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An efficient route into synthetically challenging bridged achiral 1,2,4,5-tetraoxanes with antimalarial activity

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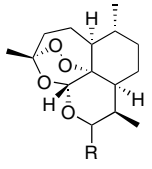
Abstract—Here we present an efficient route into synthetically challenging bridged 1,2,4,5-tetraoxanes. The key to the success of this route is the use of H_2O_2 and catalytic I_2 to form the *gem*-dihydroperoxide followed by a Ag_2O mediated alkylation using 1,3-diiodopropane. Using this methodology a range of bridged tetraoxanes which display good in vitro antimalarial activity were synthesized.

Artemisinin is an extract of the Chinese wormwood *Artemisia annua* and has been used since ancient times to treat malaria. The active pharmacophore within this drug is the endoperoxide bridge. Semi-synthetic artemisinin derivatives such as artesunate and artemether are highly potent antimalarials and exhibit little or no cross resistance with other antimalarials, however, they have a limited availability, high cost, poor bioavailabilty, poor pharmacokinetic properties and all derivatives are chiral and are synthesized from the natural product artemisinin (Fig. 1).[1–4]

Some of the key developments within antimalarial drug discovery have concentrated on incorporating the peroxide pharmacophore into structurally simpler compounds. Vennerstrom's extensive work in this field has moved from initial amine peroxides[5] containing one peroxide bridge into 1,2,4,5-tetraoxanes[6] and most recently 1,2,4-trioxolanes and the development of OZ277.[7] Kim's elegant work in the 1990s included the synthesis of spiro-1,2,4,5-tetraoxanes and yingzhaosu A analogues.[8,9] Solaja and co-workers have more recently concentrated on the synthesis of steroidal 1,2,4,5-tetraoxanes.[10] Fully synthetic 1,2,4-trioxanes and trioxolanes have been successfully synthesized,[7,11–19] and these compounds are also found to be potent antimalarials but have recently been found to be less stable than their 1,2,4,5-tetraoxane counterparts (see Fig. 2).



Artemisinin, R = =O
Artesunate, R = α-OC(O)$CH_2CH_2CO_2Na$
Artemether, R = β-OMe

Figure 1. Artemisinin and its semi-synthetic analogues artesunate and artemether.

Advantages of the tetraoxane heterocycle include ease of synthesis, potential achirality and a low cost of synthesis from readily available reagents.

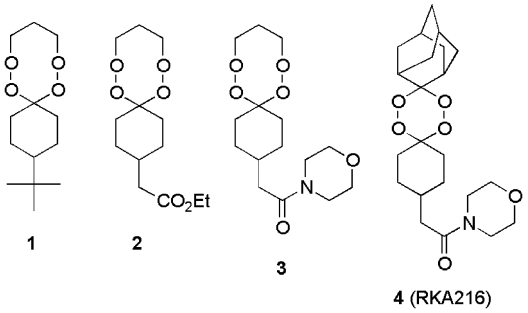


Figure 2. 1,2,4,5-Tetraoxanes **1**, **2**, **3** and **4** (RKA 216).

Keywords: Antimalarial; Artemisinin; 1,2,4-Trioxane; Tetraoxane; *Plasmodium falciparum*.
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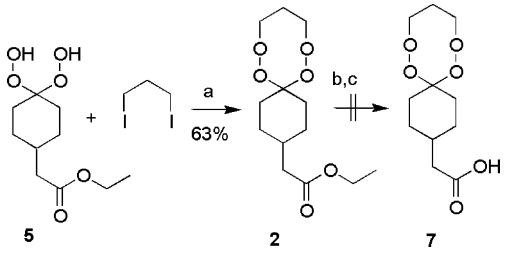
There are many methods available for the synthesis of dispiro-1,2,4,5-tetraoxanes but these reactions are highly dependant on substrate, temperature, concentration, pH, addition mode, solvent and type of substrate.[6,8,20–29] Bridged tetraoxanes are a particularly challenging synthetic target with few literature methods available for their synthesis. A recent publication by Kim et al. identified tetraoxane **1** as a highly active antimalarial both in vitro (IC_{50} = 3 nM) and in vivo (ED_{50} = 15 mg/kg).[30] Our initial studies focused on the synthesis of tetraoxanes **2** and **3** as these compounds would have enhanced polarity. In addition, tetraoxane **2** was viewed as a particularly attractive target as the ester group can be converted into a range of polar solubilizing functional groups. The presence of a morpholino group within the tetraoxane template has previously been found to greatly enhance activity as is exemplified by tetraoxane **4** (RKA 216).[28]

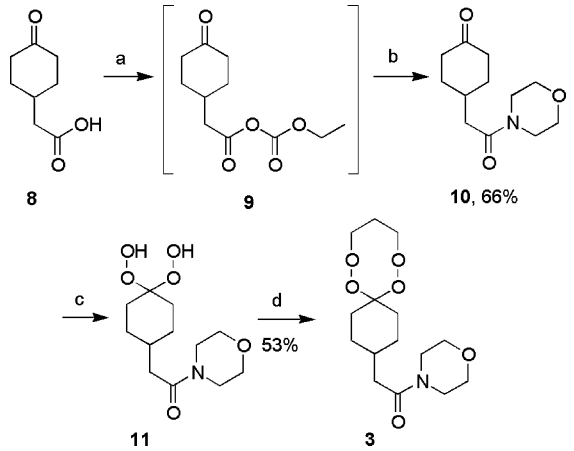
Tetraoxane **2** was synthesized in 63% yield from *gem*-dihydroperoxide **5**[28] by Ag_2O mediated alkylation using 1,3-diiodopropane. In order to convert the ester **2** to the amide **3** it was necessary to hydrolyze the ester to the acid **7** (Scheme 1). This reaction was unsuccessful as a 'Kornblum-de La Mare' type rearrangement occurred resulting in formation of ketone **8** as the major product.

An alternative synthetic route was required that involved synthesis of the amide prior to formation of the bridged tetraoxane. Ketone **8** was converted to the amide **10** via a mixed anhydride intermediate **9**. This was then transformed into the *gem*-dihydroperoxide **11** using formic acid and hydrogen peroxide. Formation of the tetraoxane **3** was achieved using the alkylation reaction described previously (Scheme 2).[31]

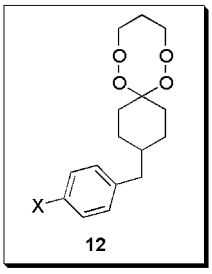
The in vitro antimalarial activity of tetraoxanes **2** and **3** was measured versus the 3D7 strain of *Plasmodium falciparum*. Tetraoxane **2** had an IC_{50} of 117.5 nM and tetraoxane **3** an IC_{50} of 689.3 nM. It became evident that there were several problems with this initial template, as the ester was not amenable to hydrolysis and the synthesis was longer than anticipated. Given the low activity of these two derivatives the more lipophilic template **12** became a new synthetic target (Fig. 3). Varying the nature of the X group on the phenyl ring would give a greater diversity as well as allow some control over the lipophilicity and polarity of the tetraoxanes (see Scheme 3).



Scheme 1. Synthetic route for synthesis of tetraoxane **7**. Reagents and conditions: (a) Ag_2O, EtOAc; (b) KOH, MeOH, 70 °C; (c) DCM, H_2O, HCl.

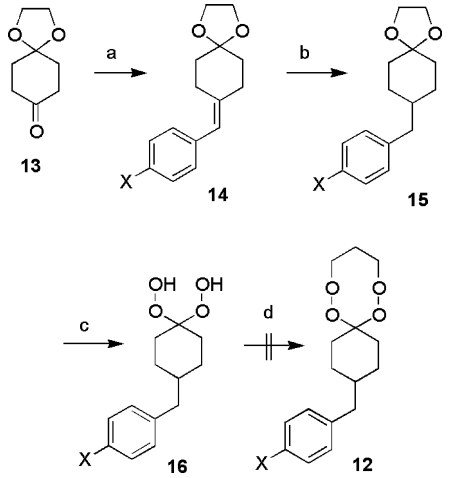


Scheme 2. Synthetic route for synthesis of tetraoxane **3**. Reagents and conditions: (a) Et_3N, $ClCO_2C_2H_5$, DCM, 0 °C; (b) morpholine, rt −0 °C; (c) formic acid, H_2O_2, 0 °C; (d) $ICH_2CH_2CH_2I$, Ag_2O, EtOAc.



X = H, F, CF_3 lipophilic
X = CN, CO_2Me, SO_2Me polar

Figure 3. New template for tetraoxanes **12**.



Scheme 3. Proposed synthetic route for synthesis of tetraoxanes **12**. Reagents: (a) NaH, DMSO, X-$PhCh_2P^+(Ph)_3Br^-$; (b) H_2, Pd/C; (c) H_2O_2, tungstic acid; (d) $I(CH_2)_3I$, Ag_2O.

Initially a four-step synthetic route to achieve the synthesis of target tetraoxanes **12** was proposed. Ketal **13** underwent a Wittig reaction using the respective Wittig reagent, NaH and DMSO, to give alkene **14**. The alkene functionality was then removed using catalytic hydrogenation to give the protected ketone **15**. Direct treatment

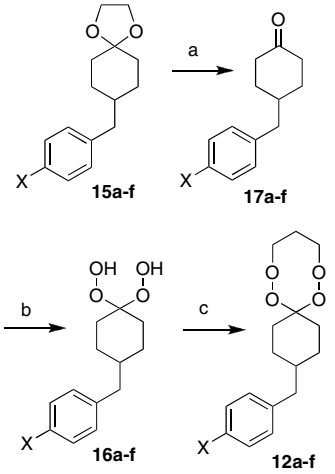
of **15** with H_2O_2/THF (1:1) and tungstic acid gave the *gem*-dihyroperoxide **16** which was then to be alkylated using 1,3-diiodopropane and Ag_2O to give the tetraoxanes **12** (Scheme 1).[28] Synthesis of the protected ketone **15** was achieved in high yields, however, conversion to the *gem*-dihydroperoxide **16** gave a complex mixture of products and the *gem*-dihydroperoxide could not be isolated in a pure form. Use of the impure *gem*-dihydroperoxide in the alkylation step resulted in a further mixture of complex products and although in one case some of the tetraoxane was isolated the yield was less than 5% and some impurities were still present.

It was apparent from the results of investigating this method that it was possible to form these bridged tetraoxanes but that a better, higher yielding, cleaner methodology was required for synthesis of the *gem*-dihydroperoxides. A further review of the literature revealed a paper by Žimitek and co-workers using only 2 equivalents of H_2O_2 and catalytic iodine to form *gem*-dihydroperoxides from ketones in excellent yields.[32] This method would involve deprotection of the ketone prior to conversion to the *gem*-dihydroperoxide but as the deprotection step was believed to be high, yielding this methodology was adopted.

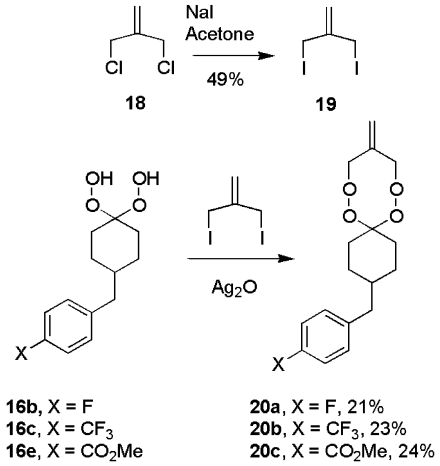
Conversion of commercially available ketal **13** to alkenes **14a–f** was achieved using a Wittig reaction as described previously in 44–79% yield. Catalytic hydrogenation of alkenes **14a–f** gave near quantitative conversion to **15a–f** in all cases. Acetal deprotection using 10% aq HCl in acetone gave ketones **17a–f** in 68–93% yield. *gem*-Dihydroperoxides **16a–f** were synthesized from ketones **17a–f** using the H_2O_2, catalytic iodine system described above in yields of 67–87%. The final alkylation step was initially carried out using 1,3-diiodopropane and Ag_2O in ethyl acetate at 0 °C. However, yields on the first two reactions were less than 20%, this number was significantly improved if the reaction was carried out at room temperature. Tetraoxanes **12a–f** were therefore synthesized in yields of 32–42% (Scheme 4 and Table 1).

One further point of diversity explored was altering the bridged alkyl group. In this case 2-methylene-1,3-diiodopropane was used as the bridging alkyl reagent. This first was synthesized from the dichloro compound **18** using NaI in acetone in 49% yield. The resulting diiodo compound **19** was then used in the alkylation step as described previously for 1,3-diiodopropane to give compounds **20a–c** in 21–24% yield (Scheme 5).[34] The yield of these reactions was generally lower than for 1,3-diiodopropane although again the best yields were achieved at room temperature. This observation is perhaps unsurprising due to the more reactive nature of vinyl system resulting in more side reactions.

The average log *P* and tPSA of all the target compounds synthesized can be seen below (Table 2). Interestingly compounds **2** and **3** which exhibited poor antimalarial activity have considerably lower average log *P* than the phenyl tetraoxanes **12a–f** and **20a–c** (see Table 3).



Scheme 4. Synthesis of propane bridged 1,2,4,5-tetraoxanes **12a–f**. Reagents: (a) 10% aq HCl, acetone; (b) H_2O_2, I_2, MeCN; (c) $I(CH_2)_3I$, Ag_2O.



Scheme 5. Synthesis of bridged 1,2,4,5-tetraoxanes **20a–c**. Reagents: **16b**, X = F **16c**, X = CF_3 **16e**, X = CO_2Me **20a**, X = F, 21% **20b**, X = CF_3, 23% **20c**, X = CO_2Me, 24%.

Table 1. Yields for the synthesis of tetraoxanes **12a–f** and all intermediates

Compound	X	% Yield **14**	% Yield **15**	% Yield **17**	% Yield **16**	% Yield **12**
a	H	74	96	93	79	34[33a]
b	F	79	97	68	68	42
c	CF_3	44	95	84	67	40
d	CN	52	95	83	87	35
e	CO_2Me	54	97	79	71	34[33b]
f	SO_2Me	48	95	81	73	32

Table 2. Average log *P* and tPSA of all target compounds

Compound	X	Av log *P*[35]	tPSA[36]
2	—	2.20 ± 1.12	63.24
3	—	1.16 ± 0.91	66.48
12a	H	3.93 ± 1.10	36.94
12b	F	4.01 ± 1.17	36.94
12c	CF_3	4.79 ± 1.16	36.94
12d	CN	3.46 ± 1.14	60.73
12e	CO_2Me	3.75 ± 1.11	63.24
12f	SO_2Me	2.65 ± 1.35	71.08
20a	F	4.20 ± 1.24	36.94
20b	CF_3	4.95 ± 1.24	36.94
20c	CO_2Me	4.05 ± 1.30	63.24

Table 3. In vitro antimalarial activity of the bridged 1,2,4,5-tetraoxanes against the 3D7 strain of *Plasmodium falciparum*

Compound	Mean IC_{50} (nM ± SD)
Artemether	3.20 ± 1.97
Artemisinin	9.20 ± 1.97
12a	51.85 ± 22.84
12b	68.25 ± 25.24
12c	52.35 ± 18.17
12d	99.65 ± 2.19
12e	93.50 ± 27.86
20a	43.40 ± 1.41
20b	42.80 ± 3.68
20c	116.18 ± 40.52

The in vitro antimalarial activity of these compounds was measured versus the 3D7 strain of *Plasmodium falciparum*.[37] The majority of these 1,2,4,5-tetraoxanes are active in the 40–100 nM IC_{50} range. Work is currently underway to further enhance potency within this 1,2,4,5-tetraoxane template.

To conclude, an efficient five-step synthesis of bridged 1,2,4,5-tetraoxanes has been achieved to generate a range of tetraoxanes with good antimalarial activity. The compounds synthesized have two points of potential diversity allowing several different functional groups to be investigated in future research.

Acknowledgments

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31. *General procedure for the synthesis of bridged tetraoxanes: gem*-Dihydroperoxide (1.09 mmol) was dissolved in EtOAc (7.25 ml) and Ag_2O (2.18 mmol, 2.0 equiv) added. A solution of diiodo alkane/alkene (1.09 mmol, 1.0 equiv) in EtOAc (3.60 ml) was added dropwise over 15 min. The reaction was then stirred at room temperature overnight and filtered through a *Celite* pad. Ether (50 ml) was added and the resulting solution was washed with 3% aq sodium thiosulfate, $NaHCO_3$ aq and brine. The organic layer was dried over $MgSO_4$, evaporated and purified by flash column chromatography. Procedure for the synthesis of compound **3**. This product was prepared in 53% as a light yellow powder according to the general procedure for the preparation of bridged tetraoxanes. The product was purified by flash column chromatography using ethyl acetate/DCM (1:1, v/v, Rf = 0.17) as the eluent. Mp 90–92 °C υ_{max} ($CHCl_3$)/cm^{-1} 1433.2, 1632.5, 2858.9, 2913.2, 3016.6 ^1H NMR (400 MHz, $CDCl_3$) δ_H, 1.11–1.42 (m, 2 H, CH_2), 1.45–1.80 (m, 4H, CH_2), 1.85–1.89 (m, 1H, CH), 2.08–2.31 (m, 4H, CH_2), 2.22 (d, 2H, J = 6.7 Hz, CH_2), 3.48 (t, 2H, J = 4.8 Hz, NCH_2), 3.55–3.72 (br s, 6H, NCH_2/CH_2O), 3.98–4.18 (m, 2H, CH_2O), 4.28–4.47 (m,

2H, CH_2O) ^{13}C NMR (100 MHz, $CDCl_3$), δ_C 29.3, 31.1, 34.2, 39.3, 42.4, 46.6, 67.2, 74.3, 108.1, 171.0 MS (ES+), 315.36 $[M+Na]^+$ (100), 338.1, [2M+Na]+ 653.3 HRMS calculated for 338.1580 $C_{12}H_{20}O_3N$, found 338.1574. Elemental analysis C: 56.99, H: 7.91, N: 4.39 (required values C: 57.13, H: 7.99, N: 4.39).

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33. (a) Procedure for the preparation of compound **12a**. This product was prepared in 34% as a white powder according to the general procedure for the preparation of bridged tetraoxanes. This product was purified by flash column chromatography gradient elution hexane to 3% hexane in EtOAc. ^1H NMR (400 MHz, $CDCl_3$) δ_H, 1.11–1.40 (m, 4H, $2\times CH_2$), 1.45–1.70 (m, 3H, CH_2, CH), 2.05–2.22 (m, 4H, $2\times CH_2$), 2.50 (d, 2H, J = 6.9 Hz, $CHCH_2$), 4.00–4.20 (m, 2H, $2\times CHHO$), 4.29–4.51 (m, 2H, $2\times CHHO$), 7.10–7.30 (m, 5H, aromatics); ^{13}C NMR (100 MHz, $CDCl_3$), δ_C 28.4, 29.2, 30.8, 39.1, 43.2, 74.4, 108.5, 126.2, 128.6, 129.5, 141.3; MS (CI+), 296 $[M+NH_3]^+$ (100), HRMS calculated for 296.18619 $C_{16}H_{26}O_4N$, found 296.18672.; (b) Procedure for the preparation of compound **12e**. This product was prepared in 34% as a white powder according to the general procedure for the preparation of bridged tetraoxanes. Purification was achieved by flash column chromatography using hexane to 5% hexane in EtOAc. ^1H NMR (400 MHz, $CDCl_3$) δ_H, 1.15–1.40 (m, 4H, $2\times CH_2$), 1.45–1.70 (m, 3H, CH_2, CH), 2.05–2.25 (m, 4H, $2\times CH_2$), 2.57 (d, 2H, J = 6.7 Hz, $CHCH_2$), 4.00–4.20 (m, 2H, $2\times CHHO$), 4.29–4.51 (m, 2H, $2\times CHHO$), 7.20 (m, 2H, aromatics), 7.95 (m, 2H, aromatics); ^{13}C NMR (100 MHz, $CDCl_3$), δ_C 28.8, 29.2, 31.3, 39.0, 43.2, 52.3 74.4, 108.3, 128.3, 129.5, 130.0, 146.8, 167.5; MS (ES+), 359 $[M+Na]^+$ (100), HRMS calculated for 359.1471 $C_{18}H_{24}O_6Na$, found 359.1460.
34. Procedure for the preparation of compound **20b**. This product was prepared in 23% as a white powder according to the general procedure for the preparation of bridged tetraoxanes and was purified by flash column chromatography gradient elution hexane to 3% hexane in EtOAc. ^1H NMR (400 MHz, $CDCl_3$) δ_H, 1.15–1.40 (m, 4H, $2\times CH_2$), 1.45–1.70 (m, 3H, CH_2, CH), 2.00–2.25 (m, 2H, CH_2), 2.55 (d, 2H, J = 6.7 Hz, $CHCH_2$), 4.40 (m, 2H, $2\times CHHO$), 4.80 (m, 2H, $2\times CHHO$), 5.25 (s, 2H, $=CH_2$), 7.20 (m, 2H, aromatics), 7.52 (m, 2H, aromatics); ^{13}C NMR (100 MHz, $CDCl_3$), δ_C 28.3, 29.1, 32.0, 43.0, 78.4, 106.9, 108.4, 115.2, 115.4, 119.3, 130.7, 136.8, 151.7; MS (CI+), 376 $[M+NH_3]^+$ (100), HRMS calculated for 376.17358 $C_{18}H_{25}O_4F_3N$, found 376.17470.
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