Two-Step Synthesis of Achiral Dispiro-1,2,4,5-tetraoxanes with Outstanding Antimalarial Activity, Low Toxicity, and High-Stability Profiles†

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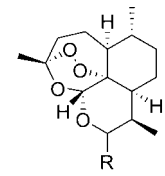
A rapid, two-step synthesis of a range of dispiro-1,2,4,5-tetraoxanes with potent antimalarial activity both in vitro and in vivo has been achieved. These 1,2,4,5-tetraoxanes have been proven to be superior to 1,2,4-trioxolanes in terms of stability and to be superior to trioxane analogues in terms of both stability and activity. Selected analogues have in vitro nanomolar antimalarial activity and good oral activity and are nontoxic in screens for both cytotoxicity and genotoxicity. The synthesis of a fluorescent 7-nitrobenza-2-oxa-1,3-diazole (NBD) tagged tetraoxane probe and use of laser scanning confocal microscopy techniques have shown that tagged molecules accumulate selectively only in parasite infected erythrocytes and that intraparasitic formation of adducts could be inhibited by co-incubation with the iron chelator desferrioxamine (DFO).

Artemisinin **1** is an extract of the Chinese wormwood *Artemisia annua* and has been used since ancient times to treat malaria. Artemisinin is a highly potent antimalarial that exhibits little or no cross-resistance with other antimalarials.[1,2] It does, however, have a limited availability, high cost, and poor bioavailabilty. Semi-synthetic derivatives such as artesunate **2** and artemether **3** also display poor pharmacokinetic properties (Figure 1).[3–5] The key pharmacophore within these drugs is the 1,2,4-trioxane functionality. Recently several fully synthetic trioxanes and 1,2,4-trioxolanes/ozonide (OZ[a]) analogues have been synthesized incorporating this key peroxide bridge pharmacophore.[6–14]

In this article we describe for the first time a series of stable, orally active 1,2,4,5-tetraoxane based antimalarials that can be prepared in only two synthetic steps. To our knowledge, this represents the shortest sequence reported for the synthesis of nonsymmetrical tetraoxanes with activity profiles similar to that of the natural product artemisinin. While impressive activity profiles have been recorded for molecules within the 1,2,4-trioxolane class, we reasoned that the achiral tetraoxane template **5** would provide molecules with enhanced stability compared with ozonides such as **4** and **6**. This is based on the observation that simple 1,2,4-trioxolanes such as **4** are antimalarially inactive and unstable, whereas the close 1,2,4,5-tetraoxane **5** is relatively stable and expresses antimalarial activity in the nanomolar range (IC_{50} = 25 nM).[7] Given the observation that the adamantane group is capable of stabilizing an ozonide **6**, it seemed logical to explore 1,2,4,5-tetraoxanes that also incorporate this endoperoxide stabilizing motif (Figure 2).



Artemisinin **1**, R = =O
Artesunate **2**, R = α-OC(O)$CH_2CH_2CO_2Na$
Artemether **3**, R = β-OMe

Figure 1. Artemisinin and its semisynthetic analogues.

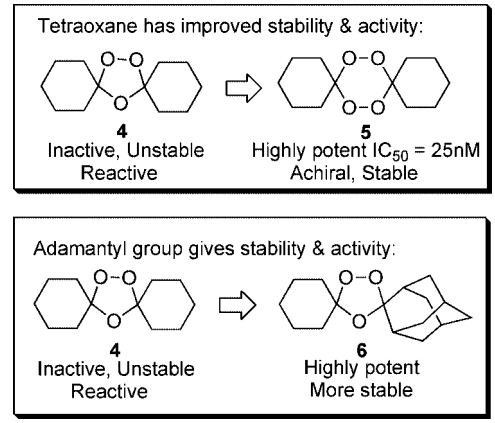


Figure 2. Stability and reactivity of endoperoxides **4**−**6**.

1,2,4,5-Tetraoxanes were initially used industrially for the production of macrocyclic hydrocarbons and lactones. They are achiral and easily prepared from inexpensive materials. There are many methods available for the synthesis of 1,2,4,5-tetraoxanes, and these reactions are highly dependent on substrate, temperature,

† This paper is dedicated to Kevin Barry, deceased October 3, 2007.

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[a] Abbreviations: OZ, ozonide; NBD, 7-nitrobenza-2-oxa-1,3-diazole; HFIP, hexafluoroisopropanol; MTO, methyltrioxorhenium(VII); AM, amodiaquine; CQ, chloroquine; TI, therapeutic index; 4NNQO, 4-nitroquinoline 1-oxide; 2Aan, 2-aminoanthracene; DFO, desferrioxamine.

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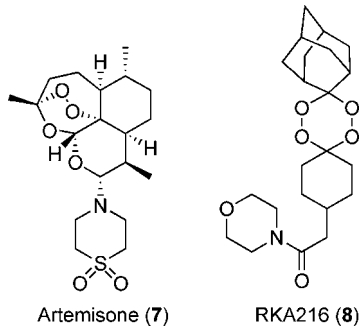


Figure 3. Structures of artemisone and **8** (RKA216).

concentration, pH, addition mode, solvent, and type of substrate.[15–27]

Here, we present a two-step synthesis of a range of achiral dispiro 1,2,4,5-tetraoxanes with highly potent antimalarial activity. These compounds are rapidly synthesized from inexpensive, readily available starting materials. The stability of the 1,2,4,5-tetraoxane pharmacophore compared to other synthetic endoperoxides has been investigated. This chemistry has also been employed to synthesize a fluorescent 4-chloro-7-nitrobenza-2-oxa-1,3-diazole (NBD) tagged compound which has been used to probe the non-heme "chelatable iron" dependent accumulation mechanism of 1,2,4,5-tetraoxanes.

Recent work on semisynthetic artemisinin derivatives by the Haynes group has revealed that incorporation of metabolically inert polar groups into the artemisinin framework provides analogues (for example, artemisone **7** (Figure 3)) with significantly reduced neurotoxicity and superior activity profiles when compared with currently used artemisinin derivatives.[2] Vennerstrom et al. have also explored polar, synthetic 1,2,4-trioxolanes containing sulfonamide, urea, and amide functionalities. Previously within the O'Neill group the synthesis of a range of 1,2,4,5-tetraoxanes with the amide functionality has been achieved including **8** (RKA216),[26] a highly potent antimalarial (IC_{50} = 5.2 nM, ED_{50} = 3.18 mg/kg). The synthesis of **8** is a six-step process. Following from these initial studies, we were keen to develop a shorter synthetic route. In order to achieve the minimal number of synthetic steps, the sulfonamide functional group and targeted derivatives **11a**−**9i** and **12a**−**c**,**f** were selected.[28]

Results and Discussion

Dispiro 1,2,4,5-tetraoxanes **11a**−**i** and **12a**−**c**,**f** were prepared using the two-step synthesis depicted in Scheme 1. Sulfonylpiperidones **10a**−**i** were prepared from piperidinone **9** using the respective sulfonyl chloride and K_2CO_3 in a biphasic reaction.[29] Conversion to the tetraoxanes **11a**−**i** was achieved in a one-pot reaction. Ketones **10a**−**i** were treated with hydrogen peroxide and methyltrioxorhenium(VII) (MTO) using a method developed by Iskra and co-workers to give the *gem*-dihydroperoxide. Adamantanone and HBF_4 were then added to complete conversion to the dispiro-1,2,4,5-tetraoxanes **11a**−**i** in reasonable to good yields (Tables 1 and 2), the key to the selectivity of this reaction being the use of the fluorous solvent hexafluoroisopropanol (HFIP).[23,30] Tetraoxanes **12a**−**c**,**f** were prepared in a similar manner using cyclododecanone rather than adamantanone.

From preliminary in vitro data it was rapidly apparent that the presence of the adamantyl ring greatly improved activity. One possible explanation for this is that the rigidity of the ring imparts greater stability. With this in mind the tropinone derivative below was synthesized (Scheme 2). In this case commercially available tropinone **13** was demethylated,[31] sulfonated, and subjected to the one-pot procedure described above to give tetraoxane **16**.

This one-pot methodology was also applied to the synthesis of a urea based dispiro 1,2,4,5-tetraoxane analogue of **8**, a derivative previously prepared in our group (Scheme 3). Piperidinone **9** was reacted with acid chloride **17** to give urea **18**. The one-pot methodology was then applied to give tetraoxane **19** in a 6% yield. Because of the poor yield of this reaction, no further ureas were synthesized. Alternative routes into these urea based compounds are currently under investigation.

Antimalarial Activity. Table 3 lists the in vitro antimalarial activity of all the dispiro-1,2,4,5-tetraoxanes synthesized versus the 3D7 strain of *Plasmodium falciparum*.[32] Most of these compounds have activity in the 3–30 nM region. There are clear trends in the SAR required for maximum activity. The presence of an adamantyl group greatly increases activity. Smaller alkyl groups at R^1 as opposed to larger aromatic groups also increase activity.

From this in vitro data and taking into account other factors such as cost and ease of synthesis, compound **11b** was selected as a lead for further biological studies with **11d** selected as a backup. **11b** was then tested versus seven strains of *Plasmodium falciparum* (Table 4).

In vivo antimalarial activity of **11b** and **11d** was measured in *P. berghei* ANKA infected mice.[33] The results can be seen in Table 5. While the activities of **11b** and **11d** are slightly lower than artesunate and **8**, it is clear that these molecules are potent antimalarials.

When this is taken into consideration along with their rapid two-step synthesis (tetraoxane **8** is a six-step synthesis),[27] they show excellent potential as candidate antimalarial drugs. The calculated log *P* and PSA of the two lead tetraoxanes **11b** and **11d** can be seen in Figure 4.

Cytotoxicity and Genotoxicity Screens. Tetraoxanes **11b** and **11d** were subjected to cytotoxicity and therapeutic index screens (Table 6). In the screens cytotoxicity was measured by Resazurin reduction. Single full dose response curves were generated using 10 independent drug concentrations. The 100 therapeutic index (TI) is the ratio of the TOX_{50} to the IC_{50} for the specific compound against the 3D7 *P. falciparum* isolate.[34] The tetraoxane derivatives **11b** and **11d** are remarkably nontoxic in these screens with in vitro TI values between 5000 and 17 000.

The potential genotoxicity of selected lead compounds **11b** and **11d** was determined by the *Salmonella typhimurium* SOS/*umu* assay in two strains: TA1535/pSK1002 and NM2009 (Table 7). This assay is based on the ability of DNA damaging agents to induce the expression of the *umu* operon. The *Salmonella* strains have a plasmid pSK1002 that carries an *umu*C-lacZ fused gene that produces a hybrid protein with β-galactosidase activity and whose expression is controlled by the *umu* regulatory region. Since many compounds do not exert their mutagenicity effect until they have been metabolized, the assay was also performed in the presence of rat liver S9-mix. Positive control agents (4-nitroquinoline 1-oxide (4NNQO) and 2-aminoanthracene (2Aan)) were used to test the response of the tester strains. Negative results were obtained for tetraoxanes **11b** and **11d** at the highest concentration tested (50 μM), both in the absence and in the presence of an in vitro metabolic activation system (S-9 mix).[35] (For detailed figures, see Supporting Information.)

Stability and Reactivity Studies. To investigate the activity, stability, and reactivity of the 1,2,4,5-tetraoxane pharmacophore

Scheme 1. Two-Step Synthesis of Dispiro-1,2,4,5-tetraoxanes

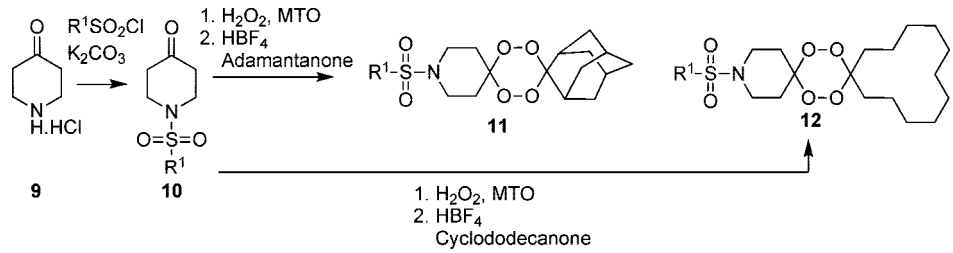


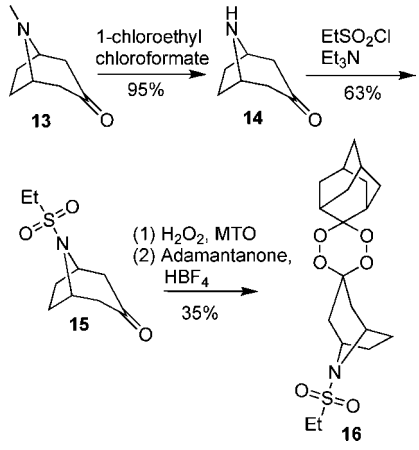
Table 1. Yields of Adamantyl Dispiro-1,2,4,5-tetraoxanes **11a−i**

compd	R1	% yield	% yield **11**
11a	Me	62	61
11b	Et	59	60
11c	^{i}Pr	52	56
11d	Cp	59	53
11e	CH_3CF_3	62	51
11f	Ph	98	36
11g	*p*-ClPh	98	41
11h	*p*-FPh	99	38
11i	*p*-CF_3Ph	95	25

Table 2. Yields of Cyclododecyl Dispiro-1,2,4,5-tetraoxanes **12**

compd	R^1	% yield **12**
12a	Me	36
12b	Et	32
12c	^{i}Pr	38
12f	Ph	20

Scheme 2. Synthesis of Tropinone Derived Dispiro-1,2,4,5-tetraoxane



compared to other synthetic endoperoxides, a series of structurally similar endoperoxides were synthesized varying only in the nature of their endoperoxide cores (Figure 5). Compounds **20** and **21** were prepared by literature methods; for synthetic routes see Supporting Information (phenyl derivatives selected because of UV chromophore to aid TLC analysis).[28]

The synthesis of 1,2,4-trioxane **22** can be seen below (Scheme 4). BOC-protected piperidinone **23** was converted to epoxide **24** in 82% yield, and the synthesis of the peroxide **25** was achieved using $Mo(acac)_2$ and H_2O_2. The 1,2,4-trioxane core **26** was formed using *p*-toluenesulfonic acid and 2-adamantanone in 83% yield. The BOC protecting group was then removed to give the hydrochloride salt **27** in 62% yield. Subsequent reaction with benzenesulfonyl chloride gave the desired 1,2,4-trioxane **22** in 92% yield.

Scheme 3. Two-Step Synthesis of Morpholine Urea 1,2,4,5-Tetraoxane

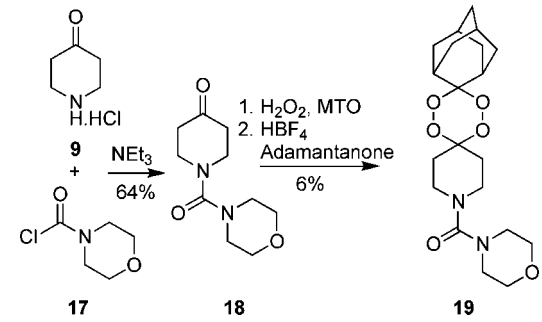


Table 3. In Vitro Antimalarial Activities of the Dispiro-1,2,4,5-tetraoxanes versus 3D7 *Plasmodium falciparum*

tetraoxane drug	R^1	mean IC_{50} (nM) vs 3D7
artemether		3.20 ± 1.97
artemisinin		9.20 ± 1.97
11a	Me	10.18 ± 6.80
11b	Et	5.55 ± 4.09
11c	^{i}Pr	5.87 ± 4.34
11d	Cp	3.52 ± 3.45
11e	CH_2CF_3	14.35 ± 5.42
11f	Ph	8.10 ± 1.21
11g	*p*-ClPh	22.73 ± 4.04
11h	*p*-FPh	16.73 ± 4.80
11i	*p*-CF_3	20.73 ± 7.79
12a	Me	27.75 ± 9.84
12b	Et	29.13 ± 15.50
12c	^{i}Pr	86.37 ± 17.50
12f	Ph	131.07 ± 33.18
16	Et	60.57 ± 22.00
19	N/A	4.70 ± 3.04

Table 4. In Vitro Antimalarial Activities of Tetraoxane **11b** versus Seven Strains of *Plasmodium falciparum*[a]

	IC_{50} ± SD (nM)			
strain	tetraoxane **11b**	artesunate	AM	CQ
DD2	3.0 ± 1.0	1.5 ± 0.9	6.1 ± 2.9	80.5 ± 3.1
K1	3.0 ± 0.9	0.7 ± 0.5	10.2 ± 1.3	73.9 ± 2.5
GC03	3.0 ± 0.6	1.1 ± 0.3	4.5 ± 1.0	8.1 ± 2.6
V1S	2.7 ± 1.6	0.7 ± 0.3	7.7 ± 1.9	83.7 ± 7.8
HB3	3.8 ± 2.7	1.7 ± 0.3	5.9 ± 0.4	6.6 ± 1.2
PH3	1.9 ± 0.4	1.9 ± 0.4	4.7 ± 0.7	72.0 ± 10.8
TM4	2.4 ± 0.3	0.5 ± 0.1	6.4 ± 0.7	91.3 ± 10.5

[a] AM = amodiaquine. CQ = chloroquine.

In vitro antimalarial activity of the endoperoxides shows that tetraoxane **11f** and OZ analogue **20** are potent antimalarials with comparable activity. Interestingly both **11f** and **20** are nearly 100-fold more active than both the 1,2,4-trioxanes **21** (>1000 nM) and **22** (710 nM) (Table 8). This observation underscores the advantage of the 1,2,4,5-tetraoxane core over the 1,2,4-trioxane core structure.

Table 5. In Vivo (Oral) Antimalarial Activities of Tetraoxanes **11b** and **11d** versus *Plasmodium berghei*

tetraoxane drug	ED_{50} (mg/kg)
11b	6.61
11d	7.93
8	3.18
artesunate	3.20
artemisinin	8.42

The stability and reactivity of the endoperoxides in the presence of iron were investigated. Tetraoxane **11f**, OZ analogue **20**, and trioxane **22** were subjected to 1.0 equiv of $FeBr_2$ in THF for the set time periods layed out in the table (this combination leads to complete degradation of artemisinin after 24 h).[36,37] The resulting residue was purified by flash column chromatography and the percent recovery of starting endoperoxide calculated (Table 9).

Table 9 shows that the 1,2,4,5-tetraoxanes exhibit remarkable stability with 69% recovery of starting material after 48 h. At this time the OZ analogue **20** has been completely degraded. The 1,2,4,5-tetraoxanes also have greater stability than the trioxane **22** because only 43% of the starting trioxane was recovered after 48 h. Tetraoxane **11f** was also treated with $FeSO_4 \cdot 7H_2O$ and $FeCl_2 \cdot 4H_2O$, and no degradation was seen after 24 h, further confirming the remarkable stability of these 1,2,4,5-tetraoxanes. Chemical stability tests in aqueous solution and acid were also carried out and again resulted in no significant degradation (see Supporting Information).

Mechanism of Accumulation. In order to investigate the intraparasitic sites of accumulation, it was necessary to synthesize an NBD tagged dispiro-1,2,4,5-tetraoxane for confocal microscopy studies (Scheme 5).[38] Tetraoxane **29** was synthesized using sulfonation followed by one-pot synthesis of the tetraoxane as described earlier. The methyl ester **29** was then converted to acid **30** using NaOH in ethanol. Coupling of the acid **30** with the mono-BOC protected diamine and removal of the BOC group provided the free amine that was coupled with NBD-Cl to give the desired NBD tagged tetraoxane **33**. This compound was found to have an IC_{50} of 16 nM versus the 3D7 strain of *Plasmodium falciparum*.[39]

The NBD tagged tetraoxane **33** was then used in single-cell confocal imaging of malaria infected erythrocytes, the results of which can be seen in Figure 6.[38] It can be seen from these images that the NBD tagged 1,2,4,5-tetraoxane accumulates only in infected erythrocytes (see panel a) within the cytoplasm and the digestive vacuole of the parasite; washing the cells with buffer failed to remove the tagged molecule as depicted in panel b. The formation of stable adducts produced within the parasite was inhibited by co-incubation with the iron chelator desferrioxamine (DFO). In the presence of DFO, there is significant intraparasitic accumulation (see panel c), the majority of which can be removed by cell washing (see panel d). This implies that DFO, by chelation of non-heme iron, reduces the reductive bioactivation and covalent adduct formation between the drug and parasite protein targets. It is also noted that accumulation of the probe can be seen within the acidic hematin-containing food vacuole and that there is evidence of some association of **33** with malaria pigment as seen in Figure 6d.

Conclusions

To conclude, rapid two-step syntheses of a range of dispiro-1,2,4,5-tetraoxanes with potent antimalarial activity both in vitro and in vivo have been reported. The lead compounds within this series have been proven to be remarkably nontoxic and to exhibit no genotoxicity. The 1,2,4,5-tetraoxanes have been proven to be superior to the ozonide analogues in terms of stability and to be superior to 1,2,4-trioxane analogues in terms of both stability and activity. The synthesis of a fluorescent NBD-tagged tetraoxane probe and subsequent single-cell confocal imaging have shown that the NBD probe accumulates in the parasite and that formation of stable adducts was inhibited by co-incubation with the iron chelator DFO. These results encourage further preclinical development of these candidates as cost-effective alternatives to the artemisinin derivatives.

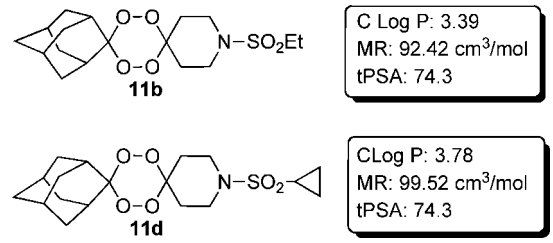


Figure 4. Calculated log *P* and PSA of lead compounds **11b** and **11d**.

Table 6. Cellular Cytotoxicity Screens (μM) and Therapeutic Index (TI) (μM) for Tetraoxanes **11b** and **11d**[a]

drug	Hep2G	L6	MRC-5	VERO	H9c(2-1)
11b TOX_{50}	>50	31	>50	>50	>50
TI	>9090	5636	>9090	>9090	>9090
11d TOX_{50}	>50	>50	>50	>50	>50
TI	>14285	>14285	>14285	>14285	>14285
doxorubicin	0.3	>5	2	>5	3

[a] HepG2: human Caucasian hepatocyte carcinoma. H9c2(2-1): myocardium, heart, rat. L6: skeletal muscle myoblast, rat. Vero: kidney, African green monkey, *Cercopithecus aethiops*. MRC-5: embryonal lung, diploid, male, human.

Table 7. Mutagenic Potential on *Salmonella typhimurium* Strains TA 1535/pSK1002 and NM2009 in the Absence and Presence of S9-Mix

	TA1535/pSK1002		NM2009	
compd	−S9	+rat S9	−S9	+rat S9
11b	neg	neg	neg	neg
11d	neg	neg	neg	neg
4NNQO	pos	pos	pos	pos
2Aan	neg	pos	neg	pos

Experimental Section

All reactions that employed moisture sensitive reagents were performed in dry solvent under an atmosphere of nitrogen in oven-dried glassware. All reagents were purchased from Sigma Aldrich or Alfa Aesar chemical companies and were used without purifica-

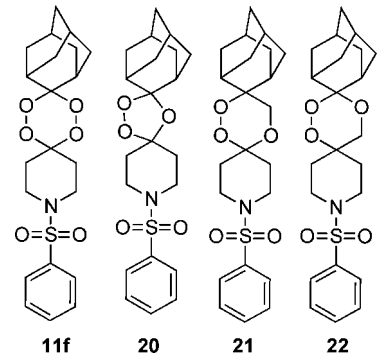


Figure 5. Tetraoxane **11f**, OZ analogue **20**, and trioxanes **21** and **22** used in stability studies.

Scheme 4. Synthesis of 1,2,4-Trioxane **22**

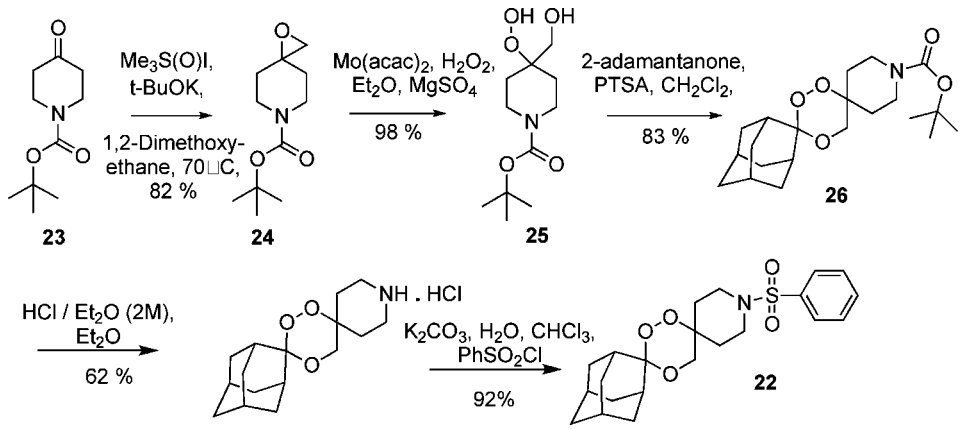


Table 8. *In Vitro* antimalarial activities of the phenyl sulfonamide endoperoxides

Endoperoxide Drug	Mean IC_{50}(nM)3D7
Artesunate	0.60 ± 0.035
11f	5.46 ± 1.19
20	4.98 ± 0.60
21	>1000
22	710 ± 115

Table 9. Iron degradation studies on endoperoxides **11f**, **20** and **22**

	% Recovery of Endoperoxide			
Drug	4 h	8 h	24 h	48 h
Tetraoxane **11f**	88.7	80.5	72.0	69.0
OZ analogue **20**	11.0	9.0	2.6	0.0
1,2,4-Trioxane **22**	96.8	84.9	56.7	43.2

tion. Thin layer chromatography (TLC) was carried out on Merck silica gel 60 F-254 plates, and UV inactive compounds were visualized using iodine or anisaldehyde solution. Flash column chromatography was performed on ICN Ecochrom 60 (32–63 mesh) silica gel, eluting with various solvent mixtures and using an air line to apply pressure. NMR spectra were recorded on a Brucker AMX 400 (^{1}H, 400 MHz; ^{13}C, 100 MHz) spectrometer. Chemical shifts are described in parts per million (δ) downfield from an internal standard of trimethylsilane. Microanalyses were performed in the University of Liverpool Microanalysis Laboratory. Mass spectra were recorded on a VG analytical 7070E machine and Fisons TRIO spectrometers using electron ionization (EI) and chemical ionization (CI).

General Procedure for Preparation of Adamantyl-1,2,4,5-tetraoxanes 11a−i. A solution of 1-R^1-sulfonylpiperidin-4-one **10** (1.13 mmol), 30% H_2O_2 (0.26 mL, 2.26 mmol, 2.0 equiv), and MTO (trace) in HFIP (2.27 mL) was stirred at room temperature for 2 h. After this time 2-adamantanone (339 mg, 2.26 mol, 2.0 equiv) was added followed by dropwise addition of a 54% ethereal solution of HBF_4 (368 mg, 2.26 mmol, 2.0 eq). The reaction was then allowed to stir at room temperature for 1 h. Dichloromethane (10 mL) was added. The organic layer was washed with a saturated solution of $NaHCO_3$ and dried over $MgSO_4$ and the solvent removed in vacuo. The resulting residue was purified by flash column chromatography (SiO_2, hexane/EtOAc = 9:1) to give the desired dispiro-1,2,4,5-tetraoxane **11a−i**.

11a: R = Me, 61%, white solid. ^{1}H NMR ($CDCl_3$), δ: 3.22–3.45 (4H, m, 4H1), 2.80 (3H, s, C*H*$_3$), 2.52 (2H, s, 2H2a), 1.51–2.23 (16H, m, 2H2b and adamantane). ^{13}C NMR ($CDCl_3$), δ: 111.6, 105.8, 41.6, 37.2, 36.2, 34.2, 33.5, 31.4, 27.4, 26.2. MS *m*/*z* (ES, +ve, CH_3OH), 382 ([M + Na]$^+$, 100%). Found [M + Na]$^+$, 382.1313. $C_{16}H_{25}NO_6NaS$ requires 382.1300. Anal. ($C_{16}H_{25}NO_6S$) C, H, N.

11b: R = Et, 60%, white solid. ^{1}H NMR ($CDCl_3$), δ: 3.22–3.45 (4H, m, 4H1), 2.95 (2H, q, J = 7.4 Hz, C*H*$_2$CH$_3$), 2.50 (2H, s, 2H2a), 1.51–2.23 (16H, m, 2H2b and adamantane), 1.35 (3H, t, J = 7.4 Hz, CH$_2$C*H*$_3$). ^{13}C NMR ($CDCl_3$), δ: 111.5, 106.0, 44.9, 37.2, 36.2, 33.5, 31.35, 30.6, 27.4, 8.3. MS *m*/*z* (ES, +ve, CH_3OH), 396 ([M + Na]$^+$, 100%). Found [M + Na]$^+$, 396.1447. $C_{17}H_{27}NO_6NaS$ requires 396.1457. Anal. ($C_{17}H_{27}NO_6S$) C, H, N.

11c: R = ^{i}Pr, 56%, white solid. ^{1}H NMR ($CDCl_3$), δ: 3.22–3.35 (4H, m, 4H1), 3.15 (1H, m, CH$_3$C*H*CH$_3$), 2.50 (2H, s, 2H2a), 1.51–2.10 (16H, m, 2H2b and adamantane), 1.31 (6H, d, J = 6.9 Hz, C*H*$_3$CHC*H*$_3$). ^{13}C NMR ($CDCl_3$), δ: 111.5, 106.0, 54.1, 44.9, 37.3, 33.5, 32.0, 27.4, 23.0, 17.1, 14.5. MS *m*/*z* (ES, +ve, CH_3OH), 410 ([M + Na]$^+$, 100%). Found [M + Na]$^+$, 410.1600. $C_{18}H_{29}NO_6NaS$ requires 410.1613. Anal. ($C_{18}H_{29}NO_6S$) C, H, N.

11d: R = Cp, 53%, white solid. ^{1}H NMR ($CDCl_3$), δ: 3.30–3.50 (4H, m, 4H1), 2.50 (2H, s, 2H2a), 2.20 (1H, m, CH$_2$C*H*CH$_2$), 1.51–2.10 (16H, m, 2H2b and adamantane), 1.15 (2H, m, C*H*$_2$CHCH$_2$), 0.95 (2H, m, CH$_2$CHC*H*$_2$). ^{13}C NMR ($CDCl_3$), δ: 111.5, 106.0, 54.1, 44.9, 37.3, 33.5, 32.0, 27.4, 26.5, 4.8. MS *m*/*z* (ES, +ve, CH_3OH), 408 ([M + Na]$^+$, 100%). Found [M + Na]$^+$, 408.1438. $C_{18}H_{27}NO_6NaS$ requires 408.1457. Anal. ($C_{18}H_{27}NO_6S$) C, H, N.

11e: R = CH_2CF_3, 51%, white solid. ^{1}H NMR ($CDCl_3$), δ: 3.70 (2H, q, J = 9.3 Hz, C*H*$_2$CF$_3$), 3.30–3.60 (4H, m, 4H1), 2.50 (2H, s, 2H2a), 1.51–2.23 (16H, m, 2H2b and adamantane). ^{13}C NMR ($CDCl_3$), δ: 110.2, 104.3, 52.2, 51.9, 35.9, 32.1, 31.35, 30.6, 27.4, −1.0. MS *m*/*z* (ES, +ve, CH_3OH), 450 ([M + Na]$^+$, 100%). Found [M + Na]$^+$, 450.1156. $C_{17}H_{24}NO_6F_3NaS$ requires 450.1174. Anal. ($C_{17}H_{24}NO_6F_3S$) C, H, N.

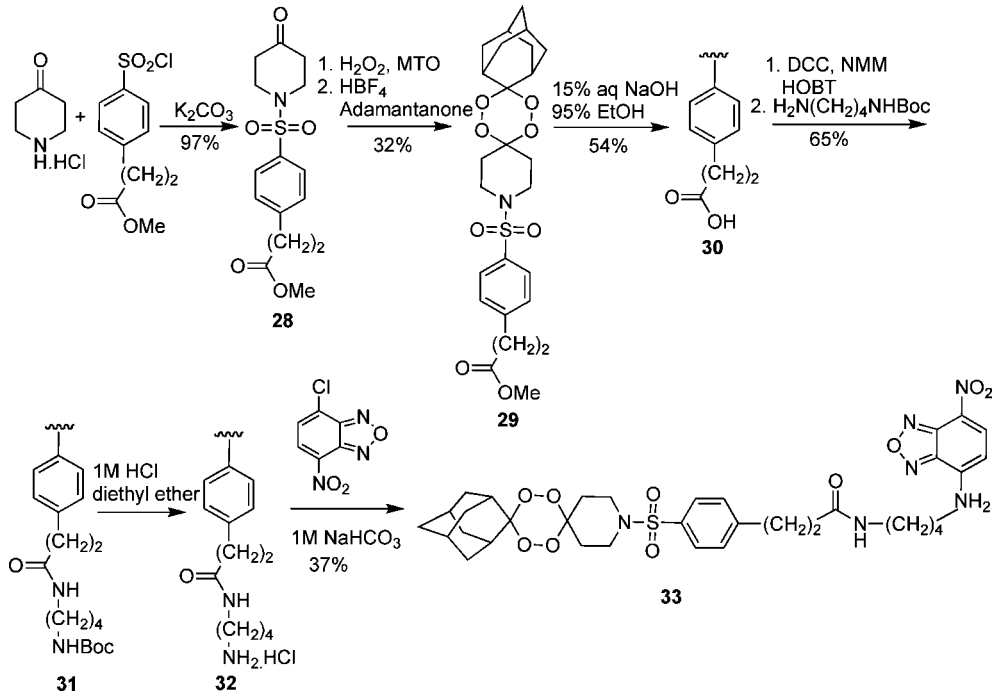
11f: R = Ph, 35%, white solid. ^{1}H NMR ($CDCl_3$), δ: 7.50–7.85, (5H, aromatics), 3.01–3.25, (4H, m, 4H1), 2.51, (2H, s, 2H2a), 1.51–2.20 (16H, m, 2H2b and adamantane). ^{13}C NMR ($CDCl_3$), δ: 136.6, 133.4, 129.6, 128.0, 111.5, 105.8, 47.4, 39.7, 37.2, 36.7, 33.4, 27.8, 27.3. MS *m*/*z* (ES, +ve, CH_3OH), 444 ([M + Na]$^+$, 100%). Found [M + Na]$^+$, 444.1445. $C_{21}H_{27}NO_6NaS$ requires 444.1457. Anal. ($C_{21}H_{27}NO_6S$) C, H, N.

11g: R = *p*-ClPh, 38%, white solid. ^{1}H NMR ($CDCl_3$), δ: 7.50–7.85, (4H, aromatics), 3.05–3.25, (4H, m, 4H1), 2.55, (2H, s, 2H2a), 1.55–2.10 (16H, m, 2H2b and adamantane). ^{13}C NMR ($CDCl_3$), δ: 140.0, 129.9, 129.4, 124.0, 111.5, 105.6, 47.4, 39.7, 37.2, 36.7, 33.4, 32.0, 27.3. MS *m*/*z* (ES, +ve, CH_3OH), 478 ([M + Na]$^+$, 100%). Found [M + Na]$^+$, 478.1081. $C_{21}H_{26}NO_6NaSCl$ requires 478.1067. Anal. ($C_{21}H_{26}NO_6S$) C, H, N.

11h: R = *p*-FPh, 41%, white solid. ^{1}H NMR ($CDCl_3$), δ: 7.10–7.80, (4H, aromatics), 3.05–3.25, (4H, m, 4H1), 2.55, (2H, s, 2H2a), 1.55–2.10 (16H, m, 2H2b and adamantane). ^{13}C NMR ($CDCl_3$), δ: 130.7, 130.6, 117.0, 116.7, 111.5, 105.6, 47.4, 39.7, 37.2, 36.7, 33.4, 32.0, 27.3. MS *m*/*z* (ES, +ve, CH_3OH), 462 ([M + Na]$^+$, 100%). Found [M + Na]$^+$, 462.1341. $C_{21}H_{26}NO_6FNaS$ requires 462.1363. Anal. ($C_{21}H_{26}NO_6FS$) C, H, N.

11i: R = *p*-CF_3Ph, 25%, white solid. ^{1}H NMR ($CDCl_3$), δ: 7.75–7.95, (4H, aromatics), 3.05–3.25, (4H, m, 4H1), 2.55, (2H, s,

Scheme 5. Synthesis of NBD−Tetraoxane Conjugate **33**



2H2a), 1.55–2.10 (16H, m, 2H2b and adamantane). ^{13}C NMR ($CDCl_3$), δ: 128.4, 128.0, 127.3, 127.3, 126.9, 116.8, 111.4, 47.4, 39.7, 37.2, 36.7, 33.4, 32.0, 27.3. MS *m*/*z* (ES, +ve, CH_3OH), 512 ($[M + Na]^+$, 100%). Found $[M + Na]^+$, 512.1346. $C_{22}H_{26}NO_6F_3NaS$ requires 512.1331. Anal. ($C_{22}H_{26}NO_6F_3S$) C, H, N.

General Procedure for Preparation of Cyclododecyl-1,2,4,5-tetraoxanes 12a−c,f. A solution of 1-R^1-sulfonylpiperidin-4-one **10** (1.13 mmol), 30% H_2O_2 (0.26 mL, 2.26 mmol, 2.0 equiv), and MTO (trace) in HFIP (2.27 mL) was stirred at room temperature for 2 h. After this time cyclododecanone (412 mg, 2.26 mol, 2.0 equiv) was added followed by dropwise addition of a 54% ethereal solution of HBF_4 (368 mg, 2.26 mmol, 2.0 equiv). The mixture was then stirred at room temperature for 1 h. Dichloromethane (10 mL) was added, and the organic layer was washed with a saturated solution of $NaHCO_3$ and dried over $MgSO_4$. The solvent was removed in vacuo. The resulting residue was purified by flash column chromatography (SiO_2, hexane/EtOAc = 9:1) to give the desired dispiro-1,2,4,5-tetraoxane **12a−c,f**.

12a: R = Me, 36%, white solid. ^{1}H NMR ($CDCl_3$), δ: 3.25–3.42, (4H, m, 4H1), 2.80, (3H, s, C*H*$_3$), 2.50 (2H, bs, 2H2a), 2.25 (2H, m, 2H3a), 1.85 (2H, bs, 2H2b), 1.60 (2H, m, 2H3b), 1.21–1.49 (18H, m, dodecane ring). ^{13}C NMR ($CDCl_3$), δ: 113.5, 105.7, 43.3, 42.1, 35.5, 29.9, 29.5, 26.3, 22.6, 19.8, 18.4. MS *m*/*z* (ES, +ve, CH_3OH), 414 ($[M + Na]^+$, 100%). Found $[M + Na]^+$, 414.1926. $C_{18}H_{33}NO_6NaS$ requires 414.1926. Anal. ($C_{18}H_{33}NO_6S$) C, H, N.

12b: R = Et, 32%, white solid. ^{1}H NMR ($CDCl_3$), δ: 3.31–3.50, (4H, m, 4H1), 2.95, (2H, q, J = 7.4 Hz, C*H*$_2$CH$_3$), 2.50 (2H, bs, 2H2a), 2.25 (2H, bs, 2H3a), 1.85 (2H, bs, 2H2b), 1.60 (2H, m, 2H3b), 1.28 (3H, t, J 7.4, CH_2CH_3), 1.31–1.49 (18H, m, dodecane ring). ^{13}C NMR ($CDCl_3$), δ: 113.5, 106.0, 44.9, 42.1, 35.5, 29.9,

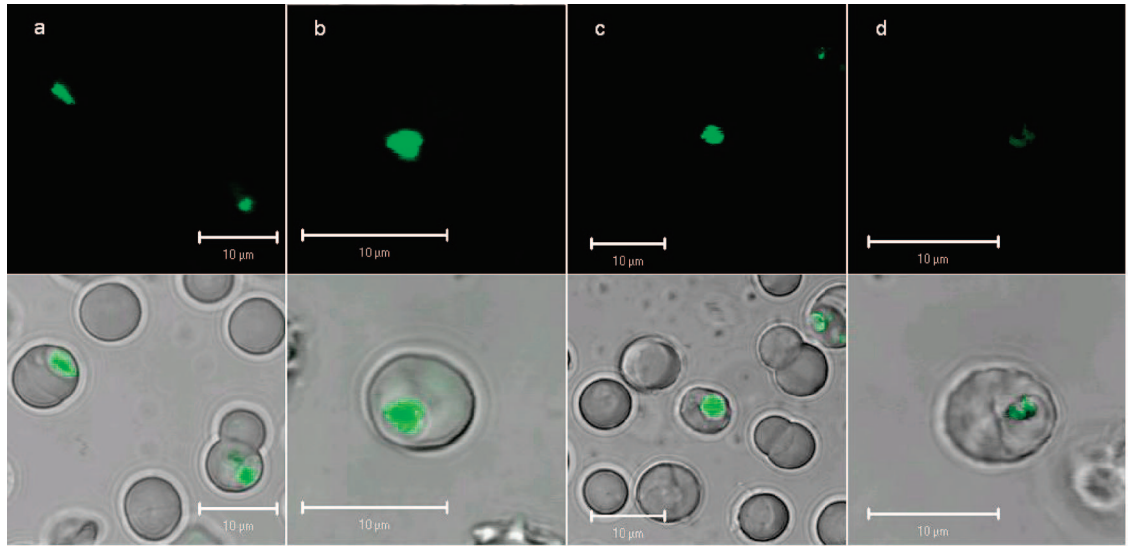


Figure 6. Confocal microscope images of infected red blood cells with NBD conjugates (1 μM) in the presence or absence of DFO (100 μM). The NBD-labeled compound was added 10 min before imaging. For imaging experiments using DFO, the parasite cultures were preincubated at 37 °C for 30 min with DFO prior to use. After uptake of drug, the perfusion chamber was washed with 5000× its volume of media to examine the retention of fluorescence: Tetraoxane−NBD adduct **33** without DFO before (a) and after wash (b) and with DFO (100 μM) before (c) and after wash (d).

26.4, 26.2, 22.6, 19.8, 8.26. MS *m*/*z* (ES, +ve, CH_3OH), 428 ([M + Na]$^+$, 100%). Found [M + Na]$^+$, 428.2100. $C_{19}H_{35}NO_6NaS$ requires 428.2083. Anal. ($C_{19}H_{35}NO_6S$) C, H, N.

12c: R = ^{i}Pr, 38%, white solid. ^{1}H NMR ($CDCl_3$), δ: 3.31–3.50, (4H, m, 4H1), 3.15, (2H, m, CH_3CHCH_3), 2.50 (2H, bs, 2H2a), 2.25 (2H, bs, 2H3a), 1.85 (2H, bs, 2H2b), 1.60–1.20 (26H, m, 2H3b, CH_3CHCH_3, dodecane ring). ^{13}C NMR ($CDCl_3$), δ: 113.5, 105.9, 54.2, 44.8, 42.0, 35.3, 29.9, 26.3, 26.1, 22.6, 19.7, 17.7. MS *m*/*z* (ES, +ve, CH_3OH), 442 ([M + Na]$^+$, 100%). Found [M + Na]$^+$, 442.2256. $C_{20}H_{37}NO_6NaS$ requires 442.2239. Anal. ($C_{20}H_{37}NO_6S$) C, H, N.

12f: R = Ph, 20%, white solid. ^{1}H NMR ($CDCl_3$), δ: 7.95–7.45 (5H, m, aromatics), 3.05–3.35 (4H, m, 4H1), 2.50 (2H, bs, 2H2a), 2.25 (2H, bs, 2H3a), 1.85 (2H, bs, 2H2b), 1.62 (2H, m, 2H3b), 1.21–1.49 (18H, m, dodecane ring). ^{13}C NMR ($CDCl_3$), δ: 136.5, 133.4, 129.6, 128.0, 113.4, 105.7, 43.7, 42.4, 31.6, 29.7, 26.5, 26.2, 23.1, 19.7. MS *m*/*z* (ES, +ve, CH_3OH), 476 ([M + Na]$^+$, 100%). Found [M + Na]$^+$, 476.2097. $C_{23}H_{35}NO_6NaS$ requires 476.2083. Anal. ($C_{23}H_{35}NO_6S$) C, H, N.

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Supporting Information Available: Experimental details for the synthesis of **10a**−**i** and tetraoxane **33**, combustion analysis results, additional detailed information on cytotoxicity and geneotoxicity studies, synthesis procedures for compounds **20**−**22** used in the section Stability and Reactivity Studies and additional details on stability tests, and details of the confocal microscopy procedure. This material is available free of charge via the Internet at http://pubs.acs.org.

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