# CHARPTER V

# CONCLUSION

The use of *N*-(2-aminoethyl) glycine (aeg), pseudo peptide nucleic acid backbone, has proven their powerful usefulness in various applications. In this research, we have demonstrated the use of *N*-(2-aminoethyl) glycine to synthesize the C-10 non-acetal deoxoartemisinin oligomer. The C-10 non-acetal deoxoartemisinin was prepared from dihydroartemisinin using known procedures.[77,78,100]

Fmoc-aeg-deoxoartemisinin-*t*Bu monomer (**132**) was obtained in 91% yield from the coupling reaction of carboxylalkyldeoxoartemisinin (**131**) and *tert*-butyl *N*-[2-(*N*-9-fluorenylmethoxy-carbonyl) aminoethyl] glycinate hydrochloride (**133**) by using HATU as a coupling reagent. The desired monomer (**132**) was used as a precursor for synthesis of Fmoc-aeg-deoxoartemisinin-*t*Bu oligomers. Deprotection of Fmoc-aeg-deoxoartemisinin-*t*Bu monomer (**132**) using TFA/$CH_2Cl_2$ and 20% piperidine in DMF gave Fmoc-aeg-deoxoartemisinin-OH monomer (**135**) and $NH_2$-aeg-deoxoartemisinin-*t*Bu monomer (**136**) in quantitative yields. Fmoc-aeg-deoxoartemisinin-*t*Bu dimer (**134**) was synthesized from coupling of Fmoc-aeg-deoxoartemisinin-OH monomer (**135**) and $NH_2$-aeg-deoxoartemisinin-*t*Bu monomer (**136**) by using HATU as a coupling reagent to give dimer **134** in 88% yield. By using the same deprotection-coupling strategy was obtained Fmoc-aeg-deoxoartemisinin-*t*Bu trimer (**137**) and Fmoc-aeg-deoxoartemisinin-*t*Bu tetramer (**139**) in 73% yield, 60% yield, respectively.

Fmoc-lys(Boc)-OH was added at the N-terminus of these new the C-10 non-acetal deoxoartemisinin monomer and oligomers series to investigate the effect of

amino acid to their biological activities. The oligo peptide with one lysine attached to the N-terminal end, Fmoc-lys(Boc)-aeg-deoxoartemisinin-*t*Bu monomer (**141**), dimer (**143**), trimer (**144**) and tetramer (**146**) were synthesized by using standard coupling protocol in solution phase and obtained in 50-70% yield. The *N*Boc protection of Fmoc-lys(Boc)-aeg-deoxoartemisinin-*t*Bu monomer (**141**), dimer (**143**), trimer (**144**) and tetramer (**146**) to give Fmoc-lys-aeg-deoxoartemisinin-*t*Bu monomer (**148**), dimer (**149**), trimer (**150**) and tetramer (**151**) were achieved in 23-73% yield by using TFA in $CH_2Cl_2$. In addition, subsequentially deprotection of Fmoc-lys(Boc)-aeg-deoxoartemisinin-*t*Bu monomer (**141**) and Fmoc-lys(Boc)-aeg-deoxoartemisinin-*t*Bu dimer (**143**) using 20% piperidine in DMF and 4 M HCl in dioxane, respectively gave lys-aeg-deoxoartemisinin-*t*Bu monomer (**152**) and lys-aeg-deoxoartemisinin-*t*Bu dimer (**154**) in quantitative yield. These compounds were tested for their biological activities.

In addition to explore the possibility to improve the biological activities due to conformational rigidity of the C-10 non-acetal deoxoartemisinin oligomers, cyclization of the C-10 non-acetal deoxoartemisinin oligomers were performed and investigated for against the chloroquine-resistance K1 strain of *Plasmodium falciparum*. The cyclization using the same sequential deprotection–coupling strategy under high dilution condition gave the corresponding Fmoc-aeg-deoxoartemisinin-*t*Bu oligomers the corresponding cyclic-aeg-deoxoartemisinin oligomers. The cyclic-aeg-deoxoartemisinin-*t*Bu dimer (**156**) was achieved by head-to-tail cyclization of $NH_2$-aeg-deoxoartemisinin-OH dimer (**157**) by using HATU and HOAt as coupling reagents to give 42% yield. Under the same conditions, the corresponding cyclic-aeg-

deoxoartemisinin-*t*Bu trimer (**158**) and cyclic-aeg-deoxoartemisinin-*t*Bu tetramer (**161**) were obtained in 33% yield and 51% yield, respectively.

The biological activities of all synthesized compounds have been investigated and the results revealed that Fmoc-aeg-deoxoartemisinin-*t*Bu monomer (**132**) was the most active compound with $IC_{50}$ of 4.46 nM against the chloroquine-resistance K1 strain of *P. falciparum*. In contrast, tetramer derivatives (**139**), trimer derivatives (**137**) and dimer derivatives (**134**) showed less potent against malaria parasites than monomer derivatives (**132**). Futhermore, adding Lys amino acid to the N-terminus of C-10 non-acetal deoxoartemisinin monomer and dimer did not increase their activity against the chloroquine-resistance K1 strain of *P. falciparum.* In contrast with our expectation, the cyclic oligomers showed poorer activity than Fmoc-aeg-deoxoartemisinin-*t*Bu monomer (**132**) against the chloroquine-resistance K1 strain of *Plasmodium falciparum* with $IC_{50}$ of 3.28 μM.

The results also revealed that the extent of anticancer activity depends upon the extent of the number of peroxide units. Thus, dimer derivatives showed more potent against cancer cell lines than the corresponding monomer derivatives. In addition, introducing one lysine-based monomer into C-10 non-acetal monomer and dimer derivatives can increase anticancer activity which presumably due to hydrophilic compounds increase their aqueoue solubility. The results revealed that Fmoc-lys-aeg-deoxoartemisinin-*t*Bu dimer (**149**) was the most active oligomer with $IC_{50}$ of 0.18, 2.04 and 2.97 μM against HT-29, Caco-2 and A549 cancer cell lines, respectively. Moreover, dimer **149** also showed low toxicity toward the normal cell with $LD_{50}$ of 38.50 μM. Furthermore, these resulted could be concluded that Fmoc-aeg-deoxoartemisinin-*t*Bu dimer (**132**) and Fmoc-lys(Boc)-aeg-deoxoartemisinin-*t*Bu

dimer (**143**) are selectivity toward B16F10 cancer cell line. Fmoc-lys-aeg-deoxoartemisinin-*t*Bu dimer (**149**) is selectivity toward HT-29, Caco-2 and A549 cancer cell lines. On the contrary, lys-aeg-deoxoartemisinin-*t*Bu dimer (**154**) is selectivity toward Caco-2 and A549 but not selectivity toward HT-29 cancer cell lines.

These findings suggested that that employment of pseudo peptide nucleic acid (PNA) monomer containing C-10 non-acetal deoxoartemisinin moiety appears to be a very promising strategy to assemble Fmoc-aeg-deoxoartemisinin-*t*Bu oligomers and cyclic-aeg-deoxoartemisinin-*t*Bu oligomers. The finding suggests that some of these compounds might serve as potential candidates for anticancer and antimalarial agents, particularly, the analog Fmoc-aeg-deoxoartemisinin-*t*Bu monomer (**132**) and Fmoc-lys-aeg-deoxoartemisinin-*t*Bu dimer (**149**) are quite a promising lead compounds.