Angewandte Chemie
Eine Zeitschrift der Gesellschaft Deutscher Chemiker

# Supporting Information



69451 Weinheim, Germany

# Evidence for A Common Non-Heme "Chelatable Iron" Dependent Activation Mechanism for Semi-synthetic and Synthetic Endoperoxide Antimalarials

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[∗∗] This work was supported by grants from the BBSRC (UK) (PAS, PON, PGB, VB, MJ, SAW) (BB/C006321/1, BBS/B/05508, BBS/Q/Q/2004/06032, BBS/S/P/2003/10353), Romark, Florida (RA) and in part by the EU (GE, RA) (Antimal. FP6 Malaria Drugs Initiative)

## 1) In vitro sensitivity assays.

Drug susceptibilities were assessed by the measurement of fluorescence after the addition of SYBR Green I as previously described by Smilkstein et al .[1] Drug $IC_{50}$s were calculated from the log of the dose/response relationship, as fitted with Grafit software (Erithacus Software, Kent, United Kingdom). Results are given as the means of at least three separate experiments.

For the fluorescence assay, after 48 h of growth, 100 µl of SYBR Green I in lysis buffer (0.2 µl of SYBR Green I/ml of lysis buffer) was added to each well, and the contents were mixed until no visible erythrocyte sediment remained. After 1 h of incubation in the dark at room temperature, fluorescence was measured with a Varioskan fluorescence multiwell plate reader from Thermo Electron Corporation with excitation and emission wavelengths of 485 and 530 nm, respectively.

## 2) Drug combination assays (Isobologram Analysis)

To analyze the combined effect of the artemisone and the iron chelators (DFO and 3-Hydroxy-1,2-dimethyl-4(1*H*)-pyridone ) the $IC_{50}$ for each drug alone was obtained as described above. From these values, a stock solution of each drug was prepared such that the $IC_{50}$ of each drug would fall around the fourth serial dilution. Combinations of the stock solutions were prepared in constant ratios of 0:10, 1:9, 3:7, 5:5, 7:3, 9:1, and 10:0. Each combination was serially diluted across a microtiter plate and processed as for the standard sensitivity assay. The fractional inhibitory concentration (FIC; FIC = $IC_{50}$ of the drug in the combination/$IC_{50}$ of the drug when tested alone) of each drug was calculated and plotted as an isobologram . [2, 3]

## 3) Confocal microscopy

Parasitized erythrocytes (*P. falciparum* strain 3D7) were imaged using a LSM 510 scanning confocal microscope (Carl Zeiss, Jena, Germany), with excitation at 488 nm attenuated to 0.2–1 % intensity with an acousto-optical tunable filter. Pinhole size was adjusted to give optical sections of 1.2 µm, emission was detected at 505nm and analysed with LSM Pascal 510 software (v.2.01, Carl Zeiss). For imaging, midterm trophozoite-infected erythrocytes were immobilized using poly-L-lysine coated coverslips in a Bioptechs FCS2 perfusion chamber (maintained at 37ºC in RPMI 1640 media). Fluorescent-labeled compounds (1µM in media) was added 10min before imaging. For imaging experiments using DFO and DFP, the parasite cultures were preincubated at 37$^{o}$C for 30 minutes with the iron chelators (at 100µM in media) prior to use

For studies with peripheral blood mononuclear **cells** (PBMC) cells images are as follows for normal and high gain using NBD conjugate shown.

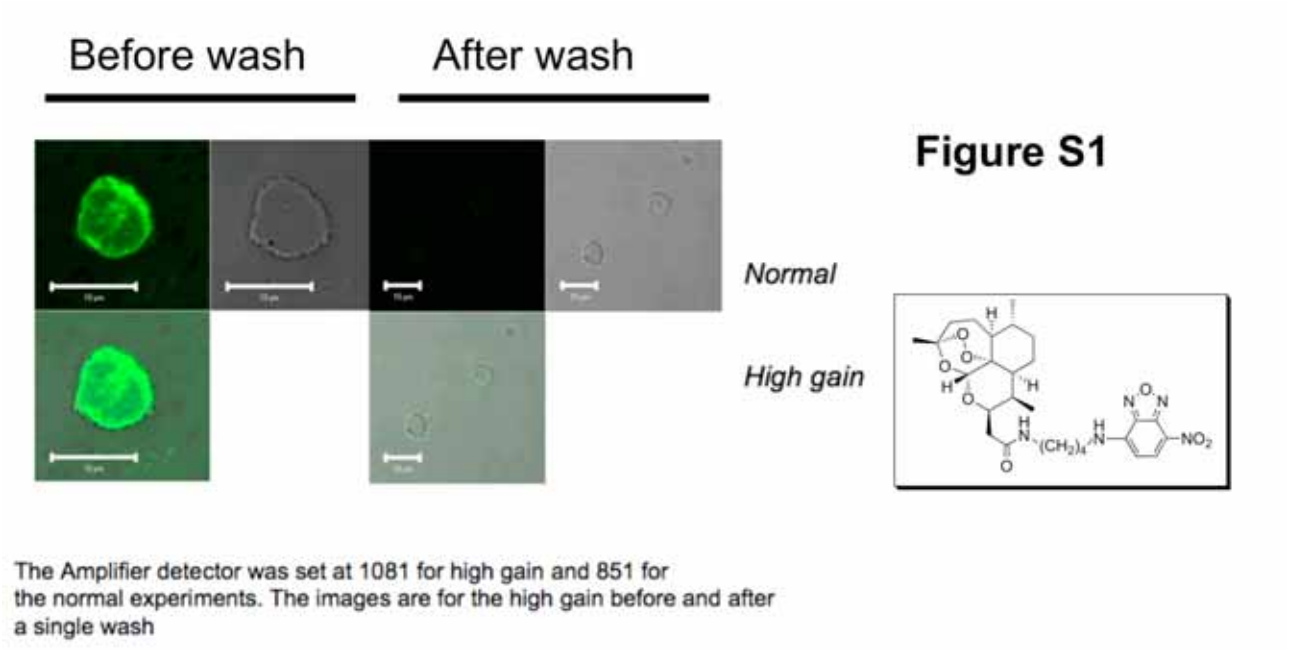


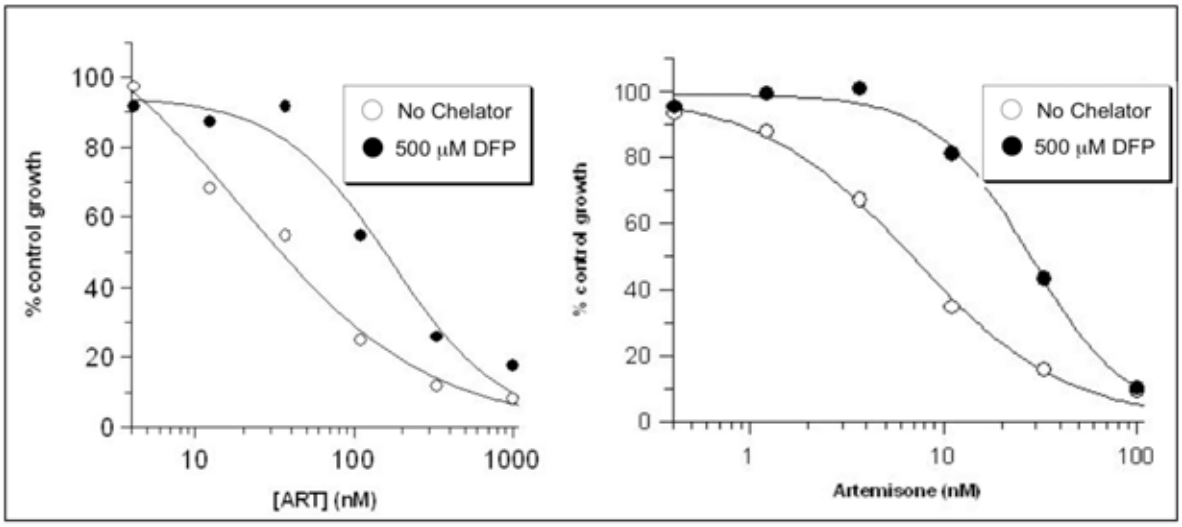

**Figure S1**

The Amplifier detector was set at 1081 for high gain and 851 for the normal experiments. The images are for the high gain before and after a single wash

**4) Fixed-dose effect of DFP on *P. falciparum* endoperoxide drug sensitivity**

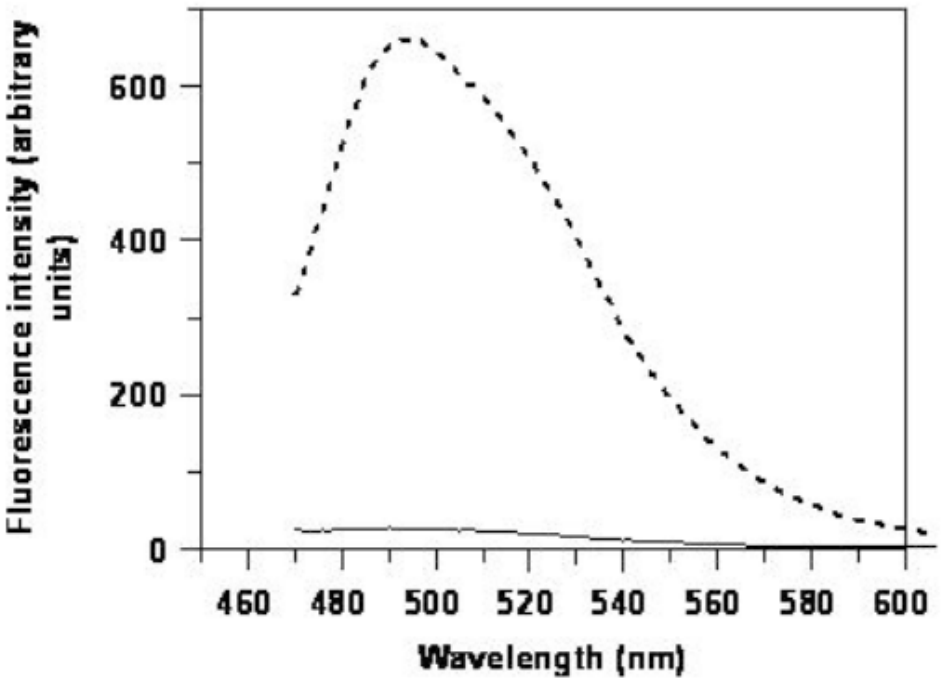
The chloroquine-sensitive laboratory adapted 3D7 isolate of *Plasmodium falciparum* was used throughout the study and cultured in vitro using standard methods.[4] Drug sensitivity was carried out essentially as described by Bennet et al.,[5]with the following modifications. Synchronized [6] trophozoite stage parasites cultures (2% parasitaemia, 1% heamtocrit) were pre-incubated with and without DFP (500 μM) for 30 minutes at 37ºC before incubation with serial drug dilutions (3 h, 37 ºC). Following incubation with drug dilutions, the inoculums were washed three times in 20 volumes of RPMI with DFP (500 μM) and once in RPMI alone. After washing, inoculums were re-suspended in complete culture media and grown in 96 well plates for 48 h at 37 ˚C in 1%$O_2$/4%$CO_2$ in $N_2$. Growth was determined fluorimetrically as described by Bennet et al., (2). All experiments were performed in triplicate on at least three independent occasions. $IC_{50}$ values were calculated using the four-parameter logistic method (Grafit program ; Erithacus Software, UK). Representative DOSE RESPONSE curves are shown below, [ART] = artesunate;

# Figure S2




# Figure S3

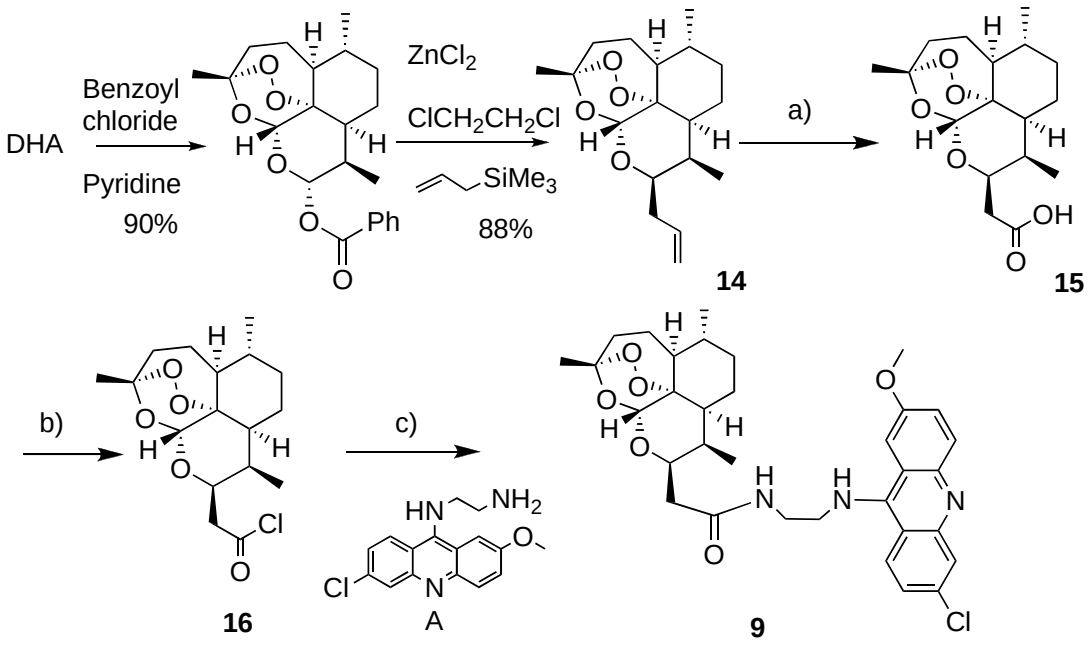
Fluorescence emission spectrum of 10μM MJ51 in the presence (solid line) and absence (dashed line) of 10 μM FPIX.




5) **Synthetic Chemistry**

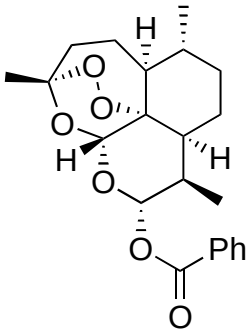
Artemisone was prepared according to the method reported by Haynes .[7]

**Synthesis of Acridine Conjugate 4d**




a) $NaIO_4$ (0.004 eq), $KMNO_4$ (0.002 eq), Acetone: Water 1:1, 25°C 60% b) Oxalyl Chloride (2 eq), $CH_2Cl_2$, 0° C , 100% c) $Et_3N$ (0.3 eq), *N*1-(6-chloro-2-methoxyacridin-9-yl) ethane-1,2-diamine A (2 eq), $CH_2Cl_2$, 0 °C , 25°C, 75%

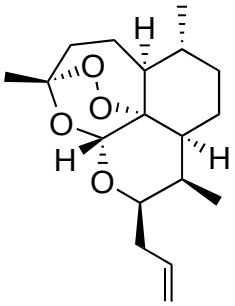
**Dihydroartemisinin 10α- benzoate Synthesis:**



A solution of dihydroartemisinin (2g, 7.044mmol) and anhydrous pyridine (3.60 ml, 44.5 mmol) in anhydrous dichloromethane (40ml) was cooled to 0℃ under $N_2$. Benzoyl Chloride (1.2 ml, mmol) was added via syringe, and the mixture was allowed to stir at room temperature for 16 hours. The reaction mixture was then analysed by TLC (9:1 Hexane / Ethyl Acetate). On completion, the reaction was worked up by dissolving the reaction mixture in ethyl acetate, and washing successively with 7% citric acid solution, saturated $NaHCO_3$ solution and distilled water. The organic phase was then dried over magnesium sulfate, filtered and then solvent removed under vacuum to give an off white solid as crude product. Using flash column chromatography the product was purified as a white solid (90%) ; Melting Point: 109-112℃; $^1H$ NMR: ($CDCl_3$, 400MHz) δ 8.13 (2H, m, aromatic), 7.57 (1H, m aromatic), 7.45 (2H, m, aromatic), 6.05 (1H, d,

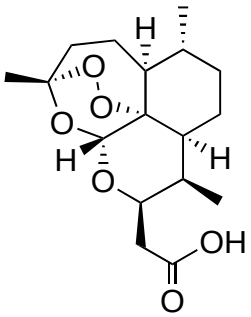
J=9Hz), 5.53 (1H, s), 2.76 (1H, m), 2.40 (1H, m), 2.07-1.20 (10 H, m), 1.43 (3H, s), 0.99 (3H, d, J=5.91 Hz), 0.93 (3H, d, J=7.14 Hz); $^{13}$ C NMR: ($CDCl_3$, 400MHz) δ165.41, 133.72, 130.54, 130.03, 128.71, 104.86, 92.94, 92.01, 80.61, 52.06, 45.76, 37.70, 36.67, 34.54, 32.41, 26.37, 25.00, 22.48, 20.66, 12.65; Mass Spectrometry: CI ($+NH_3$) :requires 406.22. Found: $[MNH_3]^+$ = 406.22320 Microanalysis: $C_{22}H_{28}O_6$ Mr: 388, Theory: C: 68.10% H: 7.27%; Found: C: 68.18%, H: 7.28%

**10-β-Allyldeoxoartemisinin:**



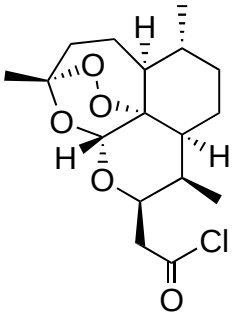

A solution of DHA-10-α-benzoate, (0.4g, 1mmol) in DCE (4 mL) was added via cannula to a mixture of allyltrimethysilane (0.76ml, 4.8mmol, 4.8eq.) Zinc chloride (0.16g, 1.2mmol, 1.2 eq.) and 4A mol sieves in 5ml DCE. The mixture was stirred at 0℃ under $N_2$ for 1hr before TLC analysis. On completion (TLC), the reaction mixture was diluted with ethyl acetate, washed with 5% citric acid solution, sat. $NaHCO_3$, and brine. The organic phase was dried over magnesium sulphate and concentrated under vacuum. The resulting crude colourless oil was purified by column chromatography to give pure product as a white solid (78%); Melting Point: 76-78℃; [8] $^1$H NMR: (400MHz, $CDCl_3$) δ 5.93 (1H, m), 5.33 (1H, s) 5.12 (2H, m) 4.31 (1H, m) 2.68 (1H, sex) 2.45-2.17 (3H, m) 2.07-1.20 (10H, m) 1.41 (3H, s) 0.96 (3H, d, J = 5.9 Hz) and 0.89 (3H, d, J= 7.67 Hz); $^{13}$C NMR: (400MHz, $CDCl_3$) δ136.88, 116.48, 103.54, 89.49, 81.48, 52.74, 44.72, 37.87, 36.99, 34.88, 34.61, 30.59, 26.49, 25.29, 23.10, 20.59, 13.38. Mass Spectrometry: $C_{18}H_{28}O_4$ CI requires: 325.413 Found $[MNH_3]$+ = 325.41432 Microanalysis: $C_{18}H_{28}O_4$ Mr: 308.41, Theory: C: 70.1% H: 9.16% Found: C: 69.65%, H:9.30%

**Synthesis of Carboxylic Acid 15:**



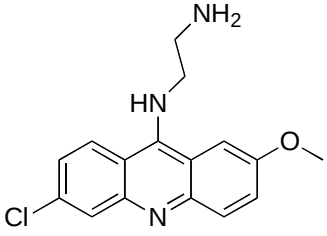
To a stirred solution of C10-allyldeoxoartemisinin (0.2g, 0.63mmol) in 40ml acetone and 40ml water was added sodium periodate (0.54g, 2.55mmol, 4eq.) and potassium permanganate (0.06g, 0.39mmol, 0.63eq.). The mixture was stirred for an additional 12 hrs. On completion (TLC), the reaction mixture was filtered and the filtrate concentrated under vacuum. The concentrate was treated with NaOH until basic and washed with ether. The aqueous phase was then acidified to pH 1 with conc. HCl. The aqueous phase was extracted with ether. The combined organic extracts were dried over magnesium sulphate and concentrated to give **15** as an oil; $^{1}$H NMR: (400 MHz, $CDCl_3$) δ 9.01 (1H, s, carboxylic acid) 5.02 (1H, s) 3.91 (1H, m) 2.48 (1H, m) 2.45-2.17 (3H, m) 2.07-1.20 (10H, m) 1.41 (3H, s) 0.96 (3H, d, J= 5.9 Hz) and 0.89 (3H, d, J= 7.67 Hz); $^{13}$C NMR: (400MHz, $CDCl_3$) δ176.23, 103.78, 88.70, 81.18, 69.15, 52.01, 43.23, 39.83, 36.99, 36.42, 34.5, 29.62, 26.04, 25.00, 24.87, 18,76, 12.86; Mass Spec: $C_{17}H_{26}O_6$ CI ($NH_3$) Requires: 343.415. Found: 343.41721; Microanalysis: $C_{17}H_{26}O_6$ Mr: 326.38 Theory: C:62.56% H:8.03% Found: C: 62.58% H: 8.00%

**Artemisinin-carboxylic acid chloride 16:**




Carboxylic acid **15** (1.24g, 3.8 mmol) was dissolved in 67 ml anhydrous dichloromethane at 0℃ under $N_2$. Oxalyl chloride (0.66 ml, 5.2 mmol) was added drop-wise over 10 minutes. The mixture was left stirring for 12 hours. After twelve hours the reaction mixture was stripped of solvent and the resulting acid chloride was used with no further purification or characterisation.

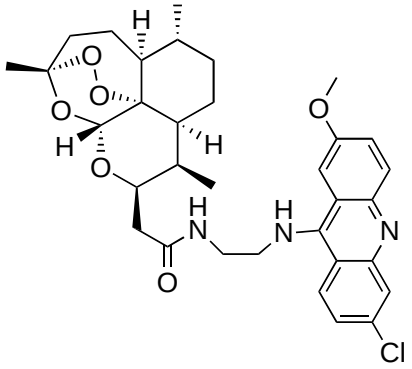
***N*1-(6-chloro-2-methoxyacridin-9-yl) ethane-1,2-diamine A**




A solution of 6,9-dichloromethoxyacridine (1g, 3.6mmol) and 1,2-diaminoethane(10.7ml, 160mmol) in phenol (4g, 42mmol) was heated to reflux for 4 hours with stirring and then allowed

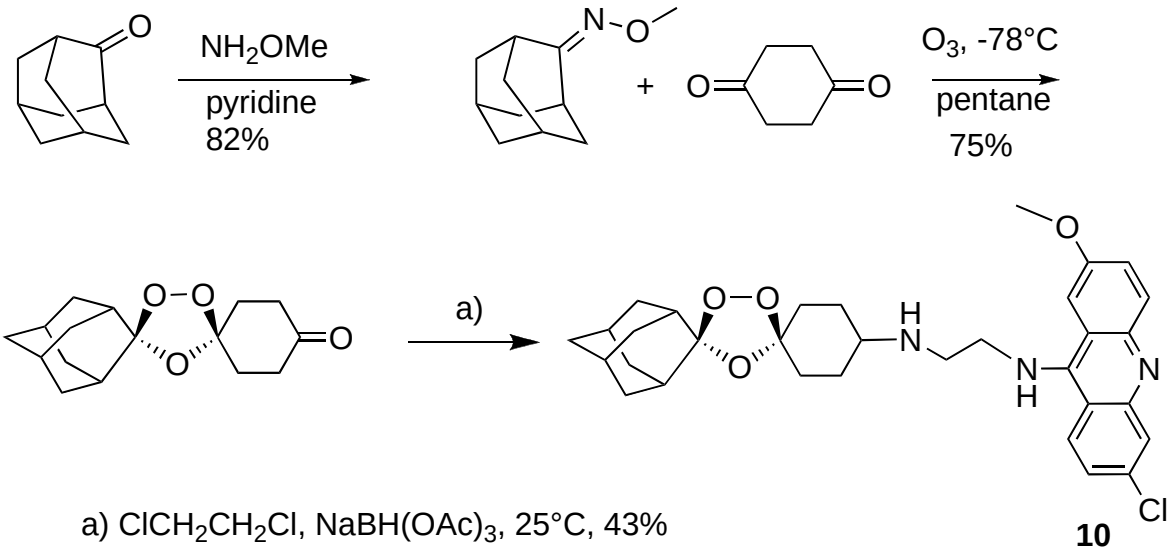
to cool to room temperature. The reaction mixture was concentrated under vacuum to remove any excess amine to give a crude solid. The solid was dissolved in dichloromethane and washed with 1N sodium hydroxide and brine. The organic phase was dried with sodium sulphate and concentrated to give an off-white solid as product in 53%. M.pt. 107ºC $V_{max}$ ($CHCl_3$)/$cm^{-1}$ 921.1, 1027.2, 1254.6, 1431.5, 1466.9, 1527.5, 1652.9, 1628.6,2884.7, 2955.2, 3015.8 $^1$H NMR (400 MHz, $CDCl_3$) δ 3.01(m, 2H, $CH_2N$), 3.71(m, 2H, $NCH_2$), 3.97(s, 3H, $OCH_3$), 7.30(dt, 1H, J = 2.06Hz, Ar), 7.38(d, 1H, J = 2.54Hz, Ar), 7.41(dd, 1H, J = 2.86Hz, 2.7Hz, Ar), 7.99(d, 1H, J = 9.53Hz, Ar), 8.07(d, 1H, J = 2.06Hz, Ar), 8.14(d, 1H, J = 9.06Hz, Ar) $^{13}$CNMR (100MHz, $CDCl_3$), $\delta_C$ 42.40, 52.10, 55.91, 99.89, 124.85, 124.92, 128.54, 131.76, 135.20, 146.37, 148.80, 150.73, 156.53 MS (CI) m/z 301.77 $[M + H]^+$ (10) 302.1. HRMS m/z calculated for 302.1060 $C_{11}H_{13}O_5N_3Cl$ (100), found, 302.1067.

**Conjugate 9**



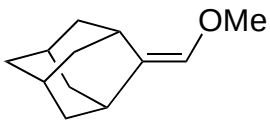

To a stirred solution of acid chloride **16** (0.30g, 0.87mmol) and *N*1-(6-chloro-2-methoxyacridin-9-yl) ethane-1,2-diamine (0.26g, 0.87mmol) in anhydrous dichloromethane at 0ºC was added anhydrous triethylamine (0.1ml, 0.99mmol). The reaction mixture was left stirring overnight at room temperature. Solvent was then removed *in vacuo* and the resultant yellow oil was purified by column chromatography eluted in 4:1 dichloromethane:MeOH to provide the product as a yellow solid in 70% yield; $^1$H NMR: ($CDCl_3$, 400MHz) δ8.4 (1H, s), 8.05-6.95 (6H, m aromatic),5.02 (1H, s), 4.0 (1H, s), 3.91 (1H, m), 3.70, (3H, s), 3.43, (2H, m), 3.28 (2H, m), 2.48 (1H, m) 2.45-2.17 (3H, m) 2.07-1.20 (10H, m) 1.41 (3H, s) 0.96 (3H, d, J= 5.9 Hz) and 0.89 (3H, d, J= 7.67 Hz); $^{13}$C NMR: ($CDCl_3$, 400MHz) δ174.7, 156.8, 148.6, 148.0, 134.9, 131.0, 128.9, 128.0, 127.3, 121.9, 118.6, 104.6, 98.0, 96.2, 92.8, 84.5, 67.5, 56.2, 54.0, 44.6, 37.3, 36.3, 30.8, 30.4, 29.5, 25.3, 23.7, 22.8, 19.0, 18.4, 11.8, 11.4.; Mass Spectrometry: $C_{33}H_{40}ClN_3O_6$: requires 610.14 Found: 610.13998 Microanalysis:$C_{33}H_{40}ClN_3O_6$ Theory: C: 64.96% H:6.61% N:6.89%; Found: C: 65.00%; H:6.78%; N:6.93%

**Synthesis of OZ Acridine Conjugate 10**



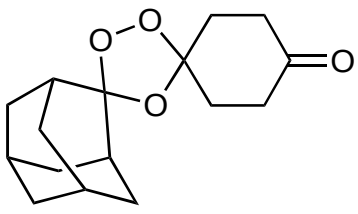

**10**

**Synthesis of *O*-methyl 2-adamantanone oxime**



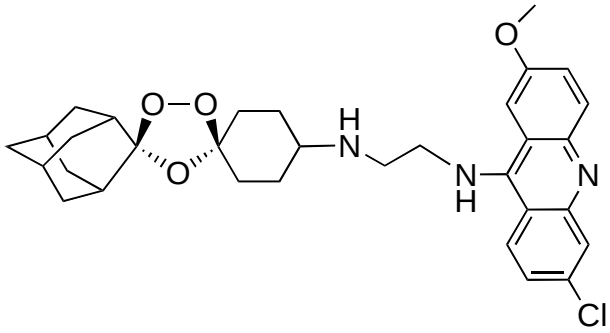

To a solution of 2-adamantanone (2.00g, 13.3mmol), in methanol (20ml) was added pyridine (2ml) and methoxylamine hydrochloride (1.66g, 19.9mmol, 1.5 eq). The reaction mixture was stirred under nitrogen for 50 hours, concentrated in vacuo, diluted with $CH_2Cl_2$ (30ml) and water (30ml). The organic layer was separated and the aqueous layer extracted with $CH_2Cl_2$ (15ml). The combined organic extracts were washed with 1M HCl (20ml x 2) and saturated NaCl (20ml) and dried over $MgSO_4$. Evaporation in vacuo afforded 2.09g (88%) as a white solid ; m.p 70-71°C, (lit. m.p 70-71°C)[9] ; $\nu_{max}$ /cm$^{-1}$ (nujol) 1641 (C=N) ; $^1$H NMR (400MHz, $CDCl_3$) δ 3.80 (3H, s , C<u>H</u>$_3$), 3.46 (1H, s, C-<u>H</u> Bridgehead), 2.53 (1H, s, C-<u>H</u> Bridgehead), 2.43-1.79 (12H, m) ; δC (100MHz, $CDCl_3$) 166.9 (C=N), 61.3, 39.5 (2x<u>C</u>H$_2$), 38.0 (2x<u>C</u>H$_2$), 37.9, 36.9, 29.8, 28.2 (2x<u>C</u>H$_2$); *m/z* (CI) 180.13865 $[M+H]^+$ ; $C_{11}H_{18}NO$ requires 180.13884; $C_{11}H_{17}NO$ requires C 73.74%, H 9.49%, N 7.82% found C 73.40%, H 9.52%, N 7.84%.

**Adamantane-2-spiro-3'-8'-oxo-1',2',4'-trioxaspiro[4.5]decane**



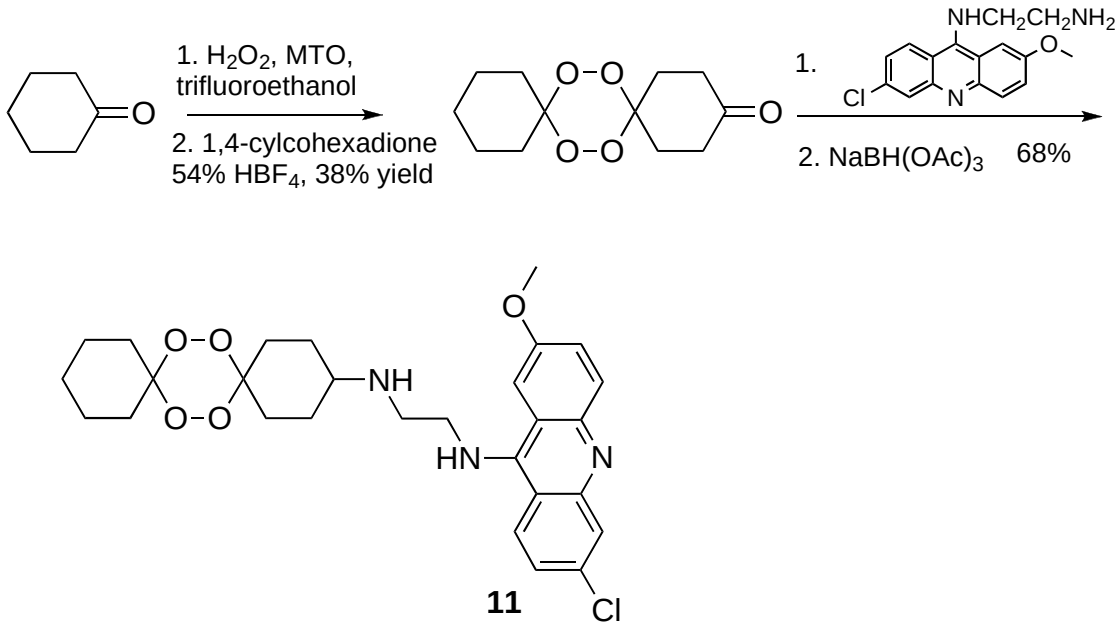
According to the method of Vennerstrom et al.[10] a solution of *O*-methyl-2-adamantanone oxime (1 g, 5.5 mmol) and 1,4-cyclohexadione (1.23 g, 10 mmol) in pentane (60 ml) and dichloromethane (40 ml) was treated with ozone. The crude product was purified ($SiO_2$, ethyl acetate/n-hexane, 35%) to afford the expected trioxolane as a white solid (1 g, 75%). m.p. 114-115ºC. ν (film)/$cm^{-1}$ 1720 (C=O), 1112 (C3'-O-C5'), 899 (C3'-O-O-C5'). $^1$H NMR (400 MHz, $CDCl_3$) δ 2.55 (t, J= 7.1 Hz, 4H), 2.17 (t, J= 7.1 Hz, 4H), 1.70-2.03 (m, 14H). $^{13}$C NMR (100 MHz, $CDCl_3$) δ 26.80, 27.21, 33.48, 35.14, 35.24, 36.68, 38.19, 39.64, 107.35, 112.83, 209.62 (C8'). Anal. Calcd. for $C_{16}H_{22}O_4$: C, 69.04; H, 7.97%. Found: C, 69.24; H, 8.07%.

**Adamantane-2-spiro-3'-8'-(9-(3-amino-1-ethylamino)-6-chloro-2-methoxyacridine)-1',2',4'-trioxaspiro[4.5]decane (10)**

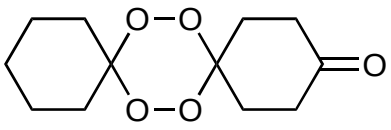



The target trioxolaquine was achieved by reductive amination of adamantane-2-spiro-3'-8'-oxo-1',2',4'-trioxaspiro[4.5]decane (5b) (0.37 g, 1.4 mmol) with *N*1-(6-chloro-2-methoxyacridin-9-yl) ethane-1,2-diamine (0.64 g, 2.1 mmol) and sodium trioacetoxyborohydride (0.45 g, 2.1 mmol) in dichloroethane (10 ml) to give the product as an orange solid (0.33 g, 43%); m.p. 136ºC. ν (film)/$cm^{-1}$1247 (R-O-$CH_3$), 1113 (C3'-O-C5'), 819 (C3'-O-O-C5'). $^1$H NMR (400 MHz, $CDCl_3$) δ 8.06 (d, J= 7.9 Hz, 1H), 7.96 (d, J= 1.8 Hz, 1H), 7.92 (d, J= 9.2 Hz, 1H), 7.33 (dd, J= 2.5 Hz, J= 9.3 Hz, 1H), 7.23 (d, J= 2.4 Hz, 1H), 7.19 (dd, J= 1.9 Hz, J= 9.2 Hz, 1H), 3.93 and 3.91 (2xs, 3H), 3.83 (t, 2H), 3.02 (t, 2H), 2.68-2.66 (m, 1H), 2.05-1.58 (m, 22H). $^{13}$C NMR (100 MHz, $CDCl_3$) δ 24.03, 24.64, 25.09, 27.85, 28.18, 29.14, 30.39, 31.99, 32.92, 33.12, 33.95, 34.58, 34.94, 44.70, 47.19, 52.75, 53.69, 97.87, 106.40, 109.98, 122.15, 123.14, 134.08, 149.13, 154.09. MS *m/z* (CI, +ve) 564 ($[M+H]^+$, 100). Anal. Calcd. for $C_{32}H_{38}N_3O_4Cl$: C, 68.11; H, 6.78; N, 7.48%. Found: C, 67.78; H, 6.50; N, 7.05%.

**Synthesis of Tetraoxane Acridine Conjugate 11**

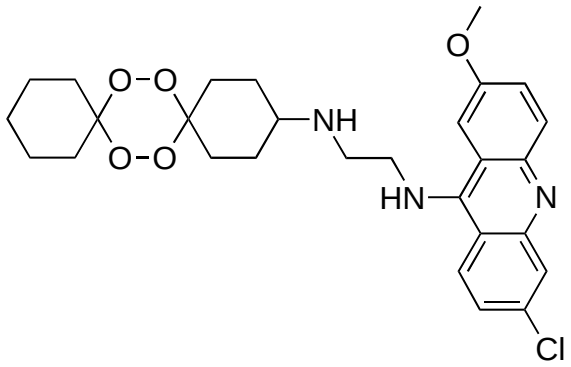



**7,8,15,16-Tetraoxa-dispiro[5.2.5.2]hexadecan-3-one (6)**



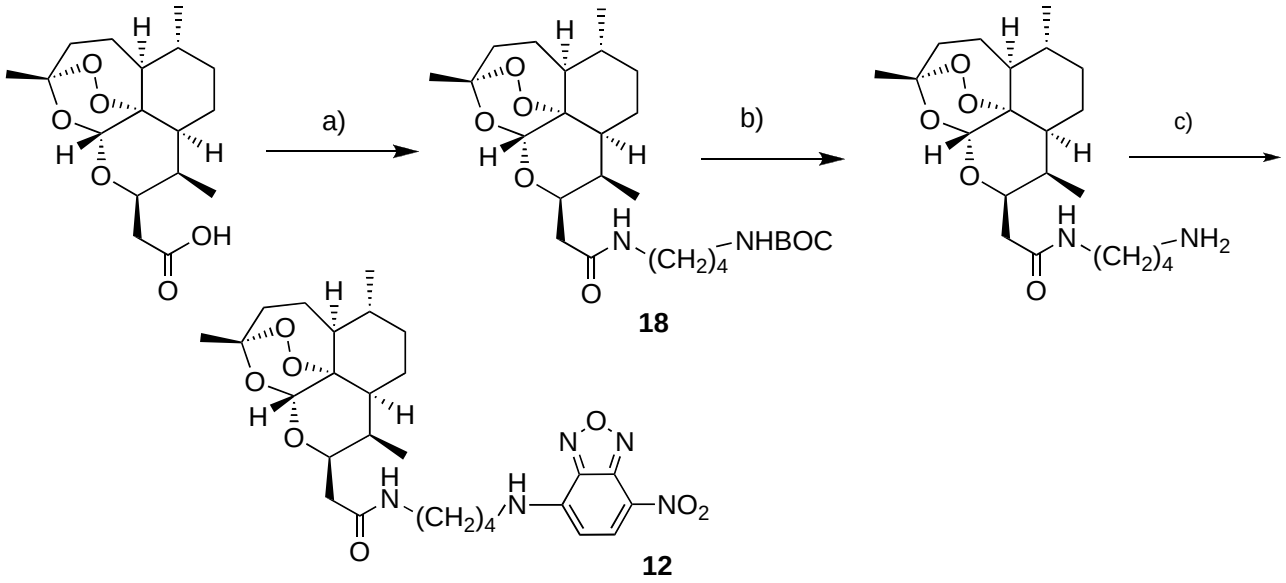
A solution of of cyclohexanone (0.12g, 2mmol), 30% $H_2O_2$ (0.05g, 4mmol), and a catalytic amount of methyltrioxorhenium (MTO, 0.0005g, 0.002mmol)) in 2,2,2-trifluoroethanol (TFE, 4 ml) was allowed to stir for 2 hours at room temperature. 1,4-Cyclohexanedione (0.4485g, 4mmol) was added to the solution followed by 54% ethereal solution of tetrafluoroboric acid (0.095g, 2mmol). The reaction mixture was left stirring for an additiona l hour. Dichloromethane was added and the organic phases washed with dilute $NaHSO_4$, dried over $MgSO_4$ and the solvent evaporated under reduced pressure. The residue was purfied by flash chromatography ($SiO_2$, $CH_2Cl_2$: Hexane = 9:1) to give the tetraoxane in 38% yield. Mpt. 78-80$^oC$; $V_{max}$ ($CHCl_3$)/$cm^{-1}$1719.8, 2856.2, 2942.3, 3012.7 $^1$HNMR (400MHz, $CDCl_3$) $\delta_{H,}$ 1.5(m, 6H, cyclohexyl), 1.80(s, 4H, cyclohexyl), 2.15(t, 2H, J = 7.42Hz, $CH_2$), 2.30(t, 2H, J= 7.08Hz, $CH_2$), 2.5(m, 4H, $CH_2$), $^{13}$CNMR (100MHz, $CDCl_3$), $\delta_C$ 14.0, 23.07, 25.84, 31.98, 37.25, 106.60, 108.56, 210.77, MS (ES+) $[M + Na]^+$ (100), 265.0, $[M + Na + CH_3OH]^+$ (60) 297.1.

***N*-(6-Chloro-2-methoxy-acridin-9-yl)-*N*'-(7,8,15,16-tetraoxa-dispiro-[5.2.5.2]-hexadec-3-yl)-ethane-1,2-diamine. (11)**



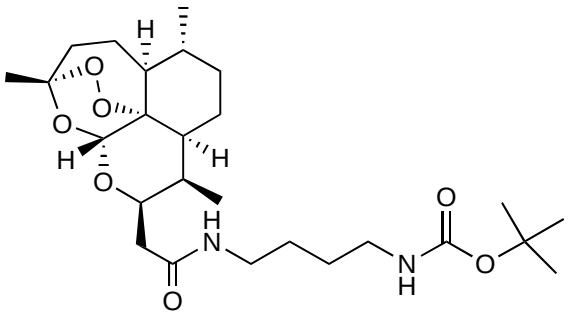

Tetraoxa-dispiro[5.2.5.2]hexadecan-3-one **6** (0.2g, 0.8mmol) and *N*1-(6-chloro-2-methoxyacridin-9-yl) ethane-1,2-diamine (6.06mmol) were mixed in dichloromethane (30ml) before addition of sodium triacetoxyborohydride (1.2g, 6.06mmol). The reaction was stirred at room temperature for 18hrs and then washed with distilled water. The organic layer was dried and evaporated under vacuum to dryness. Purification by chromatography (10-25% methanol/ dichloromethane) afforded the product as an orange solid in 68% yield.; IR $V_{max}$ ($CHCl_3$)/$cm^{-1}$ 926.1, 956.5, 1062.6,1087.8, 1442.5, 1558.5, 1624.4, 2858.9, 2931.3, 3012.8, 3366.0 ; $^1$HNMR (400MHz, $CDCl_3$) $\delta_H$ 1.36-1.66(m, 10H, cyclohexyl), 1.66-1.99(m, 8H, cyclohexyl), 2.20-2.37(m, 2H, cyclohexyl), 2.20-2.37(m, 2H, cyclohexyl), 2.66-2.79(m, 1H, CH), 3.16-3.24(m, 2H, $CH_2NH$), 3.91(s, 3H,$OCH_3$), 4.02-4.10(m, 3H, $NHCH_2$), 5.04(bs, 1H, NH), 7.07(dt, 1H, J = 2.09Hz, Ar), 7.17(d, 1H, J = 2.46Hz, Ar), 7.21(bs, 1H, NH), 7.28(t, 1H, J = 2.46Hz, Ar), 7.79(dd, 1H,J = 5.13Hz, 5.32Hz, Ar), 7.85(t, 1H, J = 2.09Hz, Ar), 7.96(dd, 1H, J = 8.35Hz, 8.16Hz, Ar) ; $^{13}$CNMR (100MHz, $CDCl_3$), $\delta_C$ 22.65, 25.46, 27.34, 30.65, 45.99, 48.44, 50.54, 55.57, 100.05, 106.92, 107.74, 108.39, 112.88, 115.97, 123.68, 125.40, 126.70, 136.81, 141.25, 144.58, 151.60, 155.88, 165.00 MS (CI) m/z 528.04 $[M + H]^+$ (100) 528.1/530.1 HRMS m/z calculated for 528.2265 $C_{28}H_{35}O_5N_3Cl$, found, 528.2270

**Synthesis of C-10 Carba NBD Conjugate 12**




a) i) DCC (1.25 equiv.), HOBt (1.2 equiv.), 4-NMM (2.5 equiv.), DCM, rt, 24h. ii) $H_2N(CH_2)_4NHBoc$ (2 equiv.), 25°C,16h, 58% b) HCl (4M), 1,4 Dioxane, 0°C, 24h, 48% c) NBD-Cl (1.1 equiv.), $NaHCO_3$ (0.1M), MeOH, 60°C, 2h, 70% )

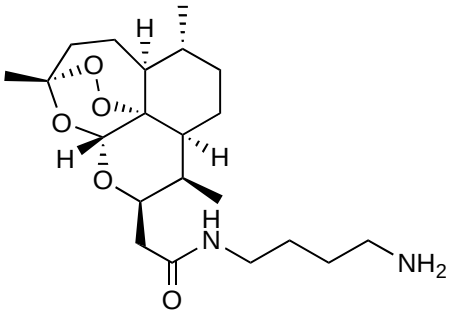
**Synthesis of BOC protected analogue 18**



DCC (0.20g, 0.9mmol, 1.2eq), HOBt (0.12g, 0.9mmol) and *N*-methyl morpholine (4-*N*mm) (0.21ml, 2.5eq), was added to a stirring solution of carboxylic acid **15** (0.25g, 0.8mmol) in $CH_2Cl_2$ (30ml). The flask was left to stir at room temperature overnight before adding mono BOC protected butylamine (*tert*-butyl 4-aminobutylcarbamate) (0.28g, 1.5mmol, 2eq,). After 24h the white precipitate was filtered and the filtrate washed with $H_2O$ (30ml) and organic layer separated with $CH_2Cl_2$ (3x30ml). The combined organic extracts were washed with brine (30ml), dried ($MgSO_4$) filtered and concentrated to give a yellow oil. The crude product was purified by column chromatography (20%EtOAc/Hex) to give **18** as a pale yellow solid (0.22g, 58%). Mp 133-135°C; $\nu_{max}/cm^{-1}$; (nujol) 3383 (N-H), 3346 (N-H), 1710 (C=O), 1681 (HNC=O), 875 (O-O) and 821 (O-O). *m/z* $^1$H NMR (400MHz, $CDCl_3$) δ 5.87 (1H, s, 12-H), 4.86-4.81 (1H, dt, *J*=7.9 and 6.1Hz, 10-Hα), 4.15-4.13 (1H, m, NH), 3.52-3.32 (2H, m, $CH_2$-CONH), 2.58-1.60 (26H, m), 1.54-1.52 (4H,

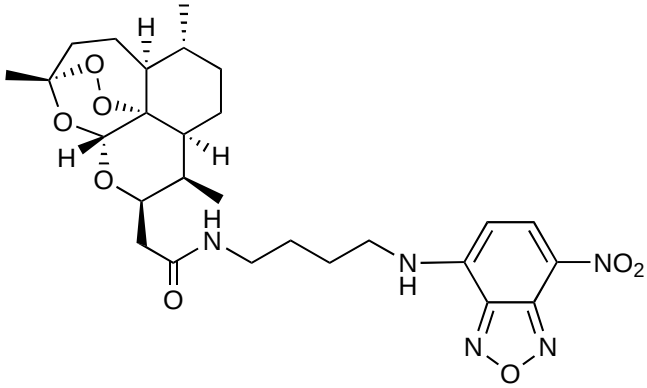
m, 2xC<u>H</u>$_2$), 1.33 (3H, s, 3-C<u>H</u>$_3$), 0.97-0.95 ( 3H, d, *J*=5.8Hz, 9-C<u>H</u>$_3$), 0.89-0.87 (3H, d, J=7.5Hz, 6-C<u>H</u>$_3$). $^{13}$C NMR (100MHz, $CDCl_3$) δ 171.9 (<u>C</u>ONH), 103.3 (<u>C</u>-3), 90.5 (<u>C</u>-12), 81.2 (<u>C</u>-5), 70.2 (<u>C</u>-10), 52.2, 49.5, 43.9, 40.6, 39.3, 37.9, 37.7, 36.9, 36.5, 36.3, 34.6, 30.7, 30.1, 28.8, 26.2, 25.1, 25.0 (3-<u>C</u>H$_3$), 21.4, 20.9, 20.3 (6-<u>C</u>H$_3$), 12.4 (9-<u>C</u>H$_3$). (ES+); 519.3022 $[M+Na]^+$ $C_{26}H_{44}O_7N_2$ requires 519.3046.

**Deprotection of 18**



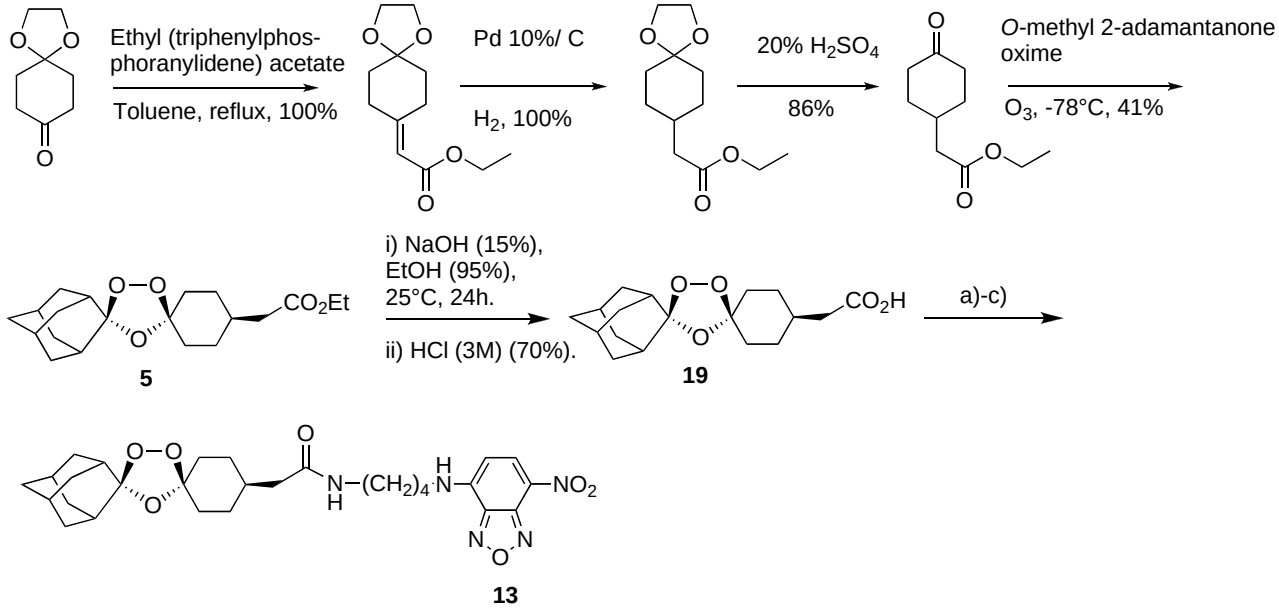
To a solution of **18** (0.22g, 0.45mmol), in 1,4 dioxane (5ml) was added 4M HCl (10ml) and the reaction was allowed to stir under nitrogen for 24 hours. The solvent was evaporated in vacuo and a solution of $K_2CO_3$ added until the solution was neutralized (~10ml). The solution was washed with $CH_2Cl_2$ (3x30ml), water (30ml) and the organic extracts washed with brine (20ml), dried ($Na_2SO_4$), filtered and solvent evaporated in vacuo to give a yellow crystalline solid. The crude product was purified by column chromatography (10%MeOH/DCM) to give a yellow solid (0.09g, 47%); $^1$H NMR (400MHz, $CDCl_3$) δ 5.31 (1H, s, 12-H), 4.76-4.69 (1H, m, 10-<u>H</u>α), 4.54-4.49 (1H, m, N<u>H</u>), 3.38-3.24 (2H, m, C<u>H</u>$_2$-CONH), 2.58-1.60 (19H, m), 1.86-1.52 (4H, m, 2xC<u>H</u>$_2$), 1.40 (3H, s, 3-C<u>H</u>$_3$), 0.96-0.95 ( 3H, d, *J*=5.8Hz, 9-C<u>H</u>$_3$), 0.87-0.85 (3H, d, J=7.5Hz, 6-C<u>H</u>$_3$) ; $^{13}$C NMR (100MHz, $CDCl_3$) δ 172.4(C=O), 103.4, 81.0, 71.6, 45.3, 44.0, 39.9, 38.6, 36.5, 35.6, 35.5, 34.4, 30.9, 29.7, 29.5, 26.2, 25.9, 20.1, 18.7, 13.1, 12.5. *m/z* (ES+); 397.2718 $[M+H]^+$ $C_{26}H_{44}O_7N_2$ requires 397.2702.

**Synthesis of C-10 Carba NBD Conjugate 12**



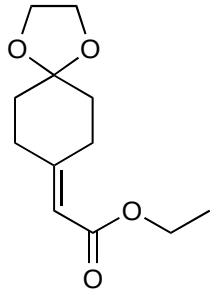
To a solution of the amine (74mg, 0.2mmol) in methanol (5mL) and $NaHCO_3$ (10mL, 0.1M) was added NBD-Cl (4-chloro-7-nitrobenzo[*c*][1,2,5]oxadiazole) (40.8mg, 0.2mmol, 1.1eq) in methanol (5mL) and heated to 55°C. After 2 hours the dark brown mixture was cooled and the solvent evaporated. The crude product was purified by column chromatography (30%EtOAc) to give **12** (73mg, 70%) as a dark brown solid. $^1$H NMR (400MHz, MeOD) δ 8.53 (1H, d, *J*= 9Hz, HC=C-$NO_2$), 6.38 (1H, d, *J*=9Hz, HC=C-NH) 5.33(1H, s, 12-H), 4.61-4.52 (1H, m, 10-Hα ) 3.57 (1H, m, NH), 3.43-3.36 (2H, m, $CH_2$-NH), 3.26-3.22 (2H, m,) 2.69-2.53 (2H, $CH_2$-CO-NH), 2.58-1.60 (2H, $CH_2$-NH-CO), 2.28-2.20 (2H, m, $CH_2$), 1.92-1.10 (13H, m), 1.26 (3H, s, 3-$CH_3$), 0.97-0.95 ( 3H, d, *J*=5.8Hz, 9-$CH_3$), 0.89-0.87 (3H, d, J=7.5Hz, 6-$CH_3$). $^{13}$C NMR (100MHz, MeOD) δ 177.3 (CONH), 141.4 (C=N), 111.5, 107.4, 101.2, (C-3), 93.3(C-12), 86.7, 85.0, 81.2 (C-5), 70.5 (C-10), 56.0, 48.9, 44.5, 42.7, 41.4, 40.3, 38.5, 37.7, 34.2, 30.7, 28.9, 26.8, 21.4, 23.4, 21.9, 16.2, 15.4. ). *m/z* (ES+); 582.2549 $[M+Na]^+$ $C_{27}H_{37}O_8N_5{}^{23}Na$ requires 582.2540.

**Synthesis of OZ NBD Conjugate**



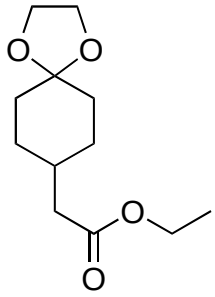

a) i) DCC (1.25 equiv.), HOBt (1.2 equiv.), 4-NMM (2.5 equiv.), DCM, rt, 24h. ii) $H_2N(CH_2)_4NHBoc$ (2 equiv.), 25°C,16h, 75% . b) HCl (4M), 1,4 Dioxane, 0$^o$C, 24h, 38% c) NBD-Cl (1.1 equiv.), $NaHCO_3$ (0.1M), MeOH, 60$^o$C, 2h, 80%

**Synthesis of Ethyl-2-[4-Ethylenedioxy)cyclohexylidene)acetate**



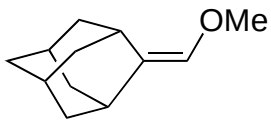
A solution of 1,4-cyclohexadione mono-ethylene ketal (5.00g, 32.0 mmol) and ethyl (triphenylphosphoranylidene) acetate (12.27g, 35.2mmol, 1.1eq) in toluene (80ml) was allowed to relux for 24 hours under nitrogen. The solvent was concentrated and residue purified by flash chromatography (10%EtOAc/Hexane) to give a colourless oil (7.24g, 100%). $\nu_{max}$ /cm$^{-1}$ (neat) 2950 (C=CH), 1713 (C=O), 1651 (C=CH) ; $^{1}$H NMR (400MHz, $CDCl_3$) δ 5.66 (1H, s, C<u>H</u>=C), 4.17-4.12 (2H, q, *J*=7Hz, C<u>H</u>$_2$CH$_3$), 3.97 (4H, s, OC<u>H</u>$_2$C<u>H</u>$_2$O), 3.01-2.98 (2H, t, *J*=7Hz, C<u>H</u>$_2$C=C), 2.39-2.35 (2H, t, *J*=7Hz, C<u>H</u>$_2$C=C), 1.79-1.73 (4H, m, 2xC<u>H</u>$_2$CO), 1.29-1.25 (3H, t, *J*=7Hz, C<u>H</u>$_3$); δC (100MHz, $CDCl_3$) 166.9(C=O), 160.5(C=<u>C</u>H), 114.7, 108.4, 64.8, 59.9, 36.2 (2x<u>C</u>H$_2$), 35.4, 35.0, 26.4, 14.6; *m/z* (CI) 227.12826 $[M+H]^+$ ; $C_{12}H_{19}O_4$ requires 227.12834; $C_{12}H_{18}O_4$ requires C 63.69%, H 8.02%, found C 63.78%, H 8.07%.

**Ethyl 2-[4-4-(Ethylenedioxy)cyclohexyl]acetate**



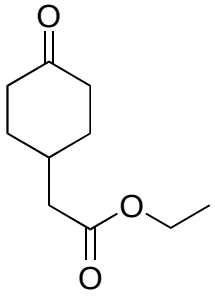
A suspension of the alkene (7.24g, 31.0 mmol) and 10% palladium on charcoal in ethyl acetate (140ml) was stirred under a hydrogen atmosphere for 24 hours. The reaction mixture was filtered using celite under vacuum and the filtrate concentrated to give colourless oil (7.18g, 100%). $\nu_{max}$/cm$^{-1}$ (neat) 1729 (C=O) ; $^{1}$H NMR (400MHz, $CDCl_3$), δ 4.15-4.09 (2H, q, *J*=7Hz, C<u>H</u>$_2$CH$_3$) 3.92 (4H, s, OC<u>H</u>$_2$C<u>H</u>$_2$O), 2.22-2.21 (2H, d, *J*=7Hz, C<u>H</u>$_2$C=O) 1.88-1.78 (1H, m, C<u>H</u>), 1.76-1.70 (4H, m, 2xC<u>H</u>$_2$C-O), 1.60-1.52 (4H, td, *J*=14, 4, 2xC<u>H</u>$_2$CH$_2$CO), 1.38-1.28 (4H, td, *J*=14, 4Hz, 2xC<u>H</u>$_2$CH$_2$CO), 1.27-1.23 (3H, t, *J*=7Hz, C<u>H</u>$_3$). $^{13}$C NMR (100MHz, $CDCl_3$) δ 173.3 (C=O), 114.7, 109.0, 64.6, 60.9, 40.6, 34.6, 33.8, 32.7 (2x<u>C</u>H$_2$), 30.3, 14.6 ; *m/z* (CI) 229.14393 $[M+H]^+$ ; $C_{12}H_{21}O_4$ requires 229.14398.

**Synthesis of *O*-methyl 2-adamantanone oxime**



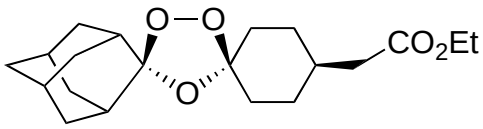

To a solution of 2-adamantanone (2.00g, 13.3mmol), in methanol (20ml) was added pyridine (2ml) and methoxylamine hydrochloride (1.66g, 19.9mmol, 1.5 eq). The reaction mixture was stirred under nitrogen for 50 hours, concentrated in vacuo, diluted with $CH_2Cl_2$ (30ml) and water (30ml). The organic layer was separated and the aqueous layer extracted with $CH_2Cl_2$ (15ml). The combined organic extracts were washed with 1M HCl (20ml x 2) and saturated NaCl (20ml) and dried over $MgSO_4$. Evaporation in vacuo afforded 2.09g (88%) as a white solid ; m.p 70-71°C, (lit. m.p 70-71°C) ; $\nu_{max}$ /$cm^{-1}$ (nujol) 1641 (C=N) ; $^1$H NMR (400MHz, $CDCl_3$) δ 3.80 (3H, s , C<u>H</u>$_3$), 3.46 (1H, s, C-<u>H</u> Bridgehead), 2.53 (1H, s, C-<u>H</u> Bridgehead), 2.43-1.79 (12H, m) ; δC (100MHz, $CDCl_3$) 166.9 (C=N), 61.3, 39.5 (2xC<u>H</u>$_2$), 38.0 (2xC<u>H</u>$_2$), 37.9, 36.9, 29.8, 28.2 (2xC<u>H</u>$_2$); *m/z* (CI) 180.13865 $[M+H]^+$ ; $C_{11}H_{18}NO$ requires 180.13884; $C_{11}H_{17}NO$ requires C 73.74%, H 9.49%, N 7.82% found C 73.40%, H 9.52%, N 7.84%.

**Ethyl 2-(4-oxocyclohexyl) acetate**



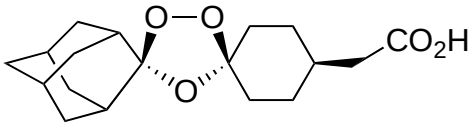

The acetal ethyl 2-[4-4-(ethylenedioxy)cyclohexyl]acetate (1.43g, 5.7mmol) in ethanol (30ml) was treated with 20% $H_2SO_4$ (10ml) for 24 hours, stirring at room temperature. The acid was neutralized with $NaHCO_3$ (10ml). The organic layer was extracted with ether (3x30ml), washed with water (30ml) and dried over $MgSO_4$. The residue was purified using flash chromatography (10%EtOAc/Hexane) to give 0.9g (86 %) of colourless oil. $\nu_{max}$ /$cm^{-1}$ (neat) 1731 (C=O) ; $^1$H NMR (400MHz, $CDCl_3$) δ 4.18-4.12 (2H, q, *J*=7Hz, C<u>H</u>$_2$CH$_3$) 2.39-2.31 (4H, m, 2xC<u>H</u>$_2$), 2.12-2.06 (2H, m, C<u>H</u>$_2$), 1.88-1.78 (1H, m, C<u>H</u>), 1.72 (1H, m, C<u>H</u>), 1.53-1.43 (2H, m, C<u>H</u>$_2$), 1.28-1.25 (3H, t, *J*=7Hz, C<u>H</u>$_3$) ; $^{13}$C NMR (100MHz, $CDCl_3$) δ 211.4 (C=O), 172.7 (C=O), 60.8, 64.6, 40.9 (2xC<u>H</u>$_2$CH$_2$), 40.6, 33.5, 32.7, 14.6 ; *m/z* (CI) 202.14472 $[M+NH_4]^+$ ; $C_{10}H_{20}O_3N$ requires 202.14430.

**Adamantane-2-spiro-3-8-ethoxycarbonylmethyl-1,2,4-trioxaspiro[4.5]decane 5**



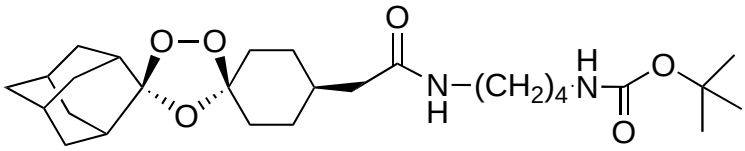

Ozone (3-4%) was produced with an ozone generator first passed through $CH_2Cl_2$ (80ml) cooled to -78°C then bubbled through a solution of oxime (0.75g, 4.2mmol) and ethyl 2-(4-oxocyclohexyl) acetate (0.80g, 4.3mmol) in pentane (80ml) and anhydrous $CH_2Cl_2$ (20ml). The reaction was left for 2.5 hours. After completion the solution was flushed with $O_2$ for 5 min then concentrated *in vacuo.* The residue was purified by column chromatography (2%EtOAc/Hexane) to give 0.60g (41%) as a white solid. Mp 63-64°C (62-64°C lit ref.) $\nu_{max}$ /cm$^{-1}$ (nujol) 1739 (C=O), 885(O-O), 852(O-O); $^1$H NMR (400MHz, $CDCl_3$) δ 4.20-4.18 (2H, q, *J*=7Hz, $CO_2C\underline{H}_2$) 2.21-2.18 (2H, d, *J*=7Hz, $C\underline{H}_2CO_2$), 2.31-1.58 (22H, m), 1.30-1.25 (3H, t, *J*=7Hz, $C\underline{H}_3$); $^{13}$C NMR (100MHz, $CDCl_3$) δ173.1 ($\underline{C}$=O), 111.7, 108.8, 60.6, 37.2, 34.3, 30.3, 35.1, 35.3, 33.5, 27.3, 26.9, 14.6 ; *m/z* (ES+) 373.1981 $[M+Na]^+$ ; $C_{20}H_{30}O_5{}^{23}Na$ requires 373.1991; $C_{20}H_{30}O_5$ requires C 68.54%, H 8.62%, found C 68.60%, H 8.66%

**Adamantane-2-spiro-3-8-(carboxymethyl)-1,2,4-trioxaspiro[4.5]decane 19**



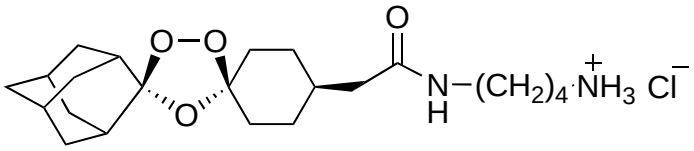

To a solution of **5** (0.63g, 1.8mmol) in 95% ethanol (10ml) was added 15% aq. NaOH (10ml). The mixture was heated to 55-60°C for 3h, cooled to rt, and acidified with 3M HCl (~10ml). The suspension was kept at 0-5°C for 1h. The solution was washed with $H_2O$ (30ml) and organic layer separated with $CH_2Cl_2$ (3x30ml). The combined organic extracts were washed with brine (30ml), dried ($MgSO_4$) filtered and concentrated. The crude product was purified by column chromatography (10-50% EtOAc/Hexane) to afford 0.41g (70%) as a white solid; m.p 138-139°C (lit. ref. 146-148°C); $\nu_{max}$ /cm$^{-1}$ (nujol) 3223 (OH) 1718 (C=O) 894 (O-O), 856 (O-O); $^1$H NMR (400MHz, $CDCl_3$) δ2.26-2.23 (2H, d, *J*=7Hz, $C\underline{H}_2CO_2H$) 2.25-1.67 (21H, m), 1.33-1.19 (2H, m) ; $^{13}$C NMR (100MHz, $CDCl_3$) δ 178.8 (C=O), 111.7, 108.7, 40.8, 37.2, 36.8, 35.2, 34.3, 33.5, 30.3, 27.3, 26.9 ; *m/z* (ES-) 321.1693 $[M-H]^-$ ; $C_{18}H_{25}O_5$ requires 321.1702 ; $C_{18}H_{26}O_5$ requires C 67.05%, H 8.12%, found C 67.09%, H 8.14%.

**BOC protected cis-Adamantane-2-spiro-3'-8'-(butylamido)-1',2',4'-trioxaspiro[4.5]decane**



DCC (0.16g, 0.8mmol, 1.2eq), HOBt (0.10g, 0.7 mmol, 1.2eq) and 4-*N*mm (0.17ml, 1.5mmol 2.5eq), was added to a stirring solution of the OZ acid (0.20g, 0.6mmol) in dry $CH_2Cl_2$ (30ml). The flask was left to stir at room temperature overnight before adding the Boc protected butylamine (0.24g, 1.5mmol, 2eq,) in dry $CH_2Cl_2$ (15mL). After 24 h the white precipitate was filtered and the filtrate washed with $H_2O$ (30ml) and organic layer separated with $CH_2Cl_2$ (3x30ml). The combined organic extracts were washed with brine (30ml), dried ($MgSO_4$) filtered and concentrated to give a yellow oil. The crude product was purified by column chromatography (20%EtOAc/Hex) to give a pale yellow solid (0.23g, 75%). $^1$H NMR (400MHz, $CDCl_3$) δ 5.74 (1H, s, NH), 4.60 (1H, s, NH), 3.51-3.45 (2H, m, $CH_2$-NHCO), 3.27-3.23 (2H, m, $CH_2$-NHCO), 2.04-2.03 (2H, m, $CH_2$CONH), 1.96-1.04 (27H, m), 1.44 (9H, s, $C(CH_3)_3$). $^{13}$C NMR (100MHz, MeOD) δ 172.3 (CONH), 156.5 (OC=ONH), 111.7, 108.9, 79.7, 49.5, 43.9, 40.4, 39.4, 37.2, 36.8, 35.3, 35.2, 35.1, 34.4, 34.3, 34.1, 33.8, 30.4, 30.2, 28.8, 28.1, 27.2, 27.1, 26.9, 26.0, 25.3 *m/z* (ES+); 515.3087 $[M+Na]^+$ $C_{27}H_{44}O_6N_2{}^{23}Na$ requires 515.3097.

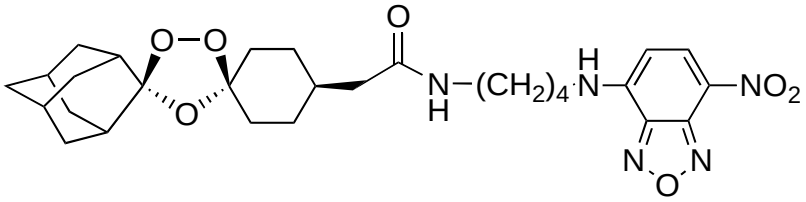
**Cis-Adamantane-2-spiro-3'-8'-(butylamido)-1',2',4'-trioxaspiro[4.5]decane hydrochloride**



To a solution of the above BOC derivative (450mg, 0.9mmol) in ether (5mL) was added 1M ethereal HCl (11mL, 91mmol). The mixture was stirred for 3h at room temperature forming a pale pink precipitate. The ether was removed and the crude product purified by column chromatography (4-15%MeOH/DCM) to yield 148mg (38%) as white crystals. $^1$H NMR (400MHz, MeOD) δ 3.36 (1H, s, NH), 3.24 (2H, t, *J=7Hz*, $CH_2$NHCO), 2.98-2.95 (2H, t, *J=7Hz*, $CH_2$-$NH_3^+Cl^-$), 2.12-2.10 (2H, d, *J=7Hz*, $CH_2$-CONH), 2.03-1.56 (28H, m), 1.30-1.19 (2H, m, $CH_2$). $^{13}$C NMR (100MHz, MeOD) δ 174.4 (CONH), 118.8, 111.7, 108.9, 42.9, 40.4, 39.5, 38.6, 37.1, 37.1, 35.8, 34.9, 34.4,

34.2, 34.1, 30.1, 29.9, 27.5, 27.1, 26.6, 25.1. *m/z* (ES+); 393.2751[M+Na]$^+$ $C_{22}H_{37}O_4N_2{}^{23}Na$ requires 393.2753.

**_Cis_-adamantane-2-spiro-3'-8'-(butylamido-7-nitrobenzo-2-oxa-1,3-diazole) )-1',2',4'-trioxaspiro[4.5]decane 13**



To a solution of cis-Adamantane-2-spiro-3'-8'-(butylamido)-1',2',4'-trioxaspiro[4.5]decane hydrochloride (124mg, 0.3mmol) in methanol (5mL) and $NaHCO_3$ (1M, 1mL, 0.5mmol) was added NBD-Cl (167mg, 0.9mmol, 3 eq) in methanol (5mL) and the reaction was heated to 55°C. After 3 hours the dark brown mixture was cooled and the solvent evaporated. The crude product was purified by column chromatography (30%EtOAc/Hex) to give **13** (125mg, 80%) as a dark orange solid. $^1$H NMR (400MHz, MeOD) δ 8.52-8.50 (1H, d, *J=9Hz*, C<u>H</u>C-$NO_2$) 6.37-6.35 (1H, d, *J=9Hz*, C<u>H</u>-CNH) 3.60 (2H, m, C<u>H</u>$_2$NHCO), 3.28-3.25 (2H, t, *J=7Hz*, C<u>H</u>$_2$NH), 2.09-2.08 (2H, d, *J=7Hz*, C<u>H</u>$_2$-CONH), 2.01-0.89 (29H, m) $^{13}$C NMR (100MHz, MeOD) δ 175.5 (<u>C</u>ONH), 146.2 (C=N), 138.8 (C=N), 112.7, 110.0, 50.0, 44.1, 40.2, 39.5, 38.2, 36.1, 35.4, 33.4, 31.3, 31.1, 30.8, 28.7, 28.3, 27.1, 24.1. *m/z* (ES-); 554.2594[M-H]$^-$ $C_{28}H_{36}O_7N_5$ requires 554.2591.

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