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Synthesis and biological evaluation of extraordinarily potent C-10 carba artemisinin dimers against *P. falciparum* malaria parasites and HL-60 cancer cells

James Chadwick [a], Amy E. Mercer [b], B. Kevin Park [b], Richard Cosstick [a], Paul M. O'Neill [a,b,*]

[a] Department of Chemistry, University of Liverpool, Liverpool, L69 7ZD, UK
[b] Department of Pharmacology and Therapeutics, University of Liverpool, Liverpool, L69 3GE, UK

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ABSTRACT

A series of artemisinin dimers incorporating a metabolically stable C-10 carba-linkage have been prepared, several of which show remarkable in vitro antimalarial activity (as low as 30 pM) versus *Plasmodium falciparum* and in vitro anticancer activity in the micromolar to nanomolar range versus HL-60 cell lines.

1. Introduction

Artemisinin **1**, also known as *qinghaosu*, is a tetracyclic 1,2,4-trioxane occurring in *Artemisia annua*.[1] Artemisinin and its derivatives are currently recommended as front-line antimalarials for regions experiencing *Plasmodium falciparum* resistance to traditional antimalarial drugs. In addition to their well-known antimalarial activity, artemisinin derivatives possess potent activity against cancer cells.[2–6]

A potential drawback with many of these derivatives is the presence of a metabolically susceptible C-10 acetal linkage; which is unstable in vivo due to the potential for metabolism to dihydroartemisinin[7] and subsequent rapid clearance from the body by glucuronidation.[8] It has been noted that the replacement of oxygen at the C-10 position with carbon produces compounds not only with greater hydrolytic stability but also with a longer half-life and potentially lower toxicity.[9,10]

The observation that artemisinin dimers possess significant antimalarial and anticancer activity[2] prompted previous efforts, both within our group[11] and elsewhere,[12–17] to synthesize C-10 non-acetal artemisinin dimers. We prepared a series of dimers, which were all found to display low nanomolar antimalarial activity against K1 and HB3 strains of *P. falciparum*.[11] In contrast to their potent antimalarial activities, most of the dimers possessed poor anticancer activity apart from two phosphate ester dimers **2** and **3** (Fig. 1), which expressed nanomolar growth inhibitory (GI_{50}) values against a range of cancer cells in the NCI 60-cell line assay. Detailed investigations in human promyelocytic leukemia HL-60 and Jurkat cell lines demonstrated that these compounds possessed similar activity to doxorubicin, which is used clinically to treat acute leukemias.

These promising results encouraged us to prepare a further series of C-10 dimers to explore what effects varying the nature and length of linker would have upon activity.

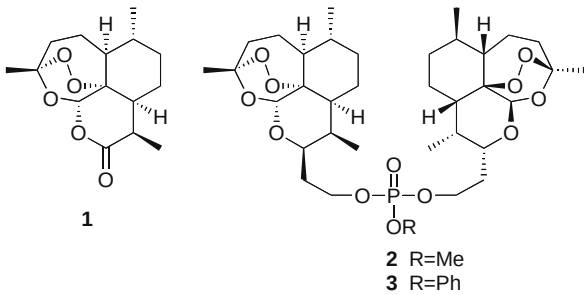

Figure 1. Artemisinin (**1**) and phosphate ester dimers (**2** and **3**).

* Corresponding author. Tel.: +44 151 794 3553, fax: +44 151 794 3588.
E-mail address: P.M.Oneill01@liv.ac.uk (P.M. O'Neill).

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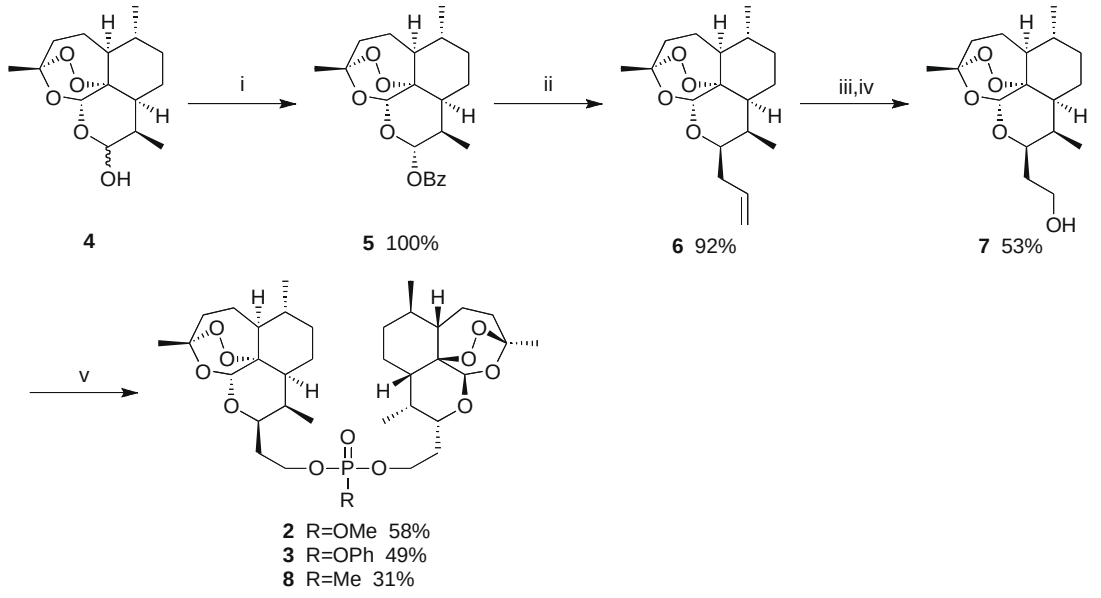


Scheme 1. Reagents and conditions: (i) BzCl, py, CH_2Cl_2, 0 °C, 16 h; (ii) allyl trimethylsilane, $ZnCl_2$, 4 Å molecular sieves, DCE, 0 °C, 3 h; (iii) O_3, CH_2Cl_2, −78 °C, 1 h; (iv) $NaBH_4$, THF/MeOH (9:1), 0 °C, 4 h; (v) P(O)(R)Cl_2, NaHMDS, THF, −78 °C→t, 16 h.

2. Chemistry

The synthesis of dimers **2** and **3** from dihydroartemisinin is outlined in Scheme 1.[11] Acylation of dihydroartemisinin **4** with benzoyl chloride employing pyridine as a nucleophilic catalyst gave dihydroartemisinin 10α-benzoate **5**. Reaction of **5** with allyl trimethylsilane using zinc chloride as catalyst gave 10β-allyldeoxoartemisinin **6**, ozonolysis of the terminal double bond in **6** followed by sodium borohydride reduction of the ozonide giving alcohol **7**. Deprotonation of this alcohol with NaHMDS and reaction with the appropriate dichlorophosphate gave dimers **2** and **3** in reasonable yield.

The potential of methyl phosphate ester dimer **2** as an anticancer agent may be somewhat limited by the stability of the phosphate ester linkage. To address this potential problem we decided to replace this linkage with a methyl phosphonate group, which should result in a compound with increased hydrolytic stability.[18] Deprotonation of **7** with NaHMDS and subsequent reaction with methanephosphonic dichloride gave methyl phosphonate linked dimer **8**.

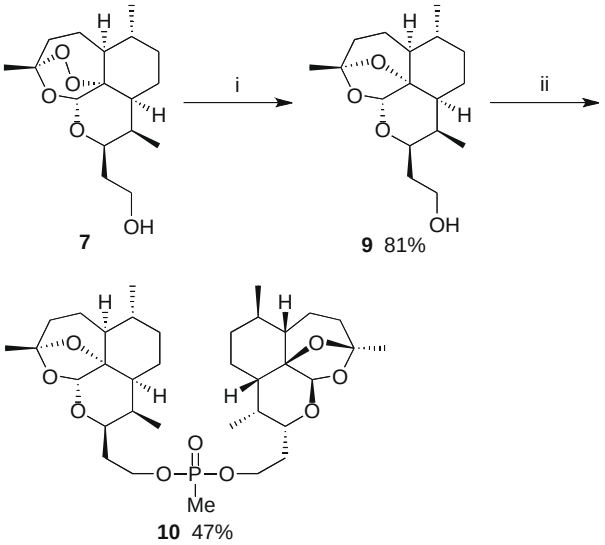
To determine whether removal of the endoperoxide bridge would have an effect on the activity, the corresponding deoxygenated methyl phosphonate linked dimer **10** was also synthesized (Scheme 2). Reduction of the endoperoxide bridge of C-10 carba alcohol **7** with zinc powder in acetic acid gave **9**, which was subsequently used to prepare **10**.

In order to examine whether the length of the spacer between artemisinin moieties has an effect on activity we decided to synthesize the analogs of **2** and **3** containing one less and one more carbon atom, respectively, in the linker either side of the phosphate ester group.

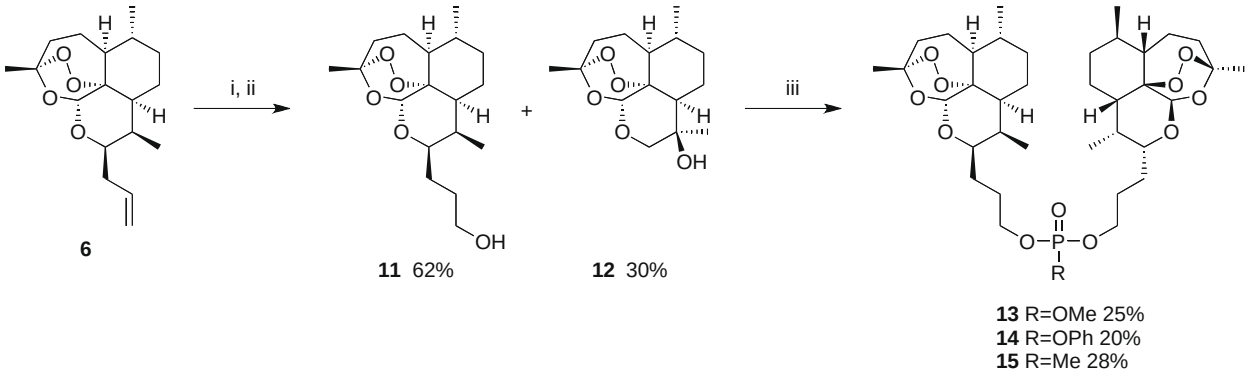
The synthesis of the chain lengthened analogs **13** and **14** are outlined in Scheme 3. Hydroboration of the terminal double bond in **6** with borane dimethyl sulfide complex followed by oxidation of the resulting organoborane with sodium perborate gave alcohol **11** as the major product along with a minor product **12**. Alcohol **11** was allowed to react with the appropriate dichlorophosphate to give **13** and **14**, or with methanephosphonic dichloride to give **15**.

Synthesis of an analog of C-10 carba alcohol **7** with one fewer carbon atom in the side chain proved more problematic. Synthesis of 10β-vinyldeoxoartemisinin **16** and ozonolysis-reduction of the double bond offered an attractive route. Initially, we endeavored to employ our optimized procedure for the synthesis of **6**[19] to the synthesis of 10β-vinyldeoxoartemisinin **16**, by replacing allyl trimethylsilane with vinyl trimethylsilane (Scheme 4). Unfortunately, vinyl trimethylsilane is not nucleophilic enough and as such allows the competing dehydration reaction to predominate, meaning that the only product isolated from the reaction was anhydroartemisinin **17**.

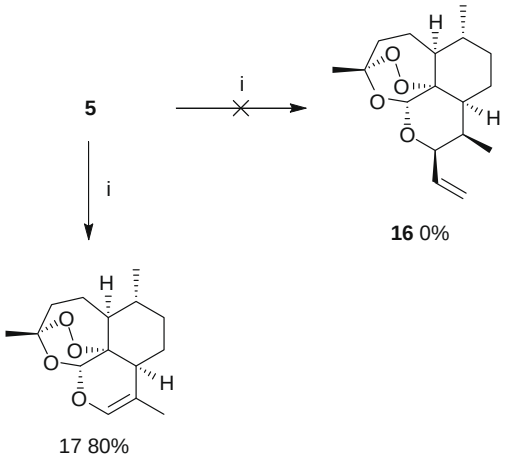
The C-10 position of artemisinin can be regarded as a sugar anomeric center. The synthesis of *C*-glycosyl allenes by the addition of propargyl trimethylsilane to carbohydrate derivatives is well known in carbohydrate chemistry; ozonolysis of the allene leading directly to aldehydes without the need for an additional reducing step.[20,21] Based on this idea, it was decided to employ this route



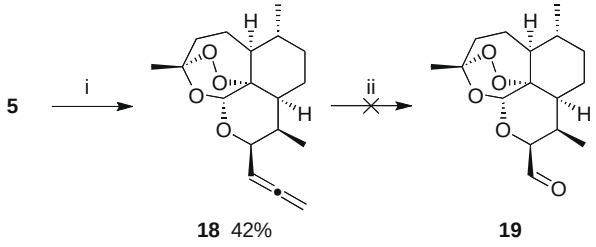
Scheme 2. Reagents and conditions: (i) Zn, AcOH, rt, 72 h; (ii) P(O)MeCl_2, NaHMDS, THF, −78 °C→rt, 24 h.



Scheme 3. Reagents and conditions: (i) $BH_3{\cdot}SMe_2$, THF, 0 °C→rt, 24 h; (ii) $NaBO_3{\cdot}4H_2O$, THF/H_2O, rt, 24 h; (iii) P(O)(R)Cl_2, NaHMDS, THF, −78 °C→rt, 16 h.



Scheme 4. Reagents and conditions: (i) vinyl trimethylsilane, $ZnCl_2$, 4 Å molecular sieves, DCE, 0 °C, 18 h.



Scheme 5. Reagents and conditions: (i) propargyl trimethylsilane, $ZnCl_2$, 4 Å molecular sieves, DCE, 0 °C, 24 h; (ii) O_3, CH_2Cl_2, −78 °C, 1 h.

to synthesise the required aldehyde **19** which could then be reduced to the corresponding alcohol.

Initially, the conditions employed in carbohydrate chemistry were used; dihydroartemisinin was converted into its acetate and allowed to react with propargyl trimethylsilane in the presence of TMSOTf. Unfortunately, these conditions led exclusively to the formation of anhydroartemisinin with none of the required product being observed.

The failure of the conditions employed in carbohydrate chemistry prompted us to combine this approach with our procedure for controlled oxonium ion formation (Scheme 5). Dihydroartemisinin 10α-benzoate **5** was allowed to react with propargyl trimethylsilane in the presence of $ZnCl_2$ to give the required allene **18** in moderate yield along with trace amounts of anhydroartemisinin. Ozonolysis of the allene appeared to give the required aldehyde;

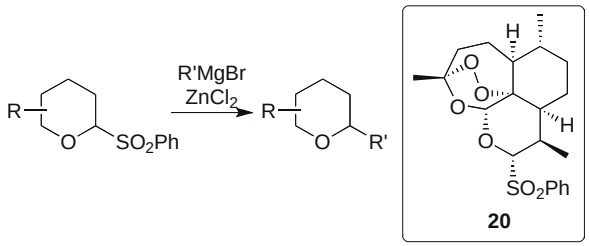
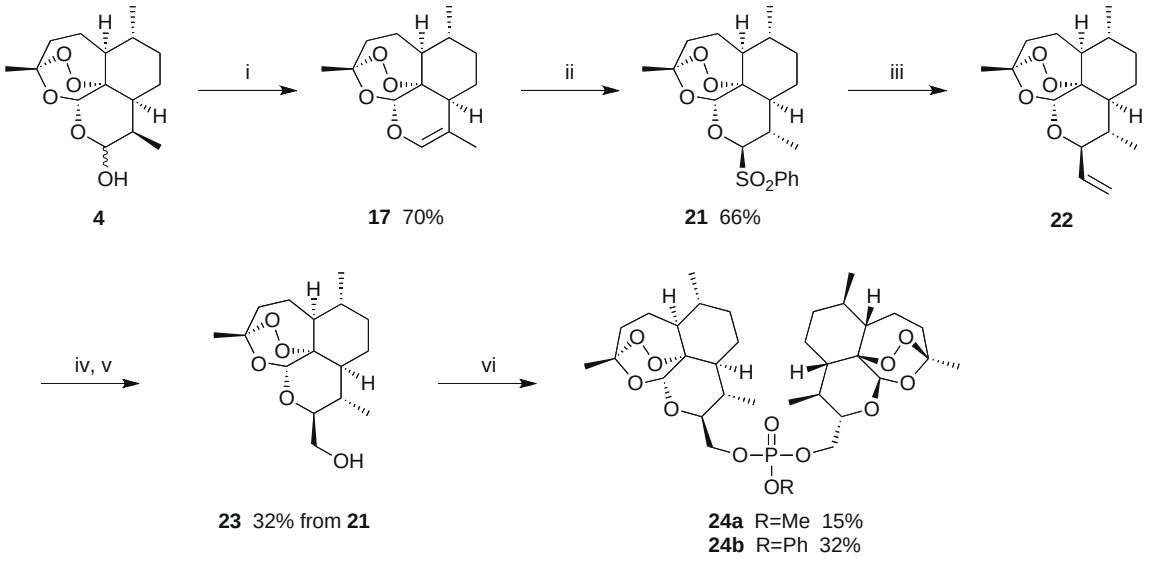


Figure 2. Substitution reaction of 2-benzenesulfonyl ethers with carbon nucleophiles and Posner's artemeisinin C-10 sulfone (**20**).

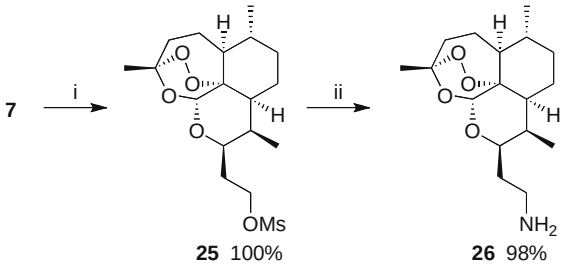
thin layer chromatography of the reaction mixture showed almost complete consumption of the allene and the ^1H and ^{13}C NMR spectra of the concentrated reaction mixture showed signals at 10 ppm and 202 ppm, respectively, indicating the presence of an aldehyde functionality. However, following reaction workup and silica gel flash column chromatography, none of the aldehyde **19** was isolated.

The comparatively high cost of propargyl trimethylsilane and the modest yield of **18** obtained, prompted us to look at an alternative approach. Brown et al. have employed the benzenesulfonyl moiety as a leaving group for direct nucleophilic replacement by organozinc reagents on 2-benzene sulfonyl cyclic ethers derived from tetrahydropyrans or tetrahydrofurans (Fig. 2).[22] Posner et al. prepared a series of sulfide, sulfone and sulfonamide trioxanes and assayed them for antimalarial activity.[23] One of these derivatives **20**, prepared by reacting anhydroartemisinin with benzenesulfinic acid, incorporated the benzenesulfonyl group at the C-10 position of artemisinin.

It was decided to prepare **20** and react it with the organozinc reagent derived from vinyl magnesium bromide and zinc chloride to hopefully give 10β-vinyl artemisinin which could then be converted into the required alcohol. Reacting DHA **4** with boron trifluoride diethyl etherate gave anhydroartemisinin **17**, which was subsequently allowed to react with benzenesulfinic acid (Scheme 6). Unfortunately, this reaction led to epimerization at C-9 and the product obtained was 10β-benzenesulfonyl-9-*epi*-dihydroartemisinin **21**. Nucleophilic replacement of the sulfone with the organozinc reagent, derived from vinylmagnesium bromide and zinc chloride, introduced the required vinyl substituent at the C-10 position. In addition to **22**, significant amounts of anhydroartemisinin **17** were also formed and since it proved impossible to separate these products by chromatography the mixture was subjected to ozonolysis and reduction. The required alcohol **23** was cleanly isolated in reasonable yield (32% over two steps) and allowed to react with the appropriate dichlorophosphate to give **24a** and **24b**.



Scheme 6. Reagents and conditions: (i) $BF_3{\cdot}OEt_2$, THF, 66 °C, 2.5 h; (ii) $PhSO_2H$, CH_2Cl_2, rt, 30 min; (iii) vinylmagnesium bromide, $ZnCl_2$, THF, rt, 48 h; (iv) O_3, CH_2Cl_2, −78 °C, 1 h; (v) $NaBH_4$, THF/MeOH (9:1), 0 °C, 4 h; (vi) $P(O)(OR)Cl_2$, NaHMDS, THF, −78 °C→rt, 16 h.



Scheme 7. Reagents and conditions: (i) MsCl, NEt_3, CH_2Cl_2, 0 °C, 2 h; (ii) NH_4OH, EtOH, rt, five days.

As well as looking at varying the length of the linker between the artemisinin moieties we also wished to look at varying the nature of the linker to see the effects that this might have on cytotoxicity. It is noteworthy that the most active dimers previously synthesized contain polar groups in the linker. For example, phosphate ester dimers **2** and **3**[11] and several of the dimers prepared by Posner[14,15] and Jung[16] either contain water solubilizing groups or polar functionality capable of acting as a H-bond donor or acceptor in the linker. Such functionality may enhance binding to the biological target molecule(s).

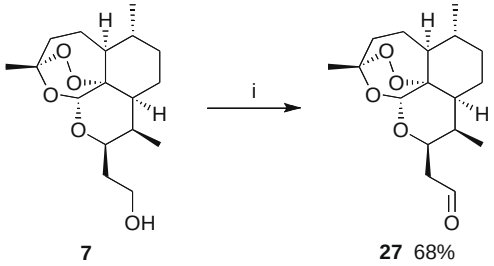
It was thought that an amide linked dimer would be readily accessible from our C-10 carba alcohol **7** via a few simple transformations to give a C-10 carba carboxylic acid and C-10 carba amine **26** which could then be joined by amide bond formation. Mesylation of **7** and reaction with ammonia solution gave the required C-10 carba amine **26** (Scheme 7). Upon storage the peroxide bridge in **26** appeared to degrade and therefore for prolonged storage this compound was formulated as its hydrochloride salt.

The required carboxylic acid **28** has been prepared previously by oxidation of the terminal double bond in **6** with $NaIO_4$ and $KMnO_4$, however the product obtained from this procedure appeared contaminated with a number of other products and also degraded quite rapidly. Therefore, it was decided to oxidize alcohol **7** first to the aldehyde **27** and then to the required carboxylic acid **28**. Previously aldehyde **27** has been prepared within this group by Swern oxidation of **7** but this procedure gave unsatisfactory results; the dimethyl sulfide generated during the reaction seemed to lead to product degradation and led to very complicated reaction mixtures which proved impractical to purify. Oxidation with Dess–Martin periodinane gave aldehyde **27** without the problems of degradation (Scheme 8).

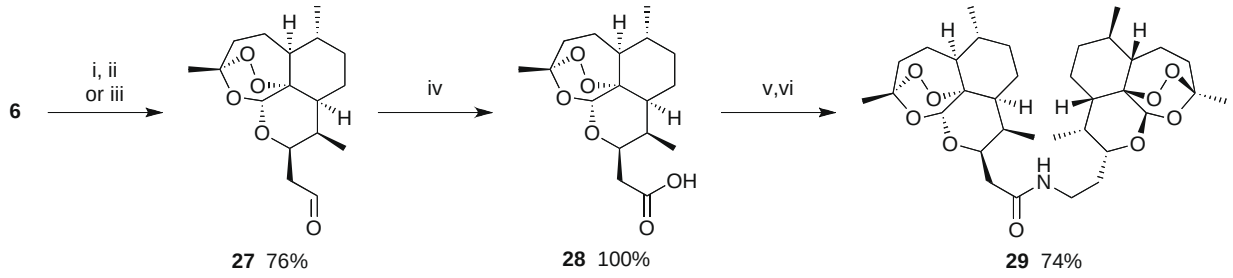
Aldehydes can be prepared directly from alkenes by a number of procedures; ozonolysis of the alkenes and in situ reduction of the ozonide with dimethyl sulfide or triphenyl phosphine is a popular route. However, previous attempts to prepare aldehyde **27** by ozonolysis-reduction proved unsuccessful. Simply carrying out the ozonolysis in methanol, rather than the customary dichloromethane, and employing triphenyl phosphine as the reducing agent led to **27** being isolated in 76% yield (Scheme 9). Aldehyde **27** was also prepared directly from **6** by the oxidative cleavage of the double bond with OsO_4 and $NaIO_4$ in the presence of 2,6-lutidine. Oxidation of **27** with sodium chlorite gave **28** in quantitative yield. Conversion of **28** to its acid chloride with oxalyl chloride and reaction with **26** gave **29** (Scheme 9).

A carbonate-linked dimer **30** was prepared by reaction of 10β-(2-hydroxyethyl)-deoxoartemisinin **7** with trichloromethyl chloroformate (Scheme 10). The corresponding reaction with 10β-(2-aminoethyl)deoxoartemisinin **26** gave the urea-linked dimer **31**, albeit in low yield (18%) (Scheme 10).

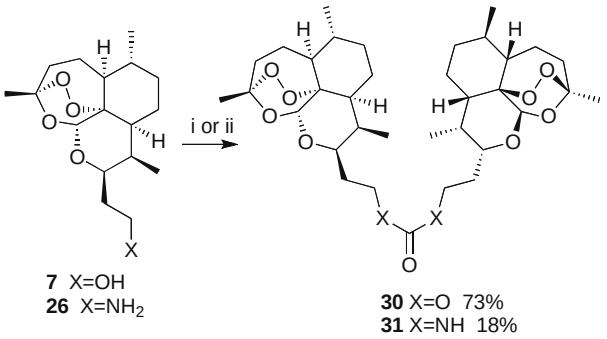
A 4,4′-bipiperidine-linked dimer **32** was prepared by reacting 4,4′-bipiperidine with 10β-(2-methanesulfonylethyl)deoxoartemisinin **25** in refluxing benzene for 48 h (Scheme 11). One valuable property of the 4,4′-bipiperidine-linked dimer is the presence of



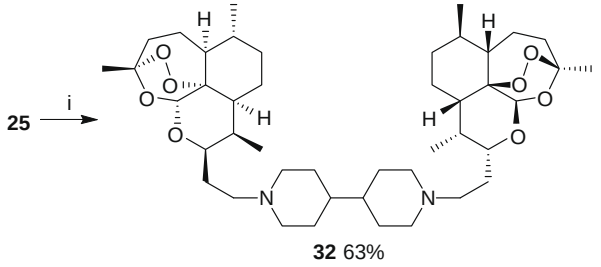
Scheme 8. Reagents and conditions: (i) Dess–Martin periodinane, CH_2Cl_2, rt, 30 min.



Scheme 9. Reagents and conditions: (i) O_3, MeOH, −78 °C, 60 min; (ii) PPh_3, MeOH, −78 °C→rt, 18 h or (iii) OsO_4 (cat.), $NaIO_4$, 2,6-lutidine, dioxane/H_2O, rt, 24 h; (iv) $NaClO_2$, 2-methyl-2-butene, NaH_2PO_4, *t*-BuOH/H_2O, rt, 2 h; (v) $(CO)_2Cl_2$, CH_2Cl_2, 0 °C→rt, 90 min; (vi) **26**, NEt_3, CH_2Cl_2, 0 °C→rt, 16 h.



Scheme 10. Reagents and conditions: (i) **7**, $CCl_3OC(O)Cl$, py, Et_2O, 0 °C→rt 48 h or (ii) **26**, $CCl_3OC(O)Cl$, NEt_3, Et_2O, 0 °C→rt 16 h.



Scheme 11. Reagents and conditions: (i) 4,4′-bipiperidine, benzene, 75 °C, 48 h.

two protonatable nitrogen atoms that could allow this compound to be formulated as a water soluble salt.

3. Results and discussion

3.1. Anticancer activity

The cytotoxic properties of artemisinin dimers were evaluated against human promyelocytic leukemia HL-60 cells in vitro using the MTT assay (Table 1). This cell line was chosen because leukemic cell lines are known to be particularly sensitive to artemisinin derivatives. The MTT assay measures the activity of dehydrogenase enzymes within the mitochondria of metabolically active cells.

As we have previously reported, **2** and **3** were studied in detail in HL-60 cells using dihydroartemisinin and doxorubicin as positive controls.[11] Both demonstrated excellent activity with IC_{50} values comparable to doxorubicin and in terms of cytotoxicity to non-cancerous mammalian cells these compounds were non-toxic to peripheral blood mononuclear cells at doses above 100 μM.

To address the potential problem with the stability of the phosphate ester linkage in **2** the methyl phosphonate-linked dimer **8** was prepared and also proved to possess potent activity. Confirmation that the endoperoxide is essential was provided by the significant drop in activity for the corresponding deoxygenated compound **8**.

The influence of the linker length on activity was examined by preparing dimers **13**, **14** and **15** which contain one additional carbon atom on either side of the linker and also **24a** and **24b** which has one fewer carbon atom on each side. The dimers with three carbon atoms in each of the artemisinin C-10 side chains (**13**, **14** and **15**) are of the same order of magnitude but slightly more active than those with two (**2**, **3** and **8**). On reducing the number of carbon atoms in each C-10 side chain from two to one the activity is decreased, although the stereochemistry at the C-9 position has been inverted and the influence that this might have on the activity is unknown.

The amide-linked dimer **29** also shows significant activity against HL-60 cells. It is interesting to note the similarity of **29** to one of the most potent compounds prepared by Jung et al.,[16] which shows activity against a range of murine and human cancer cell lines.

Of the remaining dimers synthesized in this study, the 4,4′-bipiperidine-linked dimer **32** possesses good activity against HL-60 cells whereas the carbonate and urea-linked dimers, **30** and **31**, respectively, are essentially devoid of activity.

3.2. Antimalarial activity

The antimalarial activity of artemisinin-dimers was evaluated in vitro against either chloroquine-resistant K1 or chloroquine-sensitive 3D7 strains of *P. falciparum* by [^{3}H]-hypoxanthine incorporation using artemisinin and artemether as positive controls (Table 2). Several of the dimers have remarkable activity against *P. falciparum* malaria.

As reported previously, dimers **2** and **3** are among the most potent compounds to have been tested against the K1 strain of *P. falciparum*.

The other dimers prepared were assayed against the chloroquine-sensitive 3D7 strain of *P. falciparum*. Methyl phosphonate dimer **8** and amide dimer **29** are the two most active compounds to have been tested and are among the most potent antimalarials that have been prepared at The University of Liverpool. The necessity of the endoperoxide bridge is demonstrated by the complete absence of activity for the deoxygenated methyl phosphonate dimer **10**. The lack of activity observed for urea-linked dimer **31** may be attributable to its poor solubility, which was observed during the testing procedure.

4. Conclusion

In conclusion, we have prepared a series of C-10 carba-linked artemisinin dimers, several of which show remarkable antimalarial and anticancer activity and deserve closer examination as potential

Table 1
In vitro anticancer activity of artemisinin-dimers against HL-60 cells

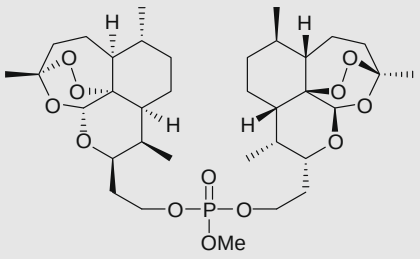
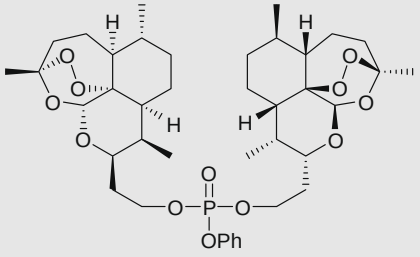
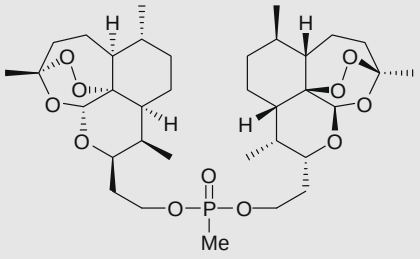
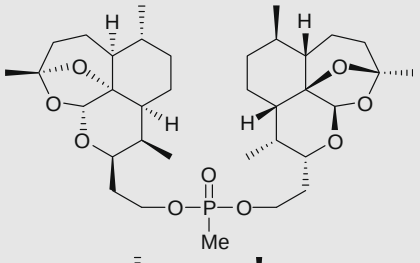
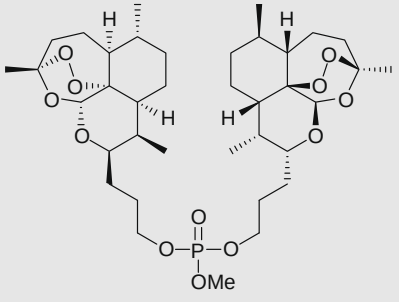
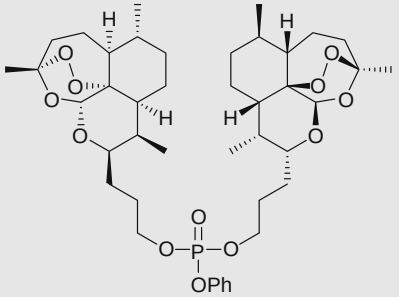
	Compound	IC_{50} (μM)	±SD (μM)
2		0.07	0.02
3		0.27	0.11
8		0.16	0.06
10		28.14	3.56
13		0.05	0.02
14		0.25	0.05

Table 1 (*continued*)

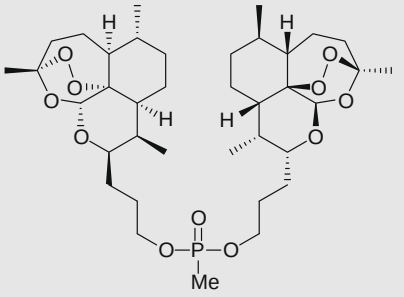
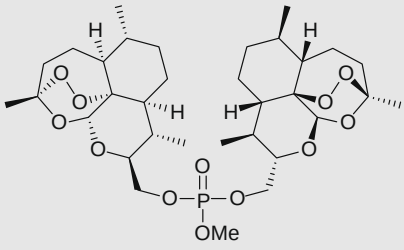
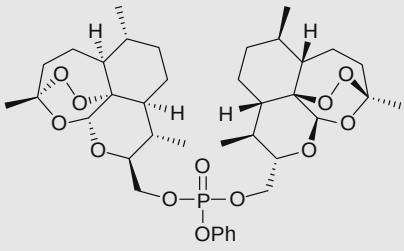
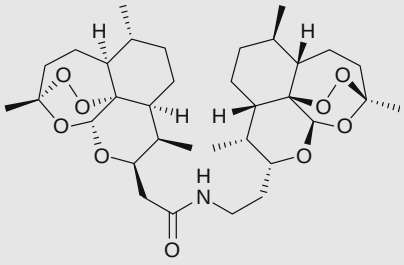
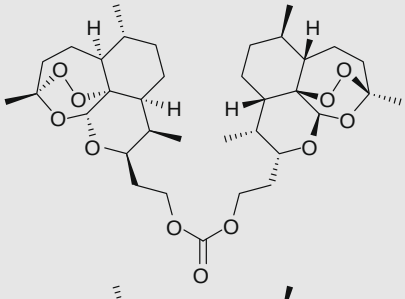
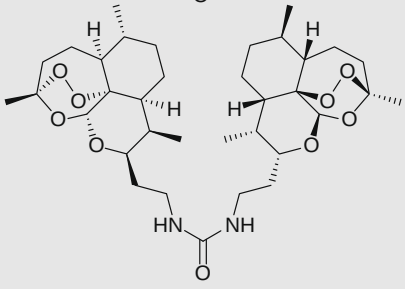
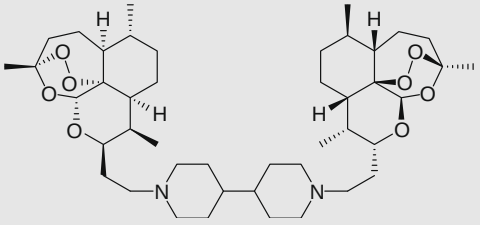
	Compound	IC_{50} (μM)	±SD (μM)
15		0.10	0.04
24a		0.77	0.18
24b		1.04	0.28
29		0.10	0.07
30		>100	
31		>100	

Table 1 (*continued*)

	Compound	IC_{50} (μM)	±SD (μM)
32		0.40	0.17
4	Dihydroartemisinin	2.41	0.71
	Doxorubicin	0.018	0.002

antimalarials or anticancer agents. Amide-linked dimer **29** was the most potent compound assayed against 3D7 *P. falciparum* (IC_{50} = 0.03 nM), as well as having significant activity against HL-60 cancer cells (IC_{50} = 0.10 μM). Methyl phosphate dimer **13** was the most potent of the compounds assayed against HL-60 cancer cells (IC_{50} = 0.05 μM). In addition to their excellent antimalarial and anticancer activity, these compounds also appear to be non-toxic to normal cell lines; compounds **2** and **3** are not toxic to peripheral blood mononuclear cells (PBMCs) at concentrations >250 μM giving a therapeutic index of >500.

5. Experimental

5.1. Chemistry

Air- and moisture-sensitive reactions were carried out in oven-dried glassware sealed with rubber septa under a positive pressure of dry nitrogen or argon from a manifold or balloon, unless otherwise indicated. Similarly sensitive liquids and solutions were transferred via syringe or stainless steel cannula. Reactions were stirred using Teflon-coated magnetic stir bars. Organic solutions were concentrated using a Buchi rotary evaporator with a diaphragm vacuum pump.

Anhydrous solvents were either obtained from commercial sources or dried and distilled immediately prior to use under a constant flow of dry nitrogen. THF and diethyl ether were distilled from Na, CH_2Cl_2 and NEt_3 from CaH_2. All other reagents were used as received from commercial sources unless otherwise indicated.

Analytical thin layer chromatography was performed with 0.25 mm silica gel 60F plates with 254 nM fluorescent indicator from Merck. Plates were visualised by ultraviolet light or by treatment with iodine, *p*-anisaldehyde, ninhydrin or potassium permanganate followed by gentle heating. Chromatographic purification of products was accomplished by flash chromatography, as described by Still et al.[24]

Melting points were determined on a Gallenkamp apparatus and are uncorrected. NMR spectra were measured on Bruker (400 MHz and 250 MHz) nuclear magnetic resonance spectrometers. Solvents are indicated in the text. Data for ^{1}H NMR spectra are reported as follows: chemical shift (δ, ppm) relative to tetramethylsilane as the internal reference, integration, multiplicity (s = singlet, br s = broad singlet, d = doublet, t = triplet, q = quartet, sex = sextet, td = triplet of doublets, m = multiplet), coupling constant (*J*, Hz), assignment. Data for ^{13}C NMR are reported in terms of chemical shift (δ, ppm) relative to residual solvent peak. Infrared spectra were recorded on a PerkinElmer RX1 FT-IR spectrometer and are reported in wavenumbers (cm^{-1}). Mass spectra (MS) and high resolution mass spectra (HRMS) were recorded on either a Trio 1000 quadrupole GC mass spectrometer (CI) or a Micromass LCT mass spectrometer (ESI). Reported mass values are within error limits of ±5 ppm. Elemental analyses (%C, %H, %N) were determined by the University of Liverpool Microanalysis Laboratory. Reported atomic percentages are within error limits of ±0.5%. In instances where purity was not determined by elemental analysis, compounds displayed only one observable spot by T.L.C. at the reported R_f.

5.1.1. General procedures

5.1.1.1. Synthesis of bisphosphate ester dimers: general procedure 1 (GP1). NaHMDS [1.0 M in THF] (1.2 equiv) was added to a stirring solution of the appropriate alcohol (1.0 equiv) in anhydrous THF (0.05 M) at −78 °C. After stirring at −78 °C for 10 min, the appropriate dichlorophosphate (0.5 equiv) was added. The reaction mixture was allowed to warm up to room temperature and stirred overnight, before being quenched at 0 °C with water (5 mL). The mixture was extracted with ether (2 × 10 mL) and the organic extracts washed with brine (10 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure to give the crude product, which was purified by column chromatography on Florisil.

5.1.1.2. Synthesis of methylphosphonate dimers: general procedure 2 (GP2). NaHMDS [1.0 M in THF] (1.2 equiv) was added to a stirring solution of the appropriate alcohol (1.0 equiv) in anhydrous THF (0.05 M) at −78 °C. After stirring at −78 °C for 10 min, a solution of methanephosphonic dichloride (0.5 equiv) in anhydrous THF (0.05 M) was added and the reaction mixture allowed to warm up to room temperature and stirred for 24 h. After 24 h, the reaction mixture was concentrated under reduced pressure and purified by flash column chromatography on silica gel.

5.1.2. Procedures

5.1.2.1. Dihydroartemisinin 10α-benzoate 5. Benzoyl chloride (3.2 mL, 27.6 mmol) was added to a stirring solution of dihydroartemisin **4** (5.00 g, 17.6 mmol) in anhydrous CH_2Cl_2 (54 mL) and anhydrous pyridine (9 mL) at 0 °C. After stirring at room temperature for 16 h, 7% aq citric acid solution (50 mL) was added. The organic layer was separated and the aqueous layer extracted with EtOAc (2 × 50 mL). The combined organic layers were washed with 7% aq citric acid solution (2 × 50 mL), saturated $NaHCO_3$ (50 mL) and water (50 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated under reduced pressure to give a yellow oil. Purification by flash column chromatography (silica gel, 10:90 EtOAc/*n*-Hex) gave **5** (6.83 g, 100%) as a white crystalline solid. **5**: Mp 111–112 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.13 (2H, m, Ar–H,), 7.57–7.45 (3H, m, Ar–H), 6.02 (1H, d, *J* = 9.8 Hz, H-10β), 5.53 (1H, s, H-12), 2.76 (1H, sex, H-9), 2.40 (1H, td, *J* = 14.0, 4.1 Hz, H-4α), 2.08–0.93 (19H, m) including 1.43 (3H, s, 3Me), 0.99 (3H, d, *J* = 6.0 Hz, 6Me) and 0.93 (3H, d, *J* = 7.2 Hz, 9Me); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.4, 133.3, 130.2, 129.8, 128.4, 104.5, 92.6, 91.7, 80.2, 51.7, 45.4, 37.3, 36.3, 34.2, 32.0, 25.9, 24.6, 22.1, 20.2 and 12.2; IR (Nujol)/cm^{-1} 2924, 1738 (C=O), 1491, 1452, 1377, 1272, 1114, 1100, 1037, 877 (O–O) and 831 (O–O); HRMS (CI) $C_{23}H_{32}NO_6$ $[M+NH_4]^+$ requires 406.2230, found 406.2232; Anal. calcd $C_{22}H_{28}O_6$ requires: C, 68.04; H, 7.22. Found: C, 67.79; H, 7.30.

5.1.2.2. 10β-Allyldeoxoartemisinin 6. A solution of **5** (2.13 g, 5.5 mmol) in anhydrous 1,2-dichloroethane (25 mL) was added dropwise via cannula to a stirring mixture of allyltrimethylsilane (4.4 mL, 27.7 mmol), anhydrous $ZnCl_2$ (0.90 g, 6.6 mmol) and powdered 4 Å molecular sieves in anhydrous 1,2-dichloroethane (25 mL) at 0 °C. After stirring at 0 °C for 3 h, the reaction mixture was diluted with EtOAc (150 mL) and washed with 5% aq citric

Table 2
In vitro antimalarial activity of artemisinin-dimers against chloroquine-resistant K1 or chloroquine-sensitive 3D7 strains of *P. falciparum*

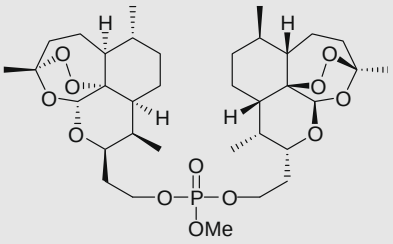
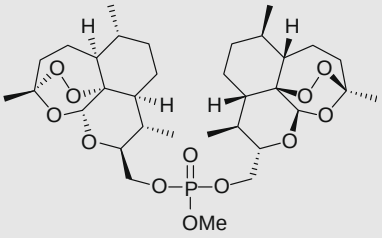
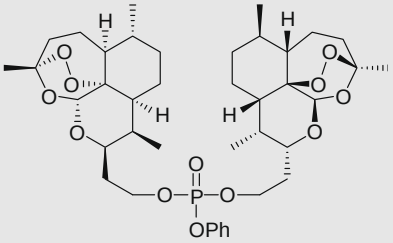
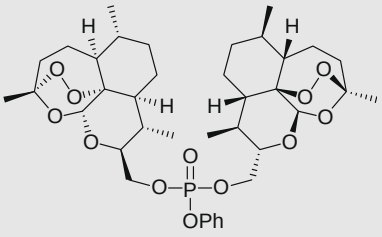
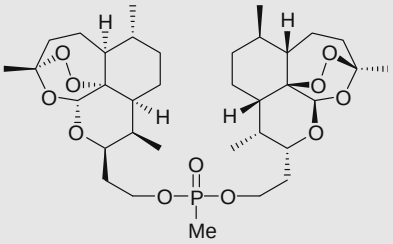
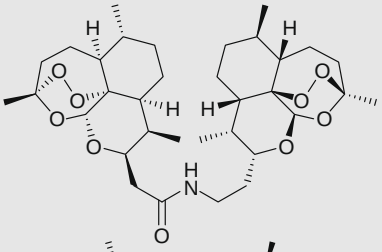
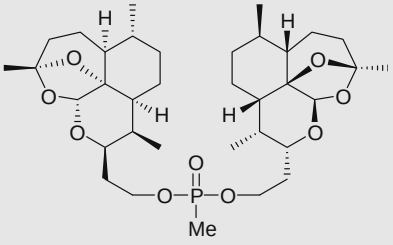
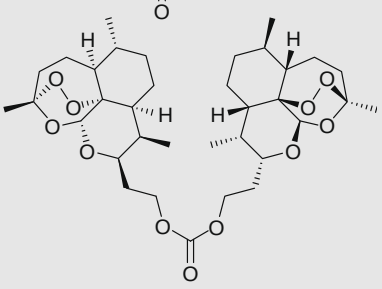
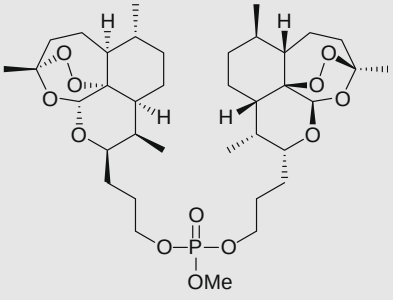
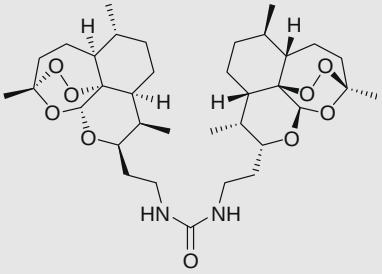
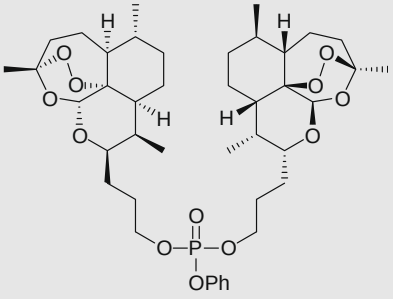
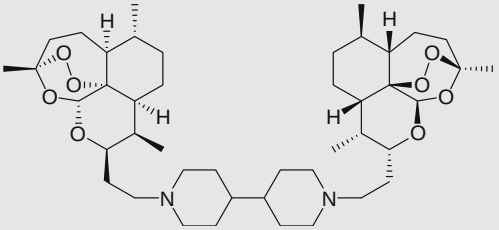
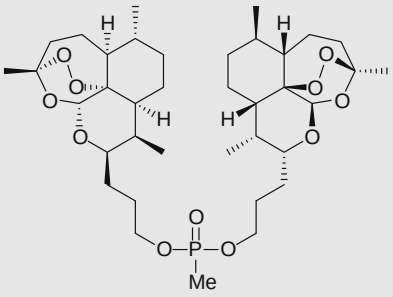
	Compound	IC_{50} (nM)	±SD (nM)		Compound	IC_{50} (nM)	±SD (nM)
2[a]		0.20	0.30	**24a**[b]		4.8	0.4
3[a]		0.50	0.10	**24b**[b]		6.0	0.30
8[a]		0.04	0.02	**29**[b]		0.03	0.01
10[a]		>1000	0.06	**30**[b]		4.60	0.40
13[b]		4.80	0.02	**31**[b]		>60	
14[b]		0.21	0.07	**32**[b]		0.46	0.08

Table 2 (*continued*)

	Compound	IC_{50} (nM)	±SD (nM)		Compound	IC_{50} (nM)	±SD (nM)
15[b]		0.50	0.3	**1**[b]	Artemisinin	12.3	1.40
					Artemether	3.53[a], 2.11[b]	1.91, 0.61

[a] IC_{50} determined against chloroquine-resistant K1 strain of *P. falciparum*.
[b] IC_{50} determined against chloroquine-sensitive 3D7 strain of *P. falciparum*.

acid (50 mL), saturated aq $NaHCO_3$ (50 mL) and brine (50 mL). The organic extracts were dried over $MgSO_4$, filtered and concentrated under reduced pressure to give a clear oil. Purification by flash column chromatography (silica gel, 10:90 EtOAc/*n*-Hex) gave **6** (1.55 g, 92%) as a white solid. Compound **6**: Mp 76–78 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.93 (1H, m, C*H*=CH_2), 5.33 (1H, s, H-12), 5.12 (2H, m, CH=CH_2), 4.31 (1H, m, H-10), 2.68 (1H, sex, J = 7.3 Hz, H-9), 2.45–2.17 (3H, m), 2.10–0.89 (19H, m) including 1.41 (3H, s, 3Me), 0.96 (3H, d, J = 6.0 Hz, 6Me), and 0.89 (3H, d, J = 7.6 Hz, 9Me); ^{13}C NMR (100 MHz, $CDCl_3$) 136.5, 116.0, 103.1, 89.2, 81.1, 74.6, 52.4, 44.4, 37.5, 36.7, 34.5, 34.3, 30.3, 26.1, 24.9, 24.7, 20.1, and 12.9; IR (Nujol)/cm^{-1} 2931, 1642, 1454, 1376, 1278, 1105, 1041, 879 (O–O), and 840 (O–O); HRMS (CI) $C_{18}H_{29}O_4$ $[M+H]^+$ requires 309.20660, found 309.20702; Anal. calcd $C_{18}H_{28}O_4$ requires: C, 70.10; H, 9.15. Found: C, 69.62; H, 9.32.

5.1.2.3. 10β-(2-Hydroxyethyl)-deoxoartemisinin 7. Ozone was bubbled through a solution of **6** (3.00 g, 9.7 mmol) in anhydrous CH_2Cl_2 (250 mL) at −78 °C for 60 min until the solution became saturated with ozone and appeared blue. Nitrogen was then bubbled through the solution for 20 min to purge excess ozone. The solvent was removed under reduced pressure and the residue taken up in anhydrous MeOH/THF (10:90, 100 mL). The solution was cooled to 0 °C and $NaBH_4$ (2.50 g, 66.1 mmol) added over 4 h. The mixture was stirred overnight at room temperature and then concentrated under reduced pressure, followed by addition of $CHCl_3$ (150 mL) and water (50 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated under reduced pressure to give a clear oil. Purification by flash column chromatography (silica gel, 40:60 EtOAc/*n*-Hex) gave **7** (1.60 g, 53%) as a white crystalline solid. Compound **7**: Mp 104–106 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.36 (1H, s, H-12), 4.46 (1H, m, H-10), 3.84 (2H, m, CH_2OH), 2.65 (1H, sex, J = 7.3 Hz, H-9), 2.33 (1H, td, J = 13.4, 4.0 Hz, H-4α), 3.07–0.87 (21H, m) including 1.42 (3H, s, 3Me), 0.96 (3H, d, J = 6.0 Hz, 6Me) and 0.87 (3H, d, J = 7.6 Hz, 9Me); ^{13}C NMR (100 MHz, $CDCl_3$) δ 103.2, 89.3, 81.1, 75.0, 62.8, 52.3, 44.2, 37.5, 36.6, 34.5, 31.7, 30.9, 30.4, 26.1, 24.8, 20.2 and 12.9; IR (Nujol)/cm^{-1} 3512 (O–H), 2928, 1462, 1379, 1273, 1093, 1048, 883 (O–O) and 838 (O–O); MS (CI) $[M+H]^+$ 313 (11), 296 (20), 295 (100), 267 (19) and 253 (27); HRMS (CI) $C_{17}H_{29}O_5$ $[M+H]^+$ requires 313.20151, found 313.20185; Anal. calcd $C_{17}H_{28}O_5$ requires: C, 65.36; H, 9.03. Found: C, 65.64; H, 9.29.

5.1.2.4. Methyl phosphate ester-linked dimer 2. Alcohol **7** (100 mg, 0.32 mmol) was reacted according to GP1. Purification by column chromatography (Florisil, 70:30 EtOAc/*n*-Hex) gave **2** (65 mg, 58%) as a white solid. Compound **2**: Mp 48–50 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.30 (2H, s, 2 × H-12), 4.49 (4H, m, 2 × OCH_2), 4.16 (2H, m, 2 × H-10), 3.76 (3H, d, J = 11.1 Hz, OMe), 2.68 (2H, sex, J = 7.3 Hz, 2 × H-9), 2.28 (2H, td, J = 13.1, 4.0 Hz, 2 × H-4α), 2.06–0.87 (42H, m) including 1.40 (6H, s, 2 × 3Me), 0.96 (6H, d, J = 6.0 Hz, 2 × 6Me) and 0.87 (6H, d, J = 7.5 Hz, 2 × 9Me); ^{13}C NMR (100 MHz, $CDCl_3$) δ 103.5, 89.3, 81.3, 71.6, 67.0, 52.7, 44.6, 37.8, 36.9, 34.8, 30.9, 30.3, 26.3, 25.1, 24.8, 20.5 and 13.2; MS (FAB) $[M+H]^+$ 701 (100), 307 (8), 237 (8), 191 (25), 154 (72), 136 (68), 107 (46), 77 (52); Anal. calcd $C_{35}H_{57}O_{12}P$ requires: C, 59.99; H 8.20. Found: C, 60.03; H, 8.56.

5.1.2.5. Phenyl phosphate ester-linked dimer 3. Alcohol **7** (100 mg, 0.32 mmol) was reacted according to GP1. Purification by column chromatography (Florisil, 70:30 EtOAc/*n*-Hex) gave **3** (60 mg, 49%) as a clear oil. Compound **3**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 (2H, t, Ar–H), 7.24 (2H,t, Ar–H), 7.14 (1H, t, Ar–H), 5.28 (2H, s, 2 × H-12), 4.38 (2H, m, 2 × H-10), 4.28 (4H, m, 2 × OCH_2), 2.67 (2H, sex, J = 7.3 Hz, 2 × H-9), 2.32 (2H, td, J = 13.0, 4.0 Hz, 2 × H-4α), 2.10–0.84 (42H, m) including 1.39 (6H, s, 2 × 3Me), 0.95 (6H, d, J = 5.9 Hz, 2 × 6Me) and 0.84 (6H, d, J = 7.4 Hz, 2 × 9Me); ^{13}C NMR (100 MHz, $CDCl_3$) δ 150.2, 130.0, 125.0, 120.5, 103.5, 89.3, 81.0, 71.5, 67.0, 52.7, 44.6, 37.8, 36.9, 34.8, 30.9, 30.3, 26.4, 25.0, 24.6, 20.5 and 13.2; HRMS (ESI) $C_{40}H_{60}O_{12}P$ $[M+H]^+$ requires 763.3822, found 763.3821; Anal. calcd $C_{40}H_{59}O_{12}P$ requires: C, 62.98; H, 7.80. Found: C, 62.75; H, 8.01.

5.1.2.6. Methylphosphonate-linked dimer 8. Alcohol **7** (260 mg, 0.83 mmol) was reacted according to GP2. Purification by column chromatography (silica gel, EtOAc) gave **8** (90 mg, 31%) as a clear oil. Compound **8**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.30 (2H, s, 2 × H-12), 4.37–4.03 (6H, m, 2 × OCH_2 and 2 × H-10), 2.70 (2H, sex, J = 7.3 Hz, 2 × H-9), 2.33 (2H, td, J = 4.0, 14.5 Hz, 2 × H-4α), 2.05–0.87 (45H, m) including 1.48 (3H, d, J = 17.5 Hz, P-Me), 1.40 (6H, s, 2 × 3Me), 0.96 (6H, d, J = 6.0 Hz, 2 × 6Me) and 0.87 (6H, d, J = 7.5 Hz, 2 × 9Me); ^{13}C NMR (100 MHz, $CDCl_3$) δ 103.6, 92.4, 81.4, 71.8, 64.1, 44.7, 37.8, 36.9, 34.8, 31.1, 30.3, 30.1, 26.4, 25.1, 20.5, 13.3, 12.0 and 10.6; HRMS (ESI) $C_{35}H_{57}NaO_{11}P$ $[M+Na]^+$ requires 707.3536, found 707.3513; Anal.

calcd $C_{35}H_{57}O_{11}P$ requires: C, 62.63; H, 8.59. Found: C, 62.17; H, 8.77.

5.1.2.7. 10β-Deoxy-(2-hydroxyethyl)-deoxoartemisinin 9. Zinc dust (1 g) was activated by washing with 5% aq HCl (3 × 10 mL), water (3 × 10 mL), EtOH (3 × 10 mL) and diethyl ether (3 × 10 mL), then thoroughly dried in vacuo. Activated zinc dust (50 mg) was added to a stirring solution of **7** (322 mg, 1.06 mmol) in glacial acetic acid (35 mL). The reaction mixture was stirred at room temperature for 72 h, with more zinc dust (50 mg) being added at 24 and 48 h, respectively. After 72 h $CHCl_3$ (50 mL) was added, the mixture filtered through a sintered glass funnel and the zinc dust washed with $CHCl_3$ (3 × 25 mL). The filtrate and washings were combined and neutralized with saturated aq $NaHCO_3$. The organic layer was separated, washed with saturated aq $NaHCO_3$ (25 mL), brine (25 mL) and water (25 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure to give a white solid. Purification by flash column chromatography (silica gel, 1:2 EtOAc/*n*-Hex) gave **9** (255 mg, 81%) as a white solid. Compound **9**: Mp 90–92 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.27 (1H, s, H-12), 4.36 (1H, m, H-10), 3.57 (2H, m, C*H*$_2$OH), 2.50 (1H, br s, OH), 2.23 (1H, sex, *J* = 7.6 Hz, H-9), 1.99–0.89 (21H, m) including 1.53 (3H, s, 3Me), 0.89 (6H, m, 6Me and 9Me); ^{13}C NMR (100 MHz, $CDCl_3$) δ 107.6 (C-3), 97.4, 82.9, 68.8, 62.1, 45.7, 40.6, 36.0, 34.9, 33.6, 30.1, 25.6, 25.1, 24.1, 22.6, 19.2 and 12.8; IR (Nujol)/cm^{-1} 3340 (O–H), 2925, 2855, 2359, 1458, 1385, 1377, 1266, 1208, 1144, 1097, 1053, 1011, 996, 984, 953, 905 and 877; HRMS (CI) $C_{17}H_{29}O_4$ [M+H]$^+$ requires 297.20660, found 297.20670; Anal. calcd $C_{17}H_{28}O_4$ requires: C, 68.89; H, 9.52. Found: C, 68.73; H, 9.42.

5.1.2.8. Deoxy methylphosphonate-linked dimer 10. Alcohol **9** (128 mg, 0.43 mmol) was reacted according to GP2. Purification by column chromatography (silica gel, EtOAc) gave **10** (66 mg, 47%) as a clear oil. Compound **10**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.25 (2H, s, 2 × H-12), 4.30–3.97 (6H, m, 2 × OC*H*$_2$ and 2 × H-10), 2.22 (2H, sex, *J* = 7.3 Hz, 2 × H-9), 2.08–0.88 (47H, m) including 1.50 (9H, m, P-Me and 2 × 3Me) and 0.88 (12H, m, 2 × 6Me and 2 × 9Me); ^{13}C NMR (100 MHz, $CDCl_3$) δ 107.4, 97.4, 82.7, 64.9, 63.6, 45.7, 40.7, 35.9, 34.9, 32.9, 29.7, 25.5, 24.1, 22.5, 19.2, 12.5 an 10.6; HRMS (ESI) $C_{35}H_{57}NaO_9P$ [M+Na]$^+$ requires 657.3638, found 657.3629; Anal. calcd $C_{35}H_{57}O_9P$ requires: C, 64.40; H, 8.80. Found: C, 64.27; H, 8.85.

5.1.2.9. 10β-(3-Hydroxypropyl)-deoxoartemisinin 11. $BH_3{\cdot}SMe_2$ [2.0 M in diethyl ether] (2.00 mL, 4.00 mmol) was added to a stirring solution of **6** (1.04 g, 3.36 mmol) in anhydrous THF (20 mL) at 0 °C. The reaction mixture was allowed to warm up to room temperature and stirred for 24 h. After 24 h, a suspension of $NaBO_3{\cdot}4H_2O$ (2.50 g, 16.25 mmol) in water (25 mL) was slowly added and the resulting suspension stirred for a further 24 h. After 24 h, water (25 mL) was added and the mixture extracted with CH_2Cl_2 (4 × 25 mL). The organic extracts were dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a colorless oil. Purification by gradient flash column chromatography (silica gel, 20:80→30:70 EtOAc/*n*-Hex) gave **11** (0.66 g, 62%) as a white solid along with a byproduct **12** (387 mg, 30%) as a white solid. Compound **11**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.33 (1H, s, H-12), 4.24 (1H, m, H-10), 3.70 (2H, C*H*$_2$OH), 2.65 (1H, sex, *J* = 7.2 Hz, H-9), 2.33 (1H, td, *J* = 14.0, 4.0 Hz, H-4α), 2.07–0.87 (23H, m) including 1.41 (3H, s, 3Me), 0.96 (3H, d, *J* = 6.0 Hz, 6Me) and 0.87 (3H, d, *J* = 7.6 Hz, 9Me); ^{13}C NMR (100 MHz, $CDCl_3$) δ 103.5, 89.7, 81.5, 75.7, 63.1, 52.7, 44.6, 40.0, 37.9, 34.8, 31.6, 30.9, 26.9, 26.4, 25.3, 25.1, 20.5 and 13.2; IR (film)/cm^{-1} 3424 (O–H), 2940, 2875, 1715, 1452, 1377, 1100 (C–OH), 877 (O–O), 825 (O–O) and 735; HRMS (CI) $C_{18}H_{31}O_5$ [M+H]$^+$ requires 327.21713, found 327.21772; Anal. calcd $C_{18}H_{30}O_5$ requires: C, 66.13; H, 9.26. Found: C, 65.65; H, 9.28. Compound **12**: Mp 131–132 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.22 (1H, s, H-12), 3.74 and 3.61 (2H, AB quartet, *J* = 11.6, 1.0 Hz, H-10), 2.32 (1H, td, *J* = 14.1, 4.0 Hz, H-4α), 2.11–0.96 (19H, m) including 1.57 (3H, s, 9Me), 1.41 (3H, s, 3Me) and 0.96 (3H, d, *J* = 5.9 Hz, 6Me); ^{13}C NMR (100 MHz, $CDCl_3$) δ 104.0, 92.3, 81.9, 70.3, 52.6, 38.1, 37.9, 37.7, 36.7, 34.2, 29.4, 26.1, 24.8, 24.2 and 20.5; IR (Nujol)/cm^{-1} 3438 (O–H), 2929, 2858, 1461, 1378, 1277, 1252, 1136, 1091, 1065, 1040, 996, 934, 911, 874 (O–O), 830 (O–O); HRMS (ESI) $C_{15}H_{24}NaO_5$ [M+Na]$^+$ requires 307.1521, found 307.1508; $C_{15}H_{24}O_5$ requires C 63.36; H 8.51. Found C, 62.89; H, 8.37.

5.1.2.10. Methyl phosphate ester-linked dimer 13. Alcohol **11** (120 mg, 0.36 mmol) was reacted according to GP1. Purification by column chromatography (Florisil, 30:70 EtOAc/*n*-Hex) gave **13** (32 mg, 25%) as a colorless oil. Compound **13**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.33 (2H, s, 2 × H-12), 4.28–4.05 (6H, m, 2 × OC*H*$_2$ and 2 × H-10), 3.78–3.64 (3H, m, OMe), 2.66 (2H, sex, *J* = 7.5 Hz, 2 × H-9), 2.32 (2H, td, *J* = 14.0, 4.0 Hz, 2 × H-4α), 2.06–0.87 (46H, m) including 1.41 (6H, s, 2 × 3Me), 0.96 (6H, d, *J* = 6.0 Hz, 2 × 6Me) and 0.87 (6H, d, *J* = 7.6 Hz, 2 × 9Me); ^{13}C NMR (100 MHz, $CDCl_3$) δ 103.2, 89.3, 81.1, 75.3, 62.8, 52.3, 44.3, 37.5, 36.6, 34.5, 31.2, 30.6, 29.7, 26.5, 26.0, 24.9, 24.8, 20.2 and 12.9; HRMS (ESI) $C_{37}H_{61}NaO_{12}P$ [M+Na]$^+$ requires 751.3798, found 751.3782; Anal. calcd $C_{37}H_{61}O_{12}P$ requires: C, 60.97; H, 8.44. Found: C, 60.43; H, 8.67.

5.1.2.11. Phenyl phosphate ester-linked dimer 14. Alcohol **11** (99 mg, 0.30 mmol) was reacted according to GP1. Purification by column chromatography (Florisil, 60:40 EtOAc/*n*-Hex) gave **14** (24 mg, 20%) as a colorless oil. Compound **14**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 (2H, t, Ar–H), 7.22 (2H, d, Ar–H), 7.15 (1H, t, Ar–H), 5.27 (2H, s, 2 × H-12), 4.29–4.11 (6H, m, 2 × OC*H*$_2$ and 2 × H-10), 2.64 (2H, sex, *J* = 7.2 Hz, 2 × H-9), 2.32 (2H, td, *J* = 14.3, 3.8 Hz, 2 × H-4α), 2.06–0.83 (46H, m) including 1.40 (6H, s, 2 × 3Me), 0.95 (3H, d, *J* = 5.9 Hz, 2 × 6Me), 0.95 (3H, d, *J* = 5.9 Hz, 2 × 6Me), 0.83 (3H, d, *J* = 7.5 Hz, 9Me) and 0.83 (3H, d, *J* = 7.5 Hz, 9Me); ^{13}C NMR (100 MHz, $CDCl_3$) δ 130.0, 125.3, 120.4, 120.4, 103.5, 89.5, 81.5, 75.1, 68.9, 52.7, 44.7, 37.8, 37.0, 34.8, 30.7, 26.5, 25.9, 25.3, 25.1, 20.5 and 13.2; HRMS (ESI) $C_{42}H_{63}NaO_{12}P^+$ [M+Na]$^+$ requires 813.3955, found 813.3948; Anal. calcd $C_{42}H_{63}O_{12}P$ requires: C, 63.78; H, 8.03, Found: C, 63.42; H, 8.19.

5.1.2.12. Methylphosphonate-linked dimer 15. Alcohol **11** (100 mg, 0.31 mmol) was reacted according to GP3. Purification by flash column chromatography (silica gel, EtOAc) gave **15** (31 mg, 28%) as a colorless oil. Compound **15**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.30 (2H, s, 2 × H-12), 4.25–3.90 (6H, m, 2 × OC*H*$_2$ and 2 × H-10), 2.67 (2H, sex, *J* = 7.0 Hz, 2 × H-9), 2.32 (2H, td, *J* = 14.1, 4.0 Hz, 2 × H-4α), 2.04–0.87 (49H, m) including 1.48 (3H, d, *J* = 17.5 Hz, P-Me), 1.41 (6H, s, 2 × 3Me), 0.96 (6H, d, *J* = 5.8 Hz, 2 × 6Me) and 0.87 (6H, d, *J* = 7.5 Hz, 2 × 9Me); ^{13}C NMR (100 MHz, $CDCl_3$) δ 103.5, 89.5, 81.5, 75.2, 65.7, 44.6, 37.8, 36.9, 34.8, 30.7, 28.9, 26.5, 25.9, 25.3, 25.0, 20.6, 13.3 and 10.5; HRMS (ESI) $C_{37}H_{61}NaO_{11}P^+$ [M+Na]$^+$ requires 735.3849, found 735.3839; Anal. calcd $C_{37}H_{61}O_{11}P$ requires: C, 62.34; H, 8.63. Found: C, 61.95; H, 8.19.

5.1.2.13. 10β-(Propa-1,2-dienyl)deoxoartemisinin 18. A solution of **5** (980 mg, 2.52 mmol) in anhydrous 1,2-dichloroethane (10 mL) was added dropwise via cannula to a stirring mixture of propargyl trimethylsilane (1.00 g, 8.91 mmol), anhydrous $ZnCl_2$ (0.45 g, 3.30 mmol) and powdered 4 Å molecular sieves in anhydrous 1,2-dichloroethane (10 mL) at 0 °C. After 2, 5 and 8 h more $ZnCl_2$ (0.45 g, 3.30 mmol) was added to the reaction mixture. After stirring overnight, the reaction mixture was

diluted with EtOAc (50 mL) and washed with 5% aq citric acid (25 mL), saturated aq $NaHCO_3$ (25 mL) and brine (25 mL). The organic extracts were dried over $MgSO_4$, filtered and concentrated under reduced pressure to give a clear oil. Purification by flash column chromatography (silica gel, 5:95 EtOAc/*n*-Hex) gave **18** (328 mg, 42%) as a pale yellow oil. **18:** ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.49 (1H, s, H-12), 5.32 (1H, ddd, J = 7.5, 6.8, 4.3 Hz C*H*=C=CH_2), 4.86 (2H, dd, J = 6.8, 4.3 Hz), 4.67 (1H, m, H-10), 2.89 (1H, m, H-9), 2.37 (1H, td, J = 14.1, 4.0 Hz, H-4α), 2.06–0.83 (19H, m) including 1.44 (3H, s, 3Me), 0.96 (3H, d, J = 6.0 Hz, 6Me) and 0.83 (3H, d, J = 7.5 Hz, 9Me); ^{13}C NMR (100 MHz, $CDCl_3$) δ 207.8 (CH=*C*=CH_2), 104.4, 89.7, 88.5, 81.3, 77.6, 74.2, 53.0, 45.6, 37.9, 36.8, 34.9, 30.8, 26.5, 25.0, 24.2, 20.7 and 14.1; IR (neat)/cm^{-1} 2239, 2875, 2245, 1956 (C=C=C), 1738, 1717, 1451, 1375, 1242, 1188, 1126, 1090, 1052, 993, 937, 876 (O–O), 845 (O–O) and 732; MS (CI) $[M+NH_4]^+$ 324 (31), 261 (100) and 247 (57); HRMS (CI) $C_{18}H_{30}NO_4$ $[M+NH_4]^+$ requires 324.21750, found 324.21808; Anal. calcd $C_{18}H_{26}O_4$ requires: C, 70.56; H, 8.55. Found: C, 70.07; H, 8.79.

5.1.2.14. Anhydroartemisinin 17. A solution of $BF_3{\cdot}OEt_2$ (8.4 mmol) was added to a stirring solution of dihydroartemisinin **4** (2.00 g, 7 mmol) in anhydrous THF (50 mL) at room temperature. The reaction mixture was stirred at 66 °C for 2.5 h, after which time it was allowed to cool to room temperature, diluted with EtOAc (50 mL) and washed with saturated aq $NaHCO_3$ (25 mL) and brine (25 mL). The organic extracts were dried over $MgSO_4$, filtered and concentrated under reduced pressure to give a pale yellow solid. Purification by flash column chromatography (silica gel, 10:90 EtOAc/*n*-Hex) gave **17** (1.47 g, 79%) as a white solid. Compound **17**: Mp 95–97 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.18 (1H, q, J = 1.3 Hz, H-10), 5.54 (1H, s, H-12), 2.45–2.35 (1H, m), 2.10–2.00 (2H, m), 1.95–0.98 (17H, m) including 1.59 (3H, d, J = 1.1 Hz, 9Me), 1.42 (3H, s, 3Me) and 0.98 (3H, d, J = 5.8 Hz, 6Me); ^{13}C NMR (100 MHz, $CDCl_3$) δ 135.6, 108.8, 105.2, 90.3, 79.6, 52.1, 45.1, 38.1, 36.9, 34.8, 30.6, 26.5, 25.1, 20.9 and 16.8; IR (Nujol)/cm^{-1} 2931, 2857, 2358, 1686, 1652, 1461, 1376, 1280, 1251, 1198, 1177, 1158, 1141, 1112, 1079, 1029, 1016, 992, 954, 904, 879 (O–O), 848 (O–O), 828 and 722; HRMS (CI) $C_{15}H_{23}O_4$ $[M+H]^+$ requires 267.15964, found 267.16045; Anal. calcd $C_{15}H_{22}O_4$ requires C, 67.64; H, 8.33. Found: C, 67.48; H, 8.35.

5.1.2.15. 10β-Benzenesulfonyl-9-*epi*-dihydroartemisinin 21. Benzene sulfinic acid was prepared from its commercially available sodium salt by treatment with 1.0 M HCl (10 mL) and extraction with CH_2Cl_2 (2 × 10 mL). The organic extracts were dried over $MgSO_4$, filtered and concentrated under reduced pressure to give a white solid which was used immediately without further purification. Benzene sulfinic acid (1.00 g, 7.0 mmol) was added to a stirring solution of anhydroartemisinin **17** (0.93 g, 3.5 mmol) in anhydrous CH_2Cl_2 (60 mL). After stirring for 30 min at room temperature, the reaction mixture was diluted with CH_2Cl_2 (100 mL) and washed with saturated $NaHCO_3$ (50 mL) and brine (50 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated under reduced pressure to give an off-white solid. Purification by flash column chromatography (silica gel, 20:80 EtOAc/*n*-Hex) gave **21** (0.95 g, 66%) as a white solid. Compound **21**: Mp 106–108 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98 (2H, t, J = 7.2 Hz, Ar–H), 7.66 (1H, t, J = 7.2 Hz, Ar–H), 7.55 (2H, t, J = 7.8 Hz, Ar–H), 5.41 (1H, s, H-12), 5.14 (1H, d, J = 10.3 Hz, H-10), 2.28–0.92 (21H, m) including 1.43 (3H, d, J = 6.9 Hz, 9Me), 1.06 (3H, s, 3Me) and 0.92 (3H, d, J = 5.7 Hz, 6Me); ^{13}C NMR (100 MHz, $CDCl_3$) δ 137.8, 133.9, 129.6, 129.2, 128.9, 102.7, 91.0, 90.1, 82.3, 51.1, 48.9, 37.5, 36.3, 35.3, 34.4, 34.1, 31.4, 25.5, 25.0, 21.1 and 20.1; IR (film)/cm^{-1} 2926, 1551 (Ar), 1384, 1250, 1210, 1000 and 1020; HRMS (ESI) $C_{21}H_{28}NaO_6S$ $[M+Na]^+$ requires 431.1504, found 431.1500; Anal. calcd $C_{21}H_{28}O_6S$ requires: C, 61.74; H, 6.91. Found: C, 61.61; H, 7.03.

5.1.2.16. 10β-Vinyl-9-*epi*-deoxoartemisinin 22. Vinylmagnesium bromide (1.0 M in THF, 8.0 mL, 8.00 mmol) was added to a stirring solution of anhydrous zinc chloride (0.63 g, 4.64 mmol) in anhydrous THF (25 mL). After stirring for 30 min at room temperature, **21** (1.58 g, 3.87 mmol) in anhydrous THF (16 mL) was added dropwise via cannula. After stirring for 48 h, 5% aq citric acid solution (50 mL) was added and the mixture extracted with EtOAc (3 × 30 mL). The organic extracts were washed with saturated $NaHCO_3$ (25 mL) and brine (25 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure to give a pale yellow oil. Purification by flash column chromatography (silica gel, 8:92 EtOAc/*n*-Hex) gave an inseparable mixture of **22** and **17** (600 mg) as a colorless oil. Compound **22**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.78 (1H, m, C*H*=CH_2), 5.55 (1H, s, H-12), 5.33 (1H, dm, J = 17.2 Hz, CH=C(H)H_{trans}), 5.19 (1H, dm, J = 10.3 Hz, CH=C(H)H_{cis}), 4.52 (1H, dd, J = 10.3, 7.0 Hz, H-10), 2.29 (1H, td, J = 13.9, 4.0 Hz, H-4α), 2.10–0.96 (20H, m) including 1.41 (3H, s, 3Me), 1.02 (3H, d, J = 6.8 Hz, 9Me) and 0.96 (3H, d, J = 6.0 Hz, 6Me); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.4 (*C*H=CH_2), 117.1 (CH=*C*H_2), 102.2, 90.4, 82.1, 77.4, 51.4, 46.7, 40.8, 37.3, 36.6, 34.1, 32.0, 24.8, 19.9 and 19.4; HRMS (CI) $C_{17}H_{27}O_4$ $[M+H]^+$ requires 295.1909, found 295.1907.

5.1.2.17. 10β-(Hydroxymethyl)-9-*epi*-deoxoartemisinin 23. Ozone was bubbled through a solution of **22** (600 mg) in anhydrous CH_2Cl_2 (50 mL) at −78 °C for 60 min until the solution became saturated with ozone and appeared blue. Nitrogen was then bubbled through the solution for 20 min to purge excess ozone. The solvent was removed under reduced pressure to give a white foam which taken up in anhydrous MeOH/THF (10:90, 100 mL). The solution was cooled to 0 °C and $NaBH_4$ (2.50 g, 66.1 mmol) added over 4 h. The mixture was stirred overnight at room temperature and then concentrated under reduced pressure, followed by addition of EtOAc (150 mL) and water (50 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated under reduced pressure to give a colorless oil. Purification by flash column chromatography (silica gel, 40:60 EtOAc/*n*-Hex) gave **23** (372 mg, 32% from **21**) as a colorless oil. Compound **23**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.45 (1H, s, H-12), 4.22 (1H, ddd, J = 10.7, 5.5, 2.6 Hz, H-10), 3.84 (1H, dd, J = 11.8, 2.6 Hz, C*H*OH), 3.53 (1H, dd, J = 11.8, 5.5 Hz, C*H*OH), 2.30 (1H, td, J = 14.0, 4.0 Hz, H-4α), 2.10–0.96 (20H, m) including 1.41 (3H, s, 3Me), 1.04 (3H, d, J = 7.0 Hz, 9Me) and 0.96 (3H, d, J = 6.0 Hz, 6Me); ^{13}C NMR (100 MHz, $CDCl_3$) δ 102.3, 90.5, 82.1, 75.7, 64.1, 51.4, 47.0, 37.3, 36.5, 35.6, 34.1, 31.8, 25.8, 24.7, 20.3 and 19.9; IR (neat)/cm^{-1} 3493 (O–H), 2927, 2875, 1740, 1457, 1375, 1280, 1239, 122, 1200, 1158, 1140, 1114, 1072, 1052, 1011, 991, 964, 944, 931, 911, 889 (O–O), 866, 836 (O–O), 818, 763, 732 and 701; HRMS (CI) $C_{16}H_{30}NO_5$ $[M+NH_4]^+$ requires 316.21240, found 316.21204; Anal. calcd $C_{16}H_{26}O_5$ requires: C, 64.41; H, 8.78. Found: C, 64.35; H, 8.91.

5.1.2.18. Methyl phosphate ester-linked dimer 24a. Alcohol **23** (104 mg, 0.35 mmol) was reacted according to GP1. Purification by column chromatography (Florisil, 50:50 EtOAc/*n*-Hex) gave **24a** (38 mg, 32%) as a colorless oil. Compound **24a**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.45 (2H, s, 2 × H-12), 4.37–4.07 (6H, m, 2 × OCH_2 and 2 × H-10), 3.82 (3H, d, J = 11.2 Hz, OMe), 2.28 (2H, td, J = 13.8, 3.8 Hz, 2 × H-4α), 2.07–1.89 (6H, m), 1.70–0.96 (34H, m) including 1.38 (6H, s, 2 × 3Me), 1.08 (6H, d, J = 6.9 Hz, 2 × 9Me) and 0.96 (6H, d, J = 5.8 Hz, 2 × 6Me); ^{13}C NMR (100 MHz, $CDCl_3$) δ 102.2, 90.5, 82.1, 74.1, 68.6, 51.4, 47.0, 37.3,

36.5, 35.8, 34.1, 31.7, 30.2, 29.7, 25.8, 24.8 and 19.9; HRMS (ESI) $C_{33}H_{53}NaO_{12}P$ $[M+Na]^+$ requires 695.3172, found 695.3147; Anal. calcd $C_{33}H_{53}O_{12}P$ requires: C, 58.92; H, 7.94. Found: C, 59.32; H, 8.33.

5.1.2.19. Phenyl phosphate ester-linked dimer 24b. Alcohol **23** (104 mg, 0.35 mmol) was reacted according to GP1. Purification by column chromatography (Florisil, 50:50 EtOAc/*n*-Hex) gave **24b** (38 mg, 32%) as a colorless oil. Compound **24b:** ^{1}H NMR (400 MHz, $CDCl_3$) δ *7.38–7.27* (4H, m, Ar–H), 7.14 (2H, m, Ar–H), 5.40 (2H, s, 2 × H-12), 4.4.46–4.18 (6H, m, 2 × O*CH*$_2$ and 2 × H-10), 2.28 (2H, td, *J* = 13.6, 3.6Hz, 2 × 4α), 2.04–1.89 (6H, m), 1.66–0.95 (34H, m) including 1.37 (6H, s, 2 × 3Me), 1.05 (6H, d, *J* = 7.0 Hz, 2 × 9Me) and 0.95 (6H, d, *J* = 5.7 Hz, 2 × 6Me); ^{13}C NMR (100 MHz, $CDCl_3$) δ 129.9, 125.1, 120.6, 114.3, 102.5, 90.8, 82.5, 74.2, 51.8, 47.4, 37.6, 36.9, 36.1, 35.9, 34.5, 32.0, 30.1, 26.2, 25.2 and 20.2; HRMS (ESI) $C_{38}H_{55}NaO_{12}P$ $[M+Na]^+$ requires 757.3329, found 757.3318; Anal. calcd $C_{38}H_{55}O_{12}P$ requires: C, 62.11; H, 7.54. Found: C, 61.79; H, 7.76.

5.1.2.20. 10β-(2-Methanesulfonylethyl)deoxoartemisinin 25. NEt_3 (0.4 mL, 2.9 mmol) and MsCl (0.2 mL, 2.6 mmol) were added to a stirring solution of **7** (0.45 g, 1.4 mmol) in anhydrous CH_2Cl_2 (20 mL) at 0 °C. After stirring at 0 °C for 2 h, water (20 mL) was added and the aqueous phase extracted with CH_2Cl_2 (3 × 10 mL). The combined organic layers were dried over $MgSO_4$, filtered and the solvent removed under reduced pressure. Purification by flash column chromatography (silica gel, 40:60 EtOAc/*n*-Hex) gave **25** (0.56 g, 100%) as a white crystalline solid. Compound **25**: Mp 123–125 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.31 (1H, s, H-12), 4.48–4.32 (3H, m, H-10 and C*H*$_2$OMs), 3.03 (3H, s, OMs), 2.69 (1H, sex, *J* = 7.3 Hz, H-9), 2.33 (1H, td, *J* = 13.8, 4.1 Hz, H-4α), 2.10–0.88 (21H, m) including 1.41 (3H, s, 3Me), 0.97 (3H, d, *J* = 6.0 Hz, 6Me) and 0.88 (3H, d, *J* = 7.6 Hz, 9Me); ^{13}C NMR (100 MHz, $CDCl_3$) δ 103.2, 89.2, 81.0, 70.9, 68.4, 52.2, 44.1, 37.3, 36.5, 34.4, 29.9, 29.6, 26.0, 24.8, 24.7, 20.1 and 12.7; IR (Nujol)/cm^{-1} 2926, 1462, 1378, 1350, 1279, 1167, 1100, 1037, 876 (O–O) and 824 (O–O); HRMS (ESI) $C_{18}H_{30}O_7S$ $[M]^+$ requires 390.17123, found 390.17127; Anal. calcd $C_{18}H_{30}O_7S$ requires: C, 55.39; H, 7.74. Found: C, 55.11; H, 7.81.

5.1.2.21. 10β-(2-Aminoethyl)-deoxoartemisinin 26. A solution of **25** (0.78 g, 2.0 mmol) in NH_4OH/EtOH (1:1, 40 mL) was stirred at room temperature for five days. The reaction mixture was then concentrated under reduced pressure, treated with 5% aq HCl until acidic and washed with CH_2Cl_2 (2 × 50 mL). The aqueous phase was then treated with 2.0 M NaOH until basic and extracted with CH_2Cl_2 (2 × 50 mL). The organic extracts were dried over $MgSO_4$, filtered and concentrated under reduced pressure to give an off-white solid. Purification by flash column chromatography (silica gel, 20:80 MeOH/CH_2Cl_2) gave **26** (0.61 g, 98%) as a white solid. Compound **26**: Mp 101–103 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.35 (1H, s, H-12), 4.29 (1H, m, H-10), 2.99–2.82 (2H, m, C*H*$_2$NH$_2$), 2.67 (1H, 1H, sex, *J* = 7.5 Hz, H-9), 2.33 (1H, td, *J* = 13.5, 4.0 Hz, H-4α), 2.09 (2H, br s, NH_2), 2.06–0.87 (21H, m) including 1.41 (3H, s, 3Me), 0.96 (3H, d, *J* = 6.1 Hz, 6Me) and 0.87 (3H, d, *J* = 7.6 Hz, 9Me); ^{13}C NMR (100 MHz, $CDCl_3$) δ 103.5, 92.3, 81.5, 74.2, 52.8, 44.7, 41.1, 37.8, 37.0, 34.9, 32.9, 30.7, 30.0, 26.5, 25.2, 20.5 and 13.3; IR (Nujol)/cm^{-1} 3365 (N–H), 2925, 2875, 1664, 1570, 1455, 1377, 1114, 1054, 1011, 944, 877 (O–O) and 839 (O–O); HRMS (CI) $C_{17}H_{30}NO_4$ $[M+H]^+$ requires 312.21747, found 312.21776; Anal. calcd $C_{17}H_{29}NO_4$ requires: C, 65.57; H, 9.39; N 4.50. Found: C, 65.38; H, 9.37; N, 4.00.

5.1.2.22. 10β-(2-Oxoethyl)deoxoartemisinin 27. Dess–Martin periodinane (15% w/v in CH_2Cl_2, 1.8 mL, 0.64 mmol) was added to a stirring solution of **7** (0.19 g, 0.61 mmol) in anhydrous CH_2Cl_2 (10 mL). After stirring for 30 min at room temperature, the reaction mixture was diluted with diethyl ether (50 mL) and poured into a stirring solution of sodium thiosulfate (2.5 g) in saturated aq $NaHCO_3$ (50 mL). After stirring for 15 min at room temperature, the organic layer was separated, washed with saturated aq $NaHCO_3$ (20 mL) and water (20 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a colorless oil. Purification by flash column chromatography (silica gel, 30:70 EtOAc/*n*-Hex) gave **27** (0.13 g, 68%) as a white solid.

5.1.2.23. 10β-(2-Oxoethyl)deoxoartemisinin 27. Ozone was bubbled through a solution of **6** (2.44 g, 7.9 mmol) in anhydrous MeOH (100 mL) at −78 °C for 60 min until the solution became saturated with ozone and appeared blue. Nitrogen was then bubbled through the solution for 20 min to purge excess ozone. PPh_3 (4.15 g, 15.8 mmol) was added to the stirring solution at −78 °C. The mixture was allowed to warm up to room temperature and stirred for 18 h. The solvent was then removed under reduced pressure and the residue purified by flash column chromatography (silica gel, 15:85 EtOAc/*n*-Hex) to give **27** (1.86 g, 76%) as a white solid.

5.1.2.24. 10β-(2-Oxoethyl)deoxoartemisinin 27. 2,6-Lutidine (0.45 mL, 3.87 mmol), OsO_4 (2.5% w/v in 2-methyl-2-propanol) and $NaIO_4$ (1.66 g, 7.74 mmol) were added to a stirring solution of **6** (597 mg, 1.94 mmol) in dioxane-water (3:1, 20 mL). After stirring at room temperature for 24 h, water (20 mL) and CH_2Cl_2 (20 mL) were added. The organic layer was separated and the aqueous phase extracted with CH_2Cl_2 (3 × 20 mL). The combined organic extracts were washed with brine (20 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a colorless oil. Purification by flash column chromatography (silica gel, 15:85 EtOAc/*n*-Hex) gave **27** (380 mg, 63%) as a white solid. Compound **27**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.80 (1H, dd, *J* = 3.2, 1.6 Hz, C*H*O), 5.32 (1H, s, H-12), 4.96 (1H, m, H-10), 2.79–2.64 (2H, m, C*H*$_2$CHO), 2.47–2.41 (1H, m), 2.33 (1H, td, *J* = 14.1, 4.1 Hz, H-4α), 2.07–2.00 (1H, m), 1.97–1.89 (1H, m), 1.84–1.76 (1H, m), 1.73–1.65 (2H, m), 1.48–0.86 (14H, m) including 1.40 (3H, s, 3Me), 0.97 (3H, d, *J* = 6.0 Hz, 6Me) and 0.86 (3H, d, *J* = 7.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 201.7 (CHO), 103.2, 89.4, 80.9, 69.4, 52.2, 44.5, 44.0, 37.5, 36.5, 34.4, 29.8, 26.0, 24.8, 24.7, 20.1 and 13.0; HRMS (CI) $C_{17}H_{30}NO_5$ $[M+NH_4]^+$ requires 328.21240, found 328.21203; Anal. calcd $C_{17}H_{26}O_5$ requires: C, 65.78; H, 8.44. Found: C, 65.62; H, 8.51.

5.1.2.25. 10β-(2-Carboxyethyl)deoxoartemisinin 28. NaH_2PO_4 (203 mg, 1.69 mmol) was added to a stirring solution of **27** (2.02 g, 6.51 mmol) in *t*-BuOH (90 mL) and water (18 mL). 2-Methyl-2-butene (2.0 M in THF, 38 mL, 76 mmol) was then added, followed by $NaClO_2$ (1.15 g, 9.58 mmol). The resulting pale yellow solution was stirred at room temperature for 2 h and then concentrated under reduced pressure. 1.0 M aq NaOH (50 mL) was added and the resulting solution washed with CH_2Cl_2 (3 × 50 mL). The aqueous phase was acidified with 1.0 M aq HCl and extracted with CH_2Cl_2 (3 × 50 mL). The combined organic extracts were dried over $MgSO_4$, filtered and concentrated under reduced pressure to give **28** (2.12 g, 100%) as a white brittle foam. Compound **28**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.70 (1H, br s, CO_2H), 5.37 (1H, s, H-12), 4.88 (1H, ddd, *J* = 9.9, 6.2, 3.3 Hz, H-10), 2.78–2.61 (2H, H-9 and C*H*HCO$_2$H), 2.50 (1H, dd, *J* = 15.7, 3.3 Hz, C*H*HCO$_2$H), 2.33 (1H, td, *J* = 14.1, 4.0 Hz, H-4α), 2.08–2.00 (1H, m), 1.99–1.90 (1H, m), 1.84–1.75 (1H, m), 1.73–1.64 (2H, m), 1.48–0.88 (14H, m) including 1.41 (3H, s, 3Me), 0.97 (3H, d, *J* = 5.9 Hz, 6Me) and 0.88 (3H, d, *J* = 7.5 Hz, 9Me); ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.1 (CO_2H), 103.3, 89.4, 80.8, 70.9, 52.1, 43.9, 37.5, 37.4, 36.5, 35.9, 34.4,

29.8, 25.8, 24.7, 20.1 and 12.7; IR (neat)/cm^{-1} 3500 (O–H), 2941, 2877, 2360, 1714 (C=O), 1452, 1378, 1281, 1192, 1126, 1092, 1055, 1014, 943, 877 (O–O), 845 (O–O), 824 and 736; HRMS (CI) $C_{17}H_{30}NO_6$ $[M+NH_4]^+$ requires 344.20734, found 344.20673; Anal. calcd $C_{17}H_{26}O_6$ requires: C, 62.56; H, 8.03. Found: C, 62.11; H, 8.53.

5.1.2.26. Amide-linked dimer 29. Oxalyl chloride (2.0 M in CH_2Cl_2, 0.30 mL, 0.60 mmol) was slowly added to a stirring solution of **28** (133 mg, 0.41 mmol) in anhydrous CH_2Cl_2 (5 mL) at 0 °C. The reaction mixture was allowed to warm up to room temperature and stirred for 90 min. After 90 min, the solvent was removed under reduced pressure and the residue dried under high vacuum for 30 min. The residue was taken up in anhydrous CH_2Cl_2 (5 mL) and added via cannula to a stirring solution of **26** (146 mg, 0.41 mmol) and NEt_3 (0.1 mL, 0.71 mmol) in anhydrous CH_2Cl_2 (5 mL) at 0 °C. The reaction mixture was allowed to warm up to room temperature and stirred overnight. The solvent was removed under reduced pressure and the residue purified by flash column chromatography (silica gel, 2:1 EtOAc/*n*-Hex) to give **29** (161 mg, 74%) as a white solid. Compound **29**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.08 (1H, br s, NH), 5.37 (1H, s, H-12), 5.33 (1H, s, H-12), 4.75 (1H, ddd, J = 11.0, 6.3, 2.1 Hz, H-10), 4.21 (1H, ddd, J = 10.8, 5.9, 2.3 Hz, H-10), 3.52–3.34 (2H, m, CH_2N), 2.74–2.60 (3H, m), 2.54–2.44 (2H, m), 2.41–2.37 (4H, m), 2.13–0.85 (37H, m) including 1.40 (3H, s, 3Me), 1.40 (3H, s, 3Me), 0.97 (3H, d, J = 6.1 Hz, 6Me), 0.96 (3H, d, J = 6.0 Hz, 6Me) and 0.85 (6Me, pseudo t, 2 × 9Me); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.8 (C=O), 103.7, 103.4, 90.0, 89.2, 81.5, 81.2, 75.3, 71.1, 52.8, 52.4, 44.9, 44.2, 39.0, 38.0, 37.8, 37.0, 34.9, 34.7, 30.7, 30.6, 29.2, 26.5, 26.3, 25.2, 25.2, 20.6, 20.4, 13.7 and 13.6; IR (Nujol)/cm^{-1} 3315 (O–H), 2919, 2727, 2359, 2340, 1717, 1665 (C=O), 1542 (C=O), 1457, 1377, 1324, 1279, 1251, 1229, 1207, 1188, 1152, 1126, 1101, 1054, 1040, 1013, 959, 934, 877 (O–O), 850, 826 (O–O), 721 and 668; HRMS (ESI) $C_{34}H_{54}NO_9$ $[M+H]^+$ requires 620.3799, found 620.3792; Anal. calcd $C_{34}H_{53}NO_9$ requires: C, 65.89; H, 8.62; N, 2.26. Found: C, 65.42; H, 9.14; N, 1.89.

5.1.2.27. Carbonate-linked dimer 30. Trichloromethyl chloroformate (41 μL, 0.33 mmol) was added to a stirring solution of **7** (206 mg, 0.66 mmol) and anhydrous pyridine (37 μL, 0.70 mmol) in anhydrous diethyl ether (25 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 1 h before being allowed to warm up to room temperature and stirred for 48 h. Water (10 mL) was then added and the mixture extracted with EtOAc (2 × 25 mL). The organic extracts were dried over $MgSO_4$, filtered and concentrated under reduced pressure to give a clear oil. Purification by flash column chromatography (silica gel, 20:80 EtOAc/*n*-Hex) gave **30** (167 mg, 73%) as white crystalline solid. Compound **30**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.30 (2H, s, 2 × H-12), 4.57–4.35 (6H, m, 2 × CH_2O and 2 × H-10), 2.65 (2H, sex, J = 7.0 Hz, 2 × H-9), 2.32 (2H, td, J = 13.9, 4.0 Hz, 2 × H-4α), 2.07–0.88 (42H, m) including 1.40 (6H, s, 2 × 3Me), 0.97 (6H, d, J = 5.9 Hz, 2 × 6Me) and 0.88 (6H, d, J = 7.5 Hz, 2 × 9Me); ^{13}C NMR (100 MHz, $CDCl_3$) δ 150.6 (C=O), 103.1, 89.4, 81.0, 70.3, 52.2, 44.0, 37.5, 36.6, 34.4, 30.0, 28.9, 26.0, 24.8, 20.1 and 12.6; IR (Nujol)/cm^{-1} 2923, 1778 (C=O), 1466, 1455, 1378, 1152, 1017, 943, 872 (O–O), 845 (O–O) and 690; HRMS (ES) $C_{35}H_{54}NaO_{11}$ $[M+Na]^+$ requires 673.3564, found 673.3585; Anal. calcd $C_{35}H_{54}O_{11}$ requires: C, 64.59; H, 8.36. Found: C, 64.51; H, 8.41.

5.1.2.28. Urea-linked dimer 31. Trichloromethyl chloroformate (50 μL, 0.42 mmol) was added to a stirring solution of NEt_3 (0.12 mL, 0.83 mmol) and **26** (260 mg, 0.83 mmol) in anhydrous diethyl ether (10 mL) at 0 °C. The solution was allowed to warm up to room temperature and stirred overnight. The reaction mixture was then diluted with EtOAc (20 mL) and washed with saturated aq $CuSO_4$ (10 mL) and brine (10 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated under reduced pressure to give a pale yellow oil. Purification by flash column chromatography (silica gel, 25:75 EtOAc/*n*-Hex) gave **31** (49 mg, 18%) as colorless oil. Compound **31**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.29 (2H, s, 2 × H-12), 4.12 (2H, ddd, J = 11.3, 6.4, 2.2 Hz, 2 × H-10), 3.58–3.41 (4H, m, 2 × CH_2N), 2.67 (2H, sex, J = 7.5 Hz, 2 × H-9), 2.33 (2H, td, J = 14.0, 4.0 Hz, 2 × H-4α), 2.07–2.00 (2H, m), 1.98–0.87 (40H, m) including 1.42 (6H, s, 2 × 3Me), 0.97 (6H, d, J = 6.0 Hz, 2 × 6Me) and 0.87 (6H, d, J = 7.6 Hz, 2 × 9Me); ^{13}C NMR (100 MHz, $CDCl_3$) δ 144.7 (C=O), 103.2, 89.3, 81.0, 71.6, 52.2, 44.1, 40.9, 37.3, 36.5, 34.4, 31.5, 29.7, 26.0, 24.8, 20.1 and 12.8; IR (neat)/cm^{-1} 3390, 2921, 1716 (C=O), 1519, 1455, 1377, 1263, 1190, 1100, 1039, 1013, 947, 879 (O–O), 843 (O–O), 824, 778, 738, 703 and 600; HRMS (ESI) $C_{35}H_{57}N_2O_9$ $[M+H]^+$ requires 649.4064, found 649.4037; Anal. calcd $C_{35}H_{56}N_2O_9$ requires: C, 64.79; H, 8.70; N, 4.32. Found: C, 64.53; H, 8.51; N, 3.91.

5.1.2.29. 4,4′-bipiperidine-linked dimer 32. 4,4′-Bipiperidine was prepared from its commercially available dihydrochloride by treatment with 1.0 M NaOH and extraction with EtOAc. The organic extracts were dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a white solid (mp 172 °C, lit. 172–173 °C) which was used without further purification. A solution of 4,4′-bipiperidine (44 mg, 0.26 mmol) and **25** (203 mg, 0.52 mmol) in anhydrous benzene (5 mL) was stirred at 75 °C for 48 h. After 48 h, saturated aq $NaHCO_3$ (10 mL) was added and the mixture extracted with diethyl ether (3 × 25 mL). The organic extracts were dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a colorless oil. Purification by reversed-phase column chromatography (octadecyl-functionalized silica gel, MeOH) gave **32** (124 mg, 63%) as a colorless oil. Compound **32:** ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.31 (2H, s, 2 × H-12), 4.11 (2H, m, 2 × H-10), 3.00 (4H, m), 2.72 (4H, m), 2.32 (4H, m), 2.07–1.18 (34H, m), 1.41 (6H, s, 2 × 3Me), 1.13 (4H, m), 0.95 (6H, d, J = 6.0 Hz, 2 × 6Me) and 0.87 (6H, d, J = 7.6 Hz, 2 × 6Me); ^{13}C NMR (100 MHz, $CDCl_3$) δ 103.8, 89.1, 81.5, 75.4, 58.1, 54.9, 52.9, 44.9, 41.3, 37.8, 36.9, 34.9, 30.6, 29.8, 29.7, 27.2, 26.6, 25.1, 20.7 and 13.7; HRMS (ESI) $C_{44}H_{73}N_2O_8$ $[M+H]^+$ requires 757.5367, found 757.5354; Anal. calcd $C_{44}H_{72}N_2O_8$ requires: C, 69.81; H, 9.59; N, 3.70. Found: C, 69.42; H, 9.83; N, 3.24.

5.2. Biology

5.2.1. Cytotoxicity studies

5.2.1.1. Materials. RPMI-1640 culture media, L-glutamine, penicillin/streptomycin solution, Hanks balanced salt solution (HBSS), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), *N*-(2-hydroxyethyl)piperazine-*N*′-(2-ethanesulfonic acid) hemisodium salt (HEPES), dimethylsulfoxide (DMSO), sodium dodecyl sulfate (SDS), *n*-dimethylformamide (DMF) and trypan blue (0.4%) solution were all purchased from Sigma (Poole, UK). Foetal bovine serum (FBS) was purchased from Bio Whittaker Europe (Verviers, Belgium). Human promyelocytic leukemia HL-60 cells were obtained from the European Collection of Cell Cultures (Salisbury, UK).

5.2.1.2. Cell culture and experimental preparation. HL-60 cells were maintained in RPMI-1640 media supplemented with 10% v/v FBS and 1% v/v L-glutamine. On reaching confluency (1×10^6 cells/mL in a 75 mL flask), 2×10^6 cells were seeded in 30 mL of fresh supplemented media. The cells were incubated under humidified air containing 5% CO_2 at 37 °C. Cell density was kept below 1×10^6 cells/mL to ensure exponential growth and to avoid differentiation. Cells were only used between passages 5 and 15 to prevent cell differentiation.

Cell viability was above 95% for all experiments. The viable cell count was based on trypan blue exclusion from the cells and was performed in a hemocytometer using a light microscope (×10; Zeiss Axioskop, Welwyn Garden City, UK). To 100 μL of cells was added 20 μL of trypan blue 0.4% solution and an aliquot was counted. During experiments cells were exposed to drug stock solutions which were made up in 100% DMSO and the final solvent concentration was below 0.5% v/v in each incubation. Each concentration in every experiment was carried out in quadruplicate and the experiments were repeated on at least three separate occasions.

5.2.1.3. MTT assay. The MTT assay is based upon the ability of dehydrogenase enzymes within viable cells to reduce the soluble MTT solution to an insoluble formazan salt.[25] The amount of formazan present is directly proportional to the number of viable cells. HL-60 cells (2.5×10^4/well) were plated in flat-bottomed 96-well plates in triplicate, and exposed to concentrations of each compound ranging from 0.01 μM to 100 μM for 72 h. Following incubation, 20 μL of MTT solution (5 mg/mL in HBSS) was added to each well. After 4 h of incubation at 37 °C, 100 μL of a lysing buffer (20% w/v sodium dodecyl sulfate; 50% v/v *n*-dimethylformamide) was added to each well to dissolve the formazan crystals, and incubated for a further 4 h. The absorbance of the wells was read using a test wavelength of 570 nm and a reference wavelength of 590 nm with a plate reader (MRX, Dynatech Laboratories). The results are expressed as a percent of vehicle only cells. IC_{50} values were estimated from individual inhibition curves plotted using GraFit software.

5.2.2. Antimalarial activity

For in vitro antimalarial assessment versus the 3D7 and K1 strains of *P. falciparum* the following protocol was employed. Parasites were maintained in continuous culture using the method of Jensen and Trager.[26] Cultures were grown in flasks containing human erythrocytes (2–5%), with parasitemia in the range of 1–10%, suspended in RPMI-1640 medium supplemented with 25 mM HEPES, 32 mM $NaHCO_3$ and 10% human serum (complete medium). Cultures were gassed with a mixture of 3% O_2, 4% CO_2 and 93% N_2. Antimalarial activity was assessed with an adaption of the 48 h sensitivity assay of Desjardins et al. using [^{3}H]-hypoxanthine incorporation as an assessment of parasite growth.[27] Stock drug solutions were prepared in 100% DMSO and diluted to the appropriate concentration using complete medium. Assays were performed in sterile 96-well microtitre plates, each plate contained 200 μL of parasite culture (2% parasitemia, 0.5% hematocrit) with or without 10 μL drug dilutions. Each drug was tested in triplicate and parasite growth compared to control wells (which constituted 100% parasite growth). After 24 h incubation at 37 °C, 0.5 μCi hypoxanthine was added to each well. Cultures were incubated for a further 24 h before they were harvested onto filter-mats, dried for 1 h at 55 °C and counted using a Wallac-1450 Microbeta Trilux Liquid scintillation and luminescence counter. IC_{50} values were calculated by interpolation of the probit transformation of the log dose–response curve.

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