:1); FT-IR (neat, cm^{-1}) 1745, 1718, 1569; ^1H NMR (400 MHz, $CDCl_3$) δ 9.55 (s, 1H), 8.32 (dd, J = 8.0, 2.0 Hz, 1H), 8.19 (J = 8.4, 1.6 Hz, 1H), 7.89 (m, 2H), 5.24 (s, 1H), 4.61 (m, 2H), 3.53 (s, 3H), 2.46 (m, 1H), 2.33 (ddd, J = 14.4, 14.0, 3.6 Hz, 1H), 2.02 (ddd, J = 14.4, 4.4, 2.8 Hz, 1H), 1.90–1.76 (m, 2H), 1.73–1.54 (m, 6H), 1.46–1.39 (m, 6H), 1.38 (s, 3H), 1.28 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.06, 145.08, 143.65, 142.69, 141.57, 132.24, 130.91, 130.67, 129.31, 105.27, 100.05, 85.16, 65.77, 56.73, 48.55, 42.43, 37.45, 30.97, 29.75, 29.46, 27.09, 25.90, 25.17; HRMS (CI) calcd for $C_{23}H_{28}N_2O_6$ (M + 1) 428.1947, found 428.1952.

Trioxane Sulfonate Ester 10. A dichloromethane (2.0 mL) solution of the trioxane alcohol **6** (29.3 mg, 0.11 mmol) and triethylamine (250 μL, 1.8 mol) was cooled to 0 °C and treated with 8-quinolinesulfonyl chloride (51 mg, 0.22 mmol) under argon atmosphere. The resulting solution was refluxed for 2 h and concentrated under reduced pressure. The residue was separated on column chromatography to give the corresponding sulfonate derivative **10** (17.2 mg, 34%): R_f = 0.5 (ethyl acetate:hexane = 1:1); FT-IR (neat, cm^{-1}) 1594, 1375, 1208; ^1H NMR (400 MHz, $CDCl_3$) δ 9.02 (m, 1H), 8.36 (m, 1H), 8.26 (m, 1H), 8.02 (m, 1H), 7.74 (m, 1H), 7.52 (m, 1H), 5.04 (d, J = 1.2 Hz, 1H), 4.97 (d, J = 1.2 Hz, 1H), 4.27 (m, 2H), 3.85 (m, 2H), 3.43 (s, 3H), 3.40 (s, 3H), 2.29–2.10 (m, 2H), 2.00–1.94 (m, 1H), 1.80–1.00 (m, 9H), 1.38 (s, 3H), 1.37 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 150.90, 145.04, 136.14, 132.02, 128.39, 126.81, 126.66, 126.04, 122.04, 105.10, 100.02, 85.03, 65.23, 56.64, 48.45, 42.04, 37.40, 30.09, 30.29, 29.37, 27.07, 25.88, 25.11.

Trioxane Amine 11a. A benzene (5.0 mL) solution of the trioxane tosylate derived from trioxane alcohol **6** (44.0 mg, 0.10 mmol) and *N*-(*p*-fluorophenyl)piperazine (150 mg, 0.83 mol) was refluxed for 5 h under argon atmosphere. The resulting solution was cooled to room temperature, treated with saturated sodium bicarbonate solution (10 mL), and extracted twice with ether (20 mL × 2). The combined ether solution was washed with saturated sodium chloride solution (10 mL), dried over anhydrous $MgSO_4$, filtered, and evaporated under reduced pressure. The residue was separated on column chromatography to give the trioxane amine **11a** (34.5 mg, 79%): R_f =

0.4 (ethyl acetate:hexane = 1:1); FT-IR (neat, cm^{-1}) 2928, 1510, 1451, 1234, 1122, 1077, 1008; ^1H NMR (400 MHz, $CDCl_3$) δ 6.97–6.92 (m, 2H), 6.89–6.84 (m, 2H), 5.15 (d, J = 1.2 Hz, 1H), 3.49 (s, 3H), 3.12 (t, J = 4.8 Hz, 4H), 2.63 (m, 4H), 2.52 (m, 1H), 2.38–2.26 (m, 2H), 2.22 (m, 1H), 2.01 (ddd, J = 14.8, 4.8, 2.8 Hz, 1H), 1.90–1.46 (m, 8H), 1.37 (s, 3H), 1.28–1.17 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.06 (d, J = 238.8 Hz), 147.95 (d, J = 2.3 Hz), 117.71 (d, J = 7.6 Hz), 115.42 (d, J = 21.3 Hz), 105.12, 100.30, 85.50, 57.26, 56.69, 53.24, 50.08, 48.54, 43.14, 37.49, 31.08, 29.06, 27.12, 26.67, 25.95, 25.26; HRMS (CI) calcd for $C_{24}H_{35}FN_2O_4$ (M + 1) 434.2581, found 434.2586.

Quaternary Amine Salt 11b. A clean 0.5 dram vial was weighed exactly and charged with a $CDCl_3$ solution of the 1,2,4-trioxane amine **11a**. Evaporation of the above solution under a vacuum pump for 3 h gave pure the amine **11a** in 11.2 mg. The vial was charged with a small clean stir bar and dry THF (0.5 mL). To the above THF solution of the amine (11.2 mg, 0.026 mmol) was injected iodomethane (20 mL, 0.32 mmol) via a gas-tight syringe. When the final solution was stirred at room temperature for 1 h, the white precipitate started to form. After being stirred for 9 h, the reaction mixture was concentrated to give a white solid quantitatively: ^1H NMR (400 MHz, $CDCl_3$) δ 7.02 (t, J = 8.6 Hz, 2H), 6.94 (dd, J = 9.2, 4.8 Hz, 2H), 5.13 (s, 1H), 4.09 (m, 2H), 3.93 (m, 1H), 3.83 (m, 1H), 3.68–3.56 (m, 2H), 3.551 (s, 3H), 3.545 (s, 3H), 3.51–3.44 (m, 4H), 2.28 (m, 2H), 2.06–1.99 (m, 1H), 1.87–1.66 (m, 6H), 1.64–1.50 (m, 2H), 1.38 (s, 3H), 1.32–1.22 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.34 (d, J = 241.8 Hz), 145.27 (d, J = 2.3 Hz), 119.04 (d, J = 7.5 Hz), 116.11 (d, J = 22.8 Hz), 105.58, 99.38, 85.32, 64.27, 60.99, 59.89, 56.97, 48.56, 47.59, 44.53 (2), 43.76, 37.40, 30.69, 29.83, 26.85, 25.83, 24.95, 22.80.

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