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Bile Acid-Based 1,2,4-Trioxanes: Synthesis and Antimalarial Assessment[1]

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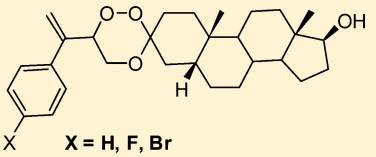
Supporting Information

ABSTRACT: A new series of bile acid-based trioxanes **23a−d**, **24a−d**, **25a−d**, **26a**, **26b**, and **26d** have been synthesized and assessed for their antimalarial activity against multidrug-resistant *Plasmodium yoelii* in Swiss mice by oral route. The antimalarial activity of these trioxanes showed a strong dependence on the side-chain length; shortening side-chain length lead to increase in activity. The antimalarial activity also showed even stronger dependence on the stereochemistry at C3 and C6 (C21 in Figure 5) of the trioxane moiety. Of the two diastereomers isolated of each of the trioxanes, more polar one was significantly more active than the less polar one. The more polar diastereomer of the trioxanes **26a**, **26b**, and **26d**, were the most active compounds of the series. All these three trioxanes provided 100% protection at 24 mg/kg × 4 days. In this model *β*-arteether provided 100% and 20% protection at 48 mg/kg × 4 days and 24 mg/kg × 4 days, respectively.



INTRODUCTION

Malaria affects around 300−500 million people in the tropical and subtropical areas of the world with an annual death toll of around two million.[2] The malaria situation is further complicated with the development of resistance against the standard and affordable drugs such as chloroquine.[3] Against this background, isolation of artemisinin **1** as the active principle of the Chinese traditional drug against malaria, *Artemisia annua*, was a major breakthrough in malaria chemotherapy.[4] Artemisinin owes its antimalarial activity due to the presence of 1,2,4-trioxane moiety and is active against both chloroquinine-sensitive and chloroquinine-resistant malaria. Artemisinin derivatives, e.g., artemether **2**, arteether **3**, and artesunic acid **4** (Figure 1), are currently the drugs of choice for the treatment of malaria caused by multidrug-resistant *Plasmodium falciparum*.[5]

As a part of our endeavor to develop synthetic substitutes for these drugs, we had recently reported a new series of steroid-based 1,2,4-trioxanes.[6] Of the several prototypes of these steroid-based trioxanes e.g. **5**, **6**, and **7** prepared by us (Figure 2), only prototype **7** had shown significant antimalarial activity, suggesting that the length of the side chain of the steroid component plays an important part in the biological activity of this class of compounds. These results prompted us to investigate the SAR of the corresponding bile acid-based trioxanes. Bile acids differ significantly from the sterols in shape, as unlike sterols, the A/B ring junction in bile acids is *cis*. Moreover, commercially available bile acids such as lithocholic acid, cholic acid, and dehydrocholic acid offer variety in the functional groups in tetracyclic ring system. Also bile acids have the advantage of having carboxyl group in the side chain which allows for a systematic degradation of the side chain. Together these unique properties of bile acids make them attractive templates for the preparation of a new series of 1,2,4-trioxanes. Several bile acid-based tetraoxanes prepared by Solaja et al., have shown promising antimalarial activity.[7] Using lithocholic acid and testosterone **20** as the starting materials, we have prepared ketones **10**, **15**, **19**, and **20a** (Figure 3) with decreasing side chain length. These ketones have been further elaborated into 1,2,4-trioxanes **23a−d**, **24a−d**, **25a−d**, **26a**, **26b**, and **26d**. Some of these new trioxanes have shown significant antimalarial activity against multidrug-resistant *Plasmodium yoelii* in mice.[8,9]

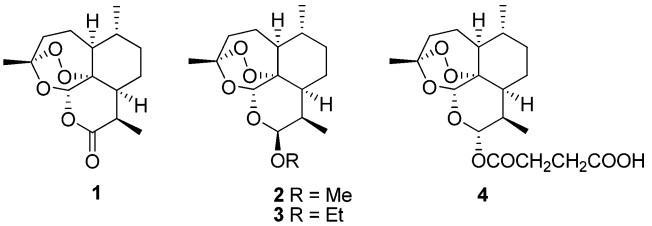


Figure 1. Artemisinin and its derivatives.

CHEMISTRY

Ketones **10**, **19**, and **20a** were prepared using reported procedures (Schemes 1, 3, and 4).[10,11] Ketone **15** was prepared from lithocholic acid **8** according to Scheme 2. Thus acetylation of lithocholic acid **8** with acetic anhydride yielded **11**, which on lead tetraacetate mediated oxidative decarboxylation furnished **12**. Olefin **12** on reaction with mercuric acetate in aqueous tetrahydrofuran, followed by addition of alkaline sodium

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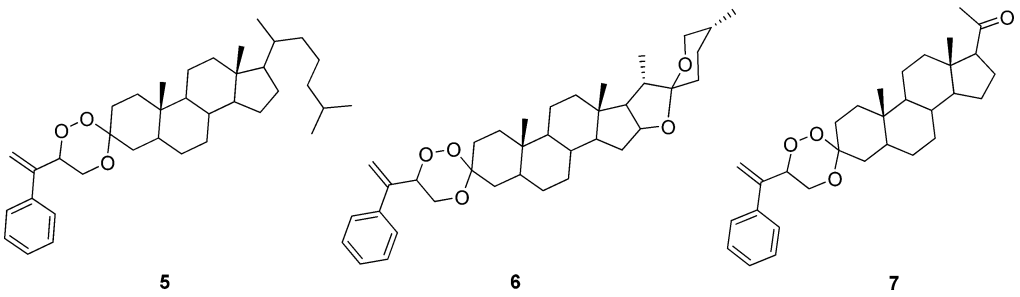


Figure 2. Steroid-based 1,2,4-trioxanes.

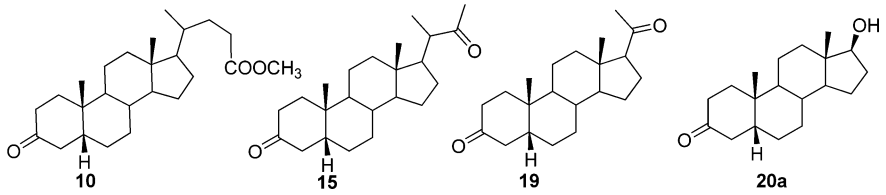
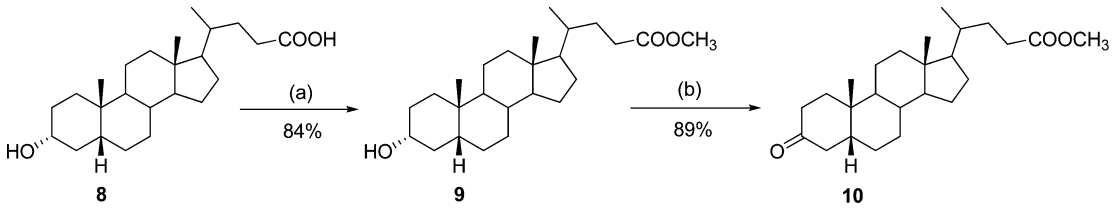


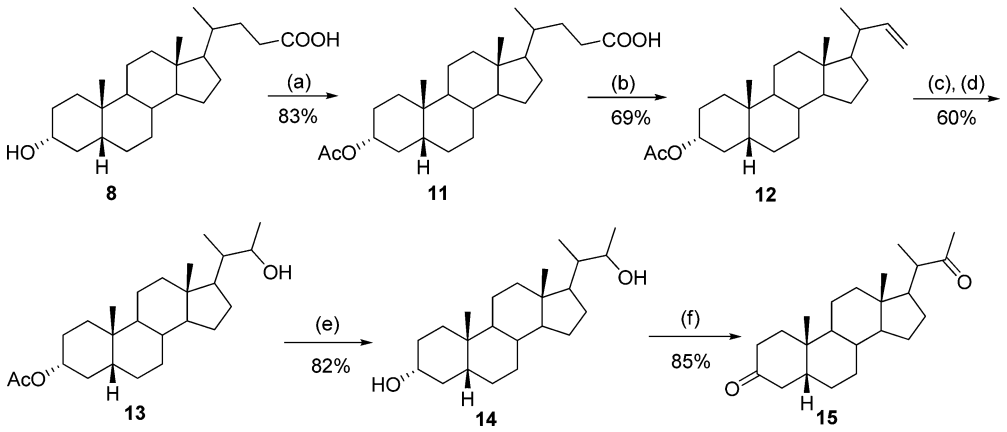
Figure 3. Bile acid-based ketones.

Scheme 1



Reagents and conditions: (a) MeOH, concd HCl, 65 °C, 4 h; (b) Jones reagent, acetone, 0 °C, 15 min.

Scheme 2



Reagents and conditions: (a) Ac_2O, Et_3N, DMAP (cat.), rt, 5 h; (b) $Pb(OAc)_4$, $Cu(OAc)_2$, C_5H_5N, C_6H_6, reflux, 12 h; (c) $Hg(AcO)_2$, THF-H_2O (1:1), rt, 30 min; (d) $NaBH_4$, NaOH, rt, 30 min; (e) MeOH, KOH, rt, 20 min; (f) Jones reagent, acetone, 0 °C, 15 min.

borohydride, furnished **13**, which on reaction with methanolic potassium hydroxide furnished **14**. Diol **14** was further oxidized with Jones reagent to **15**.

β-Hydroxyhydroperoxides **22a−d** were prepared by photooxygenation of allylic alcohols **21a−d**, using the procedure reported earlier.[12] Acid-catalyzed condensation of *β*-hydroxyhydroperoxides **22a−d** with ketones **10**, **15**, **19**, and **20a** furnished trioxanes **23a−d**, **24a−d**, **25a−d**, **26a**, **26b**, and **26d**, respectively, in 36−74% yields (Scheme 5, Table 1). All of these trioxanes were obtained as a mixture of diastereomers which were separated by column chromatography into two TLC homogeneous fractions referred to as less polar fraction (higher R_f on TLC) and more polar fraction (lower R_f on TLC). The more polar fraction in all these cases were found to be single compound as shown by ^{1}H NMR and ^{13}C NMR. A typical TLC representation of these fractions shown in Figure 4. Also the more polar fraction in all these cases was found to be significantly more active than the less polar fraction. The more polar diastereomer of **26a** furnished crystals suitable for X-ray analysis and were analyzed accordingly.

X-ray Analysis of 26a (More Polar Isomer). The conformation of compound **26a** (more polar isomer) was

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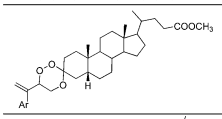
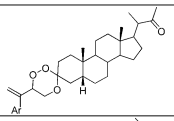
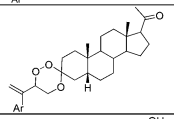
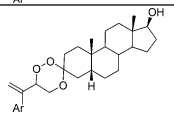
Table 1. Yields of Bile Acid-Based 1,2,4-Trioxanes

General Structure	Ar	Compound	% Yield
	Phenyl	**23a**	74
	4-fluorophenyl	**23b**	55
	4-chlorophenyl	**23c**	45
	4-bromophenyl	**23d**	51
	Phenyl	**24a**	44
	4-fluorophenyl	**24b**	67
	4-chlorophenyl	**24c**	50
	4-bromophenyl	**24d**	46
	Phenyl	**25a**	55
	4-fluorophenyl	**25b**	69
	4-chlorophenyl	**25c**	55
	4-bromophenyl	**25d**	36
	Phenyl	**26a**	39
	4-fluorophenyl	**26b**	63
	4-bromophenyl	**26d**	62

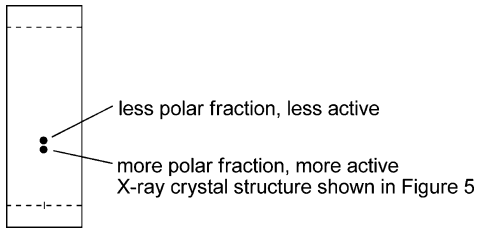


Figure 4. TLC representation of trioxane **26a**.

confirmed by single crystal X-ray diffraction studies. The compound **26a** (more polar isomer) was crystallized in $P2_1$ space group with two molecules in the unit cell. The ORTEP diagram (Figure 5) showed the molecular structure of $C_{29}H_{40}O_4$ and its conformation with atomic numbering scheme. The absolute configuration of the title compound could not be confirmed with present study. However, on the basis of the literature precedence,[11] the X-ray studies showed the molecule consists of six rings (A, B, C, D, E, and F) to which two β-equatorial methyl groups are attached at the C10 and C13 positions and one hydroxyl group at C17 substituted in ring F. The relative stereochemistry of C3 and C21 are *S* and *R*, respectively. Ring A is almost planar. Rings B, C, D, and E exist in chair conformations: the deviations of atom C3 and C21 are 0.681(5) and −0.705(5) Å, respectively, from the least-squares mean plane through atoms O1, O2, O3, and C20 for ring B, deviations of atom C3 and C10 are −0.596(5) and 0.676(5) Å, respectively, from the least-squares mean plane through atoms C1, C2, C4, and C5 for ring C, deviations of atoms C5 and C8 are −0.656(5) and 0.635(5) Å, respectively, from the least-squares mean plane through atoms C9, C10, C6, and C7 for ring D, while the deviations of atoms C9 and C13 are −0.616(5) and 0.696(6) Å, respectively, from the least-squares mean plane through atoms C11, C12, C14, and C8 for ring E. Ring F has an envelope conformation; deviations of atom C13 is 0.694(6) Å from the mean plane through atoms C14, C15, C16, and C17.

On the basis of the X-ray result of **26a** (more polar isomer), all more polar isomer of **23a−d**, **24a−d**, **25a−d**, **26b**, and **26d** were assigned the same stereochemistry as shown for **26a** in Figure 5.[13]

None of the compounds corresponding to less polar fraction of **23a−d**, **24a−d**, **25a−d**, **26a**, **26b**, and **26d** furnished crystals suitable for X-ray analysis. Also these fractions, although homogeneous on TLC, showed presence of more than one compound as shown by ^{13}C NMR. Because these fractions showed very poor antimalarial activity, no attempt was made to further purify these fractions.

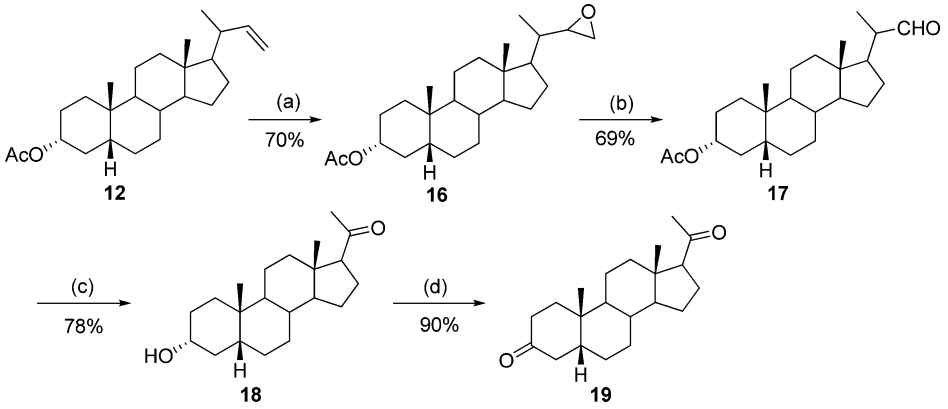
ANTIMALARIAL ACTIVITY

Both the fractions (less polar and more polar) corresponding to trioxanes **23a−d**, **24a−d**, **25a−d**, **26a**, **26b**, and **26d** were initially screened for antimalarial activity by oral route against multidrug-resistant *Plasmodium yoelii nigeriensis* in Swiss mice at a dose of 96 mg/kg × 4 days using Peter's procedure.[14] Trioxanes which showed 100% protection at 96 mg/kg × 4 days were further screened at lower doses.[15] In this model, β-arteether provided 100% and 20% protection at 48 mg/kg × 4 days and 24 mg/kg × 4 days, respectively, by oral route. The results are summarized in Table 2.

RESULT AND DISCUSSION

Reaction of β-Hydroxyhydroperoxides 22a−d with Ketones 10, 15, 19, and 20a: Stereochemical Expectation and Outcome. β-Hydroxyhydroperoxides **22a−d** used in this study were a racemic mixture and their reaction with each of the ketones **10**, **15**, **19**, and **20a** were expected to furnish trioxanes as a mixtures of four diastereomers. In the

Scheme 3



Reagents and conditions: (a) *m*-CPBA, $NaHCO_3$, CH_2Cl_2, 0 °C to rt, 5 h; (b) $HIO_4{\cdot}2H_2O$, THF, 0 °C to rt, 5 h; (c) $h\nu$, O_2, rose bengal, MeOH, 10% KOH, 0 °C, 6 h; (d) Jones reagent, acetone, 0 °C, 15 min.

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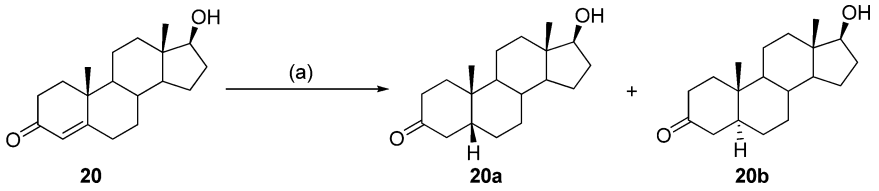
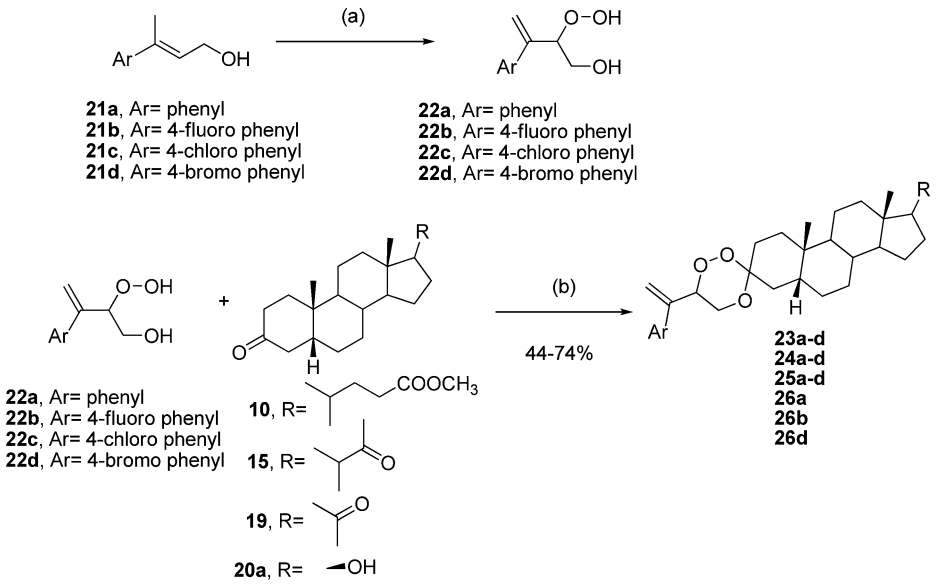
Scheme 4



Reagents and conditions: (a) Pd−C, H_2, THF, rt, 6 h.

Scheme 5



Reagents and conditions: (a) $h\nu$, O_2, methylene blue, CH_3CN, −10 to 0 °C, 4 h; (b) p−TSA, CH_3CN, rt, 6 h.

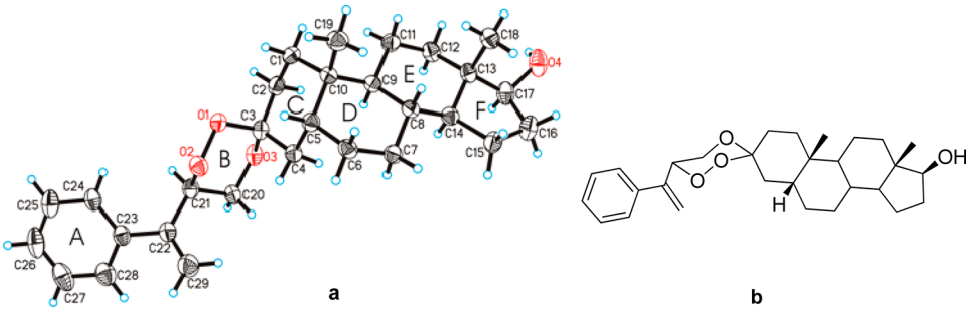


Figure 5. (a) ORTEP diagram of trioxane **26a** (more polar isomer) molecular structure of $C_{29}H_{40}O_4$ at 30% probability. (b) Stereostructure of **26a** (more polar isomer).

event, in each case the reaction furnished two TLC homogeneous fractions referred to as less polar fraction (higher R_f on TLC) and more polar fraction (lower R_f on TLC). The more polar fractions (lower R_f on TLC) of these trioxanes showed the presence of only one diastereomer as judged from their ^{1}H NMR and ^{13}C NMR data. The more polar fraction corresponding to trioxane **26a** furnished crystals suitable for X-ray analysis and were analyzed accordingly, and its stereochemistry is depicted in Figure 5. On the basis of the similarity in TLC pattern, ^{1}H NMR and ^{13}C NMR data all the other more polar isomer corresponding to trioxane **23a−d**, **24a−d**, **25a−d**, **26b**, and **26d** were assigned the same stereochemistry.

The corresponding less polar fraction of these trioxanes though homogeneous on TLC showed the presence of more than one diastereomer, at least two, as judged by ^{13}C NMR. Because none of these less polar fractions showed significant antimalarial activity, no attempt was made to further purify these fractions.

These results suggest that the reaction is partly stereospecific; one of the two enantiomers of β-hydroxyhydroperoxides favors the formation of a single diastereomer. Keeping the styrenyl side chain in an equatorial configuration could be the driving force for the selectivity. For the other enantiomer, an equatorially placed styrenyl side chain will require the ring closure from the β face of the bile acid nucleus. This energetically unfavorable requirement will result in loss of selectivity, leading to the formation of both the diastereomers comprising the less polar fraction.

Antimalarial Activity. Antimalarial activity of these trioxanes exhibited strong dependence on two factors (i)

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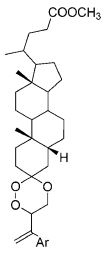
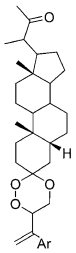
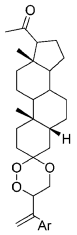
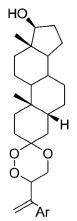
Table 2. In Vivo Antimalarial Activity of Trioxanes 23a–d, 24a–d, 25a–d, 26a, 26b, and 26d against Multidrug-Resistant *Plasmodium yoelii nigeriensis* in Swiss Mice by Oral Route

General structure	Ar	Log P	Compound	Dose	%Suppression of parasitaemia on day 4[a,b]	Cured**/ Treated
	phenyl	8.30	**23a** (less polar isomer)	96	20.60	0/5
			23a (more polar isomer)	96	43.56	0/5
	4-fluoro phenyl	8.46	**23b** (less polar isomer)	96	33.13	0/5
			23b (more polar isomer)	96	45.31	0/5
	4-chloro phenyl	8.86	**23c** (less polar isomer)	96	56.02	0/5
			23c (more polar isomer)	96	45.23	0/5
	4-bromo phenyl	9.13	**23d** (less polar isomer)	96	64.31	0/5
			23d (more polar isomer)	96	18.57	0/5
	phenyl	7.95	**24a** (less polar isomer)	96	94.31	0/5
			24a (more polar isomer)	96	100	3/5
	4-fluoro phenyl	8.11	**24b** (less polar isomer)	96	86.26	0/5
			24b (more polar isomer)	96	100	3/5
	4-chloro phenyl	8.51	**24c** (less polar isomer)	96	34.65	0/5
			24c (more polar isomer)	96	100	0/5
	4-bromo phenyl	8.78	**24d** (less polar isomer)	96	56.73	0/5
			24d (more polar isomer)	96	41.83	0/5
	phenyl	7.2	**25a** (less polar isomer)	96	79.89	0/5
			25a (more polar isomer)	96	100	5/5
				48	100	5/5
				24	100	3/5
	4-fluoro phenyl	7.36	**25b** (less polar isomer)	48	88.03	0/5
			25b (more polar isomer)	96	100	5/5
				48	100	5/5
				24	100	3/5
	4-chloro phenyl	7.76	**25c** (less polar isomer)	96	90.98	0/5
			25c (more polar isomer)	96	100	5/5
				48	100	5/5
				24	100	4/5
	4-bromo phenyl	8.03	**25d** (less polar isomer)	96	89.78	0/5
			25d (more polar isomer)	96	100	5/5
				48	100	4/5
				24	100	3/5
	phenyl	6.44	**26a** (less polar isomer)	96	69.36	0/5
			26a (more polar isomer)	96	100	5/5
				48	100	5/5
				24	100	5/5
				12	88.70	0/5
	4-fluoro phenyl	6.66	**26b** (less polar isomer)	96	100	2/5
			26b (more polar isomer)	96	100	5/5
				48	100	5/5
				24	100	5/5
				12	95.48	0/5
	4-bromo phenyl	7.27	**26d** (less polar isomer)	96	76.52	0/5
			26d (more polar isomer)	96	100	5/5
				48	100	5/5
				24	100	5/5
				12	44.50	0/5
	-	3.84	**3**	48	100	5/5
				24	100	1/5

[a]Percent suppression = $[(C - T)/C] \times 100$, where C = parasitaemia in control group and T = parasitaemia in treated group. [b]100% suppression of parasitaemia means, number of parasites if present, are below the detection limit.[15] (**) Mice that did not develop patent infection until day 28 were recorded as cured.

stereochemistry around trioxane ring and (ii) length of the side chain. As can be seen from Table 2, none of the less polar fractions corresponding to **23a–d**, **24a–d**, **25a–d**, **26a**, **26b**, and **26d** showed significant antimalarial activity. On the other hand, several of the trioxanes corresponding to the more polar fractions showed a high order of antimalarial activity by oral route and the antimalarial activity showed strong dependence on the length of the side chain.[16]

Trioxane **23a–d** (more polar isomer) with full length bile acid side chain showed very poor antimalarial activity; none of these trioxanes showed 100% clearance of parasitemia on day 4 at 96 mg/kg. Among trioxane **24a–d** (more polar isomer), with side chain shorter by one carbon, trioxane **24a–c** showed 100% clearance of parasitemia on day 4 at 96 mg/kg and two of them (**24a** and **24b**) provided 60% protection to the treated mice.

Trioxane **25a–d** (more polar isomer) with side chain length shorter by another two carbons, showed high-order antimalarial activity. Trioxanes **25a–c** provided 100% protection both at 96 mg/kg and 48 mg/kg. These trioxanes also provided 60–80% protection at 24 mg/kg. Trioxanes **26a**, **26b**, and **26d** (more polar isomer), with no carbon side chain, were the most active compounds of the series. All these three trioxanes showed 100% protection at 96, 48, and 24 mg/kg.

β-Arteether, the positive control in these studies, provided 100% protection at 48 mg/kg and 20% protection at 24 mg/kg. Thus, trioxane **24a**, **24b**, and **26d** are twice as active as β-arteether.

CONCLUSION

We have prepared a new series of trioxanes using bile acid derived ketones. The oral antimalarial activity of these trioxanes showed strong dependence both on stereochemistry around trioxane ring and length of the side chain. Trioxanes **26a**, **26b**, and **26d**, with no carbon side chain, were the most active compound of the series. All these three trioxanes showed 100% protection at 24 mg/kg and were twice as active as β-arteether.

EXPERIMENTAL SECTION

General. All glass apparatus were oven-dried prior to use. Melting points were determined on COMPLAB melting point apparatus and are uncorrected. IR spectra were recorded on a Perkin–Elmer FT–IR RXI spectrophotometer. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker DPX–200 (operating at 200 MHz for ^{1}H and at 50 MHz for ^{13}C) or DRX–300 (operating at 300 MHz for ^{1}H and at 75 MHz for ^{13}C) spectrometers using $CDCl_3$ as solvent. Tetramethylsilane (0.00 ppm) served as an internal standard in ^{1}H NMR and $CDCl_3$ (77.0 ppm) in ^{13}C NMR. Chemical shifts are reported in part per million. Splitting patterns are described as singlet (s), doublet (d), triplet (t) and multiplet (m). Electrospray mass spectra (ESMS) were recorded on a Micromass Quattro II triple quadruple mass spectrometer. Fast atom bombardment mass spectra (FAB-MS) were obtained on JEOL SX 102 spectrometer using argon/

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xenon (6 kV, 10 mA) as the FAB gas. Glycerol or m-nitrobenzyl alcohol was used as matrix. Reactions were monitored on silica gel TLC plates (coated with TLC grade silica gel, obtained from Merck). Detecting agents used (for TLC) were iodine vapors and/or spraying with an aq solution of vanillin in 10% sulfuric acid followed by heating at 150 °C. Column chromatography was performed over silica gel (60−120 Mesh) procured from Qualigens (India), flash silica gel (230−400 Mesh) procured from Spectrochem (India). All chemicals and reagents were obtained from Aldrich (USA), Lancaster (England) or Spectrochem (India) and were used without further purification. log *p* values of the compounds were calculated using Chem Draw Ultra 8.0 software. Elemental analyses of all the new compounds were recorded on Vario EL-III C H N S analyzer (Germany), and values were within 0.5% of the calculated values for all compounds and therefore these compounds meet the criteria of ≥95% purity.

Methyl-3-oxo-5β-cholan-24-oate (9). Concd hydrochloric acid (5.00 mL) was added to a suspension of **8** (5.00 g, 13.3 mmol) in methanol (200 mL), and the reaction mixture was refluxed at 65 °C for 4 h. Reaction mixture was concentrated in vacuo at rt to furnish crude product which was recrystallized with DCM−hexane to furnish **9** (4.36 g, 84% yield) as a white solid; mp 173−174 °C. IR (KBr, cm^{-1}) 1735, 3368. ^1H NMR (300 MHz, $CDCl_3$) δ 0.64 (s, 3H), 0.91−0.92 (m, 6H), 1.07−2.41 (m, 29H), 3.67 (s, 3H), 4.68−4.77 (m, 1H). ESMS (*m*/*z*) 413 $[M + Na]^+$.

Methyl-3-oxo-5β-cholan-24-oate (10). Jones reagent (5.00 mL) was added to a solution of **9** (4.00 g, 10.3 mmol) in acetone (50.0 mL), and the reaction mixture was stirred at 0 °C for 15 min. Reaction mixture was diluted with water (200 mL) and extracted with ethyl acetate (2 × 100 mL). The organic layer dried over anhyd Na_2SO_4 and concentrated in vacuo at rt to furnish crude product which was purified by column chromatography over silica gel using EtOAc−hexane (1:9) as eluent to furnish **10** (3.54 g, 89% yield) as a white solid; mp 145−146 °C. IR (KBr, cm^{-1}) 1714, 1737. ^1H NMR (300 MHz, $CDCl_3$) δ 0.65 (s, 3H), 0.88 (d, 3H, *J* = 6.3 Hz), 0.98 (s, 3H), 1.07−2.35 (m, 27 H), 2.65 (t, 1H, *J* = 14.2 Hz), 3.62 (s, 3H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 12.19 (CH_3), 18.40 (CH_3), 21.35 (CH_2), 22.75 (CH_3), 24.28 (CH_2), 25.91 (CH_2), 26.77 (CH_2), 28.25 (CH_2), 31.12 (CH_2), 31.16 (CH_2), 35.01 (C), 35.45 (CH_2), 35.70 (CH), 37.14 (CH), 37.25 (CH_2), 40.21 (CH_2), 40.94 (CH), 42.43 (CH), 42.92 (C), 44.44 (CH), 51.50 (CH_3), 56.16 (CH_2), 56.58 (CH), 174.71 (C), 213.26 (C). ESMS (*m*/*z*) 411 $[M + Na]^+$.

3α-Acetyl Lithocholic Acid (11). Acetic anhydride (10.00 mL), triethylamine (14.00 mL), and DMAP (5 mg, 0.04 mmol) were added to a solution of **8** (20.00 g, 53.11 mmol) in THF (100 mL), and reaction mixture was stirred at room temperature for 5 h. Reaction mixture was concentrated in vacuo at rt to furnish crude product which was purified by column chromatography over silica gel using MeOH−$CHCl_3$ (1:99) as eluent to furnish **11** (18.56 g, 83%). FT-IR (KBr, cm^{-1}): 1726. ^1H NMR (200 MHz, $CDCl_3$) δ 0.65 (s, 3H), 0.93 (s, 6H), 1.07−1.99 (m, 26H), 2.03 (s, 3H), 2.24−2.40 (m, 2H), 4.65−4.74 (m, 1H), 9.82 (s, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 12.45 (CH_3), 18.65 (CH_3), 21.23 (CH_2), 21.86 (CH_3), 23.73 (CH_3), 24.58 (CH_2), 26.72 (CH_2), 27.41 (CH_2), 28.57 (CH_2), 31.16 (CH_2), 31.45 (CH_2), 32.12 (CH_2), 32.62 (CH_2), 34.95 (C), 35.42 (CH_2), 35.70 (CH), 36.17 (CH), 40.53 (CH_2), 40.78 (CH), 42.26 (CH), 43.13 (C), 56.36 (CH), 56.88 (CH), 74.51 (CH), 171.19 (C), 180.73 (C). ESMS (*m*/*z*) 441 $[M + Na]^+$.

3α-Acetyl-24-nor-5β-chol-22-ene (12). Pyridine (50.0 mL) and cupric acetate (1.66 g, 8.3 mmol) were added to a solution of **11** (10.0 g, 23.9 mmol) in dry benzene (100 mL), and the reaction mixture was heated to 90 °C under N_2 atmosphere. Lead tetraacetate (30.0 g, 67.7 mmol) was added in portions during and the reaction mixture was refluxed for additional 12 h. It was cooled to room temperature and filtered through Celite; the residue was washed with benzene (75 mL). Addition of water (100 mL) and extraction with benzene yielded an organic layer that was further stirred with 1N HCl (200 mL) for 30 min. The benzene layer was separated and washed with water (100 mL) followed by saturated aq $NaHCO_3$ (100 mL). The organic layer was dried over anhyd Na_2SO_4, concentrated in vacuo at rt, and the crude product was purified by column chromatography over silica gel using EtOAc−hexane (2:98) to furnish **12** (6.15 g, 69% yield) as a white solid; mp 80−82 °C. FT-IR (KBr, cm^{-1}): 1726. ^1H NMR (200 MHz, $CDCl_3$) δ 0.67 (s, 3H), 0.93 (s, 3H), 1.05 (d, 3H, *J* = 6.2 Hz), 1.08−1.86 (m, 24H), 2.02 (s, 3H), 4.68−4.93 (m, 3H), 5.60−5.69 (m, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 12.61 (CH_3), 20.48 (CH_3), 21.22 (CH_2), 21.80 (CH_3), 23.74 (CH_3), 24.60 (CH_2), 26.74 (CH_2), 27.02 (CH_2), 27.42 (CH_2), 28.88 (CH_2), 32.64 (CH_2), 34.97 (C), 35.45 (CH_2), 36.19 (CH), 40.47 (CH_2), 40.87 (CH), 41.60 (CH), 42.28 (CH), 43.06 (C), 56.04 (CH), 56.94 (CH), 74.69 (CH), 111.96 (CH_2), 145.53 (CH), 174.25 (C). ESMS (*m*/*z*) 395 $[M + Na]^+$.

3α-Acetyl-24-nor-5β-cholan-22-ol (13). A solution of **12** (5.00 g, 13.4 mmol) in THF (10.0 mL) was added to a solution of mercuric acetate (10.2 g, 32.1 mmol) in H_2O (50.0 mL) and THF (50.0 mL). The reaction mixture was stirred at rt for 30 min. Then 3 M NaOH was added followed by the addition of 0.5 M $NaBH_4$ in 3 M NaOH. The reaction mixture was stirred at rt for another 30 min. The mercury was allowed to settle; sodium chloride was added to saturate the aqueous layer and separated. Organic layer was concentrated in vacuo at rt, and the crude product was purified by column chromatography over silica gel using EtOAc−hexane (1:9) to furnish **13** (3.16 g, 60% yield) as a white solid; mp 118−120 °C. FT-IR (KBr, cm^{-1}): 1715, 3416. ^1H NMR (200 MHz, $CDCl_3$) δ 0.79 (s, 3H), 1.06 (s, 6H), 1.17 (d, 3H, *J* = 6.7 Hz), 1.23−2.09 (m, 25H), 2.14 (s, 3H), 4.02 (bs, 1H), 4.83 (bs, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 11.96 (CH_3), 12.37 (CH_3), 16.01 (CH_3), 21.19 (CH_2), 21.73 (CH_3), 23.66 (CH_3), 24.63 (CH_2), 26.69 (CH_2), 26.92 (CH_2), 27.33 (CH_2), 27.75 (CH_2), 32.54 (CH_2), 34.85 (CH_2) 35.33 (C), 36.13 (CH), 40.50 (CH_2), 40.81 (CH), 42.15 (CH), 42.30 (CH), 43.27 (C), 54.03 (CH), 56.47 (CH), 69.02 (CH), 74.67 (CH), 170.91 (C). ESMS (*m*/*z*) 413 $[M + Na]^+$.

24-Nor-5β-cholan-3,22-diol (14). 10% methanolic KOH (20.0 mL) was added to **13** (3.00 g, 7.68 mmol) and stirred at rt for 20 min. Reaction mixture was diluted with water and filtered on a sintered funnel and dried to furnish **14** (2.18 g, 82% yield). ESMS (*m*/*z*) 371 $[M + Na]^+$.

24-Nor-5β-cholan-3,22-dione (15). Jones reagent (3.00 mL) was added to a solution of **14** (2.00 g, 5.74 mmol) in acetone (20.0 mL) and stirred at 0 °C for 15 min. Reaction mixture was diluted with water (200 mL) and extracted with EtOAc (2 × 50 mL). The organic layer was dried over anhyd Na_2SO_4 and concentrated in vacuo at rt to furnish crude product which was purified by column chromatography over silica gel using EtOAc−hexane (1:9) as eluent to furnish **15** (1.68 g, 85%) as an white solid; mp 160−162 °C. FT-IR (KBr, cm^{-1}): 1726, 2931. ^1H NMR (200 MHz, $CDCl_3$) δ 0.52 (s, 3H), 0.84 (s, 3H), 0.93 (d, 3H, *J* = 6.8 Hz), 1.11−1.83 (m, 20H), 1.91 (s, 3H), 1.98−2.32 (m, 3H), 2.52 (t, 1H, *J* = 14.1 Hz). ^{13}C NMR (50 MHz, $CDCl_3$) δ 12.61 (CH_3), 16.65 (CH_3), 21.45 (CH_2), 22.93 (CH_3), 24.70 (CH_2), 26.05 (CH_2), 26.89 (CH_2), 27.76 (CH_2), 28.32 (CH_3), 35.16 (C), 35.80 (CH), 37.30 (CH_2), 37.41 (CH_2), 40.22 (CH_2), 40.98 (CH), 42.57 (CH_2), 43.22 (C), 44.53 (CH), 50.47 (CH), 52.49 (CH), 56.12 (CH), 212.48 (C), 212.85 (C). ESMS (*m*/*z*) 367 $[M + Na]^+$.

3α-Acetyl-24-nor-5β-cholan-22-oxirane (16). *m*-CPBA (5.52 g, 40.5 mmol) was added to a mixture of **12** (4.00 g, 10.7 mmol) and $NaHCO_3$ (1.50 g, 17.8 mmol) in DCM (100 mL) at 0 °C over 20 min. The resulting mixture was stirred for additional 4 h at rt. The mixture was quenched with saturated aq $NaHCO_3$ solution (40 mL) and extracted with CH_2Cl_2 (2 × 50 mL). The organic layer dried over anhyd Na_2SO_4 and concentrated in vacuo at rt to furnish crude product which was purified by column chromatography over silica gel using EtOAc−hexane (2:98) to furnish **16** (2.92 g, 70% yield). FT-IR (KBr, cm^{-1}): 1733. ^1H NMR (300 MHz, $CDCl_3$) δ 0.62 (s, 3H), 0.84 (s, 3H), 1.02−1.08 (m, 7H), 1.22−1.94 (m, 20H), 2.00 (s, 3H), 2.55−2.57 (m, 2H), 2.70−2.76 (m, 1H), 4.70 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 10.82 (CH_3), 15.62 (CH_3), 19.46 (CH_2), 20.05 (CH_3), 22.00 (CH_3), 22.98 (CH_2), 25.02 (CH_2), 25.28 (CH_2), 25.67 (CH_2), 25.98 (CH_2), 30.90 (CH_2), 33.23 (C), 33.71 (CH_2), 34.47 (CH), 38.26 (CH), 38.58 (CH_2), 39.17 (CH), 40.52 (CH), 41.59 (C), 47.63 (CH_2), 52.74 (CH), 54.74 (CH), 55.98 (CH), 72.88 (CH), 169.03 (C). FAB-MS (*m*/*z*): 411 $[M + Na]^+$.

3α-Acetyl-23,24-dinor-5β-cholan-22-al (17). A solution of periodic acid (1.45 g, 6.38 mmol) in THF (5.00 mL) was added

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dropwise to an ice-cooled solution of **16** (2.00 g, 5.15 mmol) in THF (20.0 mL) over a period of 10 min. The resulting mixture was stirred for additional 4 h at rt. The reaction mixture was neutralized with saturated aq $NaHCO_3$ (20 mL) and water (50 mL) were extracted with diethyl ether (3 × 50 mL). The organic layer dried over anhyd Na_2SO_4 and concentrated in vacuo at rt to furnish crude product which was purified by column chromatography over silica gel using EtOAc−hexane (3:97) to furnish **17** (1.33 g, 69% yield). FT-IR (KBr, cm^{-1}): 1727. ^1H NMR (300 MHz, $CDCl_3$) δ 0.69 (s, 3H), 0.93 (s, 3H), 1.10 (d, 3H, J = 6.6 Hz), 1.21−1.94 (m, 23H), 2.01 (s, 3H), 2.30−2.37 (m, 1H), 4.65−4.76 (m, 1H), 9.55 (d, 1H, J = 3 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 11.09 (CH_3), 12.10 (CH_3), 19.19 (CH_2), 20.10 (CH_3), 21.99 (CH_3), 23.26 (CH_2), 25.03 (CH_2), 25.31 (CH_2), 25.65 (CH_2), 25.79 (CH_2), 30.93 (CH_2), 33.29 (C), 33.74 (CH_2), 34.50 (CH), 38.53 (CH_2), 39.19 (CH), 40.53 (CH), 42.00 (C), 48.12 (CH), 49.94 (CH), 54.46 (CH), 72.94 (CH), 169.20 (C), 203.59 (C); FAB-MS (m/z): 397 $[M + Na]^+$.

5β-pregnanolone (18). A slow stream of oxygen was bubbled into a solution of **17** (1.00 g, 2.67 mmol) and rose bengal (8 mg) in 10% methanolic KOH (50 mL) in a 200 mL double-jacketed round-bottom flask, maintained below 0 °C by circulating cold ethanol. The reaction mixture was irradiated with visible light by means of a tungsten−halogen lamp (500 W) for 6 h. The reaction mixture was poured into H_2O (50 mL), extracted with ether, and washed successively with 10% HCl (20 mL), saturated aq $NaHCO_3$ (20 mL), and H_2O (20 mL). The organic layer was dried over anhyd Na_2SO_4 and concentrated in vacuo at rt to furnish crude product which was purified by column chromatography over silica gel using EtOAc−hexane (15:85) to furnish **18** (0.660 g, 78% yield) as a solid; mp 126−128 °C. FT-IR (KBr, cm^{-1}): 1720, 3415. ^1H NMR (300 MHz, $CDCl_3$) δ 0.58 (s, 3H), 0.91 (s, 3H), 1.22−2.04 (m, 23H), 2.12 (s, 3H), 3.55−3.71 (m, 2H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 13.66 (CH_3), 21.14 (CH_2), 23.17 (CH_2), 23.64 (CH_3), 24.73 (CH_2), 26.72 (CH_2), 27.41 (CH_2), 30.46 (CH_2), 31.76 (CH_3) 34.92 (C), 35.67 (CH_2), 36.17 (CH), 36.35 (CH_2), 39.52 (CH_2), 40.76 (CH), 42.33 (CH), 44.76 (C), 57.05 (CH), 64.23 (CH), 71.69 (CH), 211.14 (C). ESMS (m/z) 341 $[M + Na]^+$.

5β-Pregna-3,20-dione (19). Jones reagent (2.00 mL) was added to a solution of **18** (660 mg, 2.07 mmol) in acetone (10.0 mL) at 0 °C for 15 min. Reaction mixture was diluted with water (100 mL) and extracted with EtOAc (2 × 25 mL). The organic layer was dried over anhyd Na_2SO_4 and concentrated in vacuo at rt to furnish crude product which was purified by column chromatography over silica gel using EtOAc−hexane (1:9) as eluent to furnish **19** (591 mg, 90% yield) as a white solid; mp 102−104 °C. IR (KBr, cm^{-1}): 1715. ^1H NMR (300 MHz, $CDCl_3$) δ 0.59 (s, 3H), 0.98 (s, 3H), 1.06−2.15 (m, 20H), 2.08 (s, 3H), 2.24−2.36 (m, 1H), 2.50 (t, 1H, J = 9.0 Hz), 2.64 (t, 1H, J = 14.2 Hz). ^{13}C NMR (50 MHz, $CDCl_3$) δ 13.51 (CH_3), 21.29 (CH_2), 22.70 (CH_3), 23.02 (CH_2), 24.47 (CH_2), 25.84 (CH_2), 26.62 (CH_2), 31.56 (CH_3) 35.00 (CH), 35.62 (C), 37.05 (CH_2), 37.21 (CH_2), 39.18 (CH_2), 40.83 (CH), 42.36 (CH_2), 44.26 (CH), 44.33 (C), 56.68 (CH), 63.81 (CH), 209.34 (C), 212.93 (C); ESMS (m/z) 339 $[M + Na]^+$.

17β-Hydroxy-5β-androstan-3-one (20a). Palladium charcoal (500 mg) was added to a solution of testosterone **20** (5.00 g, 17.3 mmol) in THF (25.0 mL) and hydrogenation was carried out at rt for 6 h.[11] The catalyst was filtered through Celite, solvent was removed under reduced pressure, and the crude product obtained as the mixture of 5α and 5β form was separated by column chromatography using EtOAc−benzene (2:98) as eluent to furnish **20a** (1.98 g, 39%) as a white solid; mp 120−122 °C. IR (KBr, cm^{-1}) 1708, 2862, 2927, 3421. ^1H NMR (300 MHz, $CDCl_3$) δ 0.76 (s, 3H), 1.03 (s, 3H), 1.07−2.36 (m, 22H), 2.67 (t, 1H, J = 14.2 Hz), 3.65 (t, 1H, J = 8.6 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 11.34 (CH_3), 20.97 (CH_3), 22.84 (CH_2), 23.54 (CH_2), 25.55 (CH_2), 26.64 (CH_2), 30.68 (CH_2), 35.15 (C), 35.77 (CH), 37.01 (CH_2), 37.21 (CH_2), 37.35 (CH_2), 41.10 (CH), 42.48 (CH_2), 43.29 (C), 44.48 (CH), 51.18 (CH), 81.93 (CH), 213.58 (C). ESMS (m/z) 313 $[M + Na]^+$.

General Procedure for Preparation of Bile Acid-Based 1,2,4-Trioxanes: Synthesis of Trioxane 23a. A slow stream of oxygen was bubbled into a solution of **21a** (500 mg, 3.37 mmol) and methylene blue (5 mg) in acetonitrile (25 mL) at −10 to 0 °C. The reaction mixture was irradiated with visible light by means of a tungsten−halogen lamp (500 W) for 4 h. β-Hydroxyhydroperoxide **22a** formed in the reaction was not isolated and reacted in situ with **10** (1.40 g, 3.60 mmol) in the presence of p-TSA (40 mg, 0.23 mmol) for 6 h at rt. Saturated aq $NaHCO_3$ (30 mL) was added. The aq layer was extracted with diethyl ether (2 × 100 mL), and the combined organic layer was dried over anhyd Na_2SO_4 and concentrated under vacuo at rt. The crude product was purified by column chromatography over silica gel using EtOAc−hexane (5:95) as eluent to furnish **23a** (1.37 g, 74% yield, based on **21a** as starting material).

Trioxane 23a. This was obtained in 74% yield as a diastereomeric mixture which was separated by column chromatography.

Trioxane (23a, Less Polar Isomer). This was obtained as an oil. IR (neat, cm^{-1}) 1630, 1736, 2925. ^1H NMR (300 MHz, $CDCl_3$) δ 0.63 (s, 3H), 0.89 (d, 3H, J = 6.3 Hz), 0.93 (s, 3H), 1.03−2.55 (m, 28H), 3.62 (s, 3H), 3.68−3.74 (m, 1H), 3.79−3.88 (m, 1H), 5.21 (dd, 1H, J = 10.2 and 2.3 Hz), 5.28 (s, 1H), 5.45 (s, 1H), 7.27−7.36 (m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 12.11 (CH_3), 18.32 (CH_3), 21.11 (CH_2), 23.16 (CH_3), 23.69 (CH_2), 24.22 (CH_2), 26.18 (CH_2), 26.54 (CH_2), 28.22 (CH_2), 31.01 (2 × CH_2), 32.63 (CH_2), 34.48 (CH_2), 35.08 (C), 35.38 (CH), 35.67 (CH), 39.15 (CH), 39.92 (CH), 40.13 (CH_2), 42.74 (C), 51.43 (CH_3), 55.93 (CH), 56.43 (CH), 62.70 (CH_2), 80.40 (CH), 103.72 (C), 116.28 (CH_2), 126.40 (2 × CH), 128.15 (CH), 128.56 (2 × CH), 138.60 (C), 143.61 (C), 174.59 (C). ESMS (m/z) 551 $[M + H]^+$. Anal. C for $C_{35}H_{50}O_5$: C, 76.33%, H, 9.15%. Found: C, 76.24%, H, 9.00%.

Trioxane (23a, More Polar Isomer). This was obtained as a solid; mp 80−81 °C. IR (KBr, cm^{-1}) 1630, 1735, 2920. ^1H NMR (300 MHz, $CDCl_3$) δ 0.65 (s, 3H), 0.91 (d, 3H, J = 6.2 Hz), 0.96 (s, 3H), 1.07−2.70 (m, 28H), 3.67 (s, 3H), 3.75 (dd, 1H, J = 11.6 and 2.7 Hz), 4.03 (dd, 1H, J = 11.6 and 10.6 Hz), 5.25 (dd, 1H, J = 10.6 and 2.7 Hz), 5.32 (s, 1H), 5.51 (s, 1H), 7.27−7.42 (m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 12.25 (CH_3), 18.49 (CH_3), 21.23 (CH_2), 23.16 (CH_3), 24.20 (CH_2), 24.37 (CH_2), 26.10 (CH_2), 26.43 (CH_2), 28.37 (CH_2), 31.21 (CH_2), 31.26 (CH_2), 32.97 (CH_2), 35.10 (C), 35.56 (CH), 35.82 (CH), 35.87 (CH_2), 39.37 (CH), 40.04 (CH), 40.34 (CH_2), 42.94 (C), 51.64 (CH_3), 56.19 (CH), 56.68 (CH), 63.12 (CH_2), 80.44 (CH), 103.81 (C), 116.52 (CH_2), 126.58 (2 × CH), 128.35 (CH), 128.76 (2 × CH), 138.86 (C), 143.67 (C), 174.89 (C). ESMS (m/z) 551 $[M + H]^+$. Anal. Calcd for $C_{35}H_{50}O_5$: C, 76.33%, H, 9.15%. Found: C, 76.19%, H, 9.37%.

Trioxane 23b. This was obtained in 55% yield as a diastereomeric mixture which was separated by column chromatography.

Trioxane (23b, Less Polar Isomer). This was obtained as an oil. IR (Neat, cm^{-1}) 1629, 1736, 2930. ^1H NMR (300 MHz, $CDCl_3$) δ 0.59 (s, 3H), 0.86 (d, 3H, J = 6.2 Hz), 0.89 (s, 3H), 1.00−2.49 (m, 28H), 3.58 (s, 3H), 3.63−3.69 (m, 1H), 3.75−3.85 (m, 1H), 5.10−5.13 (m, 1H), 5.24 (s, 1H), 5.37 (s, 1H), 6.94 (t, 2H, J = 8.5 Hz), 7.28−7.32 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 12.01 (CH_3), 18.24 (CH_3), 21.06 (CH_2), 22.64 (CH_2), 23.07 (CH_3), 23.12 (CH_3), 24.15 (CH_2), 26.14 (CH_2), 26.32 (CH_2), 26.50 (CH_2), 28.14 (CH_2), 30.91 (CH_2), 30.95 (CH_2), 31.57 (CH_2), 32.58 (CH_2), 34.38 (CH_2), 35.01 (C), 35.32 (CH), 35.64 (CH), 39.09 (CH), 39.27 (CH), 39.92 (CH), 40.09 (CH_2), 42.68 (C), 51.25 (CH_3), 55.93 (CH), 56.39 (CH), 62.36 (CH_2), 80.17 (CH), 80.33 (CH), 103.50 (C), 103.65 (C), 115.23 (CH), 115.51 (CH), 116.16 (CH_2), 116.31 (CH_2), 128.10 (CH), 128.21 (CH), 134.73 (C), 142.64 (C), 162.60 (C, J_{C-F} = 247.6 Hz), 174.35 (C). ESMS (m/z) 569 $[M + H]^+$. Anal. Calcd for $C_{35}H_{49}FO_5$: C, 73.91%, H, 8.68%. Found: C, 73.54%, H, 8.92%.

Trioxane (23b, More Polar Isomer). This was obtained as a white solid; mp 90−92 °C. IR (KBr, cm^{-1}) 1600, 1737, 2935. ^1H NMR (300 MHz, $CDCl_3$) δ 0.64 (s, 3H), 0.91 (d, 3H, J = 6.2 Hz), 0.94 (s, 3H), 1.04−2.67 (m, 28H), 3.66 (s, 3H), 3.74 (dd, 1H, J = 11.6 and 2.4 Hz), 4.03 (dd, 1H, J = 11.6 and 10.6 Hz), 5.18 (dd, 1H, J = 10.6 and 2.4 Hz), 5.30 (s, 1H), 5.45 (s, 1H), 7.03 (t, 2H, J = 8.6 Hz), 7.34−7.39 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 12.22 (CH_3), 18.45 (CH_3), 21.20 (CH_2), 23.13 (CH_3), 24.17 (CH_2), 24.34 (CH_2), 26.07 (CH_2), 26.39 (CH_2), 28.35 (CH_2), 31.18 (CH_2), 31.21 (2 ×

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CH_2), 32.91 (CH_2), 35.06 (C), 35.54 (CH), 35.77 (CH), 39.33 (CH), 40.00 (CH), 40.30 (CH_2), 42.90 (C), 51.64 (CH_3), 56.15 (CH), 56.64 (CH), 62.82 (CH_2), 80.38 (CH), 103.83 (C), 115.51 (CH), 115.79 (CH), 116.66 (CH_2), 128.28 (CH), 128.38 (CH), 134.89 (C), 142.58 (C), 162.84 (C, J_{C-F} = 246.1 Hz), 174.89 (C). ESMS (m/z) 569 $[M + H]^+$; Anal. Calcd for $C_{35}H_{49}FO_5$: C, 73.91%, H, 8.68%. Found: C, 73.69%, H, 8.79%.

Trioxane 23c. This was obtained in 45% yield as a diastereomeric mixture which was separated by column chromatography.

Trioxane (23c, Less Polar Isomer). This was obtained as an oil. IR (neat, cm^{-1}) 1589, 1735, 2938.1. ^1H NMR (300 MHz, $CDCl_3$) δ 0.64 (s, 3H), 0.90 (d, 3H, J = 6.1 Hz), 0.94 (s, 3H), 1.05−2.53 (m, 28H), 3.65 (s, 3H), 3.69−3.77 (m, 1H), 3.82−3.91 (m, 1H), 5.16−5.19 (m, 1H), 5.34 (s, 1H), 5.47 and 5.48 (2 × s, together integrating for 1H), 7.31 (s, 4H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 12.20 (CH_3), 18.42 (CH_3), 21.21 (CH_2), 23.25 (CH_3), 23.29 (CH_2), 24.32 (CH_2), 26.28 (CH_2), 26.46 (CH_2), 26.63 (CH_2), 27.06 (CH_2), 28.32 (CH_2), 29.16 (CH_2), 29.73 (CH_2), 31.15 (CH_2), 32.24 (CH_2), 32.72 (CH_2), 34.52 (CH_2), 35.20 (C), 35.50 (CH), 35.78 (CH), 39.29 (CH), 39.43 (CH), 40.05 (CH), 40.24 (CH_2), 42.86 (C), 51.60 (CH_3), 56.05 (CH), 56.57 (CH), 62.53 (CH_2), 80.26 (CH), 80.44 (CH), 103.84 (C), 103.99 (C), 117.07 (CH_2), 117.25 (CH_2), 127.94 (2 × CH), 128.86 (2 × CH), 134.24 (C), 137.18 (C), 142.64 (C), 174.84 (C). ESMS (m/z) 585 $[M + H]^+$. Anal. Calcd for $C_{35}H_{49}ClO_5$: C, 71.83%, H, 8.44%. Found: C, 71.88%, H, 8.03%.

Trioxane (23c, More Polar Isomer). This was obtained as a white solid; mp 105−106 °C. IR (KBr, cm^{-1}) 1591, 1738, 2936. ^1H NMR (300 MHz, $CDCl_3$) δ 0.65 (s, 3H), 0.91 (d, 3H, J = 6.2 Hz), 0.96 (s, 3H), 1.05−2.66 (m, 28H), 3.67 (s, 3H), 3.73−3.77 (m, 1H), 4.03 (dd, 1H, J = 11.4 and 10.8 Hz), 5.16−5.20 (m, 1H), 5.34 (s, 1H), 5.50 (s 1H), 7.33 (s, 4H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 12.25 (CH_3), 18.48 (CH_3), 21.22 (CH_2), 23.16 (CH_3), 24.37 (CH_2), 26.09 (CH_2), 26.42 (CH_2), 26.92 (CH_2), 28.39 (CH_2), 30.14 (CH_2), 31.21 (CH_2), 31.26 (CH_2), 32.93 (CH_2), 35.10 (C), 35.57 (CH), 35.80 (CH), 39.36 (CH), 40.03 (CH), 40.32 (CH_2), 42.94 (C), 51.70 (CH_3), 56.17 (CH), 56.67 (CH), 62.81 (CH_2), 80.25 (CH), 103.92 (C), 117.23 (CH_2), 127.96 (2 × CH), 128.95 (2 × CH), 134.33 (C), 137.28 (C), 142.52 (C), 174.98 (C). ESMS (m/z) 585 $[M + H]^+$. Anal. Calcd for $C_{35}H_{49}ClO_5$: C, 71.83%, H, 8.44%. Found: C, 71.38%, H, 8.85%.

Trioxane 23d. This was obtained in 51% yield as a diastereomeric mixture which was separated by column chromatography.

Trioxane (23d, Less Polar Isomer). This was obtained as an oil. IR (Neat, cm^{-1}) 1735, 2938. ^1H NMR (300 MHz, $CDCl_3$) δ 0.64 (s, 3H), 0.90 (d, 3H, J = 5.8 Hz), 0.94 (s, 3H), 1.04−2.51 (m, 28H), 3.65 (s, 3H), 3.68−3.76 (m, 1H), 3.80−3.89 (m, 1H), 5.14−5.18 (m, 1H), 5.34 (s, 1H), 5.47 and 5.48 (2 × s, together integrating for 1H), 7.24 (d, 2H, J = 8.3 Hz), 7.45 (d, 2H, J = 8.3 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 12.25 (CH_3), 18.47 (CH_3), 21.27 (CH_2), 23.29 (CH_3), 23.34 (CH_3), 23.90 (CH_2), 24.37 (CH_2), 26.33 (CH_2), 26.51 (CH_2), 26.68 (CH_2), 27.11 (CH_2), 28.36 (CH_2), 29.80 (CH_2), 31.23 (CH_2), 32.30 (CH_2), 32.78 (CH_2), 34.57 (CH_2), 35.26 (C), 35.55 (CH), 35.85 (CH), 39.36 (CH), 39.49 (CH), 40.12 (CH), 40.31 (CH_2), 42.93 (C), 51.64 (CH_3), 56.12 (CH), 56.64 (CH), 62.58 (CH_2), 80.30 (CH), 80.48 (CH), 103.92 (C), 104.07 (C), 117.21 (CH_2), 117.40 (CH_2), 122.48 (C), 128.32 (2 × CH), 131.87 (2 × CH), 137.74 (C), 142.79 (C), 174.90 (C). ESMS (m/z) 629 $[M + H]^+$. Anal. Calcd for $C_{35}H_{49}BrO_5$: C, 66.76%, H, 7.84%. Found: C, 66.28%, H, 7.54%.

Trioxane (23d, More Polar Isomer). This was obtained as a white solid; mp 120−121 °C. IR (KBr, cm^{-1}) 1735.4, 2938.1. ^1H NMR (300 MHz, $CDCl_3$) δ 0.64 (s, 3H), 0.90 (d, 3H, J = 5.8 Hz), 0.94 (s, 3H), 1.06−2.64 (m, 28H), 3.65 (s, 3H), 3.73−3.77 (m, 1H), 4.00−4.07 (m, 1H), 5.14−5.18 (m, 1H), 5.35 (s, 1H), 5.49 (s, 1H), 7.26 (d, 2H, J = 8.3 Hz), 7.45 (d, 2H, J = 8.3 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 12.23 (CH_3), 18.44 (CH_3), 21.18 (CH_2), 23.27 (CH_3), 24.33 (CH_2), 26.06 (CH_2), 26.45 (CH_2), 26.89 (CH_2), 28.34 (CH_2), 30.12 (CH_2), 31.20 (2 × CH_2), 32.54 (CH_2), 35.07 (C), 35.52 (CH), 35.79 (CH), 39.34 (CH), 40.03 (CH), 40.29 (CH_2), 42.90 (C), 51.60 (CH_3), 56.15 (CH), 56.68 (CH), 62.68 (CH_2), 80.14 (CH), 103.63 (C), 117.19 (CH_2), 122.46 (C), 128.22 (2 × CH), 131.85 (2 × CH), 137.74 (C), 142.55 (C), 174.81 (C). ESMS (m/z) 629 $[M + H]^+$. Anal. Calcd for $C_{35}H_{49}BrO_5$: C, 66.76%, H, 7.84%. Found: C, 67.08%, H, 8.05%.

Trioxane 24a. This was obtained in 44% yield as a diastereomeric mixture which was separated by column chromatography.

Trioxane (24a, Less Polar Isomer). This was obtained as semi solid. IR (neat, cm^{-1}) 1708, 2933. ^1H NMR (300 MHz, $CDCl_3$) δ 0.67 (s, 3H), 0.95 (s, 3H), 1.11−1.93 (m, 25H), 2.09 (s, 3H), 2.44−2.57 (m, 2H), 3.71−3.78 (m, 1H), 3.82−3.90 (m, 1H), 5.22−5.26 (m, 1H), 5.32 (s, 1H), 5.49 (s, 1H), 7.31−7.37 (m, 5H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 12.76 (CH_3), 16.80 (CH_3), 21.45 (CH_2), 23.51 (CH_3), 23.56 (CH_3), 24.07 (CH_2), 24.86 (CH_2), 26.56 (CH_2), 26.75 (CH_2), 26.86 (CH_2), 27.88 (CH_2), 28.18 (CH_3), 33.02 (CH_2), 34.86 (CH_2), 35.51 (C), 36.07 (CH), 39.53 (CH), 39.67 (CH), 40.38 (CH), 40.46 (CH_2), 43.40 (C), 50.87 (CH), 52.64 (CH), 56.33 (CH), 63.12 (CH_2), 63.18 (CH_2), 80.74 (CH), 80.88 (CH), 104.00 (C), 104.16 (C), 116.68 (CH_2), 116.82 (CH_2), 126.83 (2 × CH), 128.57 (CH), 128.97 (2 × CH), 139.01 (C), 143.94 (C), 143.98 (C), 213.19 (C). ESMS (m/z) 529 $[M + Na]^+$. Anal. Calcd for $C_{33}H_{46}O_4$: C, 78.22%, H, 9.15%. Found: C, 78.47%, H, 9.60%.

Trioxane (24a, More Polar Isomer). This was obtained as semi solid. IR (neat, cm^{-1}) 1709, 2931. ^1H NMR (300 MHz, $CDCl_3$) δ 0.67 (s, 3H), 0.95 (s, 3H), 1.09−1.94 (m, 25H), 2.09 (s, 3H), 2.45−2.70 (m, 2H), 3.74−3.79 (m, 1H), 4.05 (dd, 1H, = 11.5 and 10.5 Hz), 5.24 (dd, 1H, J = 10.2 and 2.1 Hz), 5.33 (s, 1H), 5.51 (s, 1H), 7.31−7.38 (m, 5H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 12.75 (CH_3), 16.79 (CH_3), 21.40 (CH_2), 23.52 (CH_3), 24.86 (CH_2), 26.32 (CH_2), 26.72 (CH_2), 27.11 (CH_2), 27.91 (CH_2), 28.40 (CH_3), 30.31 (CH_2), 32.81 (CH_2), 35.37 (C), 36.06 (CH), 39.57 (CH), 40.31 (CH), 40.46 (CH_2), 43.40 (C), 50.78 (CH), 52.67 (CH), 56.39 (CH), 63.29 (CH_2), 80.67 (CH), 103.78 (C), 116.76 (CH_2), 126.79 (2 × CH), 128.58 (CH), 128.98 (2 × CH), 139.06 (C), 143.82 (C), 213.20 (C). ESMS (m/z) 529 $[M + Na]^+$. Anal. Calcd for $C_{33}H_{46}O_4$: C, 78.22%, H, 9.15%. Found: C, 77.90%, H, 8.95%.

Trioxane 24b. This was obtained in 67% yield as a diastereomeric mixture which was separated by column chromatography.

Trioxane (24b, Less Polar Isomer). This was obtained as a white solid; mp 65−67 °C. IR (KBr, cm^{-1}) 1708, 2932. ^1H NMR (300 MHz, $CDCl_3$) δ 0.66 (s, 3H), 0.94 (s, 3H), 1.10 (d, 3H, J = 6.8 Hz), 1.14−1.97 (m, 22H), 2.08 (s, 3H), 2.31−2.53 (m, 2H), 3.68−3.76 (m, 1H), 3.81−3.89 (m, 1H), 5.15−5.19 (m, 1H), 5.29 (s, 1H), 5.42−5.43 (m, 1H), 7.01 (t, 2H, J = 8.6 Hz), 7.31−7.36 (m, 2H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 12.74 (CH_3), 16.78 (CH_3), 21.43 (CH_2), 23.49 (CH_3), 23.54 (CH_3), 24.09 (CH_2), 24.85 (CH_2), 26.54 (CH_2), 26.73 (CH_2), 26.84 (CH_2), 27.27 (CH_2), 27.87 (CH_2), 28.18 (CH_3), 29.45 (CH_2), 29.98 (CH_2), 32.51 (CH_2), 32.99 (CH_2), 34.80 (CH_2), 35.50 (C), 36.06 (CH), 39.53 (CH), 39.66 (CH), 40.38 (CH), 40.45 (CH_2), 43.40 (C), 50.86 (CH), 52.64 (CH), 56.33 (CH), 62.85 (CH_2), 62.91 (CH_2), 80.74 (CH), 104.06 (C), 104.21 (C), 115.65 (CH), 116.07 (CH), 116.88 (CH_2), 117.06 (CH_2), 128.54 (CH), 128.71 (CH), 135.13 (C), 142.93 (C), 142.98 (C), 163.07 (C, J_{C-F} = 246 Hz), 213.20 (C). ESMS (m/z) 547 $[M + Na]^+$. Anal. Calcd for $C_{33}H_{45}FO_4$: C, 75.54%, H, 8.64%. Found: C, 75.09%, H, 8.76%.

Trioxane (24b, More Polar Isomer). This was obtained as a white solid; mp 76−77 °C. IR (KBr, cm^{-1}) 1709, 2933. ^1H NMR (300 MHz, $CDCl_3$) δ 0.67 (s, 3H), 0.95 (s, 3H), 1.11 (d, 3H, J = 6.8 Hz), 1.26−1.94 (m, 22H), 2.09 (s, 3H), 2.45−2.67 (m, 2H), 3.74−3.79 (m, 1H), 4.05 (dd, 1H, J = 11.2 and 10.6 Hz), 5.16−5.19 (m, 1H), 5.31 (s, 1H), 5.46 (s, 1H), 7.02 (t, 2H, J = 8.6 Hz), 7.34−7.39 (m, 2H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 12.73 (CH_3), 16.77 (CH_3), 21.38 (CH_2), 23.49 (CH_3), 24.85 (CH_2), 26.30 (CH_2), 26.70 (CH_2), 27.08 (CH_2), 27.90 (CH_2), 28.42 (CH_3), 30.34 (CH_2), 32.78 (CH_2), 35.34 (C), 36.04 (CH), 39.55 (CH), 40.29 (CH), 40.44 (CH_2), 43.39 (C), 50.75 (CH), 52.65 (CH), 56.37 (CH), 63.00 (CH_2), 80.63 (CH), 103.82 (C), 115.66 (CH), 116.09 (CH), 116.92 (CH_2), 128.49 (CH), 128.64 (CH), 135.13 (C), 142.77 (C), 163.07 (C, J_{C-F} = 246 Hz), 213.22 (C). ESMS (m/z) 547 $[M + Na]^+$. Anal. Calcd for $C_{33}H_{45}FO_4$: C, 75.54%, H, 8.64%. Found: C, 75.17%, H, 8.54%.

Trioxane 24c. This was obtained in 50% yield as a diastereomeric mixture which was separated by column chromatography.

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Trioxane (24c, Less Polar Isomer). This was obtained as a white solid; mp 74−75 °C. IR (KBr, cm^{-1}) 1709, 2933. ^1H NMR (300 MHz, $CDCl_3$) δ 0.67 (s, 3H), 0.95 (s, 3H), 1.11 (d, 3H, J = 6.7 Hz), 1.19−1.99 (m, 22H), 2.09 (s, 3H), 2.30−2.50 (m, 2H), 3.69−3.77 (m, 1H), 3.82−3.91 (m, 1H), 5.16−5.19 (m, 1H), 5.34 (s, 1H), 5.48 (s, 1H), 7.31 (s, 4H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 12.41 (CH_3), 16.45 (CH_3), 21.11 (CH_2), 23.17 (CH_3), 23.22 (CH_3), 24.52 (CH_2), 26.23 (CH_2), 26.41 (CH_2), 26.53 (CH_2), 27.00 (CH_2), 27.55 (CH_2), 27.86 (CH_3), 29.09 (CH_2), 29.67 (CH_2), 32.18 (CH_2), 32.67 (CH_2), 34.45 (CH_2), 35.15 (C), 35.72 (CH), 39.17 (CH), 39.32 (CH), 40.05 (CH), 40.10 (CH_2), 43.04 (C), 50.48 (CH), 52.30 (CH), 55.98 (CH), 62.46 (CH_2), 80.17 (CH), 80.35 (CH), 103.69 (C), 103.84 (C), 116.97 (CH_2), 117.16 (CH_2), 127.85 (CH), 127.88 (CH), 128.79 (2 × CH), 134.16 (C), 137.11 (C), 142.50 (C), 142.56 (C), 212.62 (C). ESMS (m/z) 563 $[M + Na]^+$. Anal. Calcd for $C_{33}H_{45}ClO_4$: C, 73.24%, H, 8.38%. Found: C, 73.43%, H, 8.31%.

Trioxane (24c, More Polar Isomer). This was obtained as white solid; mp 90−91 °C. IR (KBr, cm^{-1}) 1709, 2934. ^1H NMR (300 MHz, $CDCl_3$) δ 0.67 (s, 3H), 0.95 (s, 3H), 1.11 (d, 3H, J = 6.7 Hz), 1.25−1.94 (m, 22H), 2.09 (s, 3H), 2.45−2.66 (m, 2H), 3.74−3.79 (m, 1H), 4.05 (dd, 1H, J = 11.3 and 10.6 Hz), 5.16−5.19 (m, 1H), 5.35 (s, 1H), 5.50 (s, 1H), 7.32 (s, 4H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 12.31 (CH_3), 16.36 (CH_3), 20.97 (CH_2), 23.08 (CH_3), 24.43 (CH_2), 25.89 (CH_2), 26.29 (CH_2), 26.67 (CH_2), 27.49 (CH_2), 28.00 (CH_2), 29.93 (CH_3), 32.37 (CH_2), 34.93 (C), 35.62 (CH), 39.14 (CH), 39.89 (CH), 40.03 (CH_2), 42.97 (C), 50.33 (CH), 52.24 (CH), 55.96 (CH), 62.53 (CH_2), 80.04 (CH), 103.44 (C), 117.01 (CH_2), 127.75 (2 × CH), 128.73 (2 × CH), 134.11 (C), 137.09 (C), 142.29 (C), 212.76 (C). ESMS (m/z) 563 $[M + Na]^+$. Anal. Calcd for $C_{33}H_{45}ClO_4$: C, 73.24%, H, 8.38%. Found: C, 73.15%, H, 8.39%.

Trioxane 24d. This was obtained in 46% yield as a diastereomeric mixture which was separated by column chromatography.

Trioxane (24d, Less Polar Isomer). This was obtained as a white solid; mp 78−79 °C. IR (KBr, cm^{-1}) 1708, 2933. ^1H NMR (300 MHz, $CDCl_3$) δ 0.59 (s, 3H), 0.88 (s, 3H), 1.04 (d, 3H, J = 6.8 Hz), 1.18−1.91 (m, 22H), 2.02 (s, 3H), 2.24−2.45 (m, 2H), 3.62−3.70 (m, 1H), 3.74−3.83 (m, 1H), 5.09−5.12 (m, 1H), 5.27 (s, 1H), 5.41 and 5.42 (2 × s, together integrating for 1H), 7.18 (d, 2H, J = 8.4 Hz), 7.39 (d, 2H, J = 8.4 Hz). ^{13}C NMR (50 MHz, $CDCl_3$) δ 12.55 (CH_3), 16.59 (CH_3), 21.23 (CH_2), 23.29 (CH_3), 23.34 (CH_3), 23.89 (CH_2), 24.65 (CH_2), 26.34 (CH_2), 26.53 (CH_2), 26.64 (CH_2), 27.07 (CH_2), 27.67 (CH_2), 27.99 (CH_3), 29.22 (CH_2), 29.78 (CH_2), 32.30 (CH_2), 32.78 (CH_2), 34.56 (CH_2), 35.30 (C), 35.85 (CH), 39.33 (CH), 39.45 (CH), 40.16 (CH), 40.24 (CH_2), 43.20 (C), 50.68 (CH), 52.43 (CH), 56.12 (CH), 62.61 (CH_2), 62.68 (CH_2), 80.51 (CH), 103.91 (C), 104.06 (C), 117.29 (CH_2), 117.49 (CH_2), 122.51 (C), 128.31 (CH), 128.34 (CH), 131.90 (2 × CH), 137.70 (C), 142.67 (C), 142.73 (C), 213.08 (C). ESMS (m/z) 586 $[M + H]^+$. Anal. Calcd for $C_{33}H_{45}BrO_4$: C, 67.68%, H, 7.75%. Found: C, 67.61%, H, 8.06%.

Trioxane (24d, More Polar Isomer). This was obtained as a white solid; mp 91−93 °C. IR (KBr, cm^{-1}) 1709, 2934. ^1H NMR (300 MHz, $CDCl_3$) δ: 0.66 (s, 3H), 0.94 (s, 3H), 1.10 (d, 3H, J = 6.8 Hz), 1.19−1.93 (m, 22H), 2.09 (s, 3H), 2.42−2.65 (m, 2H), 3.73−3.78 (m, 1H), 4.04 (dd, 1H, J = 11.5 and 10.3 Hz), 5.15−5.18 (m, 1H), 5.34 (s, 1H), 5.50 (s, 1H), 7.25 (d, 2H, J = 8.2 Hz), 7.45 (d, 2H, J = 8.2 Hz). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 12.74 (CH_3), 16.80 (CH_3), 21.39 (CH_2), 23.51 (CH_3), 24.86 (CH_2), 26.71 (CH_2), 27.10 (CH_2), 27.91 (CH_2), 28.45 (CH_3), 30.35 (CH_2), 32.79 (CH_2), 33.15 (CH_2), 35.36 (C), 36.04 (CH), 39.56 (CH), 40.29 (CH), 40.45 (CH_2), 43.40 (C), 50.77 (CH), 52.65 (CH), 56.37 (CH), 62.96 (CH_2), 80.41 (CH), 103.89 (C), 117.53 (CH_2), 122.73 (C), 128.49 (2 × CH), 132.13 (2 × CH), 137.98 (C), 142.74 (C), 213.33 (C). ESMS (m/z) 586 $[M + H]^+$. Anal. Calcd for $C_{33}H_{45}BrO_4$: C, 67.68%, H, 7.75%. Found: C, 67.28%, H, 7.91%.

Trioxane 25a. This was obtained in 55% yield as a diastereomeric mixture which was separated by column chromatography.

Trioxane (25a, Less Polar Isomer). This was obtained as a white solid; mp 62−63 °C. IR (KBr, cm^{-1}) 1702, 2934. ^1H NMR (300 MHz, $CDCl_3$) δ 0.59 (s, 3H), 0.95 (s, 3H), 1.12−2.16 (m, 22H), 2.12 (s, 3H), 2.51−2.59 (m, 1H), 3.73 (dd, 1H, J = 11.9 and 2.9 Hz), 3.86 (dd, 1H, J = 11.9 and 10.3 Hz), 5.23 (dd, 1H, J = 10.3 and 2.6 Hz), 5.32 (s, 1H), 5.49 (s, 1H), 7.29−7.39 (m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.63 (CH_3), 21.27 (CH_2), 23.10 (CH_2), 23.24 (CH_3), 23.89 (CH_2), 24.61 (CH_2), 26.32 (CH_2), 26.57 (CH_2), 31.73 (CH_3), 32.81 (CH_2), 34.64 (CH_2), 35.14 (CH_2), 35.32 (C), 35.85 (CH), 39.30 (CH), 39.39 (CH_2), 40.14 (CH), 44.50 (C), 56.89 (CH), 62.90 (CH_2), 64.02 (CH), 80.71 (CH), 103.75 (C), 103.90 (C), 116.64 (CH_2), 126.58 (CH), 126.63 (CH), 128.35 (CH), 128.75 (2 × CH), 138.80 (C), 143.78 (C), 209.77 (C). ESMS (m/z) 496 $[M + NH_4]^+$. Anal. Calcd for $C_{31}H_{42}O_4$: C, 77.79%, H, 8.84%. Found: C, 77.98%, H, 8.72%.

Trioxane (25a, More Polar Isomer). This was obtained as a white solid; mp 89−91 °C. IR (KBr, cm^{-1}) 1702, 2934. ^1H NMR (300 MHz, $CDCl_3$) δ 0.60 (s, 3H), 0.95 (s, 3H), 1.19−2.21 (m, 22H), 2.11 (s, 3H), 2.49−2.55 (m, 1H), 3.77 (dd, 1H, J = 11.6 and 2.8 Hz), 4.04 (dd, 1H, J = 11.66 and 10.3 Hz), 5.24 (dd, 1H, J = 10.3 and 2.8 Hz), 5.33 (s, 1H), 5.51 (s, 1H), 7.30−7.40 (m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.67 (CH_3), 21.27 (CH_2), 23.13 (CH_2), 23.29 (CH_3), 24.65 (CH_2), 26.52 (CH_2), 26.86 (CH_2), 30.28 (2 × CH_2), 31.75 (CH_3), 32.64 (CH_2), 35.22 (C), 35.89 (CH), 39.38 (CH), 39.47 (CH_2), 40.12 (CH), 44.57 (C), 57.04 (CH), 63.14 (CH_2), 64.13 (CH), 80.53 (CH), 103.57 (C), 116.60 (CH_2), 126.64 (2 × CH), 128.42 (CH), 128.81 (2 × CH), 138.90 (C), 143.65 (C), 209.82 (C). ESMS (m/z) 496 $[M + NH_4]^+$. Anal. Calcd for $C_{31}H_{42}O_4$: C, 77.79%, H, 8.84%. Found: C, 77.62%, H, 8.91%.

Trioxane 25b. This was obtained in 69% yield as a diastereomeric mixture which was separated by column chromatography.

Trioxane (25b, Less Polar Isomer). This was obtained as a white solid; mp 65−66 °C. IR (KBr, cm^{-1}) 1702, 2934. ^1H NMR (300 MHz, $CDCl_3$) δ 0.60 (s, 3H), 0.95 (s, 3H), 1.21−2.03 (m, 22H), 2.11 (s, 3H), 2.50−2.56 (m, 1H), 3.70−3.78 (m, 1H), 3.83−3.92 (m, 1H), 5.18 (dd, 1H, J = 10.2 and 2.8 Hz), 5.31 (s, 1H), 5.45 (s, 1H), 7.02 (t, 2H, J = 8.4 Hz), 7.33−7.38 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.65 (CH_3), 21.28 (CH_2), 23.25 (CH_2), 23.30 (CH_3), 24.61 (CH_2), 26.51 (CH_2), 26.57 (CH_2), 27.00 (CH_2), 29.25 (CH_2), 29.77 (CH_2), 31.76 (CH_3), 32.31 (CH_2), 35.32 (C), 35.86 (CH), 39.38 (CH_2), 39.43 (CH), 40.10 (CH), 44.52 (C), 56.92 (CH), 62.71 (CH_2), 64.02 (CH), 80.56 (CH), 103.81 (C), 115.53 (CH), 115.81 (CH), 116.71 (CH_2), 128.35 (CH), 128.46 (CH), 134.89 (C), 134.93 (C), 142.71 (C), 142.76 (C), 163.03 (C, J_{C-F} = 246.7 Hz), 209.83 (C). ESMS (m/z) 519 $[M + Na]^+$. Anal. Calcd for $C_{31}H_{41}FO_4$: C, 74.97%, H, 8.32%. Found: C, 74.61%, H, 8.79%.

Trioxane (25b, More Polar Isomer). This was obtained as a white solid; mp 78−80 °C. IR (KBr, cm^{-1}) 1694, 2936. ^1H NMR (300 MHz, $CDCl_3$) δ 0.59 (s, 3H), 0.94 (s, 3H), 1.20−1.89 (m, 22H), 2.10 (s, 3H), 2.45−2.54 (m, 1H), 3.75 (dd, 1H, J = 11.4 and 2.3 Hz), 4.04 (dd, 1H, J = 114 and 10.4 Hz), 5.17 (dd, 1H, J = 10.4 and 2.3 Hz), 5.31 (s, 1H), 5.46 (s, 1H), 7.02 (t, 2H, J = 8.5 Hz), 7.33−7.38 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.42 (CH_3), 21.02 (CH_2), 22.89 (CH_2), 23.04 (CH_3), 24.40 (CH_2), 26.27 (CH_2), 26.61 (CH_2), 29.96 (2 × CH_2), 31.49 (CH_3), 32.38 (CH_2), 34.96 (C), 35.63 (CH), 39.13 (CH_2), 39.21 (CH), 39.88 (CH), 44.31 (C), 56.78 (CH), 62.61 (CH_2), 63.87 (CH), 80.25 (CH), 103.37 (C), 115.32 (CH), 115.61 (CH), 116.51 (CH_2), 128.12 (CH), 128.22 (CH), 134.77 (C), 142.39 (C), 162.69 (C, J_{C-F} = 246 Hz), 209.53 (C). ESMS (m/z) 519 $[M + Na]^+$. Anal. Calcd for $C_{31}H_{41}FO_4$: C, 74.97%, H, 8.32%. Found: C, 74.52%, H, 8.88%.

Trioxane 25c. This was obtained in 55% yield as a diastereomeric mixture which was separated by column chromatography.

Trioxane (25c, Less Polar Isomer). This was obtained as a white solid; mp 72−73 °C. IR (KBr, cm^{-1}) 1705, 2933. ^1H NMR (300 MHz, $CDCl_3$) δ 0.59 (s, 3H), 0.94 (s, 3H), 1.01−2.03 (m, 22H), 2.11 (s, 3H), 2.50−2.56 (m, 1H), 3.70−3.77 (m, 1H), 3.82−3.91 (m, 1H), 5.17−5.19 (m, 1H), 5.34 (s, 1H), 5.48−5.49 (m, 1H), 7.28−7.34 (s, 4H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 13.83 (CH_3), 21.48 (CH_2), 23.30 (CH_2), 23.46 (CH_3), 23.50 (CH_3), 24.81 (CH_2), 26.53 (CH_2), 26.72 (CH_2), 26.78 (CH_2), 29.42 (CH_2), 30.01 (CH_2), 31.93 (CH_3), 32.51 (CH_2), 33.00 (CH_2), 34.77 (CH_2), 35.51 (C), 36.04 (CH), 39.49 (CH), 39.58 (CH_2), 40.34 (CH), 44.68 (C), 57.08 (CH), 62.79 (CH_2), 62.85 (CH_2), 64.20 (CH), 80.55 (CH), 80.73 (CH), 104.01 (C), 104.16 (C), 117.39 (CH_2), 117.58 (CH_2), 128.21 (2 × CH),

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129.13 (2 × CH), 134.52 (C), 137.44 (C), 142.83 (C), 142.89 (C), 209.86 (C). ESMS (m/z) 535 $[M + Na]^+$. Anal. Calcd for $C_{31}H_{41}ClO_4$: C, 72.56%, H, 8.05%. Found: C, 72.57%, H, 7.89%.

Trioxane (25c, More Polar Isomer). This was obtained as a white solid; mp 145−147 °C. IR (KBr, cm^{-1}) 1705, 2933. ^1H NMR (300 MHz, $CDCl_3$) δ 0.59 (s, 3H), 0.94 (s, 3H), 1.07−2.04 (m, 22H), 2.10 (s, 3H), 2.45−2.55 (m, 1H), 3.77 (dd, 1H, J = 11.5 and 2.3 Hz), 4.04 (dd, 1H, J = 11.5 and 10.3 Hz), 5.18 (dd, 1H, J = 10.3 and 2.3 Hz), 5.35 (s, 1H), 5.51 (s, 1H), 7.29−7.36 (m, 4H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 13.59 (CH_3), 21.18 (CH_2), 23.04 (CH_2), 23.21 (CH_3), 24.56 (CH_2), 26.43 (CH_2), 26.76 (CH_2), 30.10 (2 × CH_2), 31.68 (CH_3), 32.54 (CH_2), 35.12 (C), 35.78 (CH), 39.28 (CH), 39.36 (CH_2), 40.02 (CH), 44.47 (C), 56.93 (CH), 62.72 (CH_2), 64.01 (CH), 80.22 (CH), 103.55 (C), 117.19 (CH_2), 127.92 (2 × CH), 128.90 (2 × CH), 134.30 (C), 137.24 (C), 142.43 (C), 209.65 (C). ESMS (m/z) 535 $[M + Na]^+$. Anal. Calcd for $C_{31}H_{41}ClO_4$: C, 72.56%, H, 8.05%. Found: C, 72.94%, H, 8.32%.

Trioxane 25d. This was obtained in 36% yield as a diastereomeric mixture which was separated by column chromatography.

Trioxane (25d, Less Polar Isomer). This was obtained as a white solid; mp 79−80 °C. IR (KBr, cm^{-1}) 1703, 2931. ^1H NMR (300 MHz, $CDCl_3$) δ 0.60 (s, 3H), 0.96 (s, 3H), 1.07−2.05 (m, 22H), 2.12 (s, 3H), 2.52−2.58 (m, 1H), 3.71−3.79 (m, 1H), 3.83−3.92 (m, 1H), 5.17−5.21 (m, 1H), 5.36 (s, 1H), 5.50 and 5.51 (2 × s, together integrating for 1H), 7.25−7.28 (m, 2H), 7.46−7.49 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.45 (CH_3), 21.07 (CH_2), 22.87 (CH_2), 23.05 (CH_3), 23.10 (CH_3), 23.71 (CH_2), 24.41 (CH_2), 26.12 (CH_2), 26.30 (CH_2), 26.36 (CH_2), 26.79 (CH_2), 26.92 (CH_2), 29.85 (CH_2), 30.18 (CH_2), 31.57 (CH_3), 32.09 (CH_2), 32.58 (CH_2), 34.35 (CH_2), 35.11 (C), 35.63 (CH), 39.08 (CH), 39.17 (CH_2), 39.21 (CH_2), 39.91 (CH), 44.30 (C), 56.67 (CH), 56.70 (CH_2), 62.36 (CH_2), 63.81 (CH), 80.29 (CH), 103.64 (C), 103.78 (C), 117.09 (CH_2), 117.30 (CH_2), 122.31 (C), 128.09 (CH), 128.13 (CH), 131.70 (2 × CH), 137.47 (C), 142.45 (C), 142.51 (C), 209.63 (C). ESMS (m/z) 579 $[M + Na]^+$. Anal. Calcd for $C_{31}H_{41}BrO_4$: C, 66.78%, H, 7.41%. Found: C, 67.19%, H, 7.88%.

Trioxane (25d, More Polar Isomer). This was obtained as a white solid; mp 154−155 °C. IR (KBr, cm^{-1}) 1704, 2930. ^1H NMR (300 MHz, $CDCl_3$) δ 0.61 (s, 3H), 0.95 (s, 3H), 1.21−2.04 (m, 22H), 2.11 (s, 3H), 2.45−2.56 (m, 1H), 3.77 (dd, 1H, J = 11.5 and 2.4 Hz), 4.05 (dd, 1H, J = 11.5 and 10.3 Hz), 5.18 (dd, 1H, J = 10.3 and 2.4 Hz), 5.36 (s, 1H), 5.52 (s, 1H), 7.27 (d, 2H, J = 8.3 Hz), 7.47 (d, 2H, J = 8.3 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.45 (CH_3), 21.02 (CH_2), 22.87 (CH_2), 23.06 (CH_3), 24.41 (CH_2), 26.27 (CH_2), 26.60 (CH_2), 29.93 (2 × CH_2), 31.55 (CH_3), 32.37 (CH_2), 34.96 (C), 35.61 (CH), 39.12 (CH), 39.20 (CH_2), 39.84 (CH), 44.32 (C), 56.76 (CH), 62.55 (CH_2), 63.86 (CH), 80.00 (CH), 103.40 (C), 117.12 (CH_2), 122.32 (C), 128.07 (2 × CH), 131.71 (2 × CH), 137.54 (C), 142.30 (C), 209.58 (C). ESMS (m/z) 579 $[M + Na]^+$. Anal. Calcd for $C_{31}H_{41}BrO_4$: C, 66.78%, H, 7.41%. Found: C, 67.25%, H, 7.87%.

Trioxane 26a. This was obtained in 39% yield as a diastereomeric mixture which was separated by column chromatography.

Trioxane (26a, Less Polar Isomer). This was obtained as a white solid; mp 65−67 °C. IR (KBr, cm^{-1}) 2931, 3400. ^1H NMR (300 Hz, $CDCl_3$) δ 0.72 (s, 3H), 0.96 (s, 3H), 1.04−2.56 (m, 23H), 3.62 (t, 1H, J = 8.4 Hz), 3.69−3.77 (m, 1H), 3.81−3.90 (m, 1H), 5.23 (dd, 1H, J = 10.2 and 2.4 Hz), 5.31 (s, 1H), 5.48 (s, 1H), 7.30−7.36 (m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 11.32 (CH_3), 20.83 (CH_2), 23.27 (CH_3), 23.55 (CH_2), 23.85 (CH_2), 25.92 (CH_2), 26.52 (CH_2), 30.70 (2 × CH_2), 32.84 (CH_2), 34.61 (CH_2), 35.35 (C), 35.89 (CH), 37.07 (CH_2), 39.36 (CH), 40.34 (CH), 43.25 (C), 51.21 (CH), 62.86 (CH_2), 80.67 (CH), 81.99 (CH), 103.76 (C), 103.91 (C), 116.63 (CH_2), 126.56 (2 × CH), 128.31 (CH), 128.71 (2 × CH), 138.76 (C), 143.76 (C). ESMS (m/z) 475 $[M + Na]^+$. HRMS calcd for $C_{29}H_{40}O_4$, 452.2927; found, 452.2953. Anal. Calcd for $C_{29}H_{40}O_4$: C, 76.95%, H, 8.91%. Found: C, 76.68%, H, 9.08%.

Trioxane (26a, More Polar Isomer). This was obtained as a white solid; mp 145−147 °C. IR (KBr, cm^{-1}) 29626, 3433. ^1H NMR (300 Hz, $CDCl_3$) δ 0.72 (s, 3H), 0.96 (s, 3H), 1.02−2.52 (m, 23H), 3.61 (t, 1H, J = 8.4 Hz), 3.75 (dd, 1H, J = 11.6 and 2.7 Hz), 4.03 (dd, 1H, J = 11.6 and 10.5 Hz), 5.23 (dd, 1H, J = 10.8 and 2.7 Hz), 5.32 (s, 1H), 5.50 (s, 1H), 7.31−7.37 (m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 11.44 (CH_3), 20.90 (CH_2), 23.40 (CH_3), 23.67 (CH_2), 26.20 (CH_2), 26.87 (CH_2), 30.21 (CH_2), 30.31 (CH_2), 30.85 (CH_2), 32.75 (CH_2), 35.33 (C), 36.00 (CH), 37.20 (CH_2), 39.52 (CH), 40.41 (CH), 43.39 (C), 51.42 (CH), 63.16 (CH_2), 80.54 (CH), 82.17 (CH), 103.65 (C), 116.60 (CH_2), 126.67 (2 × CH), 128.45 (CH), 128.85 (2 × CH), 138.93 (C), 143.69 (C). ESMS (m/z) 475 $[M + Na]^+$. Anal. Calcd for $C_{29}H_{40}O_4$: C, 76.95%, H, 8.91%. Found: C, 76.91%, H, 9.22%. Crystal data: C_{29} H_{40} O_4, M = 452.61, monoclinic, $P2(1)$, a = 7.008(4) Å, b = 18.623(11) Å, c = 9.550(6) Å, β = 97.63(1)°, V = 1235.38 $Å^3$, Z = 2, D_c = 1.217 g cm^{-3}, μ (Mo Kα) = 0.08 mm^{-1}, $F(000)$ = 492, rectangular block, colorless, size = 0.20 mm × 0.225 mm × 0.30 mm, 7830 reflections measured (R_{int} = 0.0572), 5654 unique, wR_2 = 0.2261 for all data, conventional $R1$ = 0.0675 for 3215 $F_0 > 4\sigma(F_0)$ and 0.1175 for all 5654 data, S = 0.990 for all data and 302 parameters. Unit cell determination and X-ray intensity data collection were performed on Bruker SMART APEX CCD area-detector instruments. Structure solutions by direct methods and refinements by full-matrix least-squares methods on F^2. Programs: SMART (Bruker, 2001), SMART 32(Bruker), SAINT (Bruker, 2001), SHELXTL-NT [Bruker AXS Inc.: Madison, Wisconsin, USA 1997]. CCDC (deposit no.: CCDC 871201) contains the supplementary crystallographic data. These data can be obtained free of charge from www.ccdc.cam.uk/conts/retrieving.html [or from the Cambridge Crystallographic Data Center, 12 Union Road, Cambridge, CB2 1EZ, UK; fax (Internet), +44-1223/336-033; E-mail: deposit@ccdc.cam.ac.uk.

Trioxane 26b. This was obtained in 63% yield as a diastereomeric mixture which was separated by column chromatography.

Trioxane (26b, Less Polar Isomer). This was obtained as a white solid; mp 64−65 °C. IR (KBr, cm^{-1}) 2932, 3399. ^1H NMR (300 Hz, $CDCl_3$) δ 0.72 (s, 3H), 0.95 (s, 3H), 1.05−2.54 (m, 23H), 3.62 (t, 1H, J = 8.4 Hz), 3.72−3.77 (m, 1H), 3.81−3.90 (m, 1H), 5.16−5.19 (m, 1H), 5.30 (s, 1H), 5.43 and 5.44 (2 × s, together integrating for 1H), 7.01 (t, 2H, J = 8.4 Hz), 7.32−7.37 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 11.36 (CH_3), 20.86 (CH_2), 23.31 (CH_2), 23.36 (CH_3), 23.58 (CH_2), 23.90 (CH_2), 25.94 (CH_2), 26.15 (CH_2), 26.54 (CH_2), 26.99 (CH_2), 29.25 (CH_2), 29.80 (CH_2), 30.70 (CH_2), 32.37 (CH_2), 32.85 (C), 34.60 (CH_2), 35.39 (CH_2), 35.92 (CH), 37.09 (CH_2), 39.40 (CH), 39.54 (CH), 40.36 (CH), 43.28 (C), 51.23 (CH), 62.64 (CH_2), 62.69 (CH_2), 80.53 (CH), 80.72 (CH), 82.02 (CH), 103.86 (C), 104.01 (C), 115.52 (CH), 115.80 (CH), 116.69 (CH_2), 116.89 (CH_2), 128.37 (CH), 128.47 (CH), 134.90 (C), 142.71 (C), 162.79 (C, J_{C-F} = 248 Hz). ESMS (m/z) 493 $[M + Na]^+$. HRMS calcd for $C_{29}H_{39}FO_4$, 470.2832; found, 470.2808. Anal. Calcd for $C_{29}H_{39}FO_4$: C, 74.01%, H, 8.35%. Found: C, 73.84%, H, 8.81%.

Trioxane (26b, More Polar Isomer). This was obtained as a white solid; mp 161−162 °C. IR (KBr, cm^{-1}) 2932, 3407. ^1H NMR (300 Hz, $CDCl_3$) δ 0.72 (s, 3H), 0.95 (s, 3H), 1.01−2.66 (m, 23H), 3.61 (t, 1H, J = 8.4 Hz), 3.70−3.77 (m, 1H), 3.97−4.07 (m, 1H), 5.17 (dd, 1H, J = 10.2 and 2.3 Hz), 5.31 (s, 1H), 5.45 (s, 1H), 7.01 (t, 2H, J = 8.4 Hz), 7.32−7.38 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 11.28 (CH_3), 20.73 (CH_2), 23.23 (CH_2), 23.50 (CH_3), 26.03 (CH_2), 26.70 (CH_2), 30.07 (CH_2), 30.65 (CH_2), 32.57 (CH_2), 32.93 (C), 35.15 (CH_2), 35.82 (CH), 37.06 (CH_2), 39.34 (CH), 40.24 (CH), 43.22 (C), 51.25 (CH), 62.71 (CH_2), 80.34 (CH), 81.95 (CH), 103.54 (C), 115.44 (CH), 115.73 (CH), 116.60 (CH_2), 128.23 (CH), 128.34 (CH), 134.84 (C), 142.50 (C), 162.86 (C, J_{C-F} = 248 Hz). ESMS (m/z) 493 $[M + Na]^+$. HRMS calcd for $C_{29}H_{39}FO_4$, 470.2832; found, 470.2827. Anal. Calcd for $C_{29}H_{39}FO_4$: C, 74.01%, H, 8.35%. Found: C, 74.09%, H, 8.67%.

Trioxane 26d. This was obtained in 62% yield as a diastereomeric mixture which was separated by column chromatography.

Trioxane (26d, Less Polar Isomer). This was obtained as a white solid; mp 68−69 °C. IR (KBr, cm^{-1}) 2929, 3435. ^1H NMR (300 Hz, $CDCl_3$) δ 0.72 (s, 3H), 0.96 (s, 3H), 1.01−2.52 (m, 23H), 3.62 (t, 1H, J = 8.4 Hz), 3.69−3.76 (m, 1H), 3.81−3.90 (m, 1H), 5.15−5.18 (m, 1H), 5.33 (s, 1H), 5.47−5.48 (m, 1H), 7.23−7.26 (m, 2H), 7.43−7.46 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 11.27 (CH_3), 20.77 (CH_2), 23.22 (CH_3), 23.28 (CH_2), 23.50 (CH_2), 25.86 (CH_2), 26.05 (CH_2),

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26.46 (CH_2), 26.89 (CH_2), 29.73 (CH_2), 30.65 (CH_2), 32.28 (CH_2), 32.77 (CH_2), 34.48 (CH), 35.30 (C), 35.83 (CH_2), 35.94 (CH), 37.01 (CH_2), 39.31 (CH), 40.28 (CH), 43.19 (C), 51.15 (CH), 62.50 (CH_2), 80.42 (CH), 81.94 (CH), 103.80 (C), 103.95 (C), 117.18 (CH_2), 117.41 (CH_2), 122.41 (C), 128.25 (2 × CH), 131.80 (2 × CH), 137.64 (C), 142.64 (C). ESMS (m/z) 553 $[M + Na]^+$. Anal. Calcd for $C_{29}H_{39}BrO_4$: C, 65.53%, H, 7.40%. Found: C, 65.42%, H, 7.28%.

Trioxane (26d, More Polar Isomer). This was obtained as a white solid; mp 178−180 °C. IR (KBr, cm^{-1}) 2928, 3426. ^1H NMR (300 Hz, $CDCl_3$) δ 0.72 (s, 3H), 0.96 (s, 3H), 0.99−2.65 (m, 23H), 3.62 (t, 1H, J = 8.4 Hz), 3.73 (dd, 1H, J = 11.8 and 2.8 Hz), 4.01 (dd, 1H, J = 11.8 and 10.7 Hz), 5.16 (dd, 1H, J = 10.7 and 2.8 Hz), 5.33 (s, 1H), 5.49 (s, 1H), 7.25 (d, 2H, J = 8.3 Hz), 7.46 (d, 2H, J = 8.3 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 11.31 (CH_3), 20.82 (CH_2), 23.16 (CH_3), 23.58 (CH_2), 24.25 (CH_2), 25.71 (CH_2), 26.27 (CH_2), 30.81 (CH_2), 33.01 (CH_2), 35.21 (C), 35.77 (CH_2), 35.88 (CH), 37.12 (CH_2), 39.40 (CH), 40.30 (CH), 43.29 (C), 51.28 (CH), 62.80 (CH_2), 80.23 (CH), 82.13 (CH), 103.86 (C), 117.35 (CH_2), 122.53 (C), 128.29 (2 × CH), 131.92 (2 × CH), 137.75 (C), 142.59 (C). ESMS (m/z) 553 $[M + Na]^+$. Anal. Calcd for $C_{29}H_{39}BrO_4$: C, 65.53%, H, 7.40%. Found: C, 65.03%, H, 7.77%.

■ ASSOCIATED CONTENT

Supporting Information

^1H NMR and ^{13}C NMR Spectra of bile acid-based trioxanes **23a−d**, **24a−d**, **25a−d**, **26a**, **26b** and **26d**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

SAR, structure−activity relationship; TLC, thin-layer chromatography; R_f, retention factor; EtOAc, ethyl acetate; *p*-TSA, *para*-toluenesulfonic acid; *m*-CPBA, *meta*-chloroperoxybenzoic acid

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(15) (a) 100% protection means none of the treated mice developed patent infection during the 28 days observation period and hence were recorded as cured. Similarly, 20% protection means only one out of five mice was cured. (b) 100% suppression of parasitemia means no parasites were detected in 50 oil immersion microscopic fields (parasites if at all present are below the detection limit). The parasites present below the detection limit can multiply and eventually can be detected during observation on subsequent days. In such cases, though, the drug is providing near 100% suppression of the parasitaemia on day 4 but will not provide full protection to the treated mice in the 28 day survival assay. Multidrug−resistant *Plasmodium yoelii nigeriensis* used in this study is resistant to chloroquine, mefloquine, and halofantrine.

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