CHAPTER V

CONCLUSION

This investigation aimed to isolate Et 770 and Et 786 from the Thai tunicate, *Ecteinascidia thurstoni*, as starting materials, in order to do the transformation and modification of the structure to obtain the newly acylated esters of Et 770. In addition, selective *N*-demethylations of the ABC ring model compounds bearing structure related to the *A*-subunit of ecteinascidin molecule were also studied.

With KCN-pretreated method, the large-scale preparation of the labile ecteinascidins from the Thai tunicate can be achieved. This provides the advantage of increasing the yields of natural isolated Et 770 in the range 1.1×10^{-3} % yield of wet weight and Et 786 in the range 3.0×10^{-4} % yield of wet weight. The high yield of the isolated ecteinascidins is opening the opportunity in the Thai marine tunicate for further discovery of candidates for the anticancer agent.

The α-cyanoamine group of Et 770 was transformed to α-carbinolamine group of Et 743 by silver nitrate. Also Et 786 was converted to the parent Et 759B with the same manner. However, the sulfide ether group on Et 770 can be oxidized to yield the sulfoxide with m-CPBA in high yield. Moreover, diacetate esters were prepared on both phenolic hydroxyls at C-18 and C-6' of Ets 743, 770 and 786 to yield **18-20**, respectively. The biological data revealed that diacetyl analogs **18-20** possessed low activity as compared to their parent compound **2**. Oxidation of the sulfide group of Et 770 resulted in dramatically diminished cytotoxic activity. The structure of the acetylated products were elucidated using HR-FABMS, ^{1}H- and ^{13}C-NMR. Position at which the acetylation occurred was supported by the upfield shift of ^{13}C-NMR signal of the aromatic C-6' and downfield shift of the signals of carbons at C-5', C-7', C-9' of the C-subunit and C-15, C-19 of the *A*-subunit.

Aromatic ester derivatives on the phenolic hydroxyl at the *C*-subunit of Et 770 were prepared in this study, including the benzoyl (**21**), substituted benzoyl (**22-29**), pyridinoyl (**30-31**), naphthoyl (**32-33**), quinolinoyl (**34-35**), isoquinolinoyl (**36-37**). All derivatives were prepared from the reaction of Et 770 and acid anhydrides or acid chlorides or acids in the presence of DCC as the coupling reagent. In contrast, the reaction of indole-3-carboxylic acid with Et 770 in the presence of DCC provided the amide derivative **39**.

The acyl substituent at C-6' in the *C*-subunit of monoacyl derivatives **21-37** were again supported by the signal of C-6' shifting upfield, while the signals of C-5', C-7', and C-9' shifting downfield compared with those of **2**. The amide derivative **39** revealed that the signals of carbons adjacent the nitrogen atom in the *C*-subunit as C-1' and C-12' at the ten-membered sulfide bridge were shifted downfield as compared to **2** and the characteristic upfield signals of protons at 15-H, 16-CH_3, and 17-OCH_3 at the *A*-subunit confirmed that the amide derivative replaced at N-2' of the *C*-subunit.

Cytotoxic activity evaluation of these compounds on HCT116, QG56, and DU145 revealed that nitro substituted benzoyl (**22-24**), and methoxy substituted benzoyl (**28-29**) possessed similar activity to that **2.** The activity was dramatically decreased for bromo substituted benzoyl (**25-27**) and naphthoyl group (**32-33**). The nitrogen-containing heterocycles **30-31**, and **34-37** exhibited high activity. **39** showed the highest activity for these derivatives.

As a part of selective *N*-demethylation with CAN was worked on the structural models containing the ABC ring system, structure related with the *A*-subunit of ecteinascidins. The optimal condition was achieved in 5 equimolars of CAN in the presence of aqeous-acetonitrile. However, this investigation revealed that the protecting at the phenolic hydroxyl and transformation cyanoamine to amide carbonyl group are the key steps for the oxidative *N*-demethylation using CAN for those model compounds. It is worthy result for further developing method to work on the natural ecteinascidin.