CHAPTER III

EXPERIMENT

All reaction were carried out in oven-dried glassware under an inert atmosphere of nitrogen. Anhydrous solvents and reagents were either obtained from commercial source or dried and distilled over drying agent prior to use. All chemicals for organic synthesis were purchased from Sigma-Aldrich, Acros Organics, BDH Prolabo, Fluka, Merck, Carlo Erba, CXL, Lab Scan and ChemPep, Inc. Pyridine and 2,6-dimethylpyridine were dried over calcium hydride before distilled. Diisopropylethylamine were dried over potassium hydroxide before distilled. Dihydroartemisinin was obtained from borohydride reduction of artmisinin. Routine monitoring of reactions was performed using 0.25 mm Merck, silica gel 60 F_{254} aluminium TLC plates. TLC Plates were visualized using a combination of UV, *p*-anisaldehyde, ninhydrin and potassium permanganate staining. Purification of products was carried out by flash chromatography using silica gel (particle size 70-230 or 230-400 mesh ; Merck). All solvent for HPLC and doubly-distilled water were purchased from Lab-Scan. Melting points were determined on Mel-Temp apparatus in open capillary tubes and were uncorrected. Infrared absorption spectra were obtained by a Perkin-Elmer Spectrum GX. The spectra were recorded neat unless otherwise indicated and are reported in cm^{-1}. ^{1}H NMR and ^{13}C NMR spectra were obtained on a Bruker Avance (400 MHz for ^{1}H and 100 MHz for ^{13}C) spectrometer and are reported in ppm relative to tetramethylsilane and referenced internally to the residually protonated solvent. Spectra were recorded in deuterated solvent. Abbreviations for coupling patterns : s, singlet; d, doublet; t, triplet; td, triplet of doublets; tq, triplet of

quartets; b, broad resonance; m, complex pattern. Chemical shift are reported in ppm and coupling constants (*J*) are given in Hertz. High resolution mass spectrometry (HRMS) and low resolution mass spectrometry (LRMS) were obtained on a Bruker Daltonics in electrospray ionization (ESI) mode.

3.1 Chemicals

3.1.1 Chemicals for organic synthesis

Pyridine, C_5H_5N, BDH Prolabo, UK

Benzoyl chloride, C_7H_5ClO, Fluka, Germany

Citric acid, $C_6H_8O_7$, Merck, Germany

Sodium hydrogen carbonate, $NaHCO_3$, Carlo Erba, Italy

Magnesium sulphate anhydrous, $MgSO_4$, assay 97%, Sigma-Aldrich, Germany

Allyltrimethylsilane, $C_6H_{14}Si$, Sigma-Aldrich, Germany

Zinc chloride, $ZnCl_2$, CXL, Thailand

Molecular sieve 4^{o}A, Sigma-Aldrich, Germany

Sodium periodate, $NaIO_4$, Merck, Germany

Potassium permanganate, $KMnO_4$, Merck, Germany

Potassium hydroxide, KOH, BDH Prolab, UK

Sodium hydroxide, NaOH, Merck, Germany

37% Hydrochloric acid, HCl, Lab-Scan, Ireland

Acetone, C_3H_6O, BDH Prolab, UK

Dichloromethane, CH_2Cl_2, Fisher Scientific, UK

Ethyl acetate, $C_4H_8O_2$, assay 99.9%, Fisher Scientific, UK

Diethyl ether, $C_4H_{10}O$, Lab-Scan, Ireland

Hexane, C_6H_{14}, assay 95%, Carlo Erba, Italy

2,6-Dimethylpyridine, C_7H_9N, Acros Organics, USA

N,*N*-Dimethylformamide, C_3H_7NO, Lab-Scan, Ireland

N,*N*- Diisopropylethylamine, $C_8H_{19}N$, Acros Organics, USA

Piperidine, $C_5H_{11}N$, Sigma-Aldrich, Germany

Trifluoroacetic acid, $C_2HF_3O_2$, Acros Organics, USA

Fmoc-Lys(Boc)-OH, Sigma-Aldrich, Germany

Methanol, CH_3OH, HPLC grade, Lab-Scan, Ireland

Water, H_2O, HPLC grade, Lab-Scan, Ireland

Acetonitrile, C_2H_3N, HPLC grade, Lab-Scan, Ireland

Methanol, CH_3OH, assay 98%, Merck, Germany

Silica gel 60 (0.040-0.063 mm), Merck, Germany

Silica gel 60 (0.063-0.200 mm), Merck, Germany

Silica gel 60 PF_{254}, Merck, Germany

Sodium Chloride, NaCl, TRS, Thailand

1,4-Dioxane, $C_4H_8O_2$, BDH Prolabo, UK

2-(1H-7-Azabenzotriazol-1-yl)-1,1,3,3-tetramethyl uronium hexafluoro-phosphate methanaminium, $C_{10}H_{15}F_6N_6OP$, ChemPep, Inc, USA

1-Hydroxy-7-azabenzotriazole, $C_5H_4N_4O$

3.1.2 Chemicals for biological testing

RPMI-1640 culture media, Gibco, USA

L-glutamine, Gibco, USA

Penicillin/streptomycin solution, Gibco, USA

Potassium chloride, KCl, Ajex, UK

Sodium dihydrogen phosphate, Na_2HPO_4, Fluka, Germany

Potassium dihygrogen phosphate, KH_2PO_4, Carlo Erba, Italy

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, Usb, USA

Dimethyl sulfoxide, C_2H_6OS, Riedel-de Haen, Germany

N,*N*-Dimethylformamide, C_3H_7NO, Lab-Scan, Ireland

Trypan blue, Sigma-Aldrich, Germany

Foetal bovine serum, Gibco, USA

Trypsin-EDTA, Gibco, USA

3.1.3 Cell lines

Human colon adenocarcinoma cell line (HT-29)

Human colon carcinoma cell line (Caco-2)

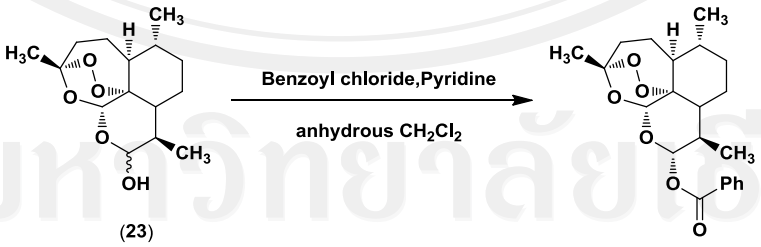
Human lung adenocarcinoma epithelial cell line (A549)

Mouse melanoma cell line (B16)

Mouse normal cell line (L929)

3.2 Modification of artemisinin derivatives at C-10

3.2.1 Preparation of Dihydroartemisinin 10*α*-Benzoate (129)

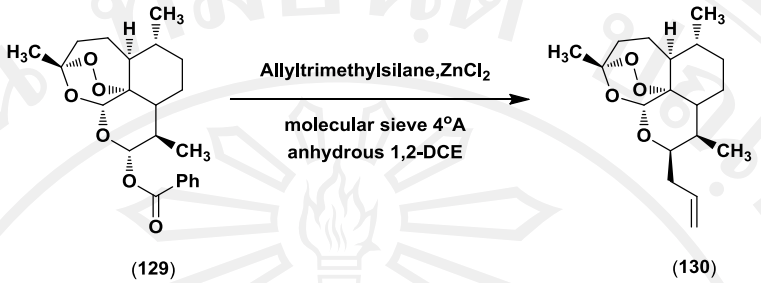


(**23**) (**129**)

To a stirred solution of dihydroartemisinin (**23**) (4.23 g, 0.015 mol) in anhydrous dichloromethane (60 ml) at 0 ^oC was sequentially added benzoyl chloride (3.45 ml, 0.029 mol) and anhydrous pyridine (7.62 ml, 0.094 mol). After 4 h, the

reaction was warmed to room temperature and stirred for overnight. The reaction was quenched by addition of 7% citric acid solution (20 ml) and extracted with ethyl acetate (3 x 20 ml). The combined organic layers were washed with saturated sodium hydrogen carbonate (3 x 20 ml), dried over anhydrous magnesium sulphate and concentrated *in vacuo*. The residue was purified by flash column chromatography over silica gel, eluting with 5-10% ethyl acetate/hexane, to give compound **129** as a white solid (5.47 g, 95%). Mp 116-118 ^{o}C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.13 (2H, m, Ar-H), 7.57-7.42 (3H, m, Ar-H), 6.02 (1H, d, *J* = 9.84 Hz, H-10), 5.53 (1H, s, H-12), 2.76 (1H, sextet, H-9), 2.40 (1H, td, *J* = 13.59, 3.98 Hz, H-4), 2.05 (m, H-4), 1.90 (1H, m, H-5), 1.85-1.76 (3H, m, H-8, H-7, H-8a), 1.47 (1H, m, H-5), 1.43 (3H, s, 3-Me), 1.40-1.30 (2H, m, H-5a, H-6), 1.10-1.08 (2H, m, H-8, H-7), 0.99 (3H, d, *J* = 6.06 Hz, 6-Me) and 0.93 (3H, d, *J* = 7.14 Hz, 9-Me); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 165.4, 133.2, 130.1, 129.6, 104.4, 92.6, 91.6, 80.2, 51.8, 45.5, 37.3, 36.3, 34.2, 32.0, 25.9, 24.6, 22.1, 20.6, 12.2; IR (KBr) 3037 ($\upsilon_{=C-H}$, aromatic), 2928 (υ_{C-H}, alkane), 1736 ($\upsilon_{C=O}$, ester), 1584 ($\upsilon_{C=C}$, aromatic), 1452 ($\upsilon_{C=C}$, aromatic), 1377 (δ_{C-H}, alkane), 1273 ($\upsilon_{C-C(O)-C}$, ester), 1037, 877 (O-O) and 831 (O-O), 714 (δ_{C-H}, aromatic) cm^{-1}; HRMS (ESI) $C_{22}H_{28}NaO_6$ $[M+Na]^+$ requires 411.1890, found 411.1784.

3.2.2 Preparation of 10β-Allyldeoxoartemisinin (130)

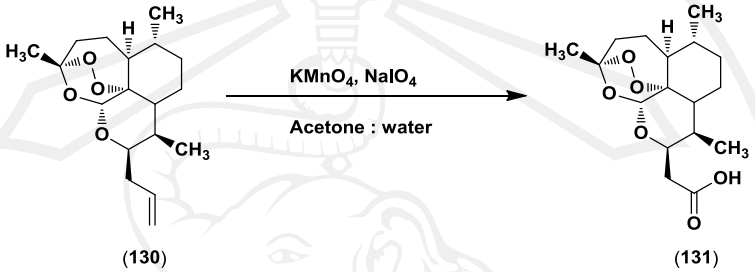


(**129**) (**130**)

To the mixture of allyltrimethylsilane (9.36 ml, 0.059 mol), anhydrous zinc chloride (2.09 g, 0.015 mol) and 4 ^{o}A molecular sieve in anhydrous 1,2-dichloroethane (12 ml) was added a solution of dihydroartemisinin 10α-benzoate (**129**) (4.58 g, 0.012 mol) in anhydrous 1,2-dichloroethane (12 ml) *via* cannular at 0 ^{o}C. After stirring for 4 h, the reaction was warmed to room temperature and stirred for 16 h. The reaction was quenched by 5% citric acid solution (20 ml) and extracted with ethyl acetate (3 x 20 ml). The combined organic layers were washed with saturated sodium hydrogen carbonate, dried over anhydrous magnesium sulphate, filtered and concentrated *in vacuo* to give the crude product as light-yellow viscous oil. The crude product was purified by flash column chromatography, eluting with 5-10% ethyl acetate/hexane, to give compound **130** as a white solid (2.50 g, 69%). Mp 70-72 ^{o}C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 5.91 (1H, m, H-14), 5.35 (1H, s, H-12), 5.12 (2H, m, H-15), 4.31 (1H, m, H-10), 2.68 (1H, sextet, *J* = 7.2 Hz, H-9), 2.35 (1H, td, *J* = 13.75, 4 Hz, H-4), 2.45-2.17 (3H, m, H-13, H-4), 1.90 (1H, m, H-5), 1.85-1.76 (3H, m, H-8, H-7, H-8a), 1.47 (1H, m, H-5), 1.43 (3H, s, 3-Me), 1.40-1.30 (2H, m, H-5a, H-6), 1.10-1.08 (2H, m, H-8, H-7), 0.94 (3H, d, *J* = 5.89 Hz, 6-Me) and 0.87 (3H, d, *J* = 7.57 Hz, 9-Me); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 136.5, 116.0, 103.1, 89.1, 81.0, 74.6, 52.3, 44.34, 37.4, 36.6, 34.5, 34.2, 30.2, 26.1, 24.9, 24.7, 20.1, 12.9; IR (KBr)

3074 ($\upsilon_{=C-H}$, alkene) 2929 (υ_{C-H}, alkane), 1642 ($\upsilon_{C=C}$, alkene), 1455 (δ_{CH2}, alkane), 1377 (δ_{CH3}, alkane), 1002 ($\delta_{oop-C=C-H}$, alkene), 924 ($\delta_{oop-C=C-H}$, alkene), 880 (O-O) and 839 (O-O) cm^{-1}.

3.2.3 Preparation of 10β-Carboxylalkyldeoxoartemisinin (131)

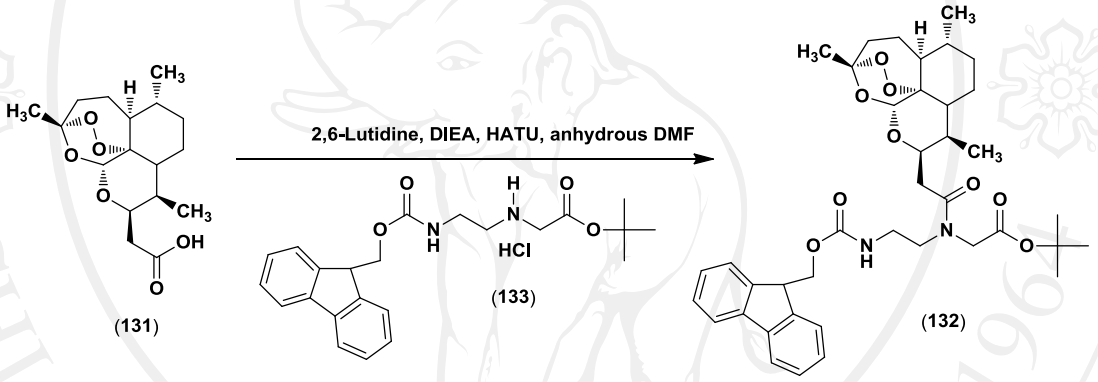


To the solution of 10β-allyldeoxoartemisinin (**130**) (0.42 g, 1 x 10^{-3} mol) in a 1 : 1 mixture of acetone/water (80 ml) was sequentially added sodium periodate (1.17 g, 5 x 10^{-3} mol) and potassium permanganate (0.14 g, 8.6 x 10^{-4} mol) at room temperature. The reaction was stirred for 12 h. The precipitate was filtered off and the solution was concentrated *in vacuo*. The concentrated was treated with 10% sodium hydroxide until basic and washed with ether (3 x 20 ml). The aqueous layer was acidified to pH 1 with concentrate hydrochloric acid. The aqueous phase was extracted with ether (3 x 20 ml). The combined organic layers were dried over anhydrous magnesium sulphate, filtered and concentrated *in vacuo* to give the crude product as light-yellow viscous oil. The crude product was purified by flash column chromatography, eluting with 25-50% ethyl acetate/hexane, to give compound (**131**) (0.35 g, 80%); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 5.40 (1H, s, H-12), 4.8 (1H, ddd, *J* = 10.48, 5.94, 3.05 Hz, H-10), 2.80-2.49 (3H, m, H-9, H-13), 2.34 (1H, td, *J* = 13.94, 3.91 Hz, H-4), 2.05 (m, H-4), 1.90 (1H, m, H-5), 1.85-1.76 (3H, m, H-8, H-7, H-8a), 1.47 (1H, m, H-5), 1.43 (3H, s, 3-Me), 1.40-1.30 (2H, m, H-5a, H-6), 1.10-1.08 (2H,

m, H-8, H-7), 0.98 (3H, d, *J* = 5.78 Hz, 6-Me) and 0.89 (3H, d, *J* = 7.20 Hz, 9-Me); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 176.3, 103.3, 89.3, 80.9, 71.0, 52.1, 43.9, 37.4, 37.3, 36.4, 35.8, 34.3, 29.7, 25.8, 24.7, 20.0 and 12.7; IR (neat) 2953 (υ_{C-H}, alkane), 2874 (υ_{C-H}, alkane), 1715 ($\upsilon_{C=O}$, carboxylic acid), 1455(δ_{CH2}, alkane), 1385 (δ_{CH3}, alkane), 1209 (υ_{C-O}, carboxylic acid), 1113, 1011, 948 (δ_{oop}, O-H) and 878 (O-O) cm^{-1}.

3.3 Synthesis of Fmoc-aeg-deoxoartemisinin-*t*Bu oligomers

3.3.1 Fmoc-aeg-deoxoartemisinin-*t*Bu monomer (132)



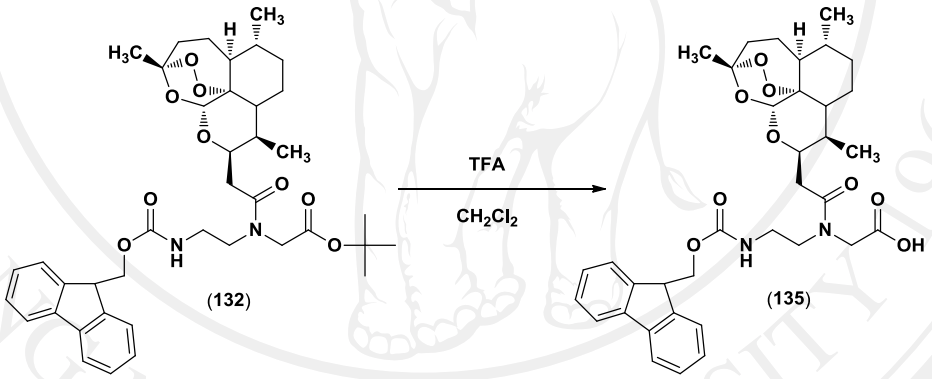
To the mixture of 2,6-lutidine (0.31 ml, 2.7 x 10^{-3} mol), 2-(1*H*-7-Azabenzotriazol-1-yl)-1,1,3,3-tetramethyl uronium hexafluorophosphate methan-aminium (0.55 g, 1.5 x 10^{-3} mol) and carboxylalkyldeoxoartemisinin (**131**) (0.35 g, 1 x 10^{-3} mol) in anhydrous *N*,*N*-dimethylformamide (5 ml) was added the mixture of *tert*-butyl *N*-[2-(*N*-9-fluorenylmethoxycarbonyl) aminoethyl] glycinate hydrochloride (**133**) (0.39 g, 9 x 10^{-4} mol) and *N*,*N*-diisopropylethylamine (0.31 ml, 1.8 x 10^{-3} mol) in anhydrous *N*,*N*-dimethylformamide (5 ml) *via* cannula at 0 ^{o}C. The mixture was stirred at 0 ^{o}C for 30 min and allowed to warm up to room temperature. After stirring at room temperature for 16 h, the reaction was concentrated *in vacuo*. The residue was dissolved in dichloromethane and washed with brine. The aqueous layer was back-extracted with dichloromethane (3 x 10 ml) and washed with brine and water. The

combined organic layers were dried over anhydrous magnesium sulphate, filtered and concentrated under reduced pressure to give the crude product as orange-yellow viscous oil. The crude product was purified by flash column chromatography (elution with ethyl acetate/hexane 3 : 2) to give the product **132** as a white solid (0.58 g, 91%). The purity of product was confirmed by reverse phase-high performance liquid chromatography (RP-HPLC) using a C18 semi-preparative column (5 µM, 19 x 150 mm) with a 30-min linear gradient of 70-80% acetonitrile in water as a mobile phase. Mp 78-80 ^{o}C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.78 (2H, d, *J* =7.5 Hz, H25, H-34), 7.63 (2H, q, *J* = 7.7, 6.6 Hz, H-28, H-31), 7.42 (2H, td, *J* = 7.32, 2.72 Hz, H-26, H-33), 7.34 (2H, td, *J* = 6.68, 2.65 Hz, H-27, H-32), 6.10 (1/2H, br, rotamer, NH), 5.90 (1/2H, br, rotamer, NH), 5.35 (1/2H, s, rotamer, H-12), 5.30 (1/2H, s, rotamer, H-12), 5.00-4.90 (1/2H, m, rotamer, H-10), 4.72-4.65 (1/2H, m, rotamer, H-10), 4.42 (1/2H, m, rotamer, H-22), 4.39 (1/2H, m, rotamer, H-22), 4.29 (1/2H, m, rotamer, H-22), 4.25 (1/2H, m, rotamer, H-22), 4.21 (1/2H, m, rotamer, H-23), 4.20 (1/2H, m, rotamer, H-23), 3.48-3.32 (2H, m, H-20), 4.25 (1/2H, m, rotamer, H-19), 4.22 (1/2H, m, rotamer, H-19), 3.70 (1/2H, m, rotamer, H-19), 3.66 (1/2H, m, rotamer, H-19), 4.37 (1/2H, m, rotamer, H-16), 4.31 (1/2H, m, rotamer, H-16), 3.79 (1/2H, m, rotamer, H-16), 3.75(1/2H, m, rotamer, H-16), 2.90-2.55 (2H, m, H-14, H-9), 2.40-2.25 (2H, m, H-14, H-4), 2.05 (1H, m, H-4), 1.90 (1H, m, H-5), 1.85-1.76 (3H, m, H-8, H-7, H-8a), 1.50 (9H, s, rotamer, H-13), 1.48 (9H, s, rotamer, H-13), 1.47 (1H, m, H-5), 1.43 (3H, s, 3-Me), 1.40-1.30 (2H, m, H-5a, H-6), 1.10-1.08 (2H, m, H-8, H-7), 0.97 (3H, d, *J* = 5.19 Hz, 6-Me) and 0.89-0.82 (3H, m, 9-Me); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 172.5, 169.5/160.0, 157.0, 144.5, 141.5, 128.0, 127.5, 125.0, 120.0, 103.5/102.9, 90.1/89.0, 83.1/82.9, 82.5/82.0, 73.0/71.0, 67.0/66.5, 53.0, 52.5,

49.9/49.5, 48.8, 47.4/47.2, 45.0/44.5, 37.5, 36.4, 34.5/34.2, 34.0, 29.9, 28.0, 26.5/25.5, 24.9, 22.5, 20.5, 13.1/12.5; IR (neat) 3412 (υ_{N-H}, amide), 3062 ($\upsilon_{=C-H}$, aromatic), 2938 (υ_{C-H}, alkane), 2874 (υ_{C-H}, alkane), 1720 ($\upsilon_{C=O}$, ester), 1644 ($\upsilon_{C=O}$, amide), 1513 ($\upsilon_{C=C}$, aromatic), 1450 ($\upsilon_{C=C}$, aromatic), 1370 (δ_{CH3}, alkane), 1231 ($\upsilon_{C-C(O)-C}$, ester), 1154, 877 (O-O) and 739 (δ_{C-H}, aromatic) cm^{-1}; HRMS (ESI) $C_{40}H_{52}N_2NaO_9$ $[M+Na]^+$ requires 727.3673, found 727.3567.

3.3.2 Fmoc-aeg-deoxoartemisinin-*t*Bu dimer (134)

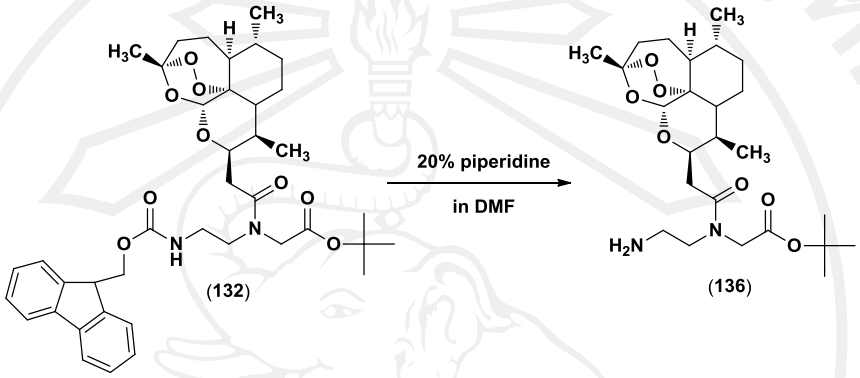
3.3.2.1 *t*-Boc deprotection of Fmoc-aeg-deoxoartemisinin-*t*Bu monomer (135)



To the solution of Fmoc-aeg-deoxoartemisinin-*t*Bu monomer (**132**) (0.50 g, 7 x 10^{-4} mol) in anhydrous dichloromethane (5 ml) was added trifluoroacetic acid (6 ml) at 0 ^oC. The reaction mixture was stirred at 0 ^oC for 15 min and warmed to room temperature. After 1 h, the reaction was neutralized by saturated sodium hydrogen carbonate and extracted with dichloromethane (3 x 20 ml). The organic layers were washed with brine and water. The combined organic layers were dried over anhydrous magnesium sulphate and concentrated *in vacuo* to give product **135** as a dark-yellow

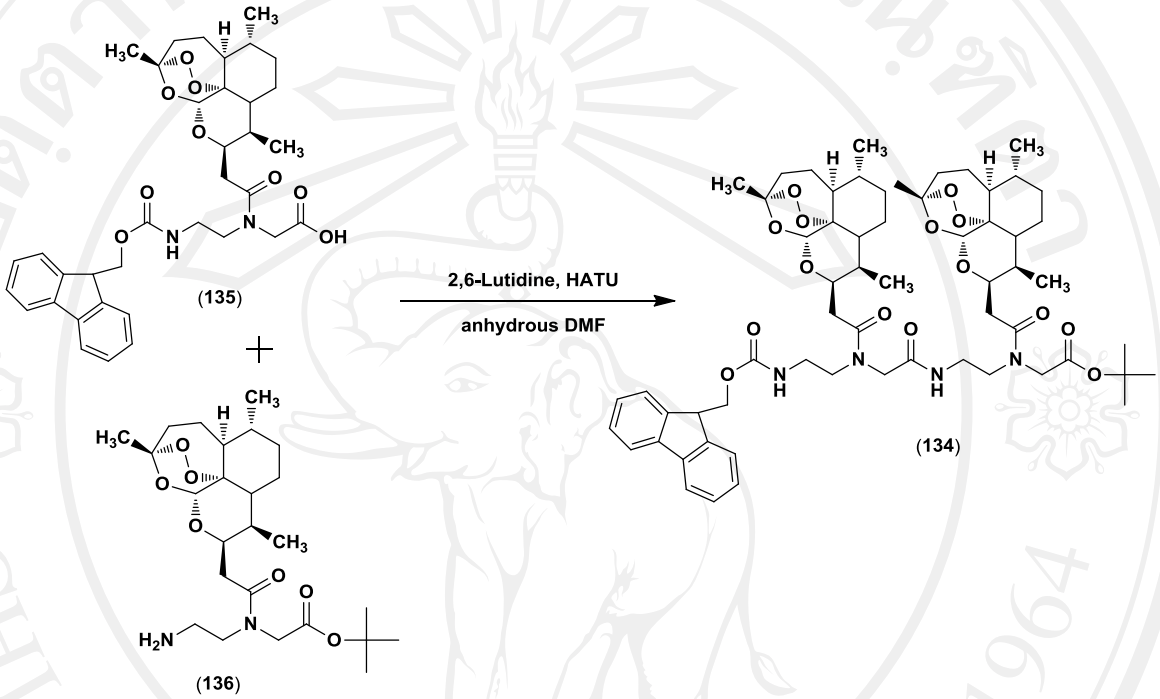
solid in quantitative yield. The crude product was used directly in the next step without further purification.

3.3.2.2 Fmoc deprotection of Fmoc-aeg-deoxoartemisinin-*t*Bu monomer (136)



To the solution of Fmoc-aeg-deoxoartemisinin-*t*Bu monomer (**132**) (0.10 g, 1 x 10^{-4} mol) in anhydrous *N*,*N*-dimethylformamide (1 ml) was added 20% piperidine in *N*,*N*-dimethylformamide (3 ml) at room temperature and stirred for 30 min at which time TLC indicated complete disappearance of the starting material. The reaction mixture was concentrated *in vacuo* to give the crude product as a light-yellow solid. The crude product was purified by flash column chromatography, eluting with 5% methanol/dichloromethane, to give NH_2-aeg-deoxoartemisinin-*t*Bu (**136**) as a white solid (0.07 g, 99%).

3.3.2.3 The coupling reaction of NH_2-aeg-deoxoartemisinin-*t*Bu monomer (136) and Fmoc-aeg-deoxoartemisinin-OH monomer (135)

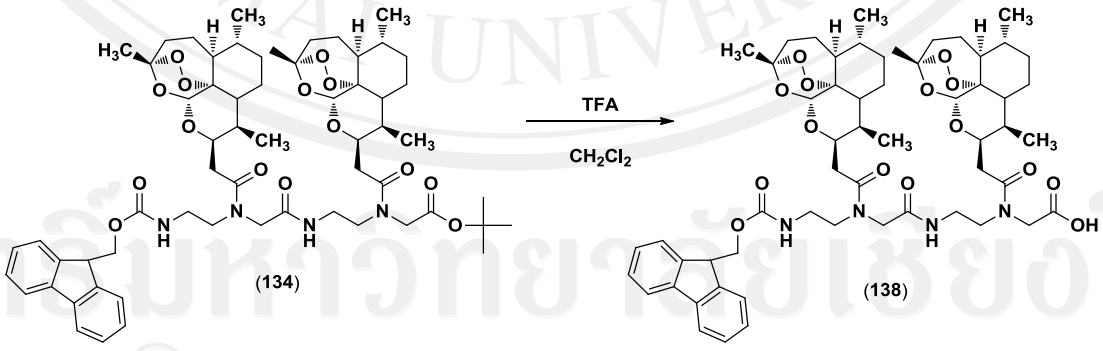


To the mixture of Fmoc-aeg-deoxoartemisinin-OH monomer (**135**) (0.34 g, 5 x 10^{-4} mol), 2,6-lutidine (0.38 ml, 3.3 x 10^{-3} mol) and 2-(1*H*-7-Azabenzotriazol-1-yl)-1,1,3,3-tetramethyl uronium hexafluorophosphate methanaminium (0.74 g, 1.9 x 10^{-3} mol) in anhydrous *N*,*N*-dimethylformamide (2 ml) was added the mixture of NH_2-aeg-deoxoartemisinin-*t*Bu monomer (**136**) (0.21 g, 4 x 10^{-4} mol) in anhydrous *N*,*N*-dimethylformamide (2 ml) *via* cannula at 0 ^{o}C. The mixture was stirred at 0 ^{o}C for 30 min and allowed to warm up to room temperature. After stirring at room temperature for 16 h, the reaction was concentrated *in vacuo*. The residue was dissolved in dichloromethane and washed with brine. The two-phase mixture was separated and the aqueous phase was back-extracted with dichloromethane (3 x 10

ml) and washed with brine and water. The combined organic layers were dried over anhydrous magnesium sulphate and followed by removal of the solvent *in vacuo* to give the crude product as orange-yellow viscous oil. The crude product was purified by flash column chromatography, eluting with 100% ethyl acetate, to give **134** as a light-yellow solid (0.43 g, 88%). The purity of product was confirmed by reverse phase-high performance liquid chromatography (RP-HPLC) technique using a C18 semi-preparative column (5 µM, 19 x 150 mm) with a 30-min linear gradient of 75-95% acetonitrile in water as a mobile phase. Mp 112-114 ^{o}C; IR (neat) 3369 (υ_{N-H}, amide), 3062 ($\upsilon_{=C-H}$, aromatic), 2938 (υ_{C-H}, alkane), 2870 (υ_{C-H}, alkane), 1720 ($\upsilon_{C=O}$, ester), 1651 ($\upsilon_{C=O}$, amide), 1520 ($\upsilon_{C=C}$, aromatic), 1451 ($\upsilon_{C=C}$, aromatic), 1376 (υ_{CH3}, alkane), 1152 ($\upsilon_{C-C(O)-C}$, ester), 878 (O-O) and 741 (δ_{C-H}, aromatic) cm^{-1}; HRMS (ESI) $C_{61}H_{84}N_4NaO_{15}$ $[M+Na]^+$ requires 1135.5933, found 1135.6098.

3.3.3 Fmoc-aeg-deoxoartemisinin-*t*Bu trimer (137)

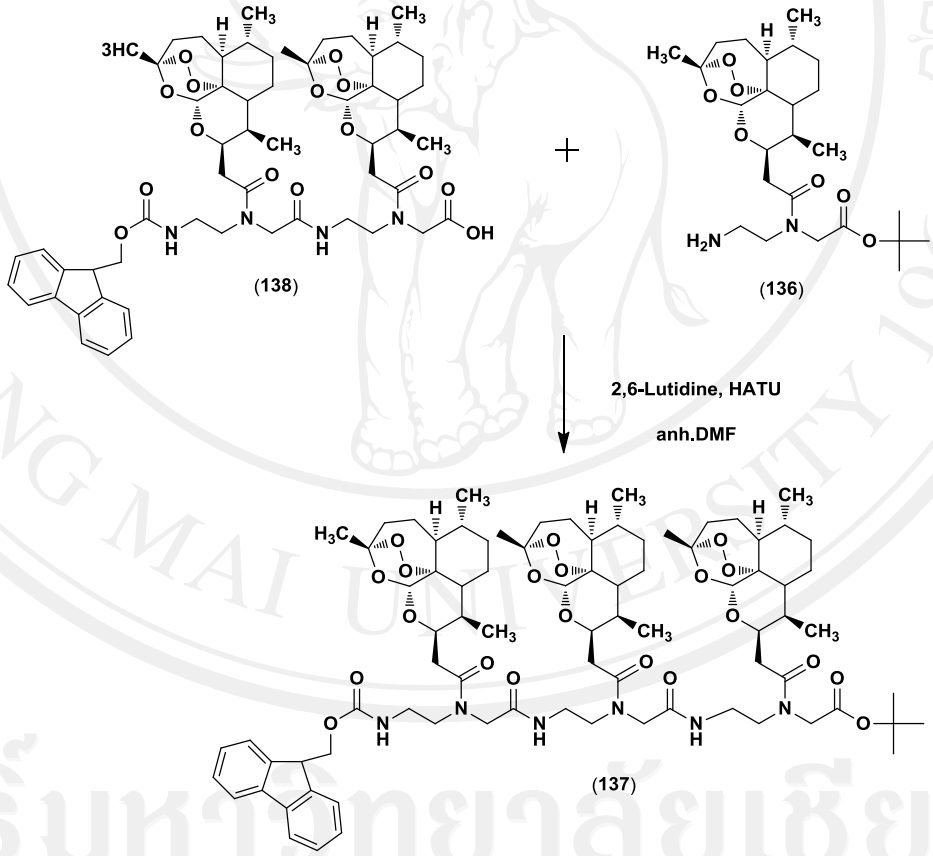
3.3.3.1 *t*-Boc deprotection of Fmoc-aeg-deoxoartemisinin-*t*Bu dimer (138)



To the solution of Fmoc-aeg-deoxoartemisinin-*t*Bu dimer (**134**) (0.16 g, 1 x 10^{-4} mol) in anhydrous dichloromethane (2 ml) was added trifluoroacetic acid (2 ml) at 0 ^{o}C. The reaction mixture was stirred at 0 ^{o}C for 15 min and warmed to room

temperature. After 1 h, the reaction was neutralized by saturated sodium hydrogen carbonate and extracted with dichloromethane (3 x 20 ml). The organic layers were washed with brine and water. The combined organic layers were dried over anhydrous magnesium sulphate and concentrated *in vacuo* to give product **138** as a dark-yellow solid (0.12 g, 77%). The crude product was used directly in the next step without further purification.

3.3.3.2 The coupling reaction of NH_2-aeg-deoxoartemisinin-*t*Bu monomer (136) and Fmoc-aeg-deoxoartemisinin-OH dimer (138)

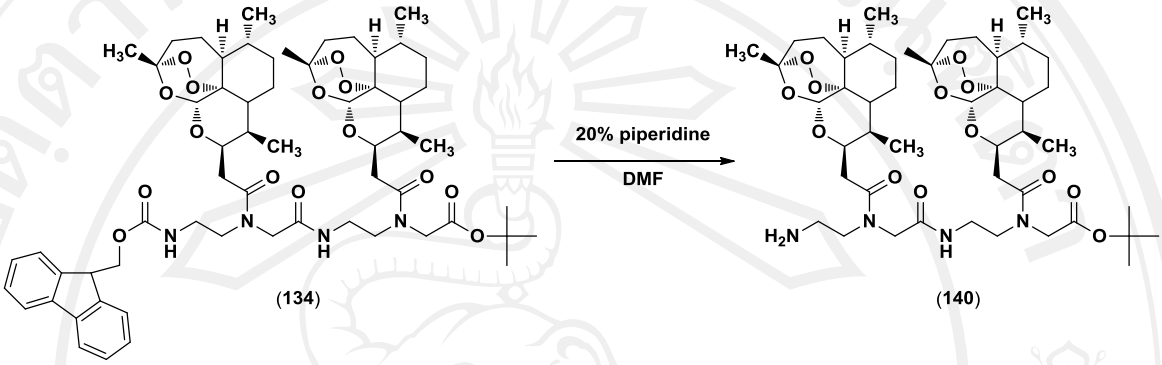


To the mixture of Fmoc-aeg-deoxoartemisinin-OH dimer (**138**) (0.13 g, 1 x 10^{-4} mol), 2-(1*H*-7-Azabenzotriazol-1-yl)-1,1,3,3-tetramethyl uronium

hexafluorophosphate methanaminium (0.17 g, 4.5 x 10^{-4} mol) and 2,6-lutidine (0.09 ml, 7.5 x 10^{-4} mol) in anhydrous *N*,*N*-dimethylformamide (2 ml) was added the mixture of NH_2-aeg-deoxoartemisinin-*t*Bu monomer (**136**) (0.05 g, 1 x 10^{-4} mol) as indicated in section **3.3.2.2** in anhydrous *N*,*N*-dimethylformamide (2 ml) *via* cannular at 0 ^oC. The mixture was stirred at 0 ^oC for 30 min and allowed to warm up to room temperature. After stirring at room temperature for 16 h, the reaction was concentrated *in vacuo*. The residue was dissolved in dichloromethane and washed with brine. The two-phase mixture was separated and the aqueous phase was back-extracted with dichloromethane (3 x 10 ml) and washed with brine and water. The organic layers were dried over anhydrous magnesium sulphate and followed by removal of the solvent *in vacuo* to give the crude product as orange-yellow viscous oil. The crude product was purified by flash column chromatography, eluting with 5% methanol/ethyl acetate, to give **137** as a light-yellow solid (0.11 g, 73%). The purity of product was confirmed by reverse phase-high performance liquid chromatography (RP-HPLC) technique using a C18 semi-preparative column (5 µM, 19 x 150 mm) with a 30-min linear gradient of 95-100% acetonitrile in water as a mobile phase. Mp 126-128 ^oC; IR (neat) 3445 (υ_{N-H}, amide), 2926 (υ_{C-H}, alkane), 1644 ($\upsilon_{C=O}$, amide), 1541 ($\upsilon_{C=C}$, aromatic), 1451 ($\upsilon_{C=C}$, aromatic), 1371 (υ_{CH3}, alkane), 1211 ($\upsilon_{C-C(O)-C}$, ester), 877 (O-O) and 749 (δ_{C-H}, aromatic) cm^{-1}; MALDI-TOF (ESI) $C_{82}H_{116}N_6O_{21}$ $[M^+]$ requires 1521.826, found 1521.939.

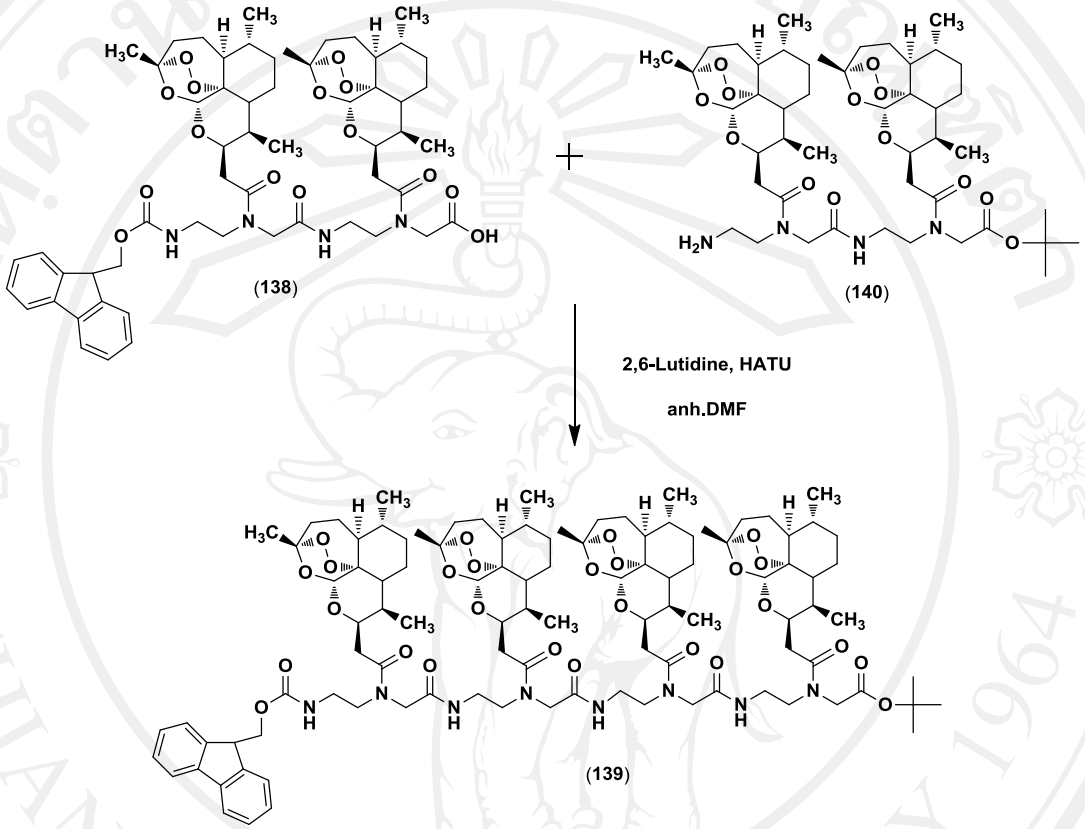
3.3.4 Fmoc-aeg-deoxoartemisinin-*t*Bu tetramer (139)

3.3.4.1 Fmoc deprotection of Fmoc-aeg-deoxoartemisinin-*t*Bu dimer (140)



To the solution of Fmoc-aeg-deoxoartemisinin-*t*Bu dimer (**134**) (0.16 g, 1 x 10^{-4} mol) in anhydrous *N*,*N*-dimethylformamide (2 ml) was added 20% piperidine in *N*,*N*-dimethylformamide (0.06 ml) at room temperature and stirred for 30 min at which time TLC indicated complete disappearance of the starting material. The reaction mixture was concentrated *in vacuo* to give the crude product as an orange-yellow solid. The crude product was purified by flash column chromatography, eluting with 10% ethyl acetate/dichloromethane, to give NH_2-aeg-deoxoartemisinin-*t*Bu dimer (**140**) as a light-yellow solid (0.12 g, 96%).

3.3.4.2 The coupling reaction of NH_2-aeg-deoxoartemisinin-*t*Bu dimer (140) and Fmoc-aeg-deoxoartemisinin-OH dimer (138)



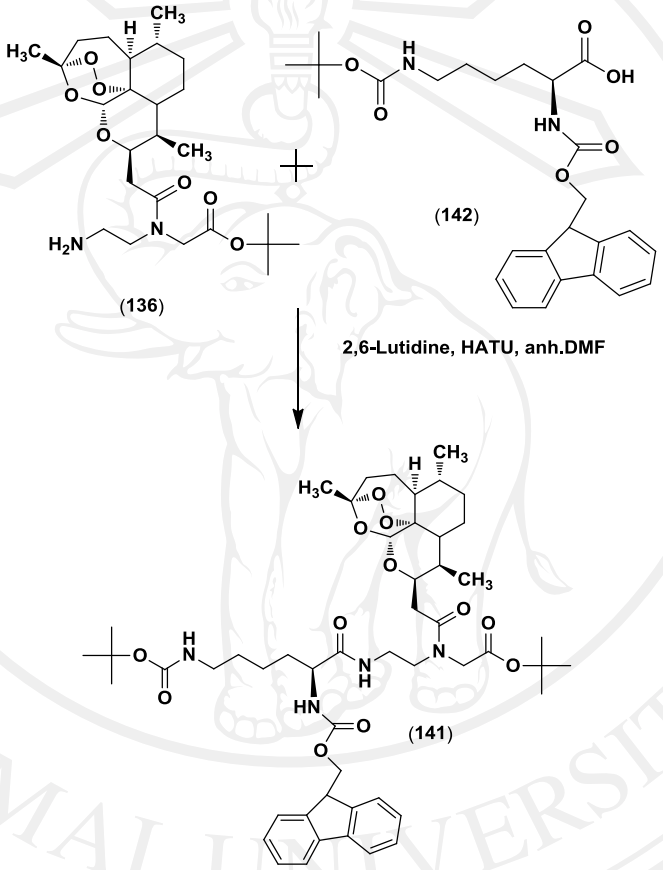
To the mixture of Fmoc-aeg-deoxoartemisinin-OH dimer (**138**) (0.21 g, 2 x 10^4 mol) as indicated in section **3.3.3.1**, 2-(1*H*-7-Azabenzotriazol-1-yl)-1,1,3,3-tetramethyl uronium hexafluorophosphate methanaminium (0.29 g, 7.5 x 10^{-4} mol) and 2,6-lutidine (0.15 ml, 1.3 x 10^{-3} mol) in anhydrous *N*,*N*-dimethylformamide (2 ml) was added the mixture of NH_2-aeg-deoxoartemisinin-*t*Bu dimer (**140**) (0.15 g, 1 x 10^{-4} mol) in anhydrous *N*,*N*-dimethylformamide (2 ml) *via* cannular at 0 ^oC. The mixture was stirred at 0 ^oC for 30 min and allowed to warm up to room temperature. After stirring at room temperature for 16 h, the reaction was concentrated *in vacuo*. The residue was dissolved in dichloromethane and washed with brine. The two-phase

mixture was separated and the aqueous phase was back-extracted with dichloromethane (3 x 10 ml) and washed with brine and water. The organic layers were dried over anhydrous magnesium sulphate and followed by removal of the solvent *in vacuo* to give the crude product as orange-yellow viscous oil. The crude product was purified by flash column chromatography, eluting with 10% methanol/ethyl acetate, to give **139** as a light-yellow solid (0.20 g, 60%). The purity of product was confirmed by reverse phase-high performance liquid chromatography (RP-HPLC) technique using a C18 semi-preparative column (5 µM, 19 x 150 mm) with a 35-min linear isocratic of 95% acetonitrile in water as a mobile phase. IR (neat) 3382 (υ_{N-H}, amide), 3063 ($\upsilon_{=C-H}$, aromatic), 2950 (υ_{C-H}, alkane), 1716 ($\upsilon_{C=O}$, ester), 1651 ($\upsilon_{C=O}$, amide), 1530 ($\upsilon_{C=C}$, aromatic), 1455 ($\upsilon_{C=C}$, aromatic), 1385 (υ_{CH3}, alkane), 1211 ($\upsilon_{C-C(O)-C}$, ester), 1155, 1138, 1107, 963, 886 (O-O) and 736 (δ_{C-H}, aromatic) cm^{-1}; LRMS (ESI) $C_{103}H_{148}N_8O_{27}$ $[M+H]^+$ requires 1930.31, found 1931.01.

3.4 Synthesis of Fmoc-lys(Boc)-aeg-deoxoartemisinin-*t*Bu oligomers

3.4.1 Fmoc-lys(Boc)-aeg-deoxoartemisinin-*t*Bu monomer (141)

3.4.1.1 The coupling reaction of NH_2-aeg-deoxoartemisinin-*t*Bu monomer (136) with Fmoc-lys(Boc)-OH (142)



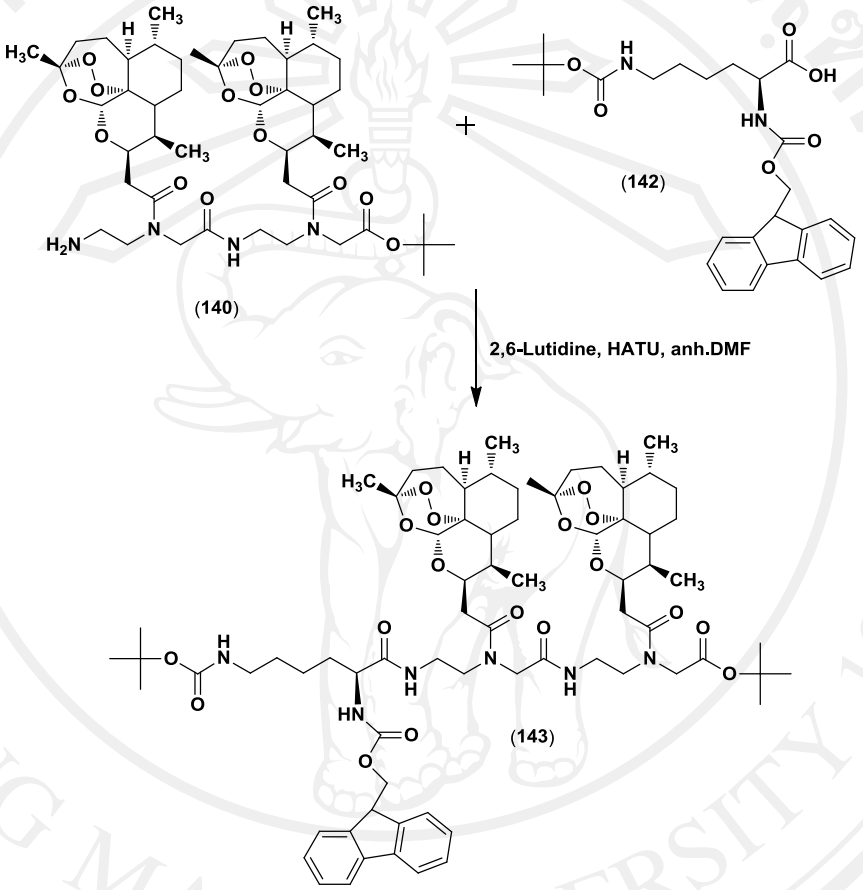
To the mixture of Fmoc-lys(Boc)-OH (**142**) (0.09 g, 1 x 10^{-4} mol), 2-(1*H*-7-Azabenzotriazol-1-yl)-1,1,3,3-tetramethyl uronium hexafluorophosphate methan aminium (0.27 g, 6.9 x 10^{-4} mol) and 2,6-lutidine (0.13 ml, 1.2 x 10^{-3} mol) in anhydrous *N*,*N*-dimethylformamide (2 ml) was added the mixture of NH_2-aeg-deoxoartemisinin-*t*Bu monomer (**136**) (0.07 g, 1 x 10^{-4} mol) as indicated in section **3.3.2.2** in anhydrous *N*,*N*-dimethylformamide (2 ml) *via* cannular at 0 ^{o}C. The mixture was stirred at 0 ^{o}C for 30 min and allowed to warm up to room temperature. After

stirring at room temperature for 16 h, the reaction was concentrated *in vacuo*. The residue was dissolved in dichloromethane and washed with brine. The two-phase mixture was separated and the aqueous phase was back-extracted with dichloromethane (3 x 10 ml) and washed with brine and water. The organic layers were dried over anhydrous magnesium sulphate and followed by removal of the solvent *in vacuo* to give the crude product as orange-yellow viscous oil. The crude product was purified by flash column chromatography, eluting with 60% ethyl acetate/hexane, to give **141** as a light-yellow solid (0.10 g, 70%). The purity of product was confirmed by reverse phase-high performance liquid chromatography (RP-HPLC) technique using a C18 semi-preparative column (5 µM, 10 x 150 mm) with a 30-min linear isocratic of 95% acetonitrile in water as a mobile phase. Mp 86-88 ^{o}C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.78 (2H, d, *J* = 7.5 Hz, H-34, H-43), 7.63 (2H, q, *J* = 7.7, 6.6 Hz, H-37, H-40), 7.42 (2H, td, *J* = 7.32, 2.72 Hz, H-35, H-42), 7.34 (2H, td, *J* = 6.68, 2.65 Hz, H-36, H-41),7.1 (br, 1H, NH), 5.7 (br, 1H, NH), 5.39 (1/2H, s, rotamer, H-12), 5.35 (1/2H, s, rotamer, H-12), 4.81 (1/2H, m, rotamer, H-10), 4.69 (1/2H, m, rotamer, H-10), 4.45 (1H, m, H-31), 4.35 (1H, m, H-31), 4.26 (1H, m, H-32), 4.25-4.15 (1H, m, H-22), 4.24 (1/2H, m, rotamer, H-16), 4.19 (1/2H, m, rotamer, H-16), 3.85 (1/2H, m, rotamer, H-16), 3.58 (1/2H, m, rotamer, H-16), 4.18-4.12 (1/2H, m, rotamer, H-19), 3.75-3.70 (1/2H, m, rotamer, H-19), 3.65 (1/2H, m, rotamer, H-19), 3.50 (1/2H, m, rotamer, H-19), 3.12 (2H, m, H-20), 3.58-3.55 (2H, m, H-26), 2.85-2.80 (1H, m, H-14), 2.72 (1H, m, H-9), 2.35-2.32 (1H, m, H-14), 2.25 (1H, m, H-4), 2.05 (1H, m, H-4), 1.51-1.49 (2H, m, H-25), 1.28 (2H, m, H-23), 1.48 (9H, s, H-29), 1.45 (9H, s, H-13), 1.38 (3H, s, 3Me), 1.90 (1H, m, H-5), 1.85-1.76 (3H, m, H-8, H-7, H-8a), 1.47 (1H, m, H-5), 1.40-1.30 (2H, m, H-5a, H-6), 1.10-1.08

(2H, m, H-8, H-7), 0.94 (3H, d, *J* = 6.07 Hz, 6Me), 0.86 (3H, d, *J* = 7.13 Hz, 9Me), 0.85 (2H, m, H-24); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 172.2/172.0, 170.0, 169.0, 156.5, 156.5, 144.5, 141.5, 128.0, 127.5, 125.0, 120.0, 103.5/102.9, 89.5/89.0, 82.9, 82.1, 80.9/80.8, 72.5/71.0, 67.0/66.9, 52.5, 52.2/52.0, 52.0/51.9, 49.9, 49.0/48.0, 47.0, 45.0/44.5, 40.0, 37.5, 36.4, 34.5/34.2, 34.0, 29.9, 28.5, 27.9, 26.5/25.5, 24.9, 22.5, 20.5, 13.1/12.5; IR (neat) 3419 (υ_{N-H}, amide), 2930 (υ_{C-H}, alkane), 2870 (υ_{C-H}, alkane), 1714 ($\upsilon_{C=O}$, ester), 1655 ($\upsilon_{C=O}$, amide), 1509 ($\upsilon_{C=C}$, aromatic), 1450 ($\upsilon_{C=C}$, aromatic), 1366 (υ_{CH3}, alkane), 1232 ($\upsilon_{C-C(O)-C}$, ester), 1154, 1053 ($\delta_{=C-H}$, aromatic), 1040 ($\delta_{=C-H}$, aromatic), 876 (O-O) and 743 (δ_{C-H}, aromatic) cm^{-1}; HRMS (ESI) $C_{51}H_{72}N_4NaO_{12}$ $[M+Na]^+$ requires 955.5147, found 955.5049.

3.4.2 Fmoc-lys(Boc)-aeg-deoxoartemisinin-*t*Bu dimer (143)

3.4.2.1 The coupling reaction of NH_2-aeg-deoxoartemisinin-*t*Bu dimer (140) with Fmoc-lys(Boc)-OH (142)

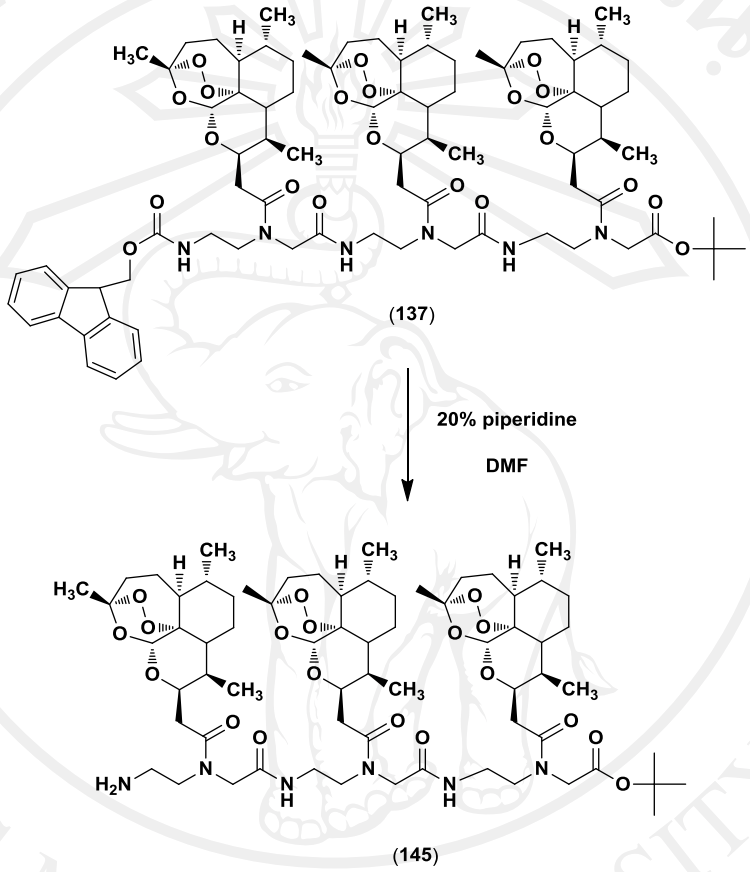


To the mixture of Fmoc-lys(Boc)-OH (**142**) (0.08 g, 1 x 10^{-4} mol), 2-(1*H*-7-Azabenzotriazol-1-yl)-1,1,3,3-tetramethyl uronium hexafluorophosphate methan aminium (0.24 g, 6.2 x 10^{-4} mol) and 2,6-lutidine (0.12 ml, 1 x 10^{-3} mol) in anhydrous *N*,*N*-dimethylformamide (2 ml) was added the mixture of NH_2-aeg-deoxoartemisinin-*t*Bu dimer (**140**) (0.13 g, 1 x 10^{-4} mol) as indicated in section **3.3.4.1** in anhydrous *N*,*N*-dimethylformamide (2 ml) *via* cannular at 0 ^{o}C. The mixture was stirred for 30 min and allowed to warm up to room temperature. After stirring at room temperature for 16 h, the reaction was concentrated *in vacuo*. The residue was dissolved in

dichloromethane and washed with brine. The two-phase mixture was separated and the aqueous phase was back-extracted with dichloromethane (3 x 10 ml) and washed with brine and water. The organic layers were dried over anhydrous magnesium sulphate and followed by removal of the solvent *in vacuo* to give the crude product as orange-yellow viscous oil. The crude product was purified by flash column chromatography, eluting with 100% ethyl acetate, to give **143** as a light-yellow solid (0.09 g, 50%). The purity of product was confirmed by reverse phase-high performance liquid chromatography (RP-HPLC) technique using a C18 semi-preparative column (5 µM, 10 x 150 mm) with a 25-min linear gradient of 75-85% acetonitrile in water as a mobile phase. Mp 124-126 ^{o}C; IR (neat) 3354 (υ_{N-H}, amide), 3063 ($\upsilon_{=C-H}$, aromatic), 2928 (υ_{C-H}, alkane), 1715 ($\upsilon_{C=O}$), 1659 ($\upsilon_{C=O}$, amide), 1525 ($\upsilon_{C=C}$, aromatic), 1452 ($\upsilon_{C=C}$, aromatic), 1366 (υ_{CH3}, alkane), 1248 ($\upsilon_{C-C(O)-C}$, ester), 1157, 1044 ($\delta_{=C-H}$, aromatic), 886 (O-O) and 739 (δ_{C-H}, aromatic) cm^{-1}; HRMS (ESI) $C_{72}H_{104}N_6NaO_{18}$ $[M+Na]^+$ requires 1363.7305, found 1363.8578.

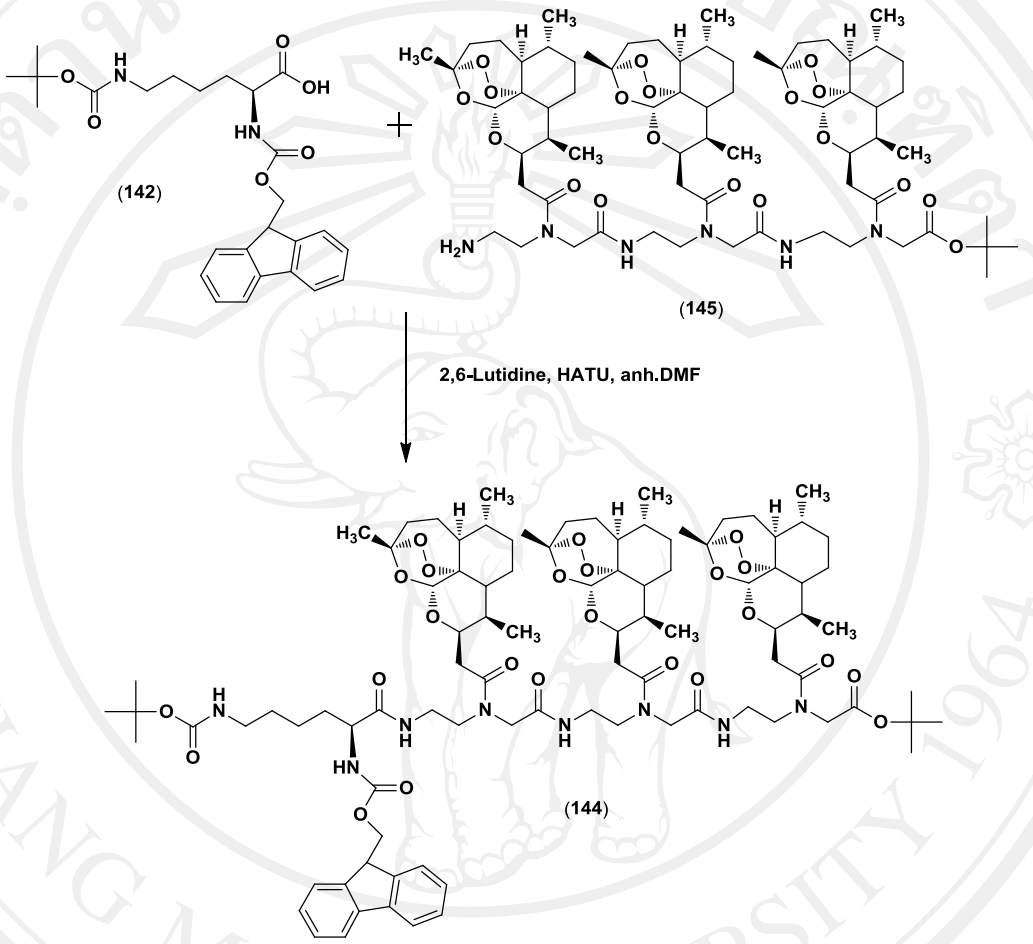
3.4.3 Fmoc-lys(Boc)-aeg-deoxoartemisinin-*t*Bu trimer (144)

3.4.3.1 Fmoc deprotection of Fmoc-aeg-deoxoartemisinin-*t*Bu trimer (145)



To the solution of Fmoc-aeg-deoxoartemisinin-*t*Bu trimer (**137**) (0.10 g, 7 x 10^{-5} mol) in anhydrous *N*,*N*-dimethylformamide (2 ml) was added 20% piperidine in *N*,*N*-dimethylformamide (0.03 ml) at room temperature and stirred for 30 min at which time TLC indicated complete disappearance of the starting material. The reaction mixture was concentrated *in vacuo* to give the crude product as a light-yellow solid. The crude product was purified by flash column chromatography, eluting with 100% ethyl acetate, to give **145** as a yellow solid (0.08 g, 95%).

3.4.3.2 The coupling reaction of NH_2-aeg-deoxoartemisinin-*t*Bu trimer (145) with Fmoc-lys(Boc)-OH (142)

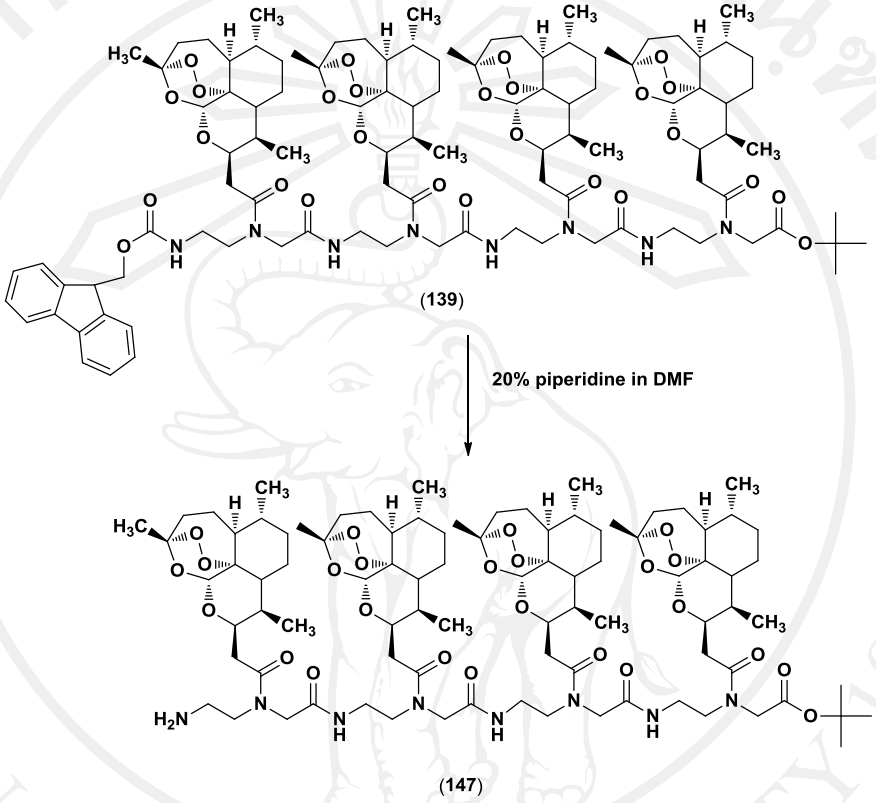


To the mixture of Fmoc-lys(Boc)-OH (**142**) (0.04 g, 8 x 10^{-5} mol), 2-(1*H*-7-Azabenzotriazol-1-yl)-1,1,3,3-tetramethyl uronium hexafluorophosphate methan aminium (0.12 g, 3 x 10^{-4} mol) and 2,6-lutidine (0.06 ml, 5 x 10^{-4} mol) in anhydrous *N*,*N*-dimethylformamide (2 ml) was added the mixture of NH_2-aeg-deoxoartemisinin-*t*Bu trimer (**145**) (0.10 g, 7 x 10^{-5} mol) in anhydrous *N*,*N*-dimethylformamide (2 ml) *via* cannular at 0 ^{o}C. The mixture was stirred at 0 ^{o}C for 30 min and allowed to warm up to room temperature. After stirring at room temperature for 16 h, the reaction was concentrated *in vacuo*. The residue was dissolved in dichloromethane and washed

with brine. The two-phase mixture was separated and the aqueous phase was back-extracted with dichloromethane (3 x 10 ml) and washed with brine and water. The organic layers were dried over anhydrous magnesium sulphate and followed by removal of the solvent *in vacuo* to give the crude product as orange-yellow viscous oil. The crude product was purified by flash column chromatography, eluting with 5% methanol/ ethyl acetate, to give **144** as a light-yellow solid (0.07 g, 53%). The purity of product was confirmed by reverse phase-high performance liquid chromatography (RP-HPLC) technique using a C18 semi-preparative column (5 μM, 10 x 150 mm) with a 20-min linear isocratic of 95% acetonitrile in water as a mobile phase. Mp 124-126 ^{o}C; IR (KBr) 3378 (υ_{N-H}, amide), 3067 ($\upsilon_{=C-H}$, aromatic), 2926 (υ_{C-H}, alkane), 1716 ($\upsilon_{C=O}$, ester), 1656 ($\upsilon_{C=O}$, amide), 1529 ($\upsilon_{C=C}$, aromatic), 1453 ($\upsilon_{C=C}$, aromatic), 1386 (υ_{CH3}, alkane), 1211 ($\upsilon_{C-C(O)-C}$, ester), 964, 886 (O-O) and 740 (δ_{C-H}, aromatic) cm^{-1}; MALDI-TOF (ESI) $C_{93}H_{136}N_8O_{24}$ $[M^+]$ requires 1750.114, found 1750.040.

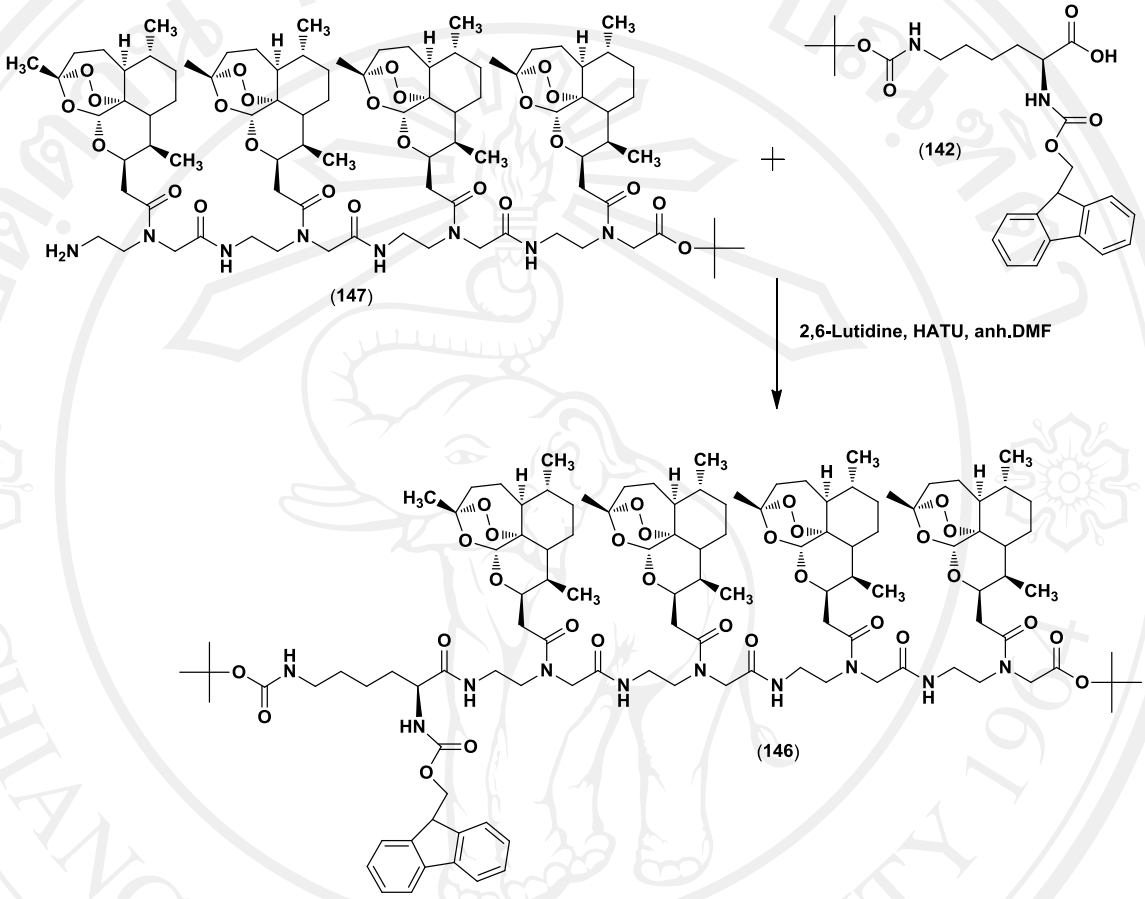
3.4.4 Fmoc-lys(Boc)-aeg-deoxoartemisinin-*t*Bu tetramer (146)

3.4.4.1 Fmoc deprotection of Fmoc-aeg-deoxoartemisinin-*t*Bu tetramer (147)



To the solution of Fmoc-aeg-deoxoartemisinin-*t*Bu tetramer (**139**) (0.17 g, 9 x 10^{-5} mol) in anhydrous *N*,*N*-dimethylformamide (2 ml) was added 20% piperidine in *N*,*N*-dimethylformamide (0.03 ml) at room temperature and stirred for 30 min at which time TLC indicated complete disappearance of the starting material. The reaction mixture was concentrated *in vacuo* to give the crude product as a light-yellow solid. The crude product was purified by flash column chromatography, eluting with 100% ethyl acetate, to obtain NH_2-aeg-deoxoartemisinin-*t*Bu tetramer **147** as a yellow solid (0.14 g, 90%).

3.4.4.2 The coupling reaction of NH_2-aeg-deoxoartemisinin-*t*Bu tetramer (147) with Fmoc-lys(Boc)-OH (142)

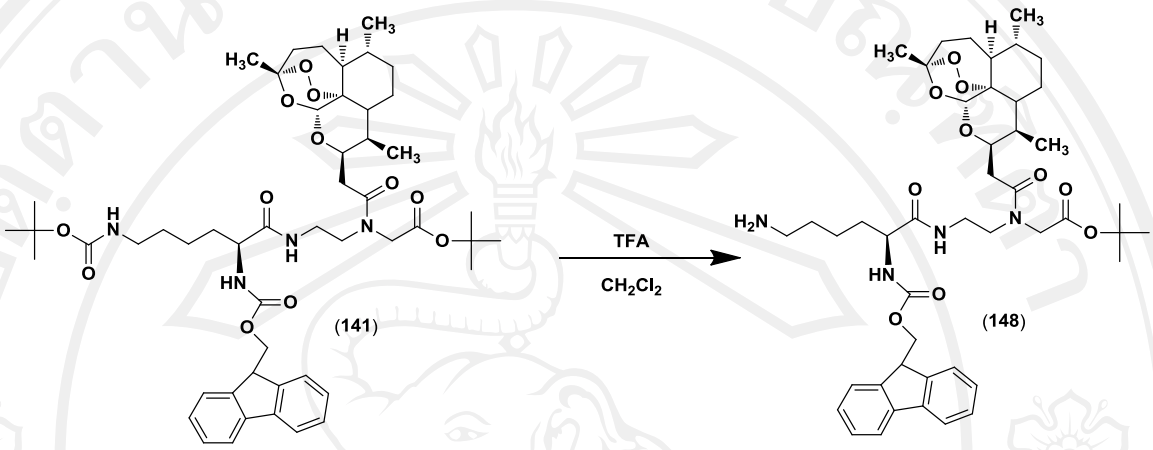


To the mixture of Fmoc-lys(Boc)-OH (**142**) (0.05 g, 1 x 10^{-4} mol), 2-(1*H*-7-Azabenzotriazol-1-yl)-1,1,3,3-tetramethyl uronium hexafluorophosphate methan aminium (0.14 g, 3.7 x 10^{-4} mol) and 2,6-lutidine (0.07 ml, 6.1 x 10^{-4} mol) in anhydrous *N*,*N*-dimethylformamide (2 ml) was added the mixture of NH_2-aeg-deoxoartemisinin-*t*Bu tetramer (**147**) (0.14 g, 8 x 10^{-5} mol) in anhydrous *N*,*N*-dimethylformamide (2 ml) *via* cannular at 0 ^{o}C. The mixture was stirred at 0 ^{o}C for 30 min allowed to warm up to room temperature. After stirring at room temperature for 16 h, the reaction was concentrated *in vacuo*. The residue was dissolved in

dichloromethane and washed with brine. The two-phase mixture was separated and the aqueous phase was back-extracted with dichloromethane (3 x 10 ml) and washed with brine and water. The organic layers were dried over anhydrous magnesium sulphate and followed by removal of the solvent *in vacuo* to give the crude product as orange-yellow viscous oil. The crude product was purified by flash column chromatography, eluting with 10% methanol/ethyl acetate, to give **146** as a light-yellow solid (0.11 g, 65%). The purity of product was confirmed by reverse phase-high performance liquid chromatography (RP-HPLC) technique using a C18 semi-preparative column (5 µM, 10 x 150 mm) with a 40-min linear isocratic of 95% acetonitrile in water as a mobile phase. Mp 128-130 ^{o}C; IR (neat) 3444 (υ_{N-H}, amide), 2925 (υ_{C-H}, alkane), 1651 ($\upsilon_{C=O}$, amide), 1644 (δ_{N-H}, amide), 1538 ($\upsilon_{C=C}$, aromatic), 1455 ($\upsilon_{C=C}$, aromatic), 1386 (υ_{CH3}, alkane), 1248 ($\upsilon_{C-C(O)-C}$, ester), 1171, 963, 886 (O-O) and 741 (δ_{C-H}, aromatic) cm^{-1}; MALDI-TOF (ESI) $C_{114}H_{168}N_{10}NaO_{30}$ $[M+Na]^+$ requires 2181.593, found 2181.073.

3.5 Fmoc-lys-aeg-deoxoartemisinin-*t*Bu oligomers

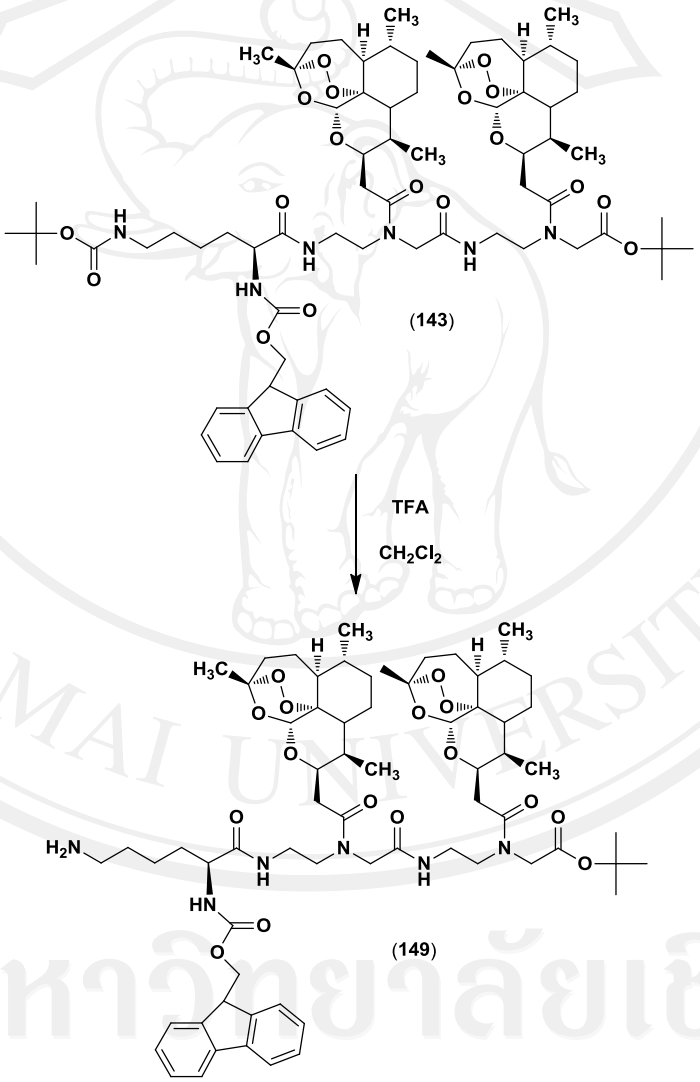
3.5.1 Fmoc-lys-aeg-deoxoartemisinin-*t*Bu monomer (148)



To a stirred solution of Fmoc-lys(Boc)-aeg-deoxoartemisinin-*t*Bu monomer (**141**) (0.15 g, 1 x 10^{-4} mol) in anhydrous dichloromethane (2 ml) was added TFA (1.19 ml) at 0 ^{o}C and stirred at 0 ^{o}C for 15 min. The reaction was warmed to room temperature and stirred for 1 h followed by neutralizing with a saturated sodium hydrogen carbonate and extracted with dichloromethane (3 x 20 ml). The organic layers were washed with brine and water. The combined organic layers were dried over anhydrous magnesium sulphate and concentrated *in vacuo* to give the crude product **148** as a dark-yellow solid. The crude product was purified by flash column chromatography, eluting with 5% methanol/dichloromethane, to give **148** as a yellow solid (0.08 g, 62%). The purity of product was confirmed by reverse phase-high performance liquid chromatography (RP-HPLC) technique using a C18 semi-preparative column (5 µM, 19 x 150 mm) with a 30-min linear gradient of 25-70% acetonitrile + 0.01% trifluoroacetic acid in water as a mobile phase. Mp 86-88 ^{o}C; IR (neat) 3421 (υ_{N-H}, amide), 3063 ($\upsilon_{=C-H}$, aromatic), 2926 ($\upsilon_{C-H,}$ alkane), 2856 ($\upsilon_{C-H,}$

alkane), 1723 ($\upsilon_{C=O}$, ester), 1660 ($\upsilon_{C=O}$, amide), 1521 ($\upsilon_{C=C}$, aromatic), 1450 ($\upsilon_{C=C}$, aromatic), 1367 (υ_{CH3}, alkane), 1202 ($\upsilon_{C-C(O)-C}$, ester), 1152, 1043 (δ_{C-N}, amine), 883 (O-O) and 743 (δ_{C-H}, aromatic) cm^{-1}; HRMS (ESI) $C_{46}H_{64}N_4NaO_{10}$ $[M+Na]^+$ requires 855.4520, found 855.4515.

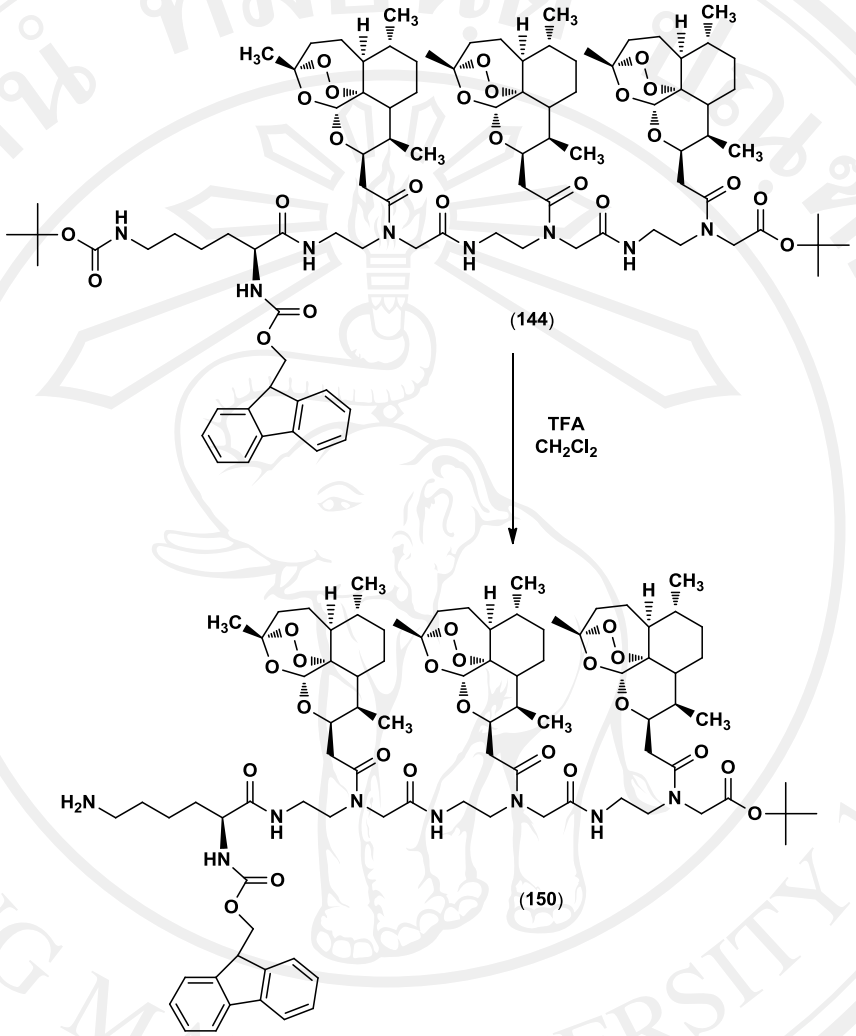
3.5.2 Fmoc-lys-aeg-deoxoartemisinin-*t*Bu dimer (149)



To a stirred solution of Fmoc-lys(Boc)-aeg-deoxoartemisinin-*t*Bu dimer (**143**) (0.04 g, 3 x 10^{-5} mol) in anhydrous dichloromethane (2 ml) was added trifluoroacetic acid (0.24 ml) at 0 ^oC and stirred at 0 ^oC for 15 min. The reaction was warmed to

room temperature and stirred for 1 h followed by neutralizing with a saturated sodium hydrogen carbonate and extracted with dichloromethane (3 x 20 ml). The organic layers were washed with brine and water. The combined organic layers were dried over anhydrous magnesium sulphate and concentrated *in vacuo* to give the crude product **149** as a dark-yellow solid. The crude product was purified by flash column chromatography, eluting with 5% methanol/dichloromethane, to give **149** as a yellow solid (0.03 g, 73%). The purity of product was confirmed by reverse phase-high performance liquid chromatography (RP-HPLC) technique using a C18 semi-preparative column (5 μM, 19 x 150 mm) with a 30-min linear gradient of 35-70% acetonitrile + 0.01% trifluoroacetic acid in water as a mobile phase. Mp 122-124 ^{o}C; IR (neat) 3391 (υ_{N-H}, amide), 3063 ($\upsilon_{=C-H}$, aromatic), 2949 (υ_{C-H}, alkane), 1719 ($\upsilon_{C=O}$, amide), 1651 ($\upsilon_{C=O}$, amide), 1536 ($\upsilon_{C=C}$, aromatic), 1451 ($\upsilon_{C=C}$, aromatic), 1371 (υ_{CH3}, alkane), 1213 ($\upsilon_{C-C(O)-C}$, ester), 1156, 1050 (δ_{C-N}, amine), 884 (O-O) and 735 (δ_{C-H}, aromatic) cm^{-1}; HRMS (ESI) $C_{67}H_{96}N_6NaO_{16}$ $[M+Na]^+$ require 1263.6781, found 1263.6775.

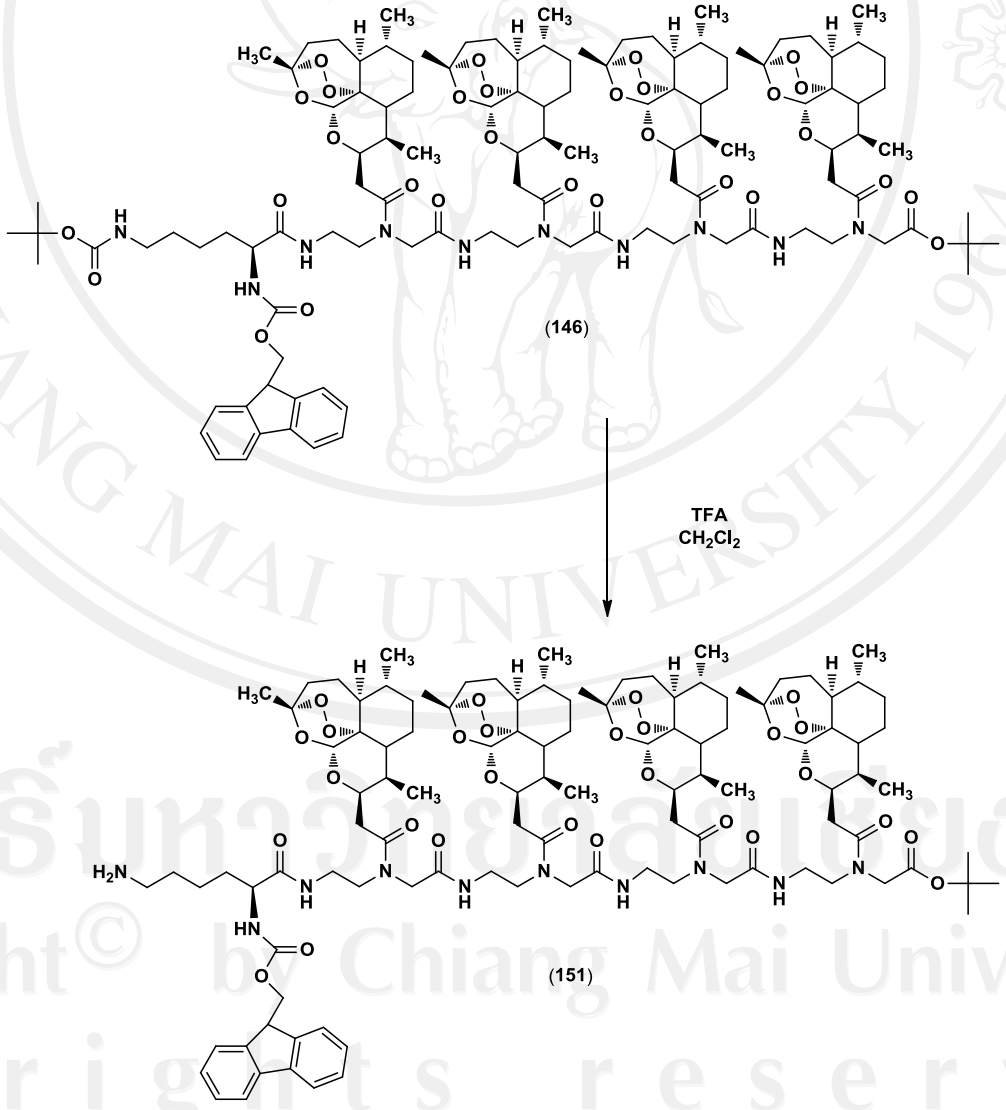
3.5.3 Fmoc-lys-aeg-deoxoartemisinin-*t*Bu trimer (150)



To a stirred solution of Fmoc-lys(Boc)-aeg-deoxoartemisinin-*t*Bu trimer (**144**) (0.05 g, 3 x 10^{-5} mol) in anhydrous dichloromethane (2 ml) was added trifluoroacetic acid (0.22 ml) at 0 ^{o}C. The reaction mixture was stirred for 15 min and warmed to room temperature. After 1 h, the reaction was neutralized by saturated sodium hydrogen carbonate and extracted with dichloromethane (3 x 20 ml). The organic layers were washed with brine and water. The combined organic layers were dried over anhydrous magnesium sulphate and concentrated *in vacuo* to give the crude product **150** as a dark-yellow solid. The crude product was purified by flash column

chromatography, eluting with 10% methanol/dichloromethane, to give **150** as a yellow solid (0.015 g, 29%). Mp 130-132 ^{o}C; IR (neat) 3437 (υ_{N-H}, amide), 2924 (υ_{C-H}, alkane), 2852 (υ_{C-H}, alkane), 1644 ($\upsilon_{C=O}$, amide), 1541 ($\upsilon_{C=C}$, aromatic), 1455 ($\upsilon_{C=C}$, aromatic), 1384 (υ_{CH3}, alkane), 1210 ($\upsilon_{C-C(O)-C}$, ester), 1139 , 1107, 1044 (δ_{C-N}, amine), 885 (O-O) and 741 (δ_{C-H}, aromatic) cm^{-1}; MALDI-TOF (ESI) $C_{88}H_{129}N_8O_{22}$ $[M+H]^+$ requires 1650.998, found 1650.842.

3.5.4 Fmoc-lys-aeg-deoxoartemisinin-*t*Bu tetramer (151)

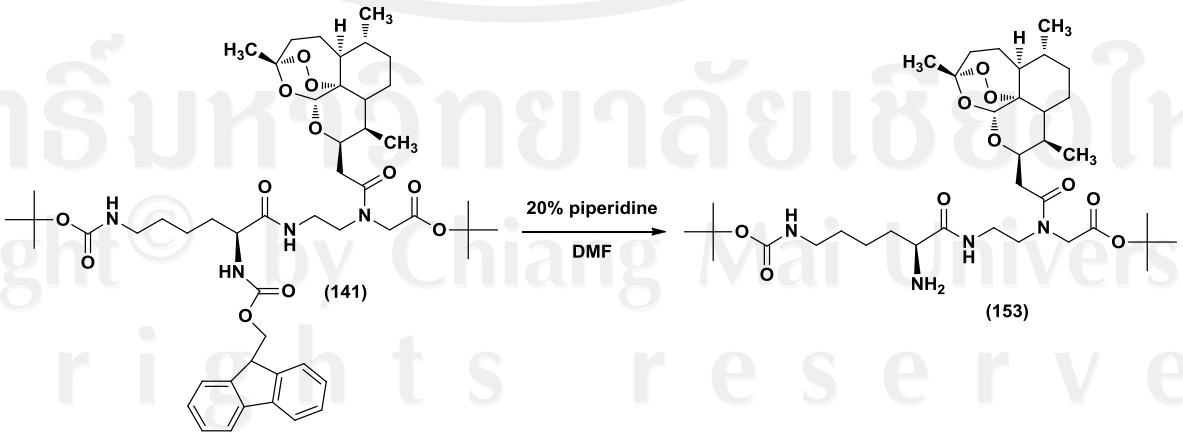


To a stirred solution of Fmoc-lys(Boc)-aeg-deoxoartemisinin-*t*Bu tetramer (**146**) (0.03 g, 1 x 10^{-5} mol) in anhydrous dichloromethane (1 ml) was added trifluoroactic acid (0.11 ml) at 0 ^{o}C. The reaction mixture was stirred for 15 min and warmed to room temperature. After 1 h, the reaction was neutralized by saturated sodium hydrogen carbonate and extracted with dichloromethane (3 x 20 ml). The organic layers were washed with brine and water. The combined organic layers were dried over anhydrous magnesium sulphate and concentrated *in vacuo* to give the crude product **151** as a dark-yellow solid. The crude product was purified by flash column chromatography, eluting with 10% methanol/dichloromethane, to give **151** as a yellow solid (0.007 g, 23%). Mp 144-146 ^{o}C; IR (neat) 3441 (υ_{N-H}, amide), 2926 (υ_{C-H}, alkane), 1644 ($\upsilon_{C=O}$, amide), 1634 (υ_{N-H}, amide), 1455 ($\upsilon_{C=C}$, aromatic), 1385 (υ_{CH3}, alkane), 1211 ($\upsilon_{C-C(O)-C}$, ester), 1171, 1138, 1081 (δ_{C-N}, amine), 1003, 963, 886 (O-O) and 739 (δ_{C-H}, aromatic) cm^{-1}; MALDI-TOF (ESI) $C_{109}H_{161}N_{10}O_{28}$ $[M+H]^{+}$ requires 2059.487, found 2059.501.

3.6 lys-aeg-deoxoartemisinin-*t*Bu oligomers.

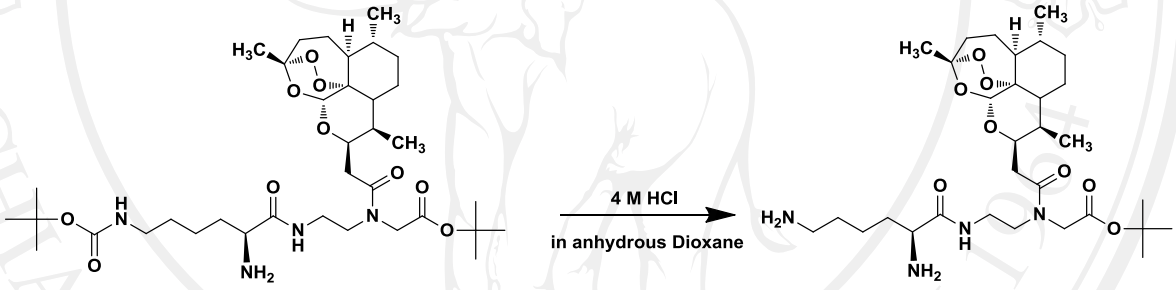
3.6.1 lys-aeg-deoxoartemisinin-*t*Bu monomer (152)

3.6.1.1 lys(Boc)-aeg-deoxoartemisinin-*t*Bu monomer (153)



To the solution of Fmoc-lys(Boc)-aeg-deoxoartemisinin-*t*Bu monomer (**141**) (0.04 g, 4 x 10^{-4} mol) in anhydrous *N*,*N*-dimethylformamide (1 ml) was added 20% piperidine in anhydrous *N*,*N*-dimethylformamide (0.02 ml) at room temperature and stirred for 30 min at which time TLC indicated complete disappearance of the starting material. The reaction mixture was concentrated *in vacuo* to give the crude product as an orange-yellow solid. The crude product was purified by flash column chromatography, eluting with 5% methanol/dichloromethane, to give lys(Boc)-aeg-deoxoartemisinin-*t*Bu monomer (**153**) as yellow oil (0.03 g, 94%).

3.6.1.2 lys-aeg-deoxoartemisinin-*t*Bu monomer (152)

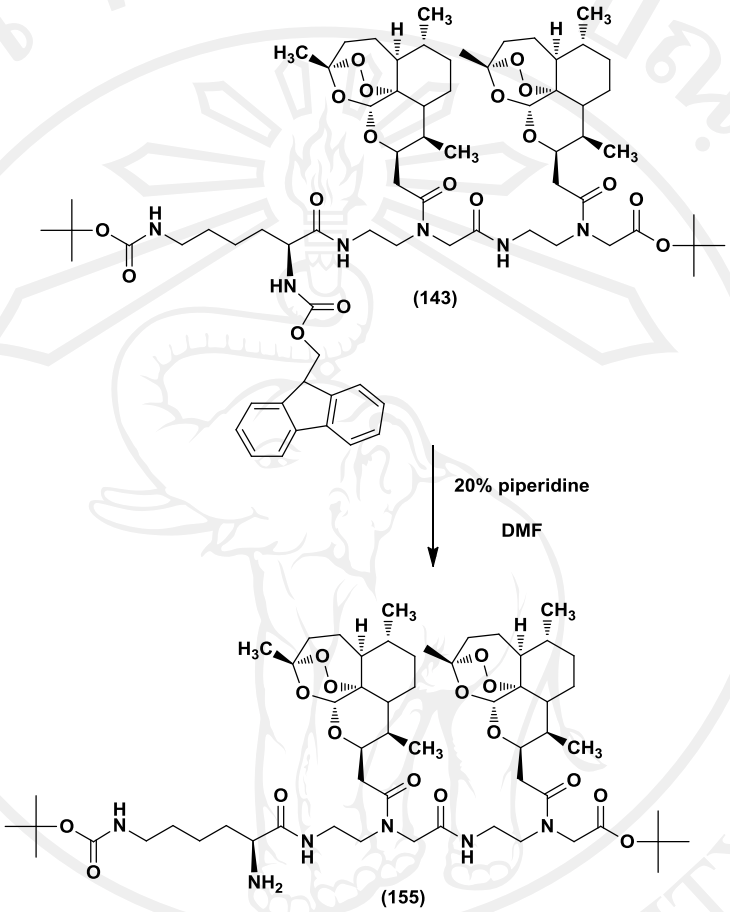


(153) (152)

The solution of lys(Boc)-aeg-deoxoartemisinin-*t*Bu monomer (**153**) (0.02 g, 3 x 10^{-5} mol) in anhydrous 1,4-dioxane 2 ml was added excess 4.0 M HCl/dioxane 2 ml. After stirring for 4 h at room temperature, the solvent was removed under reduce pressure to give the product **152** as a yellow, brittle, hygroscopic solid (17.20 mg, 100%); IR (neat) 3440 (υ_{N-H}, amide), 2959 (υC-H, alkane), 2917 (υC-H, alkane), 1644 (υC=O, amide), 1634 (δ_{N-H}, amide), 1462 (υ_{C-N}, amide), 1377 (υ_{CH3}, alkane), 1260 ($\upsilon_{C-C(O)-C}$, ester), 1157 (υ_{C-N}, amine), and 718 (δ_{N-Hoop}, amine) cm^{-1}; HRMS (ESI) $C_{31}H_{55}N_4O_8$ $[M+H]^+$ requires 611.3942, found 611.4014.

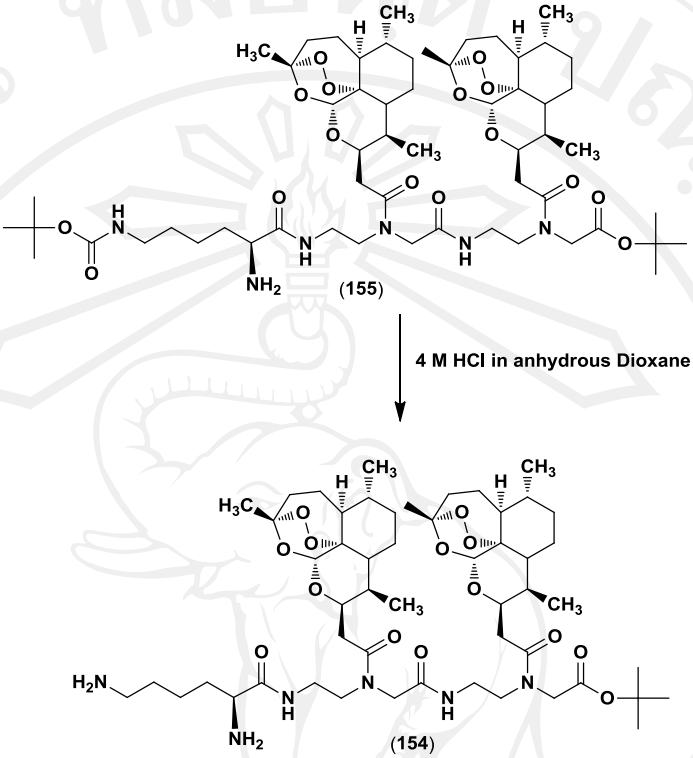
3.6.2 lys-aeg-deoxoartemisinin-*t*Bu dimer (154)

3.6.2.1 lys(Boc)-aeg-deoxoartemisinin-*t*Bu dimer (155)



To the solution of Fmoc-lys(Boc)-aeg-deoxoartemisinin-*t*Bu dimer (**143**) (0.03 g, 2 x 10^{-5} mol) in anhydrous *N*,*N*-dimethylformamide (1 ml) was added 20% piperidine in anhydrous *N*,*N*-dimethylformamide (0.01 ml) at room temperature and stirred for 30 min at which time TLC indicated complete disappearance of the starting meterial. The reaction mixture was concentrated *in vacuo* to give the crude product as an orange-yellow solid. The crude product was purified by flash column chromatography, eluting with 5% methanol/dichloromethane, to give lys(Boc)-aeg-deoxoartemisinin-*t*Bu dimer (**155**) as a light-yellow solid (0.015 g, 66%).

3.6.2.2 lys-aeg-deoxoartemisinin-*t*Bu dimer (154)

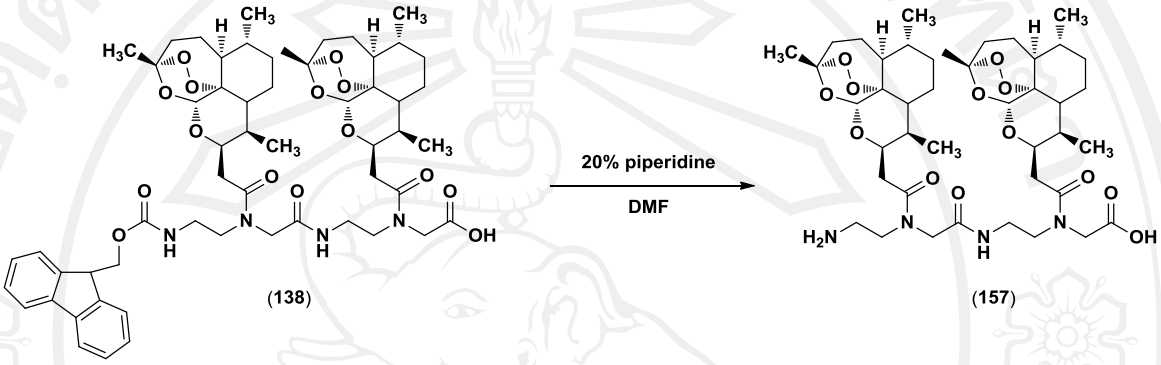


The solution of lys(Boc)-aeg-deoxoartemisinin-*t*Bu dimer (**155**) (0.02 g, 1.4 x 10^{-5} mol) in anhydrous 1,4-dioxane 2 ml was added excess 4.0 M HCl/dioxane 2 ml. After stirring for 4 h at room temperature, the solvent was removed under reduce pressure to give the product **154** as a yellow, brittle, hygroscopic solid. (13.60 mg, 92%); IR (neat) 3419 (υ_{N-H}, amide), 2926 (υ_{C-H}, alkane), 1634 ($\upsilon_{C=O}$, amide), 1539 (δ_{N-H}, amide), 1387 (υ_{CH3}, alkane), 1213 ($\upsilon_{C-C(O)-C}$, ester), 1157 (υ_{C-N}, amine), 964, 886 (O-O) and 734 (δ_{N-Hoop}, amine) cm^{-1}; HRMS (ESI) $C_{52}H_{87}N_6O_{14}$ $[M+H]^+$ requires 1019.6202, found 1019.6275.

3.7 Cyclization of NH_2-aeg-deoxoartemisinin-OH oligomers

3.7.1 Cyclization of NH_2-aeg-deoxoartemisinin-OH dimer (156)

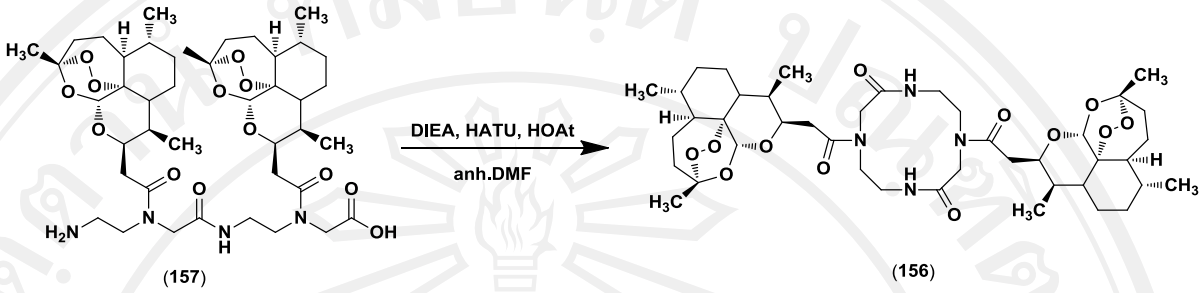
3.7.1.1 Fmoc deprotection of Fmoc-aeg-deoxoartemisinin-OH dimer (157)



(**138**) (**157**)

To the solution of Fmoc-aeg-deoxoartemisinin-OH dimer (**138**) (0.11 g, 1 x 10^{-4} mol) in anhydrous *N*,*N*-dimethylformamide (2 ml) was added 20% piperidine in *N*,*N*-dimethylformamide (0.6 ml) at room temperature and stirred for 30 min at which time TLC indicated complete disappearance of the starting material. The reaction mixture was concentrated *in vacuo* to give the crude product as a light-yellow solid. The crude product was purified by flash column chromatography, eluting with 5% methanol/dichloromethne to obtain as a eluent to give NH_2-aeg-deoxoartemisinin-OH dimer (**157**) (0.08 g, 90%).

3.7.1.2 Cyclization of NH_2-aeg-deoxoartemisinin-OH dimer (156)



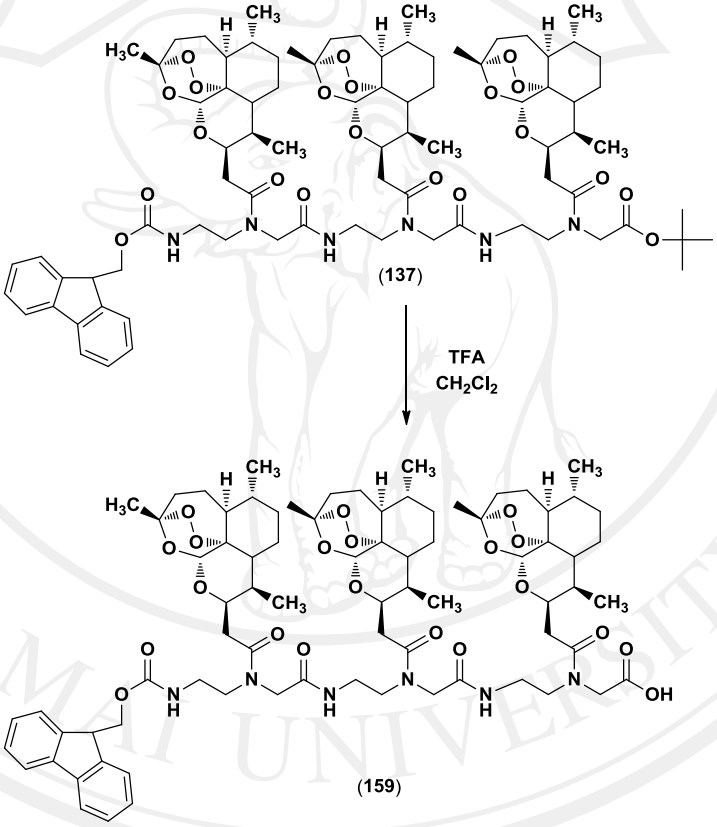
(157) (156)

To an anhydrous *N*,*N*-dimethylformamide solution of the NH_2-aeg-deoxoartemisinin-OH dimer (**157**) generated by sequentially removal of the *t*-Boc and removal of Fmoc as indicated in section **3.3.3.1, 3.7.1.1** was added 2-(1*H*-7-Azabenzotriazol-1-yl)-1,1,3,3-tetramethyl uronium hexafluorophosphate methanaminium (0.04 g, 9 x 10^{-5} mol) followed by the solution of 1-hydroxy-7-azabenzotriazole (0.01 g, 9 x 10^{-5} mol) and *N*,*N*-diisopropylethylamine (0.13 ml, 7.7 x 10^{-4} mol) in anhydrous *N*,*N*-dimethylformamide (34 ml). The reaction mixture was stirred for 16 h at room temperature and was quenched with a small amount of 5% sodium bicarbonate. The residue was dissolved in dichloromethane and the aqueous layer was back-extracted with dichloromethane. The combined organic phases were washed with brine, potassium bisulfate and brine. The solvent were dried over anhydrous magnesium sulphate and concentrated *in vacuo*. The resulting residue was purified by flash chromatography using 10% methanol/dichloromethane as the eluent to give the product **156** as a yellow solid (0.03 g, 42%). The purity of product was confirmed by reverse phase-high performance liquid chromatography (RP-HPLC) technique using a C18 semipreparative column (5 µM, 19 x 150 mm) with a 30-min linear gradient of 70% acetonitrile in water as a moblie phase. IR (neat) 3413 (υ_{N-H}, amide), 2927 (υ_{C-H},

alkane), 1719 ($\upsilon_{C=O}$, ester), 1645 (C=O, amide), 1541 (δ_{N-H}, amide), 1459 (υ_{C-N}, amide), 1382 (υ_{CH3}, alkane) and 888 (O-O) cm^{-1}; HRMS (ESI) $C_{42}H_{65}N_4O_{12}$ $[M+H]^+$ requires 817.4521, found 817.4596.

3.7.2 Cyclization of NH_2-aeg-deoxoartemisinin-OH trimer (158)

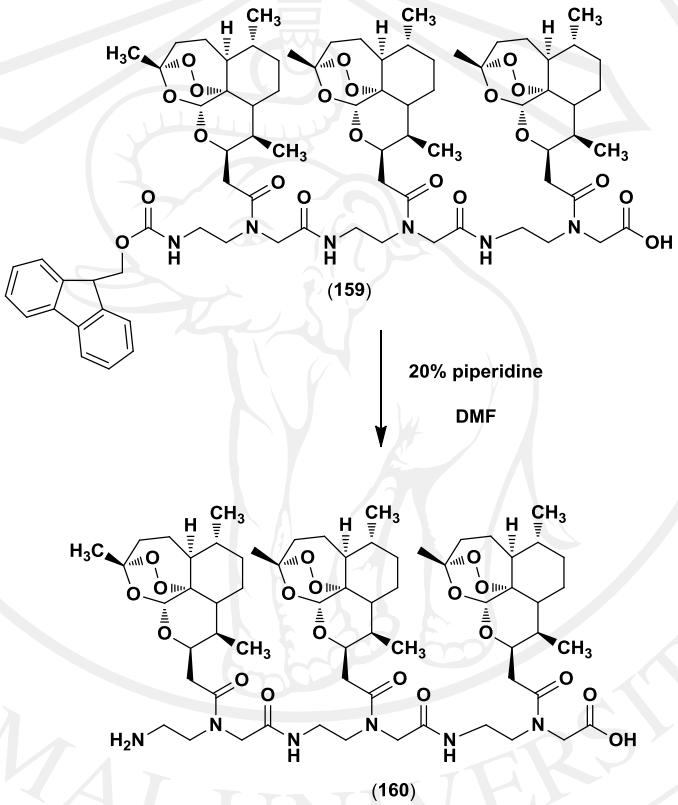
3.7.2.1 *t*-Boc deprotection of Fmoc-aeg-deoxoartemisinin-*t*Bu trimer (159)



To the solution of Fmoc-aeg-deoxoartemisinin-*t*Bu trimer (**137**) (0.10 g, 7 x 10^{-5} mol) in anhydrous dichloromethane (2 ml) was added trifluoroacetic acid (0.5 ml) at 0 ^oC. The reaction mixture was stirred at 0 ^oC for 15 min and warm to room temperature. After 1 hr, the reaction was neutralized by saturated sodium hydrogen carbonate and extracted with dichloromethane (3 x 20 ml). The organic layers were washed with brine and water. The combined organic layers were dried over anhydrous

magnesium sulphate and concentrated *in vacuo* to give product (**159**) as a dark-yellow solid in quantitative yield. The crude product was used directly in the next step without further purification.

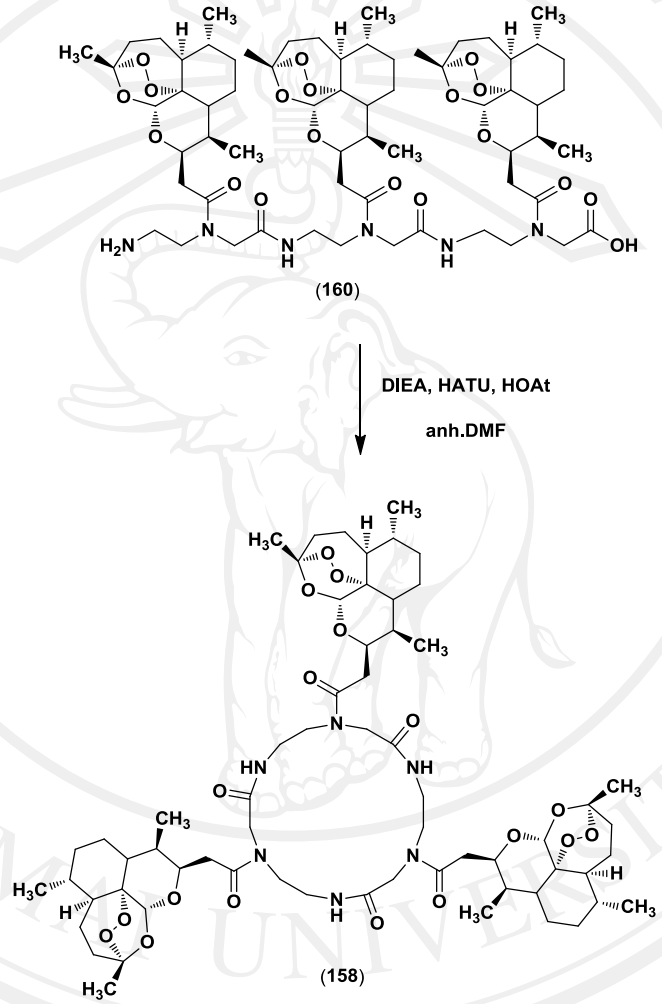
3.7.2.2 Fmoc deprotection of Fmoc-aeg-deoxoartemisinin-OH trimer (160)



To the solution of Fmoc-aeg-deoxoartemisinin-OH trimer (**159**) (0.09 g, 6 x 10^{-5} mol) in anhydrous *N*,*N*-dimethylformamide (2 ml) was added 20% piperidine in *N*,*N*-dimethylformamide (0.01 ml) at room temperature and stirred for 30 min at which time TLC indicated complete disappearance of the starting material. The reaction mixture was concentrated *in vacuo* to give the crude product as a light-yellow solid. The crude product was purified by flash column chromatography, eluting with

10% methanol/ethyl acetate to obtain as a eluent to give NH_2-aeg-deoxoartemisinin-OH trimer (**160**) (0.07 g, 91%).

3.7.2.3 Cyclization of NH_2-aeg-deoxoartemisinin-OH trimer (158)

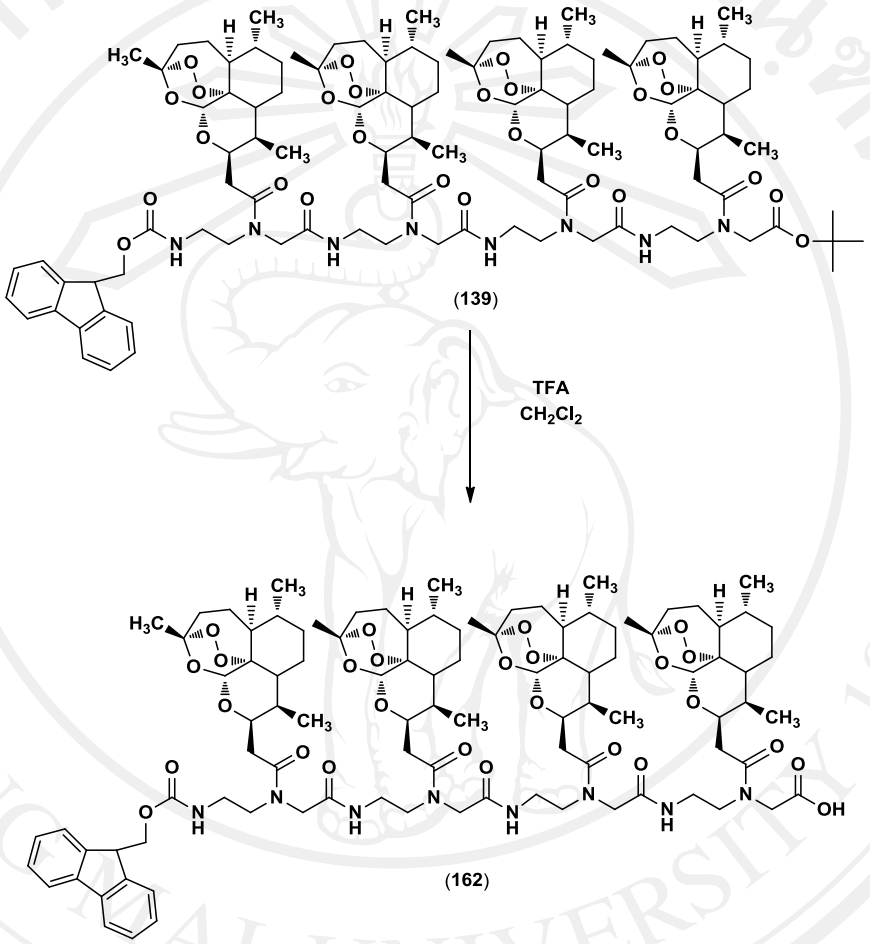


To an anhydrous *N*,*N*-dimethylformamide solution of the NH_2-aeg-deoxoartemisinin-OH trimer (**160**) generated by sequentially removal of the *t*-Boc and removal of Fmoc as indicated in section **3.7.2.1**, **3.7.2.2** was added 2-(1*H*-7-Azabenzotriazol-1-yl)-1,1,3,3-tetramethyl uronium hexafluorophosphate methan aminium (0.03 g, 7 x 10^{-5} mol) followed by the solution of 1-hydroxy-7-azabenzotriazole (0.01 g, 7 x 10^{-5} mol) and *N*,*N*-diisopropylethylamine (0.10 ml, 5.5 x

10^{-4} mol) in anhydrous *N*,*N*-dimethylformamide (35 ml). The reaction mixture was stirred for 16 h at room temperature and was quenched with a small amount of 5% sodium bicarbonate. The residue was dissolved in dichloromethane and the aqueous layer was back-extracted with dichloromethane. The combined organic phases were washed with brine, potassium bisulfate and brine. The solvent was dried over anhydrous magnesium sulphate and concentrated *in vacuo*. The resulting residue was purified by flash column chromatography using 10% methanol/dichloromethane as the eluent to give the product **158** as a yellow solid (0.02 g, 33%). LRMS (ESI) $C_{63}H_{96}N_6O_{18}$ $[M]^+$ requires 1225.47, found 1225.76.

3.7.3 Cyclization NH_2-aeg-deoxoartemisinin-OH tetramer (161)

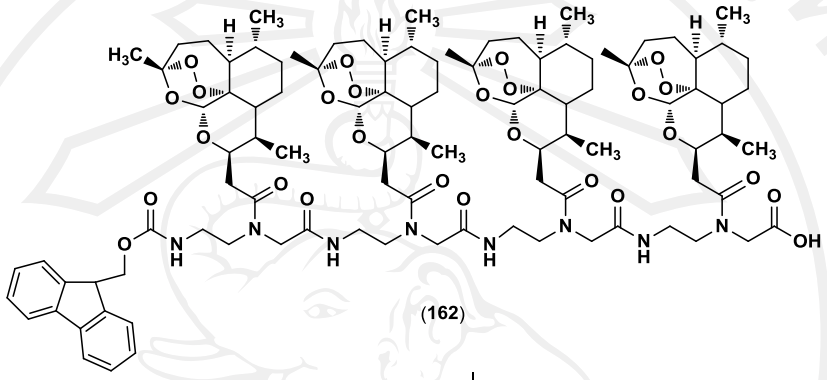
3.7.3.1 *t*-Boc deprotection of Fmoc-aeg-deoxoartemisinin-*t*Bu tetramer (162)



To the solution of Fmoc-aeg-deoxoartemisinin-*t*Bu tetramer (**139**) (0.09 g, 5 x 10^{-5} mol) in anhydrous dichloromethane (2 ml) was added trifluoroacetic acid (2 ml) at 0 ^{o}C. The reaction mixture was stirred at 0 ^{o}C for 15 min and warm to room temperature. After 1 hr, the reaction was neutralized by saturated sodium hydrogen carbonate and extracted with dichloromethane (3 x 20 ml). The organic layers were washed with brine and water. The combined organic layers were dried over anhydrous magnesium sulphate and concentrated *in vacuo* to give product (**162**) as a dark-yellow

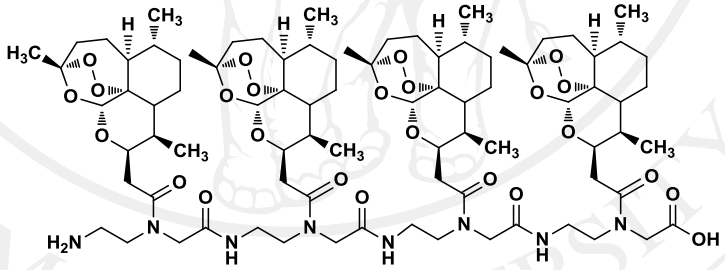
solid (0.07 g, 77%). The crude product was used directly in the next step without further purification.

3.7.3.2 Fmoc deprotection of Fmoc-aeg-deoxoartemisinin-OH tetramer (163)



(**162**)

20% piperidine in DMF

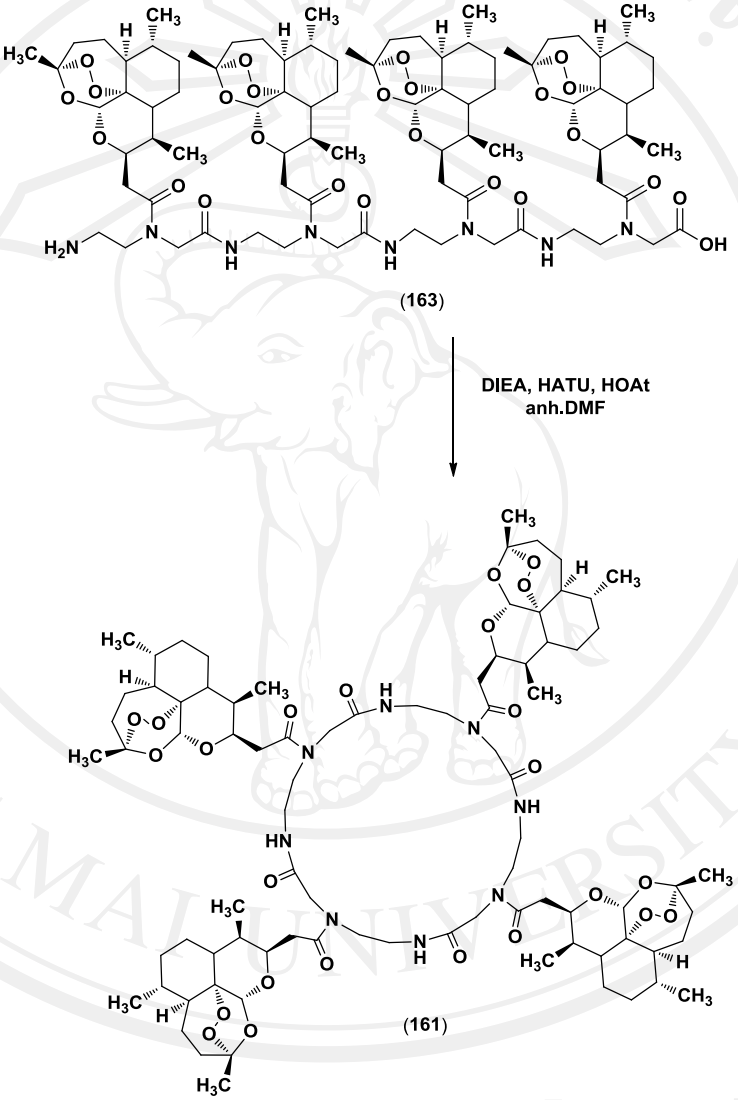


(**163**)

To the solution of Fmoc-aeg-deoxoartemisinin-OH tetramer (**162**) (0.07 g, 4 x 10^{-5} mol) in anhydrous *N*,*N*-dimethylformamide (2 ml) was added 20% piperidine in *N*,*N*-dimethylformamide (0.01 ml) at room temperature and stirred for 30 min at which time TLC indicated complete disappearance of the starting material. The reaction mixture was concentrated *in vacuo* to give the crude product as a light-yellow solid. The crude product was purified by flash column chromatography, eluting with

5% methanol/dichloromethane to obtain as a eluent to give NH_2-aeg-deoxoartemisinin-OH tetramer (**163**) (0.05 g, 90 %).

3.7.3.3 Cyclization of NH_2-aeg-deoxoartemisinin-OH tetramer (161)



To an anhydrous *N*,*N*-dimethylformamide solution of the NH_2-aeg-deoxoartemisinin-OH tetramer (**163**) generated by sequentially removal of the *t*-Boc and removal of Fmoc as indicated in section **3.7.3.1**, **3.7.3.2** was added the 2-(1*H*-7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyl uronium hexafluorophosphate methan aminium (0.02 g, 4 x 10^{-5} mol) followed by the solution of 1-hydroxy-7-

azabenzotriazole and *N*,*N*-diisopropylethylamine (0.06 ml, 3.5 x 10^{-4} mol) in anhydrous *N*,*N*-dimethylformamide (25 ml). The reaction mixture was stirred for 16 h at room temperature and was quenched with a small amount of 5% sodium bicarbonate. The residue was dissolved in dichloromethane and the aqueous layer was back-extracted with dichloromethane. The combined organic phases were washed with brine, potassium bisulfate and brine. The solvent was dried over anhydrous magnesium sulphate and concentrated *in vacuo*. The resulting residue was purified by silica gel chromatography using 10% methanol/dichloromethane as the eluent to give the product **161** as a yellow solid (0.03 g, 51%). LRMS (ESI) $C_{84}H_{128}N_8O_{24}$ $[M]^+$ requires 1633.95, found 1633.89.

3.8 Bioactivity study

3.8.1 Antimalarial activity

Plasmodium falciparum (K1, multidrug resistant strain) is cultivated *in vitro*, according to Trager & Jensen (1976) conditions [95], in RPMI 1640 medium containing 20 mM HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid), 32 mM $NaHCO_3$, and 10% heat-inactivated human serum with 3% erythrocytes, in humidified 37 °C incubator with 3% CO_2. The culture is passaged with fresh mixture of erythrocytes and medium for every day to maintain cell growth. Quantitative assessment of antimalarial activity is determined by microculture radioisotope techniques based upon the methods described by Desjardins et al. (1979) [96]. Briefly, a mixture of 200 µl of 1.5% erythrocytes with 1% parasitemia at the early ring stage is pre-exposed to 25 µl of the medium containing a test sample dissolved in 1% DMSO (0.1% final concentration) for 24 h. Subsequently, 25 µl of [3H]-hypoxanthine (Amersham, USA) in culture medium (0.5 µl) is add to each well and the plates are incubated for an additional 24 h. Levels of incorporated radioactive labeled hypoxanthine, indicating parasite growth, are determined using the TopCount microplate scintillation counter (Packard, USA).

The percentage of parasite growth is calculated using the signal count per minute of treated (CPMT) and untreated conditions (CPMu), by this formula;

$$\% \text{ parasite growth} = CPMT/CPMu \times 100$$

Inhibition concentration (IC_{50}) represents the concentration which indicates 50% reduction in parasite growth. The standard sample was dihydroartemisinin.

3.8.2 Anticancer activity

3.8.2.1 Cell culture

All cancer cell lines that used to this study were maintained in RPMI-1640 supplemented with 10% v/v fetal bovine serum (FBS), 2 mM L-glutamine, 100 IU/ml penicillin, and 100 µg/ml streptomycin. Cells were cultured at 37 ^{o}C in 5% CO_2/95% air until cells grown to 80% confluency. The medium was removed from the cell culture and cells were washed once with sterile PBS. The adherent cells were trypsinized using a 0.25 % (w/v) trypsin-EDTA solution and incubated for 2-5 min at 37 ^{o}C in 5% CO_2. Cells suspended in 2 ml of growth medium. 0.5 ml of the cell suspension was transferred to a T-25 cm^2 flask containing 5 ml of RMPI 1640 and cultured as described above.

3.8.2.2 MTT assays

The MTT assay was also used to evaluate the *in vitro* cytotoxicity.[97] Briefly, each of cancer cell lines and normal cell line were seeded at a 10,000 cells/well in 96-well plates and maintained at 37 ^{o}C in an atmosphere of 5% CO_2 in RPMI 1640 containing 10% (v/v) fetal bovine serum and penicillin (100 IU/ml) and streptomycin (100 µg/ml). After 48 h, medium was removed from the wells, and cells were washed with PBS then RPMI 1640 containing our tested compounds with concentration ranging from 0.0025 µM to 1000 µM was added and cell were incubated for 48 h. 20 µl of MTT solution (100 µg/ml in PBS) was added into individual well and then incubated for a further 4 h. The medium containing sample was removed and 100 µl of dimethylsulfoxide (DMSO) was added to solubilized the reaction products. The absorbance at 550 and 620 nm were measured using a microplate reader. Damaged or dead cells show reduced or no mitochondrial dehydrogenase activity. Percentage of

cell survival and IC_{50} values (concentration at which 50% inhibition of mitochondrial dehydrogenase occurred) were calculated from Biodatafit software. RPMI 1640 medium was used as a positive control, while 1% v/v triton X-100 was used as the negative control.