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Rationale Design of Biotinylated Antimalarial Endoperoxide Carbon Centered Radical Prodrugs for Applications in Proteomics

Victoria Barton,[†] Steven A. Ward,[‡] James Chadwick,[†] Alasdair Hill,[‡] and Paul M. O'Neill*,[†]

[†]*Department of Chemistry, University of Liverpool, Oxford Street, Liverpool L697ZD, U.K., and*
[‡]*Liverpool School of Tropical Medicine, University of Liverpool, Pembroke Place, Liverpool L35QA, U.K.*

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The semisynthetic artemisinin derivatives such as artesunate and artemether, along with the fully synthetic endoperoxide antimalarials (e.g., OZ277, *Nature* **2004**, *430*, 900−904), are believed to mediate their antimalarial effects by iron-induced formation of carbon-centered radicals capable of alkylating heme and/or protein. Here, we describe the design and synthesis of a series of biotinylated endoperoxide probe molecules for use in proteomic studies. The target molecules include derivatives of the artemisinin and OZ families, and we demonstrate that these conjugates express nanomolar in vitro activity versus cultured strains of *Plasmodium falciparum*. We also describe the synthesis of chemically cleavable linked conjugates designed to enable mild elution of labeled proteins during target protein identification.

Introduction

Endoperoxide antimalarials are now front-line replacements for traditional antimalarial remedies such as chloroquine and the antifolates, which have lost their efficacy due to the spread of the multidrug resistant mutants of the *Plasmodium* parasite.[1,2] The erratic supply and expense of artemisinin, however, has forced efforts toward simpler synthetic peroxides such as the tetraoxanes and trioxolanes.[3]

Inside the malaria parasite, it is accepted that the endoperoxide bridge is cleaved by a source of iron which releases carbon centered radicals[4] that can alkylate parasitic proteins,[5,6] the nature of which remain controversial (Figure 1).

Several studies have shown radiolabeled artemisinin can react covalently with several parasitic proteins.[5,6] Autoradiograms of SDS−polyacrylamide gels showed that six malarial proteins were radiolabeled by three different endoperoxides: arteether, DHA,[a] and arteflene. The labeling occurred at physiological concentration of the drug and was not stage or strain specific. Uninfected erythrocytes and controls treated with the inactive analogue deoxyarteether did not contain any labeled proteins.[7]

Within our research, we have focused on the preparation of activity based probes of artemisinin and trioxolane incorporating a biotin moiety for streptavidin affinity "pull down" of covalently tagged target proteins. For effective labeling of target proteins by the drug probe and successive isolation of the protein through biotin-streptavidin binding, the linkage between the active portion of the probe molecule and biotin **2** must be stable in culture medium.

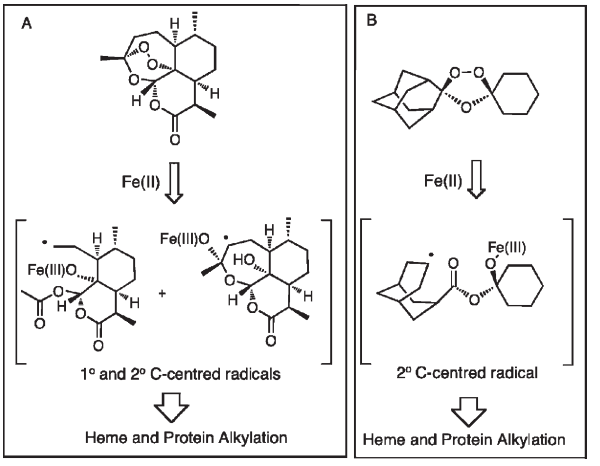

Figure 1. Iron mediated fragmentation of endoperoxides to carbon centered radicals which react with parasite proteins.

Results and Discussion

Previous studies have found the carbamate−biotin bond to be the most stable linkage followed by amide.[8] Therefore, the biotinylated artemisinin carbamate was prepared using an excess of primary alcohol, **1**, biotin **2**, diphenylphosphoryl azide (DPPA), and triethylamine in a modified Curtius rearrangement to give **3** in 68% yield (Scheme 1).

Because trioxolanes have been shown to generate a secondary carbon centered radical species predominantly within the adamantyl framework (Figure 1),[1] we have synthesized an adamantyl substituted biotinylated trioxolane. Recent evidence suggests that functionalizing this position may render the molecule inactive.[9] It was our aim to investigate if functionalization does indeed destroy activity, and if this is not the case, design a number of adamantyl functionalized trioxolanes to determine structure−activity relationships.

*To whom correspondence should be addressed. Phone: 0151 794 3553. Fax: +44 (0)151 794 3588. E-mail: p.m.oneill01@liv.ac.uk.

[a]Abbreviations: OZ, ozonide; SDS, sodium dodecyl sulfate; DHA, dihydroartemisinin; Fmoc, fluorenylmethyloxycarbonyl; Boc, tertiarybutoxycarbonyl.

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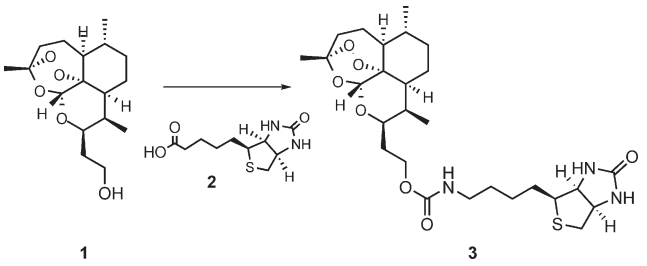
The preparation of adamantyl-substituted trioxolanes was achieved via Griesbaum co-ozonolysis (Scheme 2).[10] 5-Hydroxy adamantanone **4** was functionalized via Koch−Haaf carbonylation, which utilized fuming sulphuric acid (30%), followed by methanol quench to give the substituted adamantanone methyl ester **6** in 60% yield.[11,12]

The coupling partner *N*−*O*-methyl cyclohexanone oxime **7** was prepared in one step from cyclohexanone **5** and *O*-methylhydroxylamine hydrochloride in methanol.[13] The coupling of the two fragments was achieved via ozonolysis in pentane, conditions reported previously by Griesbaum,[14] to yield trioxolane **8** as a mixture of diastereoisomers in 30% yield. Reduction to the primary alcohol **9** was achieved in good yield using a combination of lithium borohydride and 10 mol % lithium triethylborohydride, followed by reaction with **2** in a modified Curtius reaction. However, this reaction failed to give any product, possibly due to the bulky nature of the adamantyl ring (Scheme 2). In an alternate strategy, ester **8** was hydrolyzed to carboxylic acid **11** using 15% aq NaOH Boc protected benzyl amine **12**[15] or Fmoc amine **13** was incorporated into trioxolane **11** via standard peptide coupling conditions.

Attempted removal of the Boc protecting group of **14** with mild acid (1M) caused a Hock-type fragmentation[10] to occur (Supporting Information). Because the trioxolanes are more stable to base than acid,[10] deprotection of **15** using piperidine did not cause any fragmentation of trioxolane amine **16** and therefore was the preferred route to this intermediate.

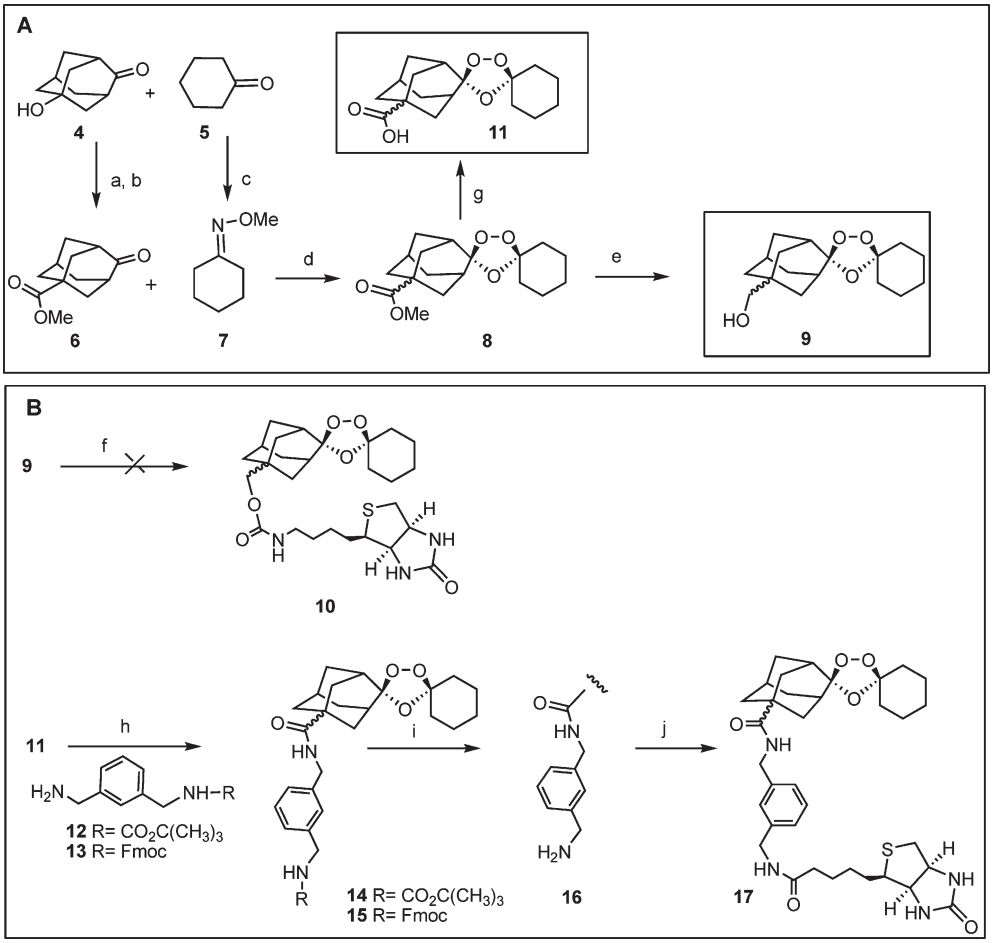
In the final step, amine **16** was coupled to **2** using standard coupling conditions to give biotinylated trioxolane **17** albeit in a low yield. The antimalarial activity of artemisinin and trioxolane biotinylated bioprobes (**3** and **17**, respectively) were evaluated against chloroquine sensitive 3D7 *P. falciparum*

Scheme 1. Synthesis of Biotinylated Artemisinin **3**[a]



[a] Reagents and conditions: biotin **2** (1.0 equiv), alcohol **1** (2.7 equiv), DPPA (1.0 equiv), Et_3N (1.0 equiv), anhyd MeCN, 55 °C, 24 h. Yield **3**, 68%.

Scheme 2. (A) Key Building Blocks **9** and **11** and (B) Attempted Synthesis of Carbamate **10** and Synthesis of **17**[a]



[a]Reagent and conditions; (a) H_2SO_4 (30%, 8.3 equiv), HCOOH (5.2 equiv), 60 °C, 2 h; (b) MeOH, 0 °C, 2 h, yield **6**, 60%; (c) NH_2·OMe·HCl (1.5 equiv), anhyd pyridine (1.5 equiv), MeOH, rt, 24 h, yield **7**, 80%; (d) O_3, cyclohexyl *O*-methyl oxime **7** (1.0 equiv), pentane, 0 °C, 3 h, yield **8**, 30%; (e) $LiBH_4$ (1.0 equiv), $LiBH(Et)_3$ (1 M in THF (0.1 equiv)), rt, 4 h, yield **9**, 73%; (f) biotin **2** (1.0 equiv), alcohol **9** (1.0 equiv), DPPA (1.0 equiv), Et_3N (1.0 equiv), MeCN, 50 °C, 24 h; (g) NaOH (15%), EtOH, rt, 3 h, yield **11**, 83%; (h) EDC·HCl (1.5 equiv), HOBt (1.2 equiv), NMM (2.5 equiv), DMF, Fmoc amine **12** (1.5 equiv), DMF, yield **15**, 78%; (i) piperidine (10.0 equiv), DMF, yield **15**, 54%; (j) EDC·HCl (1.5 equiv), HOBt (1.2 equiv), biotin (1.0 equiv), NMM (2.5 equiv), DMF, 18 h, 0 °C, yield **17**, 5%.

(Table 1). Both biotinylated endoperoxides demonstrated significant antimalarial activity and therefore were ideal antimalarial probes for protein labeling. In addition to biotinylated trioxolane **17**, several other adamantyl substituted trioxolane intermediates were tested for antimalarial activity.

Contrary to recent results which suggested that adamantyl substituted trioxolanes may be too hindered to exhibit significant antimalarial activity,[9] we found these trioxolanes displayed excellent activities. Our findings were consistent with a general SAR trend for this class of antimalarial peroxides:

Table 1. Antimalarial Activities against 3D7 *P. falciparum* Strain

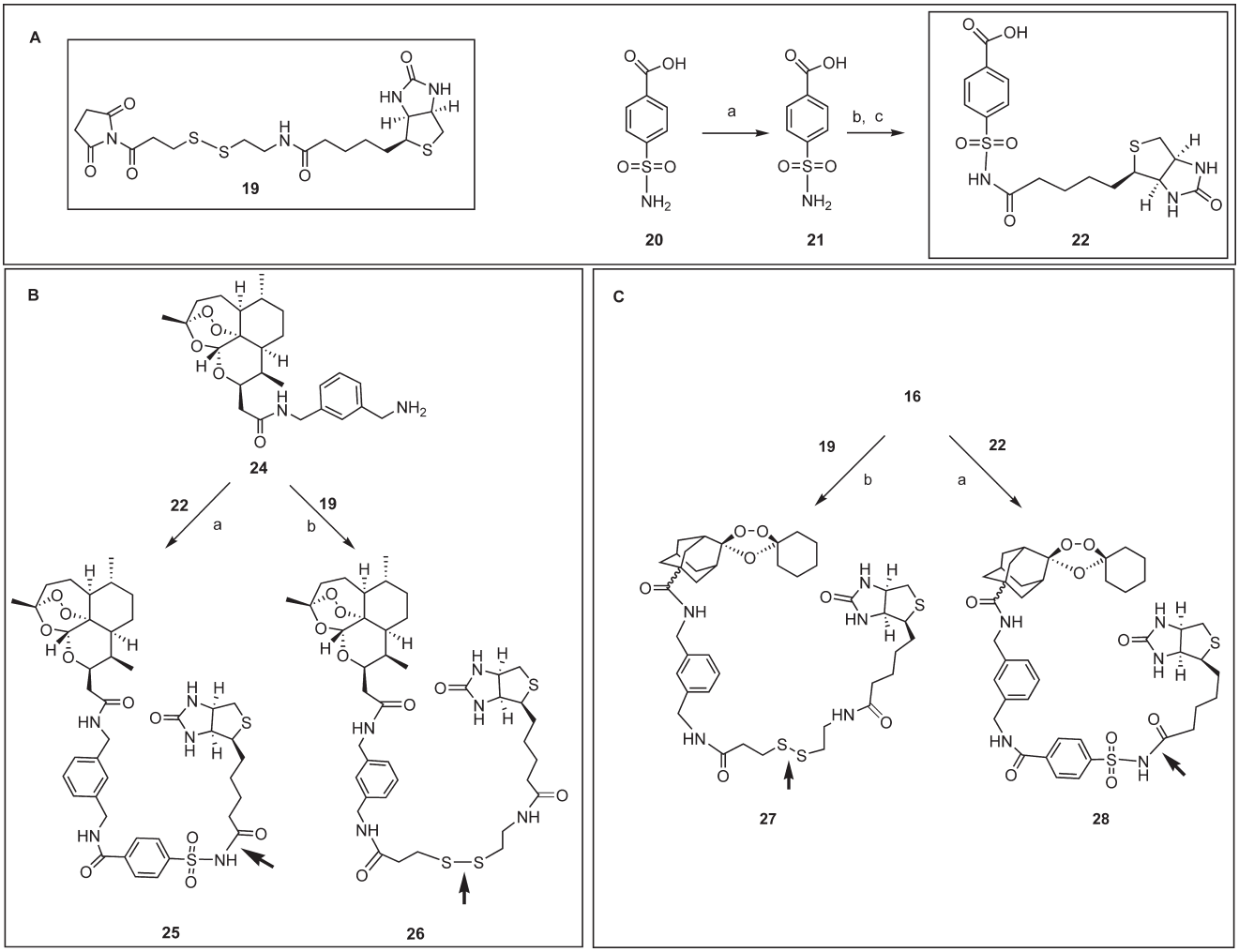
compd	*P. falciparum* (3D7) mean IC_{50} (nM)	SD (±nM)
3	1.6	
8	5.7	1.9
9	8.4	5.3
11	150	25
14	5.1	0.6
15	27	5.1
17	2.4	2.1
artemisinin	7.3	3.2

nonpolar groups such as esters had good antimalarial activities, whereas carboxylic acid **11** had very weak activity[16] (Table 1).

Recently, several investigators[17−19] have found that the use of biotinylated probes is limited due to the harsh, denaturing conditions required to disrupt the biotin−streptavidin interaction. This elution generally results in contamination of the desired probe-labeled proteins with proteins that were nonselectively bound to the streptavidin, avidin monomers, and endogenously biotinylated proteins. Hence, additional purification techniques such as gel electrophoresis are required prior to identification with mass spectrometry. As a result, cleavable strategies have been designed to circumvent these problems.

One such approach incorporates a chemically cleavable diazo-linker between biotin and the reactive group so that after the alkylated proteins were captured on streptavidin beads, only the drug bound proteins were released.[17] The mild elution conditions ensure that any endogenous biotin or other nonspecific cell material remains on the streptavidin beads. In a similar strategy, we have prepared biotinylated endoperoxides incorporating a disulfide **18**, or sulfonamide **21**, cleavable linker (Scheme 3A).

Scheme 3. (A) Chemically Cleavable Linkers: Activated Disulfide Linker **18** and Synthesis of Sulfonamide Linker **21**; (B) Synthesis of Chemically Cleavable Biotinylated Artemisinins, and (C) Trioxolane Counterparts[a]



[a]Arrows represent cleavage sites: SO_2NH^- requires ICH_2CN and NH_4OH, S−S−DTT. Reagents and conditions for (A): (i) $SOCl_2$ (2.0 equiv), MeOH, rt, 3 h, 100%; (ii) DCC (1.5 equiv), biotin (1.0 equiv), DMF, DMAP (0.25 equiv), **20** (1.0 equiv), 18 h, yield 20%; (iii) 15% NaOH, EtOH, rt, 24 h; (iv) aq HCl (2 M), yield **21**, 94%. Reagents and conditions for (B) and (C): (a) EDC·HCl (1.5 equiv), HOBt (1.2 equiv), NMM (2.5 equiv), **21** (1.0 equiv), DMF, 24 h, yield **23**, 3%, yield **26**, 14%; (b) EDIPA (1.1 equiv), sulfo-NHS-SS-biotin **18** (1.0 equiv), DMF, yield **24**, 27%, yield **25** 25%.

Table 2. Antimalarial Activities of Chemically Cleavable Biotinylated Trioxolanes and Artemisinins vs 3D7 Strain of *P. falciparum*

compd	*P. falciparum* (3D7) mean IC_{50} (nM)	SD (±nM)
23	> 1000	
24	91	24
25	33	16
26	no inhibition	

The biotinylated sulfonamide linker **21** was prepared by a four-step synthesis which involved protection of carboxysulfonamide **19**, using thionyl chloride followed by peptide coupling of **20**, with biotin **2**, and finally NaOH hydrolysis back to biotinylated carboxy sulfonamide **21** (Scheme 3A).

The biotinylated disulfide linker (commercially available as its activated *N*-hydroxysuccinimide (NHS) ester **18**) and sulfonamide linker **21** were reacted with artemisinin amine **22**, (Scheme 3B) and the trioxolane amine **16**, (Scheme 3C) to give the required probe molecules. The antimalarial activities of artemisinin and trioxolane biotinylated chemically cleavable bioprobes (**23**,**24** and **25**,**26**, respectively) were evaluated against chloroquine sensitive 3D7 *P. falciparum* (Table 2).

Addition of a polar biotinylated sulfonamide moiety into the endoperoxide framework to give trioxolane **26** and artemisinin **23** has abolished the antimalarial activity of the endoperoxides, thus limiting the use of these probes. Interestingly the trioxolane disulfide probe **25** has good antimalarial activity, almost three times more active than the corresponding artemisinin disulfide probe **24**. This suggests that the inactivity of sulfonamide **26** is predominantly due to the polarity of the sulfonamide group rather than steric bulk. This is consistent with SAR studies by Dong and co-workers[16] whereby a 4′-cyclohexyl substituted aryl sulfone trioxolane analogue was found to have a significantly higher IC_{50} and very poor in vivo antimalarial activity compared to its less polar counterparts.

Conclusion

To conclude, we have prepared biotinylated probes of artemisinin and trioxolanes that exhibit significant antimalarial activities. To circumvent potential issues in future studies with the harsh conditions required to disrupt the biotin−streptavidin bond, we have also developed methodology for the integration of a cleavable linker between the biotin tag and the site of attachment to the target protein. This strategy should permit mild elution of probe labeled proteins and direct identification by liquid chromatography mass spectrometry (LC-MS/MS).

In addition to the first examples of rationally designed probe molecules, we have also shown that 1,2,4-trioxolanes substituted on the adamantane ring retain significant antimalarial activity.

This may have an impact on future 1,2,4-trioxolane drug development initiatives because a major route of P_{450} metabolism has been shown to occur at the 5-position within the adamantyl framework of 1,2,4-trioxolanes, rendering the drug inactive.[9] Functionalization of this position may provide the key to blocking metabolism and increasing drug exposure and half-life of the drug. Future work will include proteomic studies of the active cleavable probes within malaria parasite-infected erythrocytes.

Experimental Section

General. Melting points were determined on a Gallenkamp apparatus and are uncorrected. ^{1}H NMR spectra were measured on Bruker (400 MHz) nuclear magnetic resonance spectrometer, and ^{13}C NMR spectra (100 MHz) were measured on the same instrument. 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). Infrared spectra were recorded on a PerkinElmer RX1 FT-IR spectrometer 68 (reported in wavenumbers (cm^{-1})). Elemental analyses (% C, % H, % N) were determined by the University of Liverpool Microanalysis Laboratory. For compounds for which elemental analysis was not obtained, purity (> 95%) was determined by HPLC (Gilson 321-pump coupled to a Gilson UV/vis spectrophotometer on a reverse phase semiprep column (dimension 250 mm × 20 mm; packing Eurospher 100-5 Si)). Anhydrous solvents were either obtained from commercial sources or dried and distilled immediately prior to use under a constant flow of dry nitrogen. EZ-Link NHS-SS-Biotin [succinimidyl 2-(biotinamido)-ethyl-1,3′ -dithiopropionate] **18** was obtained from Pierce (Thermo Scientific). All other reagents were used as received from Sigma Adrich, TCI, or Fisher.

Final target compounds **17**, **23**, and **26** were obtained via the following coupling procedure: To a stirring solution of the appropriate carboxylic acid (1.0 equiv) in anhydrous DMF (10 mL) at 0 °C was added EDC·HCl (1.5 equiv), HOBt (1.2 equiv), and NMM (2.5 equiv) and left to activate for 1−4 h. The appropriate amine (1−2 equiv) was added and allowed to react overnight at 0 °C. The solvent was then removed in vacuo, and crude product purified as indicated in the text.

Carbamate-Linked Artemisinin−Biotin Probe 3. A solution of alcohol **1** (500 mg, 1.6 mmol), biotin (150 mg, 0.61 mmol), DPPA (0.13 mL, 0.61 mmol), and NEt_3 (0.09 mL, 0.61 mmol) in anhydrous CH_3CN (20 mL) was stirred at 55 °C for 24 h. During the course of the reaction, nitrogen was evolved. After 24 h, the reaction mixture was allowed to cool and solvent removed under reduced pressure. The resulting residue was taken up in EtOAc (50 mL) and washed with 5% aq citric acid (2 × 30 mL), water (30 mL), satd aq $NaHCO_3$ (30 mL), and brine (30 mL). The organic extracts were dried over Na_2SO_4, filtered, and concentrated to give colorless oil. Purification by flash chromatography (5:95 EtOAc/*n*-hex) gave compound **3** (223 mg, 68%) as a colorless crystalline solid. **3**: mp 99−100 °C. ^{1}H NMR (400 MHz, MeOD) δ 8.18 (1H, br s), 5.31 (1H, s, H-12), 4.54−4.53 (2H, m), 4.39−4.09 (4H, m), 3.15 (4H, m), 2.93−2.90 (1H, dd, J = 12.7, 4.8 Hz, CH), 2.77−2.66 (3H, m), 2.32−2.28 (1H, td, J = 14.1, 3.5 Hz, CH), 1.40 (3H, s, $C\underline{H}_3$), 0.97−0.95 (3H, d, J = 5.8 Hz, 6-$C\underline{H}_3$), 0.89−0.87 (3H, d, J = 7.5 Hz, 9-$C\underline{H}_3$). ^{13}C NMR (100 MHz, MeOD) δ 165.7 (C=O), 158.4 (O$\underline{C}$(O)NH), 103.4 (C-3), 89.5 (C-12), 81.4, 71.7, 63.0 (CH), 61.6 ($\underline{C}$H), 60.9 (OCH_2), 55.3 (S−C), 52.7 (CH_2), 44.6 (HN−CH_2), 41.2, 37.9, 37.0, 34.8, 30.5, 29.9, 29.7, 28.9, 26.4, 25.7, 25.2, 21.3, 20.5, 14.5, 13.2. IR(nujol)/cm^{-1} 3239 (CONH), 2931(C−H), 1699 (C=O), 1525, 1455 (C=N), 1249, 877(O−O), 823(O−O). HRMS (ESI): 576.2725 $[M + Na]^+$ $C_{27}H_{43}N_3O_7S^{23}Na$ requires 576.2719. Anal. $C_{27}H_{43}N_3O_7S$ requires C: 58.57% H: 7.83% N: 7.59%. Found: C: 58.88% H: 7.94% N: 8.07%.

5-Adamantane-3-(amidomethyl)benzylcarbamate-2-spiro-1′-2′-4-trioxaspiro[4.5]decane Biotin 17. A solution of **16** (100 mg, 0.23 mmol) was reacted with biotin **2** (95 mg, 0.35 mmol) according to the general coupling procedure and purified by flash column chromatography (10:90 MeOH/CH_2Cl_2) to give approximately 50 mg (crude), which was further purified by reverse phase preparative HPLC (50:50 H_2O/MeOH→100% MeOH 60 min gradient, two diastereoisomers, R_t = 52 min) to give **17** (7 mg, 5%) as a colorless solid. **17**: mp 105−107 °C. ^{1}H NMR (400 MHz, MeOD) 7.28−7.23 (1H, m, CH), 7.16−7.11 (3H, m, CH), 4.48 (1H, q, J = 4.3 Hz, CH), 4.33 (4H, s, 2 × CH_2), 4.27 (1H, q, J = 4.5 Hz, CH), 3.19 (2H, dt, J = 9.2, 5.4 Hz, CH_2), 2.91 (1H, dd, J = 12.7, 5.0 Hz, CH), 2.68 (1H, d, J = 12.7 Hz, CH), 2.25 (2H, td, J = 7.4, 2.2 Hz, CH_2), 2.18 (1H, d, J = 12.0 Hz, CH), 2.06−1.39 (26H, m). ^{13}C NMR (100 MHz, MeOD) 180.1 ($\underline{C}$ONH), 176.2 (HN-$\underline{C}$O), 165.7 (C=O),

141.1, 141.0, 130.0, 127.7, 127.6, 127.5, 127.4, 111.7, 110.8 (C−O−O−C), 63.7 (CH), 62.0 (CH), 57.4 (S−C), 44.4, 44.2, 44.1, 41.5, 41.4, 39.9, 38.1, 38.0, 37.9, 37.2, 36.2, 35.2, 35.1, 30.2, 29.8, 28.7, 28.3, 27.3, 26.4, 25.3. IR (neat)/cm^{-1} 3336 (NH), 2973, 1689 (C═O), 1627 (C═C), 1419, 1164, 1049, 844. HRMS (ESI): 675.3184 $[M + H]^+$, $C_{35}H_{48}N_4O_6{}^{23}NaS$ requires 675.3192. Anal. $C_{35}H_{48}N_4O_6S$ requires C: 64.39% H: 7.41% N: 8.58%. Found C: 64.71% H: 7.64% N: 8.35%

10β-(3-(Aminomethyl)benzyl)acetamide)deoxoartemisinin Biotin Disulfide 24. To a solution of amine **22** (20 mg, 0.045 mmol) in DMF (3 mL) and EDIPA (1.1 equiv) was added NHS-SS-Biotin **18** (27 mg, 0.045 mmol). The reaction was monitored, and after 4 h, the solvent was evaporated in vacuo and residue purified by column chromatography (10:90 MeOH/CH_2Cl_2) to give a white powder (20 mg, 54%), which was further purified by reverse phase preparative HPLC (50:50 H_2O/MeOH→100% MeOH, 1 h gradient, R_t = 48 min, single peak) to give **24** (10 mg, 27%) as a colorless crystalline solid. ^{1}H NMR (400 MHz, MeOD) δ 7.29−7.17 (4H, m, 4 × CH), 5.51 (1H, s, H-12), 4.63 (1H, m, CH), 4.51−4.32 (7H, m), 4.28 (1H, dd, J = 7.8, 4.4 Hz, CH), 3.45 (2H, t, J = 6.8 Hz, CH_2), 3.18 (1H, m, CH), 2.98 (2H, t, J = 7.1 Hz, CH_2), 2.90 (1H, dd, J = 12.8, 5.0 Hz, CH_2), 2.81 (2H, t, J = 8.5 Hz, CH_2), 2.76−1.20 (26 H, m), 0.97 (3H, d, J = 6.4 Hz, 6-CH_3), 0.91 (3H, J = 7.5 Hz, 9-CH_3). ^{13}C NMR (100 MHz, MeOD) δ 176.6 (CON), 174.0 (CON), 164.6 (C═O), 139.5, 134.4, 130.1, 127.9, 127.7, 105.3 (C-3), 90.6 (C-12), 75.3, 63.7 (C−S), 62.0 (HN−CH) 54.3 (HN−CH), 46.3, 44.5, 39.8, 39.0, 38.8, 37.9, 37.3, 37.1, 37.0, 36.1, 35.6, 34.7, 30.1, 29.8, 27.4, 27.2, 26.5, 26.2, 26.0, 21.0, 14.0. HRMS (ESI): 856.3455 $[M + H]^+$, $C_{40}H_{59}N_5O_8{}^{23}NaS_3$ requires 856.3423. Anal. $C_{40}H_{59}N_5O_8S_3$ requires C: 57.60% H: 7.13% N: 8.40%. Found C: 57.42% H: 7.28% N: 8.55%.

5-Adamantane-3-(amidomethyl)benzylcarbamate-2-spiro-1′-2′-4-trioxaspiro[4.5]decane 4-(Sulfamoylbiotin) 26. Trioxolane amine **16** (88 mg, 0.2 mmol) in anhydrous DMF (5 mL) at 0 °C was reacted with 4-(biotin)sulfamoylbenzoic acid **21** (90 mg, 0.2 mmol) according to the general peptide coupling procedure. The product was purified using flash column chromatography (10:90 MeOH/CH_2Cl_2), yielding 100 mg as an off-white solid. The product was further purified by reverse phase HPLC (50−100% MeOH, 60 min gradient) R_t = 54 min (1:1 mixture of diastereoisomers) to give **26** (25 mg, 14%) as a colorless crystalline solid. **26**: mp 183−184 °C. ^{1}H NMR (400 MHz, MeOD) δ 8.03−8.00 (4H, m, 4 × Ar−H), 7.29−7.12 (4H, m, 4 × Ar−H), 4.57 (2H, s, CH_2), 4.43−4.39 (1H, m, CH), 4.36−4.33 (2H, t, J = 5.4 Hz, CH_2), 4.20−4.16 (1H, m, CH), 2.24 (2H, td, J = 7.2, 2.0 Hz, CH_2), 2.15−1.23 (32H, m). ^{13}C NMR (100 MHz, MeOD) δ 180.0 (CONH), 176.1 (NHCO), 168.7 (NHCO), 166.4 (C═O), 141.4 (C-SO_2), 140.5 (C-CO_2NH), 130.0, 129.7, 129.3, 127.6, 127.5, 127.0, 126.9, 111.6 (O−C−O),110.8 (O−C−O), 63.6 (HN−CH), 61.9 (HN−CH), 57.2 (C−S), 44.9 (CH_2), 44.1 (CH_2), 44.0 (CH_2), 41.4, 41.0, 39.9, 38.0, 37.9, 37.4, 36.2, 36.1, 35.1, 35.0, 29.8, 29.7, 28.6, 28.2, 26.4, 26.1, 26.0, 25.4, 25.3. HRMS (ESI): 858.3168 $[M + Na]^+$; $C_{42}H_{53}N_5O_9S_2{}^{23}Na$ requires 858.3182. Anal. $C_{42}H_{53}N_5O_9S_2$ requires C: 60.34% H: 6.39% N: 8.38%. Found C: 59.99% H: 6.34% N: 8.17%.

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Supporting Information Available: Full experimental details including analysis available from www.acs.co.uk. This material is available free of charge via the Internet at http://pubs.acs.org.

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