Extraordinarily Potent Antimalarial Compounds: New, Structurally Simple, Easily Synthesized, Tricyclic 1,2,4-Trioxanes†

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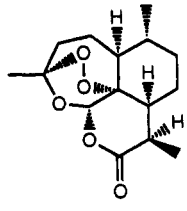
New, racemic, tricyclic trioxane alcohol **3** was designed and synthesized as a structurally simple analog of clinically useful, tetracyclic, antimalarial artemisinin. A series of 20 ester and ether derivatives of alcohol **3** were prepared easily, without destruction of the essential trioxane system. Chemical structure–antimalarial activity for each derivative was evaluated in vitro against chloroquine-resistant and chloroquine-sensitive *Plasmodium falciparum* parasites. Many of these derivatives were highly efficacious; carboxylate ester **9f**, carbamate ester **10a**, and sulfonate ester **12a** had antimalarial potency similar to that of artemisinin, and carboxylate esters **9b** and **9d**, carbamate esters **10b** and **10c**, and phosphate esters **11a–c** had antimalarial potency up to 7 times higher than that of artemisinin. Several of these most active analogs (e.g., carboxylate **9b** and carbamates **10a** and **10c**) are stable crystalline solids, a feature of considerable practical value for any new drug candidate.

Malaria is one of the most deadly diseases, affecting millions of people especially in developing countries.[1] Although alkaloids such as quinine and, more recently, chloroquine and mefloquine have been effective as antimalarial drugs, considerable resistance has been developed by malarial parasites to such alkaloids.[2,3] The rapidly increasing urgency, therefore, for discovery and design of nontraditional antimalarials has led to isolation and identification in China of a non-nitrogen-containing sesquiterpene lactone peroxide from the plant *Artemisia annua*;[4] named artemisinin (qinghaosu, **1**), this unusual 1,2,4-trioxane as well as esters and ethers of the corresponding lactol **2** have been used clinically in China,[5] and



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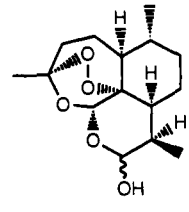

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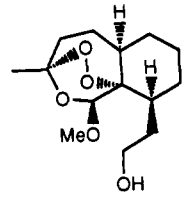
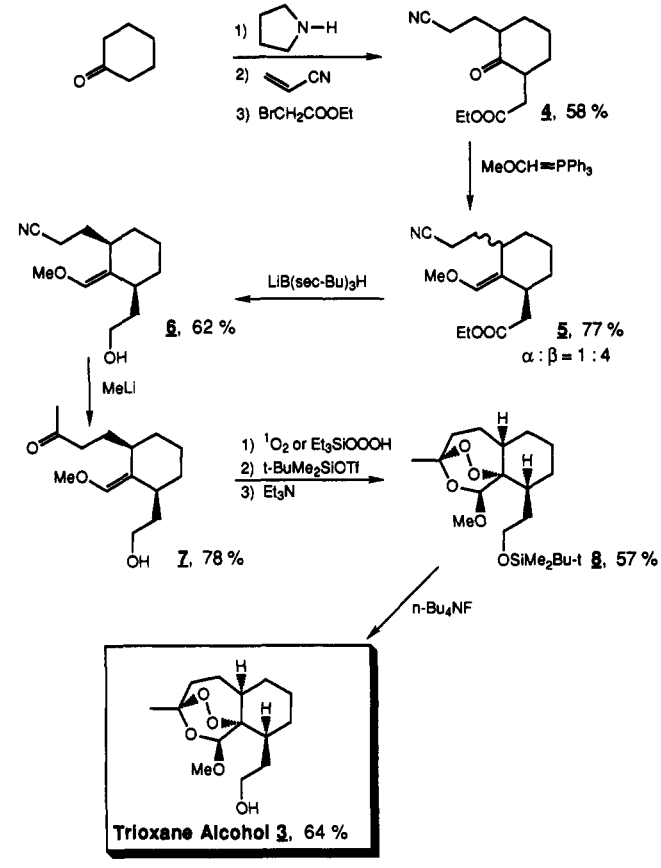


3

are being developed internationally, as potent and rapidly acting antimalarials.[6] Rational design of structurally simpler analogs of artemisinin has led to synthesis of various trioxanes, some of which possess excellent antimalarial activity.[7] Following Jefford's discovery that 1,2,4-trioxanes can be prepared via rearrangements of ketodioxetanes,[8] and based on our interest in dioxetanes,[9] we have begun a research program aimed at exploring chemical structure–antimalarial activity relationships in relatively unadorned trioxanes related structurally to artemisinin. Herein we report on the chemistry and biology of a series of new, structurally simple, easily prepared, racemic 1,2,4-trioxanes that are *tricyclic* (lacking the lactone ring present in *tetracyclic* artemisinin) and that are derivatives of trioxane alcohol **3** having the relative ster-

Scheme I



eochemistry shown as determined by single-crystal X-ray crystallography. Especially attractive features of trioxane

† These trioxanes are the subject of a pending U.S. patent application.

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(1) (a) Bruce-Chwatt, L. J. *Chemotherapy of Malaria*, 2nd ed.; WHO: Geneva, 1981; Chapter 5, p 104. (b) Wyler, D. J. Malaria-resurgence, resistance and research. *N. Engl. J. Med.* **1983**, *308*, 875–878 and 934–940. (c) Davidson, G. *New Sci.* **1982**, *96*, 1117.

(2) Wernsdorfer, W. H.; Kouznetsov, R. L. Drug-resistant malaria-occurrence, control, and surveillance. *Bull. WHO* **1980**, *58*, 341–352.

(3) (a) Peters, W. *Parasitology* **1985**, *90*, 705. (b) Moran, J. S.; Bernard, K. W. *J. Am. Med. Assoc.* **1989**, *262*, 245.

(4) For an excellent review with leading references, see: Klayman, D. L. Quinghaosu (Artemisinin): An antimalarial drug from China. *Science* **1985**, *228*, 1049–1055.

(5) (a) China Cooperative Research Group on Qinghaosu and its Derivatives as Antimalarials. Clinical studies on the treatment of malaria with Quinghaosu and its derivatives. *J. Trad. Chin. Med.* **1982**, *2*, 45–50. (b) Li, G. Q. *Chung Hua I Hsueh Tsa Chih* **1982**, *62*, 293.

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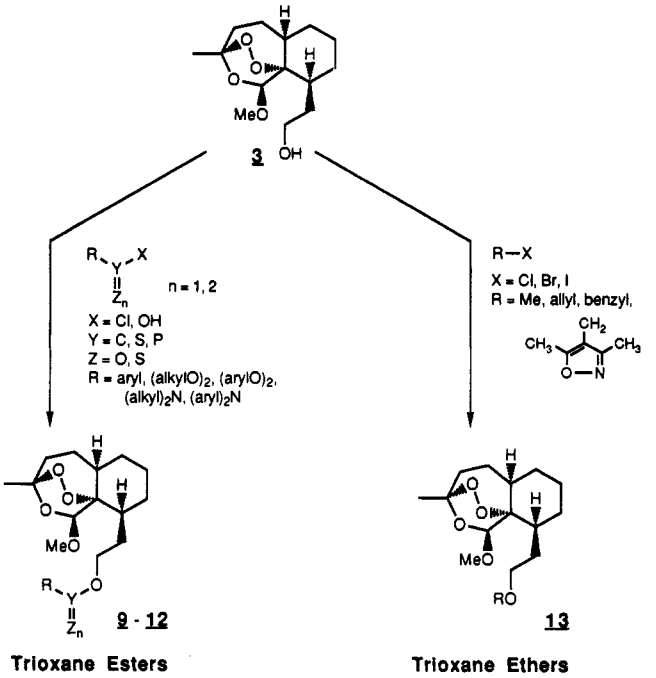
alcohol 3 are the following: (1) its straightforward and easy preparation from cheap and readily available stating materials, (2) its availability on gram scale, and (3) its easy one-step conversion, using standard chemical transformations, into alcohol derivatives such as esters and ethers, without destruction of the crucial trioxane framework.

Scheme II



Chemistry

As shown in Scheme I, trioxane alcohol 3 was prepared in only six separate operations starting with inexpensive and commercially available cyclohexanone. One-flask enamine alkylation with acrylonitrile, then in situ formation of the less-substituted enamine, and finally alkylation with ethyl bromoacetate produced 2,6-disubstituted cyclohexanone 4 in a novel and efficient one-flask process.[10] Wittig methoxymethylenation gave enol ester 5 as a 4:1 mixture of diastereomers.[11] Ester reduction provided mainly alcohol isomer 6 that underwent chemospecific addition of methyllithium to the nitrile group to afford keto olefin 7. Conversion of this electron-rich olefin into the corresponding ketodioxetane was achieved with both $Et_3SiOOOH$[9] and with $^1O_2$ generated photochemically;[8] using the $Et_3SiOOOH$ procedure, dioxetane formation was complete in less than 1 min at −78 °C as indicated by 400-MHz ^1H NMR showing disappearance of starting

(6) (a) Shmuklarsky, M. J.; Klayman, D. L.; Milhous, W. K.; Rossa, R. N.; Ager, A. L., Jr.; Tang, D. B.; Heiffer, M. H.; Canfield, C. J.; Schuster, B. G. Comparison of β-arteether against malaria parasites in vitro and in vivo. *Am. J. Trop. Med. Hyg.* **1992**, submitted. (b) Tripathi, R.; Dutta, G. P.; Vishwakarma, R. A. Comparison of antimalarial efficacy of α,β and α/β arteether against *Plasmodium cynomolgi* B infection in monkeys. *Am. J. Trop. Med. Hyg.* **1991**, *44*, 560–563.

(7) (a) Imakura, Y.; Yokoi, T.; Yamagishi, T.; Koyama, J.; Hu, H.; McPhail, D. R.; McPhail, A. T.; Lee, K.-H. Synthesis of Desethanoqinghaosu, a novel analogue of the antimalarial Qinghaosu. *J. Chem. Soc., Chem. Commun.* **1988**, 372–374. (b) Ye, B.; Wu, Y.-L. An efficient synthesis of Qinghaosu and Deoxoqinghaosu from Arteannuic acid. *J. Chem. Soc., Chem. Commun.* **1990**, 726–727. (c) Jung, M.; ElSohly, H. N.; Croom, E. M. Practical conversion of Artemisinic acid into Desoxyartemisinin. *J. Org. Chem.* **1986**, *51*, 5417–5419. (d) Roth, R. J.; Acton, N. *J. Nat. Prod.* **1989**, *52*, 1183. (e) Lin, A. J.; Li, L-Q.; Klayman, D. L.; George, C. F.; Flippen-Anderson, J. L. Antimalarial activity of new water-soluble Dihydroartemisinin derivatives. 3. Aromatic amine analogues. *J. Med. Chem.* **1990**, *33*, 2610–2614. (f) Ravindranathan, T.; Kumar, M. A.; Menon, R. B.; Hiremath, S. V. Stereoselective synthesis of Artemisinin. *Tetrahedron Lett.* **1990**, *31*, 755–758. (g) Haynes, R. K.; Von Willer, S. C. Catalyzed oxygenation of allylic hydroperoxides derived from Qinghao (Artemisinic) acid. Conversion of Qinghao acid into Dehydroqinghaosu (Artemisitene) and Qinghaosu (Artemisinin). *J. Chem. Soc., Chem. Commun.* **1990**, 451–453. (h) Lin, A. J.; Klayman, D. L.; Milhous, W. K. Preparation of Dihydroartemisinin derivatives as antimalarial agents. U.S. Pat. Appl. US 87,365, 1988; *Chem. Abstr.* **1988**, *110*, 193,144w. (i) Lin, A. J.; Lee, M.; Klayman, D. L. Antimalarial activity of new water-soluble Dihydroartemisinin derivatives. 2. Stereospecificity of the ether side chain. *J. Med. Chem.* **1989**, *32*, 1249–1252. (j) Avery, M. A.; Jennings-White, C.; Chong, W. K. M. Simplified analogues of the antimalarial Artemisinin: Synthesis of 6,9-Desmethylartemisinin. *J. Org. Chem.* **1989**, *54*, 1792–1795. (k) Singh, C. Preparation of β-hydroxyhydroperoxides by photooxygenation of allylic alcohols and their elaboration into 1,2,4-trioxanes. *Tetrahedron Lett.* **1990**, *33*, 6901–6902. (l) Avery, M. A.; Chong, W. K. M.; Detre, G. Synthesis of (+)-8a,9-Secoartemisinin and related analogs. *Tetrahedron Lett.* **1990**, *31*, 1799–1802. (m) Avery, M. A.; Chong, W. K. M.; Bupp, J. E. Tricyclic analogues of Artemisinin: Synthesis and antimalarial activity of (+)-4,5-Secoartemisinin and (−)-Nor-4,5-secoartemisinin. *J. Chem. Soc., Chem. Commun.* **1990**, 1487–1489. (n) Jung, M.; Bustos, D. A.; ElSohly, H. N.; McChesney, J. D. A concise and stereoselective synthesis of (+)-12-n-Butyldeoxoartemisinin. *Synlett* **1990**, 743–744. (o) Bunnelle, W. H.; Isbell, T. A.; Barnes, C. L.; Qualls, S. Cationic ring expansion of an ozonide to a 1,2,4-trioxane. *J. Am. Chem. Soc.* **1991**, *113*, 8168–8169. (p) Imakura, Y.; Hachiya, K.; Ikemoto, T.; Kobayashi, S.; Yamashita, S.; Sakakibara, J.; Smith, F. T.; Lee, K.-H. Antimalarial artemisinin analogs: Synthesis of 2,3-desethano-12-deoxoartemisinin-related compounds. *Heterocycles* **1990**, *31*, 2125–2129. (q) Keper, J. A.; Philip, A.; Lee, Y. W.; Morey, M. C.; Carroll, F. I. 1,2,4-Trioxanes as potential antimalarial agents. *J. Med. Chem.* **1988**, *31*, 713–716. (r) For a review, see: Zaman, S. S.; Sharma, R. P. Some aspects of the chemistry and biological activity of Artemisinin and related antimalarials. *Heterocycles* **1991**, *32*, 1593–1638. (s) For some of the first synthetic efforts, see: Li, Y.; Yu, P.; Chen, Y.; Li, L.; Gai, Y.; Wang, D.; Zheng, Y. *Acta Pharmaceut. Sinica* **1981**, *16*, 430. Schmidt, G.; Hofheinz, W. Total synthesis of Qinghaosu. *J. Am. Chem. Soc.* **1983**, *105*, 624–625. Zhou, W.-S. Synthesis of arteannuin (Qinghaosu) and related compounds. *Pure Appl. Chem.* **1986**, *58*, 817–824.

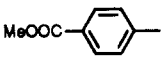
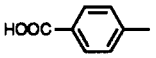
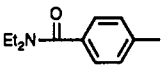
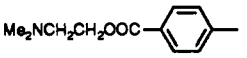
(8) (a) Jefford, C. W.; Velarde, J.; Bernardinelli, G. Synthesis of tricyclic Artemisinin-like compounds. *Tetrahedron Lett.* **1989**, *30*, 4485–4488 and references therein. (b) Jefford, C. W.; Jin, S.-J.; Bernardinelli, G. Reactions of an endoperoxide with chiral ketones. Diastereoselective formation of 1,2,4-trioxanes and 1,3-dioxolanes. *Tetrahedron Lett.* **1991**, *32*, 7243–7246.

(9) (a) Posner, G. H.; Oh, C. H.; Milhous, W. K. Olefin oxidative cleavage and dioxetane formation using triethylsilyl hydrotrioxide: Application to preparation of potent antimalarial 1,2,4-trioxanes. *Tetrahedron Lett.* **1991**, *32*, 4235–4238. Based on recent X-ray data of a related trioxane, the stereochemical assignment of the MeO group in compound 3 and of the $PhCH_2O$ group in compound 8 should be reversed to the β-orientation. (b) Posner, G. H.; Weitzberg, M.; Nelson, W. M.; Murr, B. L.; Seliger, H. H. 1,2-Dioxetanes from vinyl aromatics. *J. Am. Chem. Soc.* **1987**, *109*, 278–279.

(10) As far as we are aware, this is the first example of α,α′-dialkylation of a ketone, via its enamine, by *two different electrophiles*. For leading references to enamine formation and alkylation, see: Hickmott, P. W. Enamines: recent advances in synthetic, spectroscopic, mechanistic, and stereochemical aspects-I, II. *Tetrahedron* **1982**, *38*, 1975–2050 and 3363–3446. Whitesell, J. K.; Whitesell, M. A. Alkylation of ketones and aldehydes via their nitrogen derivatives. *Synthesis* **1983**, 517–536.

(11) (a) Johnson, A. W. *Ylid Chemistry*; Academic Press: New York, 1966. (b) Bestman, H. J.; Vostrowsky, O. Selected topics of the Wittig reaction in the synthesis of natural products. *Topics Curr. Chem.* **1983**, *109*, 85–163.

Table I. Trioxane Carboxylate Esters **9** and Carbamate Esters **10**

compd	R =	W-2 Indochina clone IC$_{50}$, nM (ng/mL)	W-2 Indochina clone relative potency[a]	D-6 African clone IC$_{50}$, nM (ng/mL)	D-6 African clone relative potency[a]
9a		8.2 (3.1)	0.2	29.5 (11.1)	0.1
9b		<1.2 (<0.5)	>1.2	<1.2 (<0.5)	>3.3
9c		15.7 (6.6)	0.1	63.3 (26.6)	0.1
9d		<1.4 (<0.7)	>1.0	<1.4 (<0.7)	>2.8
9e		3.3 (1.6)	0.4	12.6 (6.2)	0.3
9f	*t*-BuOOCNHCH$_2$–	2.5 (1.1)	0.6	3.5 (1.5)	1.1
10a	Me$_2$N–	0.8 (0.3)	1.8	2.9 (1.0)	1.3
10b	Et$_2$N–	<0.4 (<0.2)	>3.5	1.7 (0.6)	2.3
10c	Ph$_2$N–	<0.2 (<0.1)	>7.0	0.9 (0.4)	4.3
control	artemisinin	1.4 (0.4)	1.0	3.9 (1.1)	1.0

[a] Relative potency is the ratio of the artemisinin IC$_{50}$/synthetic trioxane IC$_{50}$.

Table II. Trioxane Phosphate Esters **11**

compd	R	Z	W-2 Indochina clone IC$_{50}$, nM (ng/mL)	W-2 Indochina clone relative potency[a]	D-6 African clone IC$_{50}$, nm (ng/mL)	D-6 African clone relative potency[a]
11a	Ph	O	<0.2 (<0.1)	>7.0	1.7 (0.8)	2.3
11b	Et	O	<0.9 (<0.4)	>1.6	1.1 (0.5)	3.5
11c	Et	S	0.4 (0.2)	3.5	3.7 (1.6)	1.1
control	artemisinin		1.4 (0.4)	1.0	3.9 (1.1)	1.0

[a] Relative potency is the ratio of the artemisinin IC$_{50}$/synthetic trioxane IC$_{50}$.

olefin and appearance of a singlet at δ 5.69 characteristic of the dioxetane.[9] Addition of *tert*-butyldimethylsilyl triflate to the solution of ketodioxetane at −78 °C produced trioxane **8** that was desilylated to give the desired trioxane alcohol **3**. X-ray crystallography of white crystalline alcohol **3** showed the relative stereochemistry indicated, with the methoxy group opposite in orientation to the corresponding acetal oxygen substituent in artemisinin.

Because 1,2,4-trioxanes are a recently discovered class of compounds, relatively little is known about the chemical reactivity and stability of such cyclic peroxides. It was gratifying, therefore, to find that crystalline tricyclic trioxane alcohol **3** is stable indefinitely at room temperature and that the critical trioxane portion of alcohol **3** survived exposure to basic conditions including 4-(*N*,*N*-dimethylamino)pyridine, sodium hydride, and even lithium diisopropylamide (LDA; see Experimental Section for details).[12] It was possible, therefore, to convert trioxane primary alcohol **3** into a wide range of stable and fully characterized carboxylate (**9**), carbamate (**10**), phosphate (**11**), and sulfonate (**12**) esters as well as into ethers (**13**) as summarized in Scheme II. Several of these derivatives (e.g., carboxylates **9b** and **9c**, carbamates **10a** and **10c**, and sulfonates **12a** and **12c**) are crystalline solids.

Biology

The antimalarial activities of trioxane alcohol **3** and of its ester and ether derivatives were assessed in vitro relative to the activity of chloroquine, mefloquine, and quinine in the semiautomated microdilution method of Desjardins et al.[13] as modified by Milhous et al.[14] In this assay the incorporation of [^{3}H]hypoxanthine into *Plasmodium falciparum* parasites serves as index of viability of the parasites. The drugs were tested against the African Sierra Leone (D-6) and the Indochina (W-2) clone of *P. falciparum* parasites. The African Sierra Leone clone is resistant to mefloquine but sensitive to chloroquine, quinine, sulfadoxine, and pyrimethamine whereas the Indochina clone is resistant to chloroquine, quinine, sulfadoxine, and pyrimethamine but sensitive to mefloquine. The results of these in vitro assays, summarized in Tables I–IV, were analyzed by fitting the radioactivity uptake vs drug concentration data to four parameters logistic regression. The concentration of each drug required to produce 50% inhibition (IC$_{50}$) of uptake of [^{3}H]hypoxanthine compared to control was then derived from the best fit concentration–response curve.

Results and Discussion

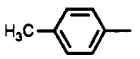
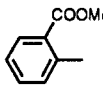
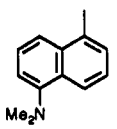
Although synthetic trioxane alcohol **3** and its derivatives have neither a lactone or a lactol ring nor the two methyl substituents forming stereogenic carbon centers found in both artemisinin (**1**) and in dihydroartemisinin (**2**), *all of these streamlined tricyclic trioxanes derived from alcohol* **3** *are active antimalarials*. As a group, trioxane ethers **13**

(12) For the conversion of artemisinin into non-lactone products via reaction with potassium carbonate in methanol at room temperature, see: (a) Academica Sinica Crystal structure and absolute configuration of qinghaosu. *Sci. Sin. (Engl. Ed.)* **1980**, *23*, 380–396; *Chem. Abstr.* **1980**, *93*, 71991e. (b) Zeng, M. Y.; Jeng, M-Y.; Li, L-N.; Chen, S-F.; Li, G-Y.; Liang, X-T.; Chen, M.; Clardy, J. Chemical transformations of Qinghaosu, a peroxidic antimalarial. *Tetrahedron* **1983**, *39*, 2941–2946.

(13) Desjardins, R. E.; Canfield, C. J.; Haynes, D. E.; Chulay, J. D. Quantitative assessment of antimalarial activity in vitro by a semiautomated microdilution technique. *Antimicrob. Agent Chemother.* **1979**, *16*, 710–718.

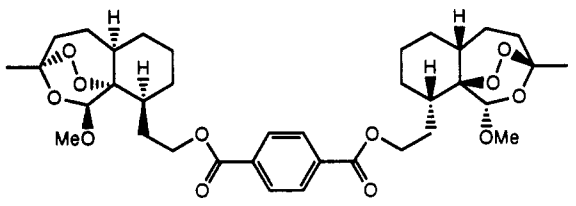
(14) Milhous, W. K.; Weatherley, N. F.; Bowdre, J. H.; Desjardins, R. E. In vitro activities of and mechanisms of resistance to antifol antimalarial drugs. *Antimicrob. Agent Chemother.* **1985**, *27*, 525–530.

Table III. Trioxane Sulfonate Esters 12

compd	R—	W-2 Indochina clone IC$_{50}$, nM (ng/mL)	W-2 Indochina clone relative potency[a]	D-6 African clone IC$_{50}$, nM (ng/mL)	D-6 African clone relative potency[a]
12a		1.9 (0.8)	0.7	5.6 (2.4)	0.7
12b		13.2 (6.2)	0.1	11.3 (5.3)	0.3
12c		1.2 (0.6)	1.2	16.3 (8.2)	0.2
control	artemisinin	1.4 (0.4)	1.0	3.9 (1.1)	1.0

[a] Relative potency is the ratio of the artemisinin IC$_{50}$/synthetic trioxane IC$_{50}$.

range from good (methyl ether **13a**) to outstanding antimalarial activity, with ethers **13b–d** substantially exceeding artemisinin in antimalarial potency in the W-2 Indochina clone. It is noteworthy that methyl ether **13a** is the least active of the ether derivatives **13**, in contrast to the ethers of dihydroartemisinin (**2**) in which the lower alkyl ethers (e.g., methyl and ethyl) are the most active.[4] Furthermore, it is remarkable that benzyl ether **13b** and allyl ether **13c**, differing considerably in lipophilic character, are so similar in relative antimalarial potency. Trioxane sulfonates **12a** and **12c**, the two most active of the sulfonate derivatives in the W-2 Indochina clone, are roughly comparable in potency to artemisinin. As a group, *trioxane phosphates* **11** *are all much more potent than artemisinin, with diphenyl phosphate* **11a** *between 2 and 7 times more potent than artemisinin.* As a group, *trioxane carbamates* **10** *also are all more potent than artemisinin, with crystalline diphenylcarbamate* **10c** *between 4 and 7 times more potent than artemisinin.* Finally, trioxane carboxylates **9** range from much less potent to 3 times more potent than artemisinin. Trioxane alcohol **3** was also converted into the corresponding symmetrical diester **14** by reaction with terephthaloyl chloride; bis-ester **14** was found to be 74 times less potent than artemisinin in the W-2 Indochina clone and 21 times less potent than artemisinin in the D-6 African clone.[15] Parent trioxane alcohol **3** itself was found to be considerably less potent than artemisinin.



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Although it is premature to formulate any single, unassailable, and comprehensive explanation for the dramatic antimalarial potency of these and some other[7–9] simplified synthetic analogs of artemisinin, we propose a tentative generalization that may be useful in guiding design of new analogs. Apparently one important factor contributing to very high antimalarial potency is the presence of a lipophilic and bulky substituent capable perhaps of sterically "protecting" the trioxane moiety from biological reducing agents, thereby making the trioxane a more selective oxidizing agent.[4] To support this generalization, we note the following two observations: (1) a benzyl ether substituent is much more efficacious than a methyl ether substituent[9a] (compare ethers **13a** and **13b** in Table IV), and (2) in carbamates **10**, antimalarial activity parallels the size of the nitrogen substituents (i.e., Ph > Et > Me, Table I).[4]

Because of their crystallinity and extraordinary antimalarial potency, carboxylate ester **9b** and especially carbamate ester **10c** appear to be the most promising candidates for preclinical evaluation in animals. Such efficacy studies are planned, and results will be reported when they become available.

In summary, disrupting the lactone and lactol rings of aretmisinin (**1**) and of dihydroartemisinin (**2**) has allowed rational design and easy synthesis of over 20 new, structurally simple, non-alkaloidal, tricyclic trioxanes having considerable antimalarial potency. Some of these ester derivatives of parent trioxane alcohol **3** are comparable or even higher in antimalarial potency relative to arteether, a very promising antimalarial that is now undergoing safety and tolerance studies under World Health Organization (WHO) sponsorship.[6]

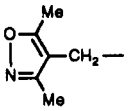
Experimental Section

General. Melting points were determined with a Thomas-Hoover capillary melting point apparatus and were uncorrected. Infrared spectra (IR) were recorded on a Perkin-Elmer 1600 FT-IR spectrometer. A Varian XL 400 spectrometer was employed for ^{1}H and ^{13}C NMR spectra with tetramethylsilane or chloroform as an internal reference. Resonances, in δ units downfield from internal Me$_4$Si, are noted 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. Elemental analyses were performed by Atlantic Microlab, Inc., Norcross, GA. The analytical GLC was carried out with a Hewlett-Packard 5880A GC gas chromatograph employing a J & W Scientific 20 m × 0.25 mm i.d. DB-1 bonded-phase methylsilicon capillary column and flame-ionization detector. Silica gel 60 (70–230 mesh, Merck) was 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.

Ethyl 3-(2-Cyanoethyl)-2-oxocyclohexaneacetate (4). An oven-dried 250-mL one-necked round-bottomed flask was charged with cyclohexanone (26.37 g, 0.27 mol) and dry benzene (50 mL). To this solution was added pyrrolidine (29 mL, 0.35 mol) over

(15) An ether dimer of dihydroartemisinin has been reported to be an active antimalarial in *P.-berghei*-infected mice: China Cooperative Research Group on Qinghaosu and its Derivatives as Antimalarials. The chemistry and synthesis of Qinghaosu. *J. Trad. Chin. Med.* **1982**, *2*, 9–16.

Table IV. Trioxane Ethers 13

compd	R	W-2 Indochina clone IC$_{50}$, nM (ng/mL)	W-2 Indochina clone relative potency[a]	D-6 African clone IC$_{50}$, nM (ng/mL)	D-6 African clone relative potency[a]
13a	Me–	3.7 (1.1)	0.4	10.7 (3.1)	0.4
13b	$PhCH_2$–	0.4 (0.2)	3.5	5.4 (1.9)	0.7
13c	CH_2=$CHCH_2$–	0.6 (0.2)	2.3	5.5 (1.7)	0.7
13d		0.5 (0.2)	2.8	7.6 (2.9)	0.5
control	artemisinin	1.4 (0.4)	1.0	3.9 (1.1)	1.0

[a] Relative potency is the ratio of the artemisinin IC$_{50}$/synthetic trioxane IC$_{50}$.

10 min through a dropping funnel. The resultant solution was refluxed for 3 h with azeotroping off water and then concentrated by distilling off benzene. The remaining crude enamine was dissolved in *p*-dioxane (100 mL) and treated with acrylonitrile (20 mL, 0.3 mol) and refluxed for 6 h, and then slowly treated with ethyl bromoacetate (31 mL, 0.28 mol), and refluxed for further 12 h. Then the reaction mixture was concentrated under reduced pressure and treated with water (30 mL) and 10% sulfuric acid (50 mL) at room temperature. The resultant solution was diluted with ether (200 mL). The organic layer was separated from the aqueous layer, further extracted twice with ether (100 mL × 2), combined, and washed with saturated NaCl solution (200 mL). The resulting organic layer was dried over anhydrous magnesium sulfate and filtered, and the solvent was removed at reduced pressure to yield a crude product. Vacuum distillation at 0.1 Torr afforded the desired product 4 (37.10 g, 58%) as a mixture of cis and trans stereoisomers: bp 160–175 °C (0.1 Torr); FT-IR (neat, cm^{-1}) 2245, 1732, 1710; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.14 (q, *J* = 7.1 Hz, 2 H), 2.92 (m, 1 H), 2.75 (dd, *J* = 16.6, 7.2 Hz, 1 H), 2.56 (m, 1 H), 2.45 (t, *J* = 7.4 Hz, 1 H), 2.43 (t, *J* = 7.5 Hz, 1 H), 2.17 (dd, *J* = 16.6, 5.7 Hz, 2 H), 2.22–2.05 (m, 2 H), 1.96–1.78 (m, 2 H), 1.55 (ddd, *J* = 17.4, 8.8, 4.7 Hz, 1 H), 1.42 (m, 2 H), 1.26 (t, *J* = 7.2 Hz, 3 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 210.92, 172.22, 119.63, 48.92, 48.81, 47.35 (47.14 for minor), 34.80, 34.55, 34.25, 34.13, 24.93, 15.09 (14.07 for minor); MS (EI, 70 eV, rel intensity) 237 (M, 17), 208 (12), 192 (B), 191 (59), 163 (27), 150 (58), 141 (35), 95 (20), 84 (27), 67 (13), 55 (20), 49 (14); HRMS calcd for $C_{13}H_{19}NO_3$ 237.1365, found 237.1361.

Ethyl 3-(2-Cyanoethyl)-2-(methoxymethylene)cyclohexaneacetate (5). An oven-dried 250-mL one-necked round-bottomed flask was charged with (methoxymethyl)triphenylphosphonium chloride (12.30 g, 35.8 mmol) and dry THF (120 mL) and then cooled to 0 °C. To this solution was added a 1.8 M phenyllithium solution (21 mL, 37.8 mmol) in cyclohexane/ether over 10 min. The resultant red ylide solution was then warmed to room temperature, stirred for 3 h, and cooled to −78 °C. To this ylide solution was added via cannula ketone 4 (5.68 g, 23.9 mmol) in THF (30 mL) over 10 min. After being stirred for 1 h at −78 °C, the reaction mixture was warmed to room temperature over 2 h, stirred for 10 h at room temperature, cooled to 0 °C, quenched with water (50 mL), and diluted with ether (50 mL). The organic layer was separated from the aqueous layer and further extracted with ether (50 mL × 2), combined, and washed with saturated NaCl solution (50 mL). The combined organic layer was dried over anhydrous magnesium sulfate and filtered, and the solvent was removed at reduced pressure to yield a crude product. Vacuum distillation at 0.05 Torr gave impure product 5 (8.99 g). Chromatography on silica gel (10:90 = hexane/ethyl acetate) afforded the pure colorless oil 5 (4.89 g, 77%) as a 4:1 mixture of stereoisomers: FT-IR (neat, cm^{-1}) 2244, 1732, 1662; ^{1}H NMR ($CDCl_3$, 400 MHz) δ (major) 5.93 (s, 1 H), 4.14 (q, *J* = 7.1 Hz, 2 H), 3.52 (s, 3 H), 2.82 (m, 1 H), 2.65 (m, 1 H), 2.42 (d, *J* = 1.9 Hz, 1 H), 2.40 (d, *J* = 1.7 Hz, 1 H), 2.46–2.24 (m, 2 H), 1.97–1.82 (m, 1 H), 1.80–1.65 (m, 1 H), 1.65–1.46 (m, 6 H), 1.28 (t, *J* = 7.1 Hz, 3 H), (minor) 5.85 (s, 1 H), 4.11 (q, *J* = 7.1 Hz, 2 H), 3.55 (s, 3 H), 3.31 (m, 1 H), 2.46–2.24 (m, 4 H), 2.22–1.96 (m, 1 H), 1.97–1.82 (m, 1 H), 1.80–1.65 (m, 1 H), 1.65–1.46 (m, 1 H), 1.26 (t, *J* = 7.1 Hz, 3 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ (major) 172.44, 144.17, 120.50, 116.29, 60.19, 59.29, 40.89, 34.79, 32.49, 31.16, 30.92, 30.13, 17.80, 14.23, (minor) 172.54, 143.68, 120.00, 115.15, 60.19, 59.45, 39.48, 36.68, 32.07, 31.95, 30.86, 30.01, 15.46, 14.11; MS (EI, 70 eV, rel intensity) 265 (M, 16), 250 (4), 233 (18), 191 (33), 178 (97), 137 (34), 123 (28), 105 (B), 88 (24), 75 (15), 67 (8); HRMS calcd for $C_{15}H_{23}NO_3$ 265.1678, found 265.1682.

Primary Alcohol 6. An oven-dried 250-mL one-necked round-bottomed flask was charged with the above stereoisomeric mixture of 5 (4.80 g, 18.1 mmol) and dry THF (100 mL) and cooled to 0 °C. To this solution was added a 1 M solution of lithium tri-*sec*-butylborohydride (L-Selectride, 50 mL, 50 mmol) in THF via cannula over 20 min. After being stirred for 10 min at 0 °C, the reaction mixture was slowly warmed to room temperature, stirred for 2 h, and cooled to 0 °C. This reaction mixture was quenched with water (50 mL) and concentrated to about 100 mL at reduced pressure (30 Torr). The resulting solution was then cooled to 0 °C and then diluted with brine (50 mL) and ether (50 mL). The organic layer was separated, and the aqueous layer was acidified with 20% sulfuric acid solution (20 mL) and extracted two times with ethyl ether (50 mL × 2). The combined organic layer was washed with saturated NaCl solution (100 mL), dried over anhydrous magnesium sulfate, and filtered, and the solvent was removed at reduced pressure to yield a crude product. Chromatography of the crude product on silica gel (50:50 = hexane/ethyl acetate) afforded the colorless oil 6 (2.50 g, 62%) as a single stereoisomer and an inseparable mixture of stereoisomers of 6 (320 mg, 8%). 6: FT-IR ($CHCl_3$) 3408, 2245, 1660; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.90 (s, 1 H), 3.64 (m, 2 H), 3.54 (s, 3 H), 2.83 (m, 1 H), 2.33 (t, *J* = 7.7 Hz, 2 H), 2.20 (m, 1 H), 1.94–1.84 (m, 1 H), 1.80–1.71 (m, 1 H), 1.70–1.62 (m, 2 H), 1.62–1.52 (m, 6 H), 1.47–1.41 (m, 1 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 143.42, 120.54, 118.14, 61.36, 59.33, 38.03, 34.07, 32.89, 31.91, 31.21, 30.71, 17.96, 15.63; MS (EI, 70 eV, rel intensity) 223 (10), 178 (B), 137 (14), 119 (16); HRMS calcd for $C_{13}H_{21}NO_2$ 223.1572, found 223.1575.

Methyl Ketone 7. An oven-dried 50-mL one-necked round-bottomed flask was charged with 6 (532.4 mg, 2.38 mmol) and dry ethyl ether (10 mL) and cooled to −78 °C. To this solution was added via a gas-tight syringe a 1.3 M methyllithium solution (10 mL, 13.0 mmol) in ethyl ether over 2 min. After being stirred for 10 min at −78 °C, the reaction mixture was slowly warmed to room temperature over 30 min and stirred for 15 h. This reaction mixture was quenched with water (10 mL) at 0 °C and then diluted with water (10 mL) and ether (20 mL). The organic layer was separated, and the aqueous layer was extracted two times with ethyl ether (20 mL × 2). The combined organic layer was washed with saturated NaCl solution (20 mL), dried over anhydrous magnesium sulfate, and filtered, and the solvent was removed at reduced pressure to yield a crude product. Chromatography on silica gel (50:50 = hexane/ethyl acetate) afforded the colorless oil 7 (444.0 mg, 78%): FT-IR ($CHCl_3$, cm^{-1}) 3417, 1713, 1661; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.85 (s, 1 H), 3.63 (m, 2 H), 3.51 (s, 3 H), 2.75 (q, *J* = 6.2 Hz, 1 H), 2.51 (m, *J* = 5.6 Hz, 1 H), 2.36 (m, *J* = 5.6 Hz, 1 H), 2.19 (q, *J* = 6.2 Hz, 1 H), 2.13 (s, 3 H), 1.74–1.65 (m, 4 H), 1.65–1.38 (m, 6 H), 1.27 (t, *J* = 5.3 Hz, 1 H, O*H*); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 209.83, 142.76, 119.57, 61.50, 59.15, 42.23, 38.00, 34.11, 32.18, 32.07, 31.29, 29.85, 28.37, 17.48; MS (EI, 70 eV, rel intensity) 240 (2), 222 (1), 208 (2), 182 (63), 150 (13), 137 (68), 119 (22), 108 (13), 105 (61), 93 (46), 91 (30), 79 (22), 67 (16), 45 (43), 43 (B), 41 (33), 39 (15); HRMS calcd for $C_{14}H_{24}O_3$ 240.1725, found 240.1729.

Trioxane Silyl Ether 8. An oven-dried 250-mL three-necked round-bottomed flask, fitted with magnetic stirring bar, serum cap, gas-needle inlet and outlet, was charged with 7 (3.03 g, 12.6 mmol), methylene blue (10 mg), and dry methylene chloride (120 mL) and cooled to −78 °C. Dry oxygen (flow rate: ca. 1 mL/s) was slowly bubbled to this solution for 4 h under UV irradiation using a medium-pressure mercury lamp as UV source. To the resultant solution, after being vigorously stirred for 10 min, was slowly added *tert*-butyldimethylsilyl trifluoromethanesulfonate (TBDMSOTf, 8 mL, 34.5 mmol) via a gas-tight syringe over 1 min. The resultant solution was stirred at −78 °C for 2 h, treated with triethylamine (12 mL), and then slowly warmed to room temperature over 4 h. The reaction mixture was concentrated to about 20 mL under reduced pressure. The short-path chromatographic separation of the crude product with ethyl acetate and hexane (5:95) gave impure 1,2,4-trioxane 8 (2.80 g, 57%), which was used in next step without further purification: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.15 (d, J = 1.2 Hz, 1 H), 3.65 (m, 2 H), 3.50 (s, 3 H), 2.32 (td, J = 13.8, 3.6 Hz, 1 H), 2.14 (q, J = 8.4 Hz, 1 H), 2.00 (ddd, J = 14.4, 4.2, 2.5 Hz, 1 H), 1.89–1.46 (m, 5 H), 1.38 (s, 3 H), 1.34–1.16 (m, 4 H), 0.90 (s, 9 H), 0.06 (s, 6 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 105.10, 100.42, 85.30, 62.30, 56.72, 48.58, 42.03, 37.50, 32.99, 31.11, 29.46, 27.18, 25.99, 25.27, 17.55, −5.27.

Trioxane Alcohol 3. To the silyl-protected trioxane 8 (2.80 g, 7.2 mmol) in dry THF (10 mL) was added a 1 M solution of tetrabutylammonium fluoride (20 mL, 20 mmol) in THF at 0 °C under argon atmosphere. The resulting solution was stirred for 10 h at room temperature, then cooled to 0 °C, and diluted with water (30 mL) and ether (30 mL). The organic layer was separated and the aqueous layer was extracted two times with ethyl ether (20 mL × 2). The combined organic layer was washed with saturated NaCl solution (30 mL), dried over anhydrous magnesium sulfate, and filtered, and the solvent was removed at reduced pressure to yield a crude product. Chromatography on silica gel (80:20 = hexane/ethyl acetate) to afford the corresponding trioxane alcohol 3 (1.25 g, 64%) as a colorless solid having the same spectroscopic properties as recrystallized material. Recrystallization of compound 3 from hexane furnished needles: mp 83–4 °C; FT-IR ($CHCl_3$, cm^{-1}) 3617, 3013, 2934, 2862, 1443, 1408, 1376, 1224, 1218, 1211, 1077, 1042, 1009, 963, 950, 909, 897, 871; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.19 (d, J = 1.6 Hz, 1 H), 3.78 (m, 1 H), 3.66 (m, 1 H), 3.50 (s, 3 H), 2.32 (ddd, J = 14.0, 13.8, 3.5 Hz, 1 H), 2.08–1.95 (m, 2 H), 1.90–1.78 (m, 1 H), 1.75 (dd, J = 6.0, 5.5 Hz, 2 H), 1.70–1.51 (m, 6 H), 1.46–1.35 (m, 2 H), 1.38 (s, 3 H), 1.30–1.20 (m, 1 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 105.27, 100.18, 85.58, 61.60, 56.70, 48.61, 41.70, 37.49, 33.91, 31.08, 30.13, 27.09, 25.61, 25.24; MS (CI, NH_3, rel intensity) 290 (M + 18, 51), 273 (1), 258 (23), 241 (18), 223 (57), 205 (15), 195 (B), 181 (62), 169 (8), 150 (13), 137 (82); HRMS (CI, NH_3) calcd for $C_{14}H_{28}NO_5$ (M + 18) 290.1967, found 290.1962. Anal. ($C_{14}H_{24}O_5$) C, H.

Trioxane Carboxylate Ester 9a. An oven-dried 5-mL one-necked round-bottomed flask was charged with dry methylene chloride (5 mL) and benzoyl chloride (50 μL, 0.4 mmol). This solution was treated with triethylamine (110 μL, 0.8 mmol) via a gas-tight syringe and trioxane alcohol 3 (27.2 mg, 0.1 mmol) in methylene chloride (10 mL) via cannula at 0 °C. After being stirred for 30 min at 0 °C, the reaction mixture was slowly warmed to room temperature and stirred for 2 h. Then the reaction mixture was concentrated to yield a crude product which was separated by silica gel column chromatography to afford the corresponding trioxane carboxylate ester **9a** (19.2 mg, 51%) as a colorless oil: FT-IR ($CHCl_3$, cm^{-1}) 1714, 1602; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.06 (d, J = 7.6 Hz, 2 H), 7.56 (t, J = 7.2 Hz, 1 H), 7.44 (t, J = 7.6 Hz, 2 H), 5.21 (s, 1 H), 4.38 (m, 2 H), 3.52 (s, 3 H), 2.44–2.30 (m, 2 H), 2.06–1.99 (m, 1 H), 1.91–1.74 (m, 2 H), 1.73–1.49 (m, 7 H), 1.39 (s, 3 H), 1.32–1.20 (m, 1 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 166.63, 132.81, 130.39, 129.59, 128.32, 105.26, 100.15, 85.22, 63.93, 56.71, 48.56, 42.33, 37.49, 31.02, 29.36, 29.16, 27.11, 25.94, 25.18; LRMS (CI, NH_3, rel intensity) 394 (M + 18, B), 377 (5), 359 (18), 345 (34), 334 (24), 317 (19), 299 (31), 286 (18), 223 (34), 205 (20), 195 (78), 137 (42), 105 (51), 94 (6), 77 (5); HRMS (CI, NH_3) calcd for $C_{21}H_{32}NO_6$ (M + 18) 394.2230, found 394.2222.

Trioxane Carboxylate Ester 9b. An oven-dried 10-mL one-necked round-bottomed flask was charged with monomethyl terephthalate (38.5 mg, 0.2 mmol), 4-(*N*,*N*-dimethylamino)-pyridine (46.1 mg), dicyclohexylcarbodiimide (54.5 mg, 0.26 mmol), and added methylene chloride (2 mL) via a gas tight syringe at 0 °C under argon atmosphere. After being stirred for 10 min, the reaction mixture was slowly warmed to room temperature, stirred for 30 min, and cooled to 0 °C. To this solution was added trioxane alcohol 3 (23.7 mg, 0.1 mmol) in methylene chloride (1 mL) via cannula. After being stirred for 10 min at 0 °C, the reaction mixture was slowly warmed to room temperature and stirred for additional 30 min, and the solvent was removed at reduced pressure to yield a crude product which was separated by silica gel column chromatography to afford the corresponding pure trioxane carboxylate ester **9b** (30.5 mg, 83%) as a white solid: mp 111–112 °C; FT-IR ($CHCl_3$, cm^{-1}) 1722; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.10 (s, 4 H), 5.21 (s, 1 H), 4.45–4.37 (m, 2 H), 3.95 (s, 3 H), 3.52 (s, 3 H), 2.45–2.30 (m, 2 H), 2.06–2.00 (m, 1 H), 1.89–1.50 (m, 9 H), 1.44–1.35 (m, 1 H), 1.39 (s, 3 H), 1.32–1.20 (m, 1 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 166.34, 165.80, 134.17, 133.80, 129.56, 105.29, 100.09, 85.19, 64.41, 56.70, 52.42, 48.56, 42.30, 37.47, 30.99, 29.42, 29.18, 27.09, 25.92, 25.17; LRMS (CI, NH_3, rel intensity) 452 (M + 18, B), 435 (M + 1, 3), 403 (20), 392 (64), 375 (23), 357 (22), 195 (45). Anal. ($C_{23}H_{30}O_8$) C, H.

Trioxane Carboxylate Ester 9c. An oven-dried 50-mL one-necked round-bottomed flask was charged with terephthaloyl chloride (494.0 mg, 2.4 mmol) and dry methylene chloride (5 mL) and cooled to 0 °C. This solution was treated with triethylamine (1.9 mL, 13.6 mmol) via a gas-tight syringe, slowly warmed to room temperature, and stirred for 2 h. To this solution, after being cooled to −78 °C, was added via cannula trioxane alcohol 3 (176.9 mg, 0.65 mmol) in methylene chloride (10 mL). After being stirred for 10 min at −78 °C, the reaction mixture was slowly warmed to room temperature over 1 h and stirred for 2 h. Then the reaction mixture was cooled to 0 °C, quenched with water (10 mL), and then acidified with saturated sodium bisulfate (10 mL). The aqueous layer was extracted three times with ethyl acetate (20 mL × 3). The combined organic layer was washed with saturated NaCl solution (20 mL), dried over anhydrous magnesium sulfate, and filtered, and the solvent was removed at reduced pressure to yield a crude product which was directly separated by silica gel column chromatography to afford the corresponding trioxane carboxylic acid **9c** (78.0 mg, 13%) as white solid whose ^{1}H NMR was essentially identical with that of recrystallized trioxane **9c**. Recrystallization from ether/hexane afforded pure trioxane **9c** as a white solid: mp 168–170 °C; FT-IR ($CHCl_3$, cm^{-1}) 3695, 3519, 1719, 1701, 1602; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.17 (d, J = 8.8 Hz, 2 H), 8.15 (d, J = 8.8 Hz, 2 H), 5.21 (s, 1 H), 4.49–4.36 (m, 2 H), 3.53 (s, 3 H), 2.45–2.30 (m, 2 H), 2.26–2.00 (m, 1 H), 1.88–1.66 (m, 6 H), 1.65–1.51 (m, 4 H), 1.40 (s, 3 H), 1.29–1.23 (m, 1 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.69, 165.69, 134.95, 132.88, 130.15, 129.66, 105.31, 100.09, 85.20, 64.49, 56.70, 48.56, 42.30, 37.47, 30.99, 29.43, 29.18, 27.09, 25.92, 25.17; LRMS (CI, NH_3, rel intensity) 438 (M + 18, 18), 403 (M − 17, 2), 389 (7), 378 (36), 361 (19), 343 (65), 223 (19), 195 (B), 176 (6), 149 (13), 137 (9); HRMS (CI, NH_3) calcd for $C_{22}H_{32}NO_8$ (M + 18) 438.2128, found 438.2136. Anal. ($C_{22}H_{28}O_8$) C, H.

Trioxane Carboxylate Ester 9d. An oven-dried 10-mL one-necked round-bottomed flask was charged with terephthaloyl chloride (61.2 mg, 0.3 mmol) and dry methylene chloride (1 mL) and cooled to 0 °C. To this solution was added triethylamine (45 μL, 0.3 mmol) via a gas-tight syringe. The reaction mixture was stirred at room temperature for 0.5 h, cooled to 0 °C, and treated with trioxane alcohol 3 (35.2 mg, 0.13 mmol) in methylene chloride (1 mL) via cannula. This solution was stirred for 2 h at room temperature, then treated with triethylamine (75 μL) and diethylamine (75 μL) at 0 °C, and then slowly warmed to room temperature. This reaction mixture was stirred for 2 h, and the solvent was removed at reduced pressure to yield a crude product which was directly separated by silica gel column chromatography to afford the corresponding pure trioxane carboxylate ester **9d** (51.7 mg, 84%) as a colorless oil: FT-IR (neat, cm^{-1}) 1719, 1636; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.08 (dd, J = 6.8, 1.6 Hz, 2 H), 7.43 (dd, J = 6.8, 1.6 Hz, 2 H), 5.21 (s, 1 H), 4.46–4.33 (m, 2 H), 3.56 (q, J = 6.0 Hz, 2 H), 3.52 (s, 3 H), 3.21 (q, J = 6.0 Hz, 2 H), 2.44–2.29 (m, 2 H), 2.06–2.00 (m, 1 H), 1.90–1.49 (m, 8 H), 1.43–1.34 (m, 1 H), 1.39 (s, 3 H), 1.32–1.20 (m, 5 H), 1.10 (t, J = 6.6 Hz, 3 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.30, 165.96, 141.47, 130.88, 129.79, 126.25, 105.26, 100.09, 85.20, 64.13, 56.69, 48.56, 43.20, 42.28,

39.29, 37.48, 30.99, 29.37, 29.16, 27.08, 25.91, 25.17, 14.19, 12.88; LRMS (CI, NH_3, rel intensity) 493 (M + 18, 1), 476 (M + 1, 15), 416 (B), 222 (6), 195 (5); HRMS (CI, NH_3) calcd for $C_{26}H_{38}NO_7$ (M + H) 476.2648, found 476.2642.

Trioxane Carboxylate Ester 9e. An oven-dried 10-mL one-necked round-bottomed flask was charged with terephthaloyl chloride (64.5 mg, 0.3 mmol) and dry methylene chloride (1 mL) and cooled to 0 °C. To this solution was added triethylamine (45 μL, 0.3 mmol) via a gas-tight syringe. After the reaction mixture was stirred at room temperature for 0.5 and cooled to 0 °C, it was treated with trioxane alcohol **3** (41.9 mg, 0.15 mmol) in methylene chloride (1 mL) via cannula. This solution was stirred for 2 h at room temperature and then treated with triethylamine (100 μL) and *N,N*-dimethylethanolamine (100 μL) at 0 °C and then slowly warmed to room temperature. This reaction mixture was stirred for 2 h and the solvent was removed at reduced pressure to yield a crude product which was directly separated by silica gel column chromatography to afford the corresponding pure trioxane carboxylate ester **9e** (51.5 mg, 68%) as a colorless oil: FT-IR (neat, cm^{-1}) 1721, 1578; ^1H NMR ($CDCl_3$, 400 MHz) δ 8.10 (s, 4 H), 5.21 (s, 1 H), 4.46 (t, *J* = 5.8 Hz, 2 H), 4.45–4.35 (m, 2 H), 3.52 (s, 3 H), 2.73 (t, *J* = 5.8 Hz, 2 H), 2.35 (s, 6 H), 2.38–2.32 (m, 1 H), 2.07–1.95 (m, 2 H), 1.91–1.50 (m, 9 H), 1.44–1.36 (m, 1 H), 1.39 (s, 3 H), 1.30–1.20 (m, 1 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 165.83, 165.79, 134.17, 133.86, 129.59, 129.53, 105.27, 100.08, 85.17, 64.39, 63.37, 57.75, 56.69, 48.55, 45.83, 42.30, 37.46, 30.97, 29.41, 29.16, 27.08, 25.92, 25.16; LRMS (CI, NH_3, rel intensity) 492 (M + H, 51), 462 (7), 433 (27), 432 (B), 71 (14), 58 (29); HRMS (CI, NH_3) calcd for $C_{26}H_{37}NO_8$ (M + H) 492.2597, found 492.2599.

Trioxane Carboxylate Ester 9f. An oven-dried 10-mL one-necked round-bottomed flask was charged with *N*-(*tert*-butoxycarbonyl)glycine (35.1 mg, 0.2 mmol), 4-(*N,N*-dimethylamino)pyridine (24.9 mg), dicyclohexylcarbodiimide (42.9 mg, 0.2 mmol), and methylene chloride (2 mL) via a gas tight syringe at 0 °C under argon atmosphere. After being stirred for 10 min at 0 °C, the reaction mixture was slowly warmed to room temperature and stirred for 30 min and cooled to 0 °C. To this solution was added trioxane alcohol **3** (26.2 mg, 0.1 mmol) in methylene chloride (1 mL) via cannula at 0 °C. After being stirred for 10 min at 0 °C, the reaction mixture was slowly warmed to room temperature and stirred for 2 h and the solvent was removed at reduced pressure to yield a crude product which was directly separated by silica gel column chromatography to afford the corresponding pure trioxane carboxylate ester **9f** (18.5 mg, 41%) as a colorless oil; FT-IR ($CHCl_3$, cm^{-1}) 3448, 1743, 1713; ^1H NMR ($CDCl_3$, 400 MHz) δ 5.14 (d, *J* = 1.2 Hz, 1 H), 5.02 (bs, 1 H), 4.29–4.16 (m, 2 H), 3.91 (d, *J* = 5.2 Hz, 1 H), 3.50 (s, 3 H), 2.32 (ddd, *J* = 14.8, 14.2, 3.0 Hz, 1 H), 2.28–2.19 (m, 2 H), 2.05 (m, 1 H), 1.90–1.72 (m, 2 H), 1.72–1.50 (m, 6 H), 1.47–1.36 (m, 1 H), 1.46 (s, 9 H), 1.38 (s, 3 H), 1.32–1.20 (m, 1 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.54, 155.67, 105.27, 100.04, 85.13, 80.10, 64.36, 56.68, 48.53, 42.45, 42.13, 37.45, 30.95, 29.48, 29.17, 28.30, 27.07, 25.90, 25.13; LRMS (CI, NH_3, rel intensity) 447 (M + 18, 14), 430 (M + 1, 2), 391 (50), 356 (7), 331 (46), 298 (53), 270 (B), 195 (22); HRMS (CI, NH_3) calcd for $C_{21}H_{36}NO_8$ (M + H) 430.2441, found 430.2434.

Trioxane Carbamate Ester 10a. An oven-dried 10-mL one-necked round-bottomed flask was charged with dry THF (1 mL) and *n*-butyllithium (1.5 M in hexane, 150 μL, 0.23 mmol) and treated with dry diisopropylamine (31 μL, 0.22 mmol) at 0 °C under argon atmosphere. This LDA solution was cooled to −78 °C and treated with trioxane alcohol **3** (20.3 mg, 0.08 mmol) in dry THF (1 mL) via cannula. After being stirred for 5 min at −78 °C, the reaction mixture was treated with *N,N*-dimethylcarbamoyl chloride (40 μL, 0.43 mmol) and then slowly warmed to room temperature over 10 min. This reaction mixture was stirred for 2 h. The reaction mixture was quenched with water (5 mL) at 0 °C. The organic layer was extracted twice with ether (10 mL × 2), combined, washed with saturated sodium chloride solution (10 mL), and dried over anhydrous magnesium sulfate, filtered, and concentrated under reduced pressure to yield a crude product which was separated by silica gel column chromatography to afford the corresponding pure trioxane carbamate ester **10a** (18.9 mg, 74%) as a colorless solid: mp (hexane) 116–116.5 °C; FT-IR ($CHCl_3$, cm^{-1}) 1687; ^1H NMR ($CDCl_3$, 400 MHz) δ 5.16 (d, *J* = 1.2 Hz, 1 H), 4.18 (ddd, *J* = 10.8, 8.0, 4.8 Hz, 1 H), 4.06 (dt, *J* = 10.8, 7.4 Hz, 1 H), 3.50 (s, 3 H), 2.90 (s, 6 H), 2.32 (ddd, *J* = 14.0, 13.5, 3.5 Hz, 1 H), 2.25 (m, 1 H), 2.05 (ddd, *J* = 14.0, 4.8, 2.6 Hz, 1 H), 1.90–1.49 (m, 8 H), 1.43–1.17 (m, 3 H), 1.38 (s, 3 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 156.74, 105.19, 100.20, 85.21, 64.18, 56.70, 48.57, 42.20, 37.49, 36.36, 35.92, 31.05, 29.41, 29.24, 27.12, 25.95, 25.21. Anal. ($C_{17}H_{29}NO_6$) C, H, N.

Trioxane Carbamate Ester 10b. To a THF solution (1 mL) of LDA (0.36 mmol) at −78 °C, generated as above, was added trioxane alcohol **3** (30.9 mg, 0.11 mmol) in dry THF (1 mL) via cannula. After the reaction mixture was stirred for 10 min, it was treated with *N,N*-diethylcarbamoyl chloride (50 μL, 0.39 mmol) and then slowly warmed to room temperature over 0.2 h. This reaction mixture was stirred for 4 h. The usual workup furnished the corresponding pure trioxane carbamate ester **34** (28.9 mg, 69%) as a colorless oil: FT-IR ($CHCl_3$, cm^{-1}) 1679; ^1H NMR ($CDCl_3$, 400 MHz) δ 5.16 (d, *J* = 1.2 Hz, 1 H), 4.18 (ddd, *J* = 10.6, 8.0, 4.8 Hz, 1 H), 4.07 (ddd, *J* = 10.6, 7.4, 7.4 Hz, 1 H), 3.50 (s, 3 H), 3.26 (bm, 4 H), 2.37–2.21 (m, 2 H), 2.05–1.99 (m, 1 H), 1.89–1.48 (m, 9 H), 1.44–1.15 (m, 2 H), 1.38 (s, 3 H), 1.12 (t, *J* = 7.0 Hz, 6 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 156.04, 105.18, 100.21, 85.20, 63.82, 56.70, 48.60, 42.23, 41.64, 41.20, 37.49, 31.06, 29.30, 29.15, 27.13, 25.96, 25.23, 14.01, 13.57; LRMS (NH_3, rel intensity) 389 (M + 18, 12), 372 (M + 1, 75), 354 (7), 340 (34), 312 (56), 294 (30), 195 (100), 137 (25); HRMS (CI, NH_3) calcd for $C_{19}H_{34}NO_6$ (M + H) 372.2386, found 372.2391.

Trioxane Carbamate Ester 10c. To a THF solution (1 mL) of LDA (0.14 mmol) at −78 °C, generated as above, was added trioxane alcohol **3** (12.3 mg, 0.05 mmol) in dry THF (1 mL) via cannula. After the reaction mixture was stirred for 10 min, it was treated with *N,N*-diphenylcarbamoyl chloride (32.0 mg, 0.14 mmol) in THF (0.5 mL) and then slowly warmed to room temperature over 0.2 h. This reaction mixture was stirred for 6 h. The usual workup furnished the corresponding pure trioxane carbamate ester **10c** (13.8 mg, 65%) as a colorless solid: mp (hexane) 149–150 °C; FT-IR ($CHCl_3$, cm^{-1}) 1706, 1594; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.35–7.18 (m, 10 H), 5.11 (s, 1 H), 4.32–4.26 (m, 1 H), 4.20–4.13 (m, 1 H), 3.47 (s, 3 H), 2.36–2.28 (m, 1 H), 2.23–2.15 (m, 1 H), 2.04–1.97 (m, 1 H), 1.85–1.30 (m, 10 H), 1.37 (s, 3 H), 1.23–1.11 (m, 1 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 154.80, 142.60, 128.86, 127.01, 126.01, 105.16, 100.11, 85.18, 65.03, 56.68, 48.62, 42.24, 37.49, 31.03, 29.25, 29.22, 27.13, 25.94, 25.19. Anal. ($C_{27}H_{33}NO_6$) C, H, N.

Trioxane Phosphate Ester 11a. To a THF solution (1 mL) of LDA (0.18 mmol) at −78 °C, generated as above, was added trioxane alcohol **3** (24.9 mg, 0.09 mmol) in dry THF (1 mL) via cannula. After the reaction mixture was stirred for 10 min, it was treated with diphenyl phosphorochloridate (40 μL, 0.19 mmol) and then slowly warmed to room temperature over 0.2 h. This reaction mixture was stirred for 2 h. The usual workup furnished the corresponding pure trioxane phosphate ester **11a** (21.4 mg, 71%) as a colorless oil: FT-IR ($CHCl_3$, cm^{-1}) 1593, 1284; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.37–7.17 (m, 10 H), 5.09 (d, *J* = 1.2 Hz, 1 H), 4.36–4.29 (m, 2 H), 3.46 (s, 3 H), 2.36–2.26 (m, 2 H), 2.04–1.99 (m, 1 H), 1.85–1.42 (m, 9 H), 1.39 (s, 3 H), 1.37–1.25 (m, 1 H), 1.25–1.14 (m, 1 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 150.58 (d, *J* = 6.8 Hz), 129.75, 125.25, 120.12 (d, *J* = 4.8 Hz), 105.26, 99.97, 85.05, 68.16 (d, *J* = 6.5 Hz), 56.66, 48.51, 41.63, 37.44, 30.90, 30.85, 29.42, 27.06, 25.90, 25.05; LRMS (NH_3, rel intensity) 522 (M + 18, 1), 505 (M + 1, 1), 473 (1), 445 (4), 309 (38), 268 (42), 195 (100); HRMS (CI, NH_3) calcd for $C_{26}H_{37}NO_8P$ (M + 18) 522.2257, found 522.2255.

Trioxane Phosphate Ester 11b. An oven-dried 10-mL one-necked round-bottomed flask was charged with diethyl phosphorochloridate (65 μL, 0.45 mmol) and dry methylene chloride (1 mL) and cooled to 0 °C. To this solution was added triethylamine (190 μL, 1.36 mmol) via a gas-tight syringe. After the reaction mixture was slowly warmed to room temperature over 0.5 h and stirred for 0.5 h, it was treated with trioxane alcohol **3** (53.3 mg, 0.20 mmol) in methylene chloride (1 mL). This solution was then refluxed for 24 h and the solvent was removed at reduced pressure to yield a crude product which was directly separated by silica gel column chromatography to afford the corresponding pure trioxane phosphate ester **11b** (41.2 mg, 52%) as a colorless oil: FT-IR (neat, cm^{-1}) 1266; ^1H NMR ($CDCl_3$, 400 MHz) δ 5.13 (d, *J* = 1.2 Hz, 1 H), 4.12 (m, 6 H), 3.50 (s, 3 H), 2.36–2.24 (m, 2 H), 2.04–1.98 (m, 1 H), 1.89–1.18 (m, 11 H), 1.38

(s, 3 H), 1.34 (t, J = 7.0 Hz, 6 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 105.22, 100.06, 85.08, 66.35 (d, J = 5.9 Hz), 63.68 (d, J = 5.8 Hz), 56.66, 48.55, 41.67, 37.45, 30.95, 30.85 (d, J = 7.1 Hz), 29.34, 27.08, 25.91, 25.12, 16.14 (d, J = 6.6 Hz); LRMS (NH_3, rel intensity) 426 (M + 18, 2), 409 (M + 1, 14), 377 (4), 349 (30), 331 (5), 195 (100), 136 (8), 119 (4); HRMS (CI, NH_3) calcd for $C_{18}H_{34}O_8P$ (M + H) 409.1991, found 409.1995.

Trioxane Phosphate Ester 11c. To a THF solution (1 mL) of LDA (0.18 mmol) at −78 °C, generated as above, was added trioxane alcohol **3** (22.8 mg, 0.08 mmol) in dry THF (1 mL) via cannula. After the reaction mixture was stirred for 10 min, it was treated with diethyl phosphorochloridothioate (30 μL, 0.19 mmol) and then slowly warmed to room temperature over 0.2 h. This reaction mixture was stirred for 4 h. The usual workup furnished the corresponding pure trioxane phosphate ester **11c** (15.0 mg, 43%) as a colorless oil: FT-IR ($CHCl_3$, cm^{-1}) 3020, 2994, 2933, 2862, 1443, 1376, 1266, 1135, 1121, 1024, 973, 898, 871; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.14 (d, J = 1.2 Hz, 1 H), 4.17–4.09 (m, 6 H), 3.50 (s, 3 H), 2.36–2.25 (m, 2 H), 2.04–1.98 (m, 1 H), 1.89–1.40 (m, 9 H), 1.38 (s, 3 H), 1.38–1.16 (m, 2 H), 1.33 (t, J = 7.0 Hz, 6 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 105.22, 100.10, 85.11, 66.93 (d, J = 5.4 Hz), 64.25 (d, J = 4.7 Hz), 56.69, 48.56, 41.71, 37.46, 30.97, 30.68 (d, J = 7.1 Hz), 29.38, 27.10, 25.92, 25.12, 15.93 (d, J = 7.5 Hz); LRMS (NH_3, rel intensity) 442 (M + 18, 13), 425 (M + 1, 30), 407 (6), 393 (12), 382 (5), 365 (11), 239 (100), 223 (26), 207 (66), 195 (98), 179 (18), 163 (13); HRMS (CI, NH_3) calcd for $C_{18}H_{34}O_7PS$ (M + H) 425.1763, found 425.1767.

Trioxane Sulfonate Ester 12a. An oven-dried 10-mL one-necked round-bottomed flask was charged with *p*-toluenesulfonyl chloride (85.4 mg, 0.45 mmol) and dry methylene chloride (1 mL) and cooled to 0 °C. To this solution was added triethylamine (180 μL, 1.3 mmol) via a gas-tight syringe. After the reaction mixture was stirred at room temperature for 0.5 h, cooled to 0 °C, and treated with trioxane alcohol **3** (100.7 mg, 0.37 mmol) in methylene chloride (1 mL) via cannula. This reaction mixture was stirred for 30 h and the solvent was removed at reduced pressure to yield a crude product which was directly separated by silica gel column chromatography to afford the corresponding pure trioxane sulfonate ester **12a** (119.4 mg, 76%) as a white solid which was spectroscopically pure. Recrystallization from ether/hexane (10:90) gave white crystals: mp 88–89 °C; FT-IR (neat, cm^{-1}) 1598, 1361, 1189, 1177; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.80 (d, J = 7.8 Hz, 2 H), 7.34 (d, J = 7.8 Hz, 2 H), 5.02 (d, J = 1.6 Hz, 1 H), 4.11 (m, 2 H), 3.45 (s, 3 H), 2.45 (s, 3 H), 2.28 (m, 1 H), 2.23–2.14 (m, 1 H), 1.99 (m, 1 H), 1.86–1.40 (m, 9 H), 1.36 (s, 3 H), 1.27–1.11 (m, 2 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 144.60, 133.25, 129.80, 127.96, 105.26, 99.80, 85.00, 69.59, 56.60, 48.52, 41.80, 37.42, 30.87, 29.87, 29.57, 27.02, 25.86, 25.03, 21.61. Anal. ($C_{21}H_{30}O_7S$) C, H, S.

Trioxane Sulfonate Ester 12b. To 2-(methoxycarbonyl)-benzenesulfonyl chloride (105.6 mg, 0.45 mmol) in dry methylene chloride (1 mL) was added triethylamine (200 μL, 1.4 mmol) via a gas-tight syringe. This reaction mixture was treated with trioxane alcohol **3** (41.6 mg, 0.15 mmol) in methylene chloride (1 mL) and then stirred for 24 h. The solvent was removed at reduced pressure to yield a crude product which was separated by silica gel column chromatography to afford the corresponding pure trioxane sulfonate ester **12b** (42.7 mg, 60%) as a colorless oil: FT-IR (neat, cm^{-1}) 1739, 1363, 1183; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.04 (dd, J = 7.6, 0.8 Hz, 1 H), 7.71–7.61 (m, 3 H), 5.07 (d, J = 1.2 Hz, 1 H), 4.29–4.20 (m, 2 H), 3.97 (s, 3 H), 3.47 (s, 3 H), 2.33–2.22 (m, 2 H), 2.00 (m, 1 H), 1.86–1.68 (m, 2 H), 1.67–1.60 (m, 1 H), 1.60–1.50 (m, 6 H), 1.35 (s, 3 H), 1.36–1.26 (m, 1 H), 1.23–1.13 (m, 1 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 167.29, 134.24, 133.38, 133.27, 130.76, 129.83, 129.39, 105.27, 99.85, 84.99, 70.44, 56.66, 53.27, 48.50, 41.63, 37.40, 30.85, 29.87, 29.49, 27.01, 25.87, 25.01; LRMS (NH_3, rel intensity) 488 (M + 18, 34), 380 (4), 272 (B), 255 (8), 234 (32), 223 (17), 195 (81), 137 (10); HRMS (CI, NH_3) calcd for $C_{22}H_{34}NO_9S$ (M + NH_4) 488.1954, found 488.1952.

Trioxane Sulfonate Ester 12c. To dansyl chloride (82.1 mg, 0.30 mmol) in dry chloroform (1 mL) was added triethylamine (50 μL, 0.4 mmol) via a gas-tight syringe. This reaction mixture was treated with trioxane alcohol **3** (23.7 mg, 0.1 mmol) in chloroform (1 mL) and then refluxed for 4 h. The solvent was removed at reduced pressure to yield a crude product which was separated by silica gel column chromatography to afford the corresponding pure trioxane sulfonate ester **12c** (30.1 mg, 68%) as a greenish gum. Recrystallization from methanol afforded greenish crystals: mp 90–91 °C; FT-IR (neat, cm^{-1}) 1614, 1589, 1357, 1174; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.60 (d, J = 8.4 Hz, 1 H), 8.30–8.26 (m, 2 H), 7.62–7.52 (m, 2 H), 7.11 (d, J = 7.6 Hz, 1 H), 4.89 (d, J = 1.2 Hz, 1 H), 4.13–4.03 (m, 2 H), 3.36 (s, 3 H), 2.89 (s, 6 H), 2.27–2.11 (m, 2 H), 1.98–1.92 (m, 1 H), 1.80–1.66 (m, 1 H), 1.65–1.56 (m, 1 H), 1.55–1.42 (m, 3 H), 1.42–1.19 (m, 4 H), 1.32 (s, 3 H), 1.17–1.09 (m, 1 H), 0.98–0.85 (m, 1 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 151.74, 131.37, 131.36, 130.52, 129.92, 129.89, 128.64, 123.04, 119.69, 115.54, 105.18, 99.80, 84.90, 69.77, 56.56, 48.38, 45.44, 41.50, 37.35, 30.77, 29.68, 29.33, 26.98, 25.82, 24.84. Anal. ($C_{26}H_{35}NO_7S$) C, H, N, S.

Trioxane Ether 13a. An oven-dried 5-mL one-necked round-bottomed flask was charged with trioxane alcohol **3** (34.2 mg, 0.13 mmol), dry *N*,*N*-dimethylformamide (0.5 mL), and methyl iodide (100 μL, 1.6 mmol) via a gas-tight syringe and cooled to 0 °C under argon atmosphere. This solution was treated with sodium hydride (60% dispersion on mineral oil, ca. 20 mg, 0.5 mmol). After being stirred for 10 min at 0 °C, the reaction mixture was slowly warmed to room temperature and stirred for 15 h. The reaction mixture was then cooled to 0 °C and quenched with water (1 mL). The organic layer was extracted twice with ether (5 mL × 2), washed with saturated NaCl solution (5 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was directly purified by silica gel column chromatography to afford the corresponding trioxane ether **13a** (17.4 mg, 48%) as a colorless oil: FT-IR ($CHCl_3$, cm^{-1}) 3020, 2931, 1442, 1408, 1376, 1266, 1120, 1078, 1008, 961, 952, 927, 870; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.15 (d, J = 1.6 Hz, 1 H), 3.49 (s, 3 H), 3.47–3.42 (m, 2 H), 3.33 (s, 3 H), 2.36–2.19 (m, 2 H), 2.04–1.98 (m, 1 H), 1.88–1.44 (m, 9 H), 1.38 (s, 3 H), 1.36–1.19 (m, 2 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 105.16, 100.32, 85.38, 71.57, 58.32, 56.67, 48.59, 42.14, 37.53, 31.11, 29.75, 29.56, 27.19, 25.96, 25.27; LRMS (NH_3, rel intensity) 304 (M + 18, 13), 287 (M + 1, 11), 255 (49), 195 (100), 137 (21); HRMS (CI, NH_3) calcd for $C_{15}H_{30}NO_5$ (M + 18) 304.2124, found 304.2130.

Trioxane Ether 13b. An oven-dried 5-mL one-necked round-bottomed flask was charged with trioxane alcohol **3** (12.1 mg, 0.04 mmol), dry *N*,*N*-dimethylformamide (0.5 mL), and benzyl bromide (25 μL, 0.2 mmol) via a gas-tight syringe, and cooled to 0 °C under argon atmosphere. This solution was treated with sodium hydride (60% dispersion on mineral oil, ca. 10 mg, 0.25 mmol). After being stirred for 10 min at 0 °C, the reaction mixture was slowly warmed to room temperature and stirred for 17 h. Then the reaction mixture was warmed to 60 °C and stirred for 1 h. The usual workup furnished the corresponding trioxane ether **13b** (5.1 mg, 32%) as a colorless oil: FT-IR ($CHCl_3$, cm^{-1}) 1602, ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.34 (d, J = 4.8 Hz, 4 H), 7.28 (m, 1 H), 5.15 (s, 1 H), 4.53 (d, J = 12.0 Hz, 1 H), 4.49 (d, J = 12.0 Hz, 1 H), 3.54 (m, 2 H), 3.49 (s, 3 H), 2.32 (m, 1 H), 2.28 (m, 1 H), 2.01 (m, 1 H), 1.89–1.48 (m, 8 H), 1.38 (s, 3 H), 1.41–1.17 (m, 1 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 138.57, 128.33, 127.68, 127.46, 105.15, 100.33, 85.36, 72.66, 69.22, 56.73, 48.55, 42.16, 37.50, 31.08, 29.86, 29.49, 27.16, 25.95, 25.23; LRMS (NH_3, rel intensity) 380 (M + 18, 11), 348 (5), 345 (8), 331 (7), 320 (20), 303 (100), 285 (88), 239 (13), 223 (26), 207 (17), 195 (96), 181 (19), 137 (31), 91 (16); HRMS (CI, NH_3) calcd for $C_{21}H_{34}NO_5$ (M + 18) 380.2437, found 380.2437.

Trioxane Ether 13c. An oven-dried 5-mL one-necked round-bottomed flask was charged with trioxane alcohol **3** (14.0 mg, 0.05 mmol), dry *N*,*N*-dimethylformamide (0.5 mL), and allyl bromide (50 μL, 0.58 mmol) via a gas-tight syringe and cooled to 0 °C under argon atmosphere. This solution was treated with sodium hydride (60% dispersion on mineral oil, ca. 10 mg, 0.25 mmol). After being stirred for 10 min at 0 °C, the reaction mixture was slowly warmed to room temperature and stirred for 4 h. The usual workup furnished the corresponding trioxane ether **13c** (9.1 mg, 57%) as a colorless oil: FT-IR ($CHCl_3$, cm^{-1}) 1644; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.92 (ddt, J = 17.2, 10.4, 5.6 Hz, 1 H), 5.27 (ddd, J = 17.2, 1.8, 1.2 Hz, 1 H), 5.17 (ddd, J = 10.4, 1.8, 1.2 Hz, 1 H), 5.15 (d, J = 1.2 Hz, 1 H), 3.97 (m, 2 H), 3.49 (s, 3 H), 3.52–3.47 (m, 2 H), 2.32 (ddd, J = 14.2, 14.2, 3.5 Hz, 1 H), 2.28–2.20 (m, 1 H), 2.01 (ddd, J = 14.2, 4.5, 3.0 Hz, 1 H), 1.89–1.48 (m, 8 H), 1.38 (s, 3 H), 1.37–1.19 (m, 3 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 135.00, 116.76, 105.15, 100.33, 85.36, 71.61, 69.30, 56.74, 48.54,

42.24, 37.49, 31.08, 29.91, 29.59, 27.15, 25.96, 25.24; LRMS (NH_3, rel intensity) 330 (M + 18, 40), 313 (M + 1, 39), 295 (19), 281 (81), 263 (46), 253 (13), 235 (48), 223 (63), 205 (67), 195 (41), 137 (21); HRMS (CI, NH_3) calcd for $C_{17}H_{29}O_5$ (M + H) 313.2015, found 313.2020.

Trioxane Ether 13d. An oven-dried 5-mL one-necked round-bottomed flask was charged with trioxane alcohol **3** (36.8 mg, 0.14 mmol), dry *N,N*-dimethylformamide (0.5 mL), and 4-(chloromethyl)-3,5-dimethylisoxazole (40 μL, 0.32 mmol) via a gas-tight syringe and cooled to 0 °C under argon atmosphere. This solution was treated with sodium hydride (60% dispersion on mineral oil, ca. 10 mg, 0.25 mmol). After being stirred for 10 min at 0 °C, the reaction mixture was slowly warmed to room temperature and stirred for 2 h. The usual workup furnished the corresponding trioxane ether **13d** (31.7 mg, 61%) as a colorless oil: FT-IR ($CHCl_3$, cm^{-1}) 1638; ^1H NMR ($CDCl_3$, 400 MHz) δ 5.13 (s, 1 H), 4.29 (d, *J* = 12.0 Hz, (1 H), 4.22 (d, *J* = 12.0 Hz, 1 H), 3.50–3.44 (m, 2 H), 3.48 (s, 3 H), 2.38 (s, 3 H), 2.38–2.27 (m, 1 H), 2.27 (s, 3 H), 2.27–2.19 (m, 1 H), 2.01 (ddd, *J* = 14.2, 5.0, 3.2 Hz, 1 H), 1.89–1.47 (m, 8 H), 1.38 (s, 3 H), 1.38–1.16 (m, 2 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 167.07, 159.92, 111.24, 105.18, 100.20, 85.30, 66.04, 68.71, 61.16, 56.68, 48.59, 41.93, 37.47, 31.02, 29.73, 29.49, 27.10, 25.93, 25.21, 11.02, 10.09; LRMS (NH_3, rel intensity) 382 (M + 1, 11), 364 (3), 350 (6), 322 (100), 239 (9), 225 (18), 195 (78), 181 (31), 137 (15), 110 (36); HRMS (CI, NH_3) calcd for $C_{20}H_{32}NO_8$ (M + H) 382.2230, found 382.2224.

Trioxane Carboxylate Ester 14. An oven-dried 10-mL one-necked round-bottomed flask was charged with terephthaloyl chloride (95.7 mg, 0.5 mmol) and dry methylene chloride (1 mL) and cooled to 0 °C. To this solution was added triethylamine (200 μL, 1.4 mmol) via a gas-tight syringe. After the reaction mixture was slowly warmed to room temperature over 0.5 h and stirred for 0.5 h, it was treated with trioxane alcohol **3** (58.5 mg, 0.22 mmol) in methylene chloride (1 mL). This reaction mixture was stirred for 1 h and the solvent was removed at reduced pressure to yield a crude product which was directly separated by silica gel column chromatography to afford the corresponding pure bis-trioxane carboxylate ester **14** (51.8 mg, 71%) as a colorless oil: FT-IR (neat) 1720; ^1H NMR ($CDCl_3$, 400 MHz) δ 8.10 (s, 4 H), 5.21 (s, 2 H), 4.48–4.35 (m, 4 H), 3.53 (s, 6 H), 2.45–2.30 (m, 4 H), 2.06–2.00 (m, 2 H), 1.91–1.66 (m, 4 H), 1.65–1.50 (m, 14 H), 1.45–1.34 (m, 2 H), 1.39 (s, 6 H), 1.34–1.21 (m, 2 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 166.85, 134.08, 129.55, 105.29, 100.11, 85.19, 64.38, 56.71, 48.56, 42.33, 37.48, 31.00, 29.43, 29.18, 27.10, 25.93, 25.18; LRMS (NH_3, rel intensity) 692 (M + 18, 9), 657 (1), 632 (11), 572 (64), 466 (11), 404 (12), 348 (22), 318 (17), 233 (16), 195 (100), 177 (11), 168 (24), 137 (14), 119 (35), 117 (23); HRMS (CI, NH_3) calcd for $C_{36}H_{54}NO_{12}$ (M + NH_4) 692.3646, found 692.3662.

Biology. Bulk drugs were dissolved in DMSO and 70% ethanol and diluted in culture medium (RPMI 1640) with 10% human plasma. Microtiter plates were prepared with 2-fold serial dilutions of the drugs over a concentration range of 0.062–256 ng/mL and the parasite suspension (at 0.5% parasitemia and a 1% hematocrit) were incubated at 37 °C in an air-tight plexiglass box which was flushed with 5% oxygen, 5% carbon dioxide, and 90% nitrogen. After 24 h of incubation, the cultures were labeled with [^3H]hypoxanthine and incubated for an additional 18–20 h. At that time particulate matter was harvested from each well by using an automated cell harvester (MACH II, TOMTEC, Orange, CT). [^3H]Hypoxanthine that was incorporated by the parasites in each well was then measured by scintillation spectrophotometry (LKB 1205 Betaplate, Wallac, Inc., Gaithersburg, MD). All tested drugs, as well as positive and negative controls were run in duplicate.

Acknowledgment. We thank the Environmental Health Sciences Center, School of Hygiene and Public Health, of The Johns Hopkins University for financial support of the chemistry program and Dr. N. Narashima Murthy and Professor Kenneth Karlin, of The Johns Hopkins University Chemistry Department, for the X-ray crystallography data on trioxane alcohol **3**. Financial support for culturing the malaria parasites and conducting drug assays was provided the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR).

NMR Studies of an FK-506 Analog, [U-^{13}C]Ascomycin, Bound to FK-506-Binding Protein

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Multidimensional, heteronuclear NMR methods were used to determine the complete ^1H and ^{13}C resonance assignments for [U-^{13}C]ascomycin bound to recombinant FKBP, including stereospecific assignment of all 22 methylene protons. The conformation of ascomycin was then determined from an analysis of NOEs observed in a ^{13}C-edited 3D HMQC-NOESY spectrum of the [U-^{13}C]ascomycin/FKBP. This structure is found to be quite different from the solution structure of the two forms of uncomplexed FK-506. However, it is very similar to the X-ray crystal structure of FK-506 bound to FKBP, rms deviation = 0.56 Å. The methods used for resonance assignment and structure calculation are presented in detail. Furthermore, FKBP/ascomycin NOEs are reported which help define the structure of the ascomycin binding pocket. This structural information obtained in solution was compared to the recently described X-ray crystal structure of the FKBP/FK-506 complex.

Introduction

The FK-506-binding protein, FKBP, is an 11.8-kDa protein that catalyzes the interconversion of the cis and trans rotamers of peptidyl-prolyl amide bonds.[1,2] This rotamase activity is inhibited by the immunosuppressant FK-506, which binds tightly (K_d ~ 0.4 nM) to this small enzyme. However, the binding of FK-506 to FKBP, although necessary, is not a sufficient condition for immunosuppressive activity. Recent studies suggest that upon formation of the FK-506/FKBP complex immunosuppressive activity is mediated by binding to and inhibiting the activity of the calcium-dependent phosphatase, calcineurin.[3]

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0022-2623/92/1835-2467$03.00/0