CHAPTER III

EXPERIMENTAL

1. Animal material

The Thai tunicate was identified by Dr T. Nishikawa of Nagoya University Museum as *Ecteinascidia thurstoni* Herdman 1981. The sample was collected by scuba diving at a depth of 1-5 meters on the East coast of Phuket Island in the Southern part of Thailand. The collection was done in four periods of time, during October 2002, January 2003, June and July 2003, and the samples were stored frozen at -20 °C until use.

2. General experimental procedures

2.1 Thin-layer chromatography (TLC)

Technique : One dimension, ascending

Adsorbent : Silica gel GF_{254} (E. Merck)

Layer thickness : 250 μm

Distance : 8 cm

Detection : 1. Ultraviolet light at wavelengths of 254 and 365 nm

2. Visible detection in iodine vapor

2.2 Column chromatography

2.2.1 Flash column chromatography

Adsorbent : Silica gel (No. 1.09385), particle size 0.040-0.063 mm (230-400 mesh ASTM) (E. Merck)

Packing method : The adsorbent was suspended in the eluant. The slurry of the adsorbent was poured into the column and then allowed to settle before use.

Sample loading : The sample was dissolved in a small volume of benzene and loaded on top of the column.

Detection : Fractions were monitored by TLC technique.

2.2.2 Gel filtration chromatography

Gel filter : Sephadex LH-20 (Pharmacia Biotech AB)

Packing method : Sephadex gel was suspended in the eluant and kept overnight to swell prior to use. The slurry of adsorbent was poured in to the column and allowed to settle before to use.

Sample loading : The sample was dissolved in a small volume of benzene and loaded on top of the column.

Detection : Fractions were monitored by TLC technique.

3 Physical constants and spectroscopy

3.1 Proton and carbon nuclear magnetic resonance (^{1}H- and ^{13}C-NMR) spectroscopy.

^{1}H-and ^{13}C-NMR , DEPT 90 and 135, ^{1}H,^{1}H-COSY, HMQC, HMBC and NOESY spectra were obtained on a JEOL-JNM-LA 500 FT-NMR spectrometer (Analytical Center of Meiji Pharmaceutical University) at 500 and 125.65 MHz, respectively, and on a Bruker Avance DPX-300 FT-NMR spectrometer (Pharmaceutical Research Instrument Center, Faculty of Pharmaceutical Sciences, Chulalongkorn University), operating at 300 MHz and 75 MHz, respectively.

The solvent for NMR spectra was deuterated chloroform ($CDCl_3$). Chemical shifts were recorded in ppm scale using the chemical shift of the solvent as the reference signal and TMS as the internal standard. Proton-detected heteronuclear correlations were measured using HMQC (optimized for $^{1}J_{HC}$ = 145 Hz) and HMBC (optimized for $^{n}J_{HC}$ = 4 and 8 Hz) pulse sequences.

3.2 Mass spectrometry

The EIMS, FABMS and HR-FABMS spectra were recorded on JMS-DX 302 and JMS-700 instruments with a direct inlet system operating at 70eV (Meiji Pharmaceutical University).

3.3 Infrared (IR) spectroscopy

IR spectra were obtained on a Perkin Elmer 2000 FT-IR 1760X spectrophotometer and on a Hitachi 260-10 spectrophotometer (Meiji Pharmaceutical University).

3.4 Optical rotation

Optical rotations were measured on a Horiba-SEPA instrument (Meiji Pharmaceutical University).

4. Chemical reagents

Acetic acid anhydride	(Fluka)
Benzoyl chloride	(TCI)
Cerium(IV)ammonium nitrate (CAN)	(Nacalai tesque)
m-Chloroperbenzoic acid (*m*-CPBA)	(Nacalai tesque)
N,N-Dicyclohexylcarbodiimide (DCC)	(Fluka)
N,N-4-dimethylaminopyridine (DMAP)	(Sigma)
Isopropyl chloroformate	(TCI)
Potassium cyanide	(Fluka)
Silver nitrate	(Sigma)
Thionyl chloride	(WAKO)
Isonicotinoyl chloride	(TCI)
Nicotinoyl chloride	(TCI)
o-, *m*-, and *p*-Bromo benzoyl chloride	(TCI)
o-, *m*-, and *p*-Methoxy benzoyl chloride	(TCI)
o-, *m*-, and *p*-Nitro benzoyl chloride	(TCI)
1 and 2-Naphthoyl chloride	(TCI)
Indole-3-carboxylic acid	(TCI)
Isoquinoline-1-carboxylic acid	(TCI)
Isoquinoline-3-carboxylic acid	(TCI)
Quinoline-2-carboxylic acid	(WAKO)
Quinoline-4-carboxylic acid	(WAKO)

5. Solvents

All solvents used were either analytical or laboratory grade and were redistilled prior to use. For chemical reaction, pyridine and dichloromethane were dried with molecular sieve type 4 Å. Tetrahydrofuran was refluxed in the presence of sodium and freshly distilled prior to use.

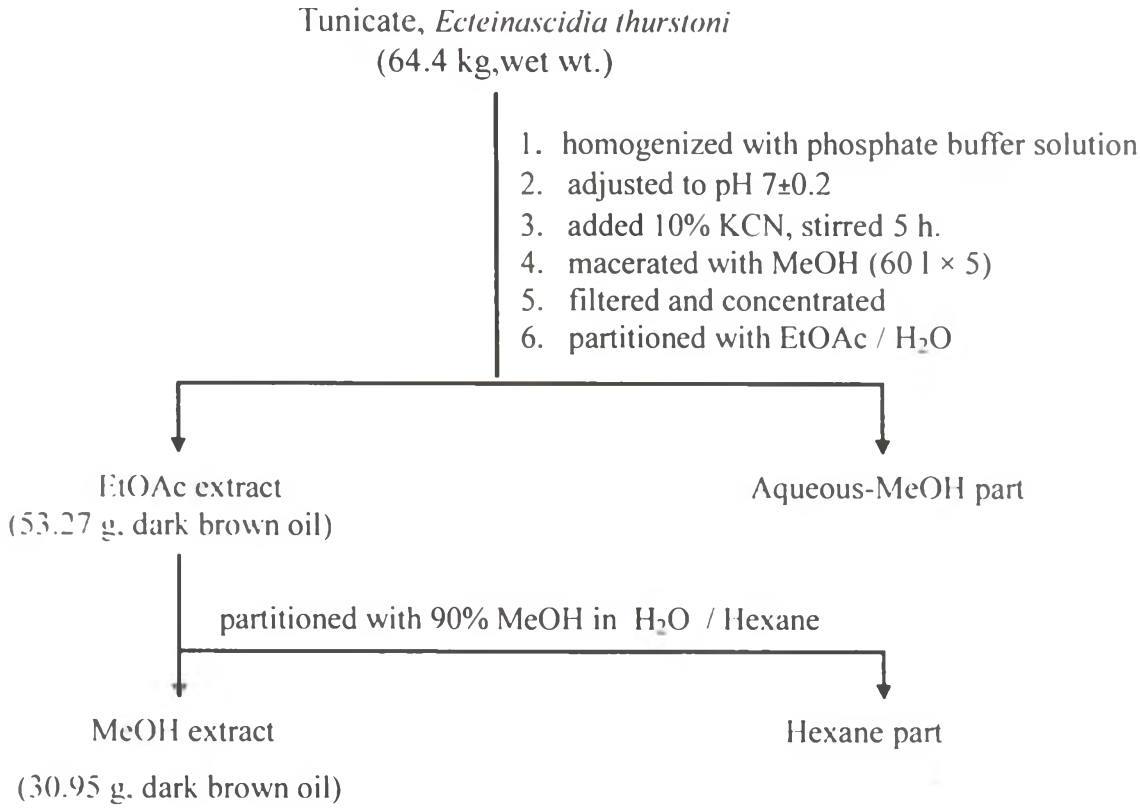
6. Extraction and isolation of ecteinascidins from the Thai tunicate, *Ecteinascidia thurstoni*

The frozen tunicate sample (64.4 kg, wet wt) was homogenized, then phosphate buffer solution was added to the result homogenized solution and the solution was adjusted to pH 7±0.2. 10 % Potassium cyanide of which 10 mmol of homogenized volume was added to the homogenized solution, and the stirring mixture was allowed to stand for 5 hours. Then the mixture was macerated with methanol 50 l (×5). The solution was filtered, and the methanolic fitrate was evaporated to the aqueous emulsion which was extracted with ethyl acetate to give a dark-brown oil residue (53.27 g). The resulting residue was re-dissolved in 90% methanol in water (100 ml) and was partitioned with hexane 100 ml (×3) to afford the methanol residue (30.95 g), upon concentration (Scheme 16).

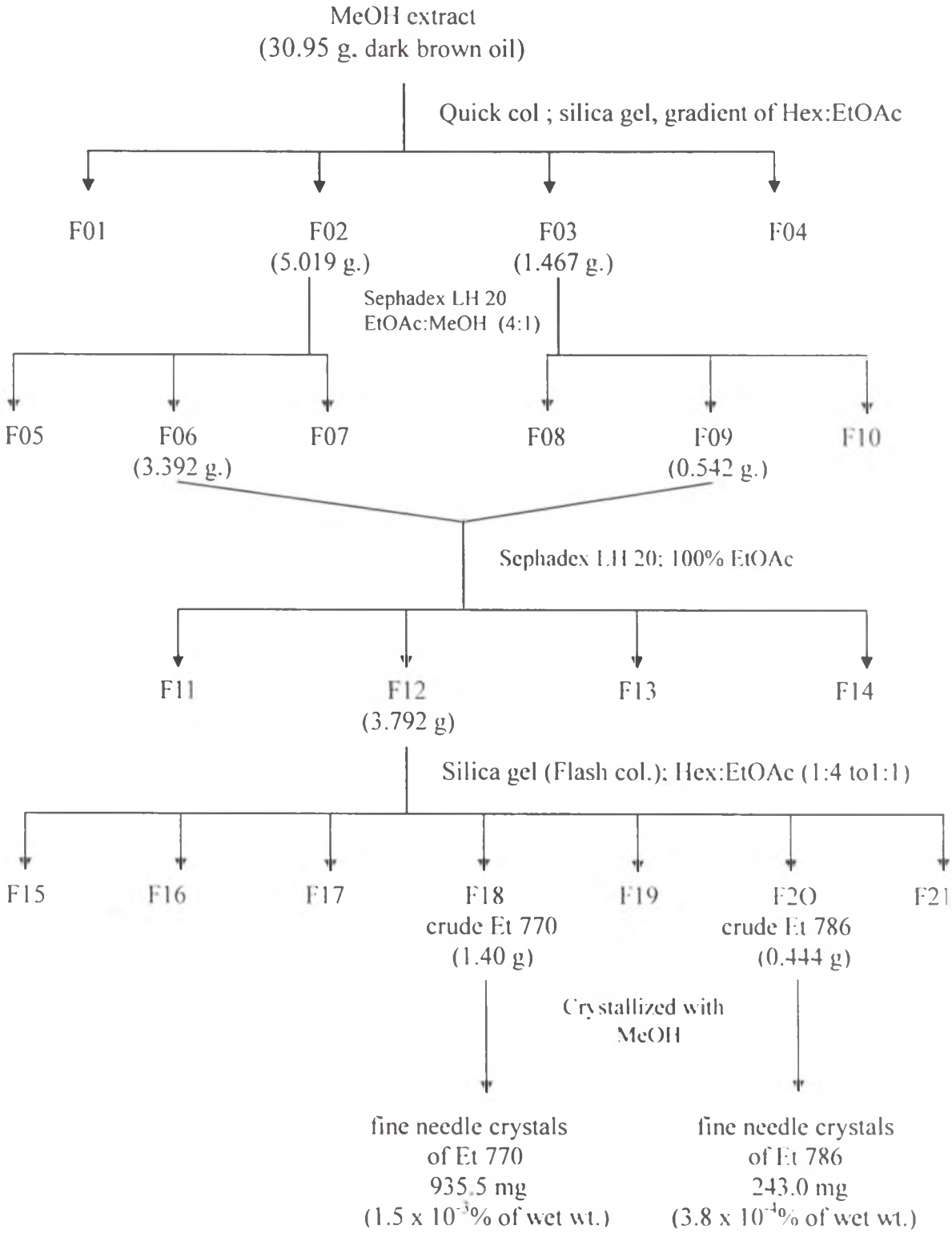
The combined methanol extract (30.95 g) was subjected to a quick column chromatography (column diameter =14 cm) using a gradient elution of hexane-ethyl acetate 1:1 to 1:5, 100% ethyl acetate and methanol, respectively. Fractions (100 ml) were collected and combined based on TLC pattern (hexane-ethyl acetate (1:4) as the eluant to obtain two fractions (F02 = 5.019 g and F03 = 1.467 g) which contained Et compounds. Each fraction was isolated by fractionated on a Sephadex LH-20 gel filtration column (column 5 × 120 cm.) using ethyl acetate-methanol (4:1) as the eluting solvent. Fractions F06 and F09 were combined and re-fractionated with a Sephadex LH-20 gel filtration column using ethyl acetate as the eluting solvent. The crude mixture of Et 770 and Et 786 was then separated by a silica gel Flash Column using an isocratic

elution of hexane-ethyl acetate (1:1). The fractions were combined according to their TLC pattern and afforded pale yellow precipitates of Et 770 (1.400 g) and Et 786 (0.444 g), respectively. Both precipitates of Et 770 and Et 786 were crystallized in methanol to give fine needle crystals of Et 770 (935.5 mg, 1.5×10^{-3} % of wet wt) and Et 786 (243.0 mg, 3.8×10^{-4} % of wet wt) as shown in Scheme 17.

Recollections of the tunicate were done in different two periods of time, June and July 2003. All collected tunicates were extracted with KCN pretreated procedure and isolated as described to afford Et 770 and Et 786 in the yields which were summarized in Table 11.



Scheme 16. Extraction of *Ecteinascidia thurstoni* collected in October 2002 and January 2003



Scheme 17. Isolation of ecteinascidin compounds from the tunicate collected in October 2002 and January 2003

7. Structural modification of ecteinascidins

7.1 Transformation of ecteinascidins

7.1.1 Transformation of Et 770 to Et 743

***Ecteinascidin 743* [1]:** Et 770 (7.7 mg, 0.01 mmol) was dissolved in a mixture of CH_3CN/H_2O [3:2 (v/v), 2.5 ml], and $AgNO_3$ (42.5 mg, 0.25 mmol, 25 equiv) was added to this solution. The suspension was stirred at 40 °C for 2 h and then cooled down to room temperature. The cool reaction mixture was filtered, concentrated, and water (10 ml) was added and extracted with $CHCl_3$ 20.0 ml (×3). The organic layers were combined and washed with brine (10 ml), dried over anh. Na_2SO_4 and concentrated to afford crude product (7.5 mg), the purification of which by flash silica gel column chromatography using an isocratic of hexane-ethyl acetate (1:3) as eluting solvent to give a colorless solid product Et 743 (**1**) (6.9 mg, 91 % yield), which was identical in all respects to that of the authentic sample (Wright *et al.*, 1990).

7.1.2 Transformation of Et 770 to Et 786

***Ecteinascidin 786* [4]:** To a solution of Et 770 (10.0 mg, 0.0129 mmol) in dry CH_2Cl_2 (2.0 ml) was slowly added *m*-CPBA (3.1 mg, 0.0143 mmol, 1.1 equiv), the mixture was stirred at 0 °C for 30 min. Then CH_2Cl_2 (50 ml) was added to the reaction mixture and was extracted with 5% $NaHCO_3$ solution 30 ml (×2). The organic layer was washed with water 30 ml (×2), dried over anh. Na_2SO_4, filtered and concentrated to give crude product (11.0 mg). The purification by flash silica gel column chromatography using the gradient elution of hexane-ethyl acetate (1:1, 1:3, 1:5 and 100 % EtOAc) afforded a colorless solid Et 786 (9.9 mg, 97 % yield), which was identical in all respects with that of the authentic sample (Suwanborirux *et al.*, 2002).

7.1.3 Transformation of Et 786 to Et 759B

***Ecteinascidin 759B* [3]:** To a solution of Et 786 (7.9 mg, 0.01 mmol) in a mixture of CH_3CN/H_2O [3:2 (v/v), 2.5 ml], was slowly added $AgNO_3$ (42.7 mg, 0.25 mmol, 25 equiv). The suspension was stirred at 40 °C for 24 hours and then cooled at room temperature, filtered, and concentrated. The residue was added water 10.0 ml and extracted with $CHCl_3$ 30.0 ml (× 3). The combined organic layers were washed with water 20.0 ml (× 3), dried over anh. Na_2SO_4 and concentrated to afford crude product (9.6 mg). Purification was done by flash silica gel column chromatography using a gradient of hexane-ethyl acetate (3:7, 2:8 and 100 % EtOAc) as eluting solvent to give Et 759B (6.6 mg, 86 % yield), which was identical in all respects with that of the authentic sample (Menchaca *et al.*, 1996).

7.2 Preparation of ecteinascidins analogs

***Ecteinascidin 770 18,6'-diacetate* [18]:** Acetic anhydride (4.7 µl, 0.05 mmol) was added to a stirred solution of Et 770 (7.7 mg, 0.01 mmol) and DMAP (0.6 mg, 0.005 mmol) in pyridine (1.0 ml) at 0°C, and the resulting solution was stirred for 4 h at 25°C. After the solvent was removed *in vacuo*, the residue was diluted with water (10 ml) and extracted with chloroform 20 ml (×3). The combined chloroform extract was washed with brine (20 ml), dried, and concentrated *in vacuo* to give a solid (10.7 mg). Purification by chromatography on a silica gel column eluting with hexane-ethyl acetate (4:1) afforded **18** as a pale yellow solid (7.3 mg, 82 % yield).

^{1}H–NMR δ: 6.95 (1H, s, 15-H), 6.62 (1H, s, 5'-H), 6.54 (1H, s, 8'-H), 6.04 and 5.98 (each 1H, s, OCHO), 5.02 (1H, d, J = 11.3 Hz, 22-H), 4.44 (1H, br s, 4-H), 4.34 (1H, br s, 1-H), 4.18 (1H, d, J = 2.8 Hz, 21-H), 4.12 (1H, dd, J = 11.3, 2.2 Hz, 22-H), 3.83 (1H, d, J = 4.6 Hz, 11-H), 3.72 (3H, s, 17-OCH_3), 3.56 (3H, s, 7'-OCH_3), 3.52 (1H, d, J = 4.6 Hz, 3-H), 3.46 (1H, dt, J = 4.9, 2.8 Hz 13-H), 3.12 (1H, m, 3'-H), 2.99 (2H, d, J = 4.9 Hz, 14-H_2), 2.83 (1H, m, 3'-H), 2.65 (1H, m, 4'-H), 2.49 (1H, m, 4'-H), 2.37 (3H, s, $OCOCH_3$), 2.33 (1H, br, 12'-H)*, 2.33 (3H, s, 16-CH_3), 2.30 (3H, s, $OCOCH_3$), 2.24 (3H, s, $OCOCH_3$), 2.24 (1H, m, 12'-H)*, 2.16 (3H, s, NCH_3), 2.04 (3H, s, 6-CH_3) (* the signals overlapped with the methyl signals).

^{13}C-NMR δ: 172.0 (11'-CO), 169.0 (s, OCOCH$_3$), 168.4 (s, OCOCH$_3$), 167.9 (s, OCOCH$_3$), 148.5 (C-7'), 148.0 (C-18), 145.6 (C-7), 143.8 (C-17), 141.3 (C-5), 140.4 (C-8), 138.6 (C-6'), 132.3 (C-9'), 131.5 (C-20), 130.6 (C-16), 128.7 (C-10'), 127.3 (C-15), 124.3 (C-19), 122.6 (C-5'), 120.8 (C-10), 117.8 (21-CN), 113.9 (C-9), 113.6 (C-6), 111.6 (C-8'), 102.0 (OCH_2O), 65.0 (C-1'), 61.1 (C-1), 60.1 (C-22), 60.1 (17-OCH_3), 59.6 (C-3), 59.2 (C-21), 55.8 (C-11), 55.0 (7'-OCH_3), 54.4 (C-13), 42.4 (C-12'), 42.1 (C-4), 41.5 (NCH_3), 39.6 (C-3'), 28.5 (C-4'), 24.0 (C-14), 20.6 (OCOCH$_3$), 20.6 (OCOCH$_3$), 20.2 (OCOCH$_3$), 15.8 (16-CH_3), 9.6 (6-CH_3).

IR ($CHCl_3$) cm^{-1} : 2931, 1766, 1433, 1371, 1263, 1197

HR-FAB-MS : *m/z* 855.2913 [M + H]$^+$ (Calcd for $C_{44}H_{47}N_4O_{12}S$, 855.2911)

FAB-MS (Glycerol) : *m/z* 855 [(M + H)$^+$]

$[\alpha]_D^{19}$: -37.6° (c = 0.56, $CHCl_3$)

***Ecteinascidin 786 18,6'-diacetate* [19]:** Using the same procedure described above, ecteinascidin 786 (7.9 mg, 0.01 mmol) was acetylated to give **19** as a pale yellow solid (6.8 mg, 78 % yield).

^{1}H–NMR δ: 6.93 (1H, s, 15-H), 6.65 (1H, s, 5'-H), 6.45 (1H, s, 8'-H), 6.08 and 6.02 (each 1H, s, OCHO), 4.92 (1H, d, *J* = 11.9 Hz, 22-H), 4.42 (1H, s, 1-H), 4.26 (1H, dd, *J* = 11.9, 2.2 Hz, 22-H), 4.20 (1H, dd, *J* = 4.9 Hz, 1.2 Hz, 11-H), 4.16 (1H, d, *J* = 2.6 Hz, 21-H), 4.02 (1H, br s, 4-H), 3.84 (3H, s, 17-OCH_3), 3.68 (1H, m, 3-H), 3.56 (3H, s, 7'-OCH_3),. 3.50 (1H, m, 13-H), 3.07 (2H, d, *J* = 5.3 Hz, 14-H_2), 3.01 (1H, br t, 3'-H), 2.80 (1H, m, 3'-H), 2.74 (1H, m, 4'-H), 2.51 (1H, m, 4'-H), 2.38 (3H, s, OCOCH$_3$), 2.33 (1H, br, 12'-H)*, 2.33 (3H, s, 16-CH_3), 2.28 (3H, s, OCOCH$_3$), 2.26 (3H, s, OCOCH$_3$), 2.24 (1H, m, 12'-H)*, 2.17 (3H, s, NCH_3), 2.09 (3H, s, 6-CH_3) (* the signals overlapped with the methyl signals).

IR ($CHCl_3$) cm^{-1} : 2927, 2854, 1768, 1448, 1369, 1197

HR-FAB-MS : *m/z* 871.2852 [M + H]$^+$ (Calcd for $C_{44}H_{47}N_4O_{13}S$, 871.2860)

FAB-MS (Glycerol) : *m/z* 871 [(M + H)$^+$]

$[\alpha]_D^{20}$: -97.3° (c = 0.16, $CHCl_3$)

***Ecteinascidin 743 18,6'-diaceate* [20]:** Et 770 (9.0 mg, 0.011 mmol) was dissolved in a mixture of acetonitrile and water [3:2 (v/v), 3.5 ml], and silver nitrate (42.7 mg, 0.25 mmol, 23.8 equiv) was added. The suspension was stirred at 40°C for 19 h. The reaction mixture was filtered and the precipitate was washed carefully with chloroform (30 ml). The combined filtrates was concentrated *in vacuo* to give a residue. The residue was diluted with water (10 ml) and extracted with chloroform 30 ml (×3). The combined organic extract was washed with brine (30 ml), dried, and concentrated *in vacuo* to give a residue (11.6 mg). The residue was purified by silica gel column chromatography with hexane-ethyl acetat (3:7) as eluant to give **20** as a pale yellow solid (3.5 mg, 41 % yield). Further elution with ethyl acetate afforded the starting material Et 770 (4.1 mg, 46 % yield).

^{1}H–NMR δ: 6.98 (1H, s, 15-H), 6.61 (1H, s, 5'-H), 6.54 (1, s, 8'-H), 6.02 and 5.95 (each 1H, d, J = 1.0 Hz, OCHO), 5.14 (1H, d, J = 11.2 Hz, 22-H), 4.88 (1H, br s, 1-H), 4.54 (1H, br s, 21-H), 4.45 (1H, br s, 4-H), 4.36 (1H, dd, J = 4.9 Hz, 1.2 Hz, 11-H), 4.04 (1H, dd, J = 11.5, 2.2 Hz, 22-H), 3.79 (3H, s, 17-OCH_3), 3.55 (3H, s, 7'-OCH_3), 3.52 (1H, m, 3-H), 3.13 (1H, m, 13-H), 2.98 (2H, d, J = 5.3 Hz, 14-H_2), 2.85 (1H, br t, 3'-H), 2.60 (1H, m, 3'-H), 2.52 (1H, m, 4'-H), 2.44 (1H, m, 4'-H), 2.38 (3H, s, 16-CH_3), 2.34 (3H, s, $OCOCH_3$), 2.34 (1H, br, 12'-H)*, 2.30 (3H, s, NCH_3), 2.24 (3H, s, $OCOCH_3$), 2.24 (1H, m, 12'-H)*, 2.03 (3H, s, 6-CH_3) (* the signals overlapped with the methyl signals).

IR ($CHCl_3$) cm^{-1} : 2925, 2854, 1743, 1431, 1369, 1261, 1195

HR-FAB-MS : m/z 828.2800 $[M + H - H_2O]^+$ (Calcd for $C_{41}H_{46}N_3O_{12}S$, 828.2802)

FAB-MS (Glycerol) : m/z 828 $[(M + H - H_2O)^+]$

$[\alpha]_D^{20}$: -47.9° (c = 0.16, $CHCl_3$)

General procedure for the preparation of aromatic ester derivatives via acid chlorides:

Et 770 (7.7 mg, 0.01 mmol) and DMAP (0.6 mg, 0.005 mmol) were dissolved in pyridine (1 ml), and equimolar quantity of each coresponding acid chloride (0.01 mmol) was added to this mixture at 0°C. The reaction mixture was stirred at 25°C for 4-12 h. After the solvent was removed *in vacuo*, the residue was diluted with water (10 ml) and extracted with $CHCl_3$ 20 ml (×3). The combined extracts were washed with brine (20 ml), dried, and concentrated *in vacuo* to give a solid. This residue was purified by silica gel column chromatography using hexane:ethylacetate mixture in appropriated ratio as an eluant to give the purified product.

***Ecteinascidin 770 6'-O-benzoate* [21]:** Yield 88%. ^{1}H-NMR δ: 8.14 (2H, d, *J* = 8.4 Hz, 2''- and 6''-H), 7.60 (1H, t, *J* = 8.4 Hz, 4''-H), 7.47 (2H, t, *J* = 8.4 Hz, 3''- and 5''-H), 6.73 (1H, s, 5'-H), 6.60 (1H, s, 8'-H), 6.48 (1, s, 15-H), 6.03, 5.97 (each 1H, br s, OCHO), 5.73 (1H, br s, 18-OH), 5.03 (1H, d, *J* = 11.6 Hz, 22-H), 4.58 (1H, br s, 4-H), 4.34 (1H, br s, 1-H), 4.29 (1H, d, *J* = 3.7 Hz, 11-H), 4.18 (1H, d, *J* = 2.7 Hz, 21-H), 4.11 (1H, dd, *J* = 11.6, 2.2 Hz, 22-H), 3.79 (3H, s, 17-OCH_3), 3.55 (3H, s, 7'-OCH_3), 3.55 (1H, d, *J* = 4.6 Hz, 3-H), 3.43 (1H, br t, 13-H), 3.14 (1H, m, 3'-H), 2.95 (2H, d, *J* = 6.1 Hz, 14-H_2), 2.84 (1H, m, 3'-H), 2.64 (1H, m, 4'-H), 2.54 (1H, m, 4'-H), 2.33 (1H, br, 12'-H), 2.33 (3H, s, 16-CH_3), 2.28 (3H, s, $OCOCH_3$), 2.21 (3H, s, NCH_3), 2.21 (1H, br, 12-H), 2.03 (3H, s, 6-CH_3)

IR ($CHCl_3$) cm^{-1} : 2931, 2854, 2810, 1743, 1602, 1508, 1431, 1371, 1263

HR-FAB-MS : *m/z* 875.2964 $[M + H]^+$ (Calcd for $C_{47}H_{47}N_4O_{11}S$, 875.2962)

FAB-MS (Dithiothreitol:Thioglycerol = 1:1) : *m/z* 875 $[(M + H)^+]$

$[\alpha]_D^{22}$: -20.2° (c = 0.33, $CHCl_3$)

***Ecteinascidin 770 6'-O-4"-nitrobenzoate* [22]:** Yield 95%. ^{1}H-NMR δ: 8.31 (4H, s, Ar-H), 6.75 (1H, s, 5'-H), 6.64 (1H, s, 8'-H), 6.61 (1H, s, 15-H), 6.02, 5.96 (each 1H, d, J = 0.9 Hz, OCHO), 5.76 (1H, br s, 18-OH), 5.04 (1H, d, J = 11.6 Hz, 22-H), 4.59 (1H, br s, 4-H), 4.35 (1H, br s, 1-H), 4.29 (1H, d, J = 3.7 Hz, 11-H), 4.19 (1H, d, J = 2.7 Hz, 21-H), 4.14 (1H, dd, J = 11.6, 2.2 Hz, 22-H), 3.80 (3H, s, 17-OCH_3), 3.55 (3H, s, 7'-OCH_3), 3.53 (1H, d, J = 4.6 Hz, 3-H), 3.43 (1H, br t, 13-H), 3.15 (1H, m, 3'-H), 2.96 (2H, d, J = 6.1 Hz, 14-H_2), 2.83 (1H, m, 3'-H), 2.67 (1H, m, 4'-H), 2.52 (1H, m, 4'-H), 2.34 (1H, br d, 12'-H), 2.33 (3H, s, 16-CH_3), 2.27 (3H, s, $OCOCH_3$), 2.21 (3H, s, NCH_3), 2.21 (1H, br d, 12-H), 2.03 (3H, s, 6-CH_3);

^{13}C-NMR δ: 172.0 (11'-CO), 168.2 ($OCOCH_3$), 162.8 (OCOPh), 150.8 (C-4"), 148.3 (C-7'), 147.9 (C-18), 145.4 (C-7), 143.1 (C-17), 141.4 (C-5), 140.1 (C-8), 138.3 (C-6'), 134.7 (C-1"), 133.2 (C-9'), 131.3 (C-2"), 131.3 (C-6"), 130.8 (C-20), 129.4 (C-16), 128.8 (C-10'), 123.6 (C-3"), 123.6 (C-5"), 122.3 (C-5'), 121.0 (C-10), 120.7 (C-15), 118.2 (C-19), 118.0 (21-CN), 114.0 (C-9), 113.5 (C-6), 111.9 (C-8'), 101.9 (OCH_2O), 64.9 (C-1'), 61.1 (C-1), 60.3 (17-OCH_3), 60.2 (C-22), 59.6 (C-21), 59.6 (C-3), 55.2 (7'-OCH_3), 54.7 (C-11), 54.6 (C-13), 42.3 (C-12'), 42.0 (C-4), 41.6 (NCH_3), 39.5 (C-3'), 29.6 (C-4'), 24.2 (C-14), 20.4 ($OCOCH_3$), 15.8 (16-CH_3), 9.7 (6-CH_3).

IR ($CHCl_3$) cm^{-1} : 2929, 2810, 1745, 1529, 1508, 1431, 1350, 1319

HR-FAB-MS : *m/z* 920.2824 $[M + H]^+$ (Calcd for $C_{47}H_{46}N_5O_{13}S$, 920.2812)

FAB-MS (Dithiothreitol:Thioglycerol = 1:1) : *m/z* 920 $[(M + H)^+]$

$[\alpha]_D^{20}$: -28.8° (c = 0.68, $CHCl_3$)

***Ecteinascidin 770 6'-O-3"-nitrobenzoate* [23]:** Yield 66%. ^{1}H-NMR δ: 8.96 (1H, t, J = 1.8 Hz, 2"-H), 8.45 (2H, dd, J = 8.1, 1.8 Hz, 3"- and 4"-H), 7.69 (1H, t, J = 8.1 Hz, 5"-H), 6.75 (1H, s, 5'-H), 6.63 (1H, s, 8'-H), 6.61 (1H, s, 15-H), 6.03 and 5.97 (each 1H, br s, OCHO), 5.73 (1H, br s, 18-OH), 5.04 (1H, d, J = 11.6 Hz, 22-H), 4.59 (1H, br s, 4-H), 4.35 (1H, br s, 1-H), 4.29 (1H, d, J = 3.7 Hz, 11-H), 4.19 (1H, d, J = 2.7 Hz, 21-H), 4.12 (1H, dd, J = 11.6, 2.2 Hz, 22-H), 3.81 (3H, s, 17-OCH_3), 3.55 (3H, s, 7'-OCH_3), 3.55 (1H, d, J = 4.6 Hz, 3-H), 3.43 (1H, br t, 13-H), 3.15 (1H, m, 3'-H), 2.95 (2H, d, J = 6.1 Hz, 14-H_2), 2.84 (1H, m, 3'-H), 2.65 (1H, m, 4'-H), 2.55 (1H, m, 4'-H), 2.34 (1H, br,

12'-H), 2.34 (3H, s, 16-CH_3), 2.29 (3H, s, $OCOCH_3$), 2.22 (3H, s, NCH_3), 2.21 (1H, br, 12-H), 2.04 (3H, s, 6-CH_3).

IR ($CHCl_3$) cm^{-1} : 2931, 2810, 1747, 1618, 1535, 1512, 1452, 1352,

HR-FAB-MS : *m/z* 920.2831 $[M + H]^+$ (Calcd for $C_{47}H_{46}N_5O_{13}S$, 920.2812)

FAB-MS (Dithiothreitol:Thioglycerol = 1:1) : *m/z* 920 $[(M + H)^+]$

$[\alpha]_D^{20}$: -18.5° (c = 0.21, $CHCl_3$)

***Ecteinascidin 770 6'-O-2"-nitrobenzoate* [24]:** Yield 83%, ^{1}H-NMR δ: 7.92 (1H, d, *J* = 8.9 Hz, 3"-H), 7.88 (1H, d, *J* = 7.4 Hz, 6"-H), 7.72 (2H, m, 4"- and 5"-H), 6.93 (1H, s, 5'-H), 6.60 (1H, s, 8'-H), 6.50 (1H, s, 15-H), 6.08 and 6.00 (each 1H, br s, OCHO), 5.82 (1H, s, 18-OH), 5.04 (1H, d, *J* = 11.6 Hz, 22-H), 4.67 (1H, br s, 4-H), 4.32 (1H, d, *J* = 4.6 Hz, 1-H), 4.24 (1H, dd, *J* = 11.6, 2.2 Hz, 22-H), 4.20 (1H, d, *J* = 3.7 Hz, 11-H), 4.17 (1H, d, *J* = 2.7 Hz, 21-H), 3.96 (3H, s, 17-OCH_3), 3.61 (3H, s, 7'-OCH_3), 3.56 (1H, d, *J* = 4.2 Hz, 3-H), 3.44 (1H, br t, 13-H), 2.96 (1H, m, 3'-H), 2.92 (2H, d, *J* = 6.1 Hz, 14-H_2), 2.84 (1H, m, 3'-H), 2.65 (1H, m, 4'-H), 2.55 (1H, m, 4'-H), 2.44 (3H, s, 16-CH_3), 2.34 (1H, br, 12'-H), 2.33 (3H, s, $OCOCH_3$), 2.24 (3H, s, NCH_3), 2.21 (1H, br, 12-H), 2.05 (3H, s, 6-CH_3)

IR ($CHCl_3$) cm^{-1} : 2927, 2854, 1757, 1535, 1508, 1458, 1350

HR-FAB-MS : *m/z* 920.2811 $[M + H]^+$ (Calcd for $C_{47}H_{46}N_5O_{13}S$, 920.2812)

FAB-MS (Dithiothreitol:Thioglycerol = 1:1) : *m/z* 920 $[(M + H)^+]$

$[\alpha]_D^{19}$: -17.8° (c = 0.15, $CHCl_3$)

***Ecteinascidin 770 6'-O-4"-bromobenzoate* [25]:** Yield 97%, ^{1}H-NMR δ: 7.99 (2H, d, *J* = 8.5 Hz, 2"- and 6"-H), 7.61 (2H, d, *J* = 8.5 Hz, 3"- and 5"-H), 6.73 (1H, s, 5'-H), 6.61 (1H, s, 8'-H), 6.60 (1H, s, 15-H), 6.03 and 5.97 (each 1H, s, OCHO), 5.73 (1H, br s, 18-OH), 5.04 (1H, d, *J* = 11.6 Hz, 22-H), 4.59 (1H, br s, 4-H), 4.35 (1H, br s, 1-H), 4.29 (1H, d, *J* = 4.6 Hz, 11-H), 4.19 (1H, d, *J* = 2.4 Hz, 21-H), 4.12 (1H, dd, *J* = 11.6, 2.2 Hz, 22-H), 3.81 (3H, s, 17-OCH_3), 3.55 (3H, s, 7'-OCH_3), 3.53 (1H, d, *J* = 4.9 Hz, 3-H), 3.43 (1H, br t, 13-H), 3.15 (1H, m, 3'-H), 2.96 (2H, d, *J* = 6.1 Hz, 14-H_2), 2.84 (1H, m,

3'-H), 2.65 (1H, m, 4'-H), 2.55 (1H, m, 4'-H), 2.34 (1H, br, 12'-H), 2.34 (3H, s, 16-CH_3), 2.29 (3H, s, $OCOCH_3$), 2.22 (3H, s, NCH_3), 2.21 (1H, br, 12-H), 2.04 (3H, s, 6-CH_3); Figure

^{13}C-NMR δ: 172.0 (11'-CO), 168.1 ($OCOCH_3$), 164.0 (OCOPh), 147.9 (C-7'), 147.9 (C-18), 145.4 (C-7), 143.2 (C-17), 141.4 (C-5), 140.1 (C-8), 138.3 (C-6'), 132.8 (C-9'), 131.8 (C-3"), 131.8 (C-5"), 131.7 (C-2"), 131.7 (C-6"), 131.4 (C-1"), 130.8 (C-20), 129.4 (C-10'), 128.7 (C-16), 128.2 (C-4"), 122.5 (C-5'), 120.8 (C-10), 120.6 (C-15), 118.1 (C-19), 118.0 (21-CN), 113.9 (C-9), 113.5 (C-6), 111.8 (C-8'), 101.9 (OCH_2O), 65.0 (C-1'), 61.1 (C-1), 60.4 (17-OCH_3), 60.2 (C-22), 59.6 (C-21), 59.6 (C-3), 55.2 (7'-OCH_3), 54.6 (C-11), 54.6 (C-13), 42.0 (C-12'), 42.0 (C-4), 41.6 (NCH_3), 39.6 (C-3'), 29.6 (C-4'), 24.1 (C-14), 20.4 ($OCOCH_3$), 15.8 (16-CH_3), 9.7 (6-CH_3)

IR ($CHCl_3$) cm^{-1} : 2929, 2854, 2810, 1743, 1519, 1508, 1431, 1369

HR-FAB-MS : *m/z* 953.2057 $[M + H]^+$ (Calcd for $C_{47}H_{46}N_4O_{11}BrS$, 953.2067)

FAB-MS (Dithiothreitol:Thioglycerol = 1:1) : *m/z* 955 $[(M + 2 + H)^+]$
953 $[(M + H)^+]$

$[\alpha]_D^{19}$: -30.9° (c = 0.75, $CHCl_3$)

***Ecteinascidin 770 6'-O-3"-bromobenzoate* [26]:** Yield 84%. ^{1}H-NMR δ: 8.27 (1H, t, *J* = 2.0 Hz, 2"-H), 8.06 (1H, d, *J* = 8.1 Hz, 6"-H), 7.73 (1H, dd, *J* = 8.1, 2.0 Hz, 4"-H), 7.35 (1H, t, *J* = 8.1 Hz, 5"-H), 6.75 (1H, s, 5'-H), 6.63 (1H, s, 8'-H), 6.61 (1H, s, 15-H), 6.02 and 5.97 (each 1H, br s, OCHO), 5.73 (1H, br s, 18-OH), 5.03 (1H, d, *J* = 11.2 Hz, 22-H), 4.57 (1H, br s, 4-H), 4.33 (1H, br s, 1-H), 4.29 (1H, d, *J* = 5.3 Hz, 11-H), 4.19 (1H, d, *J* = 2.7 Hz, 21-H), 4.13 (1H, dd, *J* = 11.2, 2.2 Hz, 22-H), 3.80 (3H, s, 17-OCH_3), 3.55 (3H, s, 7'-OCH_3), 3.51 (1H, d, *J* = 5.3 Hz, 3-H), 3.43 (1H, br t, 13-H), 3.13 (1H, m, 3'-H), 2.96 (2H, d, *J* = 6.1 Hz, 14-H_2), 2.83 (1H, m, 3'-H), 2.66 (1H, m, 4'-H), 2.55 (1H, m, 4'-H), 2.34 (1H, br, 12'-H), 2.33 (3H, s, 16-CH_3), 2.28 (3H, s, $OCOCH_3$), 2.21 (3H, s, NCH_3), 2.21 (1H, br, 12-H), 2.03 (3H, s, 6-CH_3)

IR ($CHCl_3$) cm^{-1} : 2962, 2925, 2852, 1745, 1261

HR-FAB-MS : *m/z* 953.2018 $[M + H]^+$ (Calcd for $C_{47}H_{46}N_4O_{11}BrS$, 953.2067)

FAB-MS (Dithiothreitol:Thioglycerol = 1:1) : *m/z* 955 $[(M + 2 + H)^+]$, 953 $[(M + H)^+]$

$[\alpha]_D^{21}$: -17.9° (c = 0.14, $CHCl_3$)

***Ecteinascidin 770 6'-O-2"-bromobenzoate* [27]:** Yield 74%, ^{1}H-NMR δ: 8.00 (1H, m, 6"-H), 7.70 (1H, m, 3"-H), 7.38 (2H, m, 3"- and 4"-H), 6.77 (1H, s, 5'-H), 6.63 (1H, s, 8'-H), 6.60 (1H, s, 15-H), 6.03 and 5.97 (each 1H, br s, OCHO), 5.73 (1H, s, 18-OH), 5.03 (1H, d, *J* = 11.6 Hz, 22-H), 4.57 (1H, br s, 4-H), 4.34 (1H, br s, 1-H), 4.29 (1H, d, *J* = 4.8 Hz, 11-H), 4.19 (1H, d, *J* = 2.8 Hz, 21-H), 4.12 (1H, dd, *J* = 11.6, 2.4 Hz, 22-H), 3.80 (3H, s, 17-OCH_3), 3.58 (3H, s, 7'-OCH_3), 3.53 (1H, d, *J* = 5.0 Hz, 3-H), 3.43 (1H, br t, 13-H), 3.14 (1H, m, 3'-H), 2.96 (2H, d, *J* = 6.1 Hz, 14-H_2), 2.82 (1H, m, 3'-H), 2.68 (1H, m, 4'-H), 2.52 (1H, m, 4'-H), 2.33 (1H, br, 12'-H), 2.33 (3H, s, 16-CH_3), 2.28 (3H, s, $OCOCH_3$), 2.23 (3H, s, NCH_3), 2.21 (1H, br, 12-H), 2.04 (3H, s, 6-CH_3)

IR ($CHCl_3$) cm^{-1} : 2966, 1751, 1508, 1431, 1369, 1261, 1240

HR-FAB-MS : *m/z* 953.2080 $[M + H]^+$ (Calcd for $C_{47}H_{46}N_4O_{11}BrS$, 953.2067)

FAB-MS (Dithiothreitol:Thioglycerol = 1:1) : *m/z* 955 $[(M + 2 + H)^+]$, 953 $[(M + H)^+]$

$[\alpha]_D^{21}$:-44.9° (c = 0.17, $CHCl_3$)

***Ecteinascidin 770 6'-O-4"-methoxybenzoate* [28]:** Yield 67%, ^{1}H-NMR δ: 8.09 (2H, d, *J* = 8.6 Hz, 2"- and 6"-H), 6.95 (2H, d, *J* = 8.6 Hz, 3"- and 5"-H), 6.72 (1H, s, 5'-H), 6.60 (1H, s, 8'-H), 6.60 (1H, s, 15-H), 6.03 and 5.97 (each 1H, br s, OCHO), 5.72 (1H, s, 18-OH), 5.03 (1H, d, *J* = 11.6 Hz, 22-H), 4.57 (1H, br s, 4-H), 4.33 (1H, br s, 1-H), 4.28 (1H, d, *J* = 4.8 Hz, 11-H), 4.18 (1H, d, *J* = 2.8 Hz, 21-H), 4.12 (1H, dd, *J* = 11.6, 2.4 Hz, 22-H), 3.87 (3H, s, 4"-OCH_3), 3.80 (3H, s, 17-OCH_3), 3.55 (3H, s, 7'-OCH_3), 3.55 (1H, d, *J* = 5.0 Hz, 3-H), 3.42 (1H, br t, 13-H), 3.14 (1H, m, 3'-H), 2.94 (2H, d, *J* = 6.1 Hz, 14-H_2), 2.82 (1H, m, 3'-H), 2.65 (1H, m, 4'-H), 2.51 (1H, m, 4'-H), 2.33 (1H, br, 12'-H), 2.32 (3H, s, 16-CH_3), 2.28 (3H, s, $OCOCH_3$), 2.21 (3H, s, NCH_3), 2.21 (1H, br, 12-H), 2.03 (3H, s, 6-CH_3)

IR ($CHCl_3$) cm^{-1} : 2927, 2985, 2812, 1739, 1606, 1514, 1461

HR-FAB-MS : *m/z* 905.3058 $[M + H]^+$ (Calcd for $C_{48}H_{49}N_4O_{12}S$, 905.3068)

FAB-MS (Dithiothreitol:Thioglycerol = 1:1) : *m/z* 905 $[(M + H)^+]$

$[\alpha]_D^{20}$:-34.8° (c = 0.27, $CHCl_3$)

***Ecteinascidin 770 6'-O-2"-methoxybenzoate* [29]:** Yield 72%, ^{1}H-NMR δ: 7.98 (1H, dd, *J* = 8.2, 1.5 Hz, 6"-H), 7.51 (1H, dt, *J* = 8.2, 1.5 Hz, 4"-H), 7.00 (2H, t, *J* = 8.2 Hz, 3"-, 5"-H), 6.73 (1H, s, 5'-H), 6.60 (1H, s, 8'-H), 6.60 (1H, s, 15-H), 6.03 and 5.97 (each 1H, br s, OCHO), 5.73 (1H, s, 18-OH), 5.03 (1H, d, *J* = 11.3 Hz, 22-H), 4.57 (1H, br s, 4-H), 4.33 (1H, br s, 1-H) , 4.28 (1H, d, *J* = 4.8 Hz, 11-H), 4.18 (1H, d, *J* = 2.4 Hz, 21-H), 4.12 (1H, dd, *J* = 11.6, 2.4 Hz, 22-H), 3.89 (3H, s, 4"-OCH_3), 3.81 (3H, s, 17-OCH_3), 3.55 (3H, s, 7'-OCH_3), 3.53 (1H, d, *J* = 4.6 Hz, 3-H), 3.42 (1H, br t, 13-H), 3.14 (1H, m, 3'-H), 2.94 (2H, d, *J* = 6.1 Hz, 14-H_2), 2.82 (1H, m, 3'-H), 2.66 (1H, m, 4'-H), 2.51 (1H, m, 4'-H), 2.34 (1H, br, 12'-H), 2.32 (3H, s, 16-CH_3), 2.28 (3H, s, $OCOCH_3$), 2.21 (3H, s, NCH_3), 2.21 (1H, br, 12-H), 2.03 (3H, s, 6-CH_3);

^{13}C-NMR δ: 172.0 (11'-CO), 168.0 ($O\underline{C}OCH_3$), 163.7 ($O\underline{C}OPh$), 159.9 (C-2"), 148.7 (C-7'), 147.9 (C-18), 145.4 (C-7), 143.1 (C-17), 141.4 (C-5), 140.1 (C-8), 138.3 (C-6'), 135.0 (C-4"), 132.8 (C-9'), 132.4 (C-6"), 130.8 (C-20), 129.2 (C-10'), 128.6 (C-16), 122.8 (C-5'), 120.8 (C-10), 120.6 (C-15), 120.2 (C-1"), 120.2 (C-5"), 118.1 (C-19), 118.0 (21-CN), 113.9 (C-9), 113.5 (C-6), 112.3 (C-3"), 111.6 (C-8'), 101.9 (OCH_2O), 65.0 (C-1'), 61.1 (C-1), 60.4 (17-OCH_3), 60.2 (C-22), 59.5 (C-21), 59.5 (C-3), 56.0 (7'-OCH_3), 56.0 (2"-OCH_3), 55.3 (C-11), 54.6 (C-13), 42.3 (C-12'), 42.0 (C-4), 41.6 (NCH_3), 39.6 (C-3'), 28.7 (C-4'), 24.2 (C-14), 20.4 ($OCO\underline{C}H_3$), 15.9 (16-CH_3), 9.7 (6-CH_3).

IR ($CHCl_3$) cm^{-1} : 3506, 2933, 2854, 2810, 1747, 1600, 1583, 1490

HR-FAB-MS : *m/z* 905.3074 $[M + H]^+$ (Calcd for $C_{48}H_{49}N_4O_{12}S$, 905.3068)

FAB-MS (Dithiothreitol:Thioglycerol = 1:1) : *m/z* 905 $[(M + H)^+]$

$[\alpha]_D^{19}$:-48.2° (c = 0.85, $CHCl_3$)

***Ecteinascidin 770 6'-O-nicotinate* [30]:** Yield 76%, ^{1}H-NMR δ: 9.29 (1H, dd, *J* = 2.2, 0.6 Hz, 2"-H), 8.80 (1H, dd, *J* = 4.9, 1.8 Hz, 6"-H), 8.34 (1H, ddd, *J* = 7.9, 2.2, 1.8 Hz, 4"-H), 7.40 (1H, ddd, *J* = 7.9, 4.9, 0.6 Hz, 5"-H), 6.72 (1H, s, 5'-H), 6.63 (1H, s, 8'-H), 6.59 (1, s, 15-H), 5.99 and 5.94 (each 1H, d, *J* = 1.2 Hz, OCHO), 5.77 (1H, br s, 18-OH), 5.01 (1H, d, *J* = 11.3 Hz, 22-H), 4.56 (1H, br s, 4-H), 4.32 (1H, br s, 1-H), 4.27 (1H, dd, *J* = 4.9, 1.2 Hz, 11-H), 4.16 (1H, d, *J* = 2.4 Hz, 21-H), 4.11 (1H, dd, *J* = 11.6, 2.4 Hz, 22-H), 3.78 (3H, s, 17-OCH_3), 3.53 (3H, s, 7'-OCH_3), 3.49 (1H, d, *J* = 2.7 Hz, 3-H), 3.39 (1H, br t, 13-H), 3.12 (1H, ddd, *J* = 11.6, 10.1, 3.7 Hz, 3'-H), 2.92 (2H, d, *J* = 6.1 Hz, 14-H_2), 2.84 (1H, ddd, *J* = 11.6, 5.5, 3.4 Hz, 3'-H), 2.64 (1H, ddd, *J* = 15.9, 10.1, 5.5 Hz, 4'-H), 2.50 (1H, ddd, *J* = 15.9, 3.7, 3.4 Hz, 4'-H), 2.34 (1H, d, *J* = 14.6 Hz, 12'-H), 2.31 (3H, s, 16-CH_3), 2.26 (3H, s, $OCOCH_3$), 2.18 (3H, s, NCH_3), 2.14 (1H, d, *J* = 14.6 Hz, 12'-H), 2.01 (3H, s, 6-CH_3)

^{13}C-NMR δ: 172.1 (11'-CO), 168.1 (5-O<u>C</u>OCH_3), 163.3 (O<u>C</u>OAr), 153.8 (C-6"), 151.5 (C-2"), 148.5 (C-7'), 147.9 (C-18), 145.4 (C-7), 143.2 (C-17), 141.4 (C-5), 140.1 (C-8), 138.3 (C-6'), 137.6 (C-4"), 133.0 (C-9'), 130.8 (C-20), 129.3 (C-16), 128.8 (C-10'), 125.4 (C-3"), 123.4 (C-5"), 122.4 (C-5'), 121.1 (C-10), 120.7 (C-15), 118.2 (21-CN), 118.1 (C-19), 114.0 (C-9), 113.4 (C-6), 111.9 (C-8'), 101.9 (OCH_2O), 64.9 (C-1'), 61.1 (C-1), 60.3 (17-OCH_3), 60.2 (C-22), 59.7 (C-21), 59.6 (C-3), 55.2 (7'-OCH_3), 54.7 (C-11), 54.6 (C-13), 42.4 (C-12'), 41.9 (C-4), 41.6 (NCH_3), 39.6 (C-3'), 28.7 (C-4'), 24.2 (C-14), 20.4 ($OCO\underline{CH_3}$), 15.8 (16-CH_3), 9.7 (6-CH_3)

IR ($CHCl_3$) cm^{-1} : 2921, 2854, 2808, 1743, 1618, 1591, 1508, 1431, 1369

HR-FAB-MS : *m/z* 876.2911 $[M + H]^+$ (Calcd for $C_{46}H_{46}N_3O_{11}S$, 876.2914)

FAB-MS (Dithiothreitol:Thioglycerol = 1:1) : *m/z* 876 $[(M + H)^+]$

$[\alpha]_D^{20}$:-33.7° (c = 0.45, $CHCl_3$)

***Ecteinascidin 770 6'-O-isonicotinate* [31]:** Yield 85%, ^{1}H-NMR δ: 8.80 (2H, dd, *J* = 4.5, 1.5 Hz, 2"-H), 7.92 (2H, dd, *J* = 4.5, 1.5 Hz, 3"-H), 6.72 (1H, s, 5'-H), 6.62 (1H, s, 8'-H), 6.59 (1, s, 15-H), 5.99 and 5.94 (each 1H, d, *J* = 0.9 Hz, OCHO), 5.77 (1H, br s, 18-OH), 5.01 (1H, d, *J* = 11.3 Hz, 22-H), 4.57 (1H, br s, 4-H), 4.32 (1H, br s, 1-H), 4.27 (1H, dd, *J* = 4.9, 1.2 Hz, 11-H), 4.17 (1H, d, *J* = 2.7 Hz, 21-H), 4.11 (1H, dd, *J* = 11.6, 2.4

Hz, 22-H), 3.78 (3H, s, 17-OCH_3), 3.53 (3H, s, 7'-OCH_3), 3.50 (1H, d, *J* = 4.9 Hz, 3-H), 3.40 (1H, br t, 13-H), 3.12 (1H, ddd, *J* = 11.6, 10.1, 3.7 Hz, 3'-H), 2.93 (2H, d, *J* = 6.1 Hz, 14-H_2), 2.80 (1H, ddd, *J* = 11.6, 5.5, 3.4 Hz, 3'-H), 2.64 (1H, ddd, *J* = 15.9, 10.1, 5.5 Hz, 4'-H), 2.50 (1H, ddd, *J* = 15.9, 3.7, 3.4 Hz, 4'-H), 2.34 (1H, d, *J* = 14.6 Hz, 12'-H), 2.31 (3H, s, 16-CH_3), 2.26 (3H, s, $OCOCH_3$), 2.19 (3H, s, NCH_3), 2.14 (1H, d, *J* = 14.6 Hz, 12'-H), 2.01 (3H, s, 6-CH_3)

^{13}C-NMR δ: 172.1 (11'-CO), 168.1 (5-O<u>C</u>OCH_3), 163.2 (O<u>C</u>OAr), 150.7 (C-2"), 150.7 (C-6"), 148.3 (C-7'), 147.8 (C-18), 145.3 (C-7), 143.1 (C-17), 141.3 (C-5), 140.1 (C-8), 138.3 (C-6'), 136.6 (C-4"), 133.2 (C-9'), 130.8 (C-20), 129.3 (C-16), 128.9 (C-10'), 123.3 (C-3"), 123.3 (C-5"), 122.3 (C-5'), 121.1 (C-10), 120.7 (C-15), 118.2 (21-CN), 118.1 (C-19), 114.0 (C-9), 113.4 (C-6), 111.9 (C-8'), 101.9 (OCH_2O), 64.9 (C-1'), 61.1 (C-1), 60.3 (17-OCH_3), 60.2 (C-22), 59.7 (C-21), 59.6 (C-3), 55.2 (7'-OCH_3), 54.7 (C-11), 54.6 (C-13), 42.3 (C-12'), 41.9 (C-4), 41.6 (NCH_3), 39.6 (C-3'), 28.7 (C-4'), 24.2 (C-14), 20.4 (OCO<u>C</u>H_3), 15.8 (16-CH_3), 9.7 (6-CH_3)

IR ($CHCl_3$) cm^{-1} : 3315, 2933, 2810, 1747, 1589, 1560, 1508, 1409, 1369

HR-FAB-MS : *m/z* 876.2921 $[M + H]^+$ (Calcd for $C_{46}H_{46}N_5O_{11}S$, 876.2914)

FAB-MS (Dithiothreitol:Thioglycerol = 1:1) : *m/z* 876 $[(M + H)^+]$

$[\alpha]_D^{20}$:-38.7° (c = 0.45, $CHCl_3$)

***Ecteinascidin 770 6'-O-1"-naphthoate* [32]:** Yield 96%, ^{1}H-NMR δ: 8.94 (1H, d, *J* = 8.3 Hz, 8"-H), 8.37 (1H, dd, *J* = 7.3, 1.3 Hz, 2"-H), 8.06 (1H, d, *J* = 8.1 Hz, 4"-H), 7.89 (1H, dd, *J* = 7.9, 1.5 Hz, 5"-H), 7.61 (1H, m, 7"-H), 7.58 (1H, m, 6"-H), 7.55 (1H, dd, *J* = 8.1, 7.3 Hz, 3"-H), 6.79 (1H, s, 5'-H), 6.65 (1H, s, 8'-H), 6.60 (1H, s, 15-H), 6.02 and 6.00 (each 1H, d, *J* = 1.0 Hz, OCHO), 5.76 (1H, br s, 18-OH), 5.00 (1H, d, *J* = 11.4 Hz, 22-H), 4.57 (1H, br s, 4-H), 4.34 (1H, br s, 1-H), 4.28 (1H, dd, *J* = 4.9, 1.2 Hz, 11-H), 4.19 (1H, d, *J* = 2.3 Hz, 21-H), 4.13 (1H, dd, *J* = 11.5, 2.3 Hz, 22-H), 3.79 (3H, s, 17-OCH_3), 3.58 (3H, s, 7'-OCH_3), 3.53 (1H, d, *J* = 4.4 Hz, 3-H), 3.42 (1H, br t, 13-H), 3.15 (1H, ddd, *J* = 11.6, 10.1, 3.7 Hz, 3'-H), 2.94 (2H, d, *J* = 6.1 Hz, 14-H_2), 2.85 (1H, m, 3'-H), 2.70 (1H, m, 4'-H), 2.54 (1H, *m*, 4'-H), 2.34 (1H, d, *J* = 14.6 Hz, 12'-H), 2.33 (3H, s,

16-CH_3), 2.28 (3H, s, $OCOCH_3$), 2.20 (3H, s, NCH_3), 2.20 (1H, signals overlapped with NCH_3, 12'-H), 2.03 (3H, s, 6-CH_3)

IR ($CHCl_3$) cm^{-1} : 2921, 2854, 2810, 1743, 1620, 1508, 1431, 1369

HR-FAB-MS : *m/z* 925.3128 $[M + H]^+$ (Calcd for $C_{51}H_{49}N_4O_{11}S$, 925.3118)

FAB-MS (Dithiothreitol:Thioglycerol = 1:1) : *m/z* 925 $[(M + H)^+]$

$[\alpha]_D^{20}$:-55.4° (c = 0.17, $CHCl_3$)

***Ecteinascidin 770 6'-O-2"-naphthoate* [33]:** Yield 94%. ^{1}H-NMR δ: 8.73 (1H, br s, 1"-H), 8.11 (1H, dd, *J* = 8.6, 1.5 Hz, 8"-H), 7.97 (1H, d, *J* = 7.9 Hz, 4"-H), 7.91 (1H, d, *J* = 8.6 Hz, 5"-H), 7.89 (1H, d, *J* = 7.9 Hz, 3"-H), 7.61 (1H, dt, *J* = 8.6, 1.5 Hz, 6"-H), 7.54 (1H, dt, J = 8.5, dt, *J* = 8.6, 0.9 Hz, 7"-H), 6.79 (1H, s, 5'-H), 6.64 (1H, s, 8'-H), 6.61 (1H, s, 15-H), 6.03, 5.96 (each 1H, s, OCHO), 5.76 (1H, br s, 18-OH), 5.04 (1H, d, *J* = 11.6 Hz, 22-H), 4.58 (1H, br s, 4-H), 4.34 (1H, br s, 1-H), 4.30 (1H, dd, *J* = 4.9, 1.2 Hz, 11-H), 4.20 (1H, d, *J* = 2.3 Hz, 21-H), 4.14 (1H, dd, *J* = 11.5, 2.3 Hz, 22-H), 3.80 (3H, s, 17-OCH_3), 3.56 (3H, s, 7'-OCH_3), 3.53 (1H, d, *J* = 4.3 Hz, 3-H), 3.43 (1H, br t, 13-H), 3.17 (1H, ddd, *J* = 11.6, 10.1, 3.7 Hz, 3'-H), 2.95 (2H, d, *J* = 6.1 Hz, 14-H_2), 2.85 (1H, m, 3'-H), 2.69 (1H, m, 4'-H), 2.54 (1H, *m*, 4'-H), 2.34 (1H, d, *J* = 14.6 Hz, 12'-H), 2.34 (3H, s, 16-CH_3), 2.29 (3H, s, $OCOCH_3$), 2.21 (3H, s, NCH_3), 2.20 (1H, signals overlapped with NCH_3, 12'-H), 2.04 (3H, s, 6-CH_3)

^{13}C-NMR δ: 172.1 (11'-CO), 168.1 (5-O<u>C</u>OCH_3), 164.8 (O<u>C</u>OAr), 148.7 (C-7'), 147.9 (C-18), 145.4 (C-7), 143.1 (C-17), 140.4 (C-5), 140.1 (C-8), 139.0 (C-6'), 135.8 (C-10"), 132.5 (C-9'), 132.5 (C-9"), 132.0 (C-1"), 130.8 (C-20), 129.5 (C-8"), 129.3 (C-16), 128.8 (C-10'), 128.5 (C-6"), 128.3 (C-2", C-4"), 127.8 (C-5"), 126.7 (C-7"), 125.6 (C-3"), 122.6 (C-5'), 121.1 (C-10), 120.7 (C-15), 118.1 (21-CN), 118.1 (C-19), 114.0 (C-9), 113.4 (C-6), 111.9 (C-8'), 101.9 (OCH_2O), 64.9 (C-1'), 61.1 (C-1), 60.3 (17-OCH_3), 60.2 (C-22), 59.6 (C-21), 59.6 (C-3), 55.3 (7'-OCH_3), 54.6 (C-11), 54.6 (C-13), 41.6 (C-12'), 41.6 (C-4), 41.6 (NCH_3), 39.6 (C-3'), 28.9 (C-4'), 24.2 (C-14), 20.4 (OCO<u>C</u>H_3), 15.8 (16-CH_3), 9.7 (6-CH_3)

IR ($CHCl_3$) cm^{-1} : 2921, 2854, 2810, 1743, 1589, 1508, 1438, 1369

HR-FAB-MS : *m/z* 925.3125 $[M + H]^+$ (Calcd for $C_{51}H_{49}N_4O_{11}S$, 925.3118)

FAB-MS (Dithiothreitol:Thioglycerol = 1:1) : *m/z* 925 $[(M + H)^+]$

$[\alpha]_D^{20}$:-22.6° (c = 0.62, $CHCl_3$)

***Ecteinascidin 770 6'-O-2"-quinolinecarboxylate* [34]:** Yield 99%, ^{1}H-NMR δ: 8.34 (1H, d, *J* = 8.4 Hz, 4"-H), 8.33 (1H, d, *J* = 8.3 Hz, 8"-H), 8.25 (1H, d, *J* = 8.4 Hz, 3"-H), 7.90 (1H, d, *J* = 8.1 Hz, 5"-H), 7.80 (1H, dd, *J* = 8.3, 7.7 Hz, 7"-H), 7.67 (1H, dd, *J* = 8.1, 7.7 Hz, 6"-H), 6.86 (1H, s, 5'-H), 6.61 (1H, s, 8'-H), 6.60 (1H, s, 15-H), 6.03 and 5.97 (each 1H, s, OCHO), 5.76 (1H, br s, 18-OH), 5.05 (1H, d, *J* = 11.2 Hz, 22-H), 4.58 (1H, br s, 4-H), 4.34 (1H, br s, 1-H), 4.30 (1H, dd, *J* = 4.9, 1.2 Hz, 11-H), 4.20 (1H, d, *J* = 2.6 Hz, 21-H), 4.12 (1H, dd, *J* = 11.5, 2.3 Hz, 22-H), 3.82 (3H, s, 17-OCH_3), 3.56 (3H, s, 7'-OCH_3), 3.53 (1H, d, *J* = 4.4 Hz, 3-H), 3.43 (1H, br t, 13-H), 3.17 (1H, ddd, *J* = 11.6, 10.1, 3.7 Hz, 3'-H), 2.95 (2H, d, *J* = 6.1 Hz, 14-H_2), 2.88 (1H, m, 3'-H), 2.73 (1H, m, 4'-H), 2.60 (1H, *m*, 4'-H), 2.36 (1H, d, *J* = 14.6 Hz, 12'-H), 2.36 (3H, s, 16-CH_3), 2.30 (3H, s, $OCOCH_3$), 2.22 (3H, s, NCH_3), 2.20 (1H, signals overlapped with NCH_3, 12'-H), 2.03 (3H, s, 6-CH_3)

IR ($CHCl_3$) cm^{-1} : 2933, 2854, 1743, 1658, 1589, 1508, 1431, 1317

HR-FAB-MS : *m/z* 926.3079 $[M + H]^+$ (Calcd for $C_{50}H_{48}N_4O_{11}S$, 926.3021)

FAB-MS (Dithiothreitol:Thioglycerol = 1:1) : *m/z* 926 $[(M + H)^+]$

$[\alpha]_D^{21}$: -21.0° (c = 0.17, $CHCl_3$)

***Ecteinascidin 770 6'-O-4"-quinolinecarboxylate* [35]:** Yield 96%, ^{1}H-NMR δ: 9.07 (1H, d, *J* = 4.6 Hz, 2"-H), 8.80 (1H, dd, *J* = 8.5, 1.5 Hz, 8"-H), 8.19 (1H, dd, *J* = 8.5, 0.6 Hz, 5"-H), 8.07 (1H, d, *J* = 4.3 Hz, 3"-H), 7.79 (1H, ddd, *J* = 8.5, 7.0, 1.5 Hz, 7"-H), 7.65 (1H, ddd, *J* = 8.5, 7.0, 1.5 Hz, 6"-H), 6.82 (1H, s, 5'-H), 6.67 (1H, s, 8'-H), 6.66 (1H, s, 15-H), 6.03 and 5.97 (each 1H, d, *J* = 0.9 Hz, OCHO), 5.76 (1H, br s, 18-OH), 5.05 (1H, d, *J* = 11.3 Hz, 22-H), 4.58 (1H, br s, 4-H), 4.34 (1H, br s, 1-H), 4.30 (1H, dd, *J* = 4.9, 1.2 Hz, 11-H), 4.20 (1H, d, *J* = 2.7 Hz, 21-H), 4.12 (1H, dd, *J* = 11.5, 2.3 Hz, 22-H), 3.82 (3H, s, 17-OCH_3), 3.56 (3H, s, 7'-OCH_3), 3.54 (1H, d, *J* = 4.6 Hz, 3-H), 3.43 (1H, br

t, 13-H), 3.17 (1H, ddd, J = 11.6, 10.1, 3.7 Hz, 3`-H), 2.95 (2H, d, J = 6.1 Hz, 14-H_2), 2.88 (1H, m, 3'-H), 2.73 (1H, m, 4'-H), 2.60 (1H, ddd, *m*, 4'-H), 2.36 (1H, d, J = 14.6 Hz, 12'-H), 2.34 (3H, s, 16-CH_3), 2.30 (3H, s, $OCOCH_3$), 2.22 (3H, s, NCH_3), 2.20 (1H, signals overlapped with NCH_3, 12'-H), 2.03 (3H, s, 6-CH_3)

^{13}C-NMR δ: 168.1 (11`-<u>C</u>O), 167.7 (5-O<u>C</u>OCH_3), 164.2 (O<u>C</u>OAr), 149.8 (C-2"), 149.1 (C-9"), 148.5 (C-7'), 147.9 (C-18), 145.4 (C-7), 143.2 (C-17), 141.4 (C-5), 140.1 (C-8), 138.5 (C-6'), 134.0 (C-4"), 132.5 (C-9'), 130.8 (C-20), 130.0 (C-7"), 129.8 (C-6"), 129.5 (C-16), 128.8 (C-10'), 128.4 (C-5"), 125.6 (C-8"), 125.2 (C-10"), 122.8 (C-3"), 122.5 (C-5`), 121.0 (C-10), 120.6 (C-15), 118.1 (21-CN), 118.0 (C-19), 113.9 (C-9), 113.5 (C-6), 111.9 (C-8`), 101.9 (OCH_2O), 68.1 (C-1`), 61.1 (C-1), 60.4 (17-$OCH_3$), 60.2 (C-22), 59.6 (C-21), 59.6 (C-3), 56.2 (7`-OCH_3), 54.7 (C-11), 54.6 (C-13), 41.8 (C-12`), 41.6 (C-4), 41.6 ($NCH_3$), 39.6 (C-3'), 28.9 (C-4`), 24.1 (C-14), 20.4 ($OCO\underline{C}H_3$), 15.8 (16-CH_3), 9.7 (6-CH_3)

IR ($CHCl_3$) cm^{-1} : 2927, 2854, 2810, 1747, 185, 1508, 1431, 1369, 1263

HR-FAB-MS : *m/z* 926.3079 $[M + H]^+$ (Calcd for $C_{50}H_{48}N_4O_{11}S$, 926.3021)

FAB-MS (Dithiothreitol:Thioglycerol = 1:1) : *m/z* 926 $[(M + H)^+]$

$[\alpha]_D^{20}$:-25.3° (c = 0.17, $CHCl_3$)

General procedure for the preparation of compounds 36 and 37 via acid anhydride:

A mixture of 1-isoquinolinecarboxylic acid (173.0 mg, 1.0 mmol) and anhydrous K_2CO_3 (138.2 mg, 1.0 ml) in dichloromethane (2 ml) was stirred for 10 min. Isopropyl chloroformate (116 µl, 1.0 mmol) was added dropwise over 5 min, and the mixture was stirred overnight at room temperature. After the reaction mixture was filtered, the precipitate was washed with dichloromethane 30 ml (×3). The combined filtrates were concentrated *in vacuo* to give an anhydride **36a**.

The anhydride **37a** was prepared from 3-isoquinolinecarboxylic acid by the above procedure.

The anhydrides **36a** and **37a** were immediately reacted with Et 770 in the presence of DMAP and pyridine afforded **36** and **37**, respectively.

***Ecteinascidin 770 6'-O-1"-isoquinolinecarboxylate* [36]:** Yield 65%, ^{1}H-NMR δ: 8.86 (1H, d, *J* = 8.5 Hz, 8"-H), 8.67 (1H, d, *J* = 5.5 Hz, 3"-H), 7.90 (1H, d, *J* = 8.2 Hz, 5"-H), 7.86 (1H, d, *J* = 5.5 Hz, 4"-H), 7.74 (1H, dt, *J* = 8.5, 1.1 Hz, 7"-H), 7.68 (1H, dt, *J* = 8.5, 1.4 Hz, 6"-H), 6.78 (1H, s, 5'-H), 6.68 (1H, s, 8'-H), 6.61 (1H, s, 15-H), 6.04 and 5.97 (each 1H, d, *J* = 1.2 Hz, OCHO), 5.81 (1H, br s, 18-OH), 5.05 (1H, d, *J* = 11.6 Hz, 22-H), 4.58 (1H, br s, 4-H), 4.35 (1H, br s, 1-H), 4.29 (1H, dd, *J* = 4.9, 1.2 Hz, 11-H), 4.20 (1H, d, *J* = 2.7 Hz, 21-H), 4.14 (1H, dd, *J* = 11.6, 2.1 Hz, 22-H), 3.80 (3H, s, 17-OCH_3), 3.62 (3H, s, 7'-OCH_3), 3.53 (1H, d, *J* = 4.3 Hz, 3-H), 3.43 (1H, br t, 13-H), 3.17 (1H, ddd, *J* = 11.6, 10.1, 3.7 Hz, 3'-H), 2.95 (2H, d, *J* = 6.1 Hz, 14-H_2), 2.83 (1H, m, 3'-H), 2.69 (1H, ddd, *J* = 16.1, 10.0, 6.1 Hz, 4'-H), 2.55 (1H, dt, *J* = 15.9, 3.7 Hz, 4'-H), 2.47 (1H, d, *J* = 14.6 Hz, 12'-H), 2.34 (3H, s, 16-CH_3), 2.28 (3H, s, $OCOCH_3$), 2.21 (3H, s, NCH_3), 2.20 (1H, signals overlapped with NCH_3, 12'-H), 2.05 (3H, s, 6-CH_3)

^{13}C-NMR δ: 172.2 (11'-<u>C</u>O), 168.1 (5-O<u>C</u>OCH_3), 163.9 (O<u>C</u>OAr), 148.5 (C-7'), 147.9 (C-18), 147.8 (C- 3"), 145.4 (C-7), 143.1 (C-17), 141.8 (C-3"), 141.4 (C-5), 140.1 (C-8), 138.8 (C-6'), 137.0 (C-10"), 133.0 (C-9'), 130.8 (C-20), 130.5 (C-7"), 129.3 (C-16), 128.9 (C-10'), 128.8 (C-6"), 127.2 (C-9"), 127.1 (C-5"), 126.4 (C-8"), 124.4 (C-4"), 122.6 (C-5'), 121.1 (C-10), 120.7 (C-15), 118.2 (21-CN), 118.1 (C-19), 114.1 (C-9), 114.1 (C-6), 111.9 (C-8'), 101.9 (OCH_2O), 64.9 (C-1'), 61.1 (C-1), 60.3 (17-OCH_3), 60.2 (C-22), 59.7 (C-21), 59.6 (C-3), 55.3 (7'-OCH_3), 54.7 (C-11), 54.6 (C-13), 42.3 (C-12'), 41.9 (C-4), 41.6 (NCH_3), 39.6 (C-3'), 28.7 (C-4'), 24.2 (C-14), 20.4 ($OCO\underline{C}H_3$), 15.8 (16-CH_3), 9.7 (6-CH_3)

IR ($CHCl_3$) cm^{-1} : 2910, 2854, 2808, 1743, 1658, 1585, 1508, 1431

HR-FAB-MS : *m/z* 926.3083 $[M + H]^+$ (Calcd for $C_{50}H_{48}N_5O_{11}S$, 926.3021)

FAB-MS (Dithiothreitol:Thioglycerol = 1:1) : *m/z* 926 $[(M + H)^+]$

$[\alpha]_D^{22}$:-36.1° (c = 0.43, $CHCl_3$)

***Ecteinascidin 770 6'-O-3"-isoquinolinecarboxylate* [37]:** Yield 85%, ^{1}H-NMR δ: 9.38 (1H, s, 1"-H), 8.69 (1H, s, 4"-H), 8.08 (1H, d, *J* = 7.9 Hz, 5"-H), 8.00 (1H, d, *J* = 7.9 Hz, 8"-H), 7.80 (1H, dt, *J* = 7.9, 1.2 Hz, 6"-H), 7.70 (1H, dt, *J* = 7.9, 0.6 Hz, 7"-H), 6.82 (1H, s, 5'-H), 6.64 (1H, s, 8'-H), 6.61 (1H, s, 15-H), 6.02 and 5.96 (each 1H, d, *J* = 1.2 Hz, OCHO), 5.75 (1H, br s, 18-OH), 5.04 (1H, d, *J* = 11.3 Hz, 22-H), 4.56 (1H, br s, 4-H), 4.34 (1H, br s, 1-H), 4.28 (1H, dd, *J* = 4.9, 0.9 Hz, 11-H), 4.20 (1H, d, *J* = 2.7 Hz, 21-H), 4.14 (1H, dd, *J* = 11.6, 2.1 Hz, 22-H), 3.80 (3H, s, 17-OCH_3), 3.56 (3H, s, 7'-OCH_3), 3.52 (1H, d, *J* = 4.6 Hz, 3-H), 3.42 (1H, br t, 13-H), 3.16 (1H, dt, *J* = 10.9, 3.7 Hz, 3'-H), 2.95 (2H, d, *J* = 6.1 Hz, 14-H_2), 2.82 (1H, ddd, *J* = 11.3, 5.2, 3.7 Hz, 3'-H), 2.67 (1H, ddd, *J* = 15.9, 9.8, 5.2 Hz, 4'-H), 2.54 (1H, dt, *J* = 15.9, 3.7 Hz, 4'-H), 2.39 (1H, d, *J* = 14.3 Hz, 12'-H), 2.34 (3H, s, 16-CH_3), 2.28 (3H, s, $OCOCH_3$), 2.21 (3H, s, NCH_3), 2.22 (1H, signals overlapped with NCH_3, 12'-H), 2.04 (3H, s, 6-CH_3)

^{13}C-NMR δ: 172.2 (11'-CO), 168.1 (5-$OCOCH_3$), 163.7 (OCOAr), 152.9 (C-1"), 148.6 (C-7'), 147.8 (C-18), 145.4 (C-7), 143.1 (C-17), 141.3 (C-5), 140.8 (C-3"), 140.1 (C-8), 138.9 (C-6'), 135.4 (C-10"), 132.8 (C-9'), 131.2 (C-6"), 130.8 (C-20), 130.1 (C-7"), 129.8 (C-9"), 129.3 (C-16), 128.7 (C-10'), 128.1 (C-8"), 127.7 (C-5"), 125.0 (C-4"), 122.6 (C-5'), 121.1 (C-10), 120.7 (C-15), 118.2 (21-CN), 118.1 (C-19), 114.0 (C-9), 113.6 (C-6), 111.9 (C-8'), 101.9 (OCH_2O), 64.9 (C-1'), 61.1 (C-1), 60.3 (17-OCH_3), 60.1 (C-22), 59.7 (C-21), 59.6 (C-3), 55.2 (7'-OCH_3), 54.7 (C-11), 54.6 (C-13), 42.2 (C-12'), 41.9 (C-4), 41.6 (NCH_3), 39.6 (C-3'), 28.7 (C-4'), 24.2 (C-14), 20.4 ($OCOCH_3$), 15.8 (16-CH_3), 9.7 (6-CH_3)

IR ($CHCl_3$) cm^{-1} : 2931, 2854, 2808, 1743, 1618, 1587, 1508, 1433, 1369

HR-FAB-MS : *m/z* 926.3081 $[M + H]^+$ (Calcd for $C_{50}H_{48}N_5O_{11}S$, 926.3021)

FAB-MS (Dithiothreitol:Thioglycerol = 1:1) : *m/z* 926 $[(M + H)^+]$

$[\alpha]_D^{21}$:-35.6° (c = 0.45, $CHCl_3$)

***2'-N-3"-indolecarbonylecteinascidin 770* [39]:** Indole-3-carboxylic acid (16.1 mg, 0.1 mmol) was added to a solution of Et 770 (15.4 mg, 0.02 mmol) and 0.2 M dichloromethane solution of DCC (0.5 ml) in dichloromethane (2 ml). The reaction mixture was stirred at room temperature for 3 days, and the solvent was removed *in vacuo*. The residue was subjected to chromatography with hexane-ethyl acetate (1:1) as the eluent to give product (11.8 mg, 65% yield) as an amorphous solid.

^{1}H-NMR δ: 8.62 (1H, br s, NH), 8.06 (1H, d, *J* = 7.9 Hz, 4"-H), 7.68 (1H, d, *J* = 2.7 Hz, 2"-H), 7.64 (1H, d, *J* = 7.9 Hz, 7"-H), 7.38 (1H, ddd, *J* = 7.9, 7.0, 0.9 Hz, 5"-H or 6"-H), 7.27 (1H, ddd, *J* = 7.9, 7.0, 1.2 Hz, 5"-H or 6"-H), 6.48 (1H, s, 8'-H), 6.40 (1H, s, 5'-H), 6.09 and 5.99 (each 1H, d, *J* = 1.5 Hz, OCHO), 5.61 (1H, s, 18-OH), 5.41 (2H, br, 15-H, 6'-OH), 4.62 (1H, d, *J* = 11.3 Hz, 22-H), 4.62 (1H, signals overlapped with 22-H, 4-H), 4.54 (1H, dd, *J* = 11.3, 1.8 Hz, 22-H), 4.36 (1H, br s, 1-H), 4.20 (1H, dd, *J* = 5.0, 1.4 Hz, 11-H), 4.19 (1H, d, *J* = 2.7 Hz, 21-H), 4.00 (1H, ddd, *J* = 14.3, 5.8, 2.4 Hz, 3'-H), 3.94 (1H, d, *J* = 14.8 Hz, 12'-H), 3.71 (3H, s, 7'-OCH_3), 3.57 (3H, s, 17-OCH_3), 3.50 (1H, d, *J* = 4.9 Hz, 3-H), 3.47 (1H, m, 12'-H), 3.35 (1H, br t, 13-H), 2.85 (2H, d, *J* = 6.1 Hz, 14-H_2), 2.49 (2H, m, 3'-H, 4'-H), 2.30 (3H, s, $OCOCH_3$), 2.28 (1H, *J* = 16.8, 8.2, 3.3 Hz, 4'-H), 2.09 (3H, s, NCH_3), 2.05 (3H, s, 6-CH_3), 1.14 (3H, s, 16-CH_3)

^{13}C-NMR δ: 170.6 (11'-CO), 168.2 (5-$OCOCH_3$), 164.1 (NCOAr), 147.0 (C-18), 145.4 (C-7), 144.7 (C-6'), 144.6 (C-7'), 142.7 (C-17), 141.4 (C-5), 141.3 (C-8), 135.7 (C-8"), 129.7 (C-20), 129.3 (C-2"), 129.0 (C-16), 127.6 (C-10'), 127.5 (C-9'), 125.0 (C-9"), 122.9 (C-6"), 122.6 (C-4"), 122.4 (C-10), 121.4 (C-15), 121.3 (C-5"), 118.3 (21-CN), 117.3 (C-19), 114.7 (C-3"), 113.7 (C-5'), 113.7 (C-9), 113.3 (C-6), 111.4 (C-7"), 110.5 (C-8'), 101.9 (OCH_2O), 70.2 (C-1'), 61.1 (C-3), 60.7 (C-1), 60.3 (C-22), 59.8 (17-OCH_3), 59.8 (C-21), 55.3 (7'-OCH_3), 54.9 (C-11), 54.9 (C-13), 46.6 (C-12'), 41.8 (C-4), 41.6 (NCH_3), 38.5 (C-3'), 29.7 (C-4'), 24.9 (C-14), 20.4 ($OCOCH_3$), 14.2 (16-CH_3), 9.8 (6-CH_3)

IR ($CHCl_3$) cm^{-1} : 3357, 2925, 2852, 2810, 1751, 1595, 1508, 1431, 170

HR-FAB-MS : *m/z* 914.3063 $[M + H]^+$ (Calcd for $C_{49}H_{48}N_5O_{11}S$, 914.3071)

FAB-MS (Dithiothreitol:Thioglycerol = 1:1) : *m/z* 914 $[(M + H)^+]$

$[\alpha]_D^{20}$:+ 18.5° (c = 0.26, $CHCl_3$)

7.3 Selective *N*-demethylation on ABC ring system with CAN

General procedure for *N*-demethylation of the model compounds:

Starting material (**40-45**) (0.1 mmol) was dissolved in acetonitrile (1.0 ml) in an ice bath. Equimolar quantity of various 0.4 M Aqueous solution of cerium (IV) ammonium nitrate (0.1 mmol) was slowly added into a cool reaction mixture over 55 min at ice bath. Then, reaction mixture was stirred for overnight at rt. Adjusting reaction mixture to pH 8-9 with saturated $NaHCO_3$ was done and the basic solution was extracted with chloroform (20 ml) × 3. The combined extracts were washed with brine (20 ml), dried, and concentrated *in vacuo* to give a solid. This residue was purified by silica gel column chromatography using chloroform: methanol in appropriated ratio as eluant to give the corresponding product.

***1,2,3,4,5,6-Hexahydro-7,9,10-trimethoxy-3,8-dimethyl-4-oxo-1,5-imino-3-benzazocin* [40a]:** Yield 89 %. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 4.39 (1H, d, *J* = 4.6 Hz, 1-H), 3.90 (1H, d, *J* = 6.4 Hz, 5-H), 3.81 (3H, s, OCH_3), 3.76 (1H, dd, *J* = 11.8, 4.9 Hz, 2-Hα), 3.72 (3H, s, OCH_3), 3.60 (3H, s, OCH_3), 3.14 (1H, dd, *J* = 11.8, 0.9 Hz, 2-Hβ), 3.02 (1H, dd, *J* = 17.6, 1.3 Hz, 6-Hβ), 2.84 (1H, dd, *J* = 17.6, 6.6 Hz, 6-Hα), 2.79 (3H, s, 3-NCH_3), 2.11 (3H, s, 8-CH_3)

***1,2,3,4,5,6-Hexahydro-7,9,10-trimethoxy-3,8-dimethyl-4-oxo-1,5-imino-11-carbonyl-3-benzazocin* [40b]:** Yield 3 %.

Major rotamer: ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 8.27 (1H, s, CHO), 5.12 (1H, d, *J* = 5.1 Hz, 1-H), 4.68 (1H, d, *J* = 6.1 Hz, 5-H), 4.06 (3H, s, OCH_3), 3.95-4.01 (1H, m, 2-Hα), 3.93 (3H, s, OCH_3), 3.79 (3H, s, OCH_3), 3.22-3.46 (2H, m, 6-Hβ, 2-Hβ), 3.07-3.46 (1H, m, 6-Hα), 2.99 (3H, s, 3-NCH_3), 2.31 (3H, s, 8-CH_3)

Minor rotamer: ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 8.37 (1H, s, CHO), 5.94 (1H, d, *J* = 4.8 Hz, 5-H), 5.35 (1H, d, *J* = 6.0 Hz, 1-H), 4.08 (3H, s, OCH_3), 3.95-4.01 (1H, m, 2-Hα), 3.92 (3H, s, OCH_3), 3.78 (3H, s, OCH_3), 3.22-3.46 (2H, m, 6-Hβ, 2-Hβ), 3.07-3.46 (1H, m, 6-Hα), 2.99 (3H, s, 3-NCH_3), 2.31 (3H, s, 8-CH_3)

1,2,3,4,5,6-Hexahydro-9-methoxy-3,8-dimethyl-4-oxo-1,5-imino-3-benzazocin **[41a]:** Yield 78 %. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 6.84 (1H, s, Ar-H), 6.53 (1H, s, Ar-H), 4.22 (1H, d, *J* = 4.4 Hz, 1-H), 3.96 (1H, dd, *J* = 5.9, 1.5 Hz, 5-H), 3.90 (1H, dd, *J* = 11.5, 4.7 Hz, 2-Hα), 3.80 (3H, s, OCH_3), 3.22 (1H, dd, *J* = 11.4, 1.1 Hz, 2-Hβ), 3.04 (1H, dd, *J* = 16.9, 5.9 Hz, 6-Hα), 2.95 (1H, dd, *J* = 16.8, 1.7 Hz, 6-Hβ), 2.85 (3H, s, 3-NCH_3), 2.17 (3H, s, 8-CH_3)

1,2,3,4,5,6-Hexahydro-9-methoxy-8-methyl-3-{4-methoxy-1-phenylmetyl}-4-oxo-1,5-imino-3-benzazocin **[42a]:** Yield 86 %. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 6.82 (1H, s,Ar-H), 6.53 (1H, d, *J* = 8.6 Hz, Ar-H), 6.53 (1H, d, *J* = 8.8 Hz, Ar-H), 6.23 (1H, s, Ar-H), 4.66 (1H, d, *J* = 14.9 Hz, N-CH-), 4.10 (1H, d, *J* = 14.9 Hz, N-CH-), 4.08 (1H, s, 1-H), 3.96 (1H, dd, *J* = 4.7, 2.9 Hz, 5-H), 3.66 (3H, s, OCH_3), 3.63 (1H, d, *J* = 4.2 Hz, 2-Hα), 3.58 (3H, s, OCH_3), 2.97-3.02 (3H, m, 6-H, 2-Hβ), 2.13 (3H, s, 8-CH_3),

1,2,3,4,5,6-Hexahydro-9-methoxy-8-methyl-3-{4-methoxy-1-phenylmetyl}-4-oxo-1,5-imino-11-carbonyl-3-benzazocin **[42b]:** Yield 4 %.

Major rotamer: ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 8.16 (1H, s, CHO), 6.93 (1H, d, *J* = 6.6 Hz, Ar-H), 6.56-6.66 (4H, m, Ar-H), 6.31 (1H, d, *J* = 11.0 Hz, Ar-H), 5.27 (1H, d, *J* = 5.3 Hz, 1-H), 4.81 (1H, d, *J* = 14.9 Hz, N-CH-), 4.56 (1H, d, *J* = 5.3 Hz, 5-H), 4.06 (1H, dd, *J* = 14.7 Hz, N-CH-), 3.74 (3H, s, OCH_3), 3.71-3.74 (1H, m, 2-Hα), 3.66 (3H, s, OCH_3), 3.08-3.22 (3H, m, 6-H, 2-Hβ), 2.21 (3H, s, 8-CH_3)

Minor rotamer: ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 8.21 (1H, s, CHO), 6.93 (1H, d, *J* = 6.6 Hz, Ar-H), 6.56-6.66 (4H, m, Ar-H), 6.31 (1H, d, *J* = 11.0 Hz, Ar-H), 5.54 (1H, d, *J* = 4.2 Hz, 1-H), 4.81 (1H, d, *J* = 14.9 Hz, N-CH-), 4.77 (1H, d, 1-H), 4.56 (1H, d, *J* = 5.3 Hz, 5-H), 4.09 (1H, dd, *J* = 14.7 Hz, N-CH-), 3.74 (3H, s, OCH_3), 3.71-3.74 (1H, m, 2-Hα), 3.65 (3H, s, OCH_3), 3.08-3.22 (3H, m, 6-H, 2-Hβ), 2.21 (3H, s, 8-CH_3),

***1,2,3,4,5,6-Hexahydro-9-methoxy3,-8-dimethyl-7,10-quinone-4-oxo-1,5-imino-3-benzazocin* [43a]:** Yield 70 %. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 4.39 (1H, d, *J* = 4.4, 1-H), 4.00 (3H, s, OCH_3), 3.97 (1H, d, *J* = 6.6, 5-H), 3.80 (1H, dd, *J* = 12.4, 5.2, 2-Hα), 3.17 (1H, d, *J* = 12.5, 2-Hβ), 2.90 (3H, s, 3-NCH_3), 2.87 (1H, dd, *J* = 16.9, 1.1, 6-Hβ), 2.69 (1H, dd, *J* = 19.3, 6.3, 6-Hα), 1.96 (3H, s, 8-CH_3)

Bis-*1,2,3,4,5,6-hexahydro-10-hydroxy-9-methoxy-3,8,11-trimethyl-4-oxo-1,5-imino-3-benzazocin* [44b]: Yield 4 %. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 5.82 (each 1H, br s, OH), 4.25 (1H, d, *J* = 4.6 Hz, 1'-H), 4.23 (1H, d, *J* = 4.6 Hz, 1-H), 3.95 (1H, dd, *J* = 11.9, 4.6 Hz, 2'-Hα), 3.91 (1H, dd, *J* = 10.7, 4.5 Hz, 2'-Hα), 3.81 (3H, s, 9'-OCH_3), 3.78 (3H, s, 9-OCH_3), 3.46 (each 3H, s, 5-H, 5'-H), 3.20 (each 1H, d, *J* = 5.8 Hz, 2-Hα, 2'-Hα), 2.87 (3H, s, 3'-NCH_3), 2.85 (3H, s, 3-NCH_3), 2.49 (3H, s, 11'-NCH_3), 2.45 (3H, s, 11-NCH_3), 2.54 (1H, d, *J* = 6.6 Hz, 6'-Hα), 2.30 (1H, dd, *J* = 17.5, 6.6 Hz, 6-Hα), 2.14 (1H, d, *J* = 17.5 Hz, 6-Hβ), 1.97 (1H, d, *J* = 17.5 Hz, 6-Hβ).

***1,2,3,4,5,6-Hexahydro-10-acetate-9-methoxy-3,8,11-trimethyl-4-oxo-1,5-imino-3-benzazocin* [45]:** To a solution of **44** (27.6 mg, 0.1 mmol) in dry pyridine 0.5 ml was slowly added acetic anhydride (0.1 ml, 0.5 mmol, 5.0 equiv) in ice bath. Then, the mixture reaction was stirred at room temperature for overnight. The completely reaction was removed pyridine under distill reduced pressure. The residue was added water (15.0 ml) and extracted with chloroform 15.0 ml (×3). The combined chloroform layers were washed with brine (20.0 ml) and dried over anh. Na_2SO_4, filtered and the solvent was removed in *vacuo* to afford the crude product (34.8 mg). After purified with column chromatography to give product **45** (30.8 mg, 97 % yield).

^{1}H-NMR (300 MHz, $CDCl_3$) δ : 6.85 (1H, s, 7-H), 3.90 (1H, s, 1-H), 3.87 (1H, dd, *J* = 14.7, 5.0 Hz, 2-Hα), 3.74 (3H, s, OCH_3), 3.64 (1H, d, *J* = 6.6 Hz, 5-H), 3.16 (1H, dd, *J* = 17.4, 6.8 Hz, 6-Hα), 3.04 (1H, d, *J* = 10.3 Hz, 2-Hβ), 2.84 (3H,s, 3-NCH_3), 2.81 (1H, d, *J* = 15.9 Hz, 6-Hβ), 2.45 (3H, s, 11-NCH_3), 2.37 (3H, s, $OCOCH_3$), 2.27 (3H, s, 8-CH_3)

***1,2,3,4,5,6-Hexahydro-10-acetate-9-methoxy-3,8-dimethyl-4-oxo-1,5-imino-3-benzazocin* [45a]:** Yield 72 %. ^{1}H-NMR (300 MHz, $CDCl_3$) δ : 7.20 (1H, s, 7-H), 4.20 (1H, d, J = 4.6 Hz,, 5-H), 3.90(1H, t, J = 3.9 Hz, 5-H), 3.77 (1H, d, J = 4.8 Hz, 2-Hα), 3.73 (1H, d, J = 4.8 Hz, 2-Hβ), 3.65 (3H, s, OCH_3), 3.10 (1H, d, J = 0.9 Hz, 6-Hα), 3.04 (1H, d, J = 0.9 Hz, 6-Hβ), 2.78 (3H, s, 3-NCH_3), 2.29 (3H, s, $OCOCH_3$), 2.20 (3H, s, 8-CH_3)

8. Biological assay

A single-cell suspension of each cell (2×10^3 cells/well) was added to the serially diluted test compounds in a microplate. The cell were then cultured for 4 days. Cell growth was measured with a cell counting kit (DOJINDO, Osaka, Japan). IC_{50} was expressed as the concentration at which cell growth was inhibited by 50% compared with untreated control.