# Synthesis, *in vitro* and *in vivo* antimalarial assessment of sulfide, sulfone and vinyl amide-substituted 1,2,4-trioxanes prepared *via* thiol-olefin co-oxygenation (TOCO) of allylic alcohols†

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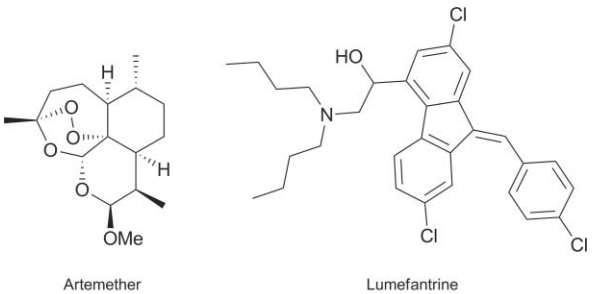
Thiol-Olefin Co-Oxygenation (TOCO) methodology has been applied to the synthesis of a small library of weak base and polar 1,2,4-trioxanes. The 1,2,4-trioxane units synthesised exhibit remarkable stability as they survive base catalysed hydrolysis and mixed anhydride/amine coupling reactions. This unique stability feature has enabled a range of novel substitution patterns to be incorporated within the spiro 1,2,4-trioxane unit. Selected analogues express potent *in vitro* nM antimalarial activity, low cytotoxicity and oral activity in the *Plasmodium berghei* mouse model of malaria.

## Introduction

Malaria is one of the world's most prevalent infectious diseases, remaining a major health problem and, therefore, a developmental barrier for many countries. It is estimated that about 40% of the world's population is at risk of contracting malaria, leading to around 350–500 million infections coupled with a staggering million deaths *per annum*. The widely affordable antimalarial drugs such as chloroquine[1] (**1**) and sulfadoxine (**2**),[2] either as monotherapies or in combination, are now largely ineffective due to drug resistance.

The potential impact of artemisinin (**3**), a Chinese herbal extract, on malaria chemotherapy is hampered by supply and cost; the majority of infected people needing treatment for malaria cannot afford drugs containing an artemisinin derivative.[3] Nevertheless, artemisinin and its derivatives remain the most effective antimalarials and are currently used in combination with other drugs recommended by the World Health Organization.[4] As an example, Coartem®, a combination of Lumefantrine–Artemether (Novartis), has been registered and is currently used for the treatment of uncomplicated malaria.[5]




Artemether Lumefantrine

Coartem components = (1:6 artemether to lumefantrine)

Although the actual mechanism of action of artemisinin and related compounds remains a topic of intense debate,[6] SAR studies have revealed that the 1,2,4-trioxane heterocycle is the key pharmacophore.[7] In line with this, various groups have designed and synthesized a variety of compounds containing this functional group, some of which show promising activity towards the malaria parasite. Several methodologies have been developed for the synthesis of the 1,2,4-trioxane pharmocophore, and many of these involve the preparation of an α-peroxy alcohol followed by *in situ* reaction with a carbonyl component in the presence of an acid catalyst.[7–10] Other approaches include synthesis from nitriles,[11] reaction of dioxetanes with carbonyls in the presence of Lewis acids,[12] acid-catalyzed cyclization of hydroxyperoxyacetals with olefins[13] and reactions of α-peroxy aldehydes with carbonyl compounds.[14]

An alternative method for preparing 1,2,4-trioxanes involves the free-radical sequential thiol-olefin co-oxygenation (TOCO) reaction. TOCO chemistry is particularly attractive because it allows functional group manipulation at various stages. In terms of versatility, there are no available methods in the literature for the preparation of sulfide- or sulfone-functionalised spiro 1,2,4-trioxanes in just two synthetic steps from readily available building blocks. Furthermore, this chemistry enables rapid access to 1,2,4-trioxane aldehydes in only three synthetic steps; such targets are not accessible by alternative published synthetic methodologies.

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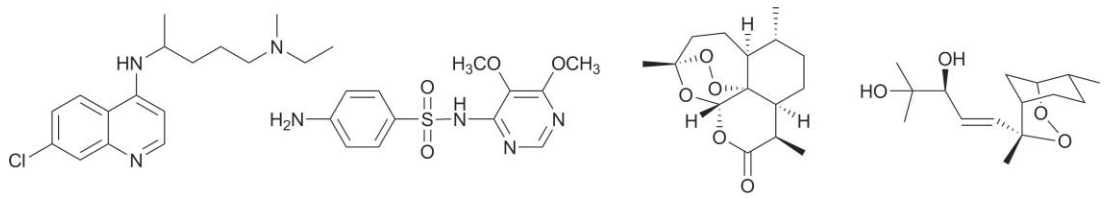
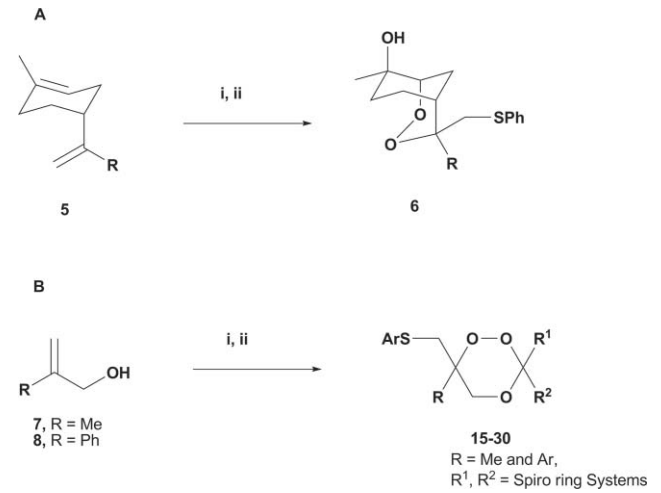
† Electronic supplementary information (ESI) available: Spectroscopic and analytical data for compounds **16–30** (sulfide trioxanes), **32–42** (trioxane sulfones), **50–54** (trioxane carbaldehydes), **64–65** (trioxane acrylamides), **67–73** (trioxane acrylic acid methyl esters), **75–76** (trioxane acrylic acids) and **79–85** (trioxane amides). CCDC reference numbers 680267, 680268. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/b924319d




TOCO chemistry has been used by various groups[15–19] for the synthesis of a wide range of cyclic peroxides, including *Yingzhaosu A* (**4**), a potent series of bicyclic yingzhaosu A analogues and a series of endoperoxide chalcone pro-drugs.[20] The reactions are usually initiated by AIBN and ultra violet (UV) irradiation or by di-*tert*-butylperoxalate (DBPO). We and others have previously used the TOCO methodology to prepare endoperoxides from terpene derivatives (Scheme 1A).[18] Replacement of the terpene with an allylic alcohol (**7** and **8**) led to the synthesis of 1,2,4-trioxane derivatives (Scheme 1B).[19] By using the corresponding homo-allylic alcohol analogue of **7**, 1,2,4-trioxepanes can also be readily prepared.[21]

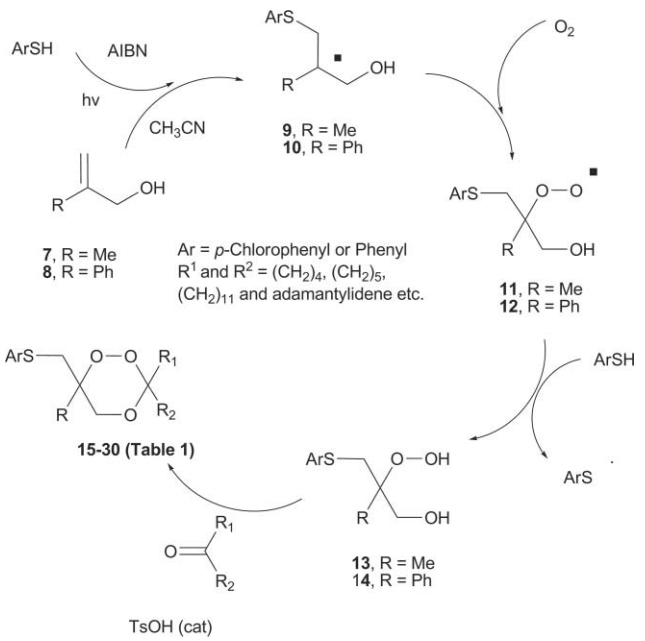



**Scheme 1** A) (i) AIBN, ArSH, $O_2$, h$\nu$. (ii) $PPh_3$, $CH_2Cl_2$, 0–5 °C, 2 h then r.t. 1 h. B) (i) AIBN, ArSH, $CH_3CN$, $O_2$, 0 °C, 4–6 h (ii) $R^1R^2C{=}O$, TsOH, $CH_2Cl_2$, r.t.

We report herein the application of the thiol-olefin co-oxygenation to the synthesis of a library of novel 1,2,4-trioxane analogues. Subsequent conversion of the newly incorporated sulfide functional group into more polar functional groups has been performed in a systematic fashion to reduce the Clog*P* and improve the aqueous solubility profiles of hit molecules (*vide infra*).

## Results and discussion

With AIBN as a radical initiator and UV irradiation, we performed a one-pot reaction of the allylic alcohol (**7** or **8**) and 4-chlorothiophenol in acetonitrile. In this process, a thiyl radical is generated which subsequently adds onto the double bond of the allylic alcohol in a Markovnikov fashion to form a tertiary carbon-centred radical **9**. The carbon-centered radical then reacts with molecular oxygen to form a peroxy radical **11**. Abstraction of hydrogen by **11** from the 4-chlorothiophenol species produces a racemic mixture of α-hydroperoxyl intermediate **13** and liberates a second thiyl radical to propagate the reaction (Scheme 2). It was anticipated that stabilizing the carbon-centred radical could improve the reaction; hence the commercially available 2-methyl-2-propen-1-ol (**7**) was replaced with 2-phenyl-2-propen-1-ol (**8**). The reaction of the allylic alcohol **8** occurs *via* the same mechanism to produce the peroxy radical **12** and a racemic mixture of the hydroperoxy intermediate **14**. Intermediate **14** undergoes smooth condensation with a wide range of ketones in the presence of tosic acid catalyst to afford the 1,2,4-trioxanes **15–30** (Table 1) in good yields.

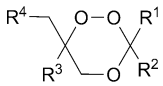



**Scheme 2**

Replacement of the allylic alcohol **7** with **8** had marginal effect on the yield but purification was much easier. Considering the multi-step nature of the reaction, and the sequence of events leading to the formation of several new bonds, the TOCO reactions proceed in very good yield.

Room temperature $^1$H NMR analysis of the sulfides **15–30** revealed the $OCH_2$ and $SCH_2$ protons as broad singlets. This results from interconversion of different conformations due to ring flipping of the six-membered trioxane ring system. The peaks were resolved into two doublets (d) for **15** and **18** by performing variable temperature analysis. While **18** was resolved at 0 °C; 3.49 (d, 1H, $J = 13.1$ Hz, $SCH_2$), 3.61 (d, 1H, $J = 13.1$ Hz, $SCH_2$), 3.68 (d, 1H, $J = 12.0$ Hz, $OCH_2$), 3.82 (d, 1H, $J = 12.0$ Hz, $OCH_2$),

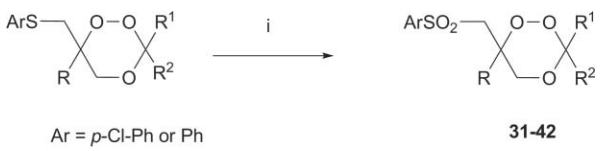
**Table 1** Trioxanes synthesized *via* the TOCO reaction with cyclic ketones



| Compound | $R^1$ and $R^2$ | $R^3$ and $R^4$ | Yield (%) |
|---|---|---|---|
| **15** | $(CH_2)_5$ | **$R^3$** = Me; **$R^4$** = *p*-Cl-Ph-S- | 64 |
| **16** | $(CH_2)_4$ | **$R^3$** = Me; **$R^4$** = *p*-Cl-Ph-S- | 78 |
| **17** | $(CH_2)_{11}$ | **$R^3$** = Me; **$R^4$** = *p*-Cl-Ph-S | 50 |
| **18** | Adamantylidene | **$R^3$** = Me; **$R^4$** = *p*-Cl- Ph-S- | 70 |
| **19** | $(CH_2)_5$ | **$R^3$** = Ph; **$R^4$** = *p*-Cl-Ph-S- | 80 |
| **20** | $(CH_2)_{11}$ | **$R^3$** = Ph; **$R^4$** = *p*-Cl-Ph-S- | 46 |
| **21** | $(CH_2)_5$ | **$R^3$** = Ph; **$R^4$** = Ph-S- | 68 |
| **22** | $(CH_2)_4$ | **$R^3$** = Ph; **$R^4$** = Ph-S- | 54 |
| **23** | $(CH_2)_5$ | **$R^3$** = Me; **$R^4$** = Ph-S- | 53 |
| **24** | $(CH_2)_3$ | **$R^3$** = Me; **$R^4$** = Ph-S- | 61 |
| **25** | Adamantylidene | **$R^3$** = Me; **$R^4$** = Ph-S- | 42 |
| **26** | 4-*t*-Bu-cyclohexylidene | **$R^3$** = Me; **$R^4$** = *p*-Cl-Ph-S- | 80 |
| **27** | $(CH_2)_{11}$ | **$R^3$** = Me; **$R^4$** = Ph-S- | 68 |
| **28** | $(CH_2)_{11}$ | **$R^3$** = Me; **$R^4$** = 2-naphthalenethiol | 64 |
| **29** | *cis*-Bicyclo[3.3.0]octane-3,7dione | **$R^3$** = Me; **$R^4$** = *p*-Cl-Ph-S- | 65 |
| **30** | 1,4-Cyclohexanedione | **$R^3$** = Me; **$R^4$** = Ph-S- | 25 |

**15** required further cooling to –20 °C; 3.48 (d, 1H, $J$ = 13.1 Hz, $SCH_2$), 3.62 (d, 1H, $J$ = 13.1 Hz, $SCH_2$), 3.69 (d, 1H, $J$ = 12.1 Hz, $OCH_2$), 3.88 (d, 1H, $J$ = 12.1 Hz, $OCH_2$). This suggests that the cut off temperature at which the flipping begins is governed by the nature of the groups attached to the ring.

Posner and co-workers observed that within a series of tricyclic 1,2,4-trioxanes, the sulfone group imparted high antimalarial potency even in instances where the corresponding sulfide displayed no activity.[22,23] Thus, we prepared the sulfones **31–42** from the sulfides **15–30** using 2.2 equivalents of *m*CPBA [24] (Scheme 3, Table 2) in high yields. For compound **35**, a single X-ray crystal structure (as dimethyl formamide solvate) was solved and is depicted in Fig. 1.




**Scheme 3** (i) *m*CPBA (2.2 equiv.), $CH_2Cl_2$, 4–6 h, r.t.

A major limitation with many synthetic endoperoxide antimalarials is their poor oral bioavailability which stems in part from limited aqueous solubility. Our next focus was to make

**Table 2** Trioxanes sulfones synthesized

| Compound | *R* | $R^1$ and $R^2$ | Ar | Yield (%) |
|---|---|---|---|---|
| **31** | Me | $(CH_2)_4$ | *p*-Cl-Ph | 76 |
| **32** | Me | $(CH_2)_5$ | *p*-Cl-Ph | 80 |
| **33** | Me | $(CH_2)_{11}$ | *p*-Cl-Ph | 80 |
| **34** | Me | Adamantylidene | *p*-Cl-Ph | 80 |
| **35** | Ph | $(CH_2)_5$ | *p*-Cl-Ph | 77 |
| **36** | Ph | $(CH_2)_{11}$ | *p*-Cl-Ph | 79 |
| **37** | Me | $(CH_2)_5$ | Ph | 75 |
| **38** | Me | $(CH_2)_3$ | Ph | 91 |
| **39** | Me | Adamantylidene | Ph | 56 |
| **40** | Me | 4-*t*-Butylcyclohexyl | *p*-Cl-Ph | 92 |
| **41** | Me | 1,4-Cyclohexanedione | Ph | 82 |
| **42** | Me | $(CH_2)_{11}$ | Ph | 93 |

the 1,2,4-trioxanes more polar by incorporation of amide side chains. First, the sulfides **15–20** were oxidised with a stoichiometric amount of *m*CPBA to generate the corresponding sulfoxides **43–48** (Scheme 4). Secondly, **43–48** were exposed to Pummerer conditions according to the method reported by Arroyo-Gomez and co-workers[25,26] to give the aldehydes **49–54** in high yield.

Manipulation of the resulting aldehydes led to mixed results; oxidation of aldehyde **52**[27,28] to the corresponding carboxylic acid **55** failed, as did reductive amination[29,30] with morpholine ((Scheme 5) to give amine **57**). For substrates **49–54**, it appears

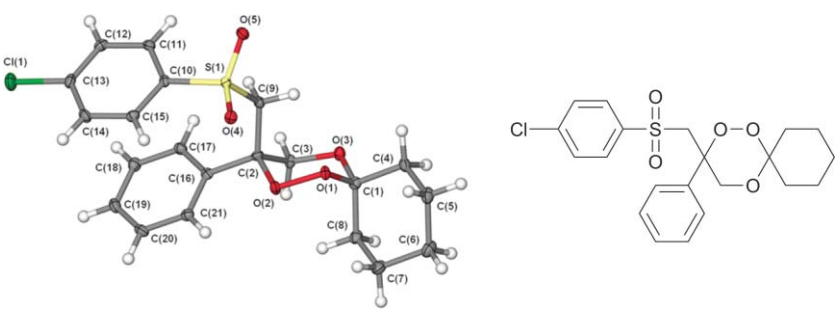
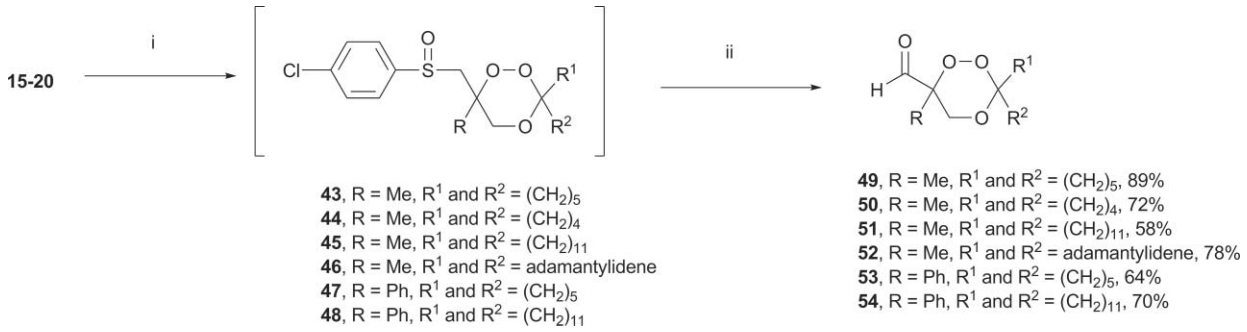
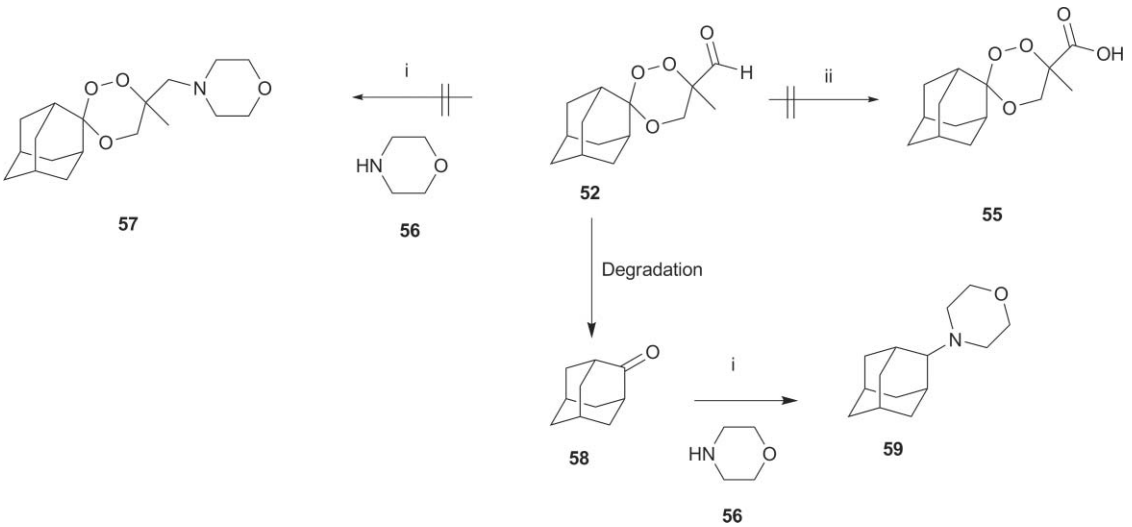



**Fig. 1** Single-crystal X-ray structure of the trioxane sulfone **35** (CCDC 680267). Non-H atoms are represented by thermal ellipsoids at the 50% probability level and H-atoms are shown as circles with arbitrary radii.




**Scheme 4** (i) *m*CPBA (1 equiv.), $CH_2Cl_2$, 4–6 h, r.t., (ii) TFAA, $CH_2Cl_2$, 2,6-lutidine, 3 h, r.t.




**Scheme 5** (i) $NaBH(OAc)_3$, $CH_2Cl_2$, 18 h, r.t. (ii) $^t$BuOH, $H_2O$, 2-methylbutene, $NaH_2PO_4$, $NaClO_2$, 2 h, r.t.

that the close proximity of the aldehyde to the endoperoxide bridge results in decomposition in the presence of tertiary butoxide ($^t$-BuO$^-$) or cyclic amines.

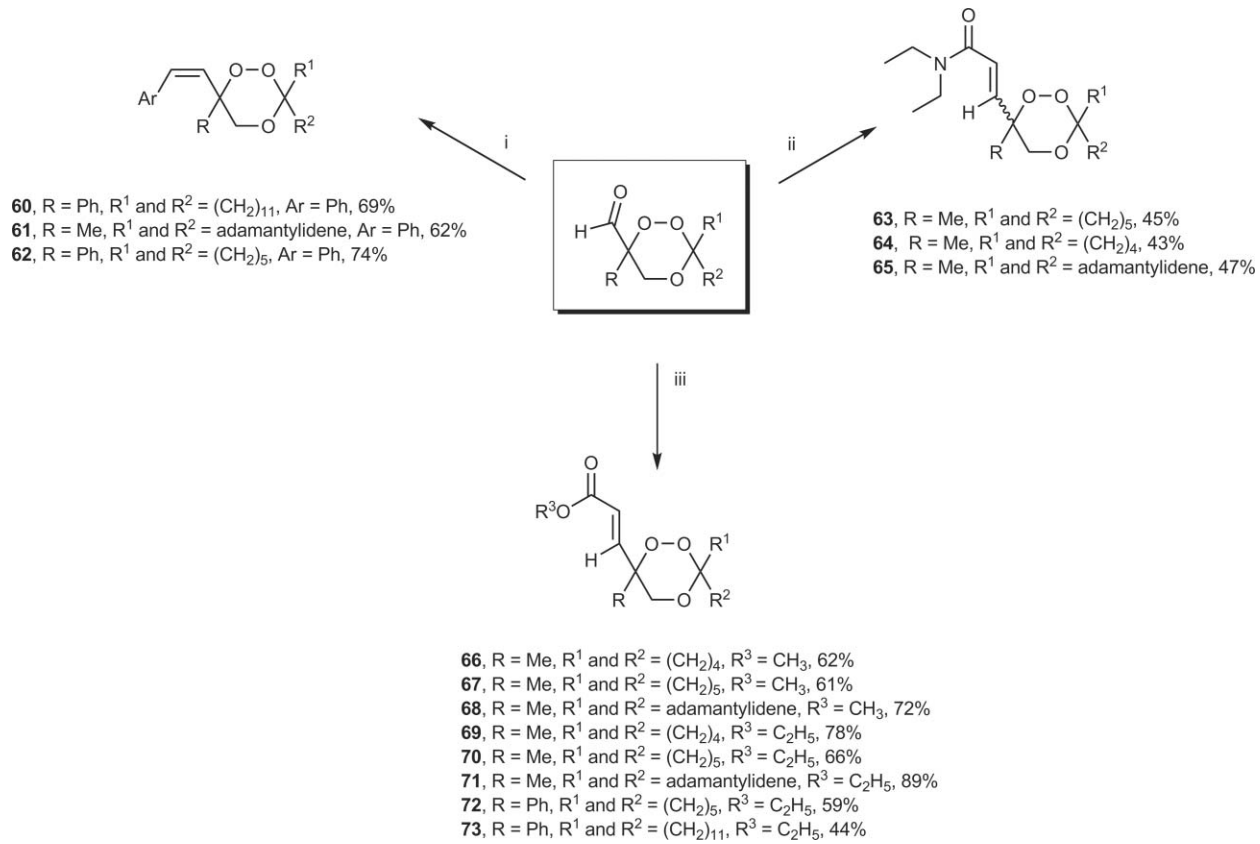
While the oxidation led to a complex mixture of products, none of which were the desired carboxylic acid **55**, the reductive amination reaction led to the formation of **59** (*via* reductive amination of **58**), presumably generated from the breakdown of **52**. A series of vinyl-substituted trioxanes **60–73** was prepared from the aldehydes **49–54** *via* Wittig reactions (Scheme 6)[31] with various ylides in very good yield, with the exception of $Ph_3P{=}CHCON(C_2H_5)_2$. ($Ph_3P{=}CHCON(C_2H_5)_2$ was prepared by refluxing *N*,*N*-diethylchloroacetamide with triphenylphosphine in nitromethane.[32]

Reaction of a range of aldehydes with the methyl and ethyl ester Wittig reagent gave **66–73** predominantly as *trans*-geometric isomers ($J > 15$ Hz). However, in contrast, the reaction with $Ph_3P{=}CHCON(C_2H_5)_2$ resulted in **63–65** as a mixture of geometric isomers with the *trans*-isomer being the major product. The ratio of the *trans* : *cis*, determined from the fraction of major and minor isolated products, is 9 : 1, 4 : 1, 4 : 1 for **63**, **64** and **65**, respectively. The *cis*- and *trans*-geometric isomers were assigned based on the coupling constants of the vinyl protons ($J \sim 13$ and 16 Hz, respectively). Broadening of proton signals observed with the $OCH_2$ and $CH_2S$ of **15–30** was encountered with the vinyl protons of the amides **63–65**. The broadening was resolved by running the $^1$H NMR at –20 °C. For example, the vinyl protons for **65** gave their chemical shifts as 6.66 (d, 1H, $J = 15.4$ Hz, CH) and 6.89 (d, 1H, $J = 15.4$ Hz, CH) for the major fraction, and 5.52 (d, 1H, $J = 13.1$ Hz, CH) and 6.07 (d, 1H, $J = 13.1$ Hz, CH) for the minor fraction.

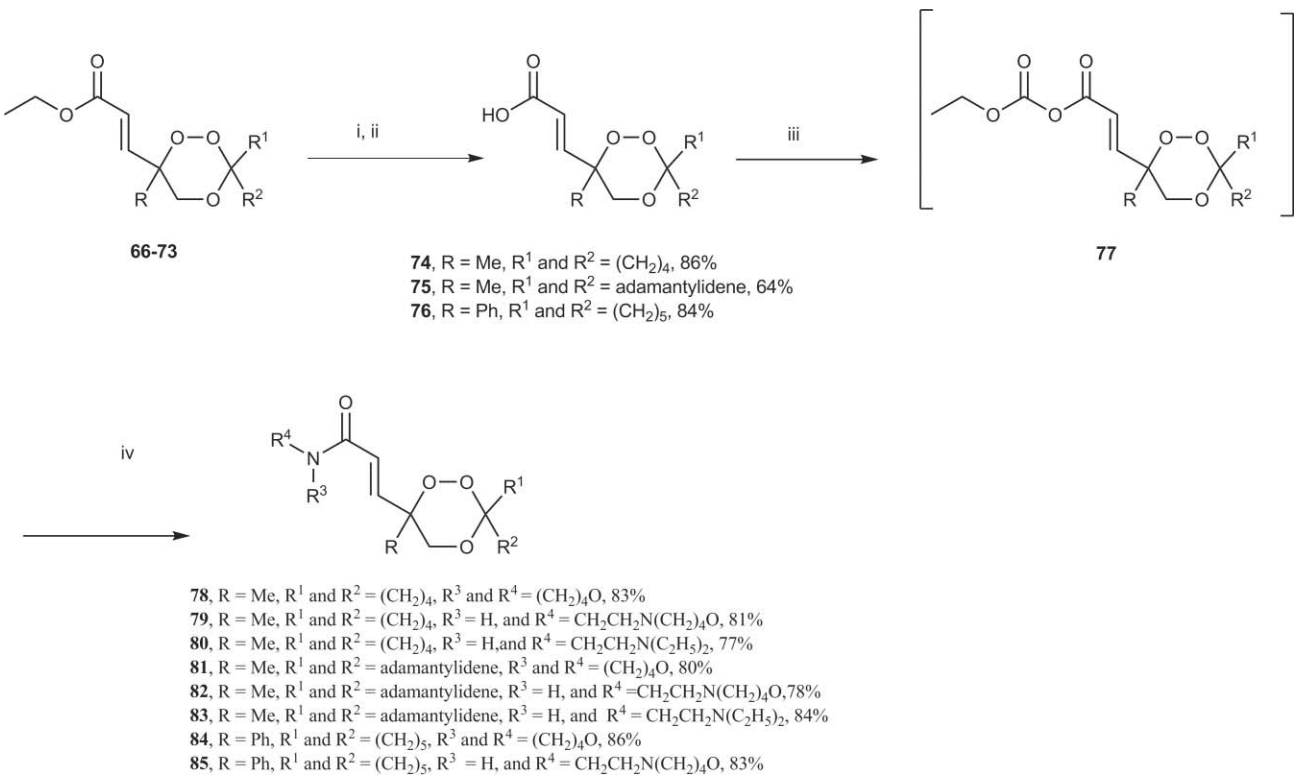
The molecules **60**, **61** and **62** have the capacity to release chalcone derivatives, while analogues **63–73** can release fumarate-like structures following reductive cleavage of the endoperoxide bridge with Fe(II). Part of our future investigations will focus on the mechanistic studies of the iron-mediated degradation chemistry of this class of compounds.

Further manipulation of the ester functionality of **63–73** was also attempted. The most important observation was that the vinyl esters readily undergo base-catalysed hydrolysis to the corresponding carboxylic acids (Scheme 7).[33,34] The reactions were surprisingly successful despite the use of potassium hydroxide (KOH) at high temperatures indicating the remarkable stability of the 1,2,4-trioxane within this series of trioxanes.

Opsenica and co-workers observed that inclusion of an amide moiety as an auxiliary functional group increases their antimalarial activity.[35] This observation was made during their work exploring the influence of steroid carriers on the antimalarial and antiproliferative activity of cholic acid-derived tetraoxanes. Our recent work on 1,2,4,5-tetraoxanes also demonstrated that the presence of an amide bond and basic amine side-chains significantly improves antimalarial activity and disposition by enhancing aqueous solubility.[36] Based on this principle, a series




**Scheme 6** (i) ArCH = $^{+}P(Ph)_3Br^{-}$, NHMDS, THF, 75 min, r.t. (ii) $Ph_3P^{+}CH_2CON(C_2H_5)_2Br^{-}$, $CHCl_3$, $H_2O$, NaOH, r.t., 1 h. (iii) $Ph_3P{=}CHCOR^3$, $CH_2Cl_2$, 3 h, r.t.




**Scheme 7** (i) KOH, MeOH, 70 °C, 1 h. (ii) $CH_2Cl_2$, $H_2O$, HCl, r.t. (iii) $(C_2H_5)_3N$, $ClCO_2C_2H_5$, $CH_2Cl_2$, 0 °C, 1 h. (iv) $NHR^1R^2$, 0 °C, 30 min then r.t., 90 min.

of amides were prepared by reacting carboxylic acids **74–76** with ethyl chloroformate and $Et_3N$ in dichloromethane to give mixed anhydrides **77**, which were coupled with a selection of amines to give amides **78–85** (Scheme 7).[35,37] Both the acid hydrolysis and the amide couplings proceeded in high yields, typically 75–86%.

Room temperature $^1$H NMR analysis of the amides **78–85** revealed the vinyl protons mostly as broad singlets, a similar phenomenon observed with the sulfide $OCH_2$ and $SCH_2$, due to interconversion of different conformations of the six-membered endoperoxide ring system. As before, cooling to −20 °C revealed the vinyl protons as the expected doublets.

A crystal was grown for compound **83** by slow evaporation of a $CHCl_3$–hexane mixture and the single-crystal X-ray structure is depicted in Fig. 2 below. The X-ray crystal structure confirms our $^1$H NMR assignment of *trans* geometry for the double bond in trioxane **83**. (As clearly shown in the X-ray crystal structure, recrystallisation of **83** from chloroform–hexane has produced the hydrochloride hydrate, and a plausible explanation for this observation is the presence of HCl in the chloroform solvent.)

## Antimalarial properties

*In vitro* analysis on 36 selected trioxanes was performed on the 3D7 chloroquine-sensitive parasite isolate of *Plasmodium falciparum* (Table 3).[38] Artemisinin and chloroquine were used as standard positive controls.

From the large volume of data recorded against the 3D7 strain of *Plasmodium falciparum*, several SAR trends emerge. For spiro 1,2,4-trioxane sulfides **15–18**, the best activity is conferred by fusion to a cyclopentyl or adamantyl ring system. Replacement of the methyl function with a phenyl ring system reduces activity for most analogues prepared (see, for example, **19**). An unexpected result was the high potency conferred by fusion of the 1,2,4-trioxane heterocycle to a *cis*-bicyclo[3.3.0]octane: this molecule (**29**) had excellent activity of 24 nM. In spite of this observation, further studies were not performed due to the expense of the *cis*-bicyclo[3.3.0]octane-3,7-dione starting material.

In general, the sulfones expressed reduced activity when compared with the corresponding sulfides (see **16** *versus* **31**, and **15** *versus* **34**). For the vinyl-substituted dispiro 1,2,4-trioxanes, excellent activity was noted for diethyl amides **63**, **64** and **65**. Additional analogues with low nanomolar activity include the morpholine analogues **81** and **82**, and alkyl amine-linked derivative **83**. As noted elsewhere,[10] for the polar vinyl 1,2,4-trioxane series, the adamantylidene fused derivatives express the best level of antimalarial activity.

From the calculated Clog*P* and aqueous solubilities it is clear that analogues **63–65** and **79–85** have significantly reduced lipid

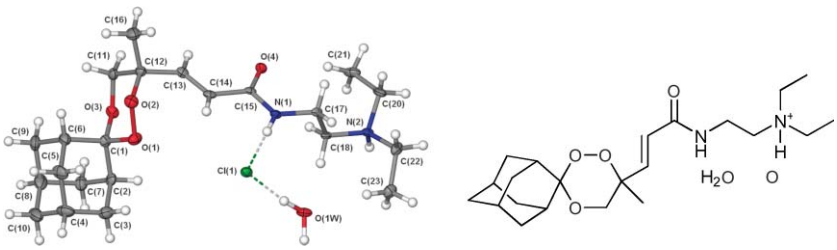



**Fig. 2** The asymmetric unit of the single-crystal X-ray structures of the trioxane amide **83** ($HCl{\cdot}H_2O$) (CCDC 680268). Non-H atoms are represented by thermal ellipsoids at the 50% probability level and H-atoms are shown as circles with arbitrary radii.

**Table 3** *In vitro* antimalarial activity *versus* the chloroquine sensitive 3D7 strain of *Plasmodium falciparum* and calculated log*P* and aqueous solubilities (Log*S*). Lead compounds are in *italics*

| Comp. | $IC_{50}$/nM[a] | Log*P* | Solubility/mg mL$^{-1}$ | Comp. | $IC_{50}$/nM[a] | Log*P* | Solubility/mg mL$^{-1}$ |
|---|---|---|---|---|---|---|---|
| **15** | 110.50 | 4.90 | 5.72 | **63** | 48.32 | 2.76 | 553.86 |
| ***16*** | *37.91* | *4.46* | *8.47* | ***64*** | *38.82* | *2.29* | *1356.48* |
| **17** | 388.99 | 7.71 | 0.40 | ***65*** | *19.41* | *3.57* | *18.34* |
| **18** | 59.07 | 5.68 | 0.50 | **66** | 79.21 | 1.98 | 1057.68 |
| **19** | 361.50 | 6.01 | 0.86 | **67** | 129.22 | 2.42 | 499.80 |
| **23** | 113.91 | 4.25 | 12.85 | **68** | 68.29 | 3.20 | 31.56 |
| **24** | 314.00 | 3.41 | 33.54 | **69** | 124.88 | 2.39 | 829.68 |
| **26** | 88.18 | 6.41 | 0.58 | **70** | 112.35 | 2.84 | 390.79 |
| ***29*** | *24.63* | *3.77* | *7.36* | **71** | 79.21 | 3.61 | 20.82 |
| **30** | 154.00 | 2.73 | 47.77 | **78** | 250.80 | 1.40 | 3920.36 |
| **31** | 92.90 | 3.07 | 37.17 | **79** | 111.75 | 0.94 | 4188.70 |
| **32** | 136.90 | 3.51 | 25.55 | ***80*** | *37.03* | *2.03* | *1636.33* |
| **34** | 131.80 | 4.30 | 2.73 | ***81*** | *27.95* | *2.60* | *85.21* |
| **37** | 108.88 | 2.89 | 87.87 | ***82*** | *37.80* | *2.19* | *131.57* |
| **39** | 72.00 | 3.70 | 8.28 | ***83*** | *36.70* | *3.31* | *36.64* |
| **40** | 140.06 | 4.38 | 13.27 | **84** | 121.08 | 3.03 | 100.53 |
| **41** | >1000 | 1.37 | 348.26 | **85** | 71.70 | 2.56 | 194.84 |
| **60** | 103.19 | 8.21 | 0.41 | **1** | 19.20 | 4.57 | 44.16 |
| **61** | 101.90 | 5.09 | 1.13 | **3** | 10.20 | 2.72 | 39.89 |

[a] See the Experimental section for the description of the assays. IC50 values were averaged values determined from three independent experiments. The log*P* and solubilities were calculated using Virtual Computational Chemistry Laboratory (VCCLAB), http://www.vcclab.org, 2005.

**Table 4** *In vitro* cytotoxicity of selected analogues[a]

| Compound | IC50/μM |
|---|---|
| **16** | >100 |
| **29** | >100 |
| **31** | >100 |
| **82** | >100 |
| **83** | >100 |

[a] Freshly isolated PBMC, 72 h ($n = 3$), cytotoxicity assessed by MTT assay.

**Table 5** Peter's suppressive test results *versus Plasmodium yoelli* in mice[a]

| Compound | % of inhibition at 30 mg $kg^{-1}$ |
|---|---|
| **16** | 5 |
| **31** | 12.0 |
| **34** | 25.1 |
| **65** | 65 |
| **81** | 50 |
| **82** | 80 |
| **Artesunate** | 100 |
| **Artemether** | 100 |

[a] Parasitaemia was determined by microscopic examination of Giemsa-stained blood films taken on day 4. Microscopic counts of blood films from each mouse were processed using spreadsheet (Microsoft Corp.) and expressed as percentage of inhibition from the arithmetic mean parasitaemia of each group in relation to the untreated group.

solubility profiles compared with the initial hit molecule **16**. The concept of reducing Clog*P* in antimalarial endoperoxide drug design has been discussed,[39] and here the benefits are evident in terms of producing improved lead compounds with encouraging *in vitro* and *in vivo* (*vide infra*) antimalarial activity profiles.

Table 4 lists the *in vitro* cytotoxicity of analogues **16**, **31**, **82** and **83** *versus* human peripheral mono-nuclear type B cells ($n = 3$), where the IC50 values were all greater than 100 μM. The MTT assays were carried out after 72 h of drug treatment and it is clear that representative molecules from the sulfide (**16**), sulfone (**31**) and vinyl amides (**82** and **83**) have very high therapeutic indices.

A 4-day Peters'[40] test was performed on **16**, **31**, **34**, **81** and **82**, and the results are summarized in Table 5 below. The compounds were administered orally by dissolving in standard suspending formula (SSV) (0.5% sodium carboxymethylcellulose, 0.5% benzyl alcohol, 0.4% Tween 80, 0.9% NaCl), followed by treatment with 0.2 mL of a solution of the test compound two hours (day 0) and on days 1, 2, and 3 post infections.

The sulfides **15–18** were shown to have poor oral antimalarial activity following administration to mice (**15**, **17** and **18** <5% inhibition (data not shown)). Since oxidative metabolism may have been an issue with this series, we next examined the sulfone derivatives **31** and **34**. Although activity was improved, maximum inhibition was never above 20–25%. The vinyl amide adamantanone analogues **65**, **81** and **82** performed somewhat better, with analogue **82** expressing activity approaching the semi-synthetic derivatives artesunate and artemether by oral administration.

## Conclusions

This study exemplifies the value of TOCO chemistry for the synthesis of a diversely functionalised set of 1,2,4-trioxane derivatives. The initial hit sulfide series, although active *in vitro*, proved to have poor oral antimalarial activity as did their sulfone counterparts. Further medicinal chemistry optimisation of the side chain was successful in terms of reducing the Clog*P*, with analogues **63–65** having significantly reduced lipophilicity compared with initial hit molecule **16**. Additional manipulation of the side-chain to enhance polarity (**79–85**) provided analogues with acceptable Clog*P* values, low nanomolar activity, low cytotoxicity and, importantly for trioxane **82**, good oral activity in the *Plasmodium yoelli* model of malaria. Based upon the synthetic chemistry reported here, further optimisation of this template should now be possible in order to enhance the oral activity of these molecules further.

## Experimental

All reagents were obtained from commercial suppliers including Aldrich Chemical Co. and Lancaster. Dichloromethane (DCM), triethylamine ($Et_3N$) and tetrahydrofuran (THF) were freshly distilled before use. The analytical thin layer chromatography was performed on pre-coated silica gel (0.25 mm layer of silica gel F254) aluminium sheets. UV light (254 nm) was used for all visualizations and flash column chromatography was performed using Merck 938S Kieselgel 60 Silica gel. All of the IR spectra were run using the Perkin-Elmer 298 infrared spectrophotometer. Solid samples were dissolved in $CHCl_3$ and liquids/oils neat on sodium chloride discs.

$^1$H NMR spectra were recorded using the Bruker (400, 250, and 200 MHz) NMR spectrophotometers. Spectra were referenced to the residual solvent peak and chemical shifts expressed in ppm from TMS as the internal reference peak. With exception of those stated, all of the NMR was performed at room temperature. The following notations were used to describe the multiplicity; b, broad, s, singlet, bs, broad-singlet, d, doublet, dd, double-doublet, t, triplet, q, quartet, m, multiplet, and coupling constants were recorded in hertz.

Mass spectra were recorded between 20–70 eV using a VG7070E and/or Micromass LCT mass spectrometers. The molecular ion $M^+$ with intensities in parenthesis is given followed by peaks corresponding to major fragment losses. Melting points are expressed in degree Celsius (°C) and performed using the Gallemkamp melting point apparatus and capillary tubes. The antiparasitic properties (IC50, 4 day Peters' experiment) were performed at the University of Liverpool and London Schools of Tropical Medicine. X-ray crystal structure measurements were performed at the Department of Chemistry the University of Liverpool.

### *In vitro* sensitivity assays

Drug susceptibilities were assessed by the measurement of fluorescence after the addition of SYBR Green I as previously described by Smilkstein *et al.*[41] Drug IC50s were calculated from the log of the dose/response relationship, as fitted with Grafit software (Erithacus Software, Kent, United Kingdom). Results are given as the mean of at least three separate experiments. For the fluorescence assay, after 48 h of growth, 100 μL of SYBR Green I in lysis buffer (0.2 μL of SYBR Green I/mL of lysis buffer) was added to each well, and the contents were mixed until no visible erythrocyte sediment remained. After 1 h of incubation in the dark at room temperature, fluorescence was measured with

a Varioskan fluorescence multiwell plate reader from Thermo Electron Corporation with excitation and emission wavelengths of 485 and 530 nm, respectively.

### Representative procedure for the preparation of trioxane sulfides 15–30

**Preparation of 3-(4-chloro-phenylsulfanylmethyl)-3-methyl-1,2,4-trioxa-spiro[5.5]undecane (15).** A 2-necked 500 mL round bottom flask was charged with a solution of 2-methyl-2-propenol (500 mg, 0.58 mL, 0.69 mmol) and AIBN (77.5mg, 4.72mmol) in $CH_3CN$ (115 mL). The reaction vessel was flushed with oxygen for several minutes at 0 °C then stoppered and kept under a positive pressure of pure oxygen, with the aid of two oxygen balloons. The reaction mixture was vigorously stirred and UV irradiated at 0 °C using an externally mounted 100 W BLACK-RAY UV lamp at a distance of 5–7 cm, with the simultaneous addition of 4-chlorothiophenol (1250 mg, 8.64 mol, 3.13 equiv.) solution in $CH_3CN$ (65 mL) over a period of 30 min. After completion of the addition, the reaction was stirred at 0 °C for 4–6 h or until consumption of starting materials (monitored by tlc). The reaction vessel was then cooled to −10 °C, flushed with nitrogen and a solution of cyclohexanone (1703 mg, 17.35 mmol, 1.63 mL) in DCM (65 mL) was added, followed by a catalytic amount of tosic acid. The mixture was stirred at −10 °C, and then allowed to warm slowly to room temperature overnight. Concentration *in vacuo* and purification by flash column chromatography using ethyl acetate–hexane (1 : 5, v/v, $R_f$ 0.44) gave **15** (1.46 g, 64%) as a white powder. Mp 57–59. °C $\nu_{max}$ ($CHCl_3$)/cm$^{-1}$ 810.2, 920.1, 1007.2, 1090.5, 1446.6, 1473.1, 2855.3, 2931.1, 3006.8. $^1$H NMR (400 MHz, $CDCl_3$, 0 °C) $\delta_H$ 1.14 (s, 3H, $CH_3$), 1.33-1.57 (m, 8H, cyclohexyl), 1.90 (bs, 1H, cyclohexyl), 2.14 (bs, 1H, cyclohexyl), 3.48 (d, 1H, $J = 13.1$ Hz, $SCH_2$), 3.62 (d, 1H, $J = 13.1$ Hz, $SCH_2$), 3.69 (d, 1H, $J = 12.1$ Hz, $OCH_2$), 3.88 (d, 1H, $J = 12.1$ Hz, $OCH_2$), 7.24 (d, 2H, $J = 8.6$ Hz, Ar), 7.35 (d, 2H, $J = 8.6$ Hz, Ar); $^{13}$C NMR (100 MHz, $CDCl_3$). $\delta_C$ 20.6 0, 22.6 0, 22.7, 25.9, 33.8, 64.2, 79.4, 102.7, 129.4, 129.7, 131.0, 131.7. MS (ES+) $[M + Na]^+$ (100) 367.1, $[2M + Na]^+$ 679.2. HRMS calculated for 351.0798 $C_{16}H_{21}O_3NaS^{35}Cl$, found 351.0803. Elemental analysis C: 58.62, H: 6.48 (required values C: 58.44, H:6.44).

### Representative procedure for preparation of sulfones 31–42

**Preparation of 8-(4-chloro-benzenesulfonylmethyl)-8-methyl-6,7,10-trioxa-spiro[4.5]-decane (31).** A solution of sulfide **16** (0.1 g, 0.32 mmol, 1 equiv.) and *m*CPBA (0.16 g, 0.95 mmol, 2.2 equiv.) in DCM (2 mL) was stirred for 4–6 h at room temperature. After consumption of the more polar intermediate (monitored by tlc), the mixture was poured into a saturated solution of 5% $K_2CO_3$. The mixture was extracted with DCM and the organic layer separated, dried over $MgSO_4$ and evaporated. Purification of the residue by flash column chromatography using ethyl acetate–hexane (1 : 4, v/v, $R_f$ 0.58) gave **31** in (0.08 g, 76%) as white powder. Mp 114–116 °C. $\nu_{max}$ ($CHCl_3$)/cm$^{-1}$820.0, 963.6, 1008.7, 1086.6, 1144.0, 1316.2, 1390.0, 1472.0, 1578.6, 2871.3, 2969.7, 3010.7.$^1$H NMR (400 MHz, $CDCl_3$) $\delta_H$ 1.46 (s, 3H, $CH_3$), 1.52-1.84 (m, 8H, cyclopentyl), 3.67 (d, 2H, $J = 11.9$ Hz, $OCH_2$), 3.90 (d, 1H, $J = 14.1$ Hz, $SO_2CH_2$), 4.00 (d, 1H, $J = 12.1$ Hz, $SO_2CH_2$), 7.55 (d, 2H, $J = 8.7$ Hz, Ar), 7.90 (d, 2H, $J = 8.7$ Hz, Ar). $^{13}$C NMR (100 MHz, $CDCl_3$), $\delta_C$ 20.2, 23.5, 24.9, 30.1, 58.9, 67.6, 114.9, 129.8, 139.9, 140.8. MS (ES+) $[M + Na]^+$ (100) 369, $[2M + Na]^+$ 715/717; HRMS calculated for 369.0539 $C_{15}H_{19}O_5NaSCl$, found 369.0532. Elemental analysis C: 51.96, H: 5.48 (required values C: 51.95, H: 5.52).

### General procedure for the preparation of sulfoxides 43–48

A solution of the sulfide (1 equiv.) and *m*CPBA (1 equiv.) in DCM was stirred for 4–6 h at room temperature. The mixture was poured into a saturated solution of 5% $K_2CO_3$ and extracted with DCM. The organic layer was separated, dried over $MgSO_4$ and evaporated. The mixtures of the two enantiomers were used without purification for the Pummerer reaction.

### General procedure for the preparation of aldehydes 49–54

**Preparation of 8-methyl-6,7,10-trioxa-spiro[4.5]decane-8-carbaldehyde (49).** To a solution of the sulfoxide **44** (1 g, 3.02 mmol) at 0 °C in $CH_3CN$ (5 mL), 2,6-lutidine (1.5 g, 12.8 mmol) and trifluoroacetic anhydride (TFAA) (1.63 g, 11.65 mmol), in $CH_3CN$ (12mL) were added. The mixture was stirred at room temperature for 3 h and extracted with ethyl acetate (50 mL). The organic layer was dried in $MgSO_4$ and the solvent removed under reduced pressure to give the aldehyde. Purification was achieved by flash column chromatography using ethyl acetate–hexane (1 : 9, v/v, $R_f$ 0.70) as eluent to give **49** (0.53 g, 89%) as a colourless oil. $^1$H NMR (400 MHz, $CDCl_3$) $\delta_H$ 1.07 (s, 3H, $CH_3$), 1.60-1.82 (m, 6H, cyclohexyl), 1.86-1.96 (m, 1H, cyclopentyl), 2.41-2.51 (m, 1H, cyclopentyl), 3.70 (d, 1H, $J = 11.6$ Hz, $CH_2O$), 4.18 (d, 1H, $J = 11.6$ Hz, $CH_2O$), 9.90 (s, 1H, CHO) $^{13}$C NMR (400 MHz, $CDCl_3$), $\delta_C$ 16.7, 23.5, 37.3, 64.3, 84.7, 114.7, 203.2 MS (ES+) $[M + Na]^+$ (100) 209.1, HRMS calculated for 209.0814 $C_9H_{14}O_4Na$, found 209.0817.

### Representative procedure for the preparation of 60–62

**Preparation of 3-methyl-3-styryl-1,2,5-trioxa-spiro[5.11]heptadecane 60.** To a stirred suspension of benzyltriphenylphosphonium bromide (0.51 g, 1.18 mmol) in THF (2 mL) was added NHMDS (1.18 mL, 1.18 mmol, 1 M solution in THF) *via* syringe. The reaction mixture was allowed to stir at room temperature for 15 min, and then a solution of 3-methyl-1,2,5-trioxa-spiro[5.11]heptadecane-3-carbaldehyde (0.21 g, 0.738 mmol) in THF (2 mL) was added. After an additional 1 h, the reaction was quenched with saturated aq. $NaHCO_3$, extracted with ether, washed with brine, dried ($Na_2SO_4$) and concentrated *in vacuo*. The product was purified by flash chromatography to give the desired compound as a white solid in 69% yield. The product was purified by column chromatography using ethyl acetate–hexane (25 : 75, v/v, $R_f$ 0.78) to give the product as a white solid. Mp 86–87 °C. $^1$H NMR (400 MHz, $CDCl_3$) $\delta_H$ 7.32 (m, 5H, aromatic), 7.11 (d, $J = 12.67$ Hz, 1H, *trans* olefin–C=C*H*), 6.72 (d, $J = 12.67$ Hz, *trans* olefin), 3.90 (bs, 1H, -OC$H_2$), 3.61 (bs, 1H, -OC$H_2$), 1.28 (m, 22H, cyclodeodecane moiety), 1.23 (s, 3H, -C$H_3$). $^{13}$C NMR (100 MHz,$CDCl_3$) 134.17, 129.17, 128.90, 128.29, 126.92, 126.84, 106.55, 79.41, 60.71, 40.70, 31.94, 26.36, 22.99 and 21.34. MS (ES+) *m/z* 381.3 $[M + Na]^+$ (98), 413.3 $[M + CH_3OH + Na]^+$ (62). HRMS *m/z* calcd for $C_{23}H_{34}O_3Na$ $[M + Na]^+$ 381.2406 found,

381.2419. Elemental analysis C: 77.03, H: 9.58 (required values C: 77.05, H: 9.56).

### Representative procedure for the preparation of acrylamides 63–65

**Preparation of (*E*/*Z*)-*N*,*N*-diethyl-3-(3-methyl-1,2,5-trioxa-spiro[5.5]undec-3-yl)-acrylamide (63).** To a mixture of $Ph_3P^+$=CHCON$(C_2H_5)_2Br^-$ (0.37 g, 0.99 mmol) in water (3 mL) and aldehyde **52** (0.2 g, 0.97 mmol) in DCM (10 mL), was added drop-wise a solution of NaOH (0.18 g, 0.46 mmol) in water (1 mL). The mixture was stirred for a few minutes and the organic layer was separated, dried over $MgSO_4$, filtered and evaporated. The resulting residue was passed through a silica gel column (EtOAc–Hex 1 : 1) to remove the triphenylphosphine oxide. Evaporation of the solvent gave **63** as colourless oil.

*Major fraction (*trans-*isomer).* This fraction was collected in 40% as a colourless oil. $\nu_{max}$(neat)/cm$^{-1}$ 923.4, 1088.7, 1139.8, 1277.5, 1360.1, 1430.9, 1446.7, 1619.8, 1659.1, 2860.5, 2931.3, 2962.7. $^1$H NMR (400 MHz, $CDCl_3$) $\delta_H$ 1.10-1.24 (m, 9H, $CH_3$), 1.40-1.70 (m, 8H, cyclohexyl), 1.80-2.30 (m, 2H, cyclohexyl), 3.32-3.52 (m, 4H, $NCH_2$), 3.82 (bs, 2H, $CH_2O$), 6.68 (bs, 1H, CH), 6.85 (bs, 1H, CH). $^{13}$C NMR (100 MHz, $CDCl_3$), $\delta_C$ 13.5, 15.3, 22.7, 24.3, 41.3, 42.7, 65.4, 79.2, 102.7, 122.3, 141.9, 167.3. MS (ES+), [M + Na]$^+$ (100) 366.2; HRMS calculated for 320.1838 $C_{16}H_{27}NO_4Na$, found 320.1824.

*Minor fraction (*cis-*isomer).* This fraction was collected in 5% as a colourless oil. This compound was purified by flash column chromatography using ethyl acetate–hexane (1 : 1, v/v, $R_f$ 0.44) as eluent. $\nu_{max}$(neat)/cm$^{-1}$ 923.4, 1088.7, 1143.7, 1265.7, 1446.7, 1462.4, 1619.8, 2868.3, 2939.1, 2970.6. $^1$H NMR (400 MHz, $CDCl_3$) $\delta_H$ 1.12-1.23 (m, 9H, $CH_3$), 1.30-1.65 (m, 10H, cyclohexyl), 3.31-3.51 (m, 4H, $NCH_2$), 3.82 (bs, 1H, $CH_2O$), 4.15 (d, 1H, $J$ = 11.0 Hz, $CH_2O$), 6.08 (d, 1H, $J$ = 12.9 Hz, CH), 6.25 (bs, 1H, CH). $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta_C$ 13.0, 14.3, 22.8, 25.9, 39.6, 43.1, 65.2, 81.0, 102.4, 123.7, 141.9, 167.3. MS (ES+) [M + K]$^+$ (100) 366.2, HRMS calculated for 336.1553 $C_{16}H_{27}NO_4K$, found 336.1574.

### Representative procedure for the synthesis of compounds 66–73

**Preparation of (*E*)-3-(8-methyl-6,7,10-trioxa-spiro[4.5]dec-8-yl)-acrylic acid methyl ester (66).** To a solution of the aldehyde **49** (0.38 g, 2.04 mmol) in DCM (15 mL) was added $Ph_3P$=$CHCO_2Me$ (0.73 g, 2.19 mmol) at room temperature. The reaction was stirred at this temperature for 3 h and was subsequently concentrated and chromatographed using hexane–ethyl acetate (1 : 4, v/v, $R_f$ 0.60) as eluent on a silica gel to give **66** (0.31 g, 62%) as a colourless oil. $\nu_{max}$(neat)/cm$^{-1}$ 861.0, 971.8, 1090.7, 1197.3, 1283.4, 1312.1, 1431.0, 1656.5, 1722.2, 2871.3, 2953.3. $^1$H NMR (400 MHz, $CDCl_3$) $\delta_H$ 1.15 (bs, 3H, $CH_3$), 1.45-1.90 (m, 8H, cyclopentyl), 3.78 (s, 3H, $CH_3O$), 3.85 (s, 2H, $CH_2O$), 6.15 (bs, 1H, CH), 7.18 (bs, 1H, CH). $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta_C$ 21.8, 23.8, 37.4, 52.1, 67.8, 78.9, 114.7, 122.4, 149.1, 167.0. MS (ES+), [M + Na]$^+$ (100) 265, HRMS calculated for 265.1052 $C_{12}H_{18}O_5Na$, found 265.1058.

### Representative procedure for the synthesis of carboxylic acids 74–76

**Preparation of (*E*)-3-(8-methyl-6,7,10-trioxa-spiro[4.5]dec-8-yl)-acrylic acid (74).** Ethyl ester **66** (0.6037 g, 2.4 mmol) was hydrolysed in MeOH (24 mL) at 70 °C with potassium hydroxide (0.74 g, 13.1 mmol) and water (3 mL). After an hour heating, the reaction mixture was cooled and diluted with DCM (30 mL) and water (12 mL). The aqueous layer was acidified with concentrated hydrochloric acid (3 mL) and further extracted with DCM. The combined organic layers were washed with water, brine, dried over $Na_2SO_4$ and evaporated to dryness. Purification by flash column chromatography using ethyl acetate: dichoromethane (1 : 1, v/v, $R_f$ 0.2) as eluent gave **74** (0.46 g, 86%) as white powder. $^1$H NMR (400 MHz, $CDCl_3$) $\delta_H$ 1.22 (bs, 3H, $CH_3$), 1.61-1.92 (m, 8H, cyclopentyl), 3.70-3.92 (m, 2H, $CH_2O$), 6.20 (bs, 1H, CH), 7.30 (bs, 1H, CH), 10.70 (bs, 1H, OH). $^{13}$C NMR (100 MHz, $CDCl_3$), $\delta_C$ 21.6, 23.6, 38.7, 67.7, 78.9, 114.9, 122.0, 151.4, 171.7. MS (ES–) [M – H] $^-$(100) 227.1.

### Representative procedure for the synthesis of the amides 78–85

**Preparation of (*E*)-3-(8-methyl-6,7,10-trioxa-spiro[4.5]dec-8-yl)-1-morpholin-4-yl-propenone (78).** A solution of acid **77** (0.12 g, 0.53 mmol) in dry DCM (27 mL), with added triethylamine (0.06 g, 0.008 mL, 0.53 mmol) and ethylchloroformate (0.008 g, 0.07 mL, 0.7 mmol) was stirred for 60 min at 0 °C. Morpholine (0.09 g, 0.09 mL, 1.06 mmol) was added and the solution allowed to stir for 30 min at 0 °C before being warmed to room temperature. After 90 min, it was diluted with water and extracted with DCM. The organic extract was washed with brine, and dried over anhydrous $Na_2SO_4$. The crude product was purified by flash chromatography using ethyl acetate–hexane (1 : 1, v/v, $R_f$ 0.17) as eluent to give **78** (0.13 g, 83%) as a white powder. Mp 68–70 °C. $\nu_{max}$ ($CHCl_3$)/cm$^{-1}$ 1433.2, 1614.3, 1655.1, 2858.9, 2922.3, 2976.5. $^1$H NMR (400 MHz, $CDCl_3$) $\delta_H$ 1.22 (bs, 3H, $CH_3$), 1.59-1.92 (m, 8H, cyclopentyl), 3.59 (bs, 2H, $CH_2O$), 3.65-3.74 (m, 6H, $NCH_2/CH_2O$), 3.83 (d, 1H, $J$ = 15.5 Hz, CH), 6.50 (d, 1H, $J$ = 15.5 Hz, CH), 6.80 (bs, 1H, CH). $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta_C$ 21.0, 24.5, 42.4, 46.4, 66.8, 67.5, 78.7, 114.3, 121.3, 144.7, 165.4. MS (ES+) [M + Na]$^+$ (100) 320.1, and [2M + Na]$^+$ 617.2 HRMS calculated for 320.1474 $C_{15}H_{23}NO_5Na$, found 320.1480. Elemental analysis C: 60.83, H: 7.82 (required values C: 60.59, H: 7.80).

### Crystal data for 35 dimethylformamide solvate (CCDC 680267)

$C_{24}H_{30}ClNO_6S$, $M$ = 496.00, colourless plate, 0.50 × 0.20 × 0.04 mm$^3$, triclinic, space group $P\bar{1}$ (No. 2), $a$ = 5.6889(13), $b$ = 13.461(3), $c$ = 16.663(4) Å, $\alpha$ = 67.393(4), $\beta$ = 80.926(5), $\gamma$ = 83.493(4)°, $V$ = 1161.3(5) Å$^3$, $Z$ = 2, $D_c$ = 1.418 g cm$^{-3}$, $F_{000}$ = 524, Bruker D8 diffractometer with APEX detector, Mo-K$\alpha$ radiation, $\lambda$ = 0.71073 Å, $T$ = 100(2) K, $2\theta_{max}$ = 53.5°, 6737 reflections collected, 4786 unique ($R_{int}$ = 0.0224). Final $GooF$ = 1.068, $R_1$ = 0.0457, w$R_2$ = 0.0963, $R$ indices based on 3862 reflections with $I > 2\sigma(I)$ (refinement on $F^2$), 300 parameters, 0 restraints. Lp and absorption corrections applied, $\mu$ = 0.296 mm$^{-1}$.

### Crystal data for 83 hydrochloride hydrate ($CHCl_3$/hexane) (CCDC 680268)

$C_{22}H_{39}ClN_2O_5$, $M$ = 447.00, colourless plate, 0.50 × 0.40 × 0.10 mm$^3$, monoclinic, space group $P2_1/c$ (No. 14), $a$ = 18.705(3), $b$ = 7.6876(13), $c$ = 16.257(3) Å, $\beta$ = 93.288(3)°, $V$ = 2333.9(7) Å$^3$, $Z$ = 4, $D_c$ = 1.272 g cm$^{-3}$, $F_{000}$ = 968, Bruker D8 diffractometer

with APEX detector, Mo-K$\alpha$ radiation, $\lambda = 0.71073$ Å, $T = 100(2)$ K, $2\theta_{max} = 50.7°$, 14079 reflections collected, 4241 unique ($R_{int} = 0.0506$). Final $GooF = 1.143$, $R_1 = 0.0730$, w$R_2 = 0.1611$, $R$ indices based on 3573 reflections with $I > 2\sigma(I)$ (refinement on $F^2$), 293 parameters, 3 restraints. Lp and absorption corrections applied, $\mu = 0.198$ mm$^{-1}$.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