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Piperidine dispiro-1,2,4-trioxane analogues

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ABSTRACT

Dispiro *N*-Boc-protected 1,2,4-trioxane **2** was synthesised via $Mo(acac)_2$ catalysed perhydrolysis of *N*-Boc spirooxirane followed by condensation of the resulting β-hydroperoxy alcohol **10** with 2-adamantanone. *N*-Boc 1,2,4-trioxane **2** was converted to the amine 1,2,4-trioxane hydrochloride salt **3** which was subsequently used to prepare derivatives (**4–7**). Several of these novel 1,2,4-trioxanes had nanomolar antimalarial activity versus the 3D7 strain of *Plasmodium falciparum*. Amine intermediate **3** represents a versatile derivative for the preparation of achiral arrays of trioxane analogues with antimalarial activity.

Currently, semi-synthetic derivatives of artemisinin[1,2] are the drugs of choice to treat multi-drug resistant malaria.[3] However, the commercial availability of artemisinin **1a**[1,2] (and hence its semi-synthetic derivatives) is somewhat limited since it is a natural product isolated from the herb *Artemisia annua*. As a result, extensive research into synthetic endoperoxide antimalarial drugs has been carried out in the last 15 years[4–6] to produce molecules that are structurally simpler and synthetically accessible with a projected low cost of goods[4,7] (Fig. 1).

Fully synthetic cyclic endoperoxides examined in the literature include 1,2-dioxanes, 1,2,4-trioxolanes, 1,2,4-trioxanes and 1,2,4,5-tetraoxanes, all of which retain the critical endoperoxide bond. Padmanilayam et al. recently synthesised a series of piperidine 1,2,4-trioxolanes and reported impressive antimalarial activities.[8] Inspired by this work, we were interested in exploring the antimalarial activities of dispiro-1,2,4-trioxanes with a fused piperidine ring. The advantage of incorporating a nitrogen within the C ring of a 1,2,4-trioxane lies in the fact that the resulting endoperoxide is achiral in contrast to 1,2,4-trioxanes such as **1b** and hybrid molecules such as the 1,2,4-trioxaquines,[9] for example, **1c** and this may be an advantage in terms of avoiding expensive asymmetric synthesis or having to perform resolution of racemic drug substance.

In this paper we report on the first synthesis of piperidine 1,2,4-trioxanes **2–7** and the isomeric 1,2,4- trioxane **12**.

In the synthesis of trioxanes **2–7** the key intermediate was the β-hydroperoxy alcohol. Initially we started the synthesis of **5** with the hydrochloride salt of 4-piperidone to obtain *N*-phenylsulfonylpiperidinone which was subjected to Corey–Chaykovsky epoxidation[10] to obtain *N*-phenylsulfonylpiperidinone spiroepoxide (Scheme 1). The resulting epoxide was used to synthesise *N*-phenylsulfonylpiperidine β-hydroperoxy alcohol (Scheme 1) following the procedure described by Vennerstrom et al.[11] Several attempts failed to obtain *N*-phenylsulfonylpiperidine β-hydroperoxy alcohol by treating the spiroepoxide with $MgSO_4$ and dried H_2O_2 in presence of molybdenyl acetylacetonate (Scheme 1). Later, we used commercially available *N*-Boc-4-piperidone **8** as the starting material to first obtain the *N*-Boc spiroepoxide **9** followed by perhydrolysis to get β-hydroperoxy alcohol **10** in good yield. The spiroepoxide **9** was obtained via Corey–Chaykovsky reaction in a yield of 82%.[12] When the epoxide **9** was subjected to molybdenyl acetylacetonate catalysed perhydrolysis in presence of H_2O_2, the β-hydroperoxy alcohol **10** was obtained almost quantitatively (Scheme 2).

The β-hydroperoxy alcohol **10** was used without any further purification as TLC, MS and NMR indicated the presence of only one product. Hydrogen peroxide (50%) used in the perhydrolysis reaction was dissolved in anhydrous ether and dried twice with anhydrous $MgSO_4$. It is interesting to note that β-hydroperoxy alcohol **10** was obtained almost quantitatively from **9** (Scheme 2) while *N*-phenylsulfonylpiperidine β-hydroperoxy alcohol was not accessible from *N*-phenylsulfonylpiperidine spiro epoxide (Scheme 1). Following a previously published procedure, β-hydroperoxy

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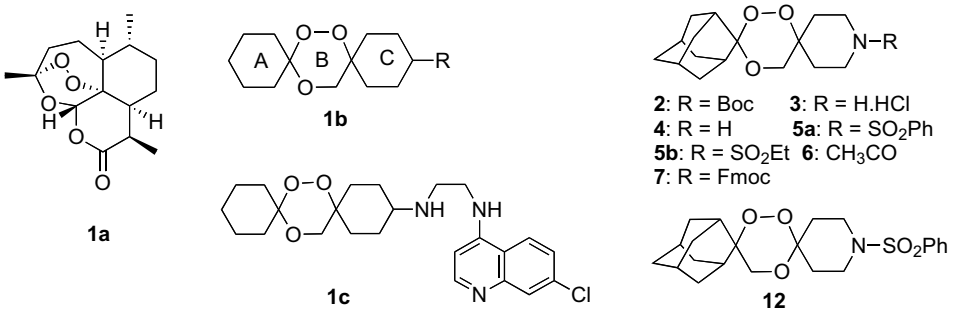
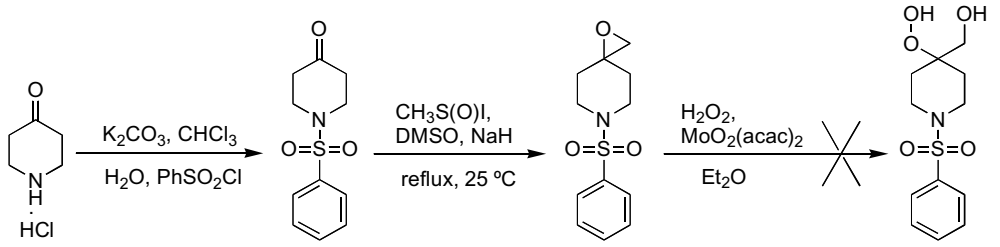
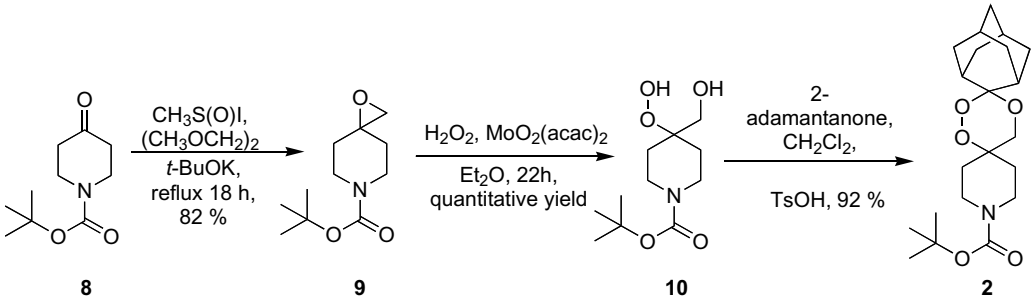


Figure 1. Artemisinin (**1a**) and synthetic piperidine dispiro-1,2,4-trioxanes (**2**–**7**).



Scheme 1. Attempted synthesis of *N*-phenylsulfonylpiperidine β-hydroperoxy alcohol.



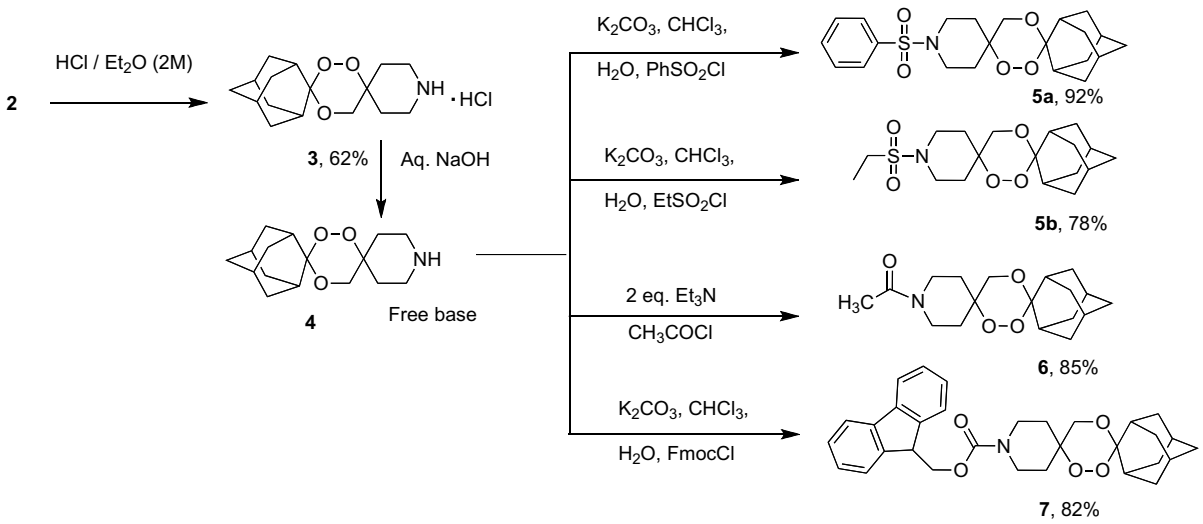
Scheme 2. Synthesis of *N*-Boc-1,2,4-trioxane (**2**).

alcohol **10** was allowed to react with 2-adamantanone in presence of catalytic amount of *p*-toluenesulfonic acid monohydrate to obtain the *N*-Boc trioxane **2**.[11] We chose 2-adamantanone as the reacting ketone since it is widely reported that spiro 1,2,4-trioxanes with an adamantane ring have better activities than spiro 1,2,4-trioxanes with cyclopentane or cyclohexane rings.[13] The *N*-Boc trioxane **2** was isolated in a yield of 92%. Once we had access to the trioxane **2** we decided to deprotect the BOC group of **2** and convert it into a trioxane hydrochloride salt **3**.

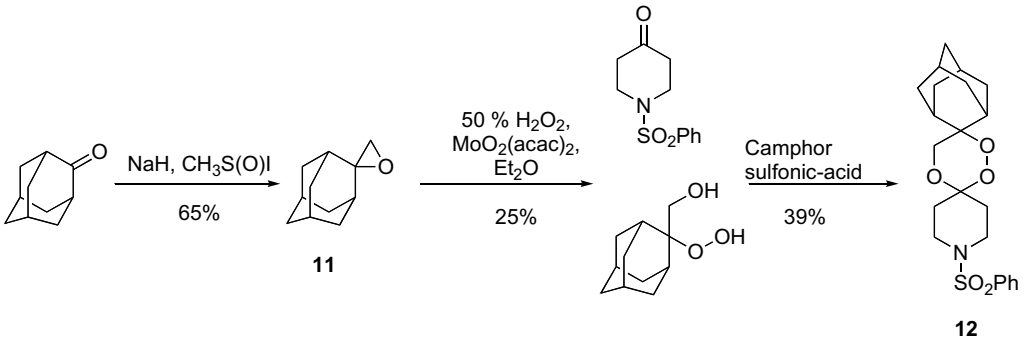
The deprotection of the BOC group of **2** was not straight forward; attempted removal of the BOC group using 1.0 M ethereal hydrochloric acid solution failed as the deprotection was incomplete and the yield was very low. We then used 2.0 M ethereal hydrochloric acid solution to successfully remove the BOC group to obtain the trioxane hydrochloride salt **3** in a yield of 62%.[14] With trioxane **3** to hand it was easy to manipulate the substituents on the *N*-atom of the spiro trioxane to obtain different derivatives and the free base amine trioxane **4** was prepared by extracting an alkaline aqueous solution of hydrochloride **3** with dichloromethane and diethyl ether. In a biphasic reaction in presence of potassium carbonate, the amine hydrochloride **3** was treated either with benzenesulfonyl chloride or ethanesulfonyl chloride to obtain the sulfonamide trioxanes **5a** (92%) and **5b** (78%) (Scheme 3).[15] In a similar reaction trioxane hydrochloride salt **3** was reacted with 9-fluorenylmethyl chloroformate to obtain *N*-Fmoc trioxane **7** (82%) (Scheme 3). The amide **6** was prepared by acylation with acetyl chloride using two equivalents of triethylamine as base to provide **6** in 85% yield. (Scheme 4).

For the purposes of comparison of antimalarial activity, the synthesis of regioisomer phenylsulfonyl 1,2,4-trioxane **12** was carried out using similar methods to that depicted in Schemes 2 and 3. Thus the epoxide **11** derived from adamant-2-one was allowed to react with hydrogen peroxide and the resultant peroxy alcohol was coupled with the phenylsulfonyl piperidinone derivative to produce **12** in moderate yield (39%).

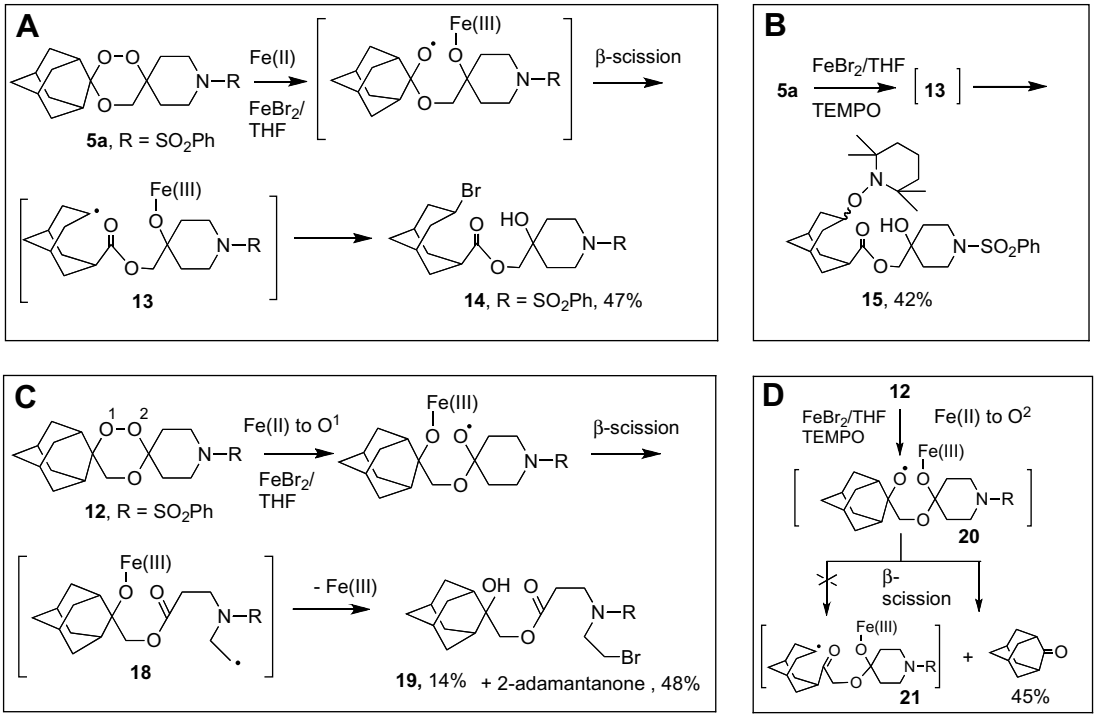
The trioxane series was tested against the 3D7 strain of *Plasmodium falciparum* and 1,2,4,5-tetraoxane **16** and 1,2,4-trioxolane **17** were included for comparison. All of the 1,2,4-trioxanes[16] tested demonstrated nanomolar antimalarial activity with amine hydrochloride having the highest potency with an IC_{50} of 120 nM. The phenylsulfonyl derivative **5a** had moderate activity of 710 nM; interestingly the regioisomer **12** was not active up to a concentration of 1000 nM. The difference in antimalarial activity between isomeric spiro 1,2,4-trioxanes has been attributed to the types of radicals that can be produced following homolytic cleavage of the endoperoxide-bridge.[17] In the case of the active adamantylid-



Scheme 3. Synthesis of N-substituted dispiro-1,2,4-trioxanes (**3**–**7**).

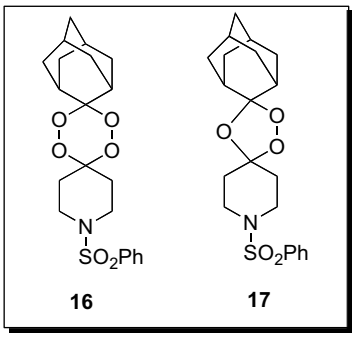


Scheme 4. Synthesis of regioisomeric 1,2,4-trioxane (**12**).



Scheme 5. (A) Generation of a secondary C-radical **13** via Fe(II) ($FeBr_2$) mediated degradation of 1,2,4-trioxane **5a**. (B) Interception of intermediate **13** with TEMPO to give Adduct **15.** (C and D) Reactivity of 1,2,4-trioxane **5b** in the presence of $FeBr_2$.

Table 1
In vitro antimalarial activity versus the 3D7 strain of *Plasmodium falciparum*[a]



Endoperoxide	IC_{50} (nM)	St. Dev.
Artemether	1.26	0.11
Artemisinin	9.54	1.10
2	197.86	21.96
3	121.33	29.70
5a	710.23	19.93
5b	179.62	19.95
6	183.45	32.78
7	174.23	23.29
12	>1000	23.29
16	10.72	2.35
17	6.22	2.12

[a] Parasites were maintained in continuous culture according to the method of Trager and Jensen.[18] IC_{50} values were measured according to the methods described by Desjardins et al.[19]

ene fused endoperoxides such as **2–7** it appeared likely, by analogy with previous work by Vennerstrom, that scission to produce an adamantane secondary carbon centred radical would occur on exposure to reducing Fe(II) (Scheme 5A).[11,17] To confirm this proposal we carried out iron mediated degradation of sulfonamide **5a** using ferrous bromide in THF. The major product was the bromo alcohol **14** (47% yield) indicating that **13** had been produced as an intermediate. In a separate experiment, definitive confirmation that the secondary C-radical was obtained by using TEMPO as the spin-trapping agent; exposure of **5a** to $FeBr_2$ in the presence of TEMPO led to formation of an adduct **15** in 42% yield (Scheme 5 B). In contrast, isomeric 1,2,4-trioxane **12** produced only a 14% yield of bromoalkane **19**, a product indicating the formation of the primary C-radical **18** as an intermediate. Attempts to trap intermediate **18** with TEMPO led to a low yield (<10%) of the spin-trapped adduct (not shown). The major product from the reaction was 2-adamantanone indicating that in this system, as in the previously studied 1,2,4-trioxolanes,[8] steric shielding of the endoperoxide bridge by the adamantly group leads to a preference for association of Fe(II) with O_2 of the endoperoxide bridge. As reported in a previous study,[11] no products supporting β-scission of **20** to the ketone secondary carbon centred radical **21** were obtained. The fact that **5b** generates minor amounts of a carbon centred radical coupled with a preference to produce inert ketone degradation products might explain the poor antimalarial activity observed with this analogue. (Table 1).

In summary, *N*-Boc β-hydroperoxy alcohol **10** was successfully obtained from its precursor in good yield. The deprotection of the *N*-Boc-protected trioxane **2** needs further optimisation to improve the yields but the current method does allow access to the hydrochloride salt of the trioxane **3**. Compound **3** and the small number of analogues prepared here have moderate activity compared with the semi-synthetic artemisinin derivatives, synthetic tetraoxanes (e.g., **16**) or ozonides (e.g., **17**);[20] however it is likely that by simple derivitisation of **3** by the chemistry employed here (and by simple reductive amination depicted in Scheme 3) many new achiral 1,2,4-trioxanes with improved potency can be prepared using this synthetic methodology including analogues of chiral trioxaquines such as **1c**.

References and notes

1. Haynes, R. K.; Vonwiller, S. C. *Acc. Chem. Res.* **1997**, *30*, 73.
2. Klayman, D. L. *Science* **1985**, *228*, 1049.
3. WHO, **2006**. Available from: http://www.who.int/malaria/docs/TreatmentGuidelines2006.pdf.
4. Jefford, C. W. *Drug Discovery Today* **2007**, *12*, 487.
5. O'Neill, P. M.; Posner, G. H. *J. Med. Chem.* **2004**, *47*, 2945.
6. Biagini, G. A.; O'Neill, P. M.; Nzila, A.; Ward, S. A.; Bray, P. G. *Trends Parasitol.* **2003**, *19*, 479.
7. Vangapandu, S.; Jain, M.; Kaur, K.; Patil, P.; Patel, S. R.; Jain, R. *Med. Res. Rev.* **2007**, *27*, 65.
8. (a) Dong, Y. X.; Chollet, J.; Matile, H.; Charman, S. A.; Chiu, F. C. K.; Charman, W. N.; Scorneaux, B.; Urwyler, H.; Tomas, J. S.; Scheurer, C.; Snyder, C.; Dorn, A.; Wang, X. F.; Karle, J. M.; Tang, Y. Q.; Wittlin, S.; Brun, R.; Vennerstrom, J. L. *J. Med. Chem.* **2005**, *48*, 4953; (b) Padmanilayam, M.; Scorneaux, B.; Dong, Y. X.; Chollet, J.; Matile, H.; Charman, S. A.; Creek, D. J.; Charman, W. N.; Tomas, J. S.; Scheurer, C.; Wittlin, S.; Brun, R.; Vennerstrom, J. L. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 5542.
9. Dechy-Cabaret, O.; Benoit-Vical, F.; Robert, A.; Meunier, B. *Chembiochem* **2000**, *1*, 281.
10. Corey, E. J.; Chaykovsky, M. *J. Am. Chem. Soc.* **1965**, *87*, 1353.
11. Tang, Y. Q.; Dong, Y. X.; Wang, X. F.; Sriraghavan, K.; Wood, J. K.; Vennerstrom, J. L. *J. Org. Chem.* **2005**, *70*, 5103.
12. Olah, G. A.; Wu, A. H. *Synthesis* **1990**, 887.
13. Tang, Y. Q.; Dong, Y. X.; Vennerstrom, J. L. *Med. Res. Rev.* **2004**, *24*, 425.
14. Cali, P.; Begtrup, M. *Synthesis* **2002**, 63.
15. Engler, T. A.; Wanner, J. *J. Org. Chem.* **2000**, *65*, 2444.
16. *(a) t-Butyl-1-oxa-6-azaspiro[2.5]octane-6-carboxylate (***9***).* A mixture of 1-Boc-4-piperidone (3.05 g, 15.3 mmol), $Me_3S(O)I$ (97% pure, 3.4 g, 15.3 mmol) and *t*-BuOK (95% pure, 1.973 g, 15.3 mmol) in 1,2-dimethoxyethane (80 ml) was refluxed for 18 h, cooled to room temperature and then quenched with water. The organic layer was separated and the aqueous layer was extracted with Et_2O (3 × 50 ml) and CH_2Cl_2 (2 × 50 ml) followed by general work up to give crude *N*-Boc spiro epoxide which was purified by flash chromatography (SiO_2, 60% EtOAc in *n*-Hexane) to give pure *N*-Boc spiro epoxide **9** (2.70 g, 82%). Mp 50–52 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 3.69–3.74 ($2H_a$, ddd, J = 4.74 Hz), 3.40–3.46 ($2H_b$, ddd, J = 9.49 Hz), 2.68 (2H, s), 1.76–1.83 (2H, ddd, J = 9.49 Hz), 1.47 (9H, s), 1.43–1.46 (2H, ddd, J = 4.74 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.01, 79.84, 57.35, 53.94, 42.81, 33.28, 28.73; IR (neat, cm^{-1}) 2972, 2925, 2864, 1682, 1421, 1363, 1317, 1238, 1161, 1122, 989, 912; m/z (CI, +ve, NH_3), 214 ($[M+H]^+$, 16%); HRMS: Found $[M+H]^+$, 214.14487, $C_{11}H_{20}NO_3$ requires 214.14430. (b) *t-Butyl 4-hydroperoxy-4-(hydroxymethyl)piperidine-1-carboxylate (***10***).* The *N*-Boc spiro epoxide **9** (2.21 g, 10.36 mmol), $MgSO_4$ dried H_2O_2–Et_2O (150 ml, see note below) and bis(acetylacetonato)-dioxomolybdenum(VI) were stirred together in a reaction flask at room temperature for 22 h after which the reaction mixture was washed with water (1 × 100 ml) and brine (1 × 100 ml). The combined aqueous layers were extracted with CH_2Cl_2 (2 × 75 ml). General workup of the combined organic layers resulted in the Boc-protected hydropeoxy product **10** (2.5 g, 98%) which was used in the next step without any further purification as the TLC indicated the presence of only one product and was also confirmed by NMR and mass spectra. ^1H NMR (400 MHz, $CDCl_3$) δ 9.41 (1H, br s), 8.64 (1H, br s), 3.69–3.89 (2H, m), 3.64 (2H, s), 3.11–3.17 (2H, m), 1.89–1.92 (2H, m), 1.47–1.50 (2H, m), 1.46 (9 H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.67, 81.66, 80.59, 66.4, 53.85, 29.51, 28.86; m/z (ES, +ve, CH_3OH), 270 ($[M+Na]^+$, 100%); HRMS: Found $[M+Na]^+$, 270.13320, $C_{11}H_{21}NO_5Na$ requires 270.13170. *Note:* Method to dry H_2O_2–Et_2O: To a mixture of H_2O_2 (42 ml, 50 wt% in H_2O) and anhydrous Et_2O (395 ml) under constant stirring, anhydrous $MgSO_4$ was added until a thick white slurry settled at the bottom of the flask. Then the supernatant was decanted and once again dried with anhydrous $MgSO_4$ and filtered. One hundred and fifty milliliters of the dried H_2O_2–Et_2O solution was used in the reaction above (Scheme 2) to obtain the Boc-protected hydroperoxy product **10**. (c) *Synthesis of N-Boc trioxane* **2**. To a mixture of β-Hydroperoxy alcohol **10** (2.5 g, 10.12 mmol) and 2-admantanone (2.42 g, 16.11 mmol) in anhydrous CH_2Cl_2 (135 ml) was added *p*-toluenesulfonic acid monohydrate (217 mg, 1.14 mmol) and the reaction mixture was stirred at room temperature for 5 h and then washed with aqueous saturated $NaHCO_3$ (80 ml), water (80 ml) and brine (80 ml). The combined aqueous phases were extracted with CH_2Cl_2 (3 × 50 ml). After general work up of combined organic layers the crude product was purified by flash chromatography (SiO_2, 30% EtOAc in *n*-Hexane) to give pure *N*-Boc trioxane **2** (3.55 g, 92.4%). Mp 74–76 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 3.65–3.86 (4H, m), 3.09–3.42 (2H, m), 1.49–2.14 (18H, m), 1.44–1.48 (9H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.16, 105.00, 79.94, 76.07, 65.65, 47.39, 39.67, 37.57, 36.73, 35.27, 33.77, 28.85, 27.88, 27.54; IR (neat, cm^{-1}) 2912, 2854, 1689, 1450, 1414, 1363, 1244, 1155, 1107, 1086, 1065, 1009; m/z (ES, +ve, CH_3OH), 402 ($[M+Na]^+$, 100%); HRMS: Found $[M+Na]^+$, 402.22470, $C_{21}H_{33}NO_5Na$ requires 402.22560. (d) *Synthesis of hydrochloride salt of trioxane* **3**. Anhydrous HCl–Et_2O (2.0 M, 25 ml) was added to a solution of *N*-Boc trioxane **2** (1.05 g, 2.76 mmol) in anhydrous Et_2O (5 ml) and the reaction

mixture was stirred at room temperature under an atmosphere of N_2. After 24 h a white solid precipitated out which was then washed with anhydrous Et_2O to give the desired hydrochloride salt of the trioxane **3** as a white solid (538 mg, 62%). ^{13}C NMR (100MHz, $CDCl_3$) δ 107.13, 74.79, 64.57, 42.06, 39.85, 37.74, 36.86, 34.93, 34.89, 33.42, 32.68, 27.12; *m*/*z* (ES, +ve, CH_3OH), 280 ($[M]^+$ cation, 100%); HRMS: Found $[M]^+$ cation, 280.19020, $C_{16}H_{26}NO_3$ requires 280.19130. (e) *Synthesis of amine trioxane* ***4.*** Trioxane hydrochloride salt **3** was dissolved in aqueous NaOH solution and then extracted three times with CH_2Cl_2 followed by general work up of the organic layer to give the amine trioxane **4**. ^1H NMR (400 MHz, $CDCl_3$) δ 3.39–3.83 (2H, br s), 2.90–3.10 ($2H_a$, m), 2.70–2.88 ($2H_b$, m), 2.17 (1H, s), 1.88–2.13 (4H, m), 1.86–1.79 (2H, m), 1.38–1.77 (10H, m), 1.15–1.35 (2H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 104.83, 76.36, 65.82, 53.78, 37.61, 33.78, 31.26, 30.07, 27.59, 27.57; IR (neat, cm^{-1}) 3367, 2914, 2854, 1718, 1624, 1541, 1446, 1425, 1286, 1267, 1107, 1084, 1066, 1043, 995; *m*/*z* (CI, +ve, NH_3), 280 ($[M+H]^+$, 100%); HRMS: Found $[M+H]^+$, 280.19166, $C_{16}H_{26}NO_3$ requires 280.19125. (f) *Synthesis of sulfonamide trioxane* ***5a****: (General procedure).* To a reaction flask containing the hydrochloride salt of trioxane **3** (200 mg, 0.63 mmol), $CHCl_3$ (7.0 ml), water (7.0 ml) and K_2CO_3 (224 mg, 1.62 mmol), benezenesulfonyl chloride (0.13 ml, 1.02 mmol) was added. After stirring the reaction mixture for 24 h the reaction was quenched by the addition of aq saturated $NaHCO_3$. The organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (3×25 ml). The combined organic layers were dried with anhydrous $MgSO_4$ and concentrated under reduced pressure. The crude product was then purified by flash chromatography using gradient elution with increasing polarity (SiO_2, EtOAc/*n*-Hexane) to give the desired sulfonamide trioxane **5** (244 mg, 92%) as a white solid. Mp 168–170 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 7.50–7.80 (5H, m, aromatic), 3.24–3.92 (4H, m, $-CH_2-O$, and $2H_{1a}$), 2.53–3.00 (2H, m, $2H_{1b}$), 1.52–1.96 (18H, $2H_{2a}$, $2H_{2b}$ and 14 adamantane, m). ^{13}C NMR (100 MHz, $CDCl_3$) δ 136.86, 133.16, 129.50, 128.02, 105.19, 74.99, 65.65, 43.79, 38.41, 37.49, 36.11, 35.15, 33.69, 27.48, 27.45; IR (neat, cm^{-1}) 2910, 2856, 1448, 1348, 1325, 1186, 1161, 1086, 1045, 931, 735, 687; *m*/*z* (ES, +ve, CH_3OH), 442 ($[M+Na]^+$, 100%); HRMS: Found $[M+Na]^+$, 442.16540, $C_{22}H_{29}NO_5SNa$ requires 442.16640; Elemental analysis ($C_{22}H_{29}NO_5S$), found C: 62.39%, H: 6.80%, N: 3.00 (requires C: 62.98%, H: 6.97%, N: 3.34).

17. For a comprehensive study of iron mediated degradation studies on 1,2,4-trioxanes, see (a) Posner, G. H.; Wang, D.; Cumming, J. N.; Oh, C. H.; French, A. N.; Bodley, A. L.; Shapiro, T. A. *J. Med. Chem.* **1995**, *38*, 2273–2275; (b) Posner, G. H.; Oh, C. H. *J. Am. Chem. Soc.* **1992**, *114*, 8328–8329.
18. Trager, W.; Jensen, J. B. *Science* **1976**, *193*, 673–675.
19. Desjardins, R. E.; Canfield, C. J.; Haynes, J. D.; Chulay, J. D. *Antimicrob. Agents Chemother.* **1979**, *16*, 710–718.
20. Ellis, G. L.; Amewu, R.; Sabbani, S.; Stocks, P. A.; Shone, A.; Stanford, D.; Gibbons, P.; Davies, J.; Vivas, L.; Charnaud, S.; Bongard, E.; Hall, C.; Rimmer, K.; Lozanom, S.; Jesus, M.; Gargallo, D.; Ward, S. A.; O'Neill, P. M. *J. Med. Chem.* **2008**, *51*, 2170–2177.