Bioorganic & Medicinal Chemistry 18 (2010) 2586–2597

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Design, synthesis and antimalarial/anticancer evaluation of spermidine linked artemisinin conjugates designed to exploit polyamine transporters in *Plasmodium falciparum* and HL-60 cancer cell lines

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ARTICLE INFO

Article history:
Received 16 October 2009
Revised 16 February 2010
Accepted 19 February 2010
Available online 23 February 2010

Keywords:
Artemisinin
Polyamine
Malaria
Cancer

ABSTRACT

A series of artemisinin–spermidine conjugates designed to utilise the upregulated polyamine transporter found in cancer cells have been prepared. These conjugates were evaluated against human promyelocytic leukaemia HL-60 cells and chloroquine-sensitive 3D7 *Plasmodium falciparum* and several show promising anticancer and antimalarial activity. Although some limitations in this vector-based approach are apparent, a number of high potency Boc-protected analogues were identified with activity against malaria parasites as low as 0.21 nM.

1. Introduction

Artemisinin, also known as *qinghaosu*, is a tetracyclic 1,2,4-trioxane occurring in *Artemisia annua*.[1] It is a highly potent antimalarial that exhibits little or no cross-resistance with other antimalarials and as such 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 also possess potent activity against cancer cells.[2–10]

Traditional cancer chemotherapy relies on the premise that rapidly proliferating cancer cells are more likely to be killed by a cytotoxic agent. However, in reality, one of the major shortcomings of current cancer therapies is the non-selective delivery of chemotherapeutic agents to both cancer cells and healthy cells. Enhanced selectivity for such drugs could diminish the associated toxicity by reducing their uptake by healthy cells. Additionally, selective delivery could also be expected to increase potency by lowering the effective dose required to kill the affected cell type. Targeted drugs, with enhanced affinity for cancer cells, would represent an important advance in cancer chemotherapy. To this aim, various drug delivery protocols and systems have been explored in the last three decades.[11] One approach that may prove successful is to utilise vectors for the cellular delivery of toxic drugs to the targeted cell type. Tumour cells overexpress many receptors and biomarkers, which can be used as targets to deliver cytotoxic agents into tumours. One example using this sort of approach to target artemisinin to cancer cells is the preparation of artemisinin–transferrin conjugates in view of the fact that cancer cells overexpress transferrin receptors due to their increased iron dependency.[12]

The polyamine transport system offers a means of targeting specific cell types via the molecular recognition events involved during the uptake of exogenous polyamines. As such, it has been proposed that polyamines may be used as vectors for the cellular delivery of chemotherapeutic and/or DNA targeted drugs.[13–20] This paper describes the preparation of artemisinin–spermidine conjugates designed to utilise the upregulated polyamine transporter found in cancer cells.

Polyamines are ubiquitous biogenic amines, found in plants, animals and microorganisms, which play a significant role in a variety of important biological processes.[21] In vivo the naturally occurring polyamines, putrescine **2**, spermidine **3** and spermine **4**, exist as polycations (as the nitrogen atoms are protonated at physiological pH) and are essential for cell growth and differentiation (Fig. 1).[22] The intracellular levels of the polyamines are under the control of complex, highly regulated pathways.[21] In addition to

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doi:10.1016/j.bmc.2010.02.035

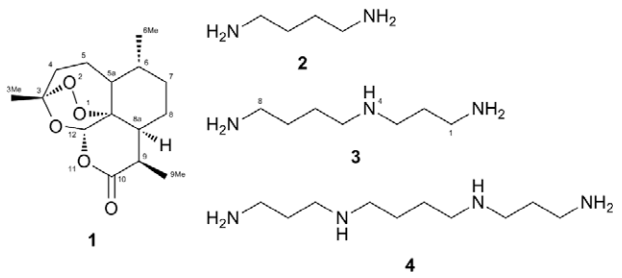


Figure 1. Artemisinin **1** and the naturally occurring polyamines: putrescine **2**, spermidine **3** and spermine **4**.

de novo biosynthesis, many cells are equipped with specific active transport systems that allow them to take up polyamines from exogenous sources. The activity of this uptake system is partially under the control of the intracellular free polyamine levels. Rapidly proliferating cells require large amounts of polyamines in order to grow, which can be internally biosynthesised and/or imported from extracellular sources. Many tumour cells have been shown to have elevated intracellular polyamine levels and an upregulated polyamine transport system; including Ehrlich ascites tumour cells,[23] human lymphocyte leukaemia cells,[24] human colon[25] and lung tumour cells,[26] neuroblastoma cells[27] and melanoma cells.[28] The high specific activity of the polyamine transporter in tumour cells is believed to be due to the inability of the biosynthetic enzymes involved to provide sufficient levels to sustain rapid cell division.[29]

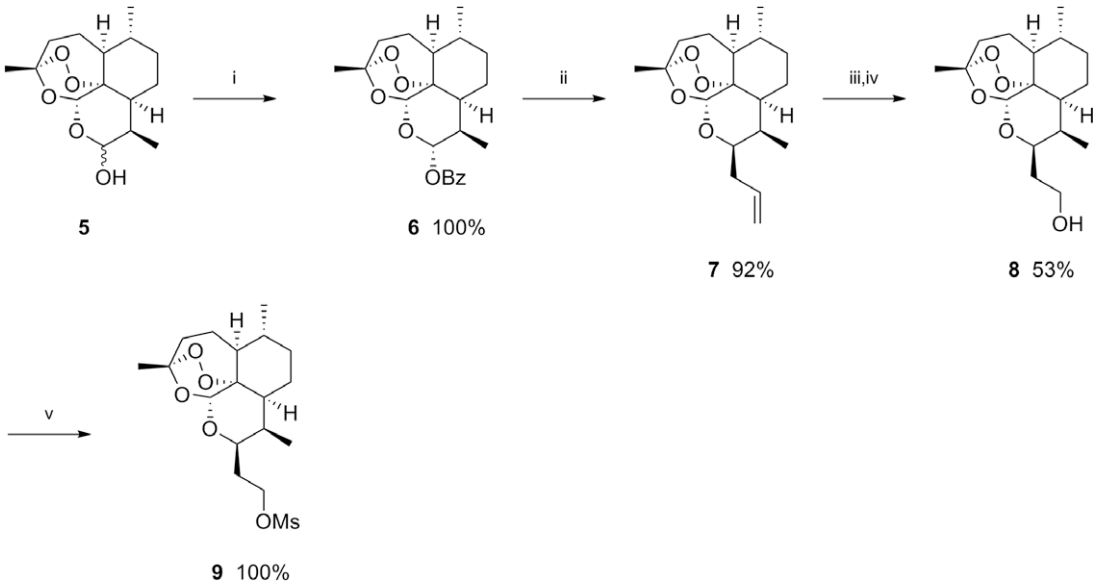
In vivo, the polyamines exist as polycations and it has been shown that charge is critical to the cellular recognition of the polyamine motif. The alignment of point charges is recognised by the polyamine transport system which facilitates their entry into cells.[30] Bergeron et al. demonstrated, by comparison of the biological properties of a series of analogues, that polyamines must be charged to be recognised by the cell.[31] Analogues with low nitrogen pK_as, such that the nitrogen atoms are inadequately protonated at physiological pH, do not compete well with spermidine for uptake.

Although the charge characteristics for recognition must be conserved, the specificity of the polyamine transport system is not stringent; as a result, a wide variety of structural derivatives are capable of utilising this uptake system.[32]

Another appealing property of the polyamines is their high affinity binding to DNA. Since DNA contains a negatively charged phosphate backbone, a major factor in polyamine–DNA interactions is electrostatic in origin (the binding constants of polyammonium cations to DNA are in the region 10^3 M^{-1}).[18] Polyamines have also been shown to interact with nucleic acid bases and to dock into the major and minor grooves of DNA,[33] although the exact manner of these interactions are unclear and are at most only slightly sequence selective. It has been demonstrated that the artemisinins are capable of causing DNA cleavage in a ferrous iron dependent reaction,[34,35] which may have implications for its biological mode of action and provides a further rationale behind conjugating artemisinin to polyamines.

2. Chemistry

In this study we wish to address whether it is feasible to target artemisinin to cancer cells by exploiting their upregulated polyamine transporter. Spermidine was chosen as the polyamine vector because the polyamine transport system exhibits preference for the naturally occurring polyamines in the order spermidine > spermine > putrescine.[19] In order to retain the relationship of point charges required for recognition by the polyamine transporter, it was decided to connect the artemisinin moiety to the spermidine backbone via an *N*-alkyl linkage. To provide a compound that has greater metabolic stability than C-10 acetal linked variants, the spermidine backbone should preferably be linked to artemisinin through a C-10 carba linkage.[36,37] With this in mind, N-alkylation of spermidine with 10β-(2-methanesulfonyl-ethyl)-deoxoartemisinin **9** offered an attractive method to conjugate the artemisinin moiety to spermidine. The synthesis of **9** is outlined in Scheme 1. Acylation of dihydroartemisinin **5** with benzoyl chloride gave dihydroartemisinin 10α-benzoate **6**. Reaction of **6** with allyl trimethylsilane using zinc chloride as catalyst gave 10β-allyldeoxoartemisinin **7**, ozonolysis of the terminal double bond in **7** followed by sodium borohydride reduction of the ozonide gave alcohol **8**, which was converted quantitatively into **9**.[38]



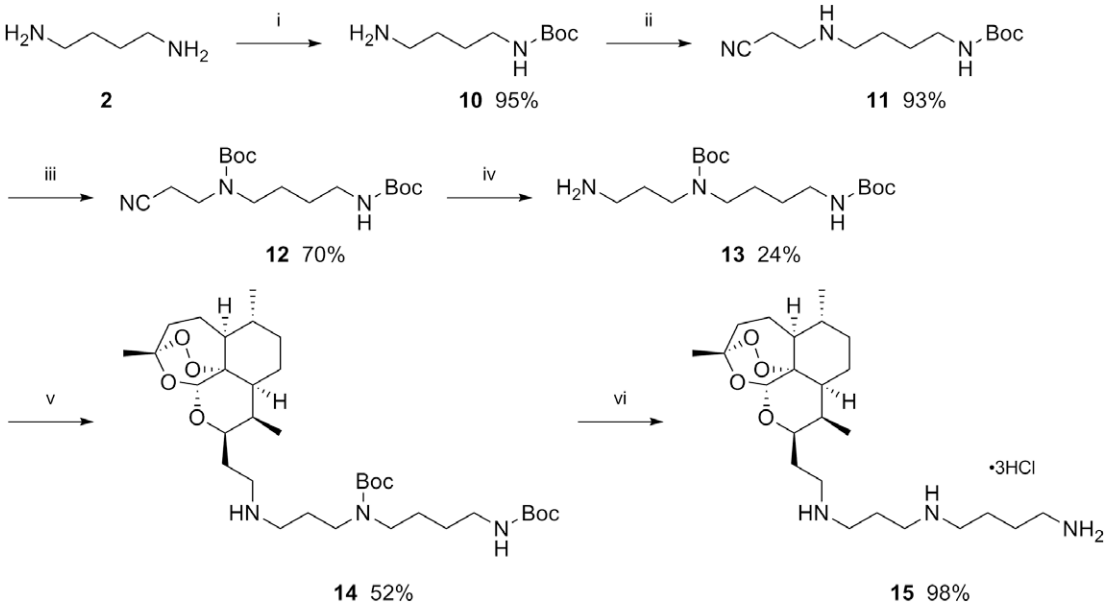
Scheme 1. Reagents and conditions: (i) BzCl, pyridine, CH_2Cl_2, 0 °C, 16 h; (ii) ally trimethylsilane, $ZnCl_2$, 4 Å molecular sieves, 1,2-dichloroethane, 0 °C, 3 h; (iii) O_3, CH_2Cl_2, −78 °C, 1 h; (iv) $NaBH_4$, THF/MeOH (9:1), 0 °C, 4 h; (v) MsCl, NEt_3, CH_2Cl_2, 0 °C, 2 h.

To compare the effects of derivatisation at the three amino centres of spermidine, we sought to synthesise the N^1-, N^4- and N^8-artemisinin–spermidine conjugates. Selective incorporation of artemisinin at the desired position requires protection of the other amino centres. One point we had to take into consideration when choosing a protecting group strategy was the stability of the endoperoxide bridge. For example, the susceptibility of the endoperoxide to catalytic hydrogenation[39] precluded the use of benzyl or carboxybenzyl (Cbz) protecting groups. It was decided to employ the *t*-butyl carbamate protecting group (Boc) and for the N^1 and N^8-conjugates to assemble the spermidine skeleton in a stepwise manner.

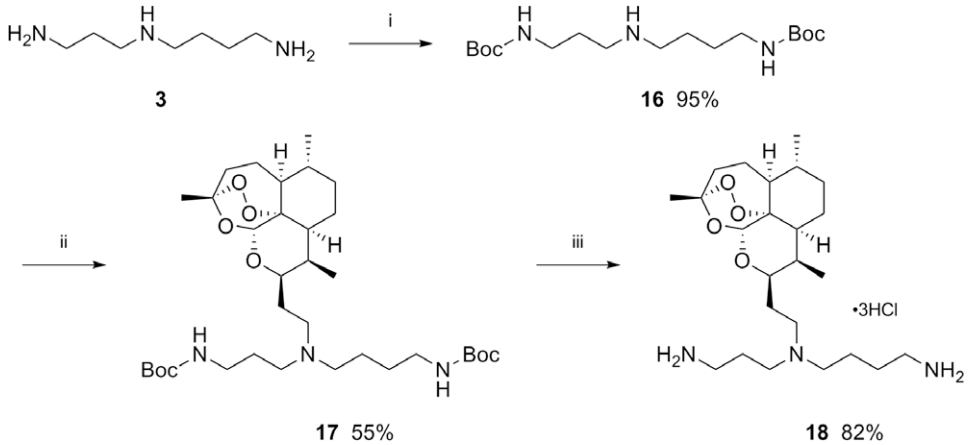
The synthesis of N^1-artemisinin spermidine conjugate is outlined in Scheme 2. 1,4-Diaminobutane **2** is mono-protected with di-*tert*-butyl dicarbonate by using a fivefold excess of the amine.[40] The free primary amine is then alkylated via a Michael-addition to acrylonitrile and an additional Boc-group introduced to protect the resulting secondary amine.[41] Reduction of the nitrile group with $LiAlH_4$ gave N^4,N^8-di-*tert*-butoxy-carbonylspermidine **13**. It is interesting to not that this reduction must be performed in diethyl ether rather than tetrahydrofuran, as the stronger basicity of the reducing agent in tetrahydrofuran led to elimination of acrylonitrile and the exclusive formation of N^1,N^4-di-*tert*-butoxycarbonyl-1,4-diaminobutane. Alkylation of **13** with **9** and removal of the Boc-protecting groups under acidic conditions gave the N^1-artemisinin–spermidine conjugate **15** as the hydrochloride salt.

The synthesis of the N^4-substituted spermidine conjugate **18** directly from spermidine is outlined in Scheme 3. Selective acylation of the primary amines of spermidine **3** with Boc-ON gave N^1,N^8-di-*tert*-butoxycarbonylspermidine **16**[18], which was alkylated at the N^4 position using 10β-(2-methanesulfonylethyl)deoxoartemisinin **9** by heating in benzene. Removal of the Boc-protecting groups under acidic conditions gave the desired compound **18** as the hydrochloride salt.

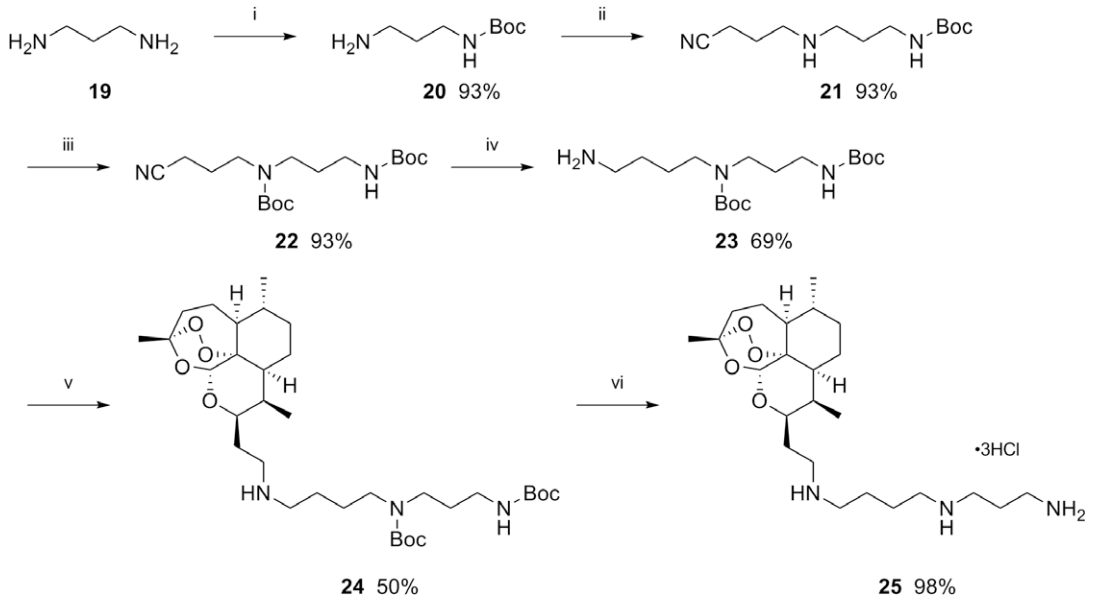
The N^8-conjugate **25** was prepared similarly to the N^1-conjugate, employing a stepwise assembly of the spermidine skeleton (Scheme 4). 1,3-Diaminopropane **19** was mono-protected with di-*tert*-butyl dicarbonate.[40] N-Alkylation of the primary amine **20** was then achieved with 4-bromobutyronitrile in acetonitrile employing potassium fluoride on Celite as a solid supported base.[42]



Scheme 2. Reagents and conditions: (i) Boc_2O, dioxane, rt, 3 h; (ii) acrylonitrile, MeOH, rt, 24 h; (iii) Boc_2O, CH_2Cl_2, rt, 2 h; (iv) $LiAlH_4$, Et_2O, 0 °C, 30 min; (v) **9**, benzene, 75 °C, 72 h; (vi) 4.0 M HCl, dioxane, rt, 4 h.



Scheme 3. Reagents and conditions: (i) Boc-ON, THF, rt, 4 h; (ii) **9**, benzene, 75 °C, 72 h; (iii) 4.0 M HCl, dioxane, rt, 4 h.



Scheme 4. Reagents and conditions: Boc_2O, dioxane, rt, 3 h; (ii) 4-bromobutyronitrile, KF–Celite, MeCN, 45 °C, 24 h; (iii) Boc_2O, CH_2Cl_2, rt, 1 h; (iv) $LiAlH_4$, Et_2O, 0 °C, 3 h; (v) **9**, benxene, 75 °C, 72 h; (vi) 4.0 M HCl, dioxane, rt, 4 h.

The resulting secondary amine **21** was protected with di-*tert*-butyl dicarbonate. Reduction of the nitrile **22** with $LiAlH_4$ gave primary amine **23**, alkylation of which with **9** and removal of the Boc-protecting groups under acidic conditions gave the N^8-conjugate as its hydrochloride salt **25**.

In addition to preparing the *N*-alkyl linked conjugates we also sought to synthesise amide-linked conjugates. A two step oxidation of the terminal double bond in **7** gave carboxylic acid **27** (Scheme 5) which underwent an EDCI–HOBt amide coupling with the selectively Boc-protected spermidines to give the N^1-,N^4- or N^8-amide-linked conjugates **31–33** following acidic deprotection.

3. Results and discussion

3.1. Anticancer activity

The cytotoxic properties of artemisinin–spermidine conjugates were evaluated against human promyelocytic leukaemia HL-60 cells in vitro using the MTT assay. This cell line was chosen because HL-60 cells are sensitive to the effects of other artemisinin derivatives[6,43,44] and are known to express an active polyamine transporter.[45] Table 1 lists the in vitro activity of the conjugates against HL-60 cells; these compounds have activity in the 18–48 μM range. As shown in Table 1, the amine-linked conjugates were approximately 1.5–2 times more active than the amide-linked conjugates. Terminal alkylation provided compounds with the greater activity; substitution at the N^8-centre giving the most active compounds, about 1.5-times more active than the N^1-conjugates, whereas the N^4-conjugates were devoid of activity (>100 μM). In addition to the amine and amide-linked artemisinin spermidine conjugates, the Boc-protected precursors were also included in the assay and somewhat surprisingly were found to be more active than the final conjugates (12–31 μM). Again the amine-linked conjugates were more active than the amide conjugates and substitution at the N^8-position was preferable to substitution at N^1, with N^4-conjugates being inactive.

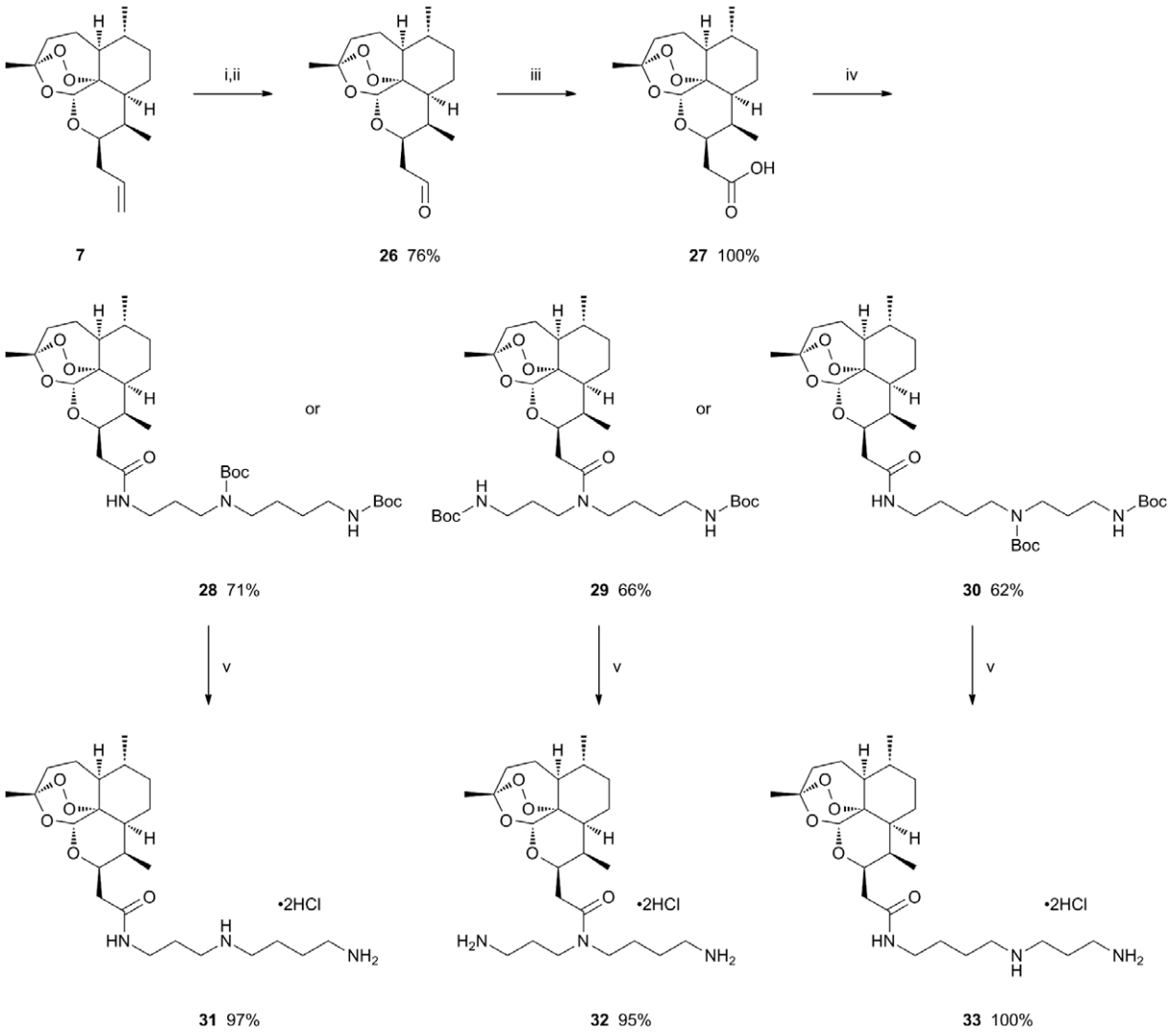
3.2. Antimalarial activity

Although these artemisinin–spermidine conjugates were designed to utilise the polyamine transporter present in many types of cancer cells, they may also have potential as antimalarials. The reasons for this are twofold; firstly, like other organisms, malaria parasites require polyamines for cell growth. It has been demonstrated that *P. falciparum* parasites possess functional polyamine biosynthetic pathways, along with salvage pathways regulated by highly specific transport systems.[46] DFMO, a polyamine biosynthesis inhibitor is effective against *Plasmodium berghei* in mice and synthetic polyamine analogues are also active as antimalarials. Secondly, artemisinin derivatives are believed to become activated within the iron-rich environment of the acidic parasite food vacuole and these analogues would be expected to become concentrated here by virtue of their basic nitrogen atoms. The potent antimalarial activity of chloroquine against chloroquine-sensitive strains can be attributed, in part, to its high accumulation in the acidic environment of the haem-rich parasite food vacuole. A key component of this intraparasitic chloroquine accumulation mechanism is a weak base ion-trapping effect whereupon the basic drug is concentrated in the acidic food vacuole in its membrane-impermeable diprotonated form. So in addition to targeting the parasites polyamine uptake system it was hoped that similar accumulation of these artemisinin–spermidine conjugates would be expected to lead to enhanced antimalarial activity.

The antimalarial activity of these artemisinin–spermidine conjugates and the Boc-protected precursors was evaluated against chloroquine-sensitive 3D7 *P. falciparum* (Table 2). Like the anticancer activity, in most cases the Boc-protected precursors (0.21–4.80 nM) had better activity than the deprotected conjugates (15–727 nM). The two most potent compounds were the Boc-protected N^1 (**14**, 0.76 nM) and N^8 (**24**, 0.21 nM) amine-linked conjugates. A similar pattern of activity to the anticancer data was observed for the Boc-protected conjugates, $N^8 > N^1 > N^4$ substitution and an amine linkage appears to be preferred over an amide one. The two most potent deprotected conjugates were the N^4 amine-linked conjugate **18** (15.2 nM) and the N^1 amide-linked conjugate **31** (81.2 nM), all of the other deprotected conjugates had activity >100 nM.

4. Conclusion

In this study we have prepared artemisinin–spermidine conjugates by attachment at the three amino centres of spermidine via



Scheme 5. Reagents and conditions: (i) O_3, MeOH, −78 °C, 60 min; (ii) PPh_3, MeOH, −78 °C to rt, 18 h; (iii) $NaClO_2$, 2-methyl-2-butene, NaH_2PO_4, *t*-BuOH/H_2O, rt, 2 h; (iv) **13**, **16** or **23**, EDCI, NMM, HOBt, CH_2Cl_2, 0 °C to rt, 18 h; (v) 4.0 M HCl, dioxane, rt, 4 h.

Table 1
In vitro anticancer activity of artemisinin–spermidine conjugates against HL-60 cells

Linkage	N^n	NHR	Compound	IC_{50} (μM)	±SD (μM)
Amine	N^1	NHBoc	**14**	16.6	3.8
		NH_2	**15**	29.2	3.6
	N^4	NHBoc	**17**	>100	
		NH_2	**18**	>100	
	N^8	NHBoc	**24**	11.6	3.5
		NH_2	**25**	17.8	4.7
Amide	N^1	NHBoc	**28**	31.2	10.0
		NH_2	**31**	47.7	10.0
	N^4	NHBoc	**29**	>100	
		NH_2	**32**	>100	
	N^8	NHBoc	**30**	22.0	3.2
		NH_2	**33**	35.2	8.1
Dihydroartemisin			**5**	2.4	0.7

Table 2
In vitro antimalarial activity of artemisinin–spermidine conjugates against chloroquine-sensitive 3D7 *P. falciparum*

Linkage	N^n	NHR	Compound	IC_{50} (nM)	±SD (nM)
Amine	N^1	NHBoc	**14**	0.76	0.19
		NH_2	**15**	350	127
	N^4	NHBoc	**17**	>100	
		NH_2	**18**	15.2	3.6
	N^8	NHBoc	**24**	0.21	0.03
		NH_2	**25**	259	85
Amide	N^1	NHBoc	**28**	4.3	0.1
		NH_2	**31**	81.0	14.3
	N^4	NHBoc	**29**	4.8	0.8
		NH_2	**32**	108	30
	N^8	NHBoc	**30**	2.6	1.0
		NH_2	**33**	727	203
Artemisinin			**1**	9.2	2.0

either an *N*-alkyl or amide linkage. The compounds were assayed for anticancer activity against HL-60 cells and were found to retain some of the activity of the parent artemisinins. Amine linkage to the spermidine skeleton was preferred over an amide one and the preferred substitution pattern is $N^8 > N^1 \gg N^4$, confirming similar finding by others that substitution of cytotoxic agents at the terminal position of the spermidine skeleton is favored over substitution at N^4.[18] Contrary to our expectations, the Boc-protected precursors were more active than the final artemisinin–spermidine conjugates, although the pattern of activity remained broadly similar.

Antimalarial activity was evaluated against chloroquine-sensitive 3D7 *P. falciparum* and again the two most potent compounds identified were the terminally substituted amine-linked Boc pre-

cursors **24** and **14** (0.21 nM and 0.76 nM, respectively). A similar pattern of activity as the anticancer data for the Boc-protected compounds was observed. However, the deprotected conjugates had much lower activity than hoped for, apart from the N^4 amine-linked conjugate **18** (15.2 nM).

The vector approach in *P. falciparum* and cancer cell lines proved not to be as successful as we anticipated though our study has revealed some very potent Boc-protected analogues. Reasons for this could include poor membrane permeability of the tricationic polyamine species, insufficient accumulation within the digestive vacuole of the malaria parasite or it could be that these conjugates are poor substrates for the polyamine transport systems present. We have yet to establish whether these conjugates utilise the upregulated polyamine uptake system present in cancer cells, although the similar pattern of activity to that reported by others is encouraging and suggests that this might be a promising approach for the delivery of artemisinins selectively to cancer cells in spite of limitations that we have described.

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.[47]

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%.

5.1.1. Procedures

5.1.1.1. Dihydroartemisinin 10-benzoate 6. Benzoyl chloride (3.2 mL, 27.6 mmol) was added to a stirring solution of dihydroartemisin **5** (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 **6** (6.83 g, 100%) as a white crystalline solid. Compound **6**: 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) $C_{22}H_{32}NO_6$ $[M+NH_4]^+$ 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.

5.1.1.2. 10-Allyldeoxoartemisinin 7. A solution of **6** (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 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 **7** (1.55 g, 92%) as a white solid. Compound **7**: 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, 69.92; H, 9.32.

5.1.1.3. 10-(2-Hydroxyethyl)-deoxoartemisinin 8. Ozone was bubbled through a solution of **7** (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 **8** (1.60 g, 53%) as a white crystalline solid. Compound **8**: 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.

5.1.1.4. 10-(2-Methanesulfonylethyl)deoxoartemisinin 9. NEt_3 (0.4 mL, 2.9 mmol) and MsCl (0.2 mL, 2.6 mmol) were added to a stirring solution of **8** (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 **9** (0.56 g, 100%) as a white crystalline solid. Compound **9**: 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.

5.1.1.5. *tert*-Butyl 4-aminobutylcarbamate 10. A solution of di-*tert*-butyl dicarbonate (10.27 g, 0.05 mol) in anhydrous 1,4-dioxane (200 mL) was added over 3 h to a stirring solution of 1,4-diaminobutane (25 g, 0.28 mol) in 1,4-dioxane (200 mL). After stirring overnight the solvent was removed under reduced pressure. Water (250 mL) was added to the residue and the insoluble bis-substituted product (0.53 g, 5%) collected by filtration. The filtrate was extracted with CH_2Cl_2 (5 × 150 mL). The organic extracts were dried over Na_2SO_4, filtered and concentrated under reduced pressure to give **10** (8.92 g, 95%) as a colourless oil. Compound **10**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.60 (1H, br s, NHBoc), 3.12 (2H, m, H-1), 2.71 (2H, t, J = 6.8 Hz, H-4), 1.55–1.35 (4H, m, H-2 and H-3) and 1.44 (9H, s, *t*-Bu); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.4 (C=O), 78.9 (*C*(CH$_3$)$_3$), 42.5, 42.2, 31.5, 31.3 and 28.8 (C(*C*H$_3$)$_3$); IR (neat)/cm^{-1} 3343 (N–H), 2960, 2920, 2852, 1700, 1687 (C=O), 1568, 1544, 1525, 1474, 1448, 1386, 1360, 1270, 1246, 1170, 1037 and 864; MS (CI) $[M+H]^+$ 189 (46), 115 (97), 89 (100), 72 (50) and 70 (74); HRMS (CI) $C_9H_{21}N_2O_2$ $[M+H]^+$ requires 189.16031, found 189.16049; Anal. Calcd $C_9H_{20}N_2O_2$ requires: C, 57.42; H, 10.71; N, 14.88. Found: C, 57.07; H, 10.75; N, 14.80.

5.1.1.6. [4-(2-Cyano-ethylamino)-butyl]-carbamic acid *tert*-butyl ester 11. A solution of acrylonitrile (2.4 mL, 36 mmol) in MeOH (150 mL) was added over 30 min to a stirring solution of *tert*-butyl 4-aminobutylcarbamate **10** (6.80 g, 36 mmol) in MeOH (100 mL). After stirring for 24 h, the solvent was removed under reduced pressure to give **11** (8.08 g, 93%) as a pale yellow oil, which was used subsequently without purification. Compound **11**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.92 (1H, br s, NHBoc), 3.12 (2H, m,C*H*$_2$ NHBoc), 2.92 (2H, t, J = 6.7 Hz), 2.65 (2H, t, J = 6.7 Hz), 2.52 (2H, t, J = 6.7 Hz, C*H*$_2$CN), 1.59–1.48 (5H, m), and 1.44 (9H, s, *t*-Bu); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.4 (C=O), 119.1 (C≡N), 79.3 (*C*(CH$_3$)$_3$), 49.0, 45.3, 40.7, 28.8 (C(*C*H$_3$)$_3$), 28.1, 27.6 and 19.0; IR (neat)/cm^{-1} 3339 (N–H), 2975, 2933, 2862, 2348, 2340, 2247 (C≡N), 1699 (C=O), 1524, 1454, 1391, 1366, 1251, 1173, 1040, 1002, 928, 868, 780 and 668; MS (CI) $[M+H]^+$ 242 (34), 186 (31), 168 (100), 142 (85), 115 (38), 89 (47) and 70 (78); HRMS (CI) $C_{12}H_{24}N_3O_2$ $[M+H]^+$ requires 242.18686, found 242.18666; Anal. Calcd $C_{12}H_{23}N_3O_2$ requires: C, 59.72; H, 9.61; N, 17.41. Found: C, 59.47; H, 9.65; N, 17.31.

5.1.1.7. (4-*tert*-Butoxycarbonylamino-butyl)-(2-cyano-ethyl)-carbamic acid *tert*-butyl ester 12. A solution of di-*tert*-butyl dicarbonate (2.26 g, 10 mol) in anhydrous CH_2Cl_2 (25 mL) was added over 1 h to a stirring solution of [4-(2-cyano-ethylamino)-butyl]-carbamic acid *tert*-butyl ester **11** (2.50 g, 10 mol) in anhydrous CH_2Cl_2 (50 mL). After stirring for 1 h, the solvent was removed under reduced pressure to give a colourless oil. Purification by flash column chromatography (silica gel, 30:70 EtOAc/*n*-Hex) gave **12** (2.37 g, 70%) as a colourless oil. Compound **12**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.65 (1H, br s, NHBoc), 3.46 (2H, t, J = 6.7 Hz), 3.28 (2H, t, J = 7.2 Hz), 3.13 (2H, m), 2.69–2.52 (2H, m, C*H*$_2$CN), 1.64–1.48 (4H, m), 1.47 (9H, s, *t*-Bu) and 1.44 (9H, s, *t*-Bu); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.4 (C=O), 155.6 (C=O), 117.9 (C≡N), 80.8 (*C*(CH$_3$)$_3$), 79.5 (*C*(CH$_3$)$_3$), 48.6, 44.2, 40.4, 28.8 (C(*C*H$_3$)$_3$), 28.7 (C(*C*H$_3$)$_3$), 27.7, 26.3 and 14.5; IR (neat)/cm^{-1} 3364 (N–H), 2977, 2934, 2868, 2359, 2339, 2250 (C≡N), 1699 (C=O), 1520, 1479, 1456, 1416, 1392, 1367, 1251, 1170, 1092, 1039, 1022, 1005, 919, 867, 776, 735, 703, 668 and 647; MS (CI) $[M+H]^+$ 342 (9), 285 (29), 268 (31), 247 (37), 242 (62), 229 (100), 168 (62) and 142 (80); HRMS (CI) $C_{17}H_{32}N_3O_4$ $[M+H]^+$ requires 342.23926, found 342.23955; Anal. Calcd $C_{17}H_{31}N_3O_4$ requires: C, 59.80; H, 9.15; N, 12.31. Found: C, 59.60; H, 9.54; N, 12.07.

5.1.1.8. {4-[(3-Amino-propyl)-*tert*-butoxycarbonyl-amino]-butyl}-carbamic acid *tert*-butyl ester 13. Lithium aluminium hydride [3.5 M in THF/toluene] (7.03 mmol) was added dropwise to a stirring solution of (4-*tert*-butoxycarbonylamino-butyl)-(2-cyano-ethyl)-carbamic acid *tert*-butyl ester (2.40 g, 7.03 mmol) **12** in diethyl ether (100 mL) at 0 °C. After stirring at 0 °C for 30 min, the reaction mixture was quenched by the sequential dropwise addition of water (2.5 mL), 15% aq NaOH (2.5 mL) and finally water (5 mL). The resulting suspension was filtered through Celite®, and the solid washed with diethyl ether (3 × 25 mL). The combined filtrates were washed with brine (10 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a pale yellow oil. Purification by flash column chromatography (silica gel, 10:90→15:85 MeOH/CH_2Cl_2) gave **13** (574 mg, 24%) as a colourless oil. Compound **13**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.70 (1H, br s, NHBoc), 3.37–3.03 (6H, m), 2.70 (2H, t, J = 6.7 Hz, C*H*$_2$NH$_2$), 2.08 (2H, br s, NH$_2$), 1.76–1.52 (4H, m), 1.46 (9H, s, *t*-Bu) and 1.44 (9H, s, *t*-Bu); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.4 (C=O), 156.2 (C=O), 79.8 (*C*(CH$_3$)$_3$), 79.5 (*C*(CH$_3$)$_3$), 47.0, 44.3, 40.1, 39.5, 31.8, 28.8 (C(*C*H$_3$)$_3$), 28.8 (C(*C*H$_3$)$_3$), 27.8 and 26.1; IR (neat)/cm^{-1} 3358 (N–H), 2975, 2932, 2867, 2359, 2340, 1687 (C=O), 1525, 1480, 1454, 1419, 1391, 1366, 1252, 1175, 1091, 1043, 1006, 870, 773 and 668; MS (CI) $[M+H]^+$ 346 (100), 272 (24), 246 (33), 172 (16) and 146 (20); HRMS (CI) $C_{17}H_{36}N_3O_4$ $[M+H]^+$ requires 346.27057, found 346.26975; Anal. Calcd $C_{17}H_{35}N_3O_4$ requires: C, 59.10; H, 10.21; N, 12.16. Found: C, 59.23; H, 10.13; N, 12.23.

5.1.1.9. 10β-[2-(N^4,N^8-Di-*tert*-butoxycarbonylspermidine)ethyl]deoxoartemisinin 14. A solution of 10β-(2-methanesulfonylethyl)deoxoartemisin **9** (146 mg, 0.37 mmol) and 4-[(3-aminopropyl)-*tert*-butoxycarbonyl-amino]-butyl}-carbamic acid *tert*-butyl ester **13** (550 mg, 1.59 mmol) in anhydrous benzene (10 mL) was stirred at 75 °C for 72 h. After 72 h, the solvent was removed under reduced pressure to give a pale yellow oil. Purification by flash column chromatography (silica gel, 10:45:45→20:40:40 MeOH/EtOAc/*n*-Hex) gave **14** (123 mg, 52%) as a pale yellow oil. Compound **14**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.42 (1H, s, H-12), 4.64 (1H, br s, NHBoc), 4.27 (1H, m, H-10), 3.25–3.08 (6H, m), 2.98–2.82 (2H, m), 2.75–2.60 (2H, m), 2.32 (1H, td, J = 13.7, 4.0 Hz, H-4α), 2.06–0.88 (46H, m) including 1.46 (9H, s, *t*-Bu), 1.45 (9H, s, *t*-Bu), 1.38 (3H, s, 3Me), 0.95 (3H, d, J = 6.2 Hz, 6Me) and 0.88 (3H, d, J = 7.5 Hz, 9Me); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.5 (C=O), 156.3 (C=O), 103.1, 88.5, 80.1, 79.8 (*C*(CH$_3$)$_3$), 79.5

($C(CH_3)_3$), 74.1, 48.2, 47.0, 46.1, 44.3, 43.4, 41.3, 36.1, 34.8, 30.6, 28.9 ($C(CH_3)_3$), 28.8 ($C(CH_3)_3$), 28.3, 27.8, 26.2, 25.2, 25.1, 20.1 and 13.5; IR (neat)/cm^{-1} 3362 (N–H), 2933, 2359, 2340, 1694 (C=O), 1519, 1455, 1418, 1366, 1251, 1172, 1043, 1012, 922, 876, 730 and 668; HRMS (ESI) $C_{34}H_{62}N_3O_8$ $[M+H]^+$ requires 640.4537, found 640.4536; Anal. Calcd $C_{34}H_{61}N_3O_8$ requires: C, 63.82; H, 9.61; N, 6.57. Found: C, 63.43; H, 10.10; N, 6.09.

5.1.1.10. 10β-[2-(N^1-Spermidine)ethyl]deoxoartemisinin trihydrochloride 15. To a stirring solution of 10β-[2-(N^4,N^8-di-*tert*-butoxycarbonylspermidine)ethyl]deoxoartemisinin **14** (100 mg, 0.16 mmol) in anhydrous 1,4-dioxane (2 mL) was added excess 4.0 M HCl in 1,4-dioxane (2 mL). After stirring for 4 h, the solvent was removed under reduced pressure to give **15** (84 mg, 98%) as a yellow, brittle, hygroscopic foam. Compound **15**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.50 (1H, s, H-12), 4.27 (1H, m, H-10), 3.30–3.00 (8H, m), 2.30–2.15 (3H, m), 2.15–0.89 (26H, m) including 1.82 (4H, m), 1.38 (3H, s, 3Me), 0.97 (3H, d, J = 6.1 Hz) and 0.89 (3H, d, J = 7.5 Hz, 9Me); ^{13}C NMR (100 MHz, $CDCl_3$) δ 101.7, 91.3, 81.2, 75.7, 54.6, 49.9, 47.4, 45.0, 44.6, 39.2, 36.1, 34.8, 32.5, 32.3, 25.1, 25.2, 24.1, 23.1, 22.8, 21.1, 20.2 and 13.7; IR (neat)/cm^{-1} 3390, 2927, 2794, 1709, 1657, 1608, 1522, 1454, 1231, 1165, 1076, 1016 and 753; HRMS (ESI) $C_{24}H_{46}N_3O_4$ $[M-(3HCl)+H]^+$ requires 440.3488, found 440.3483.

5.1.1.11. N^1,N^8-Di-*tert*-butoxycarbonylspermidine 16. To a stirring solution of spermidine (1.23 g, 8.47 mmol) in anhydrous THF (100 mL) at 0 °C was added Boc-ON (4.17 g, 16.94 mmol). After stirring for 4 h at 0 °C, the solvent was removed under reduced pressure and the residue was taken up in diethyl ether (100 mL) and washed with saturated aqueous NaOH (4 × 25 mL) until all yellow colouration was removed. The organic extracts were dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a white solid. Recrystallisation from isopropyl ether gave **16** (2.78 g, 95%) as a white crystalline solid. Compound **16**: mp 82–84 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.13 (1H, br s, NHBoc), 4.81 (1H, br s, NHBoc), 3.20 (2H, q, J = 6.1 Hz, H-1), 3.12 (2H, q, J = 5.7 Hz, H-8), 2.67 (2H, t, J = 6.6 Hz, H-3), 2.61 (2H, t, J = 6.6 Hz, H-5), 1.66 (2H, m, H-2), 1.60 (1H, br s, NH), 1.52 (4H, m, H-6 and H-7) and 1.44 (s, 18H, 2 × t-Bu); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.1 (C=O), 156.0 (C=O), 78.8 (2 × $C(CH_3)_3$), 49.8, 48.0, 39.5, 30.2, 28.8 (2 × $C(CH_3)_3$), 28.2 and 27.6; IR (Nujol)/cm^{-1} 3372 (N–H), 2924, 2853, 1686 (C=O), 1525, 1458, 1377, 1365, 1332, 1269, 1252, 1231, 1171, 1139, 1124, 1062, 1042, 1023, 987, 868 and 760; HRMS (CI); Anal. Calcd $C_{17}H_{35}N_3O_4$ requires: C, 59.10; H, 10.21; N, 12.16. Found: C, 58.94; H, 10.33; N, 12.08.

5.1.1.12. 10β-[2-(N^1,N^8-Di-*tert*-butoxycarbonylspermidine)ethyl]deoxoartemisinin 17. A solution of 10β-(2-methanesulfonylethyl)deoxoartemisin **9** (206 mg, 0.53 mmol) and N^1,N^8-di-*tert*-butoxycarbonylspermidine **16** (911 mg, 2.64 mmol) in anhydrous benzene (10 mL) was stirred at 75 °C for 48 h. After 48 h, the solvent was removed under reduced pressure to give a pale yellow oil. Purification by flash column chromatography (silica gel, 5:47.5:47.5 MeOH/EtOAc/*n*-Hex) gave **17** (182 mg, 54%) as a pale yellow oil. Compound **17**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.36 (1H, br s, NHBoc), 5.30 (1H, s, H-12), 4.82 (1H, br s, NHBoc), 4.13 (1H, m, H-10), 3.25–3.00 (4H, m), 2.68 (1H, sex, J = 7.5 Hz), 2.58–2.39 (6H, m), 2.33 (1H, td, J = 13.5, 4.1 Hz, H-4α), 2.15–0.87 (45H, m) including 1.44 (18H, s, 2 × t-Bu), 1.41 (3H, s, 3Me), 0.96 (3H, d, J = 6.0 Hz, 6Me) and 0.87 (3H, d, J = 7.5 Hz, 9Me); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.5 (2 × C=O), 103.6, 89.4, 81.5, 78.9 (2 × $C(CH_3)_3$), 74.2, 54.0, 52.8, 52.7, 44.8, 37.8, 36.9, 34.8, 30.6, 28.9 ($C(CH_3)_3$), 28.8 ($C(CH_3)_3$), 28.3, 27.1, 26.5, 25.2, 25.1, 20.1 and 13.5; IR (neat)/cm^{-1} 3358 (N–H), 2937, 2875, 1698 (C=O), 1518, 1454, 1391, 1366, 1269, 1251, 1102, 1056, 1012, 878 and 826; HRMS (ESI) $C_{34}H_{62}N_3O_8$ $[M+H]^+$ requires 640.4537, found 640.4554; Anal. Calcd $C_{34}H_{61}N_3O_8$ requires: C, 63.82; H, 9.61; N, 6.57. Found: C, 64.13; H, 9.97; N, 6.39.

5.1.1.13. 10β-[2-(N^4-Spermidine)ethyl]deoxoartemisinin trihydrochloride 18. To a stirring solution of 10β-[2-(N^1,N^8-di-*tert*-butoxycarbonylspermidine)ethyl]-deoxoartemisinin **17** (93 mg, 0.15 mmol) in anhydrous 1,4-dioxane (2 mL) was added excess 4.0 M HCl in 1,4-dioxane (2 mL). After stirring for 4 h, the solvent was removed under reduced pressure to give **18** (76 mg, 95%) as a yellow, brittle, hygroscopic foam. Compound **18:** ^{1}H NMR (400 MHz, MeOH-d_4) δ 5.30 (1H, s, H-12), 4.20 (1H, m, H-10), 3.46–3.18 (4H, m), 3.15–2.97 (4H, m), 2.27–2.08 (3H, m), 2.01–0.86 (28H, m) including 1.31 (3H, s, 3Me) and 1.00–0.86 (6H, m, 6Me and 9Me); ^{13}C NMR (100 MHz, $CDCl_3$) δ 102.3, 91.7, 81.7, 75.6, 54.6, 51.4, 51.3, 49.1, 44.6, 39.7, 38.0, 36.1, 33.5, 32.2, 29.4, 26.5, 26.3, 25.1, 23.9, 21.2, 21.1 and 13.7; IR (neat)/cm^{-1} 3380, 2935, 1696, 1612, 1464, 1391, 1084, 1000, 734 and 693; HRMS (ESI) $C_{24}H_{46}N_3O_4$ $[M-(3HCl)+H]^+$ requires 440.3488, found 440.3473.

5.1.1.14. *tert*-Butyl 3-aminopropylcarbamate 20. A solution of di-*tert*-butyl dicarbonate (15.43 g, 0.07 mol) in anhydrous 1,4-dioxane (200 mL) was added over 3 h to a stirring solution of 1,3-diaminopropane (47 mL, 0.57 mol) in 1,4-dioxane (200 mL). After stirring overnight the solvent was removed under reduced pressure. Water (250 mL) was added to the residue and the insoluble bis-substituted product (0.48 g, 2%) collected by filtration. The filtrate was extracted with CH_2Cl_2 (5 × 150 mL). The organic extracts were dried over Na_2SO_4, filtered and concentrated under reduced pressure to give **20** (11.36 g, 93%) as a colourless oil. Compound **20**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.00 (1H, br s, NHBoc), 3.20 (2H, q, H-1, J = 6.3 Hz), 2.76 (2H, t, H-3, J = 6.6 Hz),1.62 (2H, q, H-2, J = 6.7 Hz), 1.44 (9H, s, t-Bu) and 1.34 (2H, br s, NH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.5 (C=O), 79.4 ($C(CH_3)_3$), 40.1, 38.8, 33.9 and 28.8 ($C(CH_3)_3$); IR (Nujol)/cm^{-1} 3365 (N–H), 2930, 2854, 2362, 2343, 1685 (C=O), 1588, 1528, 1497, 1477, 1465, 1440, 1392, 1366, 1329, 1286, 1250, 1171, 1113, 1054, 1022, 877, 843, 820, 777, 717 and 668; MS (CI) $[M+H]^+$ 175 (21), 119 (15), 118 (39), 101 (100) and 75 (59); HRMS (CI) $C_8H_{19}N_2O_2$ $[M+H]^+$ requires 175.14465, found 175.14496; Anal. Calcd $C_8H_{18}N_2O_2$ requires: C, 55.15; H, 10.41; N, 16.08. Found: C, 55.07; H, 10.89; N, 15.97.

5.1.1.15. [3-(3-Cyano-propylamino)-propyl]-carbamic acid *tert*-butyl ester 21. Potassium fluoride on Celite (loading: 50 wt %) was prepared by shaking KF (10 g) with Celite (10 g) in water (250 mL). The water was removed under reduced pressure and the KF–Celite obtained was shaken in MeCN (100 mL), filtered and washed with MeCN (2 × 50 mL). The KF–Celite was then dried in a desiccator until constant weight (approx 20 g). A solution of *tert*-butyl 3-aminopropylcarbamate **20** (2.44 g, 14.00 mmol), 4-bromobutyronitrile (1.4 mL, 14.00 mmol) and KF–Celite (10 g) in anhydrous MeCN (50 mL) was stirred at 45 °C for 24 h. The mixture was filtered and the KF–Celite washed with MeCN (2 × 25 mL). The filtrate was concentrated under reduced pressure to give a yellow oil. Purification by flash column chromatography (silica gel, 15:85 MeOH/CH_2Cl_2) gave **21** (1.74 g, 51%) as a pale yellow oil. Compound **21**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.00 (1H, br s, NHBoc), 3.20 (2H, q, J = 6.3 Hz), 2.73 (2H, t, J = 6.6 Hz), 2.67 (2H, t, J = 6.5 Hz), 2.45 (2H, t, J = 7.2 Hz, CH_2CN), 1.83 (2H, t, J = 6.9 Hz), 1.64 (2H, t, J = 6.5 Hz) and 1.44 (9H, s, t-Bu); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.0 (C=O), 119.6 (C≡N), 79.0 ($C(CH_3)_3$), 47.8, 47.2, 41.8, 38.9, 29.8, 28.8 ($C(CH_3)_3$), 18.1 and 14.8; IR (neat)/cm^{-1} 3318 (N–H), 2975, 2934, 2359, 2340, 2245 (C≡N), 1694 (C=O), 1524, 1455, 1391, 1366, 1277, 1252, 1171, 1042, 857, 780, 735 and 668; MS (CI) $[M+H]^+$ 242 (6), 185 (13), 168 (35), 125 (39),

101 (100), 85 (35) and 75 (37); HRMS (CI) $C_{12}H_{24}N_3O_2$ $[M+H]^+$ requires 242.18686, found 242.18701; Anal. Calcd $C_{12}H_{23}N_3O_2$ requires: C, 59.72; H, 9.61; N, 17.41. Found: C, 59.53; H, 9.85; N, 17.05.

5.1.1.16. (3-[*tert*-Butoxycarbonyl-(3-cyano-propyl)-amino]-propyl)-carbamic acid *tert*-butyl ester 22. A solution of di-*tert*-butyl dicarbonate (1.45 g, 6.63 mmol) in anhydrous CH_2Cl_2 (25 mL) was added over 1 h to a stirring solution of [3-(3-cyano-propylamino)-propyl]-carbamic acid *tert*-butyl ester **21** (1.60 g, 6.63 mmol) in anhydrous CH_2Cl_2 (50 mL). After stirring for 1 h, the solvent was removed under reduced pressure to give a clear oil. Purification by flash column chromatography (silica gel, 25:75 EtOAc/*n*-Hex) gave **22** (2.11 g, 93%) as a colourless oil. Compound **22**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.15 (1H, br s, NHBoc), 3.35–3.22 (4H, m), 3.11 (2H, q, J = 6.2 Hz), 2.35 (2H, t, J = 7.2 Hz), 1.89 (2H, quintet, J = 7.0 Hz), 1.72–1.63 (2H, m), 1.48 (9H, s, *t*-Bu) and 1.44 (9H, s, *t*-Bu); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.0 (C=O), 155.7 (C=O), 119.2 (C≡N), 80.7 ($C(CH_3)_3$), 79.2 ($C(CH_3)_3$), 47.5, 45.5, 37.5, 28.8 ($C(CH_3)_3$), 28.8 ($C(CH_3)_3$), 24.4 and 14.6; IR (neat)/cm^{-1} 3362 (N–H), 2976, 2933, 2361, 2247 (C≡N), 1697 (C=O), 1517, 1479, 1454, 1420, 1391, 1367, 1251, 1167, 1085, 1057, 1041, 1001, 865, 775 and 668; MS (CI) $[M+H]^+$ 342 (37), 242 (100), 186 (69), 168 (46), 142 (92) and 125 (73); HRMS (ESI) $C_{17}H_{31}N_3O_4Na$ $[M+Na]^+$ requires 364.2212, found 364.2214; Anal. Calcd $C_{17}H_{31}N_3O_4$ requires: C, 59.80; H, 9.15; N, 12.31. Found: C, 59.72; H, 9.63; N, 12.01.

5.1.1.17. (4-Amino-butyl)-(3-*tert*-butoxycarbonylamino-propyl)-carbamic acid *tert*-butyl ester 23. Lithium aluminium hydride [3.5 M in THF/toluene] (0.87 mmol) was added dropwise to a stirring solution of (3-[*tert*-butoxycarbonyl-(3-cyano-propyl)-amino]-propyl)-carbamic acid *tert*-butyl ester **22** (296 mg, 0.87 mmol) in diethyl ether (50 mL) at 0 °C. After stirring at 0 °C for 3 h, the reaction mixture was quenched by the sequential dropwise addition of water (0.5 mL), 15% aq NaOH (0.5 mL) and finally water (1.5 mL). The resulting suspension was filtered through Celite®, and the solid washed with diethyl ether (3 × 25 mL). The combined filtrates were washed with brine (10 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a pale yellow oil. Purification by flash column chromatography (silica gel, 10:90→15:85 MeOH/CH_2Cl_2) gave **23** (206 mg, 69%) as a colourless oil. Compound **23**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.30 (1H, br s, NHBoc), 3.30–3.04 (6H, m), 2.73 (2H, t, J = 6.8 Hz, CH_2NH_2), 2.05 (2H, br s, NH_2), 1.74–1.52 (4H, m), 1.46 (9H, s, *t*-Bu) and 1.44 (9H, s, *t*-Bu); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.4 (C=O), 156.1 (C=O), 79.9 ($C(CH_3)_3$), 79.4 ($C(CH_3)_3$), 47.2, 44.3, 42.1, 38.0, 30.9, 28.8 ($C(CH_3)_3$), 28.8 ($C(CH_3)_3$), 26.4 and 25.8; IR (neat)/cm^{-1} 3362 (N–H), 2975, 2932, 2867, 2360, 2342, 1690 (C=O), 1521, 1479, 1455, 1420, 1391, 1366, 1275, 1251, 1172, 1083, 1039, 999, 923, 869, 773, 755, 734 and 668; MS (CI) $[M+H]^+$ 346 (100), 272 (27), 246 (60), 172 (31) and 146 (45); HRMS (CI) $C_{17}H_{36}N_3O_4$ $[M+H]^+$ requires 346.27057, found 346.27006; Anal. Calcd $C_{17}H_{35}N_3O_4$ requires: C, 59.10; H, 10.21; N, 12.16. Found: C, 58.87; H, 10.37; N, 12.07.

5.1.1.18. 10β-[2-(N^1,N^4-Di-*tert*-butoxycarbonylspermidine)ethyl]deoxoartemisinin 24. A solution of 10β-(2-methanesulfonylethyl)deoxoartemisin **9** (100 mg, 0.26 mmol) and (4-amino-butyl)-(3-*tert*-butoxycarbonylamino-propyl)-carbamic acid *tert*-butyl ester **23** (200 mg, 0.58 mmol) in anhydrous benzene (10 mL) was stirred at 75 °C for 72 h. After 72 h, the solvent was removed under reduced pressure to give a pale yellow oil. Purification by flash column chromatography (silica gel, 10:45:45→20:40:40 MeOH/EtOAc/*n*-Hex) gave **24** (51 mg, 50%) as a pale yellow oil. Compound 24: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.41 (1H, s, H-12), 4.78 (1H, br s, NHBoc), 4.35 (1H, m, H-10), 3.35–3.03 (6H, m), 2.70–2.58 (4H, m), 2.32 (m, 1H, td, J = 14.1, 4.0 Hz, H-4α), 2.24–0.87 (46H, m) including 1.46 (9H, s, *t*-Bu), 1.44 (9H, s, *t*-Bu), 1.39 (3H, s, 3Me), 0.96 (3H, d, J = 6.0 Hz, 6Me) and 0.87 (3H, d, J = 7.5 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.5 (C=O), 156.1 (C=O), 103.5, 89.7, 80.3, 79.4 ($C(CH_3)_3$), 78.4 ($C(CH_3)_3$), 74.9, 52.6, 48.6, 46.9, 44.6, 44.3, 38.1, 37.7, 36.9, 34.8, 30.7, 30.42, 29.7, 28.8 ($C(CH_3)_3$), 28.8 ($C(CH_3)_3$), 27.4, 26.4, 25.3, 25.1, 20.5 and 13.1; IR (neat)/cm^{-1} 3361 (N–H), 2933, 2874, 2359, 1695 (C=O), 1519, 1454, 1366, 1251, 1172, 1056, 1012, 876 and 826; HRMS (ESI) $C_{34}H_{62}N_3O_8$ $[M+H]^+$ requires 640.4537, found 640.4532; Anal. Calcd $C_{34}H_{61}N_3O_8$ requires: C, 63.82; H, 9.61; N, 6.57. Found: C, 63.71; H, 9.97; N, 6.32.

5.1.1.19. 10β-[2-(N^8-Spermidine)ethyl]deoxoartemisinin trihydrochloride 25. To a stirring solution of 10β-[2-(N^1,N^4-di-*tert*-butoxycarbonylspermidine)ethyl]-deoxoartemisinin **24** (32 mg, 0.05 mmol) in anhydrous 1,4-dioxane (2 mL) was added excess 4.0 M HCl in 1,4-dioxane (2 mL). After stirring for 4 h, the solvent was removed under reduced pressure to give **25** (27 mg, 100%) as a yellow, brittle, hygroscopic foam. Compound **25:** ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.47 (1H, s, H-12), 4.27 (1H, m, H-10), 3.28–3.00 (8H, m), 2.30 (1H, td, J = 14.3, 4.0 Hz, H-4α), 2.22–2.09 (2H, m), 2.09–0.92 (28H, m) including 1.36 (3H, s, 3Me), 0.98 (3H, d, J = 6.4 Hz, 6Me) and 0.92 (3H, d, J = 7.6 Hz, 9Me); ^{13}C NMR (100 MHz, $CDCl_3$) δ 105.4, 91.4, 83.1, 75.6, 54.6, 49.1, 46.8, 46.4, 44.6, 39.4, 38.8, 33.2, 32.2, 31.5, 29.7, 28.9, 28.2, 27.0, 26.6, 26.2, 25.1, 24.2, 21.3 and 14.0; IR (neat)/cm^{-1} 3392, 2925, 1709, 1605, 1454, 1107, 1007, 881 and 744; HRMS (ESI) $C_{24}H_{46}N_3O_4$ $[M-(3HCl)+H]^+$ requires 440.3488, found 440.3487.

5.1.1.20. 10β-(2-Oxoethyl)deoxoartemisinin 26. Ozone was bubbled through a solution of **7** (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 **26** (1.86 g, 76%) as a white solid. Compound **26**: ^{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, CH_2CHO), 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.

5.1.1.21. 10β-(2-Carboxyethyl)deoxoartemisinin 27. NaH_2PO_4 (203 mg, 1.69 mmol) was added to a stirring solution of **26** (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. One molar 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 **27** (2.12 g, 100%) as a white brittle foam. Compound **27**: ^{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.

5.1.1.22. 10β-[2-(N^4,N^8-Di-*tert*-butoxycarbonylspermidine)-2-oxoethyl]deoxoartemisinin 28. Compound 13 (369 mg, 1.07 mmol) and *N*-methylmorpholine (0.29 mL, 2.67 mmol) were added to a solution of **27** (291 mg, 0.89 mmol) in anhydrous CH_2Cl_2 (10 mL) and the solution was stirred at 0 °C for 10 min. To this solution were added EDC hydrochloride (205 mg, 1.07 mmol) and HOBt (144 mg, 1.07 mmol) and the mixture allowed to warm to room temperature and stirred overnight. The mixture was then diluted with CH_2Cl_2 (40 mL) and washed with 2.0 M aq HCl (4 × 20 mL), saturated aq $NaHCO_3$ (4 × 20 mL) and brine (20 mL). The organic phase was dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a colourless oil. Purification by flash column chromatography (silica gel, 65:35 EtOAc/*n*-Hex) gave **28** (386 mg, 66%) as a white solid. Compound **28**: mp 55–58 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.08 (1H, br s, NH), 5.39 (1H, s, H-12), 4.76 (1H, m, H-10), 4.50 (1H, br s, NH), 3.42–3.05 (8H, m), 2.70–2.44 (2H, m, H-9 and C(O)CH*H*), 2.39–2.25 (2H, m, H-4α and C(O)CH*H*), 2.16–1.91 (2H, m), 1.86–1.44 (11H, m), 1.45 (9H, s, *t*-Bu), 1.44 (9H, s, *t*-Bu), 1.39 (3H, s, 3Me), 1.35–1.18 (3H, m), 0.97 (3H, d, J = 5.4 Hz, 6Me) and 0.87 (3H, d, J = 7.5 Hz, 9Me); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.5, 155.9, 155.5, 102.8, 89.8, 80.7, 79.3, 78.9, 70.5, 51.8, 46.7, 45.0, 44.1, 43.6, 43.3, 37.4, 37.0, 36.4, 34.1, 33.9, 30.2, 28.9, 28.3, 27.9, 27.3, 25.7, 24.7, 21.0, 19.9 and 13.9; IR (neat)/cm^{-1} 3726, 3336, 2933, 1680 (C=O), 1529, 1419, 1367, 1250, 1169, 1093, 1047, 1007, 875, 771 and 732; HRMS (ES) $C_{34}H_{59}N_3NaO_9$ [M+Na]$^+$ requires 676.4149, found 676.4158; Anal. Calcd $C_{34}H_{59}N_3O_9$ requires: C, 62.46; H, 9.27; N, 6.43. Found: C, 62.84; H, 9.14; N, 6.40.

5.1.1.23. 10β-[2-(N^1,N^8-Di-*tert*-butoxycarbonylspermidine)-2-oxoethyl]deoxoartemisinin 29. Compound 16 (306 mg, 0.87 mmol) and *N*-methylmorpholine (0.24 mL, 2.22 mmol) were added to a solution of **27** (241 mg, 0.74 mmol) in anhydrous CH_2Cl_2 (10 mL) and the solution was stirred at 0 °C for 10 min. To this solution were added EDC hydrochloride (170 mg, 0.87 mmol) and HOBt (120 mg, 0.87 mmol) and the mixture allowed to warm to room temperature and stirred overnight. The mixture was then diluted with CH_2Cl_2 (40 mL) and washed with 2.0 M aq HCl (4 × 20 mL), saturated aq $NaHCO_3$ (4 × 20 mL) and brine (20 mL). The organic phase was dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a colourless oil. Purification by flash column chromatography (silica gel, 65:35 EtOAc/*n*-Hex) gave **29** (344 mg, 71%) as a white solid. Compound **29**: mp 56–58 °C; ^{1}H NMR (400 MHz, $CDCl_3$). Two rotamers δ 5.68 (1H, br t, J = 5.8 Hz, NH), 5.33 (1/3H, s, H-12), 5.31 (2/3H, s, H-12), 4.98–4.84 (1H, m, H-10), 4.73 (1H, br s, NH), 3.67–3.49 (1H, m), 3.48–3.25 (3H, m), 3.25–2.97 (5H, m), 2.79–2.63 (2H, m, H-9 and C(O)CH*H*), 2.43–2.25 (2H, m, H-4α and C(O)CH*H*), 2.08–1.89 (2H, m), 1.87–1.75 (2H, m), 1.75–1.48 (8H, m), 1.44 (2/3 × 18H, s, 2 × *t*-Bu), 1.42 (1/3 × 18H, s, 2×*t*-Bu), 1.39 (1/3 × 3H, 3Me), 1.37 (2/3 × 3H, 3Me), 1.35–1.20 (3H, m), 0.97 (3H, d, J = 5.8 Hz, 6Me) and 0.88 (3H, d, J = 7.5 Hz, 9Me); ^{13}C NMR (100 MHz, $CDCl_3$). Two rotamers δ 171.7/170.9, 156.2/156.0/155.9 (2C), 102.9/102.7, 89.8/89.6, 80.7/80.7, 79.2/78.8/78.5 (2C), 71.3/70.8, 51.9/51.8, 47.3, 45.6/45.0, 43.9/43.8, 42.0, 40.1/39.9, 37.5, 36.7, 36.5/36.5, 35.1/34.8, 34.3/34.3, 29.9, 29.4, 28.4, 28.3, 27.6/27.5, 26.0, 25.7/25.7, 24.8/24.7, 20.0/20.0 and 12.7/12.6; IR (neat)/cm^{-1} 3730, 3626, 3334, 2933, 1698 (C=O), 1630 (C=O), 1514, 1452, 1365, 1250, 1169, 1049, 1007, 941, 875 and 733;HRMS (ESI) $C_{34}H_{59}N_3NaO_9$ [M+Na]$^+$ requires 676.4149, found 676.4176; Anal. Calcd $C_{34}H_{59}N_3O_9$ requires: C, 62.46; H, 9.27; N, 6.43. Found: C, 61.99; H, 9.27; N, 6.25.

5.1.1.24. 10β-[2-(N^1,N^4-Di-*tert*-butoxycarbonylspermidine)-2-oxoethyl]deoxoartemisinin 30. Compound 23 (443 mg, 1.28 mmol) and *N*-methylmorpholine (0.35 mL, 3.21 mmol) were added to a solution of **27** (349 mg, 1.07 mmol) in anhydrous CH_2Cl_2 (10 mL) and the solution was stirred at 0 °C for 10 min. To this solution were added EDC hydrochloride (246 mg, 1.28 mmol) and HOBt (173 mg, 1.28 mmol) and the mixture allowed to warm to room temperature and stirred overnight. The mixture was then diluted with CH_2Cl_2 (40 mL) and washed with 2.0 M aq HCl (4 × 20 mL), saturated aq $NaHCO_3$ (4 × 20 mL) and brine (20 mL). The organic phase was dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a colourless oil. Purification by flash column chromatography (silica gel, 65:35 EtOAc/*n*-Hex) gave **30** (433 mg, 62%) as a white solid. Compound **30**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.10 (1H, br s, NH), 5.38 (1H, s), 5.04 (1H, br s, NH), 4.88–4.67 (1H, m, H-10), 3.48–3.32 (1H, m), 3.32–3.02 (8H, m), 2.65–2.46 (2H, m, H-9 and C(O)CH*H*), 2.40–2.26 (2H, m, H-4α and C(O)CH*H*), 2.12–2.02 (1H, m), 2.02–1.93 (1H, m), 1.85–1.43 (10H, m), 1.46 (9H, s, *t*-Bu), 1.44 (9H, s, *t*-Bu), 1.39 (3H, s, 3Me), 1.36–1.16 (3H, m), 0.98 (3H, d, J = 5.0 Hz, 6Me) and 0.88 (3H, d, J = 7.5 Hz, 9Me); NMR (100 MHz, $CDCl_3$). Two rotamers δ 171.3/170.1, 155.8, 155.2, 104.2/102.6, 91.5/89.8, 80.5, 79.2, 78.5, 69.7/69.5, 51.5/51.4, 46.3/46.0, 44.0/43.4, 43.2, 38.7/38.6, 37.5/37.1, 37.3, 36.2/35.8, 34.0/33.8, 30.1, 28.6/28.0, 28.2 (2C), 26.6, 25.8/25.6, 25.8/25.2, 24.5, 24.5, 20.0/19.8 and 13.8; IR (neat)/cm^{-1} 3730, 3626, 3342, 2929, 1678 (C=O), 1529, 1419, 1367, 1250, 1167, 1092, 1049, 1009, 876 and 733; HRMS (ESI) $C_{34}H_{59}N_3NaO_9$ [M+Na]$^+$ requires 676.4149, found 676.4178; Anal. Calcd $C_{34}H_{59}N_3O_9$ requires: C, 62.46; H, 9.27; N, 6.43. Found: C, 61.85; H, 9.29; N, 6.29.

5.1.1.25. 10β-[2-N^1-Spermidine)-2-oxoethyl]deoxoartemisinin dihydrochloride 31. To a stirring solution of **28** (150 mg, 0.23 mmol) in anhydrous 1,4-dioxane (2 mL) was added excess 4.0 M HCl in 1,4-dioxane (2 mL). After stirring for 3 h, the solvent was removed under reduced pressure to give a sticky yellow semisolid. This reside was triturated with diethyl ether to give a bright yellow solid. Filtration of the solid under a nitrogen atmosphere and washing with diethyl ether gave **31** (108 mg, 89%) as a bright yellow hygroscopic solid. Compound **31**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.53 (1H, s, H-12), 4.50 (1H, m, H-10), 3.20–2.95 (8H, m), 2.85–2.70 (2H, m, H-9 and C(O)CH*H*), 2.41–2.27 (2H, m, H-4α and C(O)CH*H*), 2.16–1.38 (14H, m), 1.33 (3H, s, 3Me), 1.31–1.19 (2H, m), 0.98 (3H, d, J = 6.3 Hz, 6Me) and 0.91 (3H, d, J = 7.3 Hz, 9Me); ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.0, 105.0, 90.0, 82.3, 75.7, 54.1, 48.3, 46.1, 45.8, 40.1, 38.2, 37.6, 37.4, 36.3, 35.8, 30.9, 27.6, 26.3, 25.8, 25.6, 24.4, 23.2, 20.7 and 13.8; IR (neat)/cm^{-1} 3830, 3730, 3624, 2952, 1709, 1645 (C=O), 1547, 1458, 1379, 1265, 1205, 1092, 1045, 876 and 731; HRMS (ESI) $C_{24}H_{44}N_3O_5$ [M– (2HCl)+H]$^+$ requires 454.3281, found 454.3301.

5.1.1.26. 10β-[2-N^4-Spermidine)-2-oxoethyl]deoxoartemisinin dihydrochloride 32. To a stirring solution of **28** (287 mg, 0.44 mmol) in anhydrous 1,4-dioxane (2 mL) was added excess 4.0 M HCl in 1,4-dioxane (2 mL). After stirring for 3 h the solvent was removed under reduced pressure to give a sticky yellow semisolid.

This reside was triturated with diethyl ether to give a bright yellow solid. Filtration of the solid under a nitrogen atmosphere and washing with diethyl ether gave **32** (214 mg, 93%) as a bright yellow hygroscopic solid. Compound **32**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.25 (1H, s, H-12), 4.71–4.58 (1H, m, H-10), 3.9–3.54 (4H, m), 3.06–2.94 (4H, m), 2.81–2.64 (2H, m, H-9 and C(O)CH*H*), 2.46–2.26 (2H, m, H-4α and C(O)CH*H*), 2.05–1.55 (14H, m), 1.50 (3H, s, 3Me), 1.34–1.16 (2H, m) and 0.95–0.89 (6H, m, 6Me and 9Me); ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.1, 109.1, 98.0, 84.3, 68.6, 48.2, 46.3, 42.5, 41.4, 40.2, 37.5, 36.5, 36.0, 35.4, 35.2, 30.4, 26.6, 26.3, 26.1, 25.6, 23.6, 22.9, 18.8 and 12.6; IR (neat)/cm^{-1} 3838, 3730, 3624, 2949, 1616 (C=O), 1462, 1383, 1095, 1005 and 729; HRMS (ESI) $C_{24}H_{44}N_3O_5$ [M−(2HCl)+H]$^+$ requires 454.3281, found 454.3320.

5.1.1.27. 10β-[2-*N*^{8}-Spermidine)-2-oxoethyl]deoxoartemisinin dihydrochloride 33. To a stirring solution of **28** (227 mg, 0.35 mmol) in anhydrous 1,4-dioxane (2 mL) was added excess 4.0 M HCl in 1,4-dioxane (2 mL). After stirring for 3 h, the solvent was removed under reduced pressure to give a sticky yellow semisolid. This reside was triturated with diethyl ether to give a bright yellow solid. Filtration of the solid under a nitrogen atmosphere and washing with diethyl ether gave **33** (174 mg, 95%) as a bright yellow hygroscopic solid. Compound **33**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09 (1H, s, C(O)N*H*), 5.54 (1H, s, H-12), 4.53 (1H, m, H-10), 3.36 (1H, dd, J = 6.7, 13.7 Hz, C(O)NHCH*H*), 3.21 (1H, dd, J = 6.7, 13.7 Hz, C(O)NHCH*H*), 3.16–3.03 (6H, m), 2.81–2.66 (2H, m, H-9 and C(O)CH*H*), 2.35–2.25 (2H, m, H-4α and C(O)CH*H*), 2.17–2.02 (4H, m), 1.93 (1H, m), 1.87–1.39 (9H, m), 1.34 (3H, s, 3Me), 1.29–1.14 (2H, m), 0.98 (3H, d, J = 6.4 Hz, 6Me) and 0.91 (3H, d, J = 7.5 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.1, 105.4, 90.6, 82.7, 75.7, 54.5, 49.0, 46.4, 46.2, 39.6, 38.7, 38.4, 38.1, 37.9, 36.2, 31.6, 27.8, 26.6, 26.3, 26.0, 25.8, 24.5, 21.1 and 14.2; IR (neat)/cm^{-1} 3834, 3732, 3624, 2954, 1645 (C=O), 1547, 1456, 1379, 1265, 1045 and 731; HRMS (ESI) $C_{24}H_{44}N_3O_5$ [M−(2HCl)+H]$^+$ requires 454.3281, found 454.3290.

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), 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 leukaemia 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 haemocytometer 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.[48] 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 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 strain of *P. falciparum* the following protocol was employed. Parasites were maintained in continuous culture using the method of Jensen and Trager.[49] 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.[50] 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% haematocrit) 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.

Acknowledgements

We would like to thank Ultrafine Chemicals (now SAFC, Synergy House, Guildhall Close, Manchester UK) for an industrial case award to J.C. and Dr. Ed Irving, Dr. Feo Scheinmann and Dr. Adrian Higson for helpful discussion concerning the synthetic chemistry.

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