Shining new light on ancient drugs: Preparation and subcellular localisation of novel fluorescent analogues of *Cinchona* alkaloids in intraerythrocytic *Plasmodium falciparum*

John G. Woodland,[a] Roger Hunter,[a] Peter J. Smith[b] and Timothy J. Egan*[a]

a. Department of Chemistry, University of Cape Town, Private Bag, Rondebosch, 7701, South Africa.

b. Division of Clinical Pharmacology, Department of Medicine, University of Cape Town, Observatory, 7925, South Africa.

* Corresponding author. Email: timothy.egan@uct.ac.za.

SUPPORTING INFORMATION

Contents

1. Supplementary figures

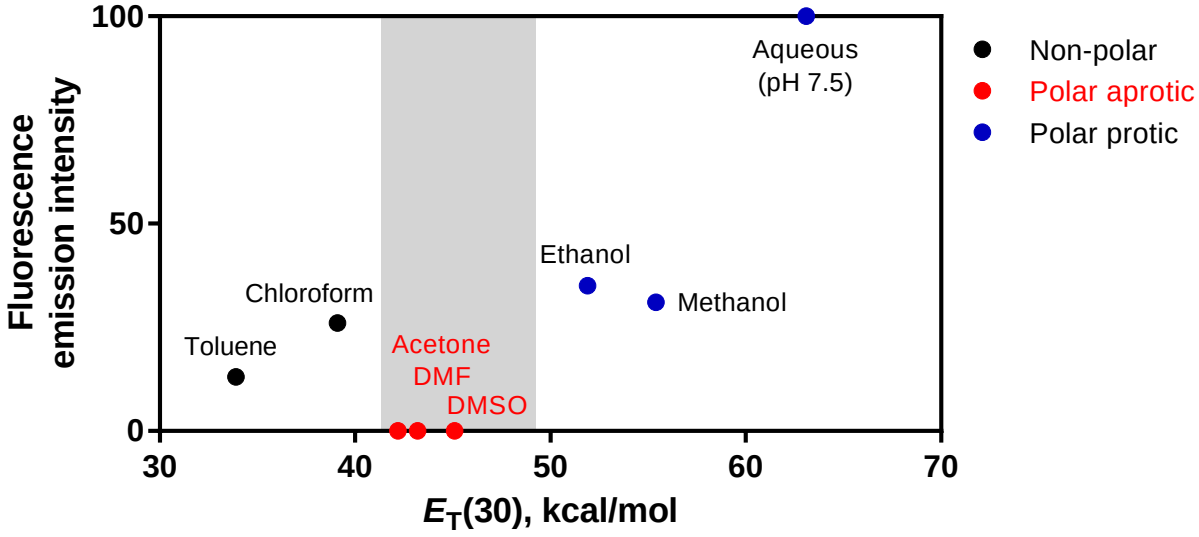


Figure S1. Relative fluorescence emission intensities of quinine in eight common solvents. 20 μM solutions of quinine were excited at 333 nm at 25°C. Emission intensities were calculated relative to water (20 mM Hepes, pH 7.5) which was set at 100%. $E_T(30)$ values taken from Reichardt.[1] Red points indicate polar aprotic solvents in which quinine fluorescence is quenched. The grey shaded region indicates the approximate range of $E_T(30)$ values over which the quinine fluorescence is quenched corresponding to polarity values of phospholipid bilayers (49-50 kcal/mol)[2] and neutral lipid bodies (45 ± 4 kcal/mol).[3]

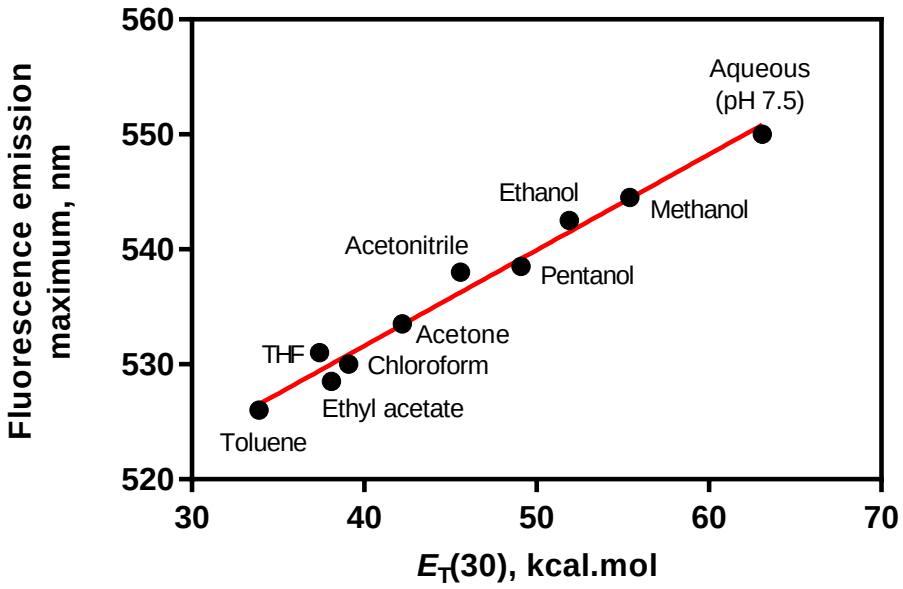


Figure S2. The relationship between the fluorescence emission maximum of the NBD fluorophore and solvent polarity in ten common solvents. $E_T(30)$ values taken from Reichardt.[9] The short-chain NBD-labelled quinidine derivative **4** was excited at 480 nm at a concentration of 20 μM at 25°C.

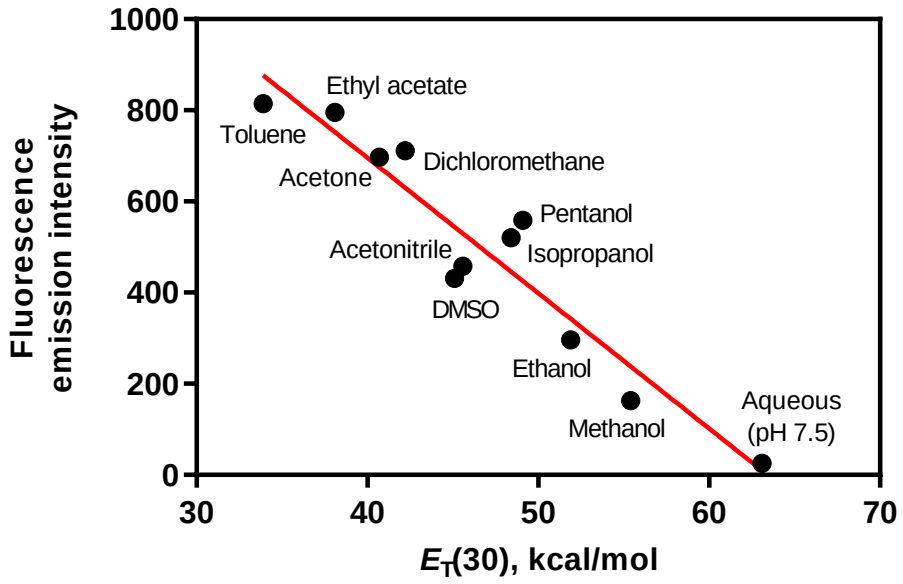


Figure S3. The relationship between fluorescence intensity and solvent polarity for eleven common solvents. $E_T(30)$ values taken from Reichardt.[9] The short-chain NBD-labelled quinidine derivative **4** was excited at 480 nm at a concentration of 20 μM at 25°C.

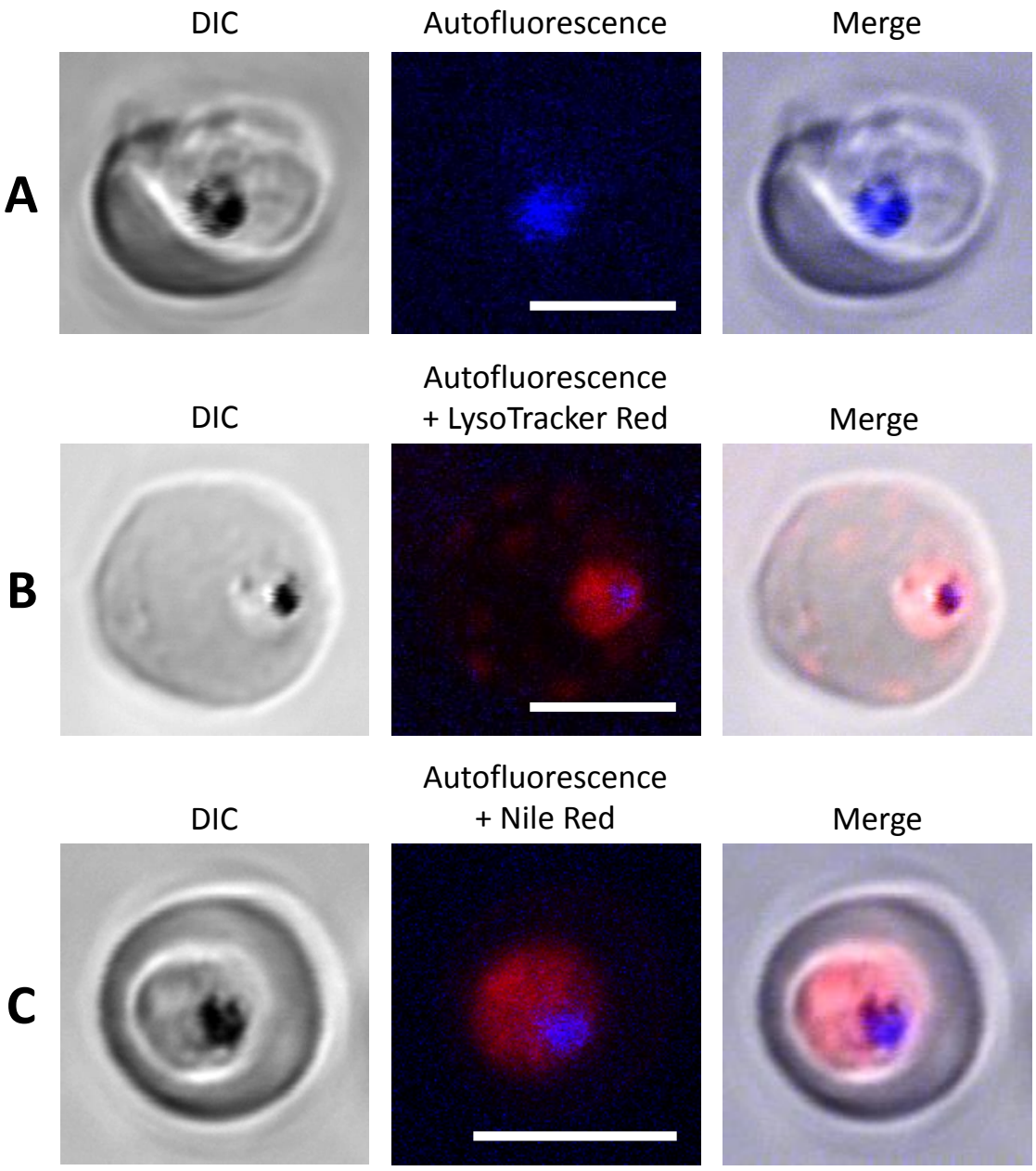


Figure S4. Erythrocytes infected with *P. falciparum*. A strong haemozoin autofluorescence (blue) corresponded to two-photon excitation at 750 nm and emission between 415-500 nm observed in untreated cells (A) and also cells treated with LysoTracker Red (B) and Nile Red (C). Scale bars represent 5 μm.

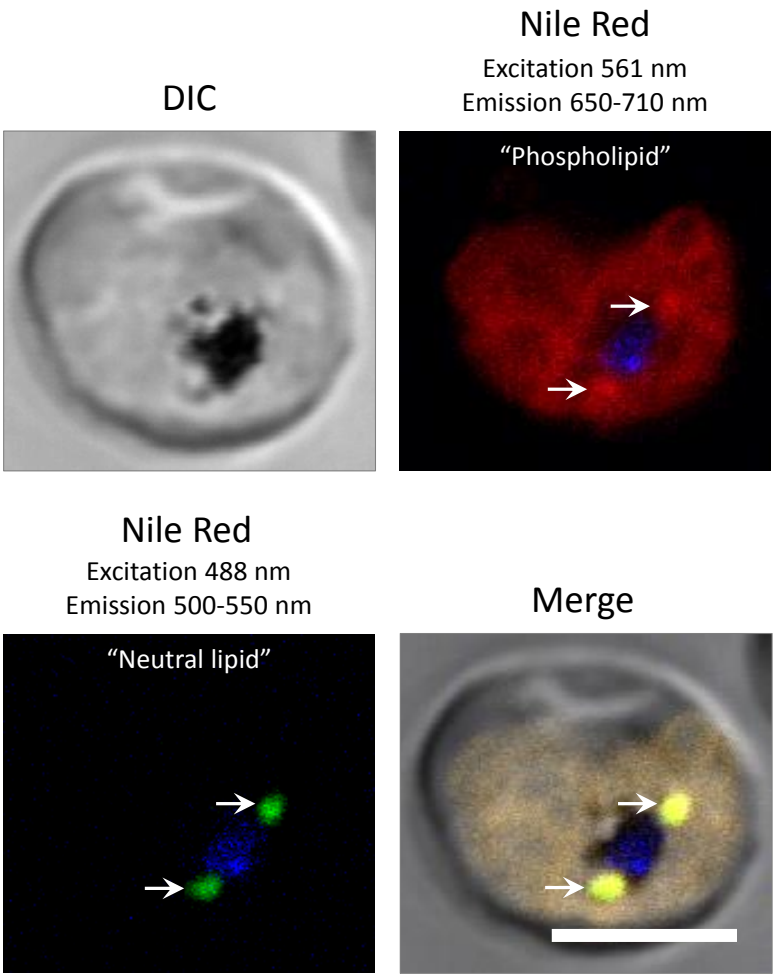


Figure S5. An erythrocyte infected with a mature *P. falciparum* parasite incubated with Nile Red and imaged using confocal microscopy at various excitation and emission wavelengths. Blue represents the haemozoin autofluorescence described in Figure S4. Phospholipid and neutral lipid environments are illuminated following the application of different excitation energies and emission filters, revealing staining patterns characteristic of neutral lipid bodies which are clearly evident in the green channel (indicated by white arrows). When imaging the NBD-labelled derivatives using super-resolution microscopy, cross-talk in the green region corresponding to the neutral lipid bodies was avoided by incubating the cells with higher concentrations of the NBD-labelled derivatives (2 μM) followed by immediate imaging. The scale bar represents 5 μm.

2. Synthetic procedures and characterisation of organic compounds

2.1. General synthetic methods

Unless otherwise stated, all reagents were purchased from Sigma-Aldrich and used without further purification. Monobromobimane was procured from Synchem UG & Co. KG. Solvents were generally purchased from Kimix Chemicals. Anhydrous $CHCl_3$ and CH_2Cl_2 were freshly distilled over $CaCl_2$ and P_4O_{10}, respectively. THF was distilled from sodium wire and benzophenone. Double-distilled deionised water (dH_2O) was provided by a Millipore Direct-Q3 water purification system.

For photoinitatied reactions, a Philips Actinic BL TL-D 15 W/10 1SL tubular low-pressure mercury vapour lamp with an emission maximum of 368 nm was used. Photosensitive reactions were protected from ambient light by aluminium foil. All purifications were performed under dim light. Thin-layer chromatography (TLC) was carried out on aluminium-backed silica gel 60 F_{254} plates (Merck). Compounds were visualised under short-wavelength UV light (254 nm) or long-wavelength UV light for fluorescence detection (365 nm). Plates were sprayed with a 2.5% solution of anisaldehyde in a mixture of sulfuric acid and ethanol (1:10 v/v) or with a 0.3% solution of ninhydrin in absolute ethanol acidified with 3% glacial acetic acid.[4]

For column chromatography, silica gel with pore size 60 Å and particle size 35-70 μm was used. Occasionally, flash chromatography was performed using a Biotage Isolera (silica gel with pore size 60 Å and particle size 63-200 μm).

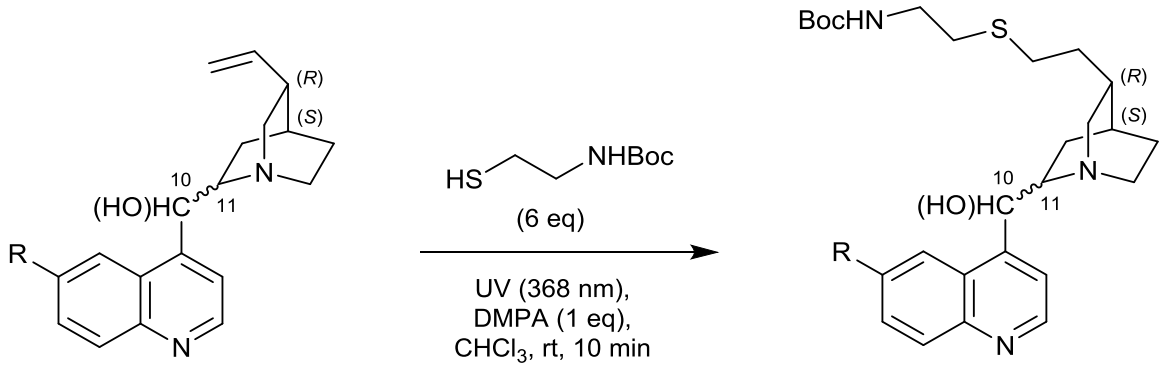
Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker 400 MHz spectrometer. Chemical shifts were recorded relative to residual chloroform in $CDCl_3$ (δ7.26 ppm in ^{1}H NMR and δ77.16 ppm in ^{13}C NMR), residual DMSO in DMSO-d_6 (δ2.50 ppm in ^{1}H NMR and δ39.52 in ^{13}C NMR) or residual methanol in CD_3OD (δ3.31 ppm in ^{1}H NMR and δ49.00 in ^{13}C NMR).[5]

Melting points were determined on a Reichert-Jung Thermovar hot-stage microscope. Optical rotation measurements were taken on a PerkinElmer Model 343 Polarimeter. Infrared (IR) spectra were recorded using either a PerkinElmer Spectrum Two FT-IR spectrometer with a PerkinElmer UATR attachment or a Bruker Tensor 27 FT-IR spectrometer with a Bruker Platinum ATR attachment. Absorption spectra were recorded at 25°C on a Varian Cary 100 UV-Vis or a Shimadzu UV-1800 spectrophotometer and were baseline-corrected.

Electron ionisation (EI) mass spectrometry was executed on a JEOL GCmate II. High-resolution mass spectrometry (HRMS) was performed at the Central Analytical Facility at Stellenbosch University using a Waters Synapt G2 with an electrospray ionisation (ESI) positive source. CHNS analysis was performed using a Thermo Flash EA 1112 series combustion analyser. High-performance liquid chromatography (HPLC) was performed using an Agilent 1220 LC System VL equipped with an Agilent ZORBAX Eclipse Plus C18 column (5 μm, 4.6 mm x 150 mm). Compounds dissolved in acetonitrile (0.3 mg/mL) were run at a flow rate of 1 mL/min with UV detection at 254 nm.

Systematic names were generated using ChemBioDraw (Version 13.0.2.3021, CambridgeSoft, 2013). Overall percentage yields were calculated from the lowest-yielding route to a final product.

2.2. General procedure for the thiol-ene 'click' reduction of *Cinchona* alkaloids



1 Quinine (10*R*,11*S*) R=OMe
2 Quinidine (10*S*,11*R*) R=OMe

7 (10*R*,11*S*) R=OMe (87%)
8 (10*S*,11*R*) R=OMe (86%)

This method was adapted from a previously-reported procedure.[6] The reaction was found not to be sensitive to oxygen or trace water; it is therefore unnecessary to use an inert atmosphere or anhydrous solvent. The reaction can be performed on a large scale (up to 2 g) enabling large amounts of thiolated derivatives to be easily prepared. Progress of the reaction is difficult to visualise using TLC due to 'smearing' of the amines across the silica substrate. Furthermore, the R_f values of the alkaloid starting materials and their products were often identical.

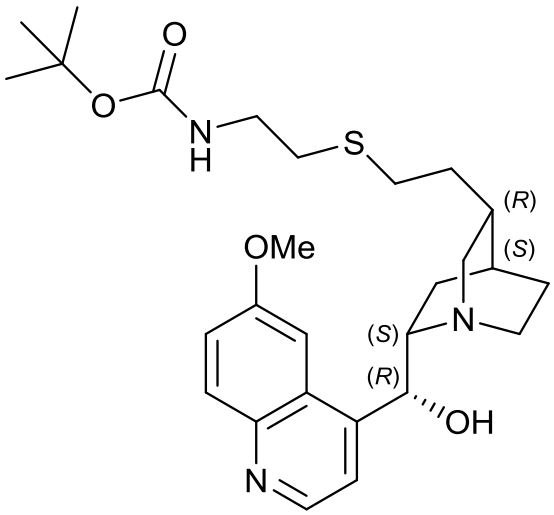
Alkaloid **1** or **2** (1 eq) and radical photoinitiator 2,2-dimethoxy-2-phenyl-acetophenone (DMPA, 1 eq) were both dissolved in $CHCl_3$ in a round-bottomed flask equipped with a stirrer bar. The thiol *tert*-butyl (2-mercaptoethyl)carbamate (6 eq) was added via syringe and the mixture was stirred thoroughly for several minutes. The flask was covered with a septum and placed on a stirrer plate against the centre of a Philips Actinic BL TL-D 15 W/10 1SL tubular low-pressure mercury vapour lamp. The entire apparatus was wrapped in aluminium foil and irradiated for ten minutes while stirring. During irradiation, the reaction mixture changed from colourless to light yellow.

The solvent was removed on a rotary evaporator and the reaction mixture was taken up in ethyl acetate (25 mL). This was transferred into a separating funnel and the organic component was washed with ice-cold 0.5 M citric acid (4 x 25 mL) to move the (protonated) product to the aqueous phase (pH 2-3). (The concentration, identity and temperature of the acid are important to avoid cleaving the *N*-Boc protecting group.)

Afterwards, the organic phase containing unreacted DMPA, the degradation products of DMPA and remaining thiol reactant was discarded. The aqueous phase was then basified to pH 11-12 with approximately ice-cold 5 M NaOH (40 mL). The neutral organic product was then extracted with

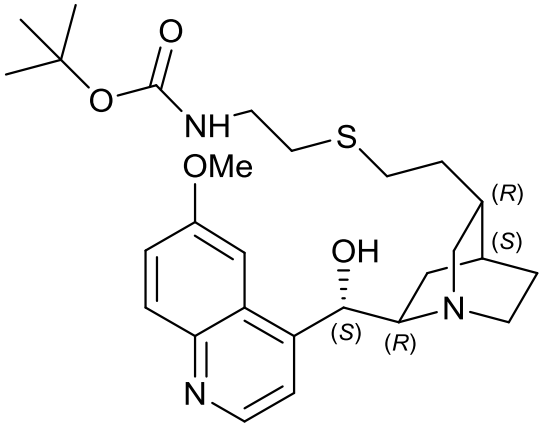
ethyl acetate (3 x 25 mL). The extracts were combined and this was then washed with citric acid, basified and extracted as before to remove further impurities.

After a final wash with brine, magnesium sulfate was added to remove trace water. Following filtration, the solvent was removed under reduced pressure and the product **7** or **8** was dried on a vacuum pump. While ^{1}H NMR analysis indicated sufficient purity for subsequent reactions (> 95%), for combustion analysis these compounds were recrystallised from aqueous ethanol or methanol as described below.



***tert*-Butyl (2-((2-((1*S*,3*R*,4*S*,6*S*)-6-((*R*)-hydroxy(6-methoxyquinolin-4-yl)methyl)quinuclidin-3-yl)ethyl)thio)ethyl)carbamate (7).**

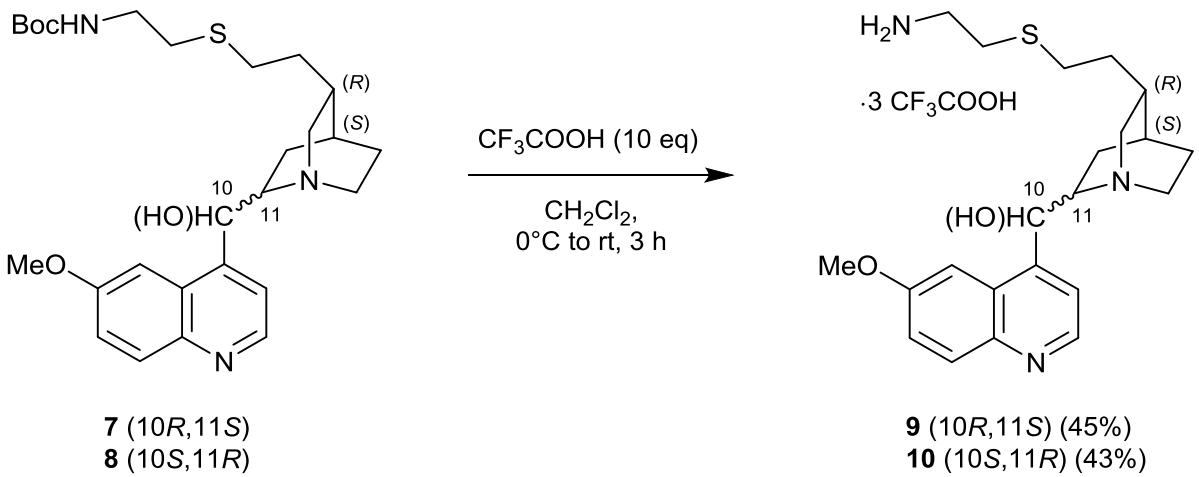
The general procedure above was followed. The alkaloid quinine (**1**, 1.000 g, 3.08 mmol), DMPA (790 mg, 3.08 mmol) and the thiol *tert*-butyl (2-mercaptoethyl)carbamate (3.12 mL, 18.48 mmol) were mixed in $CHCl_3$ (10 mL) and irradiated for ten minutes. Subsequent acid-base extraction yielded **7** as a white powder (1.340 g, 2.67 mmol, 87%). **7** was then further recrystallised from aqueous ethanol (1.084 g, 2.16 mmol, 70%). MP 69-71°C. $[\alpha]_D^{20}$ -82.6° (*c*. 0.797, EtOH). UV (EtOH) λ_{max}, nm (ε, M^{-1} cm^{-1}): 333 (5 000). ATR-FTIR ν_{max}/cm^{-1} 1705 (strong, sharp C=O stretching vibration in the region expected for a secondary carbamate).[7] ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.57 (1H, d, *J* 4.6 Hz), 7.8 (1H, d, *J* 9.2 Hz), 7.49 (1H, d, *J* 4.6 Hz), 7.20 (1H, dd, *J* 9.2, 2.7 Hz), 7.15 (1H, d, *J* 2.7 Hz), 5.71 (1H, br s), 4.88 (2H, br s), 3.80 (3H, s), 3.65 (1H, m), 3.21 (2H, q, *J* 6.1 Hz), 3.10 (2H, m), 2.68 (1H, m), 2.53 (2H, t, *J* 6.7 Hz), 2.39 (3H, m), 1.78 (4H, m), 1.46 (3H, m), 1.38 (10H, m). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 158.0, 155.9, 147.4, 147.1, 144.2, 131.5, 126.5, 121.7, 118.6, 101.3, 79.6, 70.6, 60.0, 57.9, 56.1, 43.4, 39.9, 34.4, 34.3, 32.5, 29.8, 28.5, 27.4, 25.6, 20.8. HRMS (ESI-TOF) *m*/*z*: $[M+H]^+$ calculated for $C_{27}H_{40}N_3O_4S$ 502.2740, found 502.2752. Anal. Calcd for $C_{27}H_{39}N_3O_4S$: C, 64.64; H, 7.84; N, 8.38; S, 6.39. Found: C, 64.72; H, 7.71; N, 8.52; S, 6.04.



***tert*-Butyl (2-((2-((1*S*,3*R*,4*S*,6*R*)-6-((*S*)-hydroxy(6-methoxyquinolin-4-yl)methyl)quinuclidin-3-yl)ethyl)thio)ethyl)carbamate (8).**

The general procedure above was followed. The alkaloid quinidine (**2**, 500 mg, 1.54 mmol), DMPA (395 mg, 1.54 mmol) and the thiol *tert*-butyl (2-mercaptoethyl)carbamate (1.56 mL, 9.23 mmol) were mixed in $CHCl_3$ (5 mL) and irradiated for ten minutes. Subsequent acid-base extraction yielded **8** as a white powder (668 mg, 1.32 mmol, 86%). **8** was then recrystallised from aqueous ethanol (556 mg, 1.11 mmol, 72%). MP 168-170°C. $[\alpha]_D^{20}$ +137.5° (*c*. 0.797, EtOH). UV (EtOH) λ_{max}, nm (ε, M^{-1} cm^{-1}): 333 (5 000). ATR-FTIR ν_{max}/cm^{-1} 1705 (strong, sharp C=O stretching vibration in the region expected for a secondary carbamate).[7] ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.64 (1H, d, *J* 4.6 Hz), 7.95 (1H, d, *J* 9.2 Hz), 7.51 (1H, d, *J* 4.6 Hz), 7.29 (1H, dd, *J* 9.2, 2.6 Hz), 7.18 (1H, d, *J* 2.6 Hz), 5.59 (1H, d, *J* 3.9 Hz), 5.01 (1H, br s), 3.85 (3H, s), 3.29 (2H, q, *J* 6.4 Hz), 3.14 (1H, dq, *J* 7.3, 2.4 Hz), 3.02 (1H, td, *J* 9.3, 4.0 Hz), 2.86 (2H, m), 2.74 (1H, m), 2.61 (2H, m), 2.46 (2H, t, *J* 7.7 Hz), 2.00 (1H, m), 1.81 (1H, m), 1.70 (1H, m), 1.66 (1H, m), 1.59 (1H, t, *J* 8.0 Hz), 1.47 (2H, m), 1.41 (9H, s), 1.08 (1H, m). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 157.8, 156.1, 148.0, 147.7, 144.3, 131.7, 126.7, 121.6, 118.5, 101.5, 79.7, 72.1, 59.9, 55.7, 50.8, 50.3, 40.2, 34.9, 32.8, 32.1, 29.8, 28.5, 27.2, 26.8, 20.7. HRMS (ESI-TOF) *m*/*z*: $[M+H]^+$ calculated for $C_{27}H_{40}N_3O_4S$ 502.2740, found 502.2750. Anal. Calcd for $C_{27}H_{39}N_3O_4S$: C, 64.64; H, 7.84; N, 8.38; S, 6.39. Found: C, 64.38; H, 7.79; N, 8.52; S, 6.35.

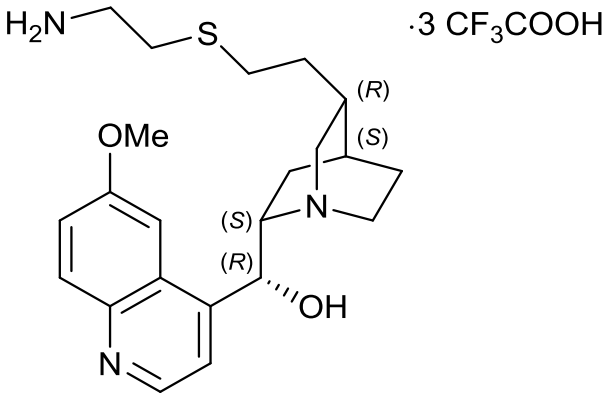
2.3. General procedure for the cleavage of *N*-Boc-protected amines



7 (10*R*,11*S*)
8 (10*S*,11*R*)

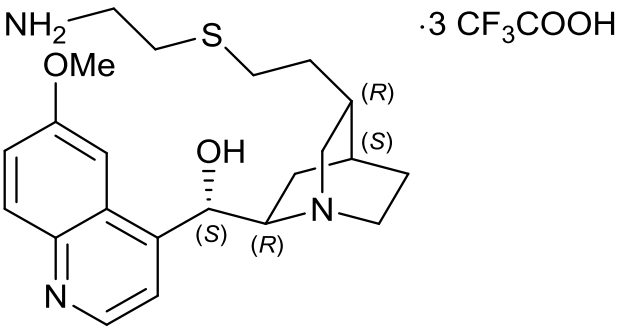
9 (10*R*,11*S*) (45%)
10 (10*S*,11*R*) (43%)

The *N*-Boc protected amine **7** or **8** (1 eq) was dissolved in CH_2Cl_2 in a round-bottomed flask equipped with a stirrer bar and cooled in an ice-bath to 0°C. Trifluoroacetic acid (TFA, 10 eq) was added dropwise over ten minutes during which time the reaction mixture changed from colourless to bright yellow. After two hours no further change was observed by TLC. During this time the ice-bath was not replenished, allowing the reaction mixture to warm slowly to room temperature. Excess TFA was removed under reduced pressure and the resulting oil was purified by column chromatography over an elution gradient of 0-25% MeOH:CH_2Cl_2. The appropriate fractions were collected, the volume was reduced and the product was dried on a vacuum pump to afford pure **9** or **10** as a trifluoroacetate salt.



Tris(trifluoroacetate) (1*S*,2*S*,4*S*,5*R*)-5-(2-((2-ammonioethyl)thio)ethyl)-2-((*R*)-hydroxy(6-methoxyquinolin-1-ium-4-yl)methyl)quinuclidin-1-ium (9).

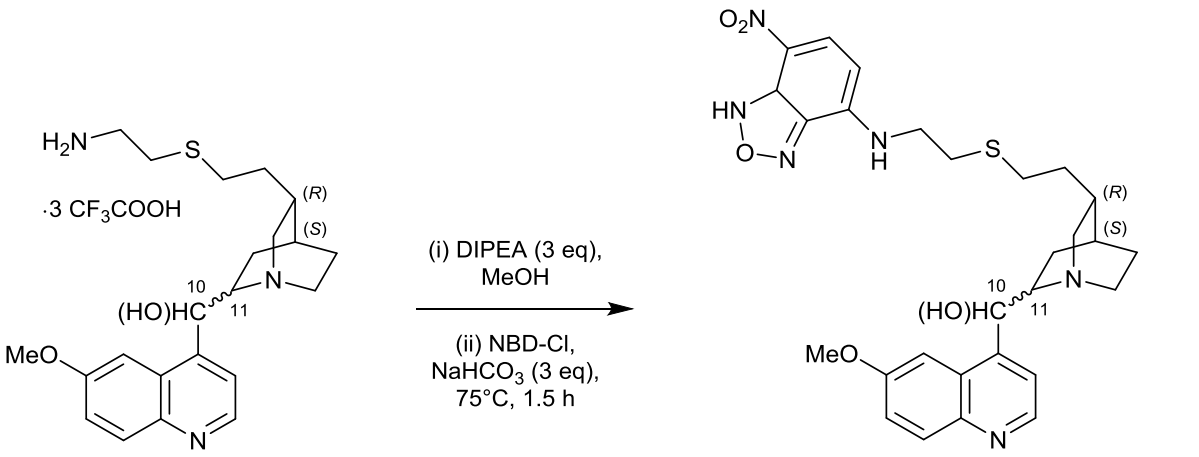
Following the general method above, **7** (0.885 g, 1.76 mmol) was dissolved in CH_2Cl_2 (10 mL) and cooled to 0°C. TFA (1.35 mL, 2.007 g, 17.6 mmol) was added dropwise. After two hours the excess solvent was evaporated and column chromatography yielded the salt **9** (0.589 g, 0.79 mmol, 45%) as a sticky white amorphous solid. UV (EtOH) λ_{max}, nm (ε, M^{-1} cm^{-1}): 333 (5 000). R_f 0.36 (MeOH:CH_2Cl_2 15:85). ATR-FTIR ν_{max}/cm^{-1} 1675 (strong, sharp C=O stretching vibration). ^{1}H NMR (400 MHz, CD_3OD) δ_H 8.87 (1H, d, *J* 5.1 Hz), 8.11 (1H, d, *J* 9.3 Hz), 8.06 (1H, d, *J* 5.1 Hz), 7.64 (1H, dd, *J* 9.3, 2.7 Hz), 7.56 (1H, d, *J* 2.7 Hz), 6.19 (1H, s), 4.25 (1H, m), 4.05 (3H, s), 3.71 (1H, m), 3.64 (1H, dd, *J* 13.0, 10.8 Hz), 3.31 (1H, m), 3.06 (3H, m), 2.74 (2H, t, *J* 6.8 Hz), 2.54 (2H, t, *J* 7.5 Hz), 2.26 (3H, m), 2.11 (1H, m), 1.91 (1H, m), 1.63 (3H, m). ^{13}C NMR (100 MHz, CD_3OD) δ_C 163.3 (C=O TFA anion), 161.2, 151.6, 145.6, 140.9, 128.6, 128.2, 129.9, 120.9, CF_3 from TFA anion not observed, 102.5, 68.3, 61.2, 57.14, 57.05, 45.2, 39.8, 33.90, 33.86, 29.72, 29.69, 26.1, 25.6, 19.0. HRMS (ESI-TOF) *m/z*: $[M+H]^+$ Calculated for $C_{22}H_{32}N_3O_2S$ 402.2215; found 402.2208. HPLC: 99.0%.



Tris(trifluoroacetate) (1*S*,2*R*,4*S*,5*R*)-5-(2-((2-ammonioethyl)thio)ethyl)-2-((*S*)-hydroxy(6-methoxyquinolin-1-ium-4-yl)methyl)quinuclidin-1-ium (10).

Following the general method above, **8** (0.963 g, 1.92 mmol) was dissolved in CH_2Cl_2 (10 mL) and cooled to 0°C. TFA (1.48 mL, 2.190 g, 19.2 mmol) was added dropwise. After two hours the excess solvent was evaporated and column chromatography yielded the salt **10** (0.617 g, 0.83 mmol, 43%) as a clear gum. UV (EtOH) λ_{max}, nm (ε, M^{-1} cm^{-1}): 333 (5 000). R_f 0.36 (MeOH:CH_2Cl_2 15:85). ATR-FTIR ν_{max}/cm^{-1} 1675 (strong, sharp C=O stretching vibration). ^{1}H NMR (400 MHz, CD_3OD) δ_H 8.87 (1H, d, *J* 5.1 Hz), 8.10 (1H, d, *J* 9.3 Hz), 8.07 (1H, d, *J* 5.1 Hz), 7.63 (1H, dd, *J* 9.3, 2.7 Hz), 7.56 (1H, d, *J* 2.7 Hz), 6.31 (1H, s), 4.05 (4H, m), 3.69 (1H, t, *J* 9.4 Hz), 3.54 (2H, m), 3.34 (1H, m), 3.19 (2H, t, *J* 6.8 Hz), 2.87 (2H, t, *J* 6.8 Hz), 2.67 (2H, m), 2.48 (1H, m), 2.21 (1H, m), 2.04 (1H, br s), 1.94 (4H, m), 1.27 (1H, m). ^{13}C NMR (100 MHz, CD_3OD) δ_C 163.4 (C=O TFA anion), 160.5, 148.1, 147.4, 143.7, 130.8, 127.6, 124.4, 120.4, CF_3 from TFA anion not observed, 102.0, 68.6, 61.2, 56.8, 51.1, 50.5, 39.9, 33.6, 32.3, 29.9, 26.6, 24.5, 18.8. HRMS (ESI-TOF) *m*/*z*: $[M+H]^+$ Calculated for $C_{22}H_{32}N_3O_2S$ 402.2215; found 402.2217. HPLC: 99.3%.

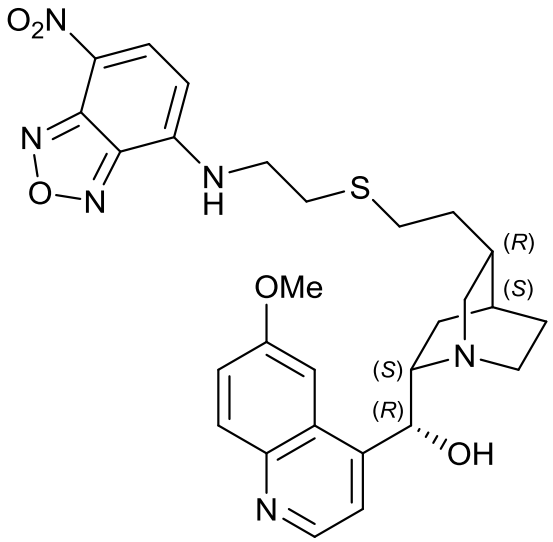
2.4. General procedure for the nucleophilic substitution of NBD-Cl by amino-functionalised alkaloids



9 (10*R*,11*S*)
10 (10*S*,11*R*)

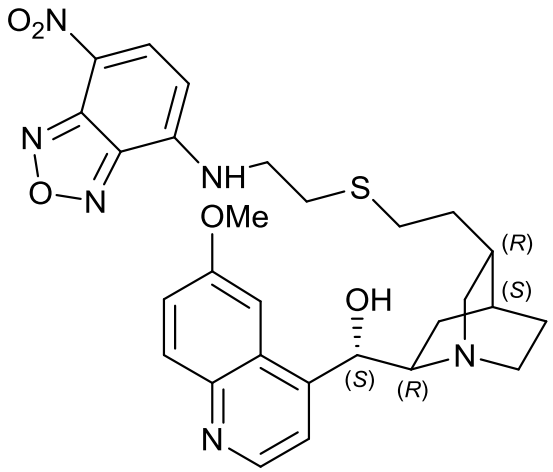
3 Quinine-NBD (10*R*,11*S*) (26%, 10% overall)
4 Quinidine-NBD (10*S*,11*R*) (27%, 10% overall)

The trifluoroacetate salt **9** or **10** (1 eq) was dissolved in MeOH in a two-necked round-bottomed flask equipped with a stirrer bar. *N*,*N*-Diisopropylethylamine (DIPEA, 3 eq) was added and the mixture was stirred for ten minutes to liberate the amines from the trifluoroacetate anions. A septum was attached to one neck of the flask while a reflux condenser was attached to the other neck and the mixture was heated to 75°C. Solid $NaHCO_3$ (3 eq) was added. 4-Chloro-7-nitrobenzo[*c*][1,2,5]oxadiazole (NBD-Cl, 1 eq) was dissolved in CH_3CN (10 mL) and added dropwise. The mixture was incubated for two hours, during which time the colour changed from bright yellow to deep brown which indicated successful displacement of the chlorine by the terminal amine of the alkaloid to form the fluorescent NBD adduct. After this time TLC indicated that all NBD-Cl was consumed. The reaction mixture was cooled and filtered through a pad of Celite. The volume was reduced on the rotary evaporator and the mixture was purified using column chromatography (0-20% MeOH:CH_2Cl_2 elution gradient). The appropriate fractions were selected, the volume was reduced and the resultant product **3** or **4** was recrystallised from aqueous MeOH.



(*R*)-(6-methoxyquinolin-4-yl)((1*S*,2*S*,4*S*,5*R*)-5-(2-((2-((7-nitrobenzo[*c*][1,2,5]oxadiazol-4-yl)amino)ethyl)thio)ethyl)quinuclidin-2-yl)methanol, "Quinine-NBD" (3).

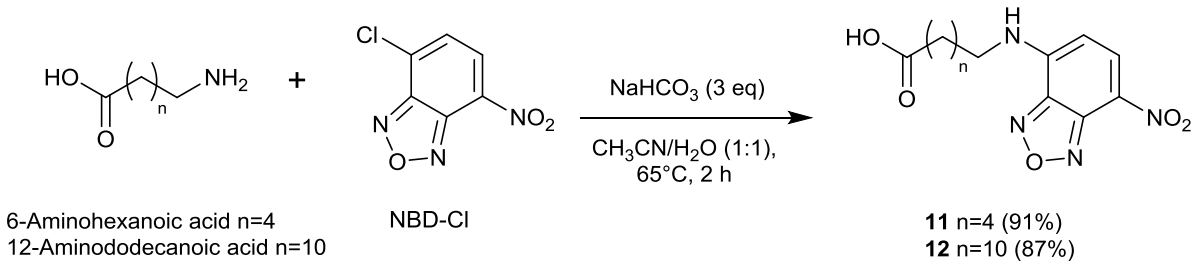
Following the general method above, **8** (1.497 g, 2.01 mmol) was dissolved in MeOH (20 mL) and neutralised with DIPEA (1.05 mL, 6.03 mmol). $NaHCO_3$ (507 mg, 6.03 mmol) was added and the mixture heated to 75°C, after which NBD-Cl (401 mg, 2.01 mmol) was added. The product **3** was isolated by column chromatography and purified by recrystallisation from aqueous MeOH (294 mg, 0.52 mmol, 26%, 10% overall). UV (EtOH) λ_{max}, nm (ε, M^{-1} cm^{-1}): 333 (11 500), 464 (18 500); UV (20 mM Hepes, pH 7.5) λ_{max}, nm (ε, M^{-1} cm^{-1}): 335 (8 500), 473 (17 500). R_f 0.61 (MeOH:CH_2Cl_2 10:90). ATR-FTIR ν_{max}/cm^{-1} 1295 (strong, sharp NO_2 symmetric stretching vibration). ^{1}H NMR (400 MHz, DMSO-d_6) δ_H 8.74 (1H, d, *J* 4.5 Hz), 8.45 (1H, d, *J* 8.9 Hz), 7.95 (1H, d, *J* 9.2 Hz), 7.63 (1H, d, *J* 4.5 Hz), 7.51 (1H, d, *J* 2.8 Hz), 7.41 (1H, dd, *J* 9.2, 2.8 Hz), 6.41 (2H, m), 6.04 (1H, d, *J* 2.6 Hz), 3.99 (4H, m), 3.60 (3H, m), 3.47 (1H, t, *J* 11.6 Hz), 3.20 (1H, m), 2.94 (1H, dd, *J* 12.1, 5.6 Hz), 2.79 (2H, t, *J* 7.0 Hz), 2.59 (2H, m), 2.05 (3H, m), 1.96 (1H, br s), 1.73 (1H, m), 1.57 (2H, m), 1.47 (1H, m). ^{13}C NMR (100 MHz, DMSO-d_6) δ_C 157.7, 147.3, 145.1, 144.7, 144.2, 143.9, 143.7, 137.6, 131.3, 125.6, 121.5, 120.8, 119.0, 101.9, 99.3, 65.9, 59.0, 56.4, 54.5, 43.2, 42.8, 32.3, 32.1, 29.3, 28.6, 24.3, 17.6. HRMS (ESI-TOF) *m*/*z*: $[M+H]^+$ Calculated for $C_{28}H_{33}N_6O_5S$ 565.2233; found 565.2229. HPLC: 96.1%.



(*S*)-(6-methoxyquinolin-4-yl)((1*S*,2*R*,4*S*,5*R*)-5-(2-((2-((7-nitrobenzo[*c*][1,2,5]oxadiazol-4-yl)amino)ethyl)thio)ethyl)quinuclidin-2-yl)methanol, "Quinidine-NBD" (4).

Following the general method above, **9** (1.542 g, 2.02 mmol) was dissolved in MeOH (20 mL) and neutralised with DIPEA (1.06 mL, 6.06 mmol). $NaHCO_3$ (509 mg, 6.06 mmol) was added and the mixture heated to 75°C, after which NBD-Cl (403 mg, 2.02 mmol) was added. The product **4** was isolated by column chromatography and purified by recrystallisation from aqueous MeOH (311 mg, 0.55 mmol, 27%, 10% overall). UV (EtOH) λ_{max}, nm (ε, M^{-1} cm^{-1}): 332 (11 000), 463 (17 000); UV (20 mM Hepes, pH 7.5) λ_{max}, nm (ε, M^{-1} cm^{-1}): 335 (9 000), 479 (19 000). R_f 0.61 (MeOH:CH_2Cl_2 10:90). ATR-FTIR ν_{max}/cm^{-1} 1300 (strong, sharp NO_2 symmetric stretching vibration). ^{1}H NMR (400 MHz, DMSO-d_6) δ_H 8.75 (1H, d, *J* 4.5 Hz), 8.51 (1H, d, *J* 8.9 Hz), 7.97 (1H, d, *J* 9.3 Hz), 7.64 (1H, d, *J* 4.5 Hz), 7.43 (1H, dd, *J* 9.3, 2.9 Hz), 7.37 (1H, d, *J* 2.9 Hz), 6.54 (1H, d, *J* 4.0 Hz), 6.50 (1H, d, *J* 8.9 Hz), 6.02 (1H, d, *J* 2.3 Hz), 3.96 (3H, s), 3.76 (3H, m), 3.56 (1H, t, *J* 9.2 Hz), 3.45 (2H, m), 3.22 (1H, m), 2.91 (2H, t, *J* 6.9 Hz), 2.65 (2H, m), 2.28 (1H, t, *J* 11.2 Hz), 2.04 (1H, quin, *J* 8.2 Hz), 1.92 (1H, br s), 1.80 (4H, m), 1.15 (1H, m). ^{13}C NMR (100 MHz, DMSO-d_6) δ_C 157.7, 147.4, 144.7, 144.3, 144.2, 144.1, 143.7, 137.7, 131.4, 125.4, 121.6, 121.0, 118.8, 101.2, 99.4, 66.4, 59.0, 55.8, 48.9, 48.3, 43.3, 31.7, 31.1, 29.4, 28.7, 24.5, 23.0, 17.2. HRMS (ESI-TOF) *m*/*z*: $[M+H]^+$ Calculated for $C_{28}H_{33}N_6O_5S$ 565.2233; found 565.2247. HPLC: 98.0%.

2.5. General procedure for the preparation of NBD-labelled amino acids

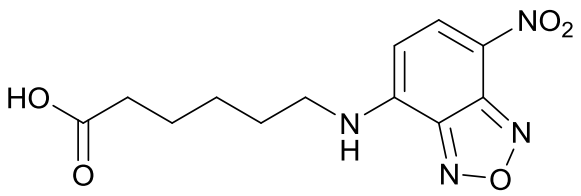


6-Aminohexanoic acid n=4
12-Aminododecanoic acid n=10

NBD-Cl

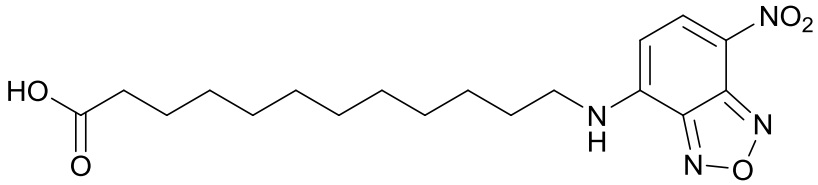
11 n=4 (91%)
12 n=10 (87%)

The procedures of Novotný et al. and Haldar et al. were followed for the preparation of the NBD-labelled amino acids of **11** and **12**.[8,9] Briefly, the amino acid (1.2 eq) and $NaHCO_3$ (3 eq) were dissolved in dH_2O (10 mL) in a two-necked round-bottomed flask equipped with a stirrer bar. A septum was attached to one neck and a reflux condenser was attached to the other. The mixture was heated to 65°C. 4-Chloro-7-nitrobenzo[*c*][1,2,5]oxadiazole (NBD-Cl, 1 eq) was dissolved in CH_3CN (10 mL) and added dropwise via syringe. The mixture was incubated for two hours, during which time the colour changed from bright yellow to deep brown. After two hours, TLC indicated that all the NBD-Cl had been consumed. The mixture was cooled and the CH_3CN was removed on the rotary evaporator. The remaining aqueous phase was acidified to approximately pH 2 with 1 M HCl. Thereafter it was extracted three times with EtOAc (20 mL), washed with brine, dried with $MgSO_4$, filtered and the solvent removed. The resultant NBD-labelled amino acid **11** or **12** was then recrystallised from aqueous MeOH.



6-((7-nitrobenzo[*c*][1,2,5]oxadiazol-4-yl)amino)hexanoic acid (11).[8]

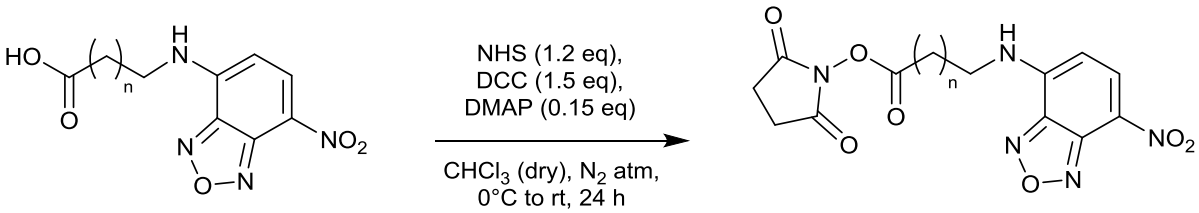
The NBD-labelled amino acid **11** was prepared from 6-aminohexanoic acid (679.7 mg, 4.81 mmol) as described above and recrystallised from aqueous MeOH to yield bright orange crystals (1.077 g, 3.66 mmol, 91%). MP 153-155°C (lit.[8] MP 158-159°C). UV (EtOH) λ_{max}, nm (ε, M^{-1} cm^{-1}): 333 (8 500), 456 (19 500). ATR-FTIR ν_{max}/cm^{-1} 1700 (strong, sharp C=O stretching vibration indicates saturated carboxylic acid). MS (EI+) *m*/*z*: $[M]^+$ 294.



12-((7-nitrobenzo[*c*][1,2,5]oxadiazol-4-yl)amino)dodecanoic acid (12).[8]

The NBD-labelled amino acid **12** was prepared from 12-aminododecanoic acid (327 mg, 1.52 mmol) as described above and recrystallised from aqueous MeOH to yield dark orange crystals (501 mg, 1.32 mmol, 87%). MP 92-94°C (lit.[8] MP 94-96°C). UV (EtOH) λ_{max}, nm (ε, M^{-1} cm^{-1}): 333 (8 000), 464 (19 000). ATR-FTIR ν_{max}/cm^{-1} 1720 (strong, sharp C=O stretching vibration indicates saturated carboxylic acid). MS (EI+) *m*/*z*: $[M]^+$ 378.

2.6. General procedure for the preparation of NBD-labelled amino acid succinimidyl esters

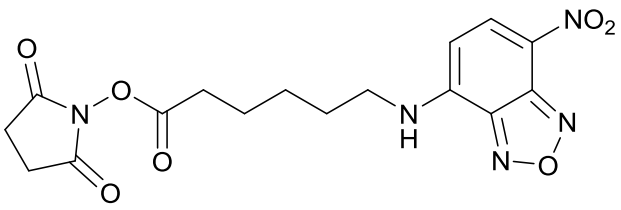


11 n=4
12 n=10

13 n=4 (79%)
14 n=10 (53%)

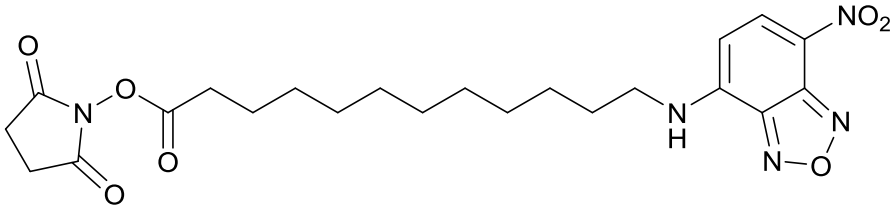
A procedure previously described for the preparation of the NBD-labelled amino acid succinimidyl esters was followed.[8] Briefly, dry $CHCl_3$ (10 mL) was added to a two-necked round-bottomed flask equipped with a stirrer bar under an inert atmosphere (N_2) to which the NBD-labelled amino acid **11** or **12** (1 eq), *N*-hydroxysuccinimide (NHS, 1.2 eq) and catalytic 4-dimethylaminopyridine (DMAP, 0.15 eq) had already been added. This was cooled in an ice-bath to 0°C. *N*,*N*'-Dicyclohexylcarbodiimide (DCC, 1.5 eq) was dissolved in dry $CHCl_3$ (2 mL) and added dropwise. If necessary, additional dry CH_3CN (5 mL) was added to ensure that the NBD-labelled amino acid was completely dissolved.

The flask was left to stir overnight and was gradually allowed to reach room temperature. After 24 hours, TLC indicated that the NBD-labelled amino acid was completed consumed. Solvents were evaporated and the residue was resuspended in a half-brine/half-water mixture to remove the urea by-product. The desired product was extracted three times with EtOAc, dried with brine and Na_2SO_4, filtered and the solvent removed. NBD-labelled amino acid succinimidyl esters **13** and **14** were labile but flash chromatography (100% EtOAc) could be used to furnish the product in a form suitable for further use (judged to be > 95% pure from ^{1}H NMR).



2,5-dioxopyrrolidin-1-yl 6-((7-nitrobenzo[*c*][1,2,5]oxadiazol-4-yl)amino)hexanoate (13).[8]

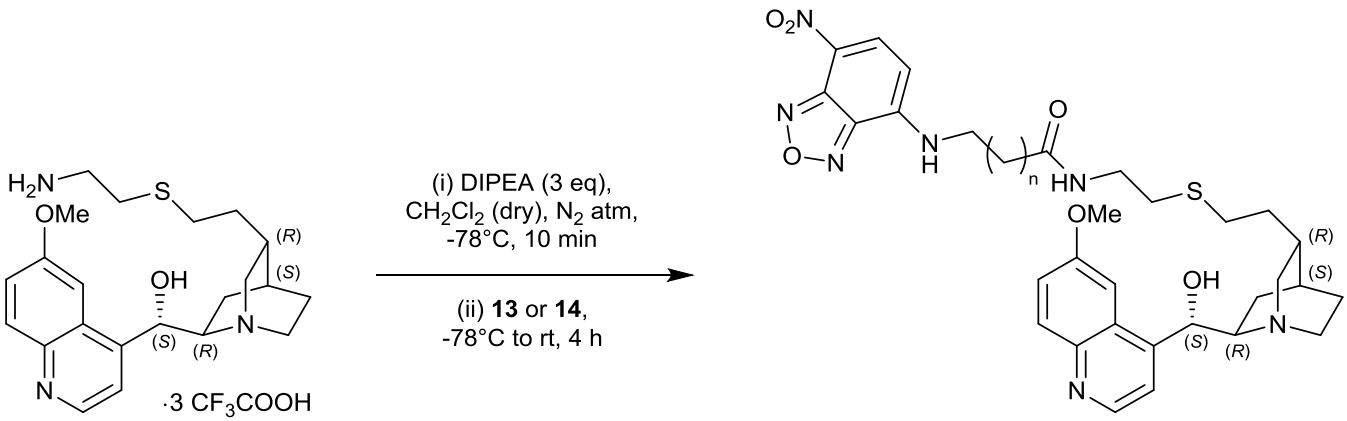
The NBD-labelled succinimidyl ester **13** was prepared from **11** (221 mg, 0.75 mmol) as described above and purified by column chromatography to yield bright orange crystals (232 mg, 0.59 mmol, 79%). MP 177-179°C (lit.[8] MP 189-191°C). UV (EtOH) λ_{max}, nm (ε, M^{-1} cm^{-1}): 333 (8 500), 456 (19 500). ATR-FTIR ν_{max}/cm^{-1} 1815 and 1785 (medium, sharp C=O stretching vibrations typical of succinimidyl carbonyls), 1730 (strong, sharp C=O stretching vibration typical of succinimidyl ester). MS (EI+) *m*/*z*: $[M]^+$ 391.



2,5-dioxopyrrolidin-1-yl 12-((7-nitrobenzo[*c*][1,2,5]oxadiazol-4-yl)amino)dodecanoate (14).[8]

The NBD-labelled succinimidyl ester **14** was prepared from **12** (504 mg, 1.06 mmol) as described above and purified by column chromatography to yield bright orange crystals (264 mg, 0.56 mmol, 53%). MP 160-162°C (lit.[8] MP 162-164°C). UV (EtOH) λ_{max}, nm (ε, M^{-1} cm^{-1}): 333 (8 000), 464 (19 000). ATR-FTIR ν_{max}/cm^{-1} 1815 and 1785 (medium, sharp C=O stretching vibrations typical of succinimidyl carbonyls), 1730 (strong, sharp C=O stretching vibration typical of succinimidyl ester). MS (EI+) *m*/*z*: $[M]^+$ 475.

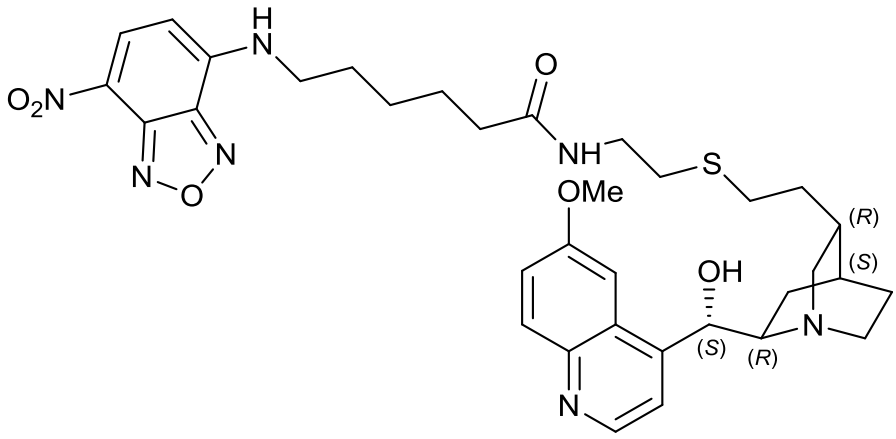
2.7. General procedure for the nucleophilic substitution of succinimidyl esters by the functionalised alkaloid



10

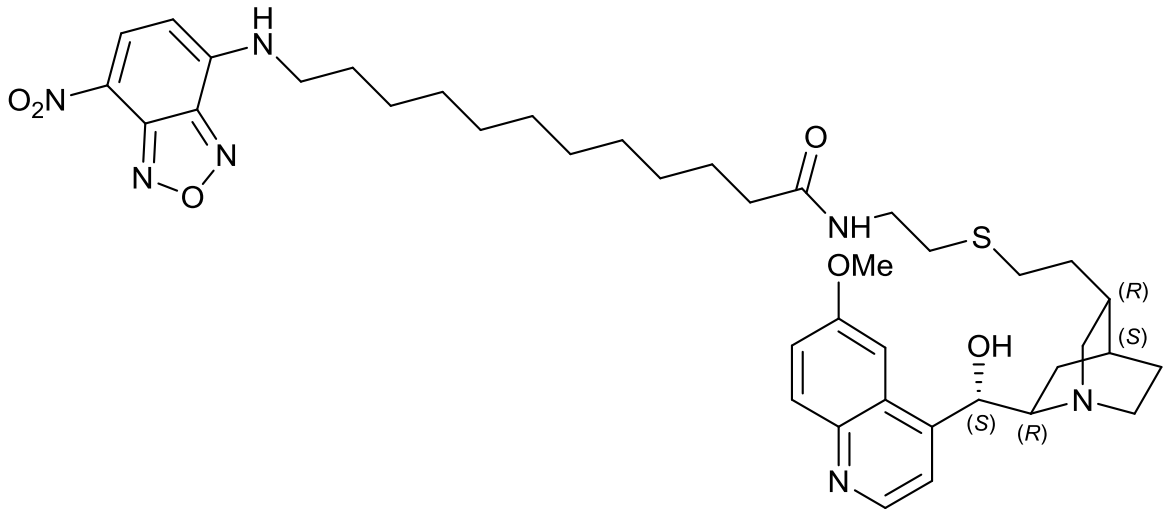
5 Quinidine-HexNBD n=4 (29%, 11% overall)
6 Quinidine-DodecNBD n=10 (68%, 25% overall)

To a round-bottomed flask equipped with a stirrer under an inert atmosphere (N_2) charged with the salt **10** (1 eq) was added dry CH_2Cl_2. This was cooled to -78°C, to which DIPEA (3 eq) was added dropwise. This was stirred for ten minutes to ensure neutralisation of the salt. Then the NBD-labelled amino acid succinimidyl ester **13** or **14** (1 eq) in dry CH_2Cl_2 was added dropwise. After three hours of continuous stirring the mixture was allowed to warm to room temperature. TLC indicated that the succinimidyl ester starting material had been completely consumed. The mixture was diluted with CH_2Cl_2 (10 mL) and washed three times with water to remove the NHS by-product. The volume was reduced. Column chromatography was performed (0-25% MeOH:CH_2Cl_2 elution gradient), the appropriate fractions collected, the volume reduced and the pure NBD-labelled alkaloid **5** or **6** was dried on the vacuum pump.



***N*-(2-((2-((1*S*,3*R*,4*S*,6*R*)-6-((*S*)-hydroxy(6-methoxyquinolin-4-yl)methyl)quinuclidin-3-yl)ethyl)thio)ethyl)-6-((7-nitrobenzo[*c*][1,2,5]oxadiazol-4-yl)amino)hexanamide, "Quinidine-HexNBD" (5)**.

Following the general method above, **10** (410 mg, 0.55 mmol) was dissolved in dry CH_2Cl_2 (10 mL) and cooled to -78°C. DIPEA (0.29 mL, 1.65 mmol) was added dropwise. **13** (215 mg, 0.55 mmol) in dry CH_2Cl_2 (5 mL) was added. After three hours of stirring, excess solvent was evaporated and, following dilution and extraction, column chromatography yielded **5** (110 mg, 0.16 mmol, 29%, 11% overall) as an orange solid. UV (EtOH) λ_{max}, nm (ε, M^{-1} cm^{-1}): 332 (10 500), 464 (15 500); UV (20 mM Hepes, pH 7.5) λ_{max}, nm (ε, M^{-1} cm^{-1}): 335 (6 500), 487 (14 500). R_f 0.40 (MeOH:CH_2Cl_2 10:90). ATR-FTIR ν_{max}/cm^{-1} 1625 (medium, sharp C=O stretching vibration for amide). ^{1}H NMR (400 MHz, DMSO-d_6) δ_H 8.66 (1H, d, *J* 4.6 Hz), 8.43 (1H, d, *J* 8.8 Hz), 7.90 (2H, m), 7.50 (1H, d, *J* 4.6 Hz), 7.46 (1H, d, *J* 2.8 Hz), 7.36 (1H, dd, *J* 9.3, 2.8 Hz), 6.34 (1H, d, *J* 8.8 Hz), 5.63 (1H, br s), 5.53 (1H, d, *J* 6.6 Hz), 3.90 (3H, s), 3.43 (2H, br s), 3.22 (2H, dt, *J* 7.8, 6.1 Hz), 2.98 (1H, m), 2.86 (1H, m), 2.72 (1H, dd, *J* 13.0, 7.2 Hz), 2.62 (1H, m, *J* 10.1 Hz), 2.55 (3H, t, *J* 7.2 Hz), 2.50 (2H, m), 2.09 (2H, t, *J* 7.3 Hz), 1.88 (1H, t, *J* 11.7 Hz), 1.68 (5H, m), 1.56 (3H, m), 1.47 (1H, m), 1.36 (4H, m). ^{13}C NMR (100 MHz, DMSO-d_6) δ_C 172.0, 156.7, 149.2, 147.4, 143.3, 144.5, 144.2, 143.8, 137.5, 131.0, 126.9, 120.9, 120.0, 118.8, 102.4, 99.1, 70.4, 60.6, 55.4, 49.7, 49.1, 43.5, 38.5, 35.2, 34.3, 32.2, 30.8, 29.1, 27.5, 26.8, 26.0, 26.0, 24.8, 22.9. HRMS (ESI-TOF) *m*/*z*: $[M+H]^+$ Calculated for $C_{34}H_{44}N_7O_6S$ 678.3074; found 678.3082. HPLC: 96.4%.



***N*-(2-((2-((1*S*,3*R*,4*S*,6*R*)-6-((*S*)-hydroxy(6-methoxyquinolin-4-yl)methyl)quinuclidin-3-yl)ethyl)thio)ethyl)-12-((7-nitrobenzo[*c*][1,2,5]oxadiazol-4-yl)amino)dodecanamide, "Quinidine-DodecNBD" (6).**

Following the general method above, **10** (404 mg, 0.53 mmol) was dissolved in dry CH_2Cl_2 (10 mL) and cooled to -78°C. DIPEA (0.28 mL, 1.59 mmol) was added dropwise. **14** (252 mg, 0.53 mmol) in dry CH_2Cl_2 (5 mL) was added. After three hours of stirring, excess solvent was evaporated and, following dilution and extraction, column chromatography yielded **6** (271 mg, 0.36 mmol, 68%, 25% overall) as an orange solid. UV (EtOH) λ_{max}, nm (ε, M^{-1} cm^{-1}): 333 (6 000), 466 (10 000); UV (20 mM Hepes, pH 7.5) λ_{max}, nm (ε, M^{-1} cm^{-1}): 339 (3 000), 485 (4 000). R_f 0.31 (MeOH:CH_2Cl_2 10:90). ATR-FTIR ν_{max}/cm^{-1} 1625 (medium, sharp C=O stretching vibration for amide). ^{1}H NMR (400 MHz, DMSO-d_6) δ_H 8.73 (1H, d, *J* 4.6 Hz), 8.47 (1H, d, *J* 9.2 Hz), 7.93 (2H, m), 7.64 (1H, d, *J* 4.6 Hz), 7.55 (1H, d, *J* 2.6 Hz), 7.40 (1H, dd, *J* 9.2, 2.6 Hz), 6.46 (1H, br s-), 6.38 (1H, d, *J* 9.2 Hz), 6.29 (1H, br s), 4.00 (3H, s), 3.74 (1H, m), 3.46 (4H, m), 3.32 (1H, m), 3.24 (2H, dt, *J* 7.8, 6.1), 3.16 (1H, m), 2.56 (4H, m), 2.29 (1H, t, *J* 11.4 Hz), 2.03 (3H, m), 1.91 (1H, br s, H-13), 1.80 (3H, m), 1.68 (3H, m), 1.48 (2H, t, *J* 7.5 Hz), 1.34 (2H, m), 1.22 (12H, m), 1.15 (1H, m). ^{13}C NMR (100 MHz, DMSO-d_6) δ_C 172.1, 157.7), 147.3, 145.2, 145.1, 144.3, 144.1, 143.7, 137.8, 131.2, 125.6, 121.7, 120.5, 118.8, 101.7, 99.0, 66.1, 59.0, 56.5, 48.6, 48.1, 43.3, 38.4, 35.4, 31.8, 31.1, 30.7, 28.9, 28.9, 28.9, 28.7, 28.6, 28.6, 28.5, 27.6, 26.3, 25.2, 24.6, 23.2, 17.2. HRMS (ESI-TOF) *m*/*z*: $[M+H]^+$ Calculated for $C_{40}H_{56}N_7O_6S$ 762.4013; found 762.4017. HPLC: 95.2%.

3. Physicochemical and in vitro evaluation of fluorescent derivatives

3.1. General physicochemical methods

Unless otherwise stated, all compounds were purchased from Sigma-Aldrich and used without further purification. Solvents were generally purchased from Kimix Chemicals. Bovine haemin (Fe(III)PPIX-Cl) was obtained from Fluka. NP-40 was obtained from Pierce Biotechnology, Rockford, IL, USA. Double-distilled deionised water (dH_2O) was provided by a Millipore Direct-Q3 water purification system. Hoechst 33342 and LysoTracker Red were obtained from Molecular Probes (Oregon, USA). Nile Red was obtained from Sigma-Aldrich.

Absorption spectra were recorded on a Varian Cary 100 UV-Vis or a Shimadzu UV-1800 spectrophotometer and were baseline-corrected. Fluorescence spectra were measured using a Varian Cary Eclipse spectrofluorimeter. Assay plates were read using a SpectraMax 340 PC 384 Absorbance Microplate Reader (Molecular Devices). Magnetic circular dichroism (MCD) spectra were recorded on a ChiraScan-Plus CD spectrophotometer with an MCD attachment (0.997 T) at 22°C.

All stock solutions were stored in the dark and working solutions were made up immediately prior to use. Unless otherwise stated, the temperature was maintained at 25.0 ± 0.2°C by means of a thermostatted water bath. Aliquots of solutions were delivered into quartz cuvettes (1.00 mm or 10.00 mm) using a Hamilton syringe. Cuvettes were scrupulously washed with 0.2 M NaOH, followed by several rinsings with water, then addition of 1 M HNO_3 and several final rinsings with water to remove adsorbed Fe(III)PPIX.[10]

Titration curves were analysed using a non-linear least squares fitting in HypSpec (Version 1.1.33, Protonic Software). Sigmoidal dose-response curves were fitted to absorbance data using GraphPad Prism (Version 6.05, GraphPad Software). All measurements were performed in triplicate.

3.2. Job plots to determine binding stoichiometries with Fe(III)PPIX

To construct Job plots to determine the binding stoichiometries of complexes with Fe(III)PPIX, the absorption spectra of solutions of varying ligand and Fe(III)PPIX mole fractions were recorded in 40% DMSO (v/v) in 20 mM Hepes, pH 7.5, according to a method previously described.[11] The total concentration of the system was constrained to 0.75 mM and absorbance was measured at 400 nm in a 1.00 mm path length cuvette. Depending on expected binding, fifteen working solutions were

prepared with mole fractions of Fe(III)PPIX between 0 and 1. Spectrophotometric data were analysed at 400 nm.

3.3. Spectrophotometric titrations to determine association constants with Fe(III)PPIX

Thermodynamic association constants were measured in 40% DMSO (v/v) in 20 mM Hepes, pH 7.5, according to a method previously described.[12] Stock solutions of quinolines were prepared at concentrations of 0.5 mM or 1 mM depending on the strength of association with Fe(III)PPIX. Each stock compound was titrated into a 5 μM Fe(III)PPIX working solution. The resulting spectrophotometric data were analysed using an appropriate complexation model with HypSpec.

3.4. Magnetic circular dichroism (MCD)

MCD spectra were recorded as previously described.[11] Solutions were prepared by diluting a stock solution of Fe(III)PPIX (1 mM in 0.1 M NaOH) to a final concentration of 7.5 μM. For the quinolines, stock solutions (20 mM in DMSO) were diluted to a final concentration of 15 μM.

3.5. NP-40 detergent-mediated assay to measure β-haematin inhibition

The β-haematin formation inhibition assay method described by Carter et al. was modified for manual liquid delivery.[13,14] 20 mM stock solutions of the samples were prepared by dissolving the pre-weighed compounds in DMSO. These were delivered to a 96-well plate in triplicate to give concentrations ranging from 0 to 1000 μM (final well concentration) with a total DMSO volume of 10 μL in each well, after which deionised water (70 μL) and NP-40 (20 μL; 30.55 μM) were added. The absorbances of plate wells were pre-read at 405 nm on a SpectraMax 340 PC384 Absorbance Microplate Reader (Molecular Devices, Sunnyvale, CA, USA). A 25 mM haematin stock solution was prepared by sonicating haemin in DMSO for one minute and then suspending 178 μL of this in a 1 M acetate buffer (pH 4.8). The homogenous suspension (100 μL) was then added to the wells to give final buffer and haematin concentrations of 0.5 M and 100 μM, respectively. The plate was covered and incubated at 37°C for 5-6 hours. After incubation, free haem was detected using the pyridine-ferrichrome method developed by Ncokazi and Egan.[15] A solution of 50% (v/v) pyridine, 30% (v/v) water, 20% (v/v) acetone and 0.2 M Hepes was prepared and 32 μL added to each well to give a final pyridine concentration of 5% (v/v). Acetone (60 μL) was then added to assist with haematin dispersion. The absorbances of plate wells were again read at 405 nm, from which the pre-read values were subtracted. Sigmoidal dose-response curves with variable slopes were fitted to the absorbance data using GraphPad Prism v6.05 to obtain a 50% inhibitory concentration (IC_{50}) for each compound.

3.6. *Plasmodium falciparum* culturing

Continuous *in vitro* cultures of asexual erythrocyte stages of chloroquine-sensitive *Plasmodium falciparum* (NF54 strain) were maintained using a modified method of Trager and Jensen.[16] The cultures were maintained between 4-8% parasitaemia and 3-6% haematocrit. The culture was synchronised at the ring stage by incubating with D-sorbitol.

3.7. In vitro activity testing against *P. falciparum*

Samples were tested in triplicate on separate occasions at the Division of Pharmacology, Department of Medicine, University of Cape Town. Quantitative assessment of antiplasmodial activity in vitro was determined via the parasite lactate dehydrogenase assay using a modified method described by Makler.[17] The test samples were prepared to a 20 mg/mL stock solution in DMSO and were tested as a suspension if not completely dissolved. A full dose-response was performed for all compounds to determine the concentration inhibiting 50% of parasite growth (IC_{50}). A starting concentration (100 µg/mL) was serially diluted two-fold in complete medium to give ten concentrations, the lowest of which was 0.2 µg/mL. Reference drugs were tested at a starting concentration of 1000 ng/mL. Active compounds were re-tested at a starting concentration of 1000 ng/mL. The highest concentration of solvent to which the parasites were exposed had no measurable effect on the parasite viability (data not shown). The IC_{50} values were obtained using a non-linear dose-response curve fitting analysis in GraphPad Prism.

4. Live-cell fluorescence microscopy

4.1. General methods

Nunc Lab-Tek II eight-well chamber slides (Thermo Fisher Scientific, Massachusetts, USA) with cover glass of No. 1.5 thickness were coated with 150 µL of 0.01% (w/v) poly-L-lysine solution. After ten minutes, the solution was removed and the plates were left to air-dry. Ringer's solution was prepared according to the recipe previously described.[18] The pH was adjusted to 7.4 after which the solution was filtered through a 0.22 µm nylon syringe filter.

Human erythrocytes infected with *P. falciparum* trophozoites (NF54 strain) were generously donated by colleagues at the Division of Pharmacology, Department of Medicine, University of Cape Town. After harvesting and centrifugation of the cells, 5 µL of the parasitised erythrocyte pellet were resuspended in 5 mL Ringer's solution and were vortexed to reduce clumping of the erythrocytes. 150 µL aliquots of suspended cells were placed in each well of the chamber slide and incubated for at least twenty minutes to allow the erythrocytes to adhere to the cover glass. After this, the Ringer's solution was removed and replaced with a fresh aliquot of Ringer's solution (150 µL) to remove non-adhered cells. This solution was then removed again and replaced with Ringer's solution (150 µL) containing appropriate concentration(s) of the fluorescent dye(s) as listed in Tables S1 and S2.

4.2. Live-cell confocal fluorescence microscopy

Confocal microscopy was performed using a Zeiss Axiovert 200 M LSM 150-META confocal microscope at the Confocal and Light Microscope Imaging Facility at the University of Cape Town. A Plan-Apochromat 63×/1.40 Oil DIC M27 objective lens was used and the cells were incubated at 37°C. Images were captured and processed with ZEN 2011 (Carl Zeiss Microscopy GmbH). Laser transmission was kept as low as possible to minimise phototoxicity to the cells.[19] Dye concentrations with respective excitation and emission settings are listed in Table S1.

Table S1. *Concentrations of fluorescent dyes used for confocal imaging of* P. falciparum *with respective excitation lasers and emission filter settings.*

Dye	**Concentration**	**Excitation laser**	**Emission filter**
Hoechst 33342	2 μM	750 nm (two-photon)	Spectral 415-500
NBD-labelled derivative	500 nM	488 nm	BP 500-550
LysoTracker Red	200 nM	561 nm	BP 575-630
Nile Red	200 nM	561 nm	BP 575-630

4.3. Live-cell super-resolution structured-illumination microscopy (SR-SIM)

SR-SIM was performed using a Zeiss LSM 780 ELYRA S1 microscope at the CAF Fluorescence Microscopy Unit at Stellenbosch University. Thin (0.1 μm) z-stacks of high-resolution image frames were collected in three rotations by utilising an alpha Plan-Apochromat 63×/1.4 Oil DIC M27 ELYRA objective lens. Images were reconstructed using ZEN 2011 (Carl Zeiss Microscopy GmbH) based on a structured-illumination algorithm. To ensure accurate colocalisation analysis, a channel alignment procedure was followed using a slide of three-colour fluorescent beads #1783-455 Tool for Calibration Multi Spek (supplied by Carl Zeiss Microscopy GmbH). Dye concentrations with respective excitation and emission settings are listed in Table S2.

Table S2. *Concentrations of fluorescent dyes used for SR-SIM imaging of* P. falciparum *with respective excitation lasers and emission filter settings.*

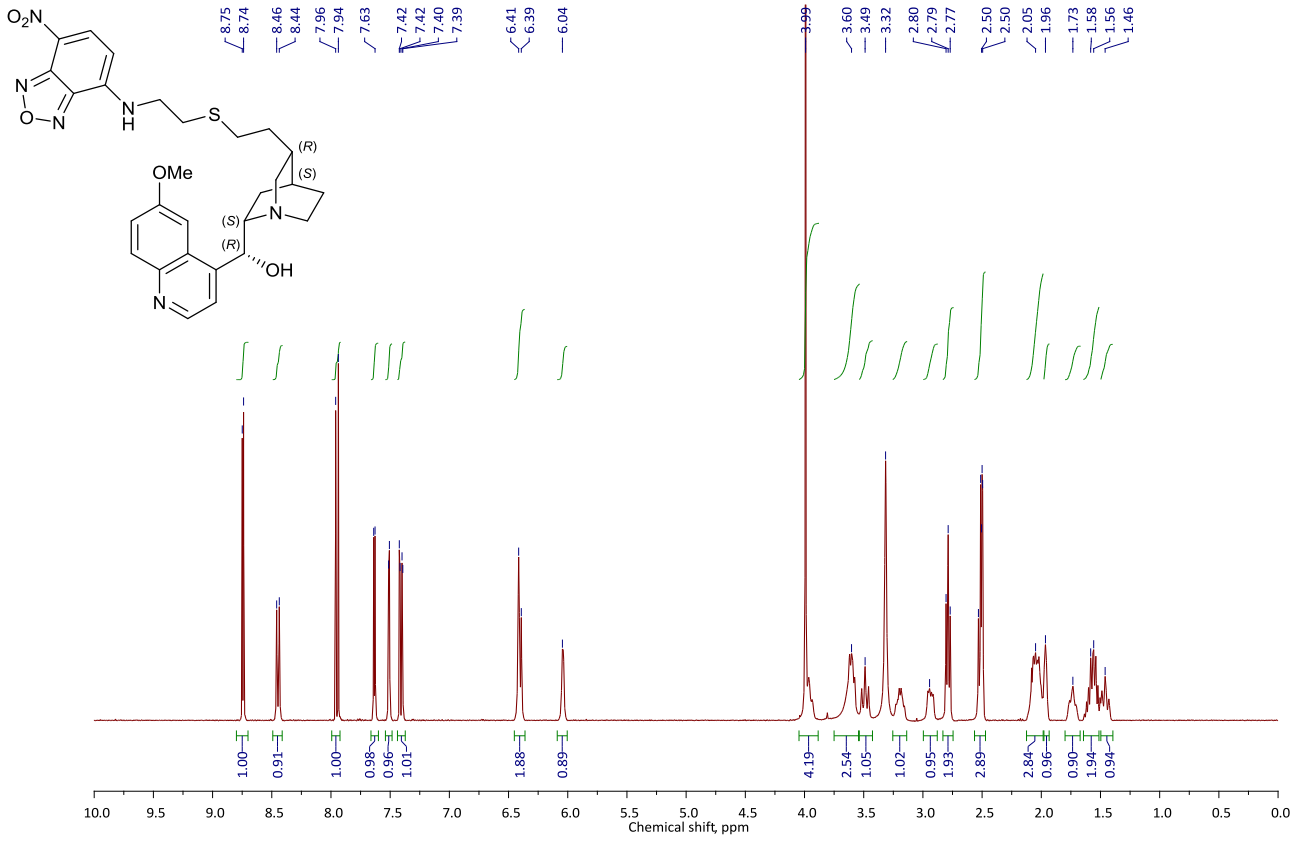
Dye	**Concentration**	**Grating**	**Excitation laser**	**Emission filter**
Hoechst 33342	2 μM	35.0 μm	405 nm	BP 420-480 + LP 750
NBD-labelled derivative	2 μM	42.0 μm	488 nm	BP 495-550 + LP 750
Nile Red	200 nM	51.0 μm	561 nm	BP 570-620 + LP 750

5. References

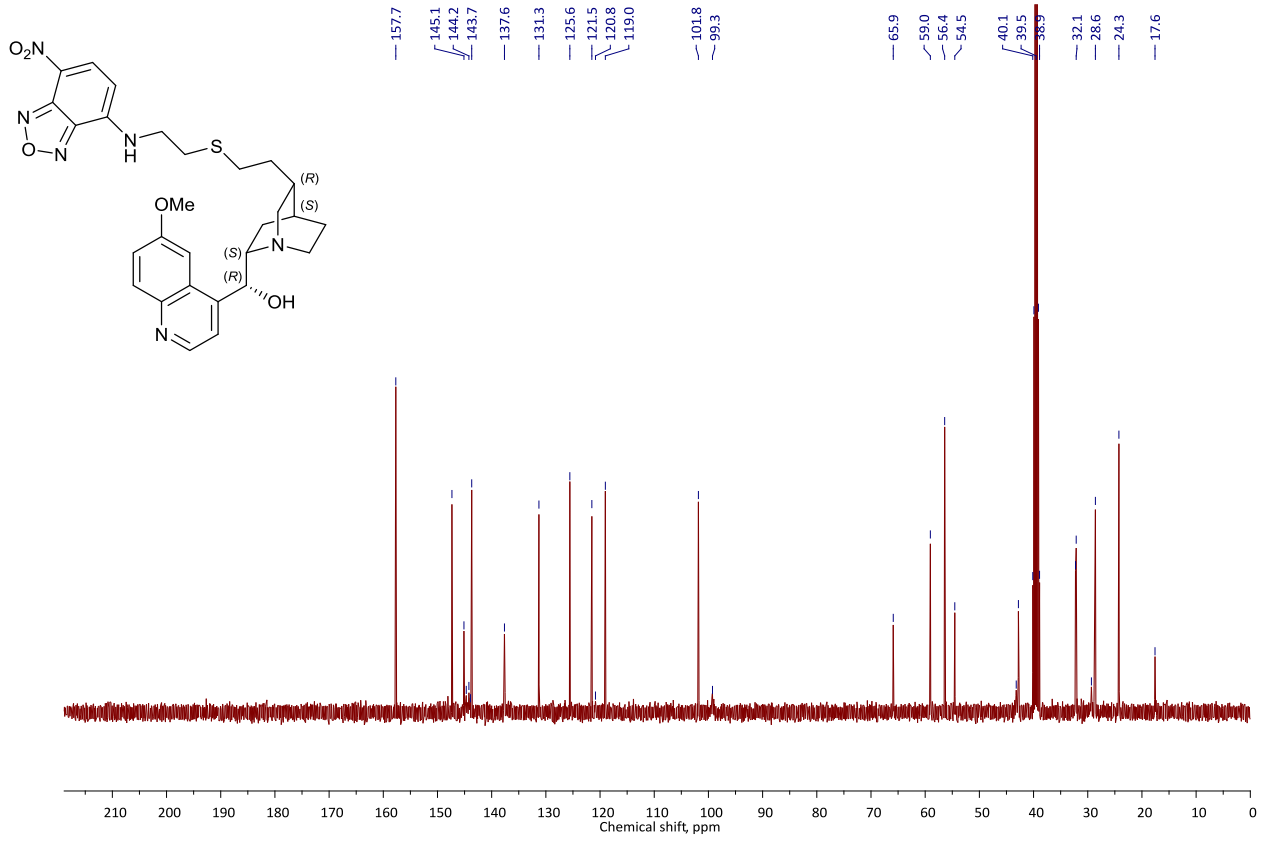
1. K. A. Zacharlasse, N. V. Phuc and B. Kozanklewicz, *J. Phys. Chem.*, 1981, **85**, 2676.
2. A. N. Hoang, R. D. Sandlin, A. Omar, T. J. Egan and D. W. Wright, *Biochemistry*, 2010, **49**, 10107.
3. C. Reichardt, *Chem. Rev.*, 1994, **94**, 2319.
4. E. Merck, in *Dyeing Reagents for Thin-Layer and Paper Chromatography*, Darmstadt, 1980.
5. H. E. Gottlieb, V. Kotlyar and A. Nudelman, *J. Org. Chem.*, 1997, **62**, 7512.
6. A. K. Tucker-Schwartz, R. Farrell and R. Garrell, *J. Am. Chem. Soc*., 2011, **133**, 11026.
7. S. Pinchas and D. Ben-Ishai, *J. Am. Chem. Soc*., 1957, **79**, 4099.
8. J. Novotný, K. Pospěchová, A. Hrabálek, R. Čáp and K. Vávrová, *Bioorg. Med. Chem. Lett.*, 2009, **19**, 6975.
9. S. Haldar, S. Kumar, S. P. Kolet, H. S. Patil, D. Kumar, G. C. Kunda and H. V. Thulasarim, *J. Org. Chem.*, 2013, **78**, 10192.
10. K. A. de Villiers, C. H. Kaschula, T. J Egan and H. M. Marques, *J. Biol. Inorg. Chem.*, 2007, **12**, 101.
11. D. Kuter, S. Benjamin and T. J. Egan, *J. Inorg. Biochem.*, 2014, **133**, 40.
12. T. J. Egan, W. M. Mavuso, D. C. Ross and H. M. Marques, *J. Inorg. Biochem.*, 1997, **68**, 137.
13. M. D. Carter, V. V. Phelan, R. D. Sandlin, B. O. Bachmann and D. W. Wright, *Comb. Chem. High Throughput Screen.*, 2010, **13**, 205.
14. R. D. Sandlin, M. D. Carter, P. J. Lee, J. M. Auschwitz, S. E. Leed, J. D. Johnson and D. W. Wright, *Antimicrob. Agents Chemother.*, 2011, **55**, 3363.
15. K. K. Ncokazi and T. J. Egan, *Anal. Biochem.*, 2005, **338**, 306.
16. W. Trager and J. B. Jensen, *Science*, 1976, **193**, 673.
17. M. T. Makler, J. M. Ries, J. A. Williams, J. E. Bancroft, R. C. Piper, B. L. Gibbins and D. J. Hinrichs, *Am. J. Trop. Med. Hyg.*, 1993, **48**, 6.
18. Y. Kuhn, P. Rohrbach and M. Lanzer, *Cell. Microbiol.*, 2007, **9**, 1004.
19. F. Wissing, C. Sanchez, P. Rohrbach, S. Ricken and M. Lanzer, *J. Biol. Chem.*, 2002, **277**, 37747.

6. ^{1}H and ^{13}C NMR spectra of new organic compounds

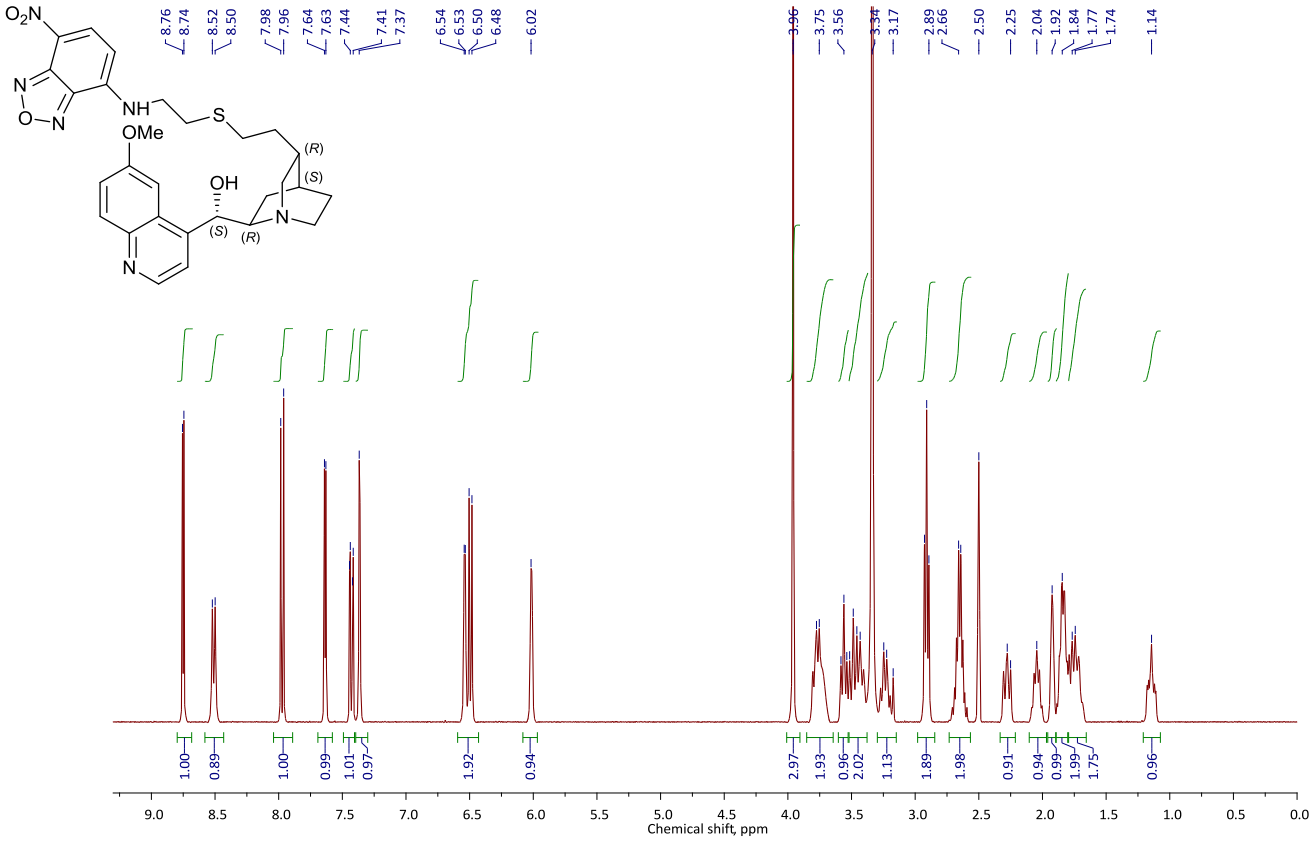
^{1}H NMR spectrum of **3** (DMSO-d_6, 400 MHz)



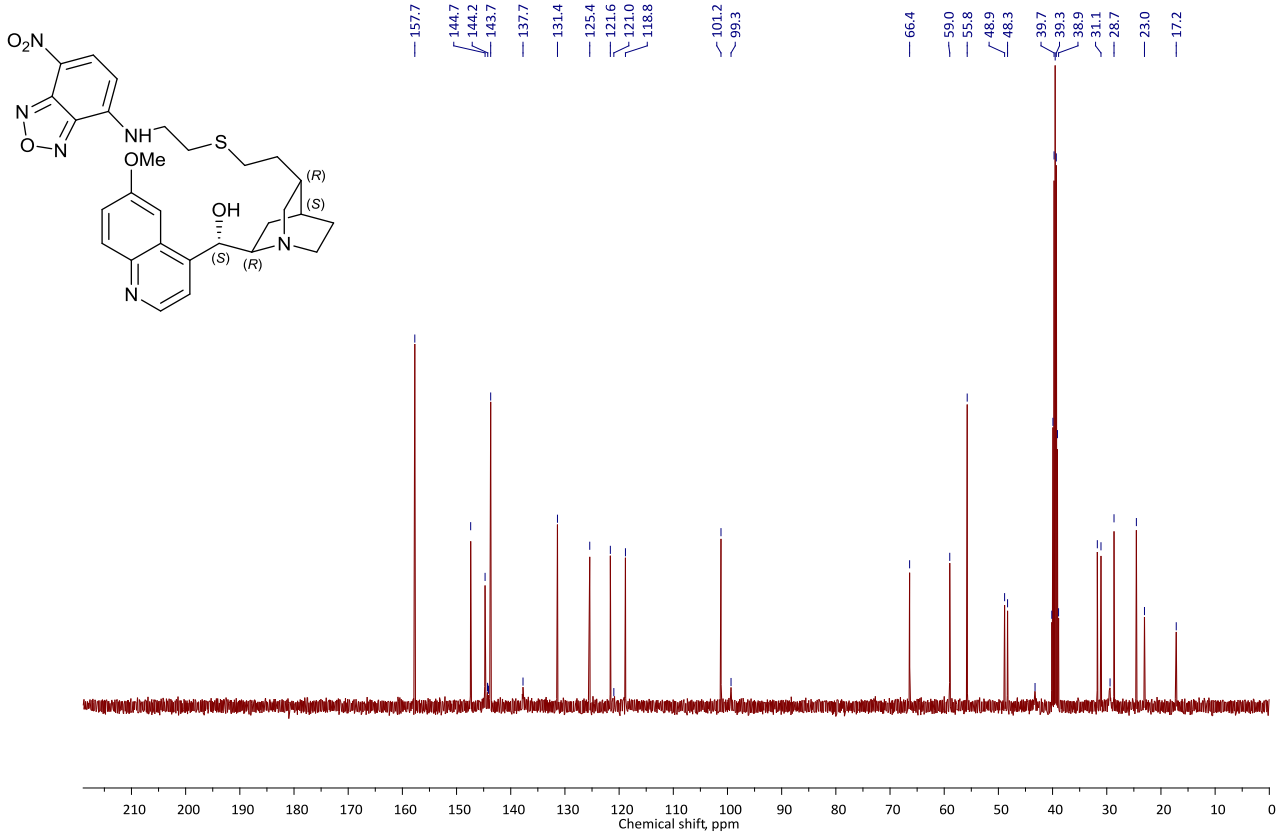
^{13}C NMR spectrum of **3** (DMSO-d_6, 100 MHz)



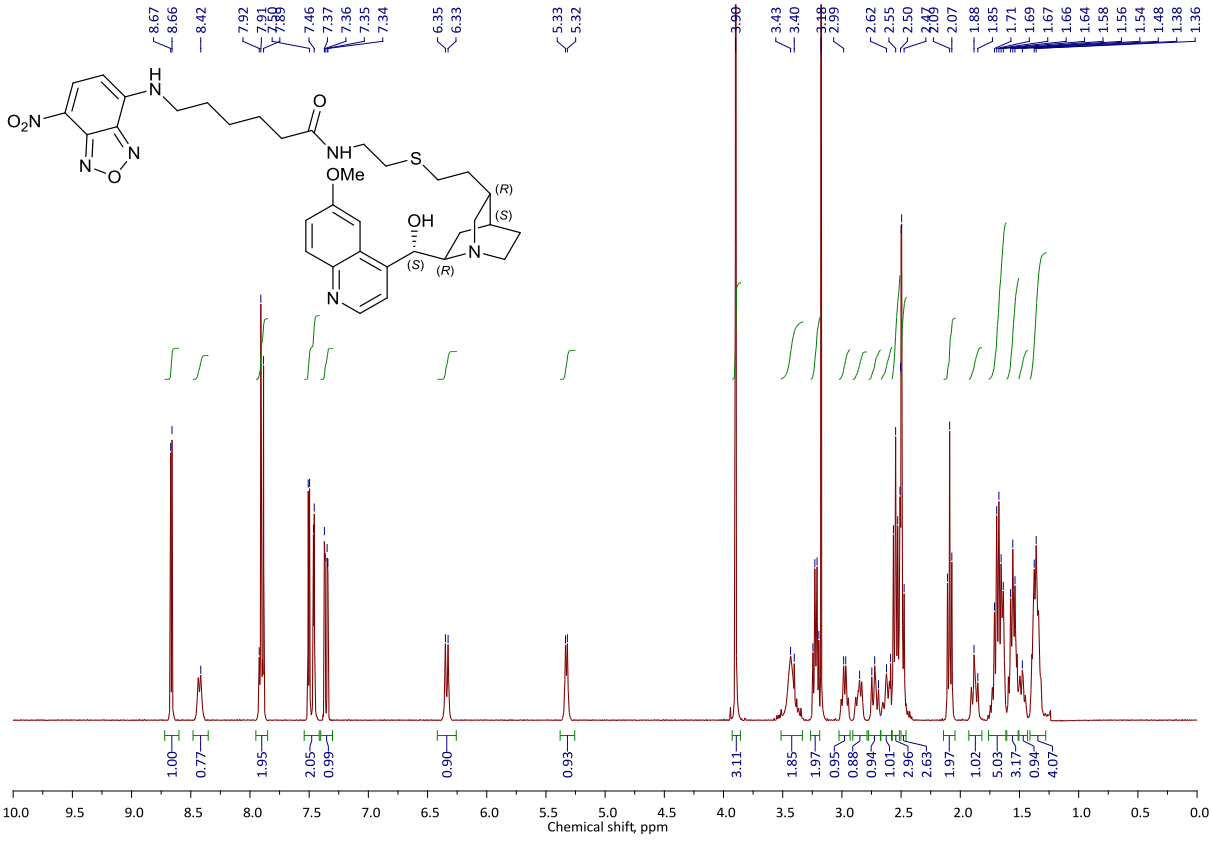
^{1}H NMR spectrum of **4** (DMSO-d_6, 400 MHz)



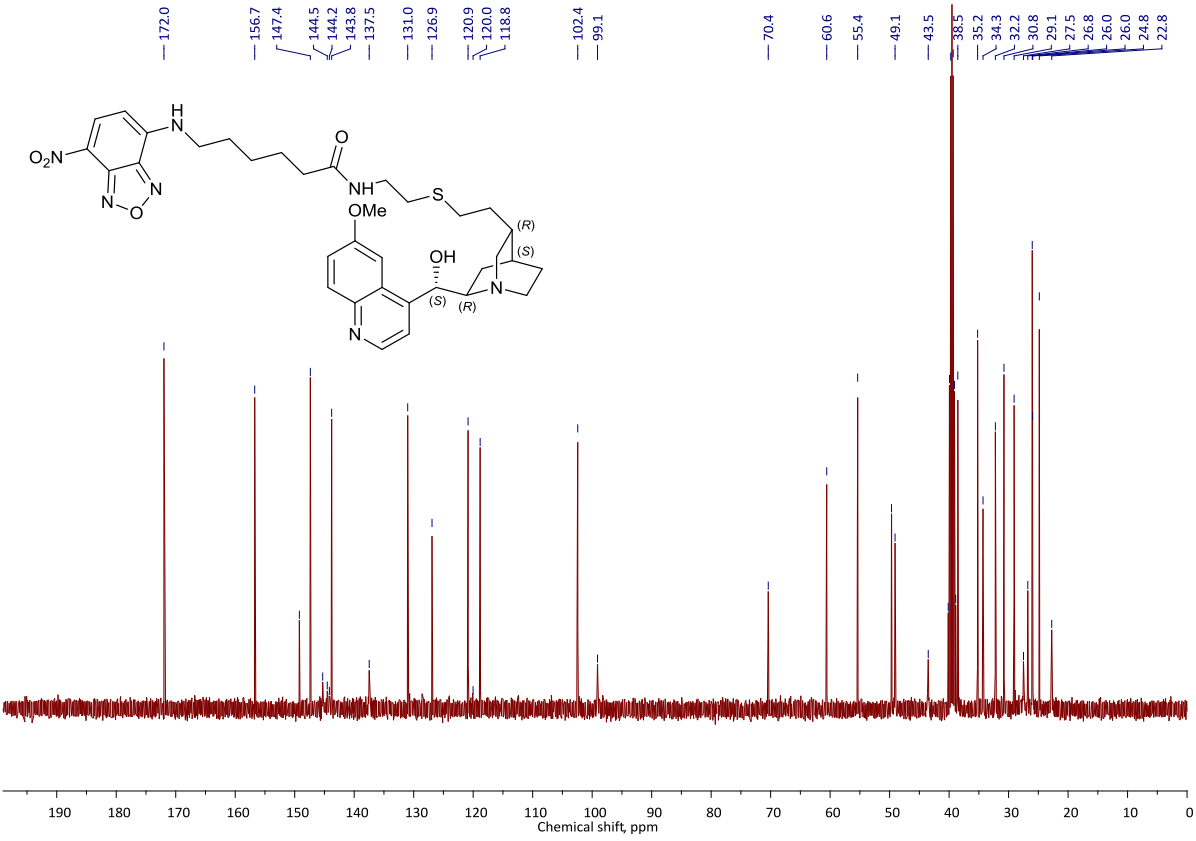
^{13}C NMR spectrum of **4** (DMSO-d_6, 100 MHz)



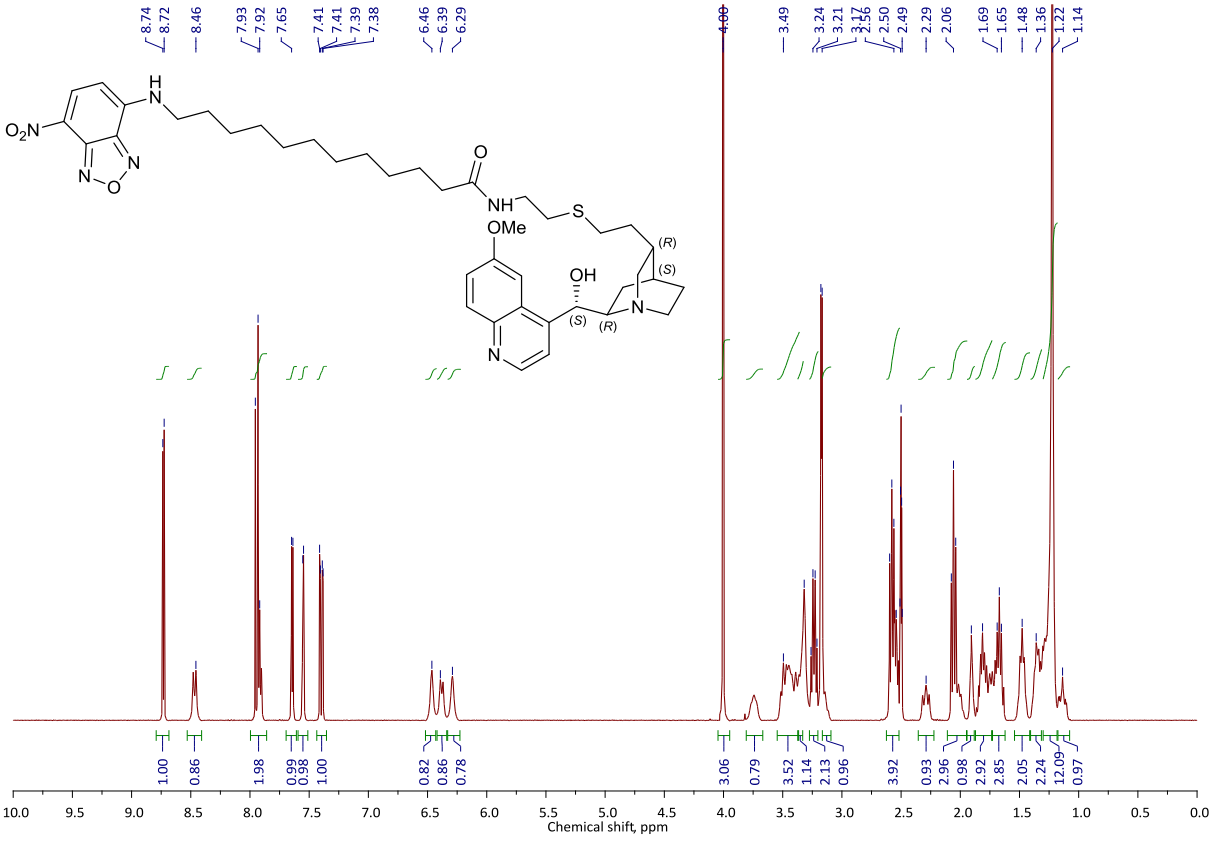
^{1}H NMR spectrum of **5** (DMSO-d_6, 400 MHz)



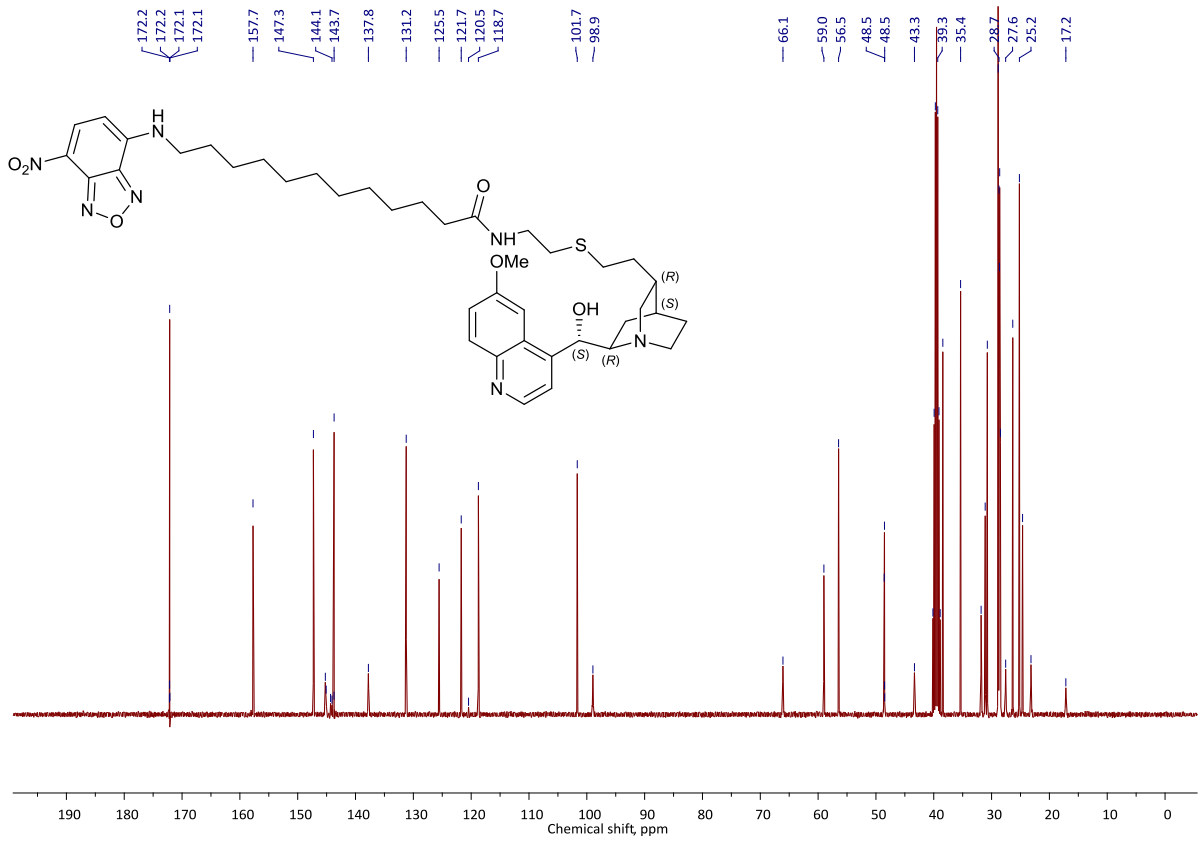
^{13}C NMR spectrum of **5** (DMSO-d_6, 100 MHz)



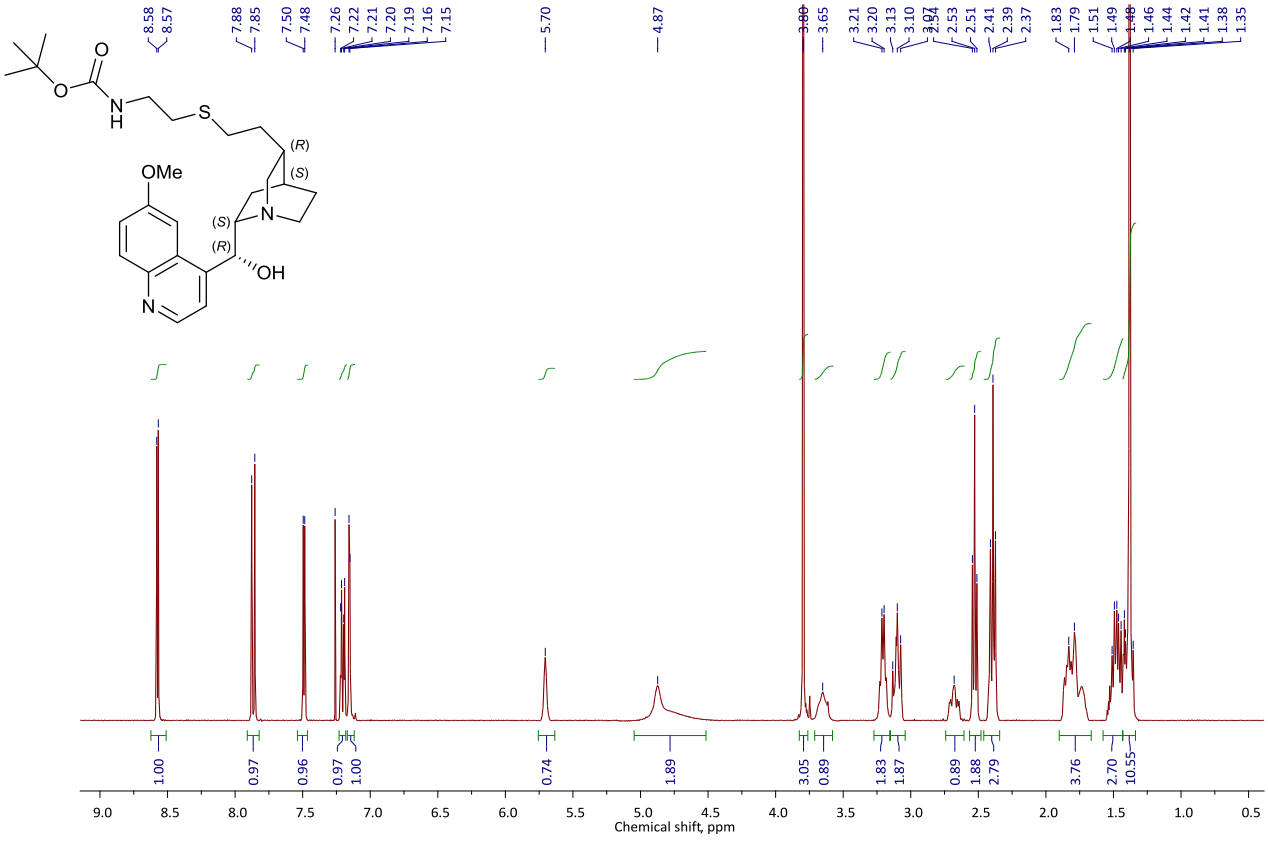
^{1}H NMR spectrum of **6** (DMSO-d_6, 400 MHz)



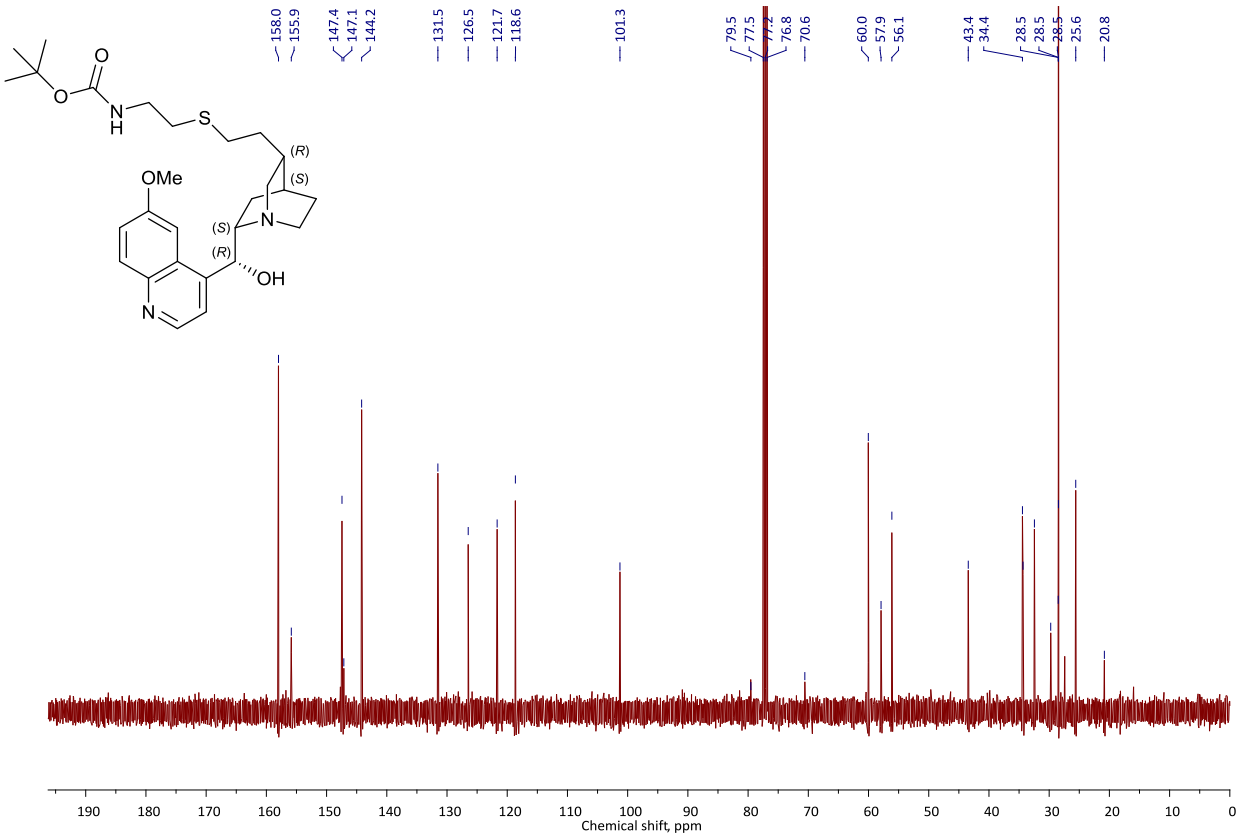
^{13}C NMR spectrum of **6** (DMSO-d_6, 100 MHz)



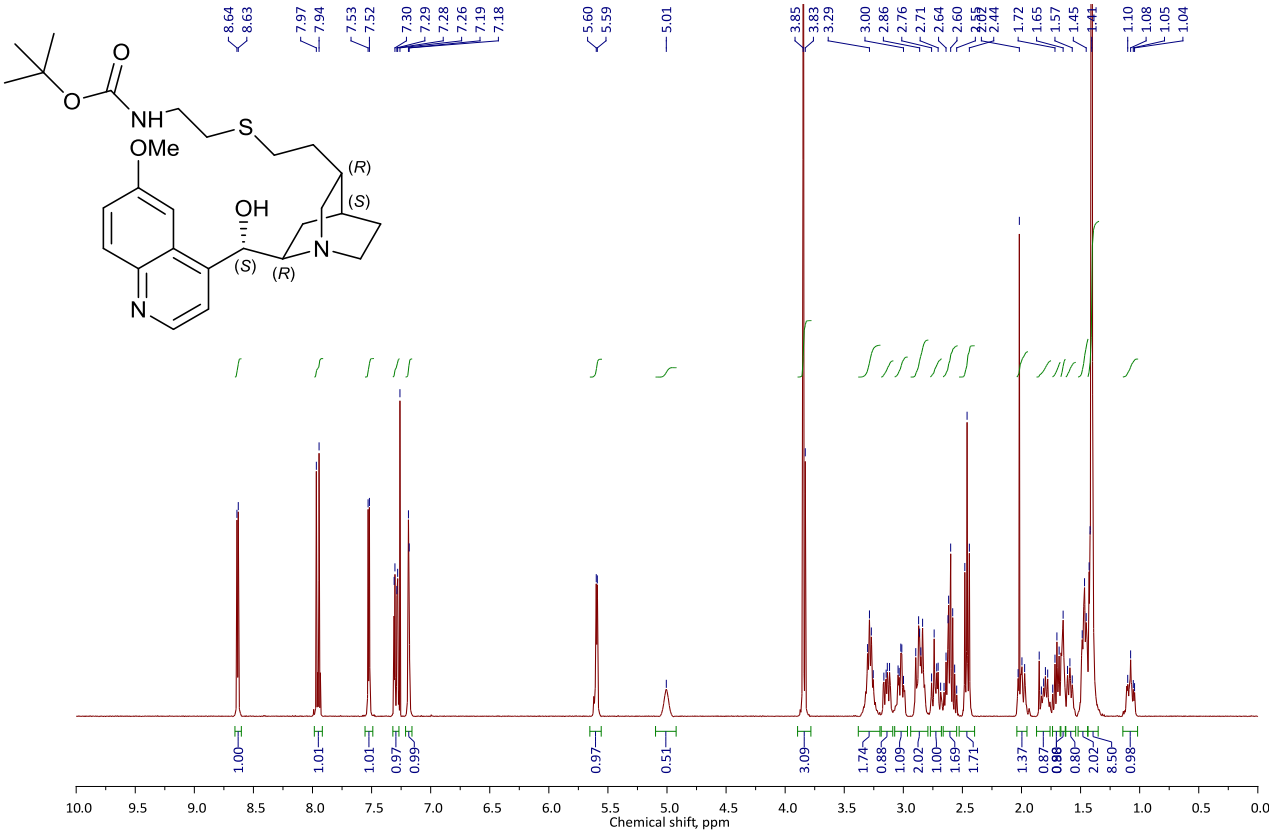
^{1}H NMR spectrum of **7** ($CDCl_3$, 400 MHz)



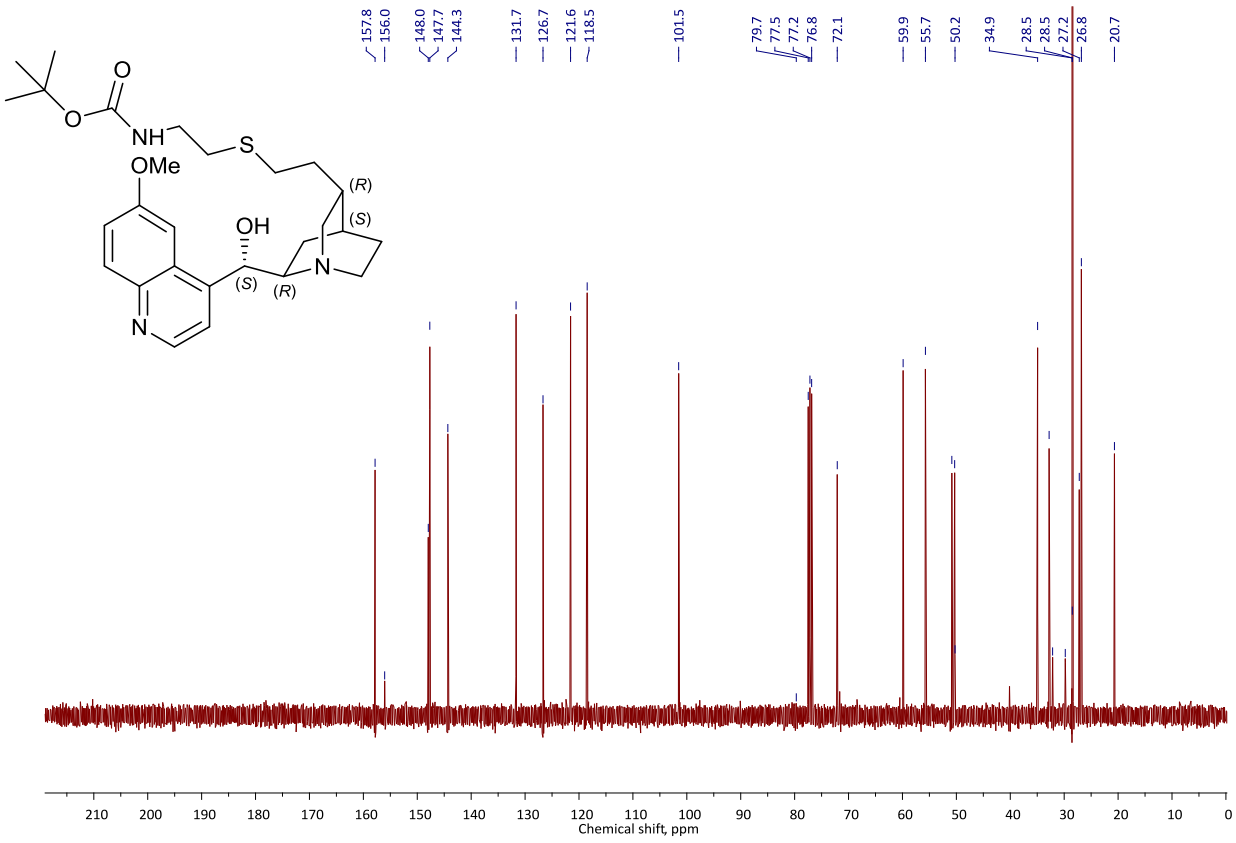
^{13}C NMR spectrum of **7** ($CDCl_3$, 100 MHz)



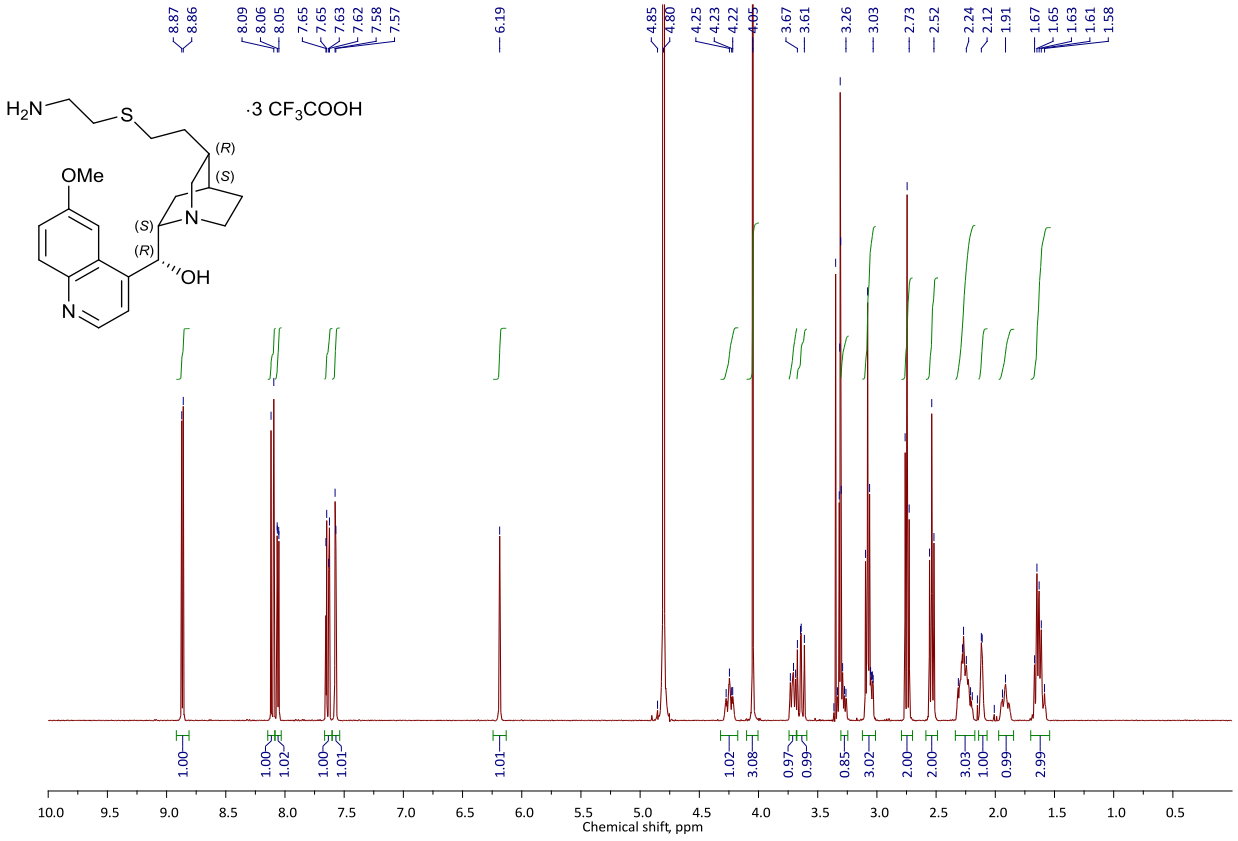
^{1}H NMR spectrum of **8** ($CDCl_3$, 400 MHz)



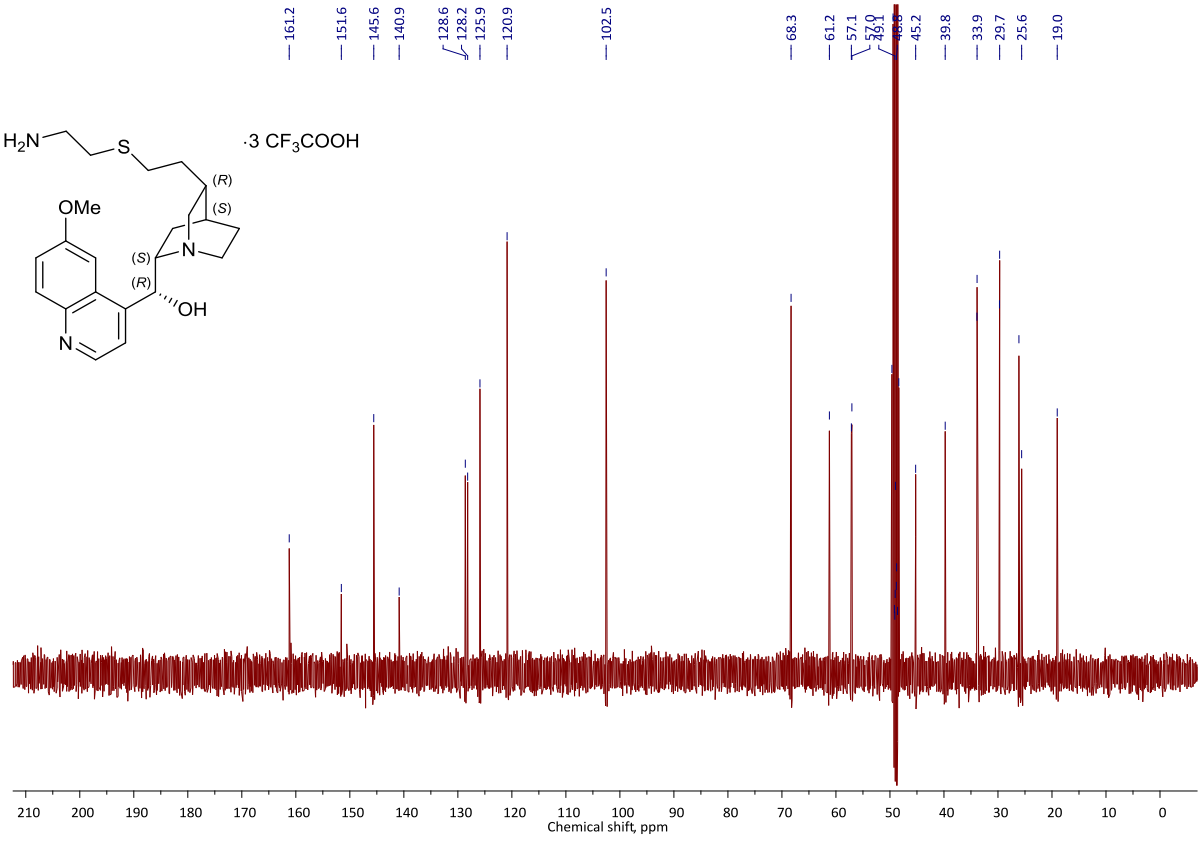
^{13}C NMR spectrum of **8** ($CDCl_3$, 100 MHz)



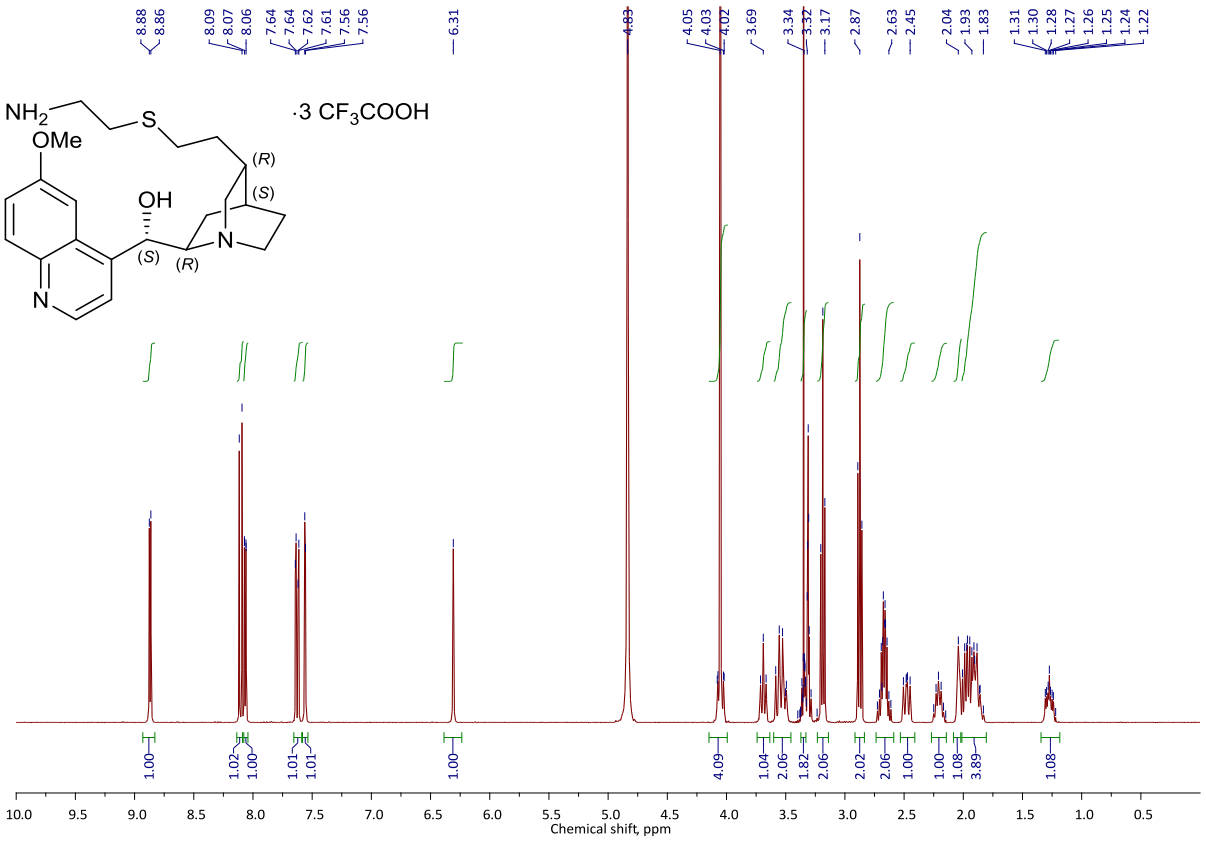
^{1}H NMR spectrum of **9** (CD_3OD, 400 MHz)



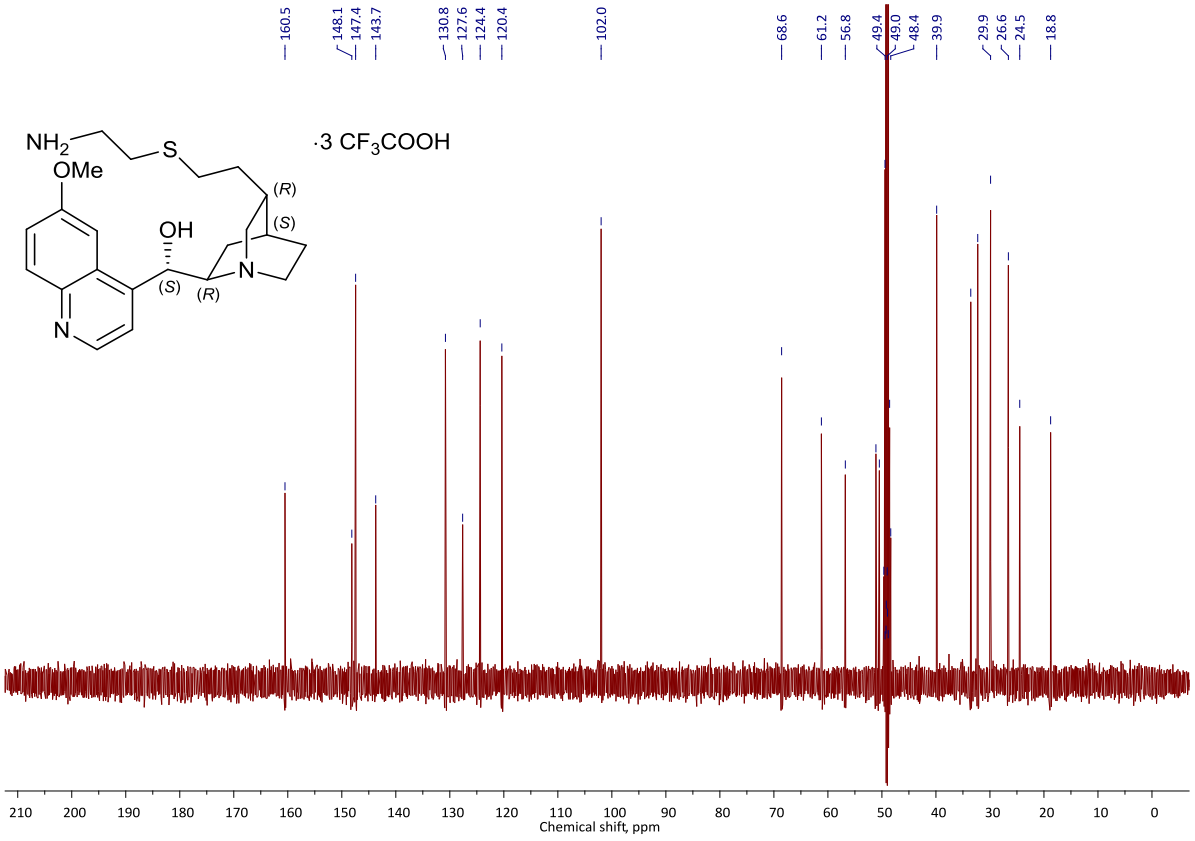
^{13}C NMR spectrum of **9** (CD_3OD, 100 MHz)



^{1}H NMR spectrum of **10** (CD_3OD, 400 MHz)

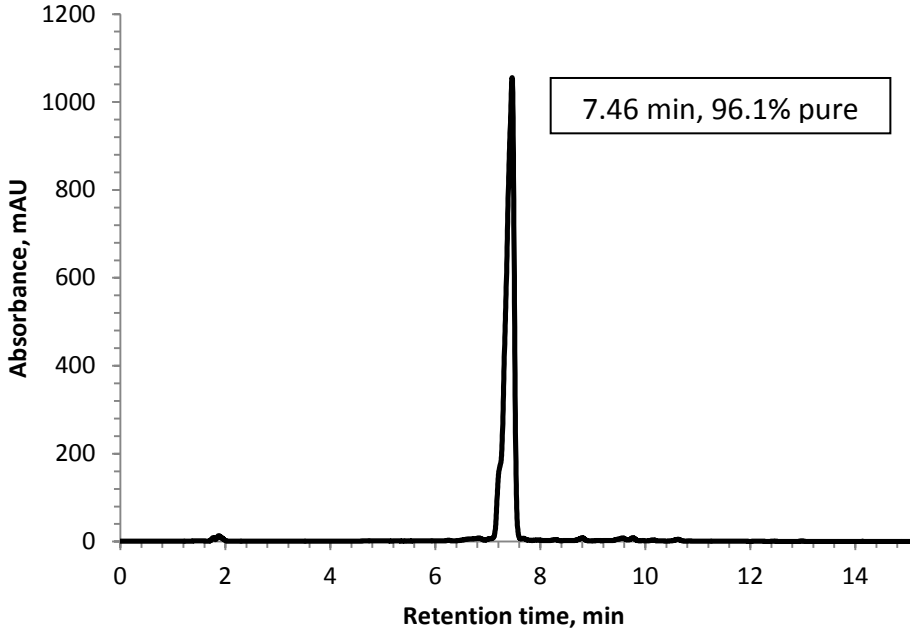


^{13}C NMR spectrum of **10** (CD_3OD, 100 MHz)

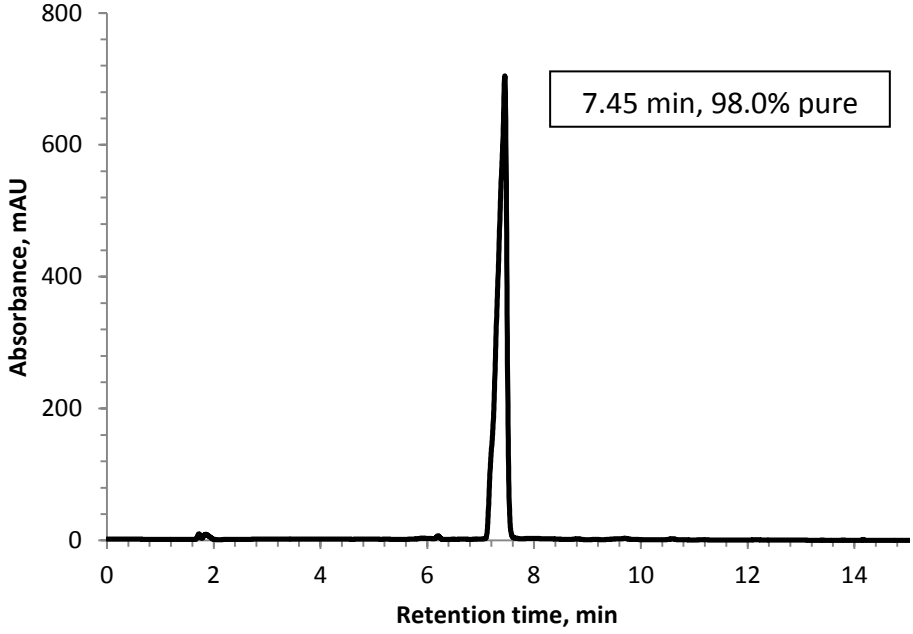


7. HPLC data

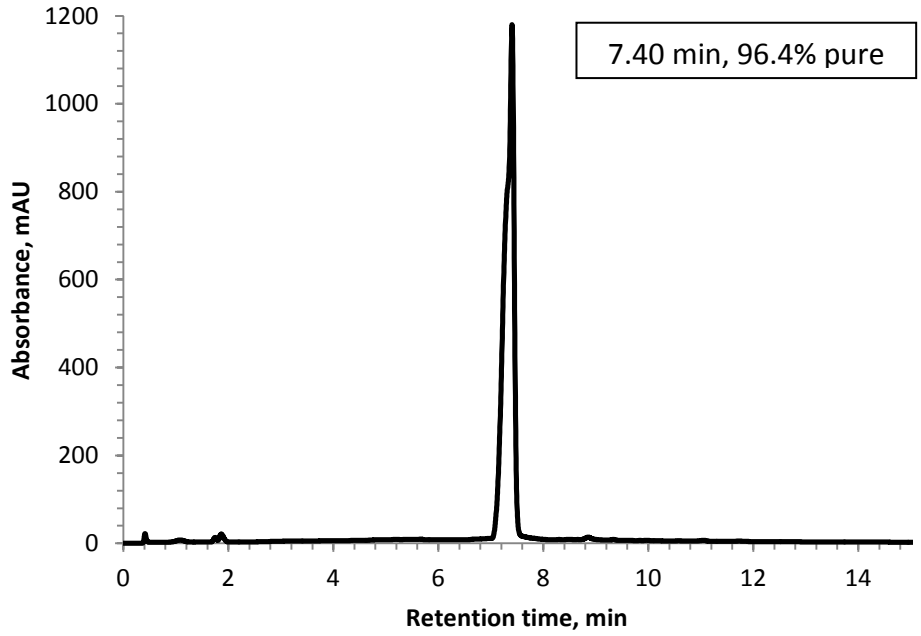
Compound **3** eluted with 5%-100% acetonitrile and 95%-0% 0.1% TFA over 15 minutes:



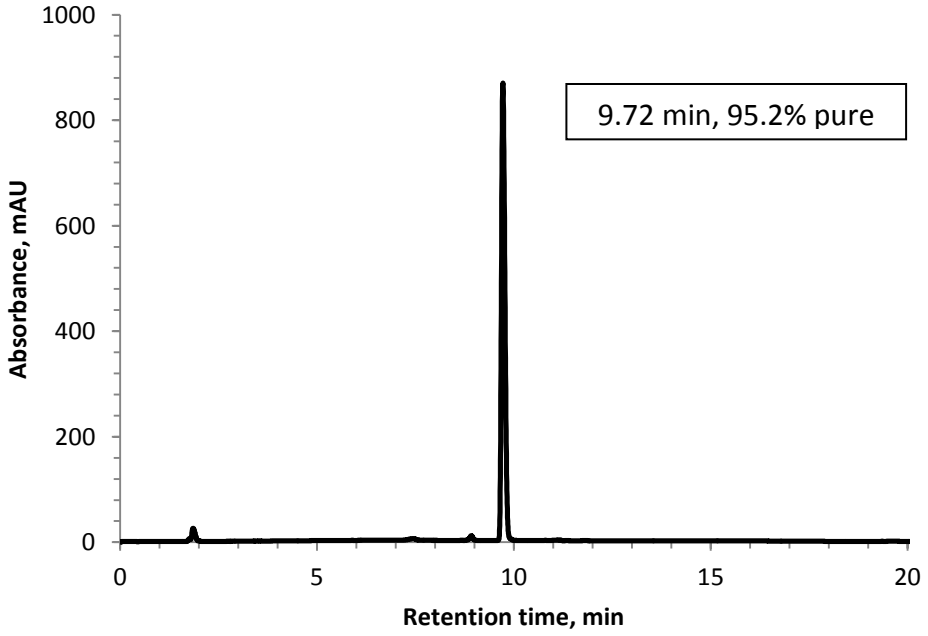
Compound **4** eluted with 5%-100% acetonitrile and 95%-0% 0.1% TFA over 15 minutes:



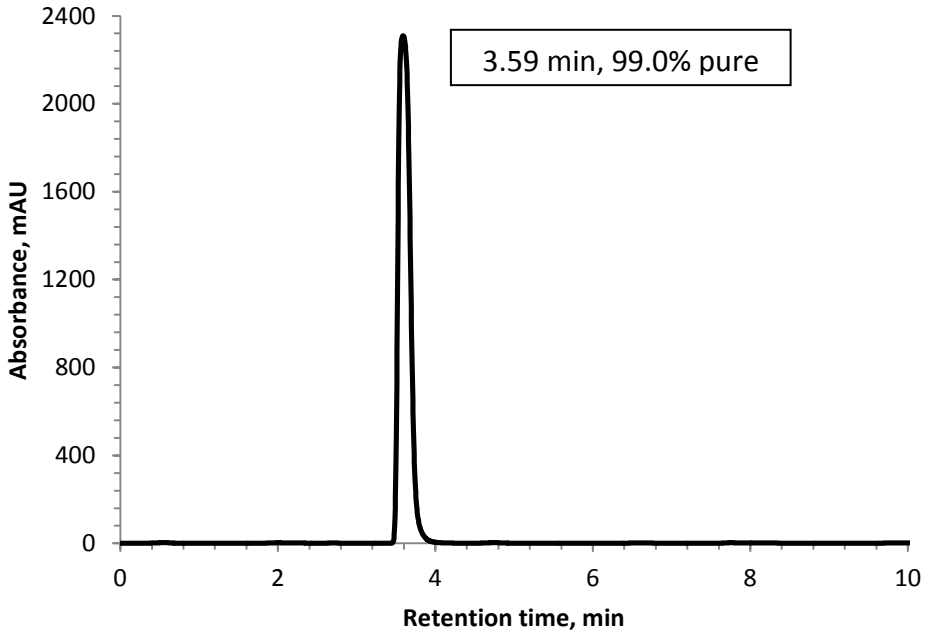
Compound **5** eluted with 5%-100% acetonitrile and 95%-0% 0.1% TFA over 15 minutes:



Compound **6** eluted with 5%-100% acetonitrile and 95%-0% 0.1% TFA over 15 minutes:



Compound **9** eluted with 10% acetonitrile and 90% 0.1% TFA over 10 minutes:



Compound **10** eluted with 10% acetonitrile and 90% 0.1% TFA over 10 minutes: