SUPPORTING INFORMATION

Synthesis of novel G factor- or chloroquine-artemisinin hybrids and conjugates with potent antiplasmodial activity

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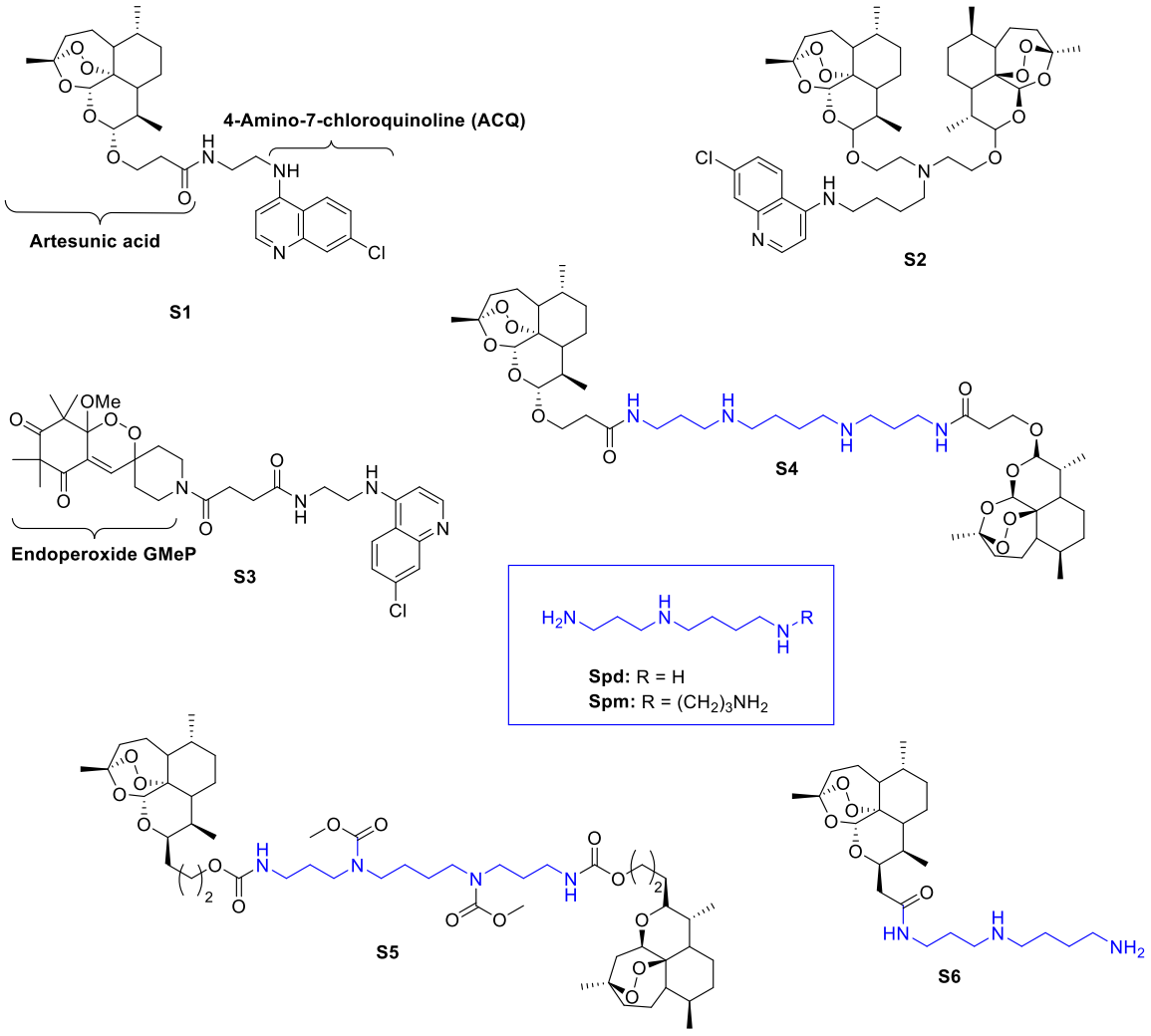


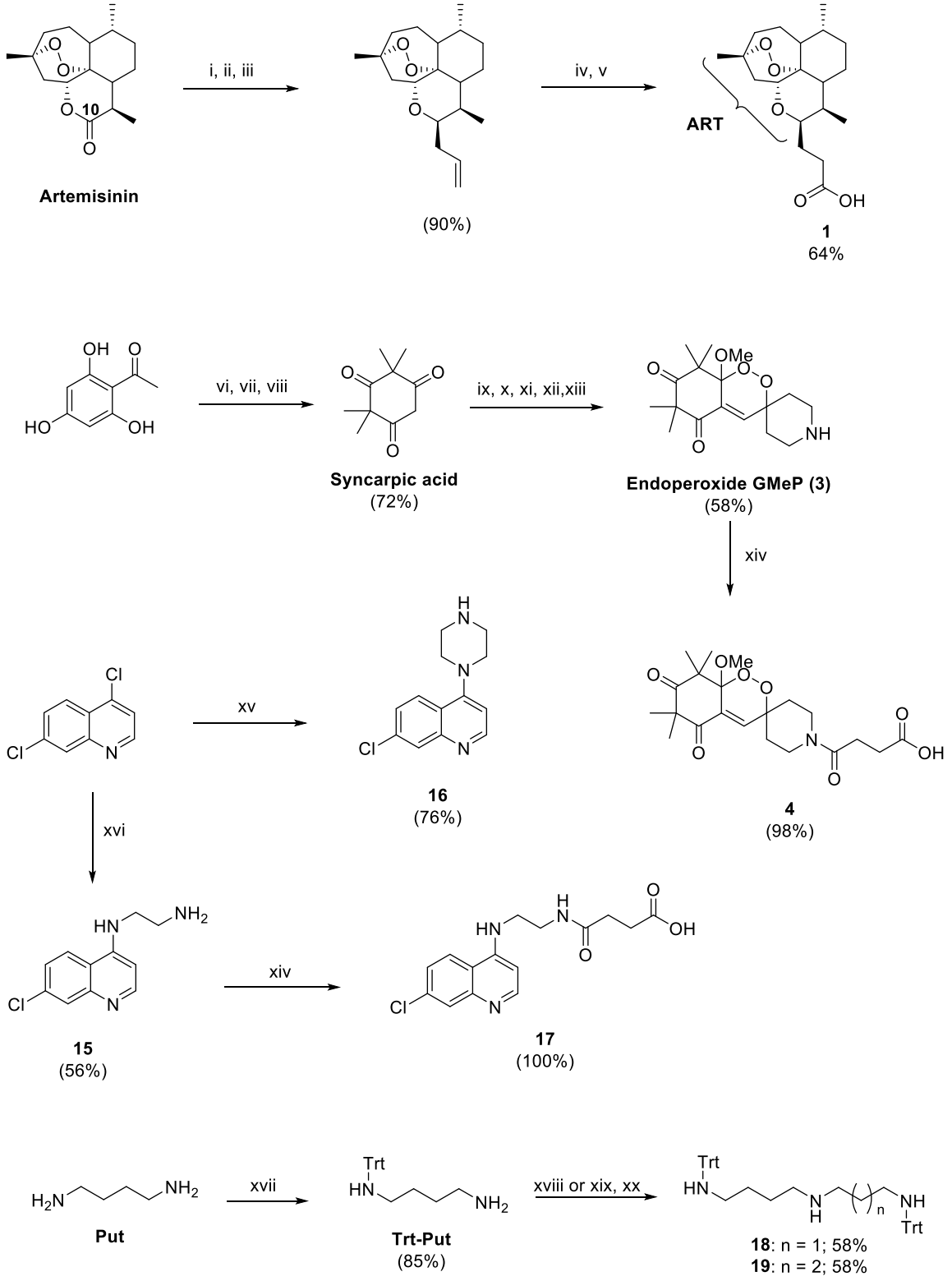
Figure S1. Examples of reported ART-CQ (**S1-S2**) and Endoperoxide GMeP-CQ (**S3**) hybrids and ART-PAs conjugates (**S4-S6**) with antimalarial or/and anticancer interest. The structures of the PAs spermidine (Spd) and spermine (Spm) are also included.

1. General

All solvents were dried and/or purified according to standard procedures prior to use. Anhydrous Na_2SO_4 was used for drying solutions and the solvents were then routinely removed at ca. 40 °C under reduced pressure using a rotary vacuum evaporator. All reagents employed in the present work were commercially available and used without further purification. ^1H NMR spectra were obtained at 600.13 MHz and ^{13}C NMR spectra at 150.90 MHz on a Bruker AVANCEIII HD spectrometer. For the compounds **15, 17 & 19**, ^1H and ^{13}C NMR spectra were obtained on a Bruker AVANCE 300 MHz or Bruker AVANCE 400DXP 400.13MHz. Chemical shifts (δ) are indicated in parts per million (ppm) upfield from TMS and coupling constants (*J*) are reported in hertz. ESI+ mass and ESI-HRMS spectra were recorded on a Micromass-Platform LC spectrometer and on a UPLC Xevo G2 QTOF (Waters) instrument, respectively.

Melting points were determined with a Buchi SMP-20 apparatus and are uncorrected. When required, reactions were carried out under an inert atmosphere (dry Ar) in pre-flamed glassware. Flash column chromatography (FCC) was performed on silica gel 60 (230-400 mesh) and analytical thin layer chromatography (TLC) on silica gel-F_{254} pre-coated aluminum foils (0.2 mm film), respectively. Spots on TLC plates were visualized with UV light at 254 nm and ninhydrine solution or charring solution. The eluent systems used for chromatography were: (A) $CHCl_3$/MeOH/conc. NH_3 8:2:0.2, (B) $CHCl_3$/MeOH/conc. NH_3 9:1:0.1, (C) $CHCl_3$/MeOH 8:2, (D) $CHCl_3$/MeOH 85:15, (E) $CHCl_3$/MeOH 9:1, (F) $CHCl_3$/MeOH 95:5, (G) $CHCl_3$/MeOH 97:3, (H) EtOAc/EtN_3 99:1, (I) EtOAc, (J) PE/EtOAc 1:2, (K) PE/EtOAc 7:3, (L) PE/EtOAc 8:2, (M) PE/EtOAc 9:1, (N) PhMe/EtOAc/AcOH 1:1:0.1, (O) PhMe/EtOAc 1:1, (P) PhMe/EtOAc 3:7, (Q) PhMe/EtOAc 7:3, (R) PhMe/EtOAc 8:2, (S) PhMe/EtOAc 9:1, (T) DCM/MeOH/conc. NH_3 9:1:0.5, (U) DCM/MeOH/conc. NH_3 8:2:0.2, (Y) Hex/EtOAc 9:1, (V) PE/EtOAc 1:1, (X) DCM/MeOH 95:5.

2. Chemistry

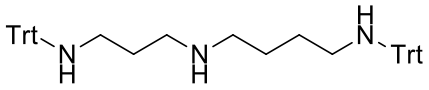

i, ii, iii
iv, v
Artemisinin
(90%)
ART
1
64%
vi, vii, viii
ix, x, xi, xii,xiii
Syncarpic acid
(72%)
Endoperoxide GMeP (3)
(58%)
xiv
xv
16
(76%)
4
(98%)
xvi
xiv
15
(56%)
17
(100%)
xvii
xviii or xix, xx
Put
Trt-Put
(85%)
18: n = 1; 58%
19: n = 2; 58%

Scheme S1. Synthesis of ART, Endoperoxide GMeP and 4-amino-7-chloroquinoline derivatives **1**, **4** and **11-15**. *Reagents and reaction conditions*: (i) $NaBH_4$, MeOH, 0-rt, 3 h, 98%; (ii) PhCOCl, pyridine, CH_2Cl_2, 0 °C, 16 h, 100%; (iii) $Me_3SiCH_2CH{=}CH_2$, $ZnCl_2$, molecular sieves 4 Å, 1,2-dichloroethane, 0 °C, 3 h, 92%; (iv) (a) $BH_3{\cdot}SMe_2$, THF, rt, 2 h, (b) H_2O_2, Na_2CO_3, H_2O, 25 °C, 30 min, 67%; (v) $RuCl_3$, $NaIO_4$, EtOAc, MeCN, H_2O, rt, 30 min, 96%; (vi) Na, MeOH, rt; (vii) MeI, reflux, 6 h, 84%; (viii) 3M HCl in H_2O, 100 °C, 6 h, 86%; (ix) *N*-Boc-piperidine-4-carboxaldehyde, piperidine, CH_2Cl_2, rt, 1h; (x) 1N HCl/NH_4Cl, CH_2Cl_2, rt, 30 min, 90%; (xi) O_2, CH_2Cl_2, rt, 14 days, 68%; (xii) nBuLi, TfOMe, THF, -78 °C, 2h, 62%; (xiii) CF_3CO_2H, TFA, CH_2Cl_2, rt, overnight, 95%; (xiv) succinic anhydride, 4-dimethylaminopyridine (DMAP), CH_2Cl_2, rt, 2 h, 98%; (xv) piperazine , MeOH, reflux, 3 h; (xvi) 1,2-diaminoethane, 80 °C, 40 min then 130 °C, 6 h; (xvii) TrtCl, CH_2Cl_2, 0 °C, 4 h; (xviii) $TrtNH(CH_2)_2CO_2Su$, Et_3N, DMF, 0 °C to rt, 2.5 h; (xix) $TrtNH(CH_2)_3CO_2H$, HBTU, DIPEA, DMF, 0 °C to rt, 2.5 h; (xx) $LiAlH_4$, THF, 70 °C, 12 h.

3. Experimental

A. Synthesis of protected polyamine derivatives

Synthesis of N^1, N^8-ditritylspermidine (18)

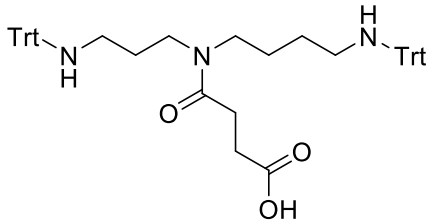


To an ice-cold solution of Trt-Putrescine (1.65 g, 5 mmol) and Et_3N (0.63 mL, 4.5 mmol) in DMF (8 mL), Trt-β-Ala-OSu (1.71 g, 4 mmol) was added in two portions. The reaction mixture was stirred at 0 °C for 30 min and at room temperature for 2h. Then, the mixture was diluted with EtOAc and washed twice with brine. The organic layer was dried over Na_2SO_4, evaporated to a minimum volume and refrigerated overnight. The precipitate was filtered, washed once with EtOAc and once with Et_2O, and the anticipated amide was obtained as a white solid (89%); R_f (O): 0.45.

The thus obtained amide (2.29 g, 3.35 mmol) was added portion-wise over 1h to a suspension of $LiAlH_4$ (0.41 g, 10.65 mmol) in THF (21 mL) at 70 °C. The reaction mixture

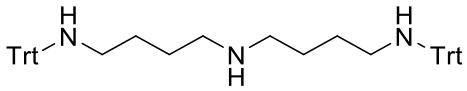
was stirred at the above-mentioned temperature overnight. Excess $LiAlH_4$ was destroyed by the careful addition at 0 °C of a saturated solution of Na_2SO_4. The precipitate was filtered off, discarded, and the filtrate was diluted in $CHCl_3$ and washed twice with brine. The organic layer was dried over Na_2SO_4, evaporated to dryness and crystallized by addition Et_2O to obtain **18** as a white solid (65%); mp 127-130 °C; R_f (F): 0.22; which had identical spectral data with the ones reported in the literature.[1]

Synthesis of Spd-derived acid 20



To a solution of **18** (600 mg, 0.952 mmol) and DMAP (120 mg, 0.952 mmol) in DCM (11.6 mL) at 0 °C, was added the succinic anhydride (150 mg, 1.5 mmol). The reaction was kept for 30 min at 0 °C and then left to be stirred at rt overnight. After completion of the reaction (monitored by TLC), the mixture was diluted with DCM, washed with cold aq. citric acid 5%, H_2O and brine, dried over Na_2SO_4, filtered and evaporated to dryness under vacuum. The residue was subjected to FCC, using solvent system Gas eluent, to give the pure product as a pale yellow oil (100%); R_f (G): 0.23; **^{1}H NMR** ($CDCl_3$, 600 MHz) δ 0.93 - 0.84 (m, 2H), 1.39 - 1.24 (m, 6H), 1.54 – 1.48 (m, 1H), 2.03 (quint., J = 6 Hz, 3H), 2.36 (t, J = 6 Hz, 2H), 2.86 (s, 3H), 3.40 (t, J = 12 Hz, 2H), 7.29 - 7.43 (m, 27H), 7.66 - 7.44 (m, 3H) ppm; **^{13}C NMR** ($CDCl_3$, 151 MHz) δ 25.43, 26.64, 28.05, 28.15, 30.54, 43.31, 48.20, 70.89, 126.24, 126.35, 127.79, 127.86, 128.55, 128.62, 145.98, 146.08 ppm; **ESI-MS** (30eV) ***m/z*:** 243.58 $[Trt]^+$, $[M+H]^+$ Calcd for $C_{49}H_{52}N_3O_3^+$ 730.40, Found 730.37, $[M+Na]^+$ Calcd for $C_{49}H_{51}N_3NaO_3^+$ 752.38, Found 752.38.

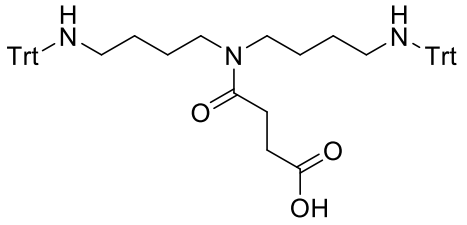
Synthesis of N^1, N^9-ditritylhomospermidine (19)



To an ice-cold solution of Trt-Putrescine (1.77 g, 5.36 mmol) and HBTU (2.44 g, 6.432 mmol) in DMF (1.7 mL), Trt-GABA-OH (2 g, 5.36 mmol) was added in two portions and Et_3N (1.73 mL, 12.86 mmol) drop-wise. The reaction mixture was stirred at 0 °C for 30 min and at room temperature for 2h. Then, the mixture was diluted with EtOAc and washed twice with brine. The organic layer was washed with cold 5% aq. citric acid, H_2O, cold 5% aq. $NaHCO_3$, H_2O and brine, dried over Na_2SO_4, filtered and evaporated to dryness under vacuum, and the desired compound was obtained as a light yellow foam (89%); R_f (J): 0.63.

The thus obtained amide (2.29 g, 3.35 mmol) was added portion-wise over 1h to a suspension of $LiAlH_4$ (0.41 g, 10.65 mmol) in THF (21 mL) at 70 °C. The reaction mixture was stirred at the above-mentioned temperature overnight. Excess $LiAlH_4$ was destroyed by the careful addition at 0 °C of a saturated solution of Na_2SO_4. The precipitate was filtered off, discarded, and the filtrate was diluted in $CHCl_3$ and washed twice with brine. The organic layer was dried over Na_2SO_4, filtered and evaporated to dryness under vacuum. The residue was subjected to FCC, using the solvent system H as eluent, to give the pure product as pale yellow oil (65%); mp 127-130 °C; R_f (H): 0.17; **^{1}H NMR** ($CDCl_3$, 400 MHz) δ 1.57 (s, 8H), 2.22 (s, 4H), 2.60 (s, 4H), 7.22 (t, J = 7.2 Hz, 6H), 7.32 (t, J = 7.5 Hz, 12H), 7.55 (d, J = 7.5 Hz, 12H) ppm; **ESI-MS** (30eV) ***m/z*:** 243.58 $[Trt]^+$, $[M+H]^+$ Calcd for $C_{46}H_{50}N_3^+$ 644.40, Found 644.39.

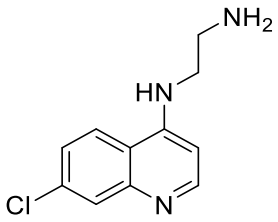
Synthesis of the Hsd-derived acid 21



To a solution of **19** (613 mg, 0.952 mmol) and DMAP (120 mg, 0.952 mmol) in DCM (11.6 mL) at 0 ºC, was added succinic anhydride (150 mg, 1.5 mmol). The reaction was kept for 30 min at 0 ºC and then left to be stirred at room temperature overnight. After completion of the reaction (monitored by TLC), the mixture was diluted with DCM, washed with cold 5% aq. citric acid, H_2O and brine, dried over Na_2SO_4, filtered and evaporated to dryness under vacuum. The residue was subjected to FCC, using the solvent system Gas eluent, to give the pure product as a pale yellow oil (100%); R_f (G): 0.23; **^{1}H NMR** ($CDCl_3$, 600 MHz) δ 1.49 – 1.44 (m, 3H), 1.61 – 1.62 (m, 4H), 2.17 – 2.13 (m, 4H), 2.63 (dd, J = 7.2, 7.2 Hz, 6H), 3.19 (t, J = 6 Hz, 2H), 3.29 (t, J = 12 Hz, 2H), 7.19 (q, J = 18 Hz, 6H), 7.29 – 7.26 (m, 15H), 7.47 (d, J = 8.4 Hz, 11H) ppm; **^{13}C NMR** ($CDCl_3$, 151 MHz) δ 29.59, 26.62, 28.05, 28.15, 30.56, 43.18, 43.31, 46.53, 48.18, 70.89, 126.24, 127.79, 127.85, 128.55, 128.62, 145.98, 146.08 ppm **ESI-MS** (30eV) *m/z***:** 243.58 $[Trt]^+$, $[M+H]^+$ Calcd for $C_{50}H_{54}N_3O_3^+$ 744.42, Found 744.38, $[M+Na]^+$ Calcd for $C_{46}H_{53}N_3NaO_3^+$ 766.40, Found 766.20.

B. Synthesis of modified 7-chloroquinoline derivatives

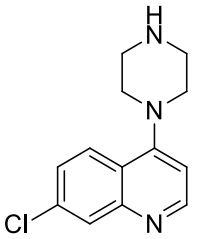
Synthesis of 4-substituted 7-chloroquinoline derivative 15



A solution of 4,7-dichloroquinoline (202 mg, 1 mmol) in 1,2-ethylenediamine (used as solvent) (326 mg, 5.4 mmol) was gradually heated under Ar until 80 ºC, stirred for 40 min and then the temperature was raised up to 130 ºC and the stirring was continued for further 6h. When the reaction was completed, the mixture diluted with DCM, washed with was H_2O, dried over Na_2SO_4, filtered and evaporated to dryness under vacuum to give the product as an orange solid (76%). The amine was used for the next reaction without further purification; R_f (T): 0.26; **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 3.12 (dd, J = 6.3, 4.5 Hz, 2H), 3.32 (dd, J = 10.8, 5.1 Hz, 2H), 5.80 (br s, 1H), 6.39 (d, J = 5.4 Hz, 1H), 7.35 (dd, J = 9, 2.1 Hz, 1H), 7.72 (d, J = 8.7 Hz, 1H), 7.94 (d, J = 2.1 Hz, 1H), 8.52 (d, J = 5.4 Hz, 1H)

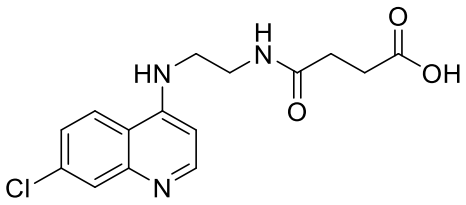
ppm; **^{13}C NMR** ($CDCl_3$, 75.47 MHz) δ 38.12, 42.94, 99.39, 117.06, 1230.74, 126.55, 127.41, 138.43, 140.52, 144.71, 156.13 ppm; **ESI-MS** (30eV) ***m/z*:** $[M+H]^+$ Calcd for $C_{11}H_{13}ClN_3^+$ 222.08, Found 222.40 .

Synthesis of 4-substituted 7-chloroquinoline derivative 16



A solution of 4,7-dichloroquinoline (202 mg, 1 mmol) and piperazine (781 mg, 4 mmol) in MeOH (10 mL) was stirred for 31 h under reflux. After completion of the reaction (monitored by TLC), the mixture was evaporated to dryness under vacuum, diluted with EtOAc, washed with solution of 5% aq. $NaHCO_3$, H_2O and brine. After been dried over Na_2SO_4, the organic extract was filtered and evaporated to dryness under vacuum to give the product as a yellow solid (56%), pure enough to be used in the next step without any further purification; R_f (A): 0.44.

Synthesis of 7-chloroquinoline-derived acid 17

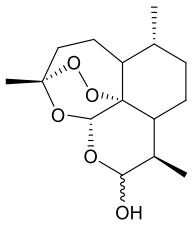


To a solution of **15** (63 mg, 0.285 mmol), DMAP (37 mg, 0.3 mmol) in dry DCM (5 mL), was added succinic anhydride (36 mg, 0.356 mmol) were stirred overnight at rt under Ar. Upon completion of the reaction (monitored be TLC), the mixture was filtered was filtered under vacuum and washed with DCM and afterwards with MeOH to give the pure product as a white solid (100%); R_f (U): 0.35; **^{1}H NMR** (CD_3OD, 300 MHz) δ 2.30 - 2.34 (m, 2H), 2.41 - 2.45 (m, 2H), 3.31 - 3.33 (m, 4H), 6.54 (d, J =5.7 Hz, 1H), 7.39 (s, 1H), 7.44 (dd, J

= 9, 2.1 Hz, 1H), 7.78 (d, *J* = 2.1 Hz, 1H), 8.15 (d, *J* = 9 Hz, 1H), 8.39 (d, *J* = 5.7 Hz, 1H) ppm; **^{13}C NMR** (CD_3OD, 75.47 MHz) δ 29.4, 30.3, 37.4, 42.3, 98.7, 117.5, 124.0, 124.4, 127.4, 133.6, 149.0, 150.3, 152.0, 172.1, 174.2 ppm; **ESI-MS** (30eV) ***m/z*:** $[M+H]^+$ Calcd for $C_{17}H_{16}ClN_3O_3^+$ 322.10, Found 322.40, $[M+Na]^+$ Calcd for $C_{17}H_{15}ClN_3NaO_3^+$ 344.08, Found 344.39 .

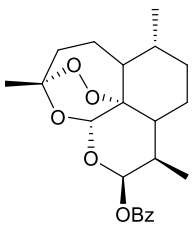
C. Procedure for the synthesis of 3-(10-deoxoartemisin-10-yl)propanoic acid (1)

<u>Step 1:</u> Synthesis of dihydroartemisinin (DHA)



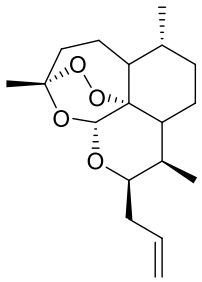
To an ice cold solution of Artemisinin (1 g, 3.5 mmol) in dry MeOH (50 mL), was added portion-wise $NaBH_4$ (331 mg, 8.75 mmol). The reaction mixture was stirred at 0 ºC for 3h. After completion of the reaction, the mixture was cooled at 0 ºC and the $NaBH_4$ excess was destroyed by treatment with dropwise addition of solution of AcOH/MeOH 1:1 (10.2 mL). Then, it was evaporated to dryness under vacuum. The white solid residue was subjected to FCC, using the solvent system O as eluant, to give the pure product as a white solid (98%); R_f (L): 0.22; which had identical spectral data with the ones reported in the literature.[2, 3]

<u>Step 2:</u> Synthesis of 10β-dihydroartemisinyl benzoate



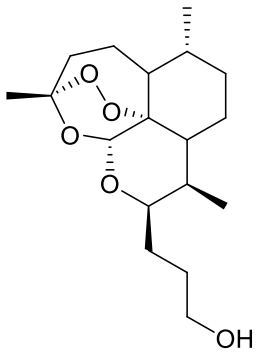
To a solution of DHA (995 mg, 3.5 mmol) in dry DCM (10.5 mL) at 0 ºC, were added pyridine (1.79 mL) and benzoyl chloride (0.638 mL, 5.51 mmol) and the mixture left to be stirred for 16h at rt. Upon completion of the reaction, the mixture was quenched with 5% aq. $NaHCO_3$ to pH 4. The aqueous layer was extracted with EtOAc three times. The combined organic phases were washed with 5% aq.citric acid, H_2O, 5% aq.$NaHCO_3$, H_2O and brine. After being dried over Na_2SO_4, the organic extract was filtered and evaporated to dryness under vacuum. The residue was subjected to FCC, using the solvent system Y as eluent to give the pure product as a white solid (100%); R_f (Y): 0.31; and identical spectral data with the ones reported in the literature.[2, 3]

<u>Step 3</u>: Synthesis of 10β-allyldeoxoartemisinin



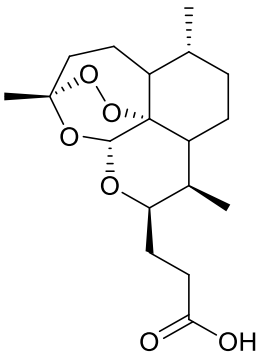
To a solution of allyltrimethylsilane (2.038 mL, 12.83 mmol) in dry DCE (11.63 mL) at 0 ºC, were added $ZnCl_2$ (431 mg, 3.16 mmol) and molecular sieves 4 Å. Then, a solution of 10β-dihydroartemisinyl benzoate (1g, 2.56 mmol) in dry DCE (11.63 mL) was added dropwise. The reaction mixture was stirred at 0 ºC for 3h. Upon completion of the reaction, the solvents were evaporated to dryness under vacuum and then the mixture was diluted with EtOAc. The organic phase was washed with 5% aq.citric acid, H_2O, 5% aq.$NaHCO_3$, H_2O and brine, dried over Na_2SO_4, filtered and evaporated to dryness under vacuum. The residue was subjected to FCC, using the solvent system Y as eluant, to give the pure product as a colorless oil (91%); R_f (Y): 0.32; with identical spectral data with the ones reported in the literature.[2, 3]

<u>Step 4:</u> Synthesis of 10β-hydroxypropyldeoxoartemisinin



To a cold solution (-20 ºC) of 10β-allyldeoxoartemisinin (1.54 g, 5.0 mmol) in THF (200 mL), a 1 M solution of BH_3•SMe_2 in THF (5.0 mL) was added dropwise. The reaction mixture was stirred at rt for 2 h. Upon completion of the reaction, the mixture was quenched with a saturated Na_2CO_3 solution (10 mL), a 30% H_2O_2 solution (5.0 mL) was added and the resulting mixture was stirred for further 30 min. Then, it was evaporated to dryness. The residue was diluted with DCM, washed once with H_2O and once with brine. The organic phase was dried over Na_2SO_4 and evaporated to dryness to afford the pure product (85%) as a colorless oil after FCC purification, using the solvent system Q as eluant; R_f (Q): 0.17; and identical spectral data with the ones reported in the literature.[4]

<u>Step 5:</u> Synthesis of 3-(10-deoxoartemisin-10-yl)propanoic acid (1)

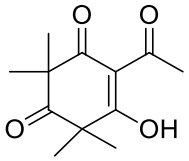


To a solution of 10β-hydroxypropyldeoxoartemisinin (200 mg, 0.6 mmol) in EtOAc/MeCN/H_2O (2.4 mL /2.4 mL /0.782 mL) at 0 ºC, were added $RuCl_3$ (2.7 mg, 0.014

mmol) and $NaIO_4$ (200 mg, 0.92 mmol). The black colored reaction mixture, was stirred for 30 min where a color change to orange was observed (indication of completion of the reaction, confirmed also by TLC analysis). Then the mixture was diluted with EtOAc and extracted with saturated solution of NH_4Cl. The aqueous phase was re-extracted two times with EtOAc, dried over Na_2SO_4, filtered and evaporated to dryness under vacuum. The residue was subjected to FCC, using the solvent system (N) purification to give the pure product as a colorless oil (96%); R_f (O): 0.21; which had identical spectral data with the ones reported in the literature.[2, 3]

D. Procedure for the synthesis of Endoperoxide GMeP (3) from 2-acetylphloroglucinol

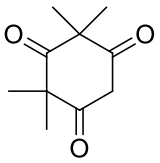
<u>Step 1:</u> Synthesis of 4-Acetyl-5-hydroxy-2,2,6,6-tetramethylcyclohex-4-ene-1,3-dione (4-acetylsyncarpic acid)



A solution of NaOMe was prepared by adding sodium (30.6 g, 1.33 mol) in several portions to absolute methanol (1 L) followed by stirring the mixture at room temperature until the solution became clear. Then 2-acetylphloroglucinol (27.46 g, 0.163 mol) was added, followed by addition of iodomethane (142 mL, 2.28 mol). The resulting mixture was refluxed for 6h (reaction progress was checked using TLC, where the product appears as a tail using petroleum ether/ethyl acetate system, 2:1 as eluent) and then cooled down to rt. The mixture was concentrated under reduced pressure and the residue was re-dissolved in H_2O, acidified with 1 M HCl, before it was extracted with diethyl ether. The combined organic layers were washed with saturated sodium sulfite solution. These washings were acidified with 3M HCl (until a white solid appeared) and extracted with diethyl ether. All organic layers were combined and after drying them over $MgSO_4$, filtration and concentration in vacuum, a yellow solid was obtained, which could be recrystallized from

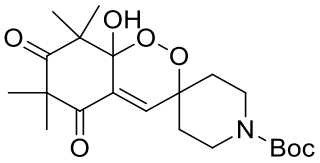
petroleum ether to provide the title compound (84%) as orange crystals; and identical spectral data with the ones reported in the literature.[5]

<u>Step 2:</u> Synthesis of Syncarpic acid



A suspension of 4-acetylsyncarpic acid (30.4 g, 0.136 mol) in 3 M HCl (1.4 L) was refluxed for 6 h until the starting material had disappeared (TLC control). Thereafter, the mixture was cooled down to room temperature and extracted with EtOAc. The combined organic layers were washed twice with H_2O, dried over Na_2SO_4, filtered and concentrated in vacuum. The crude product was recrystallized from toluene to provide syncarpic acid (74%) as pale-yellow crystals; R_f (V): 0.37; which had identical spectral data with the ones reported in the literature.[5]

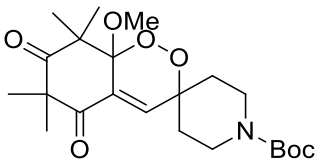
<u>Step 3:</u> Synthesis of 4-(3,5,6,7,8,8a-Hexahydro-8a-hydroxy-6,6,8,8-tetramethyl-5,7-dioxobenzo[*c*][1,2]dioxin-3-yl)piperidine-1-*tert*-butyl carboxylate



N-Boc-piperidine-4-carboxaldehyde (0.38 mg, 1.8 mmol) dissolved in anhydrous DCM (9 mL) was added to piperidine (0.09 mL, 0.9 mmol) at room temperature under argon. On the other hand, piperidine (0.09 mL, 0.9 mmol) was added to a suspension of syncarpic acid (0.32 g, 1.8 mmol) in DCM (9 mL). After 45 min, the solution of syncarpic acid was poured into the first solution of the iminium intermediate. After 30 min stirring, the mixture was concentrated under vacuum. The Mannich base, which was obtained as a white powder, was then solubilized in DCM and treated with a saturated solution of NH_4Cl in 1 M HCl. The biphasic mixture was stirred for 15 min. The organic phase was recovered,

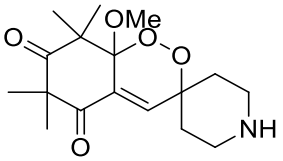
dried over MgSO4, filtered and concentrated under reduced pressure. The resulting enone was obtained as a yellow oil. It was then dissolved in DCM, and kept under air for thirteen days. After evaporation, the crude mixture was purified by FCC, using the solvent system L as eluant, to give pure product as a white solid (68%) with R_f (L): 0.46. The product had identical spectral data with the ones reported in the literature.[6]

<u>Step 4:</u> Synthesis of 4-(3,5,6,7,8,8a-Hexahydro-8a-methoxy-6,6,8,8-tetramethyl-5,7-dioxobenzo[*c*][1,2]dioxin-3-yl)piperidine-1-*tert*-butyl carboxylate



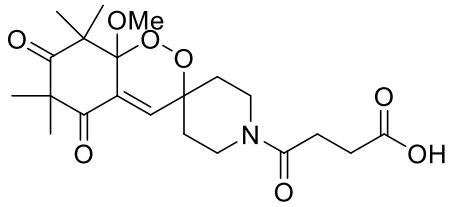
4-(3,5,6,7,8,8a-Hexahydro-8a-methoxy-6,6,8,8-tetramethyl-5,7-dioxobenzo[*c*][1,2]dioxin-3-yl)piperidine-1-*tert*-butyl carboxylate (1.25 g, 3.0 mmol) was dissolved in anhydrous THF (100 mL) under an atmosphere of argon. Then a butyllithium solution (1.3 M in hexane) (2.10 mL, 3.3 mmol) was slowly added at -78 °C. After 15 min stirring, methyl triflate (0.38 mL, 3.3 mmol) was added. The mixture was stirred for 4 h at -78 °C and then hydrolyzed with saturated NH_4Cl solution. The aqueous phase was extracted thrice with DCM. The combined organic phases were washed with brine, dried over $MgSO_4$, filtered and evaporated. Methylated endo-peroxide was obtained after purification by FCC, using the solvent system M as eluant, as a white solid (65%); R_f (L): 0.39. It had identical spectral data with the ones reported in the literature.[6]

<u>Step 5:</u> Synthesis of Endoperoxide GMeP (3)



Trifluoroacetic acid (0.66 mL, 8.8 mmol) was added to 4-(3,5,6,7,8,8a-Hexahydro-8a-methoxy-6,6,8,8-tetramethyl-5,7-dioxobenzo[*c*][1,2]dioxin-3-yl)piperidine-1-*tert*-butyl carboxylate (0.19 g, 0.4 mmol) dissolved in anhydrous DCM (22 mL) under Ar at room temperature. After 24 h, the mixture was neutralized with saturated $NaHCO_3$ solution. The aqueous phase was extracted with DCM, and the combined organic phases were washed with brine, dried over Na_2SO_4, filtered and evaporated to dryness under vacuum to give the product as a pale yellow solid (95%); R_f (E): 0.14. It had identical spectral data with the ones reported in the literature.[6]

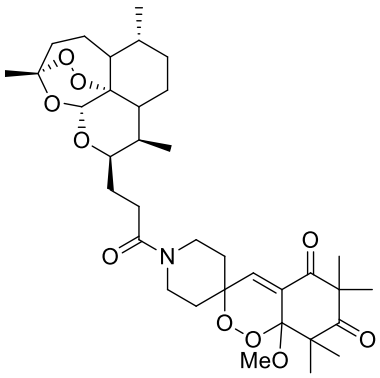
Synthesis of Endoperoxide GMeP-derived acid 4



To a solution of **3** (49.8 mg, 0.12 mmol) in dry DCM (2 mL), were added DMAP (15 mg, 0.12 mmol) and succinic anhydride (15 mg, 0.14 mmol) and stirred for 2h at room temperature. Upon completion of the reaction, the solvents were evaporated to dryness under vacuum and diluted with EtOAc. The organic phase was washed with cold 5% aq. citric acid, H_2O and brine, dried over Na_2SO_4, filtered and evaporated to dryness under vacuum. The residue was subjected to FCC, using the solvent system E as eluant, to give the pure product as a pale yellow oil (98%); R_f (E): 0.2; **^{1}H NMR** ($CDCl_3$, 600 MHz) δ 1.02 (s, 3H), 1.29 (d, J = 12 Hz, 6H), 1.33 (s, 3H), 2.63 - 2.61 (m, 7H), 2.69 - 2.66 (m, 9H), 3.47 (s, 3H), 7.24 (s, 1H) ppm; **^{13}C NMR** ($CDCl_3$, 151 MHz) δ 15.61, 21.75, 24.78, 25.92, 27.73, 28.38, 28.64, 28.84, 29.37, 51.95, 54.81, 143.05, 170.72, 172.66, 177.55, 209.96 ppm; **ESI-MS** (30eV) *m/z***:** $[M+H]^+$ Calcd for $C_{21}H_{30}NO_8^+$ 424.20, Found 424.48, $[M+Na]^+$ Calcd for $C_{21}H_{29}NNaO_8^+$ 446.18, Found 446.40, $[M+K]^+$ Calcd for $C_{21}H_{29}KNO_8^+$ 462.13, Found 462.39.

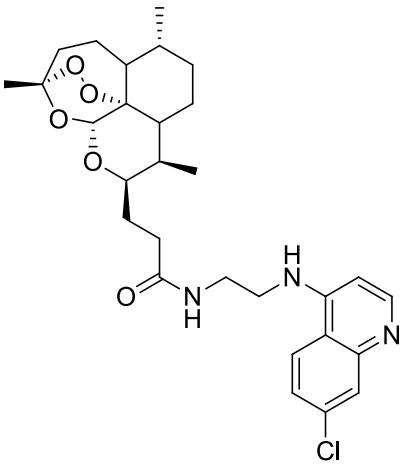
E. Synthesis of compounds 5 – 14

Synthesis of ART-GMeP hybrid 5



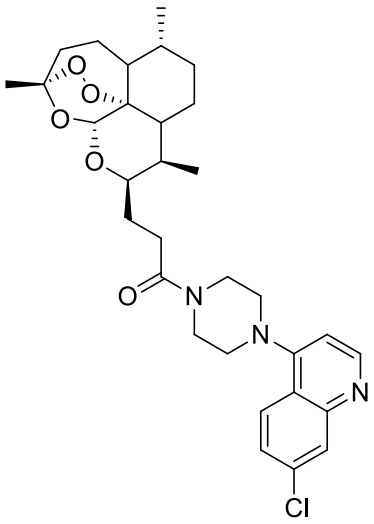
To a solution of **3** (20 mg, 0.047 mmol) and HBTU (19 mg, 0.05 mmol) in dry DCM (0.25 mL) at 0 ^{o}C, was added **1** (16 mg, 0.047 mmol) and DIPEA (0.016 mL, 0.094 mmol). The reaction was kept for 30 min at 0 ^{o}C and then left to be stirred at rt for 2h. After completion of the reaction (monitored by TLC), the mixture was diluted with DCM and placed in a separatory funnel. The organic layer was washed with cold 5% aq. citric acid, H_2O, cold 5% aq.$NaHCO_3$, H_2O and brine. After being dried over Na_2SO_4, the organic extracts were filtered and evaporated to dryness under vacuum. The residue was subjected to FCC, using solvent system O as eluant, to give the pure product as a pale yellow oil (72%); R_f (O): 0.37 ; **^{1}H NMR** ($CDCl_3$, 600 MHz) δ 0.90 (d, J = 12 Hz, 3H), 0.95 (d, J = 6 Hz, 3H), 1.03 (s, 3H), 1.29 (s, 3H), 1.31 (s, 3H), 1.34 (s, 3H), 1.42 (s, 3H), 1.50 - 1.44 (m, 2H), 1.75 - 1.52 (m, 5H), 1.84 - 1.78 (m, 3H), 1.91 - 1.86 (m, 3H), 2.03 - 1.99 (m, 4H), 2.38 - 2.31 (m, 3H), 2.80 - 2.69 (m, 2H), 3.48 (s, 3H), 3.80 (br s, 1H), 4.07 - 4.04 (m, 1H), 4.42 (br s, 1H), 5.29 (br s, 1H), 7.25 (s, 1H) ppm; **^{13}C NMR** ($CDCl_3$, 151 MHz) δ 13.40, 15.62, 20.24, 21.77, 24.52, 24.64, 24.79, 24.87, 25.94, 26.25, 30.12, 30.96, 34.48, 36.53, 37.40, 44.61, 52.55, 53.22, 54.81, 80.70, 89.16, 100.92, 103.48, 125.42, 128.37, 129.02, 129.61, 142.68, 171.37, 198.70, 209.97 ppm; **ESI-MS** (30eV) *m/z***:** 646.43 $[M+H]^+$, 668.34 $[M+Na]^+$, 684.34 $[M+K]^+$; **HRMS** (ESI/Q-TOF) *m/z*: $[M+H]^+$ Calcd for $C_{35}H_{52}NO_{10}^+$ 646.3591; Found 646.3588.

Synthesis of ART-ACQ hybrid 6



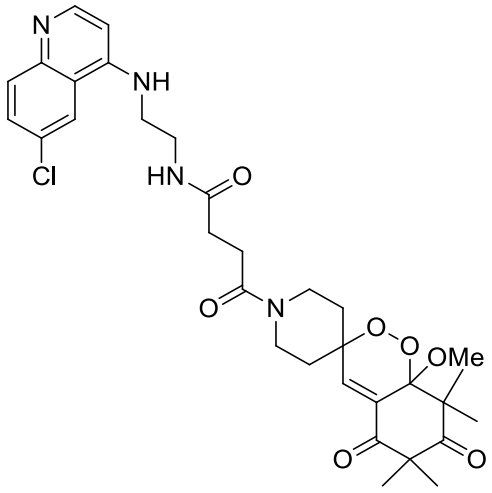
To an ice-cold solution of **15** (15.7 mg, 0.071 mmol) and HBTU (34 mg, 0.085 mmol) in dry DCM (0.25 mL), was added **1** (24.6 mg, 0.071 mmol) and DIPEA (0.039 mL, 0.22 mmol). The reaction was kept for 30 min at 0 ^{o}C and then left to be stirred overnight at rt. After completion of the reaction (monitored by TLC), it was diluted with DCM and placed in a separatory funnel. The organic layer was washed with cold 5% aq.citric acid, H_2O, cold 5% aq.$NaHCO_3$, H_2O and brine. After been dried over Na_2SO_4, the organic extracts were filtered and evaporated to dryness under vacuum. The residue was subjected to FCC, using the solvent system D as eluant, to give the pure product as a pale yellow oil (80%); R_f (D): 0.4; **^{1}H NMR** ($CDCl_3$, 600 MHz) δ 0.86 (t, J = 6 Hz, 6H), 1.20 - 1.02 (m, 4H), 1.23 (s, 3H), 1.57 - 1.46 (m, 2H), 1.77 - 1.69 (m, 3H), 1.88 - 1.85 (m, 1H), 2.24 - 2.13 (m, 2H), 2.39 - 2.34 (m, 1H), 2.49 - 2.45 (m, 1H), 2.74 - 2.70 (m, 1H), 3.54 - 3.44 (m, 2H), 3.72 - 3.69 (m, 1H), 3.86 - 3.81 (m, 1H), 4.09 - 4.06 (m, 1H), 5.21 (s, 1H), 6.37 (d, J = 6.6 Hz, 1H), 7.43 - 7.42 (m, 2H), 7.92 (s, 1H), 8.22 (t, J = 14.4 Hz, 2H) ppm; **^{13}C NMR** ($CDCl_3$, 151 MHz) δ 13.40, 20.14, 24.29, 24.64, 25.92, 30.47, 34.33, 35.67, 36.52, 37.21, 38.04, 44.15, 44.45, 52.42, 81.18, 88.79, 97.79, 103.49, 116.35, 123.36, 123.90, 126.69, 137.64, 143.55, 146.02, 153.35, 175.88 ppm; **ESI-MS** (30eV) *m/z***:** 546.41 $[M+H]^+$, 566.4 $[M+Na]^+$; **HRMS** (ESI/Q-TOF) *m/z*: $[M+H]^+$ Calcd for $C_{29}H_{39}ClN_3O_5^+$ 544.2578; Found 544.2588.

Synthesis of ART-ACQ hybrid 7



To a solution of **16** (17.6 mg, 0.071 mmol) and HBTU (34 mg, 0.085 mmol) in dry DCM (0.25 mL) at 0 °C, was added **1** (24.6 mg, 0.071 mmol) and DIPEA (0.039 mL, 0.22 mmol). The reaction was kept for 30 min at 0 °C and then left to be stirred overnight at rt. After completion of the reaction (monitored by TLC), it was diluted with DCM and placed in a separatory funnel. The organic layer was washed with cold 5% aq. citric acid, H_2O, cold 5% aq. $NaHCO_3$, H_2O and brine. After been dried over Na_2SO_4, the organic extracts were filtered and evaporated to dryness under vacuum. The residue was subjected to FCC, using the solvent system D as eluent, to give the pure product as a pale yellow oil (95%); R_f (D): 0.4; **^{1}H NMR** ($CDCl_3$, 600 MHz) δ 0.91 (d, J = 6 Hz, 3H), 0.95 (d, J = 6 Hz, 3H), 1.32 - 1.21 (m, 2H), 1.39 (s, 3H), 1.47 - 1.41 (m, 2H), 1.58 - 1.54 (m, 1H), 1.66 - 1.63 (m, 1H), 1.90 - 1.81 (m, 3H), 2.06 - 1.99 (m, 2H), 2.43 - 2.30 (m, 2H), 3.28 - 2.72 (m, 3H), 3.29 - 3.26 (m, 4H), 3.88 - 3.76 (m, 3H), 3.96 - 3.93 (m, 1H), 4.09 - 4.06 (m, 1H), 5.29 (s, 1H), 6.84 (d, J = 6Hz, 1H), 7.46 (dd, J = 1.8, 6.6 Hz, 1H), 7.94 (d, J = 6 Hz, 1H), 8.12 (s, 1H), 8.7 (d, J = 6 Hz, 1H) ppm; **^{13}C NMR** ($CDCl_3$, 151 MHz) δ 13.35, 20.23, 24.57, 24.63, 24.88, 26.27, 30.13, 31.01, 34.46, 36.31, 37.40, 38.60, 41.52, 44.57, 45.36, 51.99, 52.21, 52.51, 76.35, 81.19, 88.67, 103.48, 108.79, 121.37, 125.00, 126.77, 128.11, 135.80, 157.05, 171.83 ppm; **ESI-MS** (30eV) *m/z***:** 571.29 $[M+H]^+$, 593.32 $[M+Na]^+$, 610.35 $[M+K]^+$, 1163.25 $[2M+Na]^+$; **HRMS** (ESI/Q-TOF) *m/z*: $[M+H]^+$ Calcd for $C_{31}H_{41}ClN_3O_5^+$ 570.2735; Found 570.2734.

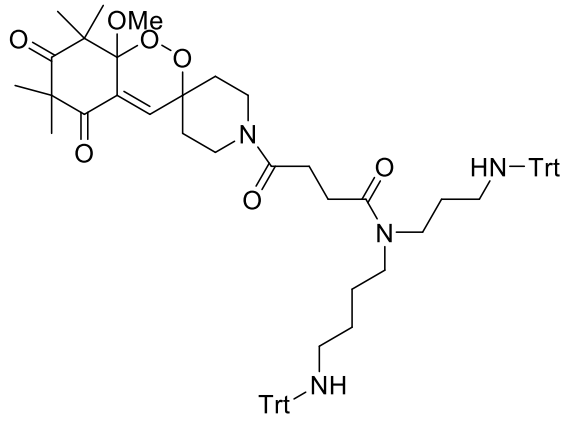
Synthesis of GMeP-ACQ hybrid 8



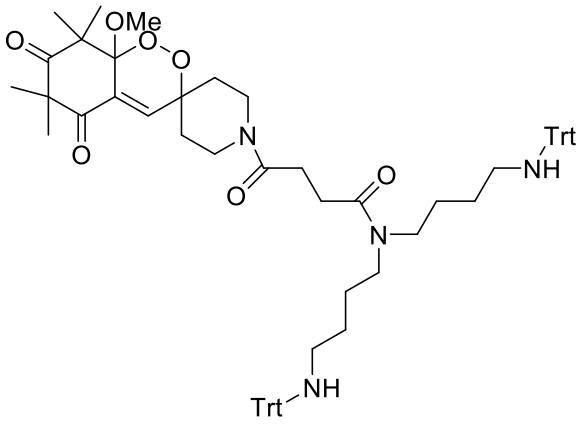
To a solution of **3** (35.53 mg, 0.110 mmol) and HBTU (50 mg, 0.123 mmol) in dry DCM (3.1 mL) at 0 ºC, was added **17** (35.4 mg, 0.110 mmol) and DIPEA (0.093 mL, 0.65 mmol). The reaction was kept for 30 min at 0 ºC and then left to be stirred overnight at rt. After completion of the reaction (monitored by TLC), was diluted with DCM washed with cold 5% aq. citric acid, H_2O, cold 5% aq.$NaHCO_3$, H_2O and brine. After been dried over Na_2SO_4, the organic extracts were filtered and evaporated to dryness under vacuum. The residue was subjected to FCC, using the solvent system X to give the pure product as a pale yellow oil (95%); R_f (X): 0.1; **^{1}H NMR** ($CDCl_3$, 600 MHz) δ 1.02 (s, 3H), 1.30 (d, J = 6 Hz, 6H), 1.35 (d, J = 12 Hz, 3H), 1.52 – 1.46 (m, 1H), 1.70 – 1.59 (m, 1H), 1.92 (t, J = 18 Hz, 1H), 2.01 (quint, J = 12 Hz, 1H), 2.36 (t, J = 6 Hz, 1H), 2.61 – 2.52 (m, 2H), 2.75 – 2.64 (m, 2H), 2.83 (s, 2H), 3.37 (t, J = 6 Hz, 1H), 3.42 (br s, 2H), 3.46 (d, J = 12 Hz, 3H), 3.76 – 3.59 (m, 3H), 4.24 (dd, J = 18, 18 Hz, 1H), 6.29 (d, J = 6 Hz, 1H), 6.99 (br s, 1H), 6.85 (br s, 1H), 7.16 (d, J = 36 Hz,1H) 7.33 (d, J = 6 Hz, 1H), 7.85 (t, J = 12 Hz, 1H), 7.92 (s, 1H), 8.48 (t, J = 4.8 Hz, 1H) ppm; **^{13}C NMR** ($CDCl_3$, 151 MHz) δ 15.62, 17.66, 21.76, 24.83, 25.92, 28.97, 29.58, 30.68, 32.26, 32.94, 36.68, 38.60, 40.58, 45.03, 49.43, 53.07, 54.83, 98.19, 100.85, 117.30, 122.25, 125.21, 128.30, 129.83, 134.90, 142.76, 150.20, 151.81, 170.07, 175.47, 198.42, 209.90 ppm; **HRMS** (ESI/Q-TOF) *m/z*: $[M+H]^+$ Calcd for $C_{32}H_{40}ClN_4O_7^+$ 627.2586; Found 627.2583.

General coupling procedure for the synthesis of compounds 22 & 23

To a solution of **3** (1 eq) and HBTU (1.2 eq) in dry DCM (0.3 M) at 0 ºC, was added **20** or **21** (1 eq) and DIPEA (3.1 eq). The reaction mixture was stirred at 0 ºC for 30 min and at room temperature for additional 2.5 h. The reaction was kept for 30 min at 0 ºC and then stirred at room temperature for 2h. After completion of the reaction (monitored by TLC), it was diluted with DCM, washed with cold 5% aq. citric acid, H_2O, cold 5% aq. $NaHCO_3$, H_2O and brine. After been dried over Na_2SO_4, the organic extracts were filtered and evaporated to dryness under vacuum. The residue was subjected to FCC, using the solvent system O as eluant, to afford the corresponding pure intermediates.



22: pale yellow oil (40%); R_f (O): 0.3; **^{1}H NMR** ($CDCl_3$, 600 MHz) δ 1.03 (s, 3H), 1.26 (s, 9H), 1.30 (s, 3H), 1.32 (s, 3H), 1.35 (s, 3H), 1.54 – 1.46(m, 2H), 1.74 – 1.63 (m, 3H), 2.04 – 1.94 (m, 1H), 2.17 – 2.10 (m, 2H), 2.36 (s, 3H), 2.82 – 2.49 (m, 5H), 3.36 – 3.06 (m, 4H), 3.48 (s, 4H), 3.82 – 3.62 (m, 1H), 4.45 – 4.32 (m, 1H), 7.25 – 7.14 (m, 13H), 7.69 – 7.27 (m, 22H) ppm; **^{13}C NMR** ($CDCl_3$, 151 MHz) δ 14.12, 15.63, 21.49, 21.78, 22.69, 24.81, 25.94, 29.39, 29.66, 29.70, 31.93, 36.97, 37.09, 40.61, 40.73, 53.11, 54.81, 78.07, 100.85, 125.28, 127.92, 128.23, 128.54, 129.04, 137.87, 143.19, 198.58, 210.01 ppm; **ESI-MS** (30eV) ***m/z*:** 243.50 $[Trt]^+$, $[M+H]^+$ Calcd for $C_{66}H_{75}N_4O_7^+$ 1035.56, Found 1035.45, $[M+Na]^+$ Calcd for $C_{66}H_{74}N_4NaO_7^+$ 1057.55, Found 1057.87, $[M+K]^+$ Calcd for $C_{66}H_{74}KN_4O_7^+$ 1073.52, Found 1073.33.



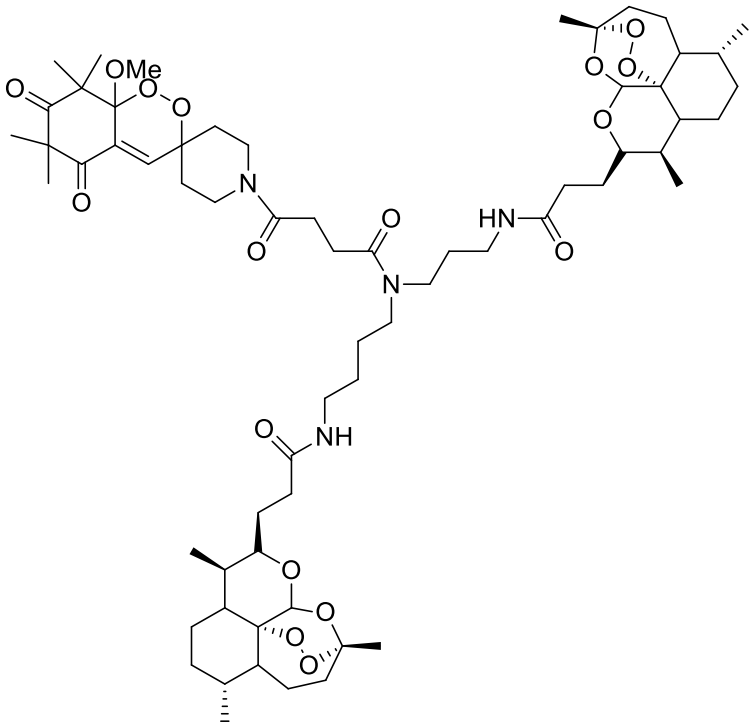
23: pale yellow oil (67%); R_f (O): 0.34; **^{1}H NMR** ($CDCl_3$, 600 MHz) δ 0.90 - 0.85 (m, 2H), 1.04 (d, J = 6 Hz, 2H), 1.27 (s, 3H), 1.30 (s, 3H), 1.32 (s, 3H), 1.35 (s, 3H), 1.75 - 1.48 (m, 9H), 2.23 - 1.92 (m, 5H), 2.36 (s, 3H), 2.80 - 2.54 (m, 4H), 3.33 - 3.21 (m, 3H), 3.49 (s, 3H), 3.83 - 3.81 (m, 1H), 7.26 - 7.15 (m, 11H), 7.28 - 7.27 (m, 8H), 7.55 - 7.43 (m, 10H) ppm; **^{13}C NMR** ($CDCl_3$, 151 MHz) δ 14.13, 15.64, 21.46, 21.78, 22.70, 24.81, 25.69, 25.94, 28.09, 29.37, 29.71, 31.93, 37.02, 40.73, 53.11, 54.81, 54.86, 54.84, 78.10, 100.86, 126.34, 127.88, 128.23, 128.66, 129.04, 129.61, 137.87, 143.24, 198.54, 210.01 ppm; **ESI-MS** (30eV) *m/z***:** 243.50 $[Trt]^+$, $[M+H]^+$ Calcd for $C_{67}H_{77}N_4O_7^+$ 1049.58, Found 1049.48, $[M+Na]^+$ Calcd for $C_{67}H_{76}N_4NaO_7^+$ 1071.56, Found 1071.90, $[M+K]^+$ Calcd for $C_{67}H_{76}KN_4O_7^+$ 1087.54, Found 1087.33.

General procedure for the synthesis of conjugates 9 and 10

To a solution of the protected intermediate **22** or **23** (1 eq) in DCM (0.2 M) at 0 ºC, was added a 3% solution of TFA (4 eq.) in DCM and TFE (2 eq.). The reaction mixture was stirred at 0 ºC for 2h and at room temperature overnight. Upon completion of the reaction, the mixture was triturated with Et_2O and Hex to give the desirable product, as a yellow oil in 100% yield; R_f (A): 0.06; ESI-MS (30eV) *m/z***:** 551.51 $[M+H]^+$, 573.37 $[M+Na]^+$ and yellow oil (100%); R_f ($CHCl_3$/MeOH/aq.NH_3 8:2:0.2): 0.07; ESI-MS (30eV) *m/z***:** 565.37 $[M+H]^+$, 587.35 $[M+Na]^+$, respectively.

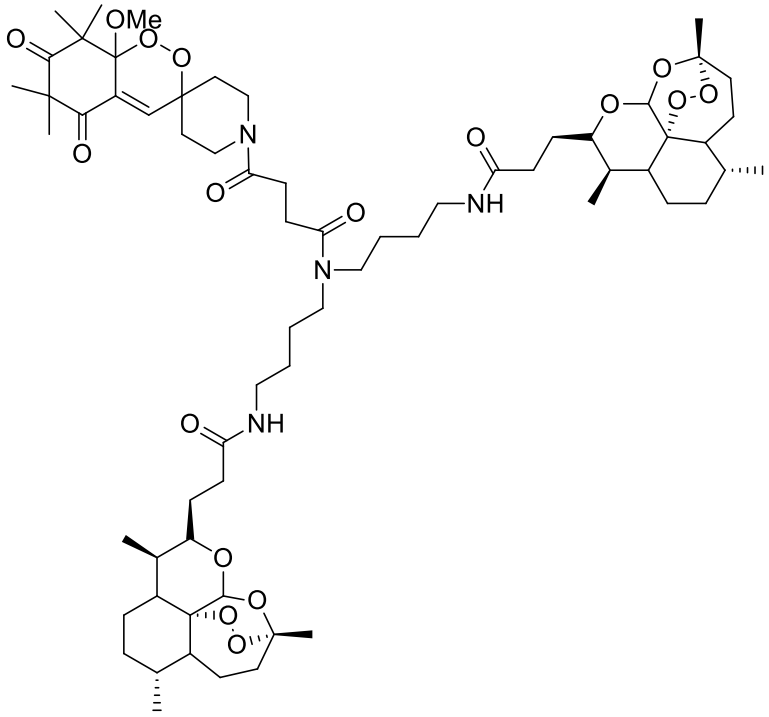
The detritylated intermediate (1 eq) was further treated (without any further purification) with HBTU (2.2 eq) in dry DCM (0.08 M) at 0 ºC and then **1** (2 eq.) and DIPEA (5.3 eq)

were sequentially added. The reaction was kept at 0 ºC for 30 min and then stirred overnight at room temperature. After completion of the reaction (monitored by TLC), it was diluted with DCM, washed with cold 5% aq. citric acid, H_2O, cold 5% aq.$NaHCO_3$, H_2O and brine, filtered, and evaporated to dryness under vacuum. The residue was subjected to FCC, using the solvent system E as eluant, to afford the pure conjugates **9** and **10**.



9: pale yellow oil (61%); R_f (E): 0.26; **^{1}H NMR** ($CDCl_3$, 600 MHz) δ 0.89 - 0.88 (m, 8H), 0.97 - 0.94 (m, 8H), 1.04 (s, 3H), 1.26 (s, 3H), 1.30 (s, 3H), 1.31 (s, 3H), 1.35 (d, J = 12 Hz, 12H), 1.60 - 1.53 (m, 5H), 1.71 - 1.65 (m, 6H), 1.81 - 1.79 (m, 4H), 1.91 - 1.88 (m, 4H), 2.04 - 2.00 (m, 4H), 2.48 - 2.30 (m, 8H), 2.72 - 2.57 (m, 10H), 3.44 - 3.30 (m, 7H), 3.48 (s, 2H), 4.11 - 4.02 (m, 3H), 4.50 - 4.38 (m, 3H), 5.34 - 5.30 (m, 2H), 7.36 (s, 1H) ppm; **^{13}C NMR** ($CDCl_3$, 151 MHz) δ 13.30, 15.60, 20.22, 24.58, 24.77, 24.85, 25.90, 2614, 29.67, 30.19, 34.42, 34.46, 36.49, 36.53, 54.78, 55.76, 69.64, 71.51, 77.41, 77.47, 77.55, 77.69, 77.79, 77.99, 78.22, 81.16, 88.80, 103.36, 157.94, 160.70, 168.48, 192.98, 194.51, 203.32, 209.94, 215.55 ppm; **ESI-MS** (30eV) *m/z***:** 1195.76 $[M+H]^+$, 1217.64 $[M+Na]^+$,

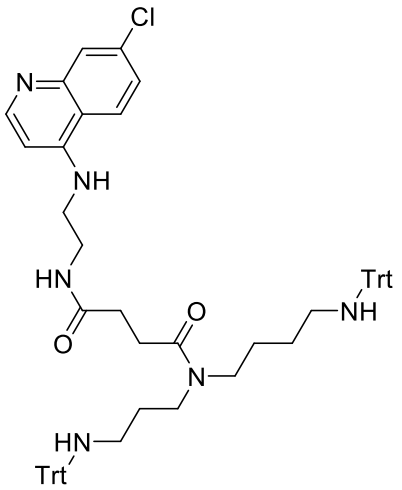
1233.78 [M+K]$^+$; **HRMS** (ESI/Q-TOF) *m/z*: [M+H]$^+$ Calcd for $C_{64}H_{99}N_4O_{17}^+$ 1195.7005; Found 1195.7024.



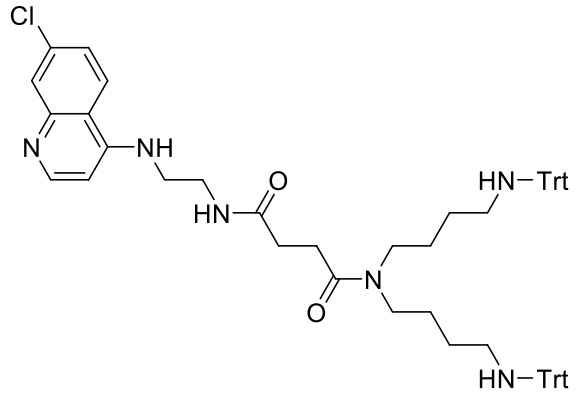
10: pale yellow oil (40%); R_f (E): 0.23; **^{1}H NMR** ($CDCl_3$, 600 MHz) δ 0.89 (d, J = 6 Hz, 8H), 0.96 (d, J = 6 Hz, 9H), 1.03 (s, 3H), 1.25 (s, 5H), 1.29 (s, 3H), 1.31 (s, 3H), 1.34 (s, 3H), 1.38 (s, 6H), 1.58 - 1.55 (m, 5H), 1.71 - 1.65 (m, 7H), 1.91 - 1.79 (m, 8H), 2.04 - 2.00 (m, 4H), 2.49 - 2.30 (m, 12H), 2.73 - 2.70 (m, 2H), 2.82 (s, 1H), 3.40 - 3.22 (m, 9H), 3.48 (s, 3H), 3.88 - 3.48 (s, 3H), 4.05 - 4.03 (m, 2H), 4.43 - 4.38 (m, 1H), 5.31 - 5.30 (m, 2H), 6.51 - 6.45 (m, 1H), 7.25 (s, 1H) ppm; **^{13}C NMR** ($CDCl_3$, 151 MHz) δ 13.20, 15.62, 20.22, 24.05, 24.80, 24.83, 24.87, 25.93, 26.15, 29.69, 30.23, 34.40, 34.45, 36.54, 37.37, 44.48, 44.52, 51.26, 52.45, 52.48, 53.12, 54.80, 54.87, 54.88, 76.16, 76.30, 78.08, 81.20, 88.72, 88.77, 103.44, 104.75, 122.27, 135.47, 143.19, 144.20, 152.80, 162.13, 169.24, 173.46, 175.20, 179.20, 210.00, 211.15, 213.12 ppm; **ESI-MS** (30eV) ***m/z*:** 1210.51 [M+H] $^+$, 1232.31 [M+Na] $^+$, 1248.94 [M+K] $^+$; **HRMS** (ESI/Q-TOF) *m/z*: [M+H]$^+$ Calcd for $C_{65}H_{101}N_4O_{17}^+$ 1209.7162; Found 1209.7185, [M+Na]$^+$ Calcd for $C_{65}H_{100}N_4NaO_{17}^+$ 1231.6976; Found 1231.7007.

General coupling procedure for the synthesis of compounds 24 and 25

To a solution of **18** or **19** (1 eq) and HBTU (1.05 eq) in dry DCM (0.3 M) at 0 °C, **17** (1 eq) and DIPEA (2 eq) were added. The reaction was kept for 30 min at 0 °C and then stirred at room temperature overnight. After completion of the reaction (monitored by TLC), it was diluted with DCM, washed with cold 5% aq. citric acid, H_2O, cold 5% aq. $NaHCO_3$, H_2O and brine. After being dried over Na_2SO_4, the organic phase was filtered and then evaporated to dryness under vacuum. The residue was subjected to FCC, using the solvent system E as eluant, to afford the corresponding intermediates.



24: pale yellow oil (86%); R_f (E): 0.38; **^{1}H NMR** ($CDCl_3$, 600 MHz) δ 1.43 - 1.34 (m, 2H), 1.75 - 1.55 (m, 2H), 2.03 (quint., J = 6 Hz, 5H), 2.38 (t, J = 6 Hz, 4H), 2.66 - 2.45 (m, 4H), 2.85 (s, 6H), 3.27 - 3.03 (m, 3H), 3.39 (t, J = 6 Hz, 4H), 3.58 (s, 3H), 6.59 (br s, 1H), 7.69 - 7.20 (m, 24H), 8.11 - 7.91 (m, 2H), 9.12 - 8.98 (m, 1H) ppm; **^{13}C NMR** ($CDCl_3$, 151 MHz) δ 7.39, 17.66, 23.37, 24.32, 29.59, 30.69, 31.30, 31.58, 38.32, 39.85, 44.70, 44.77, 49.44, 52.67, 57.28, 115.32, 120.26, 120.95, 123.42, 124.92, 125.24, 127.92, 128.01, 128.05, 128.22, 128.24, 128.53, 128.58, 128.79, 128.88, 163.72, 175.11, 189.69, 189.75; **ESI-MS** (30eV) ***m/z*:** 243.50 $[Trt]^+$, $[M+H]^+$ Calcd for $C_{60}H_{62}ClN_6O_2^+$ 933.46; Found 933.44, $[M+Na]^+$ Calcd for $C_{60}H_{61}ClN_6NaO_2^+$ 955.44; Found 955.21, $[M+K]^+$ Calcd for $C_{60}H_{61}ClKN_6O_2^+$ 971.42; Found 970.92.



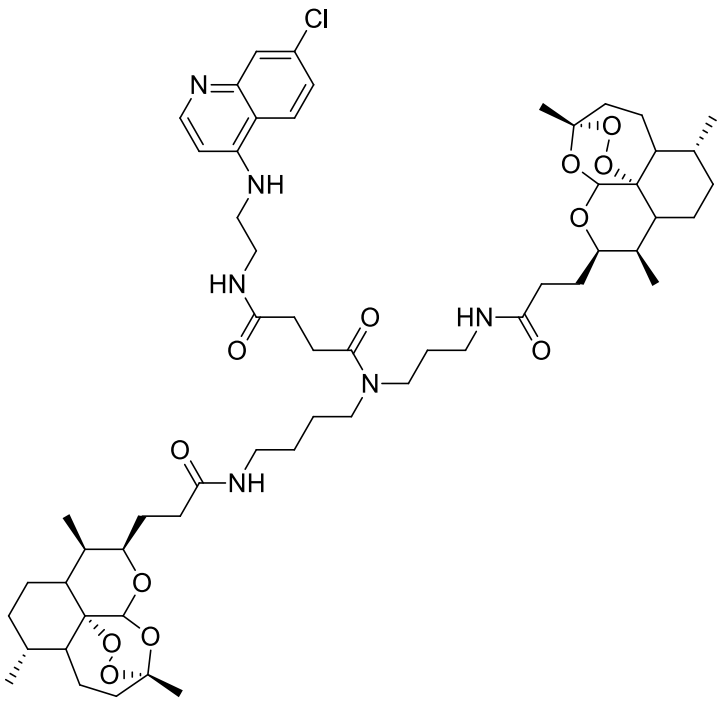
25: pale yellow oil (86%); R_f (E): 0.38; **^{1}H NMR** ($CDCl_3$, 600 MHz) δ 1.27 - 1.20 (m, 1H), 1.45 - 1.39 (m, 6H), 1.56 - 1.51 (m, 2H), 2.14 (dt, J = 6, 48 Hz, 4H), 3.13 (dt, J = 6, 30 Hz, 4H), 3.35 (s, 1H), 3.47 (s, 1H), 3.56 (s, 1H), 6.22 (s, 1H), 7.08 (br s, 1H), 7.19 - 7.13 (m, 7H), 7.26 - 7.22 (m, 9H), 7.28 (s, 1H), 7.45 (dd, J = 12, 6 Hz,21H), 7.81 (s, 1H), 7.86 (d, J = 6 Hz, 1H), 8.33 (d, J = 6 Hz, 1H) ppm; **^{13}C NMR** ($CDCl_3$, 151 MHz) δ 25.63, 26.63, 28.08, 28.33, 28.93, 31.58, 38.47, 43.22, 43.36, 44.78, 46.31, 47.95, 50.69, 70.84, 7.89, 98.19, 116.99, 122.67, 125.59, 126.23, 126.33, 127.77, 127.85, 128.59, 128.60, 135.62, 146.06, 146.16, 147.54, 150.20, 151.13, 171.54, 175.53 ppm; **ESI-MS** (30eV) *m/z***:** 243.34 $[Trt]^+$, $[M+H]^+$ Calcd for $C_{61}H_{64}ClN_6O_2^+$ 947.48; Found 947.30, $[M+Na]^+$ Calcd for $C_{61}H_{63}ClN_6NaO_2^+$ 969.46; Found 969.30, $[M+K]^+$ Calcd for $C_{61}H_{63}ClKN_6O_2^+$ 985.43; Found 985.30.

General procedure for the synthesis of conjugates 11 - 14

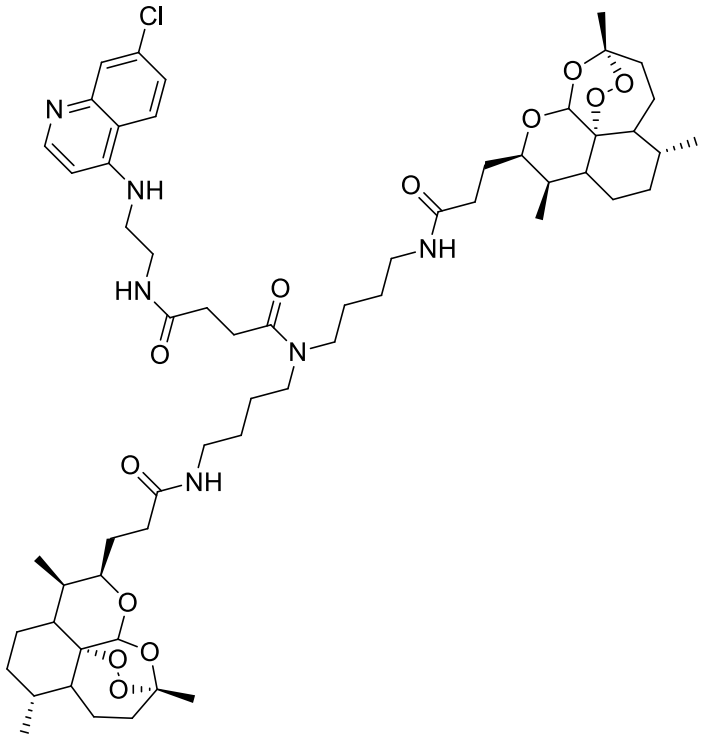
To a solution of the protected intermediate **24** or **25** (1 eq) in DCM (0.04 M) at 0 ^{o}C, was added a 6% solution of TFA (4 eq.) in DCM and TFE (2 eq.). The reaction mixture was stirred at 0 ^{o}C for 2h. Upon completion of the reaction, the mixture was triturated with Et_2O and Hex to give the desirable products, as yellow oils (100%); R_f (A): 0.04.

The detritylated intermediates (1 eq) were further treated (without any further purification) with HBTU (2.2 eq) in dry DCM (0.2 M) at 0 ^{o}C, followed by **1** or **4** (2 eq.) and DIPEA (4 eq). The reaction was kept for 30 min at 0 ^{o}C and then stirred overnight at room temperature. After completion of the reaction (monitored by TLC), the reaction mixture was diluted with DCM and placed in a separatory funnel. The organic layer was washed

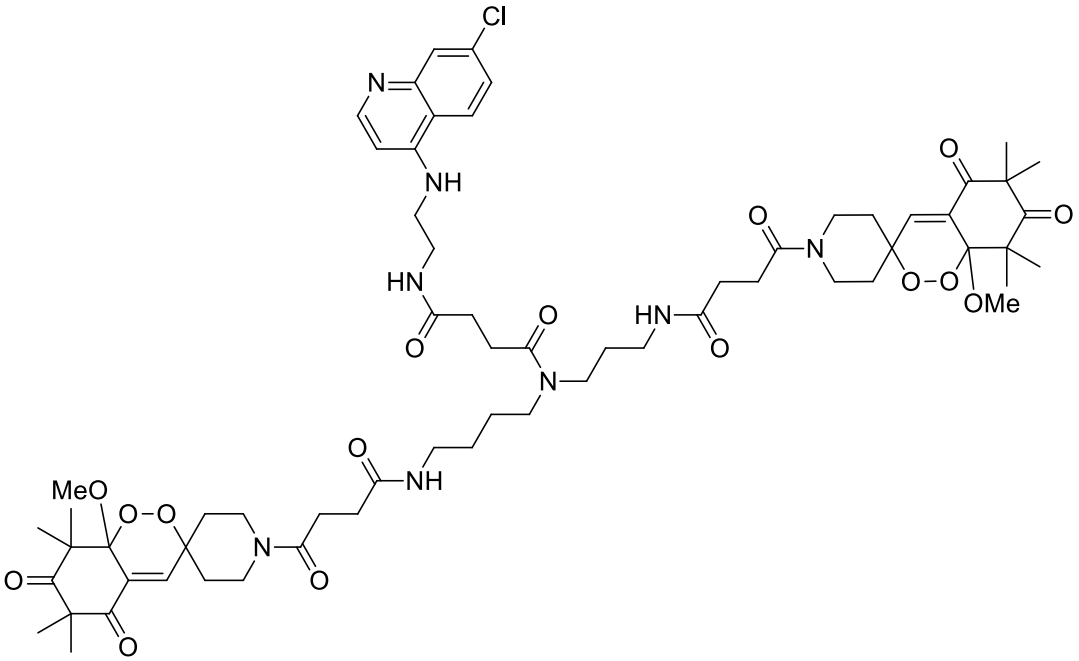
with cold 5% aq. citric acid, H_2O, cold 5% aq. $NaHCO_3$, H_2O and brine, filtered, and evaporated to dryness under vacuum. The residue was subjected to FCC affording the pure conjugates **11, 12, 13** and **14**.



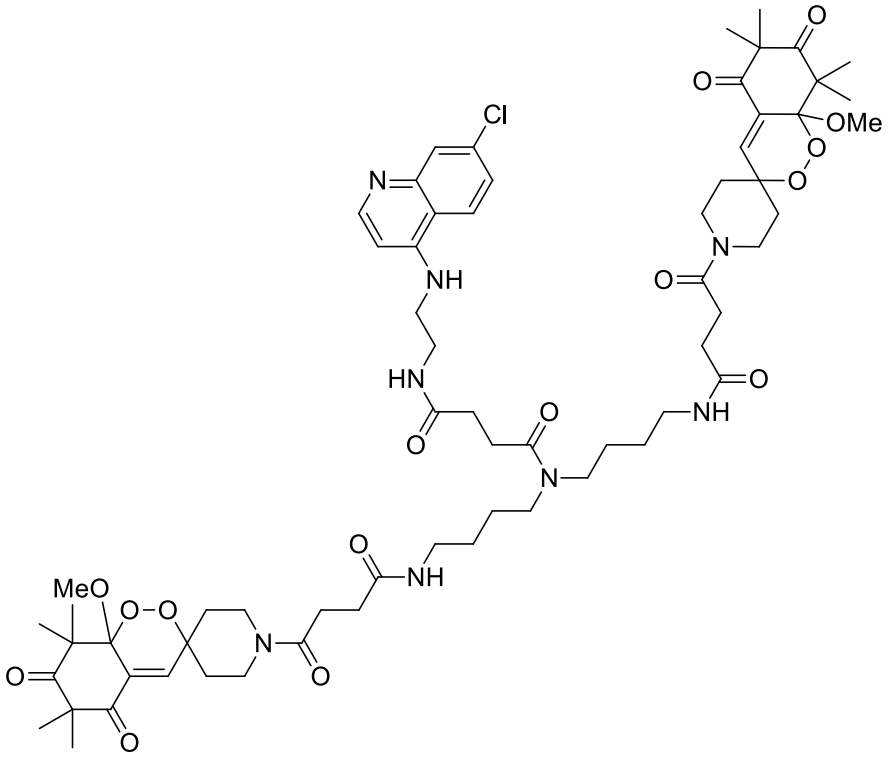
11: pale yellow oil (64%); R_f (C): 0.45; **^{1}H NMR** ($CDCl_3$, 600 MHz) δ 1.23 (s, 3H), 1.24 (s, 3H), 1.25 (s, 3H),1.35 - 1.34 (m, 10H), 1.43 - 1.39 (m, 2H), 1.57 - 1.54 (m, 4H), 1.65 - 1.63 (m, 4H), 1.80 - 1.78 (m, 6H), 2.02 - 1.99(m, 5H), 2.33 - 2.28 (m, 5H), 2.47 - 2.44 (m, 2H), 2.59 (s, 3H), 2.70 (s, 6H), 3.19 - 3.06 (m, 4H), 3.32 - 3.20 (m, 8H), 3.73 - 3.70 (m, 4H), 4.03 – 4.01 (m, 1H), 5.32 (d, J = 3.3 Hz, 2H), 6.56 (dd, J = 42, 3 Hz, 2H), 6.92 - 6.87 (m, 1H), 7.49(d, J = 6 Hz, 2H), 8.05 (s, 2H), 8.26 (s, 1H), 8.36 (s, 1H), 9.22 (s, 1H) ppm; **^{13}C NMR** ($CDCl_3$, 151 MHz) δ 13.24, 13.30, 18.43, 20.23,24.61, 24.81,24.85, 25.88, 26.12, 26.82, 27.47, 30.25, 34.43, 34.45, 36.52, 37.35, 38.20, 38.72,44.49, 44.54, 52.49, 58.44, 76.28, 76.49, 76.52, 81.25, 81.26, 88.69, 88.78, 103.50, 115.47, 119.62, 124.95, 127.84,138.41, 139.79, 142.25, 155.99, 173.44 ppm; **ESI-MS** (30eV) *m/z***:** 1093.54 $[M+H]^+$, 1115.37 $[M+Na]^+$, 1134.54 $[M+K]^+$; **HRMS** (ESI/Q-TOF) *m/z*: $[M+H]^+$ Calcd for $C_{58}H_{86}ClN_6O_{12}^+$ 1093.5992; Found 1093.5989.



12: pale yellow oil (32%); R_f (D): 0.52; **^{1}H NMR** ($CDCl_3$, 600 MHz) δ 0.87 (d, J = 12 Hz, 6H), 0.94 (d, J = 6 Hz, 7H), 1.26 - 1.21 (m, 4H), 1.36 (d, J = 12 Hz, 8H), 1.57 - 1.51 (m, 7H), 1.66 - 1.63 (m, 2H), 1.92 - 1.79 (m, 13H), 2.04 - 2.00 (m, 3H), 2.17 (s, 2H), 2.36 - 2.28 (m, 4H), 2.48 - 2.43 (m, 2H), 2.60 - 2.58 (m, 2H), 2.72 - 2.68 (m, 4H), 3.11 - 3.05 (m, 2H), 3.39 - 3.20 (m, 5H), 3.56 (s, 2H), 3.70 (s, 2H), 4.04 - 3.99 (m, 1H), 5.31 (d, J = 6 Hz, 2H), 6.43 (br s, 1H), 6.53 (br s,1H), 6.73 (br s, 1H), 7.48 - 7.43 (m, 1H), 8.22 (s, 2H), 8.30 (br s, 1H), 8.41 - 8.37 (m, 1H), 9.26 (s, 1H) ppm; **^{13}C NMR** ($CDCl_3$, 151 MHz) δ 13.24, 13.32, 20.22, 20.23, 24.62, 24.71, 24.77, 24.85, 26.11, 26.14, 28.61, 30.25, 30.92, 34.43, 34.46, 36.53, 37.38, 37.41, 38.10, 38.52, 38.62, 44.49, 44.54, 45.45, 52.48, 76.24, 76.52, 81.23, 88.69, 88.75, 97.58, 103.47, 103.55, 115.44, 119.88, 124.94, 127.80, 139.74, 142.20, 155.78, 171.52, 173.14, 173.30 ppm; **ESI-MS** (30eV) *m/z***:** 1107.45 $[M+H]^+$, 1129.41 $[M+Na]^+$; **HRMS** (ESI/Q-TOF) *m/z*: $[M+H]^+$ Calcd for $C_{59}H_{88}ClN_6O_{12}^+$ 1107.6149; Found 1107.6138.



13: pale yellow oil (40%); R_f (C): 0.3; **^{1}H NMR** ($CDCl_3$, 600 MHz) δ 1.24 (s, 23H), 1.47 (d, J = 6 Hz, 3H), 1.55 (d, J = 6 Hz, 3H), 2.09 (t, J = 6 Hz, 4H), 2.24 - 2.43 (m, 17H), 2.55 (t, J = 6 Hz, 4H), 2.91 (s, 6H), 4.27 - 4.34 (m, 3H), 4.42 - 4.53 (m, 4H), 4.51 (br s, 2H), 5.31 (s, 1H), 7.53 - 7.61 (m, 5H), 7.67 (t, J = 6 Hz, 4H), 7.88 (t, J = 6 Hz, 3H), 8.06 (d, J = 12 Hz, 3H), 8.29 (d, J = 12 Hz, 2H) ppm; **^{13}C NMR** ($CDCl_3$, 151 MHz) δ 17.39, 20.82, 25.65, 27.72, 31.15, 34.15, 38.41, 39.06, 39.33, 40.36, 42.23, 44.10, 44.67, 45.42, 48.04, 51.82, 59.49, 72.61, 99.02, 101.58, 114.21, 121.01, 124.82, 126.02, 128.43, 134.32, 146.68, 148.71, 149.07, 151.76, 169.46, 171.20, 174.59, 194.27, 209.74 ppm ; **ESI-MS** (30eV) *m/z***:** 1261.91 $[M+H]^+$, 1281.95 $[M+Na]^+$; **HRMS** (ESI/Q-TOF) *m/z*: $[M+H]^+$ Calcd for $C_{64}H_{88}ClN_8O_{16}^+$ 1259.6007; Found 1259.6040.



14: pale yellow oil (34%); R_f (C): 0.16; **^{1}H NMR** ($CDCl_3$, 600 MHz) δ 1.16 (s, 12H), 1.41 (s, 12H), 1.64 - 1.61 (m, 8H), 1.85 (dt, J = 6, 1.2 Hz, 4H), 2.17 (dt, J = 6, 1.2 Hz, 4H), 2.67 - 2.65 (m, 8H), 2.72 - 2.70 (m, 4H), 3.22 - 3.20 (m, 2H), 3.25 (s, 6H), 3.34 - 3.32 (m, 2H), 3.42 - 3.38 (m, 6H), 3.47 - 3.45 (m, 2H), 3.61 - 3.59 (m, 2H), 3.71 - 3.69 (m, 2H), 3.79 - 3.74 (m, 4H), 6.43 (d, J = 12 Hz, 1H), 6.86 (d, J = 12 Hz, 3H), 7.29 (d, J = 12 Hz, 1H), 7.89 - 7.84 (m, 2H), 8.20 (s, 2H), 8.30 (d, J = 6 Hz, 1H), 8.51 (s, 1H) ppm; **^{13}C NMR** ($CDCl_3$, 151 MHz) δ 17.39, 20.82, 25.65, 27.72, 31.13, 34.15, 39.06, 39.33, 40.56, 42.23, 44.67, 45.2, 48.04, 51.82, 59.49, 72.61, 99.02, 101.58, 114.21, 121.01, 124.82, 124.62, 126.62, 128.43, 134.32, 146.68, 148.71, 149.07, 151.76, 169.46, 172.20, 174.59, 194.27, 209.74 ppm; **ESI-MS** (30eV) ***m/z*:** 1275.95 $[M+H]^+$, 1297.13 $[M+Na]^+$; **HRMS** (ESI/Q-TOF) *m/z*: $[M+H]^+$ Calcd for $C_{65}H_{90}ClN_8O_{16}^+$ 1273.6163; Found 1273.6172.

4. Biology

4.1. Assay for *in vitro* inhibition of *P. falciparum* growth

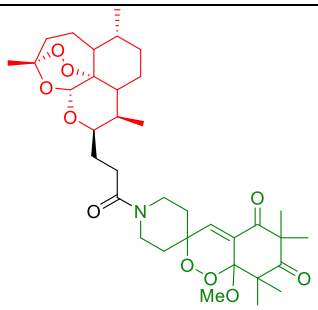
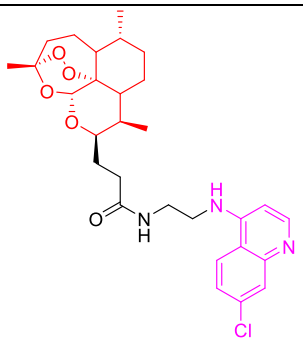
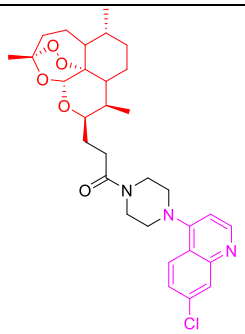
The chloroquine-resistant FcB1/Colombia strain of *Plasmodium falciparum*[1,7] was maintained in vitro on human erythrocytes in RPMI 1640 medium supplemented by 8% (v/v) heat- inactivated human serum, at 37 °C, under an atmosphere of 3% CO_2, 6% O_2, and 91% N_2. In vitro drug susceptibility assays were measured by [^{3}H]-hypoxanthine incorporation. Drug solutions were prepared in DMSO at a 10 mM concentration. Compounds were serially diluted two- fold with 100 µL culture medium in 96-well plates. Asynchronous parasite cultures (100 µL, 1% parasitaemia and 1% final hematocrite) were then added to each well and incubated for 24 h at 37 °C prior to the addition of 0.5 µCi of [^{3}H]-hypoxanthine (GE Healthcare, France, 1 to 5 Ci·mmol/mL) per well. After a further incubation of 24 h, plates were frozen and thawed. Cell lysates were then collected onto fiberglass filters and counted in a liquid scintillation spectrometer. The growth inhibition for each drug concentration was determined by comparison of the radioactivity incorporated in the treated culture with that in the control culture maintained on the same plate. The concentration causing 50% growth inhibition (IC_{50}) was obtained from the drug concentration- response curve and the results were expressed as the mean values ± standard deviations determined from several independent experiments. Chloroquine and artemisinin were used as antimalarial drug controls.

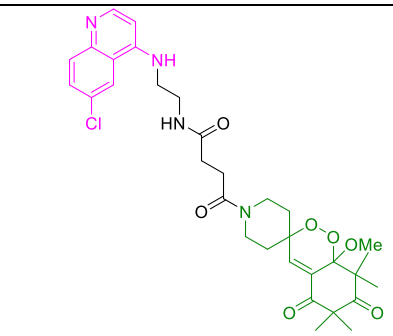
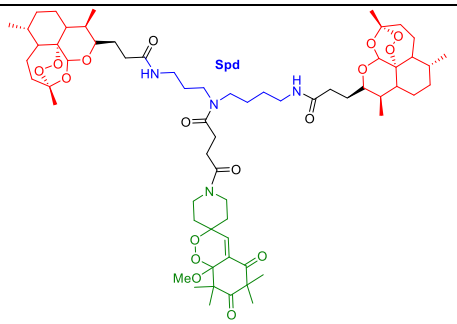
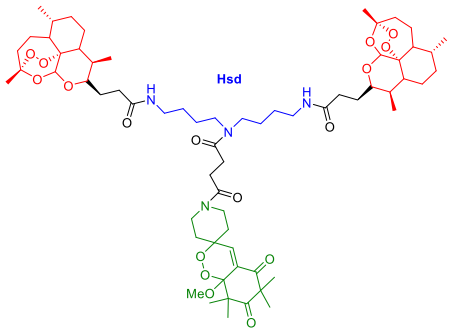
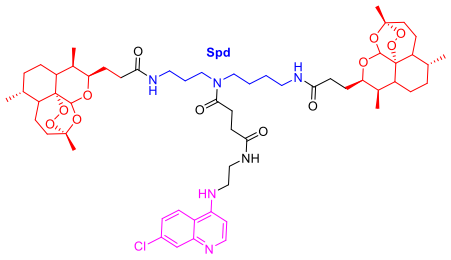
4.2. Assay for in vitro cytotoxicity assay on mammalian cell

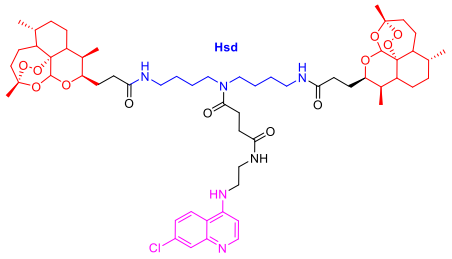
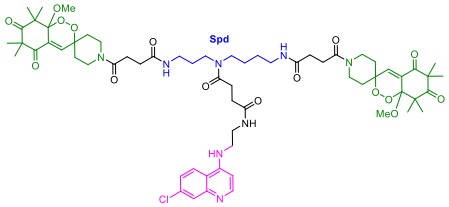
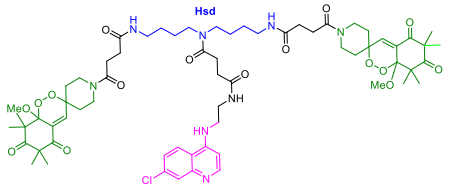
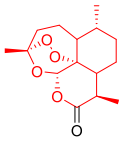
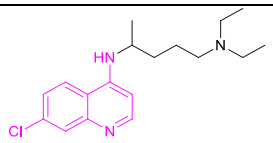
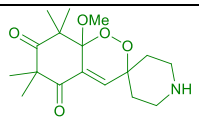
Cytotoxicity evaluation was performed upon human primary fibroblast (cell line AB943). Assays were realized in 96-well plates in RPMI 1640 medium containing 25mM HEPES, pH 7.3, 10% fetal calf serum under 5% CO2 atmosphere, at 37°C. After trypsin treatment, AB943 cells were seeded at 5000 cells per well in 100 µL. After 24 h of incubation, cells were washed and drugs diluted in culture medium were added (200 µL perwell). Drug stock solutions were prepared in pure DMSO. The final DMSO concentration in the cultures remained below 1%. Control cultures were constituted of cultures treated with pure DMSO instead of drug. The cytotoxicity assay was based on the conversion of a redox sensitive dye (resazurin) to a fluorescent product by viable cells. After 5 days of incubation,

resazurin solution was added in each well at a final concentration of 45 µM. Fluorescence was measured at 530 nm excitation and 590 nm emission wavelengths after 4 h of incubation. The percentage of inhibition of cell growth was calculated by comparing the fluorescence of cells maintained in the presence of drug to that of in the absence of drug.

Table S1. Evaluation of the antiparasitic activity of synthesized compounds on CQ-resistant *P. Falciparum* FcB1/Colombia strain and of cytotoxicity using primary human fibroblasts cell line AB943 and selectivity indexes (SI) thereof.

Compd	**STRUCTURE**	*P.falciparum* Mean[a] IC_{50} (nM) or % inhibition at 10 nM	Cytotoxocity Mean[b] IC_{50} (µM)	SI
5		2.6±1.0	42.6±2.2	16,372
6		11%	–	–
7		11%	–	–

8		124.3±40.4	17.8±0.1	144
9		8.4±3.7	17.6±0.1	2,108
10		10.6±2.3	10.4±1.9	983
11		33%	–	–

12		52.3±15.9	17.2±0.3	328
13		>167	–	–
14		>167	–	–
ART		55±13.6	>250	>4545
CQ		72±7.4	25±5	347
3		>250	–	–

[a]Number of tests: 4; [b]Number of tests: 3.

5. References

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6. Copies of ^{1}H and ^{13}C NMR spectra

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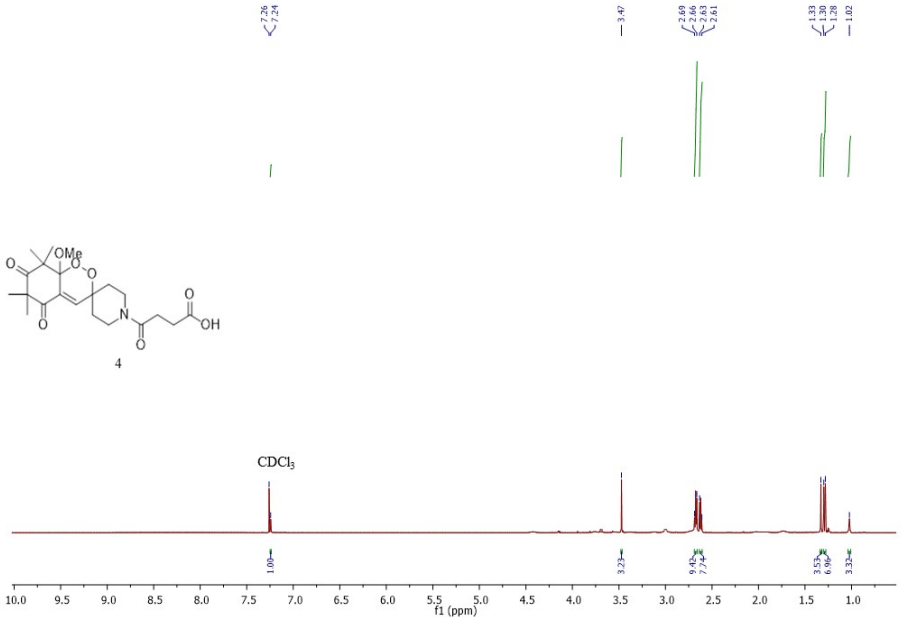

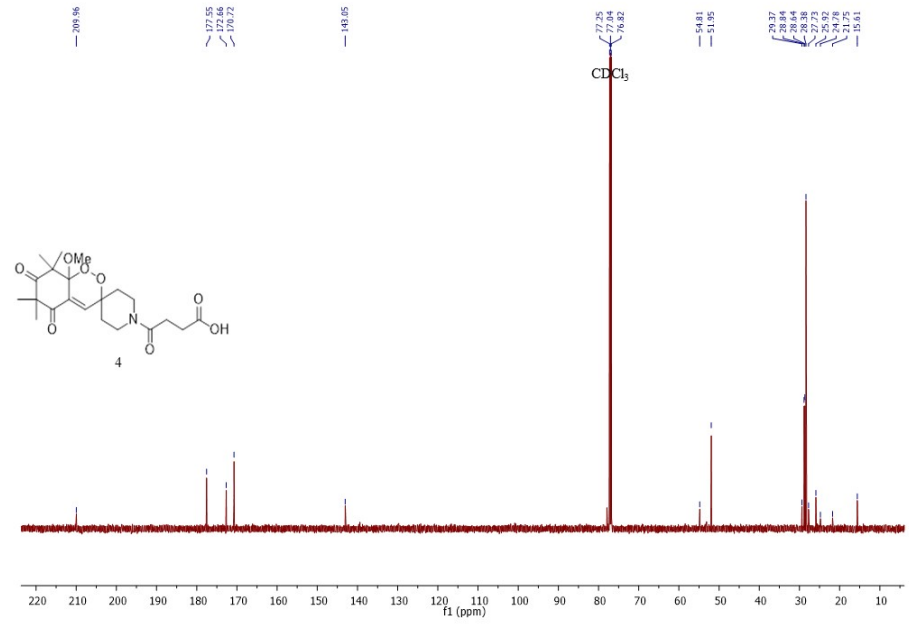

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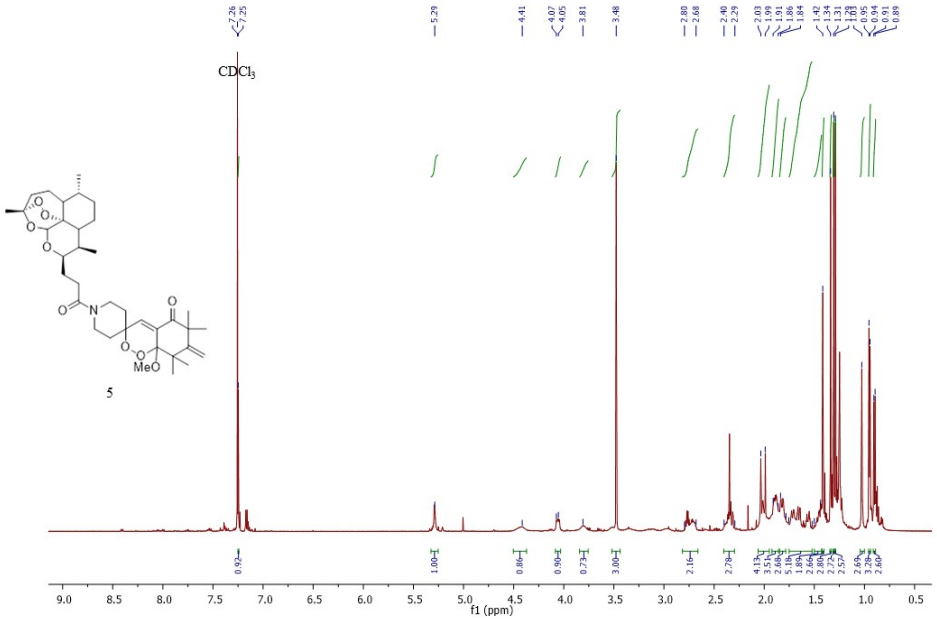

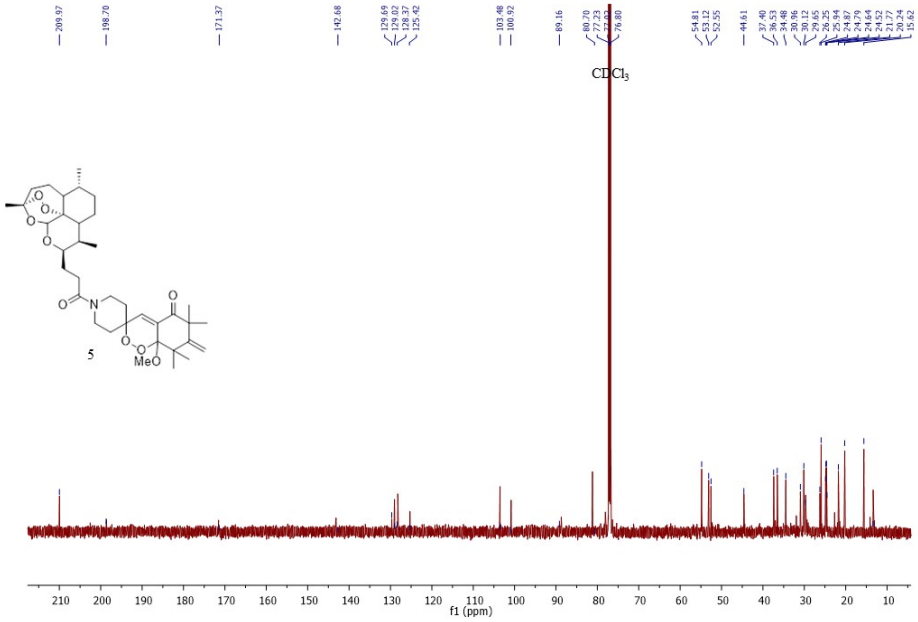

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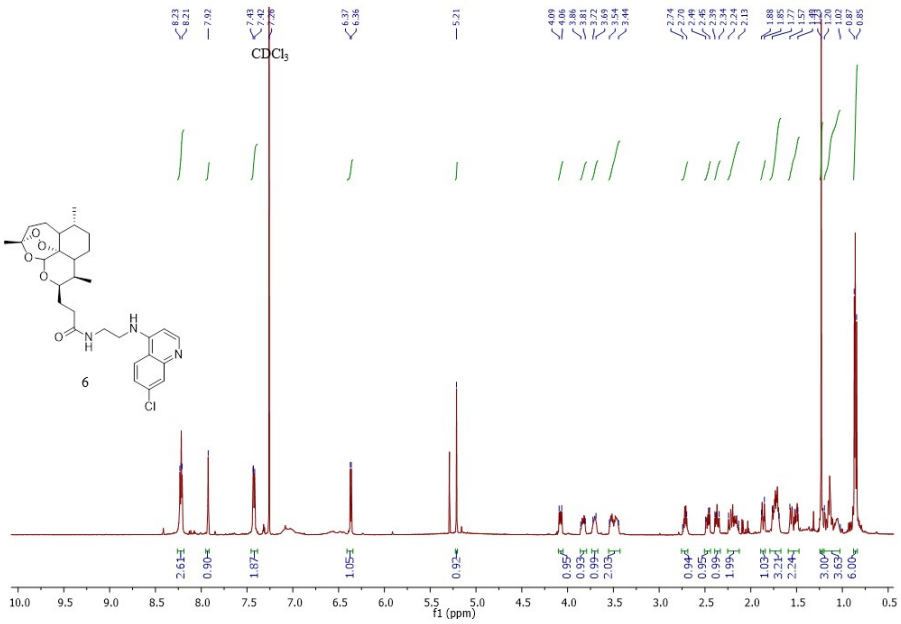

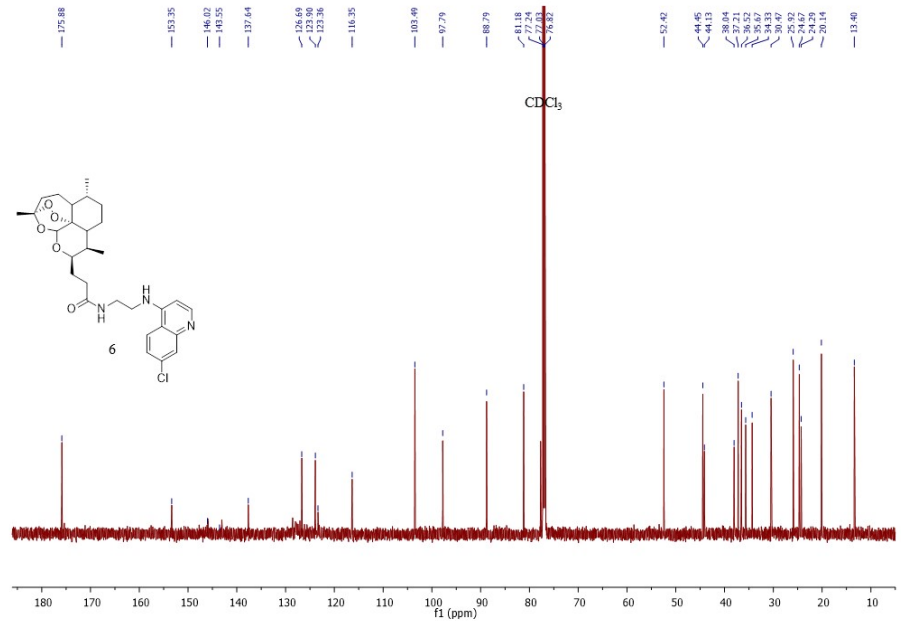

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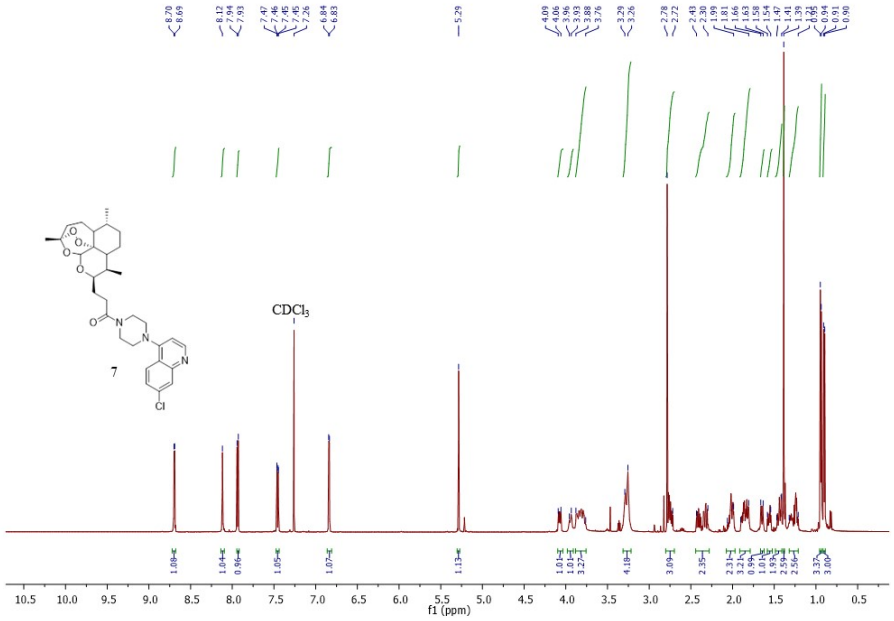

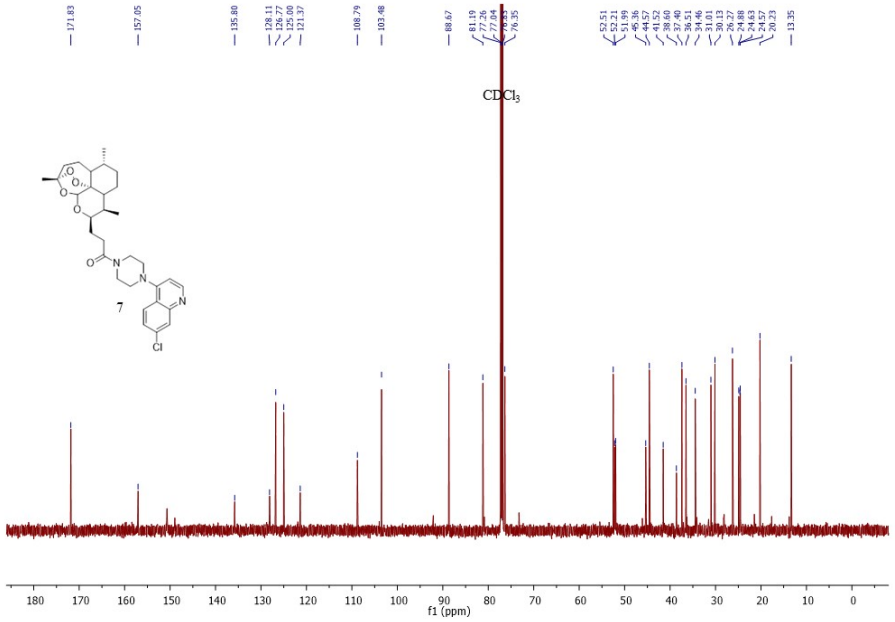

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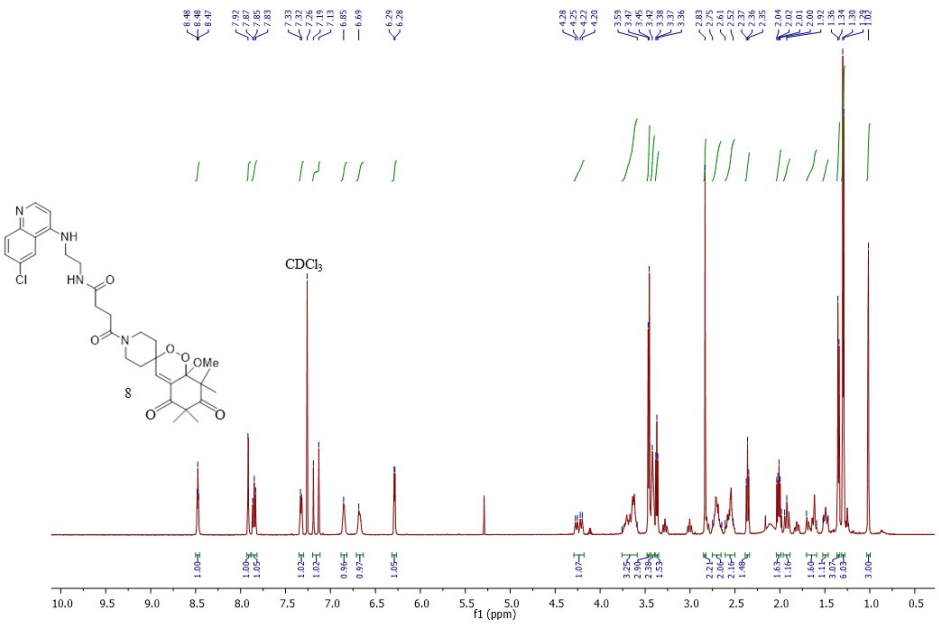

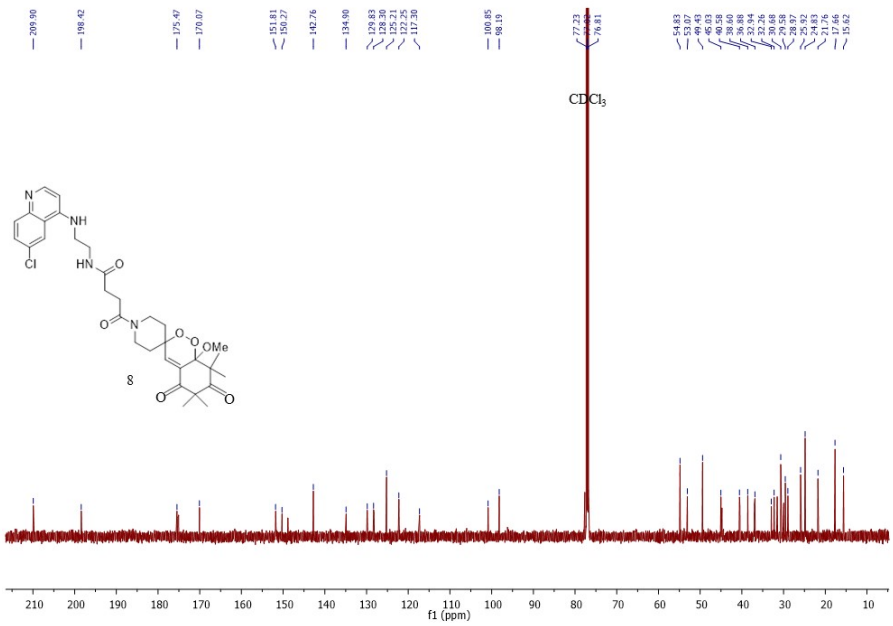

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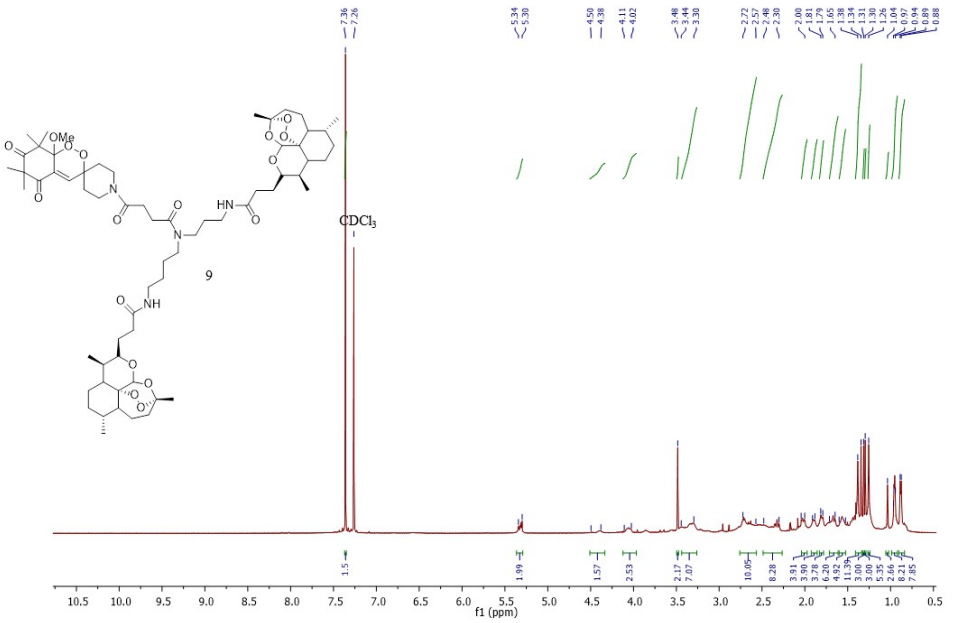

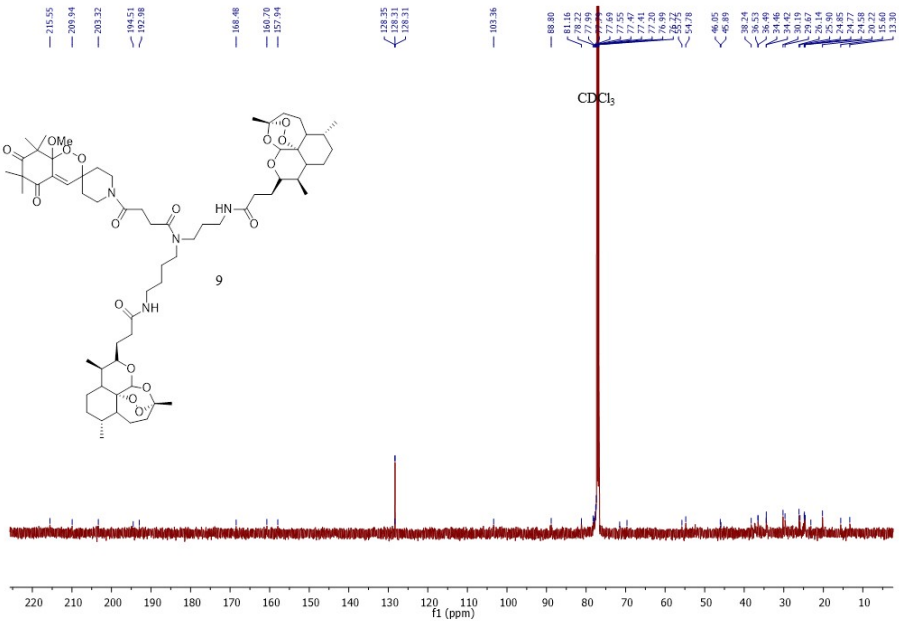

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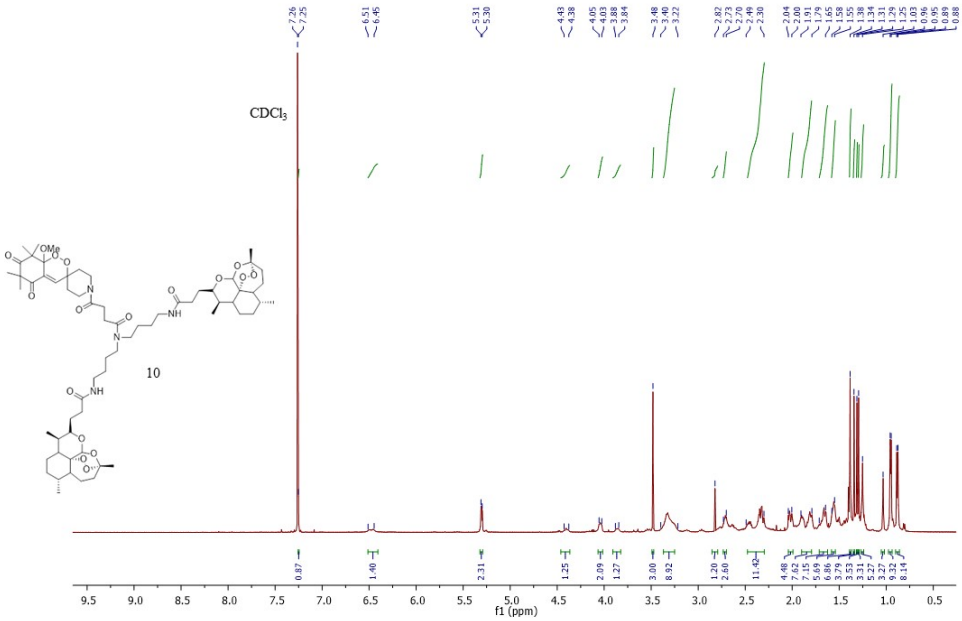

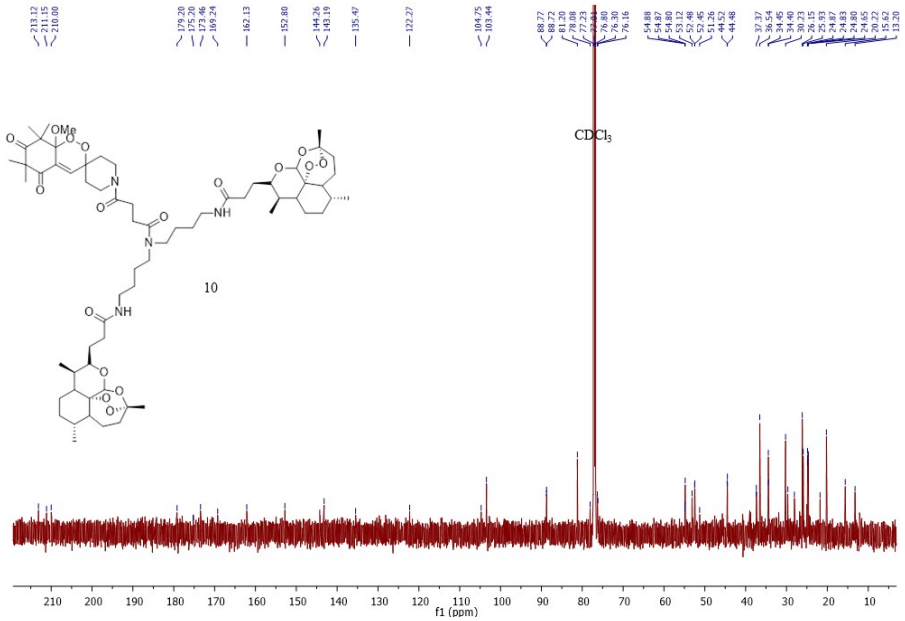

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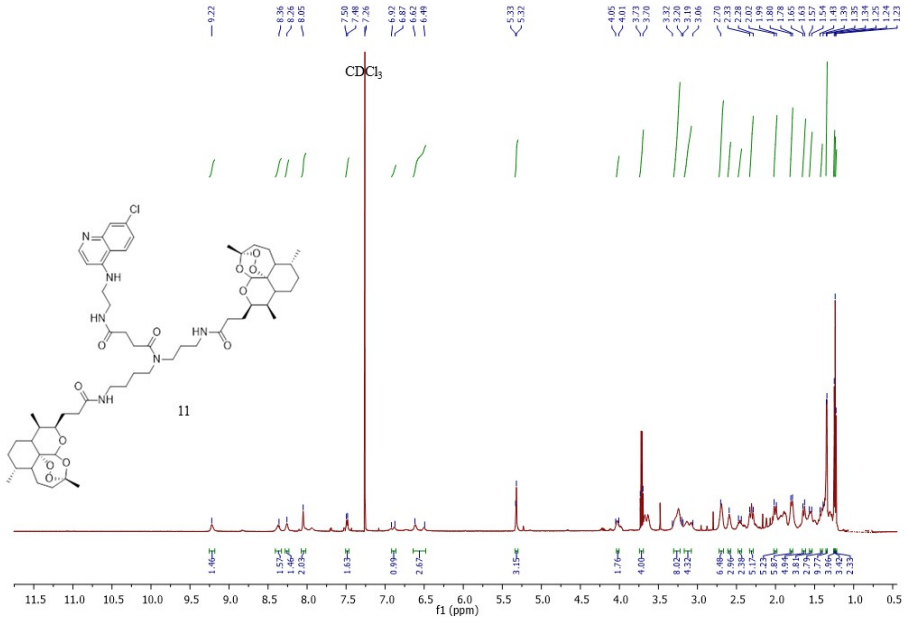

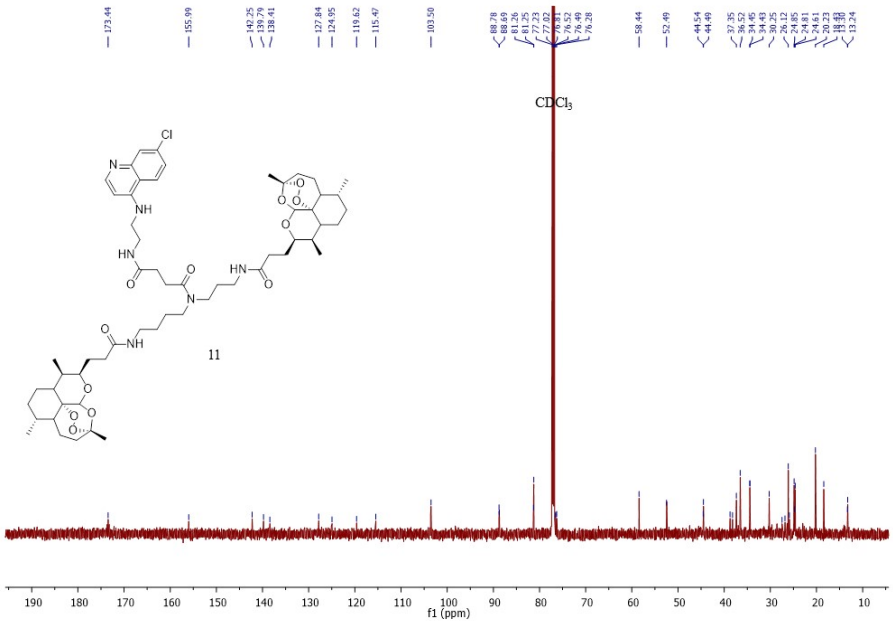

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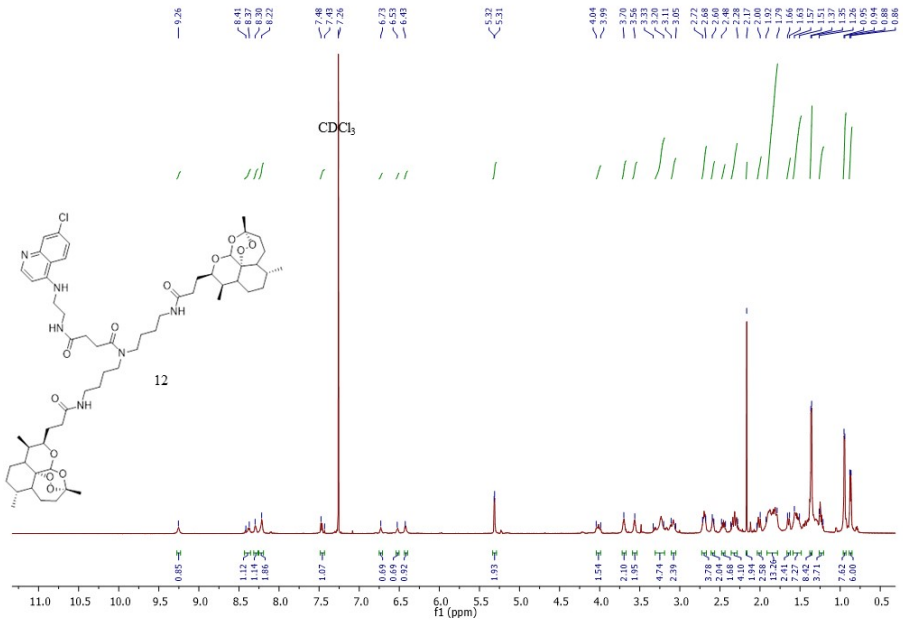

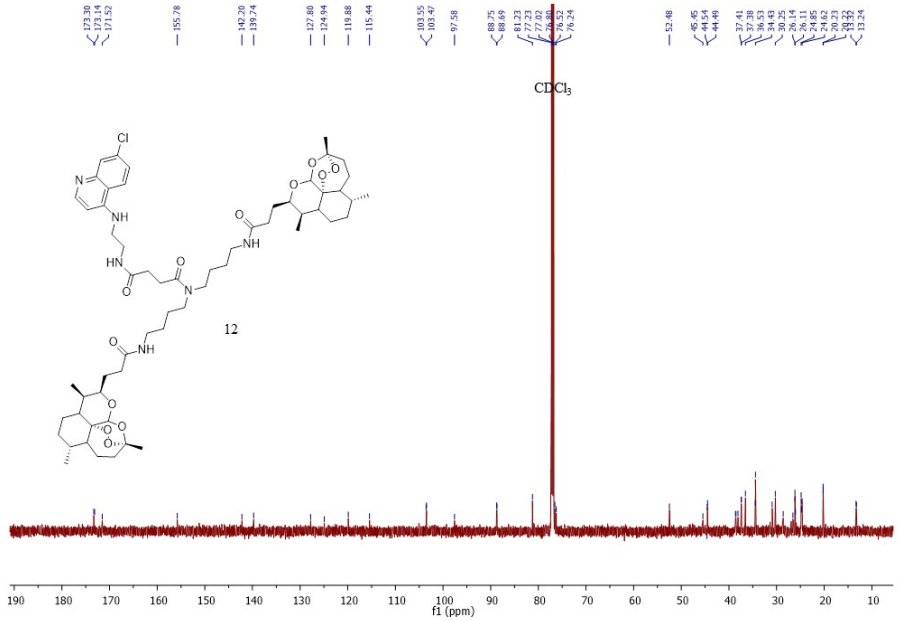

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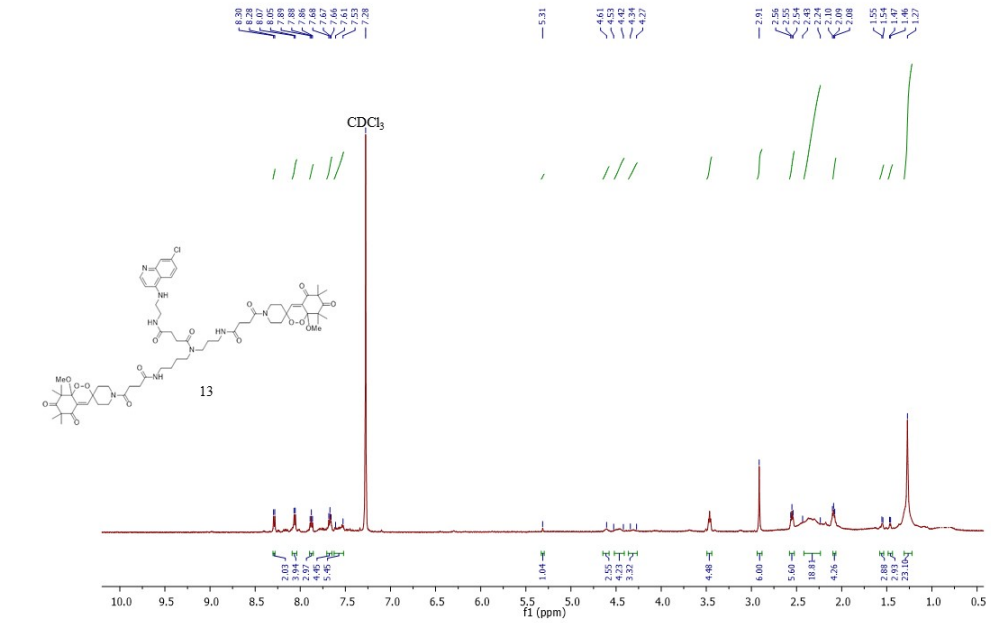

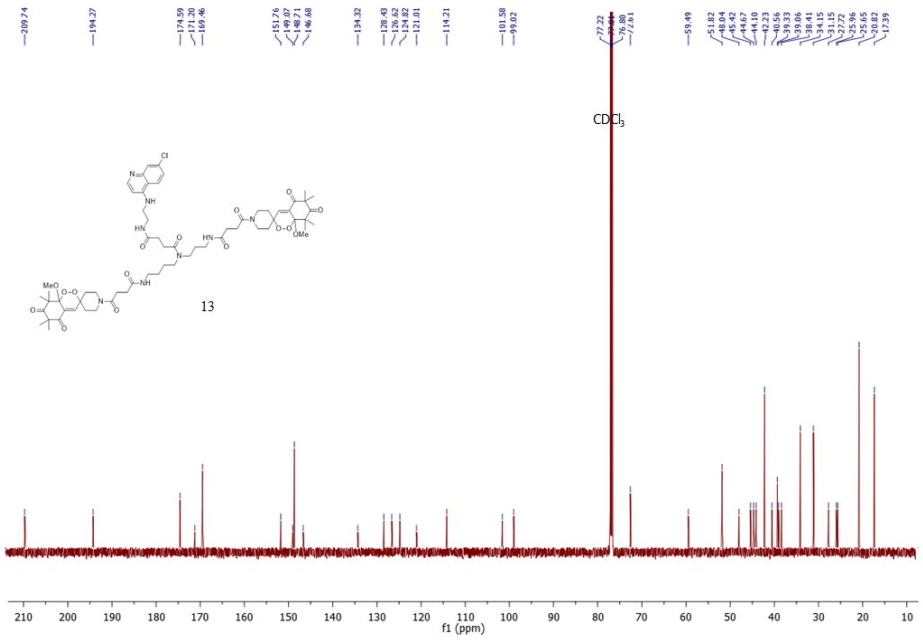

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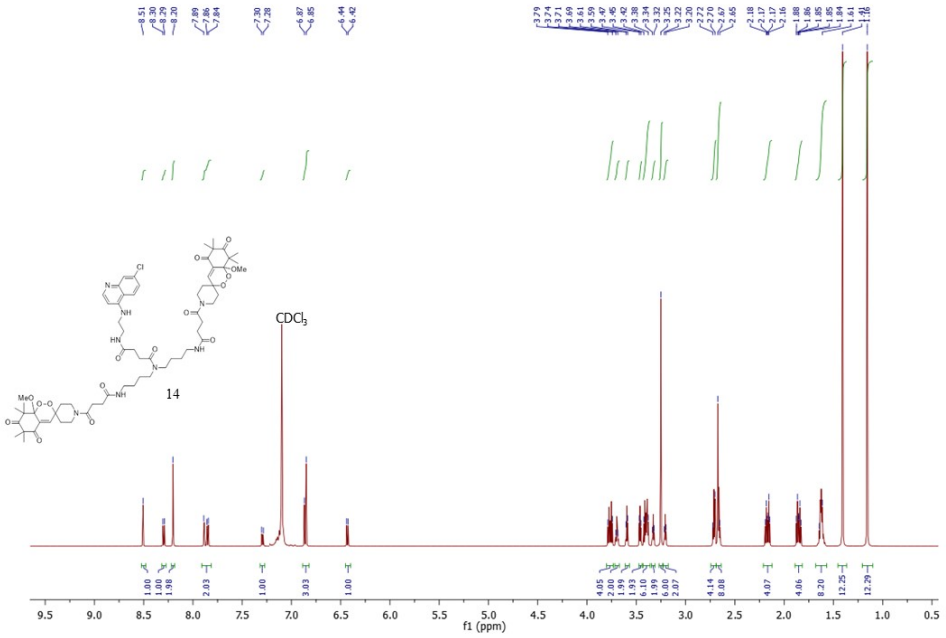

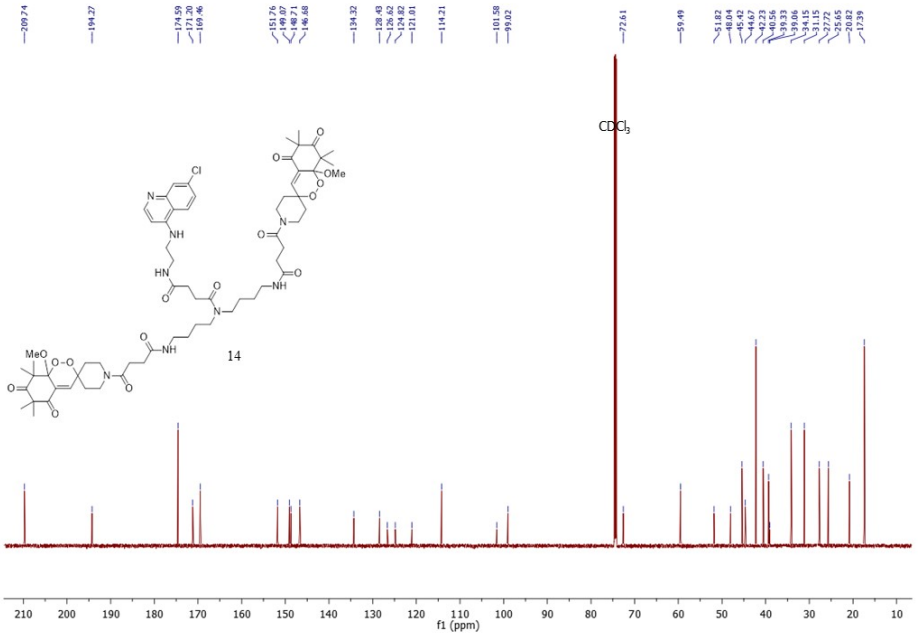

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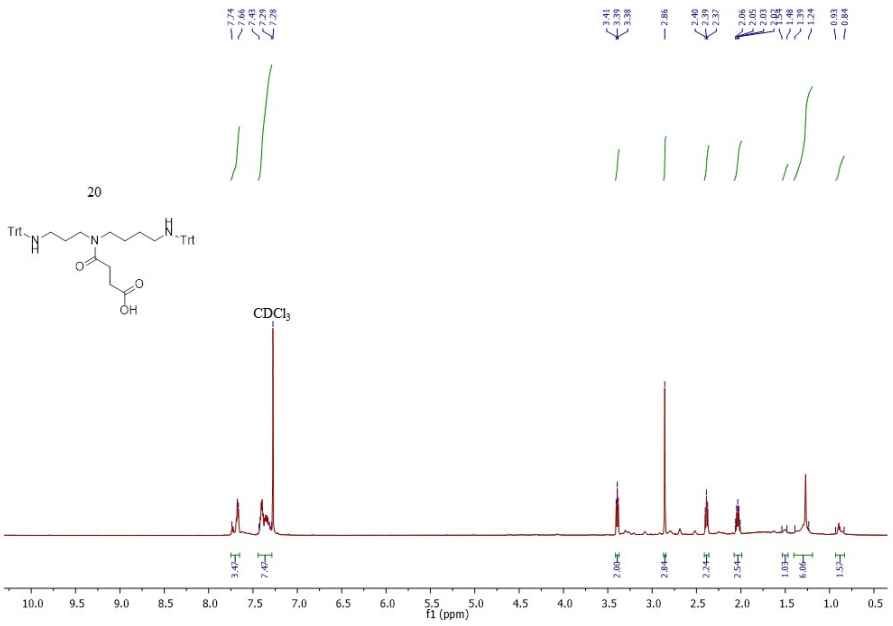

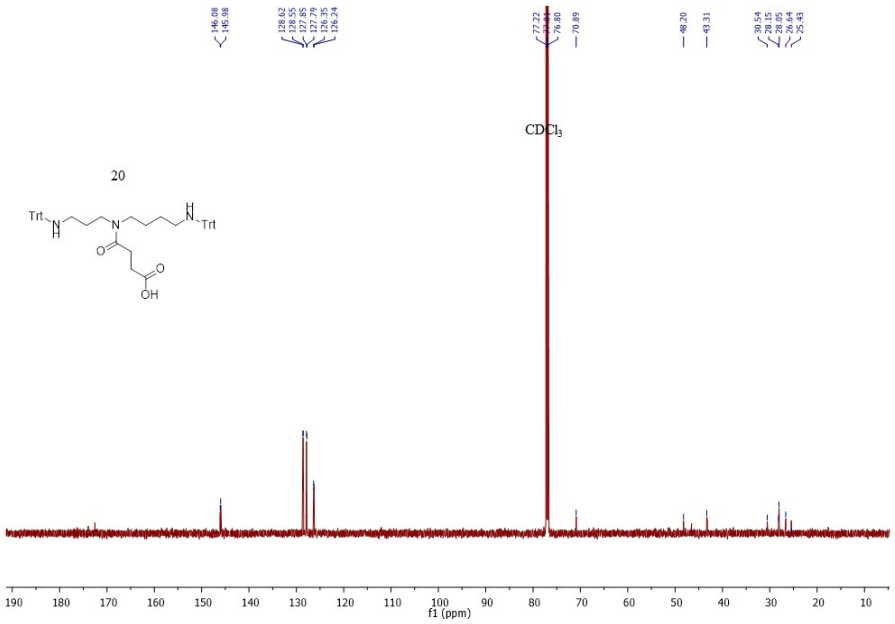

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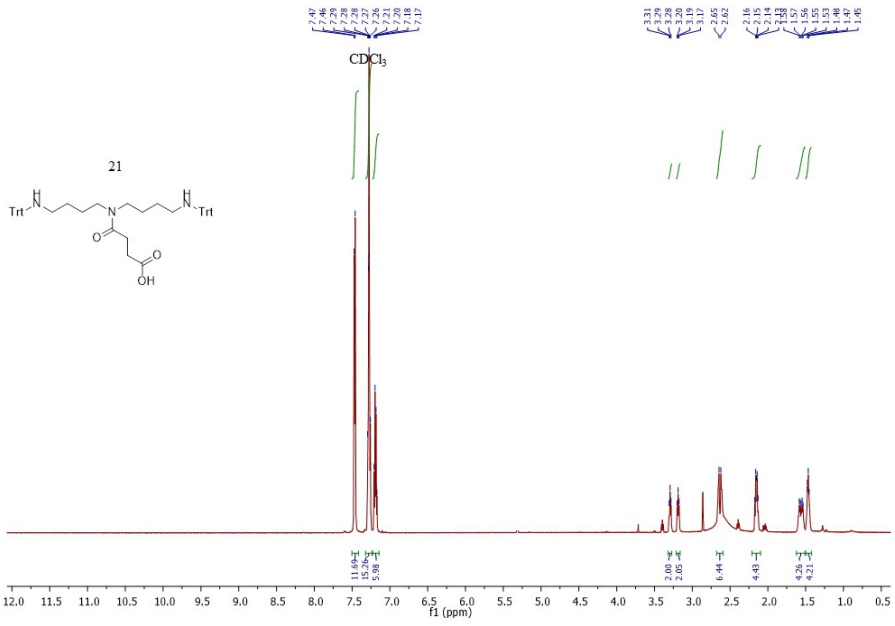

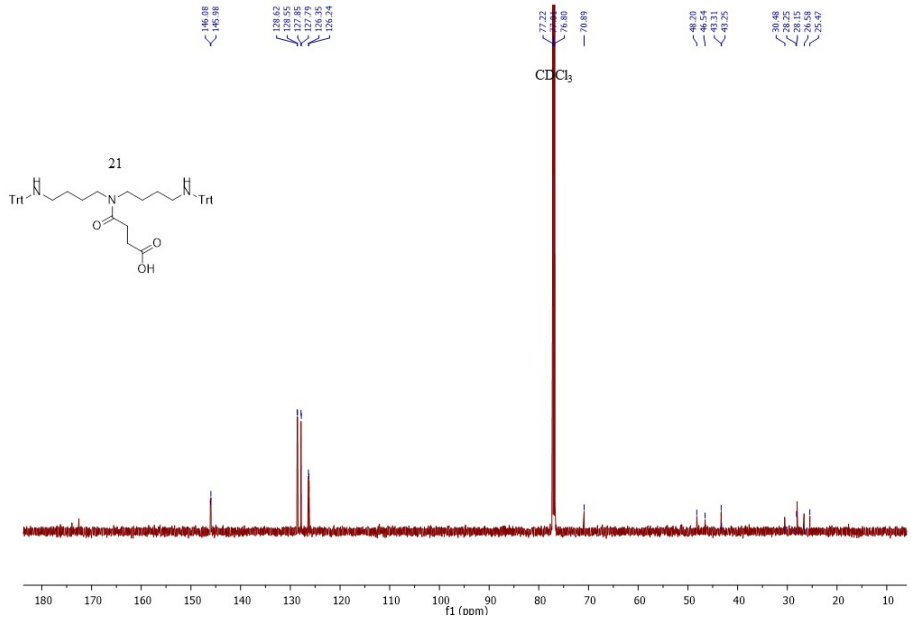

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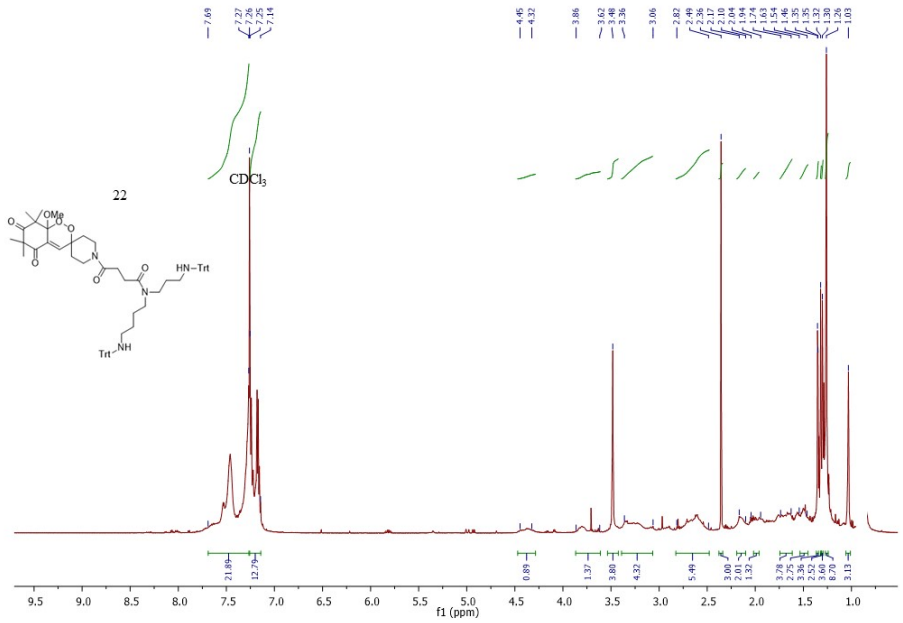
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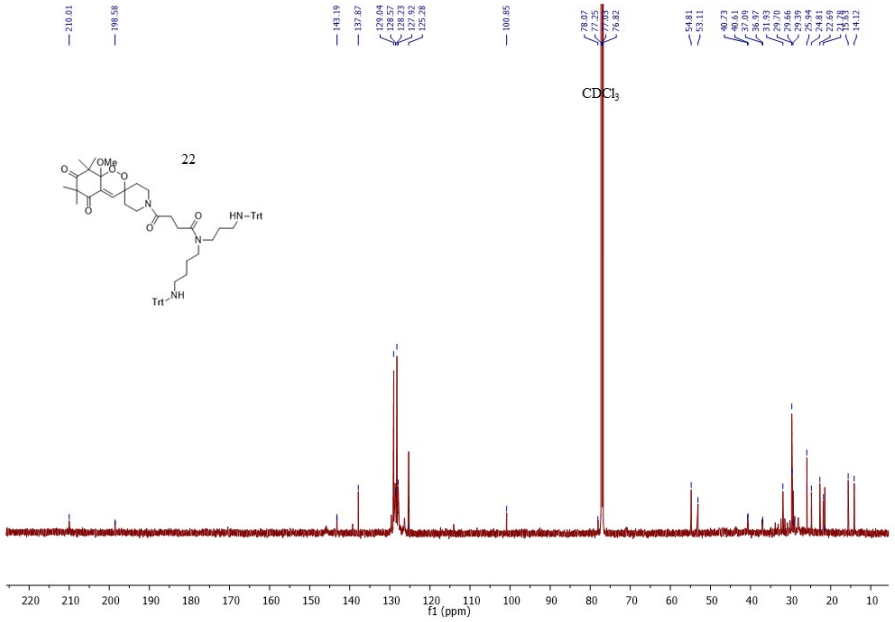
22
OMe
HN–Trt
Trt–NH
f1 (ppm)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HN–Trt
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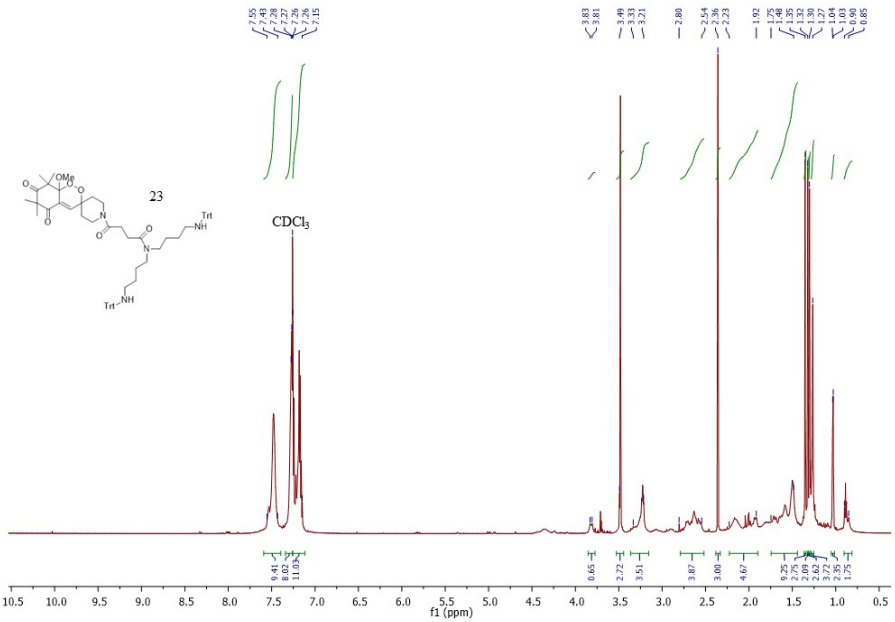

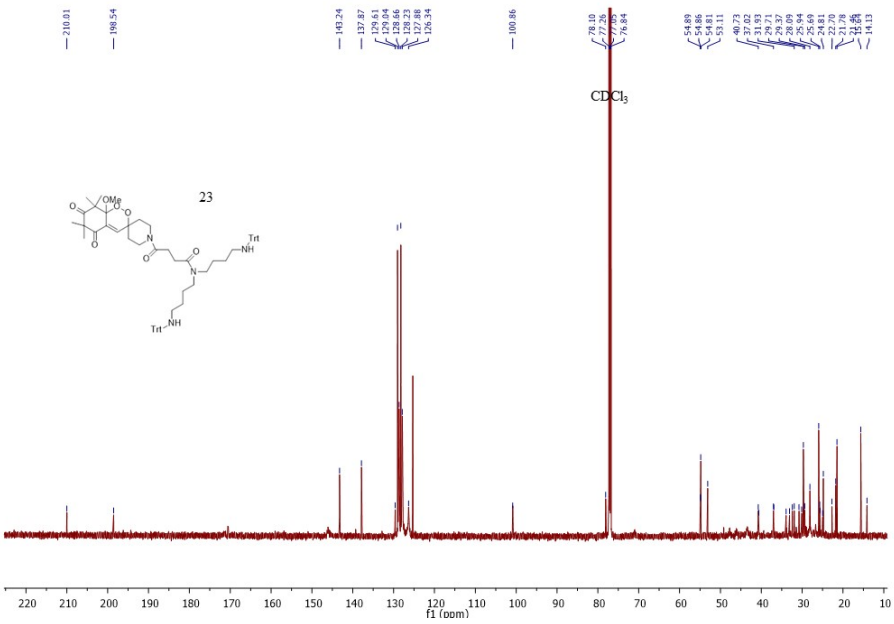

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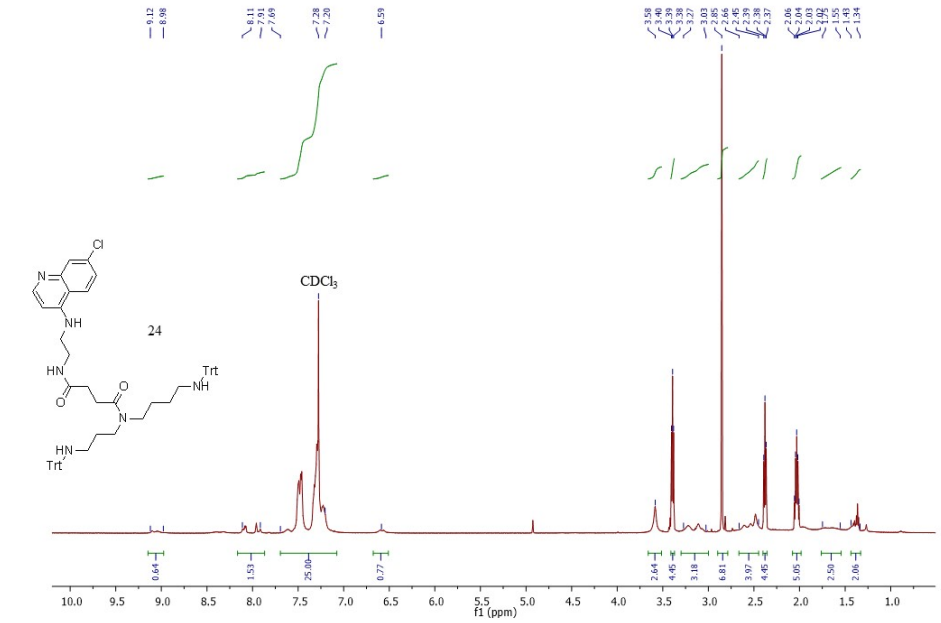
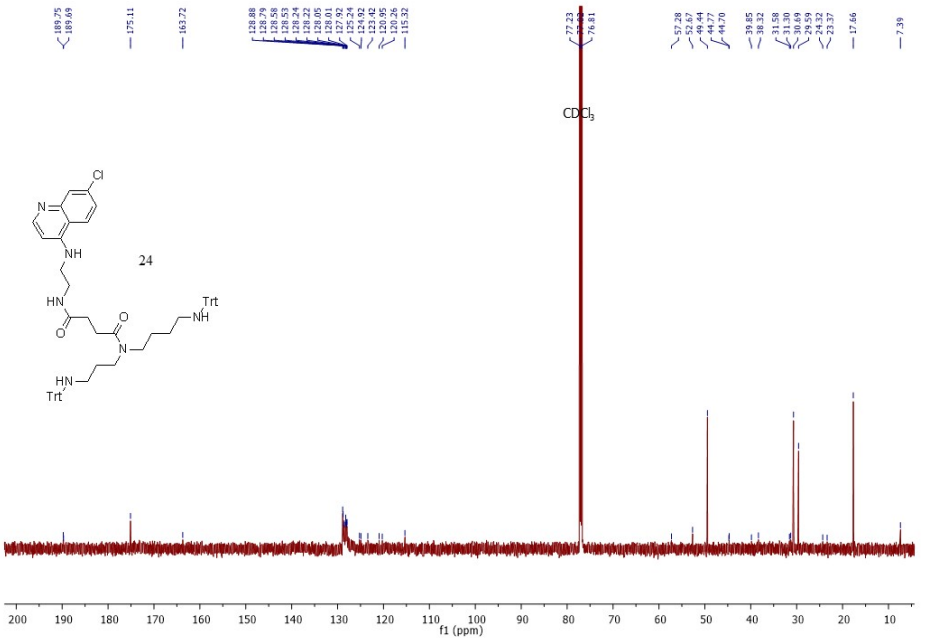

CDCl3
24
Cl
N
NH
HN
O
Trt
NH
HN
Trt
f1 (ppm)


CDCl3
24
Cl
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O
Trt
NH
HN
Trt
f1 (ppm)

25