US006124293A

United States Patent [19]

Rinehart et al.

[11] Patent Number: 6,124,293

[45] Date of Patent: Sep. 26, 2000

[54] **SEMI-SYNTHETIC ECTEINASCIDINS**

[75] Inventors: **Kenneth L. Rinehart**, Urbana, Ill.; **Jose J. Morales**, Bayamon, Puerto Rico

[73] Assignee: **The Board of Trustees of the University of Illinois**, Champaign, Ill.

[21] Appl. No.: **09/286,242**

[22] Filed: **Apr. 5, 1999**

Related U.S. Application Data

[60] Provisional application No. 60/080,802, Apr. 6, 1998.

[51] **Int. Cl.**[7] **A61K 31/495**; C07D 515/22

[52] **U.S. Cl.** **514/250**; 540/466

[58] **Field of Search** 514/250; 540/466

[56] **References Cited**

U.S. PATENT DOCUMENTS

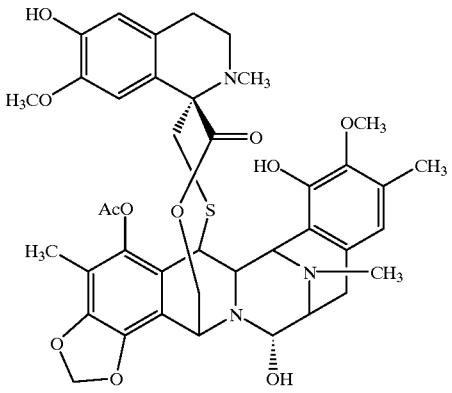
5,089,273	2/1992	Rinehart et al.	424/520
5,149,804	9/1992	Rinehart et al.	540/466
5,256,663	10/1993	Rinehart et al.	514/250
5,478,932	12/1995	Rinehart et al.	540/466
5,654,426	8/1997	Rinehart et al.	540/466
5,721,362	2/1998	Corey et al.	540/466
5,750,709	5/1998	Castor	546/348

Primary Examiner—Michael G. Ambrose

Attorney, Agent, or Firm—Ernest V. Linek; Banner & Witcoff, Ltd.

[57] **ABSTRACT**

The present invention is directed to several newly prepared semi-synthetic ecteinascidin (Et) species, designated herein as Et 757, Boc-Et 729, Iso-Et 743, Et 875, and Et 1560. The physical properties of these compounds, their preparation and bioactivities are also reported.



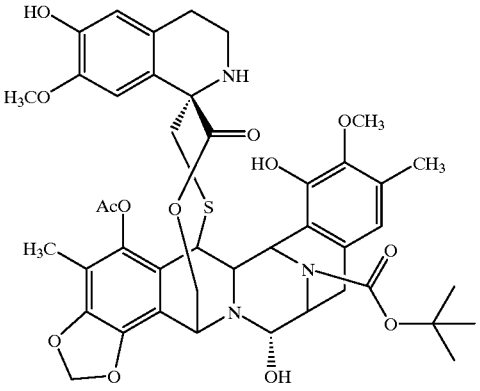
Et 757

$C_{40}H_{45}N_3O_{11}S$

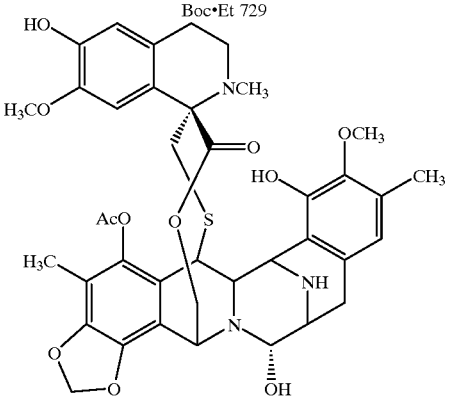
Mol. Wt. 775

HRFAB:[M + H—H_2O]$^+$ 758.2765 (Δ – 1.8 mDa)

-continued



Boc•Et 729

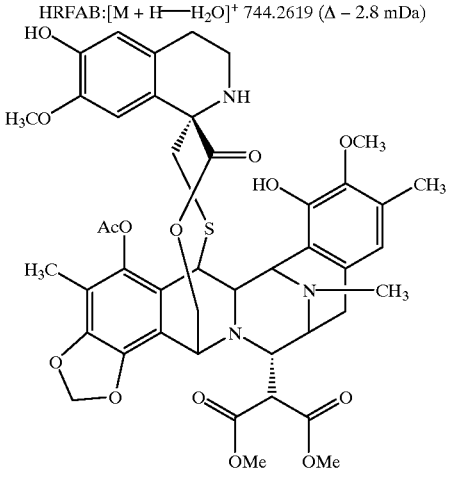


Iso-Et 743

$C_{35}H_{43}N_3O_{11}S$

Mol. Wt. 761.26

HRFAB:[M + H—H_2O]$^+$ 744.2619 (Δ – 2.8 mDa)



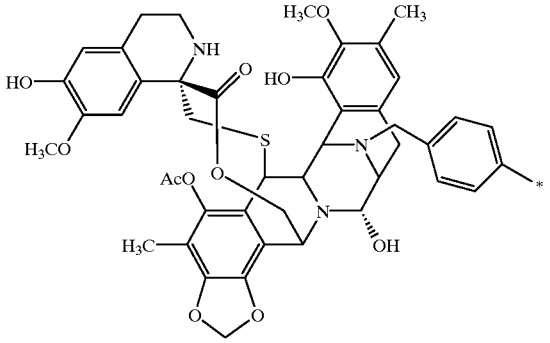
Et 875

$C_{44}H_{49}N_3O_{14}S$

Mol. Wt. 875.94

HRFAB:[M + H]$^+$ 875.2986 (Δ - 2.8 mDa)

-continued



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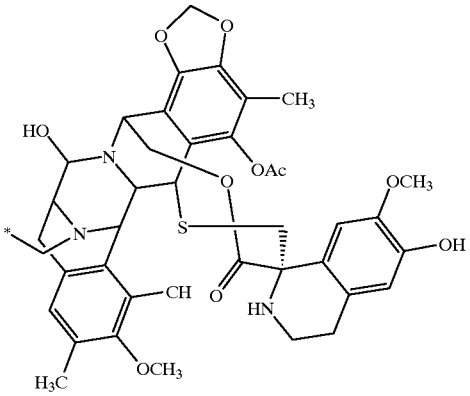


Et 1560
$C_{84}H_{38}N_6O_{22}S_2$
Mol. Wt. 1596

15 Claims, 12 Drawing Sheets

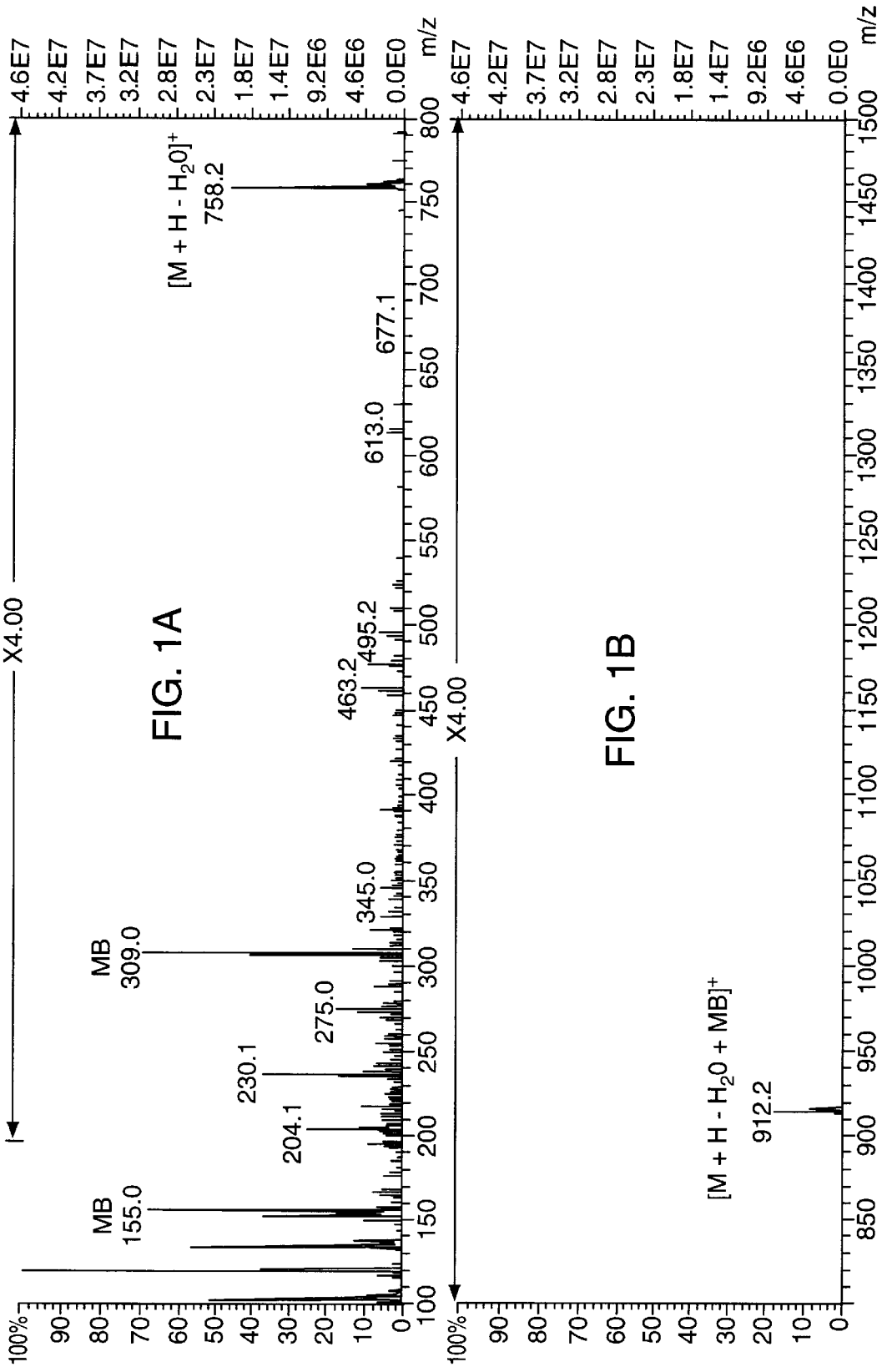

100%
90
80
70
60
50
40
30
20
10
0
4.6E7
4.2E7
3.7E7
3.2E7
2.8E7
2.3E7
1.8E7
1.4E7
9.2E6
4.6E6
0.0E0
m/z
X4.00
MB
155.0
MB
309.0
204.1
230.1
275.0
345.0
463.2
495.2
613.0
677.1
[M + H - H2O]+
758.2
[M + H - H2O + MB]+
912.2
100
150
200
250
300
350
400
450
500
550
600
650
700
750
800
850
900
950
1000
1050
1100
1150
1200
1250
1300
1350
1400
1450
1500

FIG. 1A

FIG. 1B

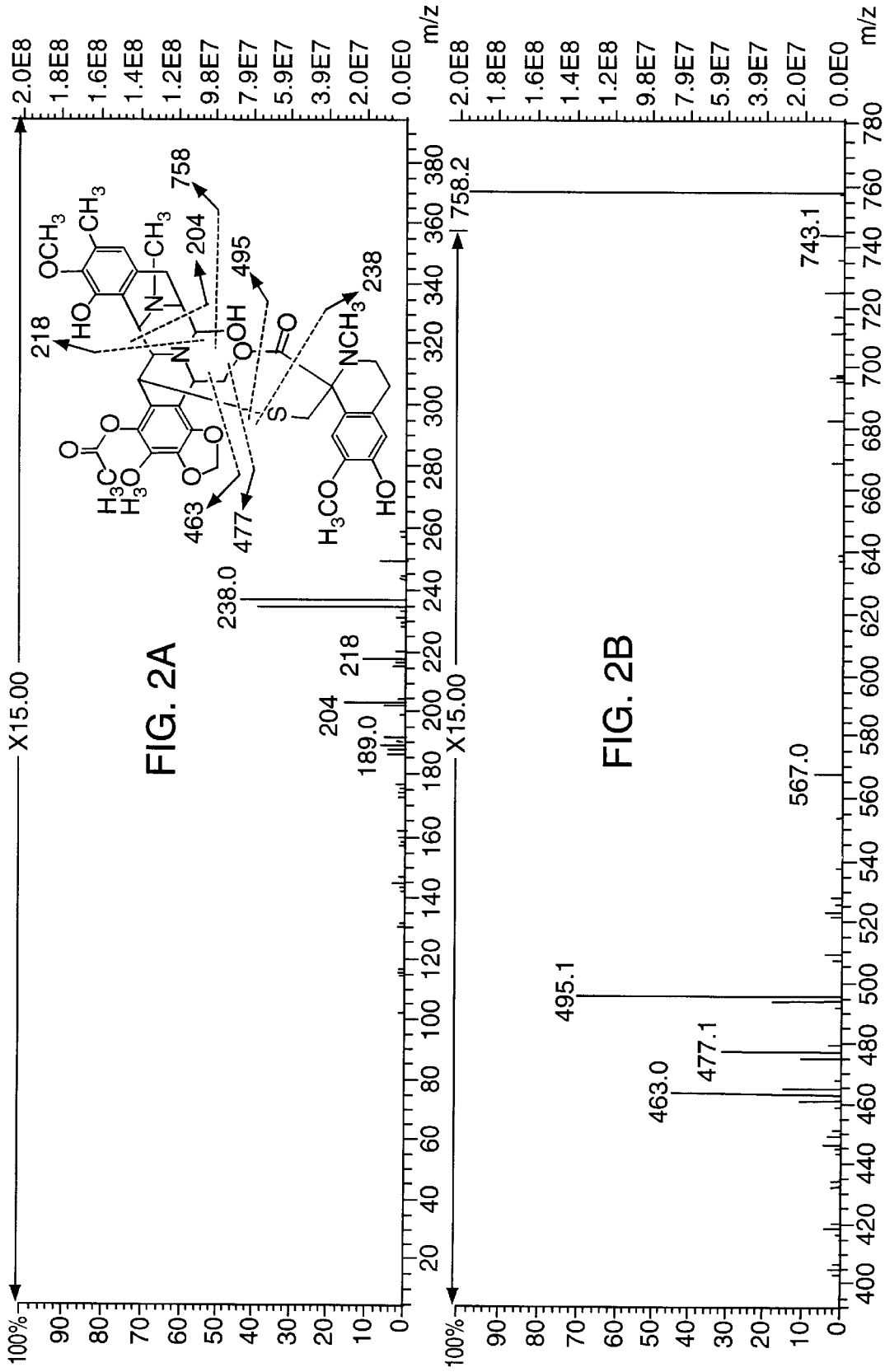
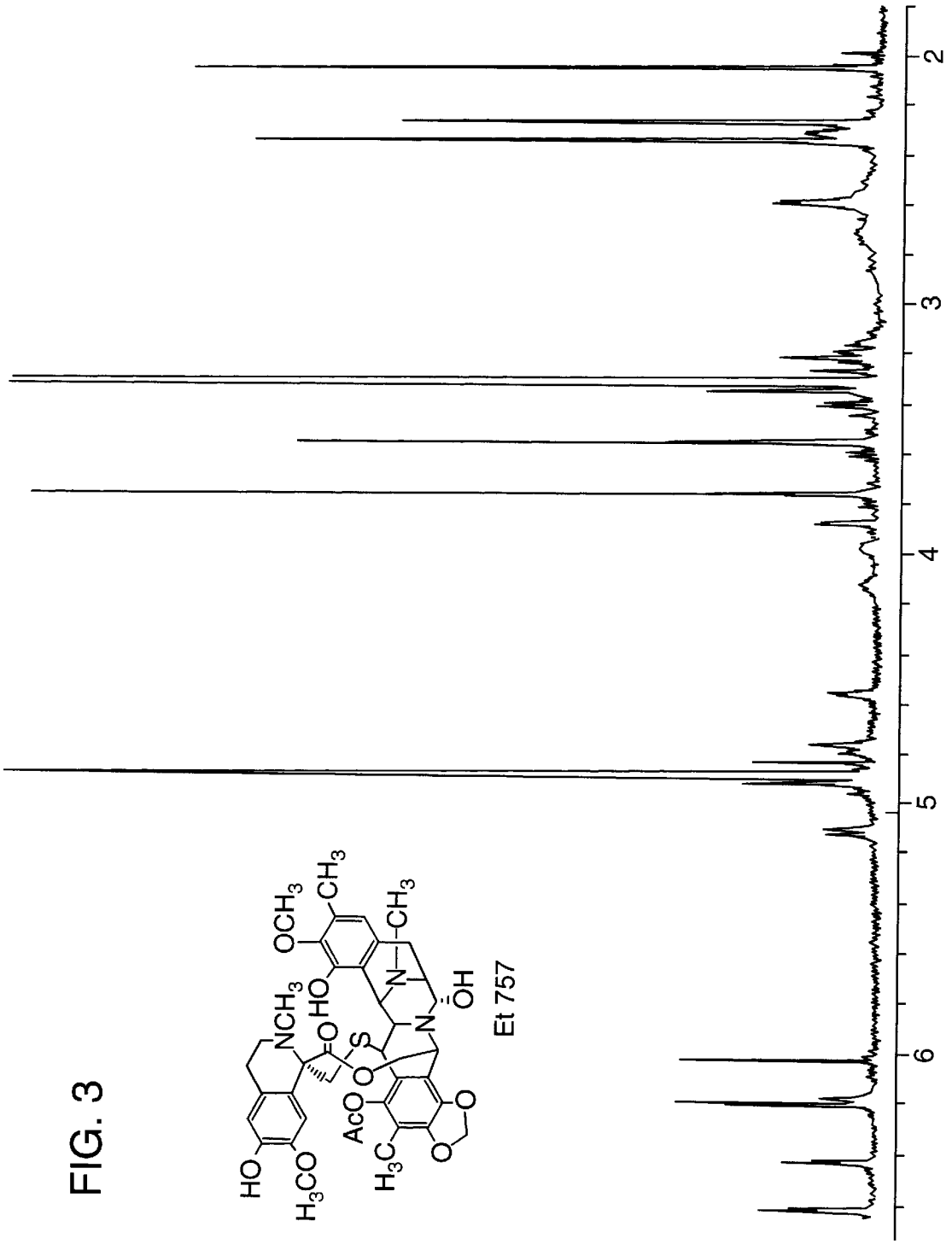

X15.00
100%
m/z
238.0
218
204
189.0
758
204
495
218
238
463
477
OCH3
CH3
CH3
HO
OH
NCH3
H3CO
HO
H3C
H3O
O
N
S
FIG. 2A
X15.00
758.2
743.1
567.0
495.1
477.1
463.0
FIG. 2B

FIG. 3



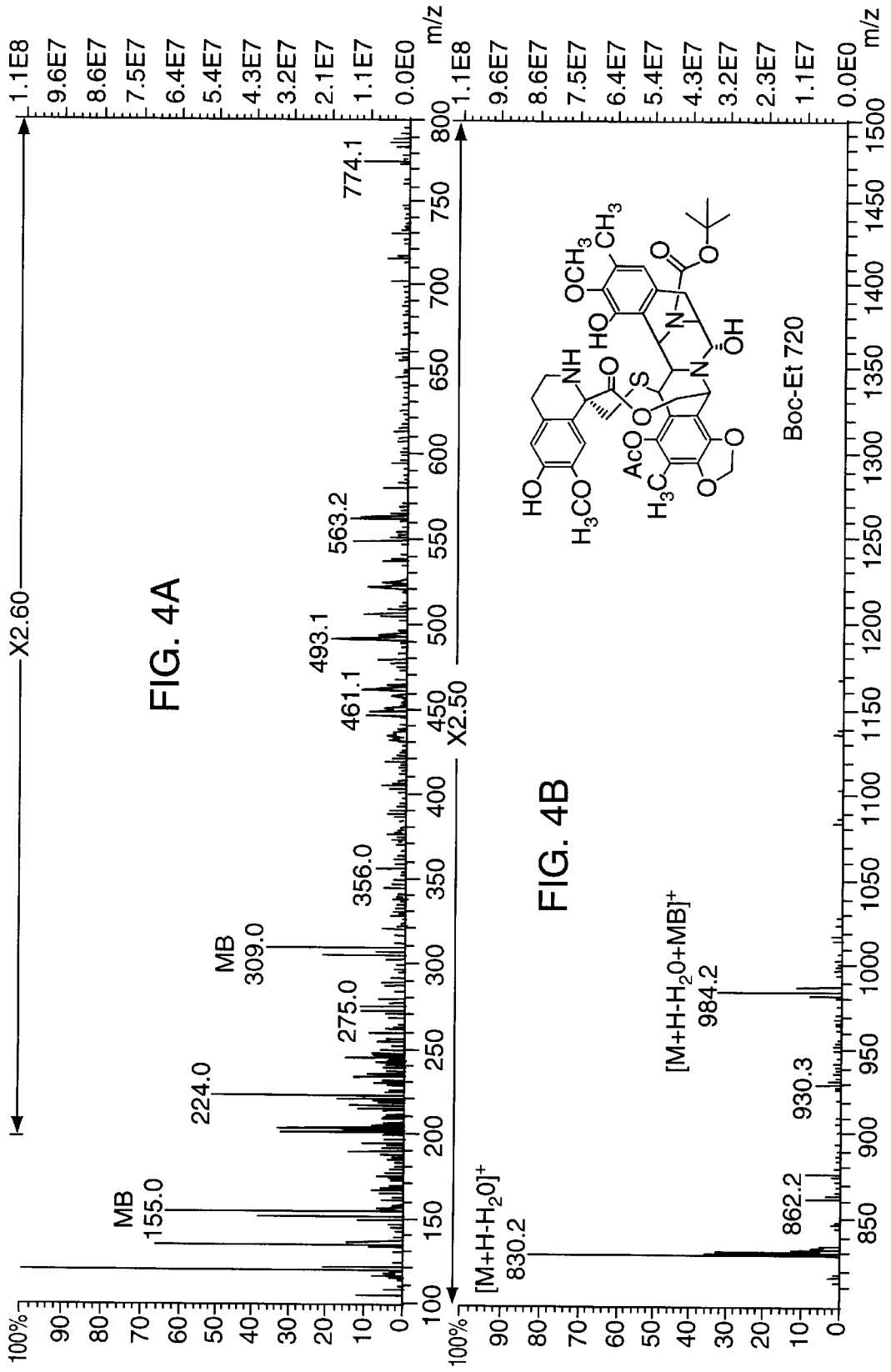

X2.60
X2.50
MB
155.0
224.0
MB
309.0
275.0
356.0
461.1
493.1
563.2
774.1
[M+H-H2O]+
830.2
862.2
930.3
[M+H-H2O+MB]+
984.2
Boc-Et 720
m/z

FIG. 4A

FIG. 4B

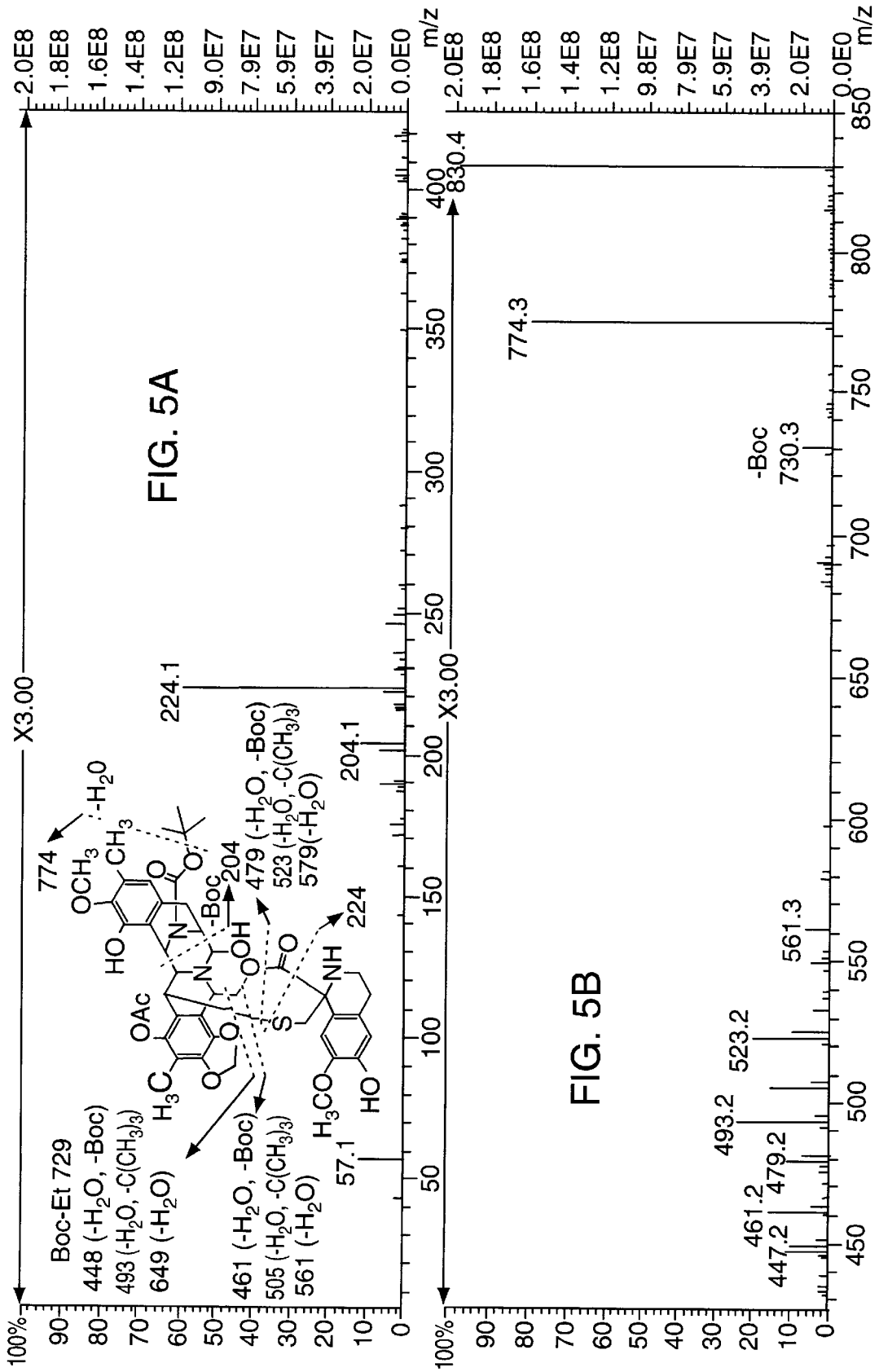


FIG. 5A
FIG. 5B
m/z
100%
X3.00
X3.00
774
-H2O
224.1
204.1
204
224
57.1
Boc-Et 729
448 (-H2O, -Boc)
493 (-H2O, -C(CH3)3)
649 (-H2O)
461 (-H2O, -Boc)
505 (-H2O, -C(CH3)3)
561 (-H2O)
479 (-H2O, -Boc)
523 (-H2O, -C(CH3)3)
579(-H2O)
-Boc
730.3
774.3
830.4
561.3
523.2
493.2
479.2
461.2
447.2

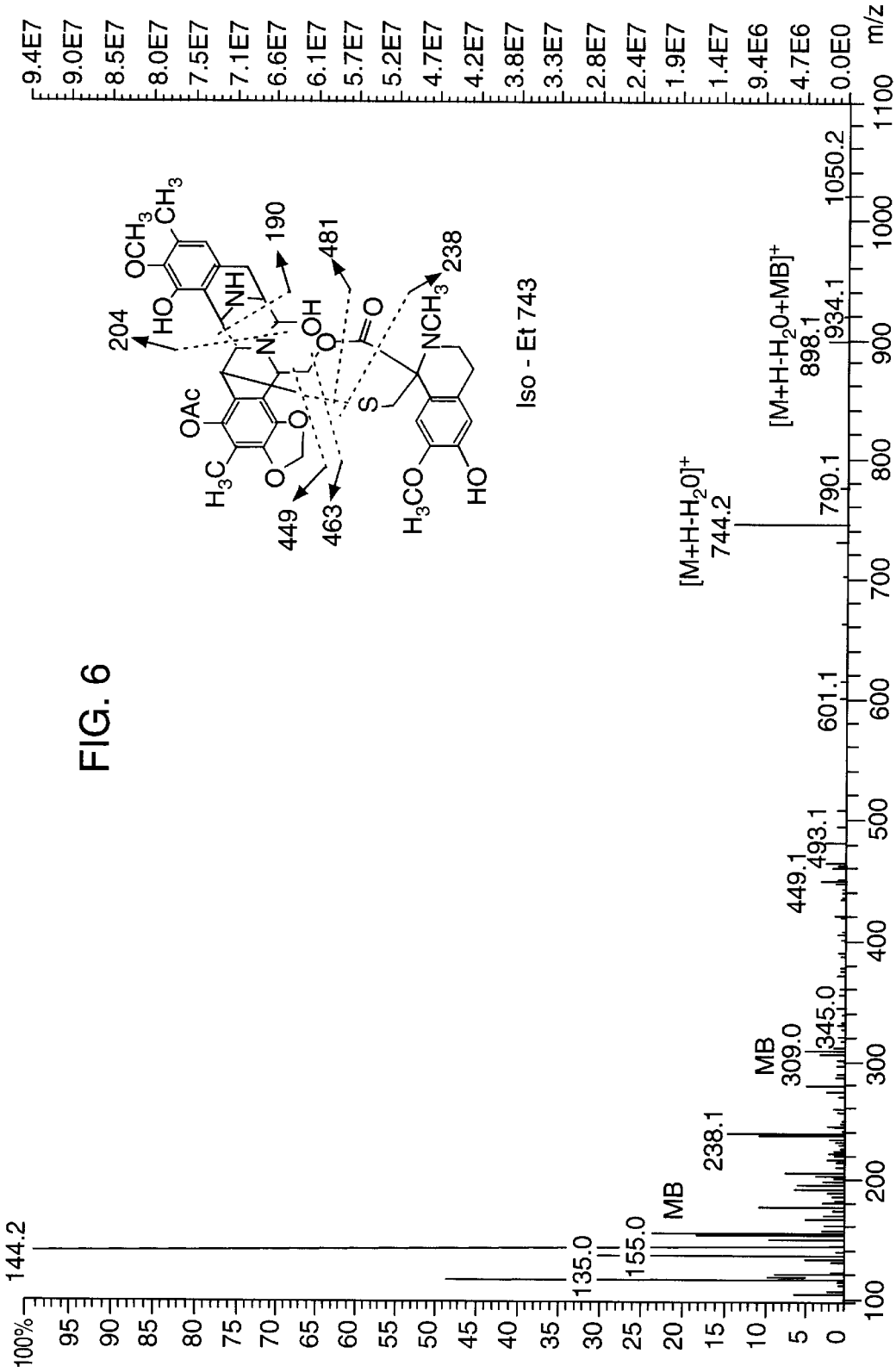

Iso - Et 743
m/z
100%
144.2
135.0
155.0
MB
238.1
MB
309.0
345.0
449.1
493.1
601.1
[M+H-H2O]+
744.2
790.1
[M+H-H2O+MB]+
898.1
934.1
1050.2
204
190
481
238
449
463
OCH3
CH3
HO
NH
OAc
H3C
OH
NCH3
H3CO
HO
S

FIG. 6

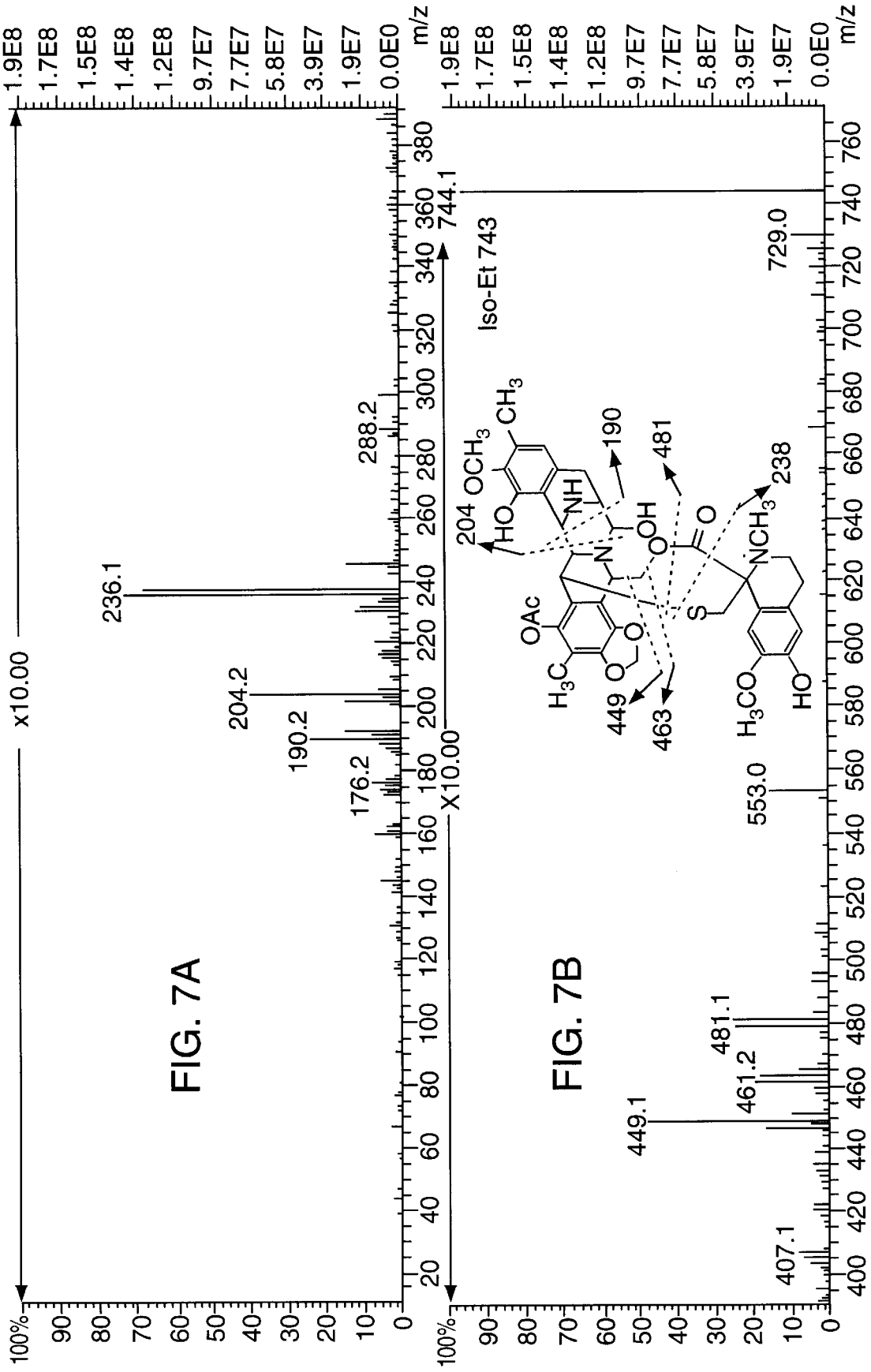

x10.00
X10.00
m/z
100%
236.1
204.2
190.2
176.2
288.2
744.1
729.0
553.0
481.1
461.2
449.1
407.1
Iso-Et 743
204
190
481
238
449
463
OAc
OCH3
CH3
NH
OH
NCH3
H3C
H3CO
HO

FIG. 7A

FIG. 7B

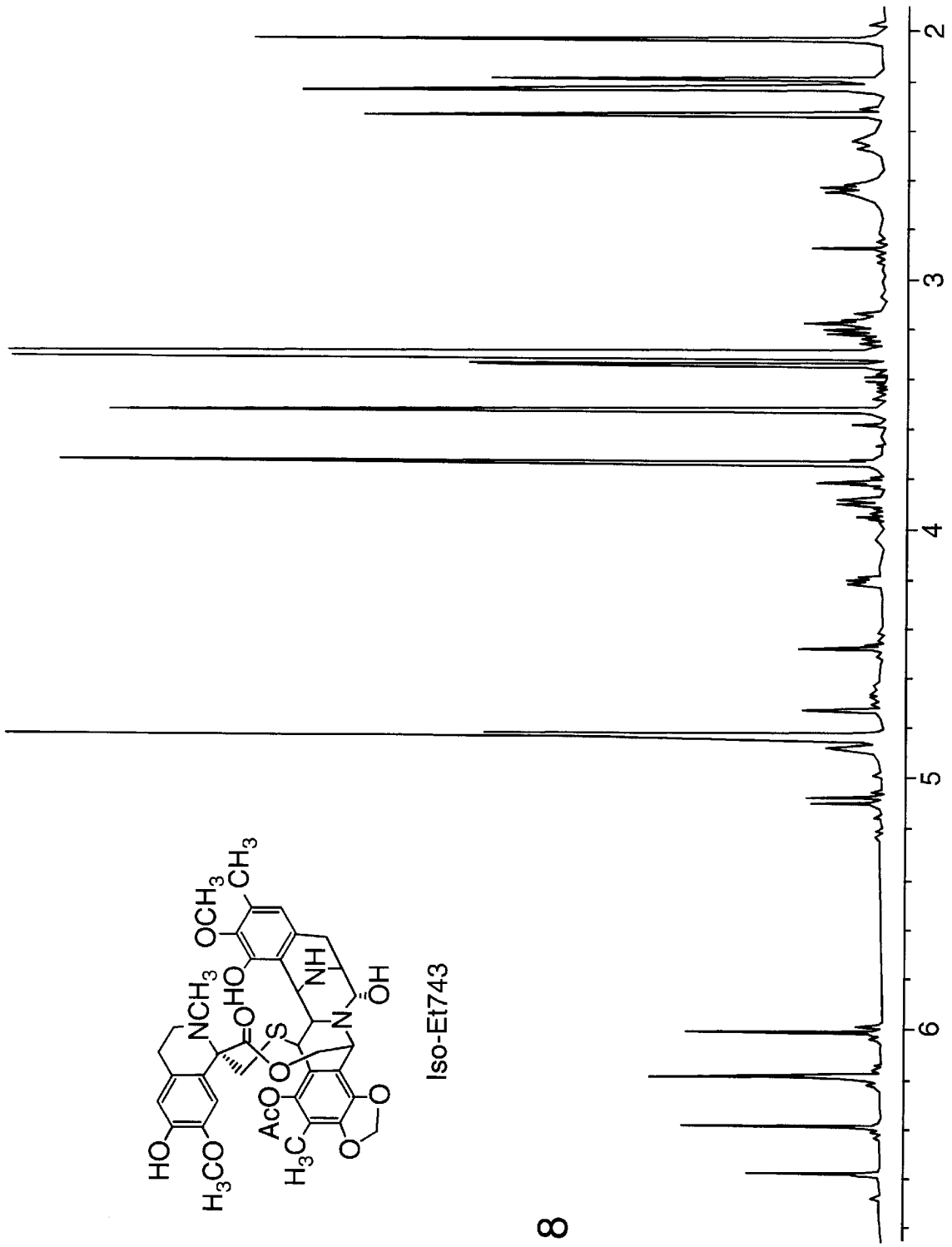


FIG. 8

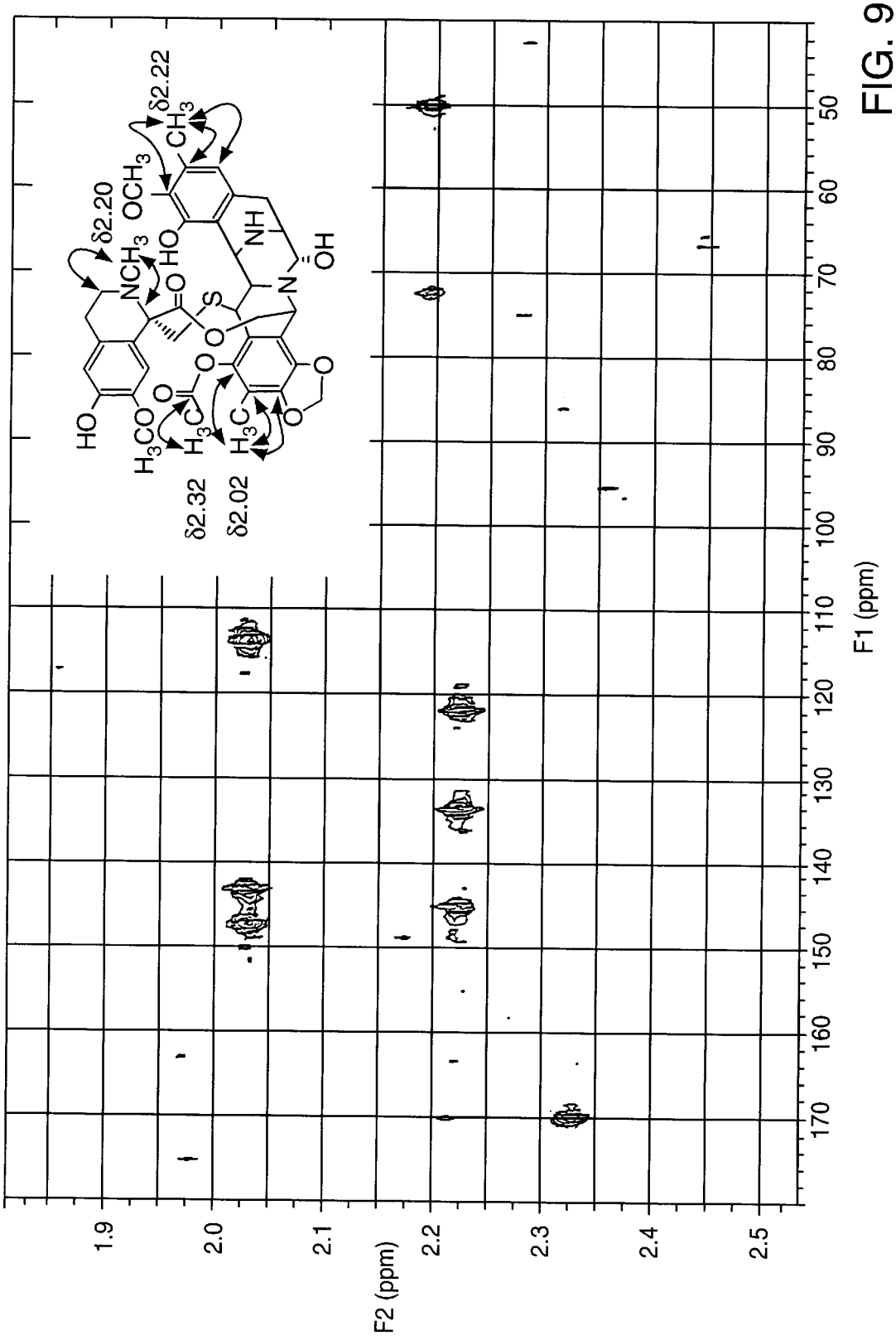


FIG. 9

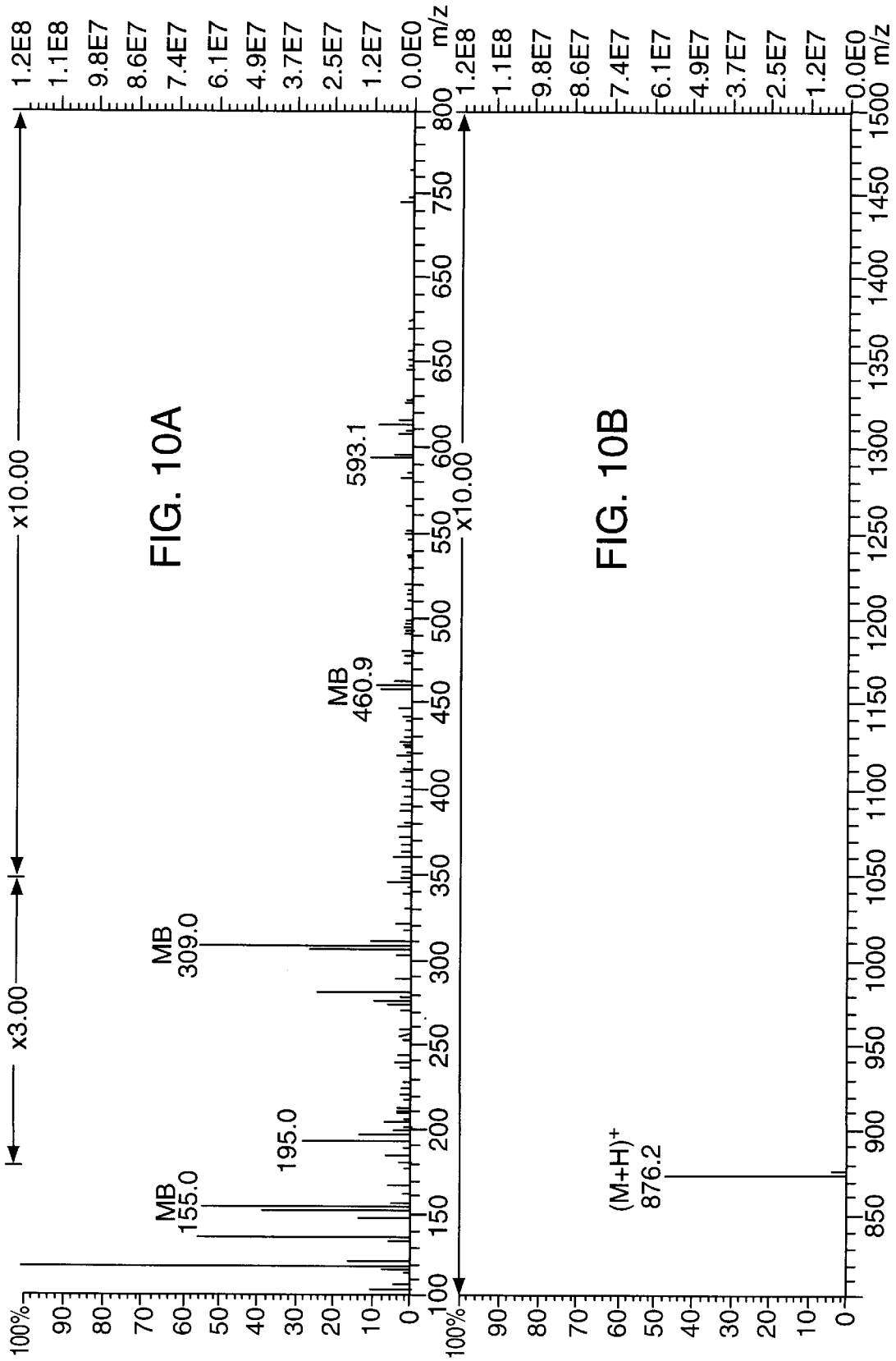


FIG. 10A

FIG. 10B

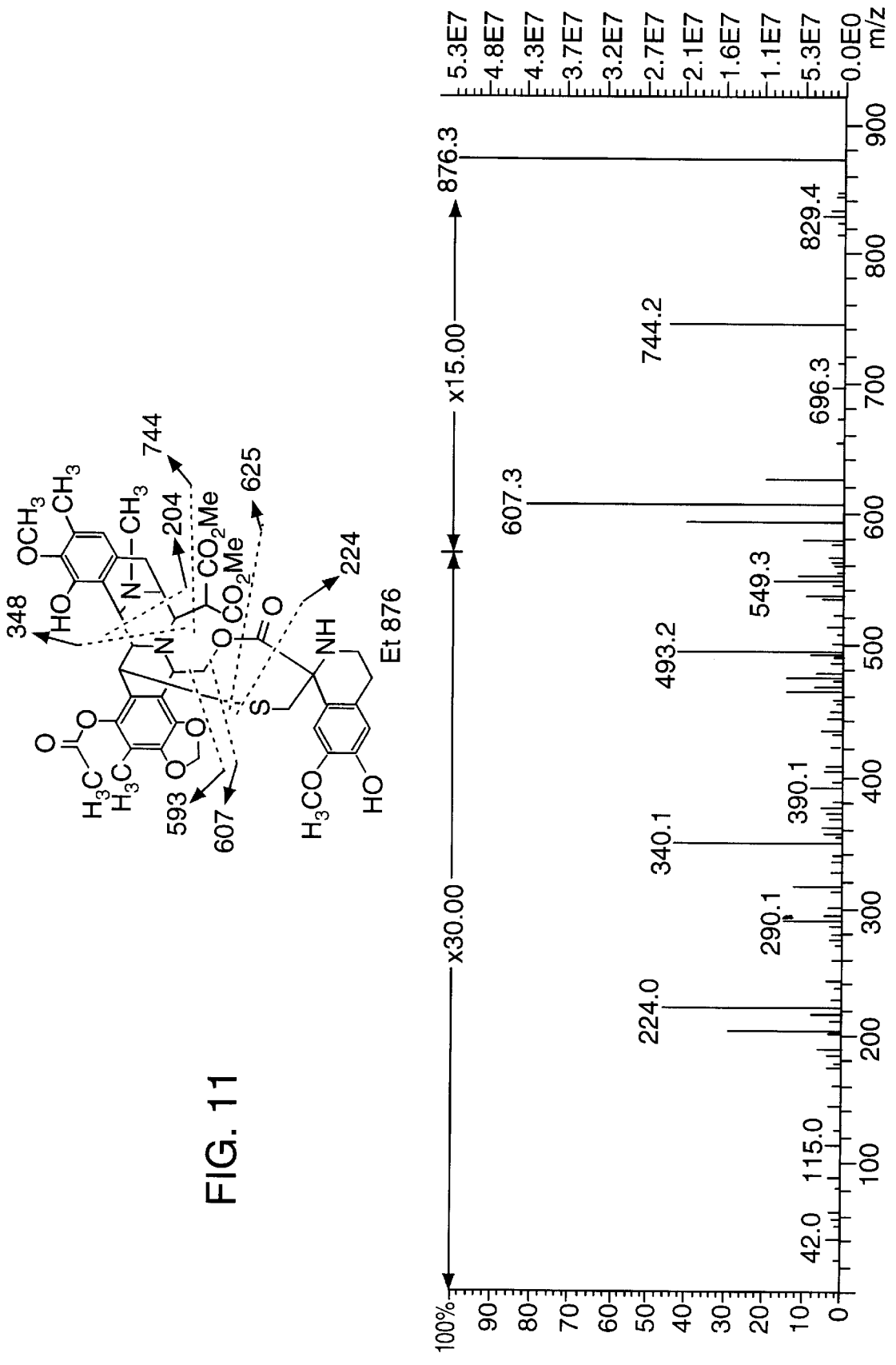

348
744
204
625
224
593
607
Et 876
x30.00
x15.00
876.3
607.3
744.2
829.4
696.3
549.3
493.2
340.1
390.1
290.1
224.0
115.0
42.0
m/z
100%
5.3E7
4.8E7
4.3E7
3.7E7
3.2E7
2.7E7
2.1E7
1.6E7
1.1E7
5.3E7
0.0E0

FIG. 11

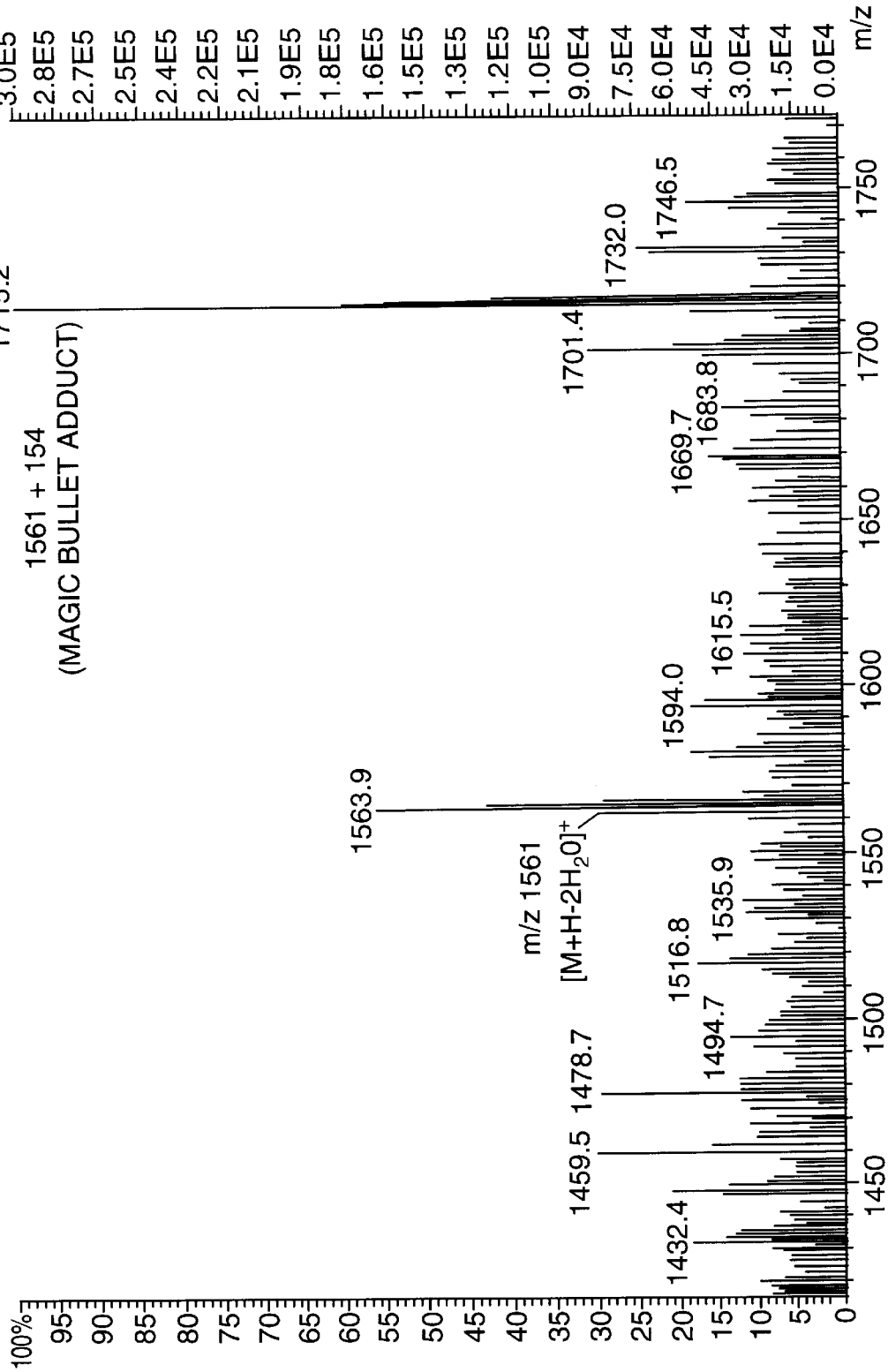

1715.2
1561 + 154
(MAGIC BULLET ADDUCT)
1732.0
1746.5
1701.4
1683.8
1669.7
1594.0
1615.5
1563.9
m/z 1561
[M+H-2H2O]+
1516.8
1535.9
1494.7
1478.7
1459.5
1432.4
m/z
100%

FIG. 12

SEMI-SYNTHETIC ECTEINASCIDINS

CROSS-REFERENCE TO RELATED APPLICATION

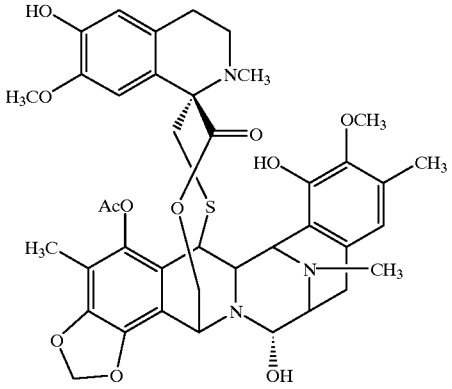
This application claims priority from provisional application, U.S. Ser. No. 60/080,802, filed Apr. 6, 1998, the disclosure of which is hereby incorporated herein by reference.

BACKGROUND OF THE INVENTION

The ecteinascidins (herein abbreviated Et or Et's) are exceedingly potent antitumor agents isolated from the marine tunicate *Ecteinascidia turbinata*. In particular, Et's 729, 743 and 722 have demonstrated promising efficacy in vivo, including activity against P388 murine leukemia, B16 melanoma, Lewis lung carcinoma, and several human tumor xenograft models in mice. The antitumor activities of Et 729 and Et 743 have been evaluated by the NCI and recent experiments have shown that Et 729 gave 8 of 10 survivors 60 days following infection with B16 melanoma. In view of these impressive results, the search for additional ecteinascidin compounds continues.

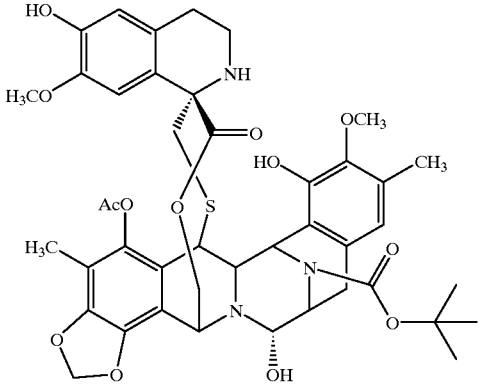
SUMMARY OF THE INVENTION

The present invention is directed to several new ecteinascidin compounds, prepared semi-synthetically, i.e., using previously discovered ecteinascidin compounds as the starting materials therefor. The structures of the new Et's of the present invention are as shown in Chart I below:



Et 757
$C_{40}H_{45}N_3O_{11}S$
Mol. Wt. 775

HRFAB:[M + H—H_2O]$^+$ 758.2765 (Δ – 1.8 mDa)



Boc•Et 729

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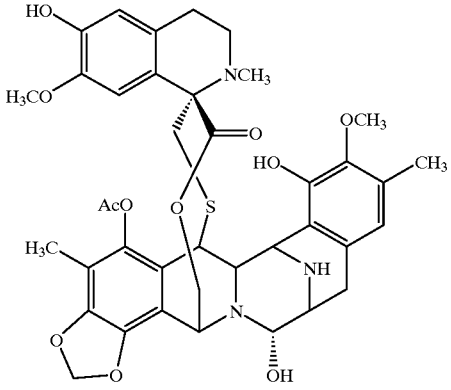
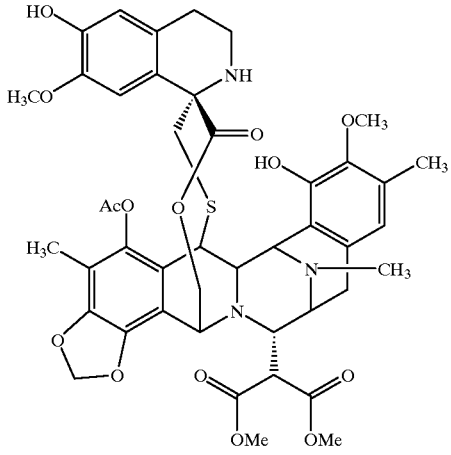


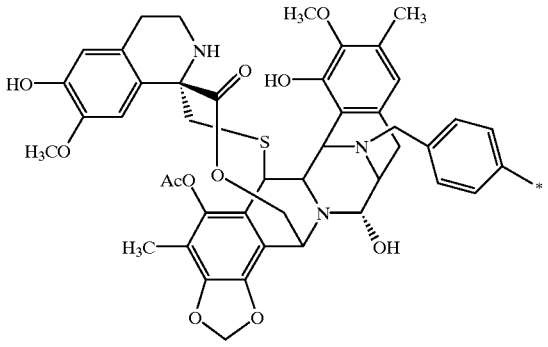
Iso-Et 743
$C_{35}H_{43}N_3O_{11}S$
Mol. Wt. 761.26

HRFAB:[M + H—H_2O]$^+$ 744.2619 (Δ – 2.8 mDa)



Et 875
$C_{44}H_{49}N_3O_{14}S$
Mol. Wt. 875.94

HRFAB:[M + H]$^+$ 875.2986 (Δ - 2.8 mDa)



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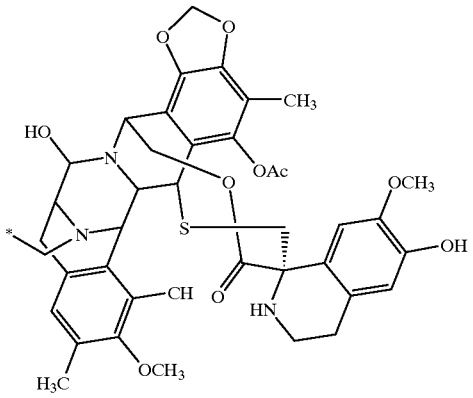


Et 1560
$C_{84}H_{38}N_6O_{22}S_2$
Mol. Wt. 1596

The new ecteinascidin compounds shown above have been found to possess similar antitumor activity profiles as the known ecteinascidin compounds, and as such they will be useful as therapeutic compounds, e.g., for the treatment of mammalian tumors including melanoma, lung carcinoma, and the like. The dosages and routes of administration will vary according to the needs of the patient and the specific activity of the active ingredient. The determination of these parameters is within the ordinary skill of the practicing physician.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGS. **1**A and **1**B show the LRFAB Mass Spectrum of Et 757 in Magic Bullet (MB). See, Rinehart et al., *Biochem, Biophys. Res. Commun.,* 1984, 124, 350.

FIGS. **2**A and **2**B show the tandem FABMS/MS spectrum of Et 757 in MB.

FIG. **3** shows the ^1H NMR (500 MHz) spectrum of Et 757 in CD_3OD.

FIGS. **4**A and **4**B show the LRFAB Mass Spectrum of Et 729 in MB.

FIGS. **5**A and **5**B show the tandem FABMS/MS spectrum of Boc-Et 729 in MB.

FIG. **6** shows the LRFAB Mass Spectrum of Iso-Et 743 in MB.

FIGS. **7**A and **7**B show the tandem FABMS/MS spectrum of Iso-Et 743 in MB.

FIG. **8** shows the ^1H NMR (500 MHz) spectrum of Iso-Et 743 in CD_3OD.

FIG. **9** shows expansion of the HMBC (750 MHz) spectrum of Iso-Et 743 in CD_3OD.

FIGS. **10**A and **10**B show the LRFAB Mass Spectrum of Et 875 in MB.

FIG. **11** shows the tandem FABMS/MS spectrum of Et 875 in MB.

FIG. **12** shows the LRFAB Mass Spectrum of Et 1560 in MB.

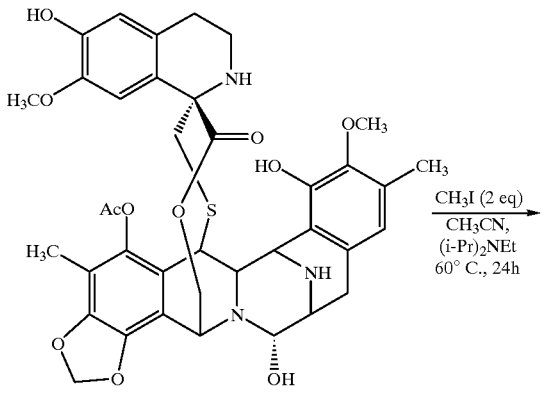
DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

As described above, a number of bioactive ecteinascidin compounds have been isolated from specimens of *Ecteinascidia turbinata*. See for example Ecteinascidins 729, 743, 745, 759A, 759B and 770, disclosed in U.S. Pat. Nos. 5,089,273 and 5,256,663, the disclosures of which are hereby incorporated herein by reference. See also, Ecteinascidins 736 and 722, disclosed in U.S. Pat. No. 5,149,804, which is hereby incorporated herein by reference. See also, U.S. Pat. Nos. 5,478,932 and 5,654,426, which are hereby incorporated herein by reference.

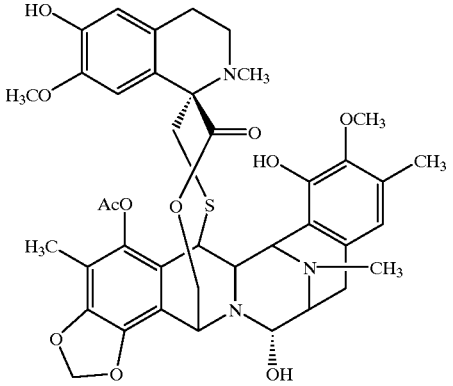
The present invention will be further illustrated with reference to the following examples which aid in the understanding of the present invention, but which are not to be construed as limitations thereof. All percentages reported herein, unless otherwise specified, are percent by weight. All temperatures are expressed in degrees Celsius.

EXAMPLE 1

Semi-synthesis of Et 757



Et 729
$C_{36}H_{41}N_5O_{11}S$
Mol. Wt. 747

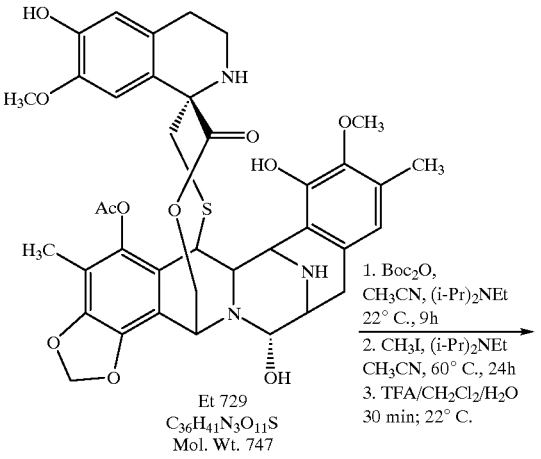


Et 757
$C_{40}H_{45}N_3O_{11}S$
Mol. Wt.: 775
HRFAB: $[M + H - H_2O]^+$ 758.2765 (Δ-1.8 mDa)

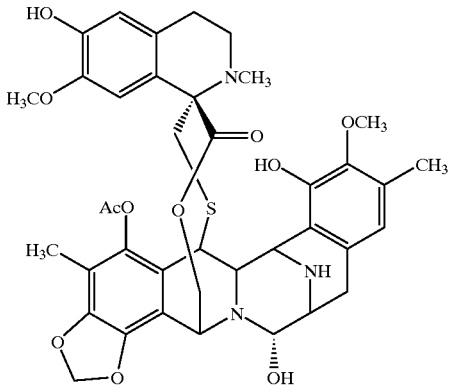
To a solution of Et 729 (9.2 mg, 0.012 mmol, 1 eq), diisopropylamine (12.9 μL, 0.074 mmol, 6 eq) and CH_3CN (300 μL) was added CH_3I (1.5 μL, 0.024 mmol, 2 eq) and the resulting solution was stirred at 60° C. for 24 hours. The reaction mixture was concentrated dryness under a nitrogen stream. The residue was purified by reversed phase HPLC (Phenomenex/Ultracarb-ODS, 2 mL/min) using 75% MeOH/H_2O containing 0.02 M NaCl as mobile phase to yield Et 757 (2.2 mg, 24%) and Et 743 (2.3 mg, 25%) and a complex mixture of permethylated products. Et 757 was further purified by HPLC (Ultracarb-ODS) using 60% MeOH/H_2O with 0.02 M NaCl as mobile phase to afford pure Et 757 (1.4 mg, 15%). HRFABMS, Calcd for $C_{40}H_{44}N_3O_{10}S$ $[M+H-H_2O]^+$ m/z 758.2747, Found 758.2765, see FIGS. **1** and **2**; ^1H NMR, see FIG. **3**.

EXAMPLE 2

Semi-synthesis of Iso-Et 743



Et 729
$C_{36}H_{41}N_3O_{11}S$
Mol. Wt. 747



Iso-Et 743
$C_{35}H_{43}N_3O_{11}S$
Mol. Wt.: 751.26
HRFAB: $[M + H - H_2O]^+$ 744.2619 (Δ-2.8 mDa)

Step A—Boc-Et 729

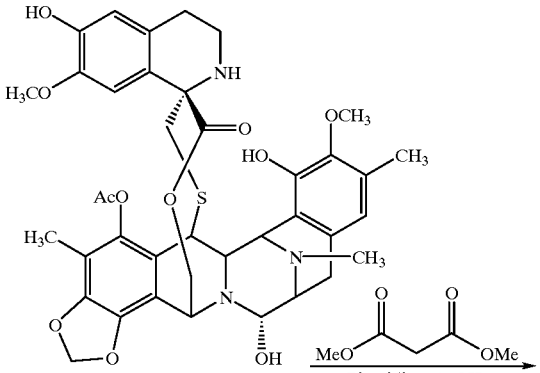
To a solution of Et 729 (12.5 mg, 0.017 mmol, 1 eq), diisopropylethylamine (1.5 μL, 0.07 mmol, 4 eq) and CH_3CN (300 μL) was added di-tert-butyl dicarbonate (3.6 mg, 0.017 mmol, 1.0 eq) and the resulting solution was stirrred at room temperature for 9 hours. The reaction mixture was concentrated to dryness under a nitrogen stream. The residue was purified by flash chromatography (gradient elution: 100% $CHCl_3$→90% $CHCl_3$/MeOH) to aford Boc-Et 729 (11.6 mg, 91%, R_f 0.53 in 90% $CHCl_3$/MeOH); HRFABMS, Calcd for $C_{43}H_{48}N_3O_{12}S$ $[M+H]^+$ m/z 830.2958, Found 830.2942, see FIGS. **4** and **5**.

Step B—Iso-Et 743

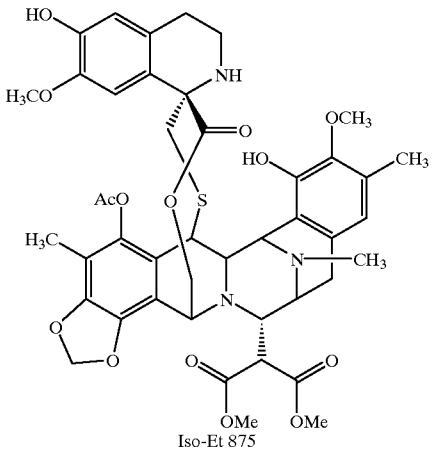
To a reaction flask containing Boc-Et 729 (11.6 mg, 0.014 mmol, 1 eq), diisopropylethyl amine (7.1 μL, 0.041 mmol, 3 eq), 500 μL of CH_3CN and a magnetic stirrer was added CH_3I (2.1 mg, 0.015 mmol, 1.1 eq), and the resulting solution was stirred at 60° C. for 24 hours. The reaction mixture was concentrated to dryness under a nitrogen stream, then 700 μL of TFA/CH_2Cl_2/H_2O (4:1:1) was added. After the mixture was stirred at room temperature for 30 minutes, it was concentrated to dryness under a nitrogen stream. The residue was purified by reversed phase HPLC (Alltech-C18, 2 mL/min) using 60% MeOH/H_2O containing 0.02 M NaCl as mobile phase to yield Iso-Et 743 (1.9 mg, 28%, based upon recovered Et 729) and unreacted Et 729 (3.6 mg). HRFABMS, Calcd for $C_{39}H_{42}N_3O_{10}S$ $[M+H-H_2O]^+$ m/z 744.2591, Found 744.2619, see FIGS. **6** and **7**; ^1H NMR and HMBC, see FIGS. **8** and **9** respectively.

EXAMPLE 3

Semi-synthesis of Et 875



Et 743
$C_{33}H_{43}N_3O_{11}S$
Mol. Wt.: 761

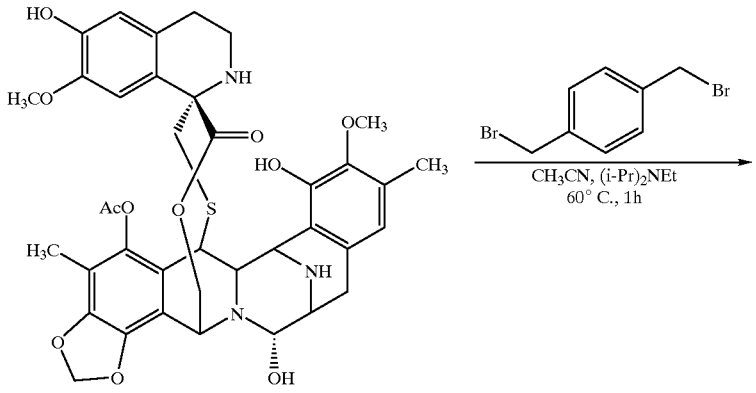


Iso-Et 875
$C_{45}H_{43}N_3O_{14}S$
Mol. Wt.: 875.94
HRFAB: $[M + H]^+$ 876.2986 (Δ2.8 mDa)

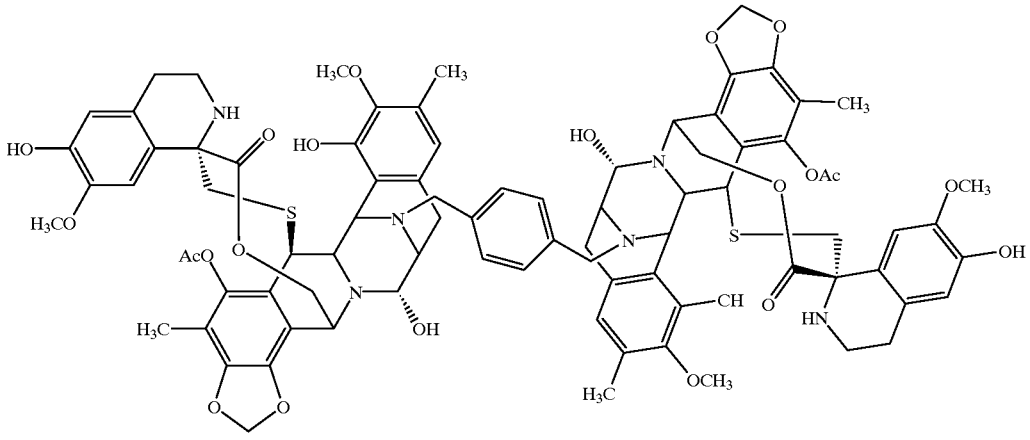
Glacial acetic acid (5 μL of a 28% AcOH/CH_3CN solution, 4 eq) was added to a mixture of Et 743 (0.9 mg, 0.001 mmol, 1 eq), piperidine (5 μL of a 2% piperidine/CH_3CN solution, 0.001 mmol, 1 eq), dimethyl malonate (5 μL of a 3% dimethyl malonate/CH_3CN solution, 0.001 mmol, 1 eq) and crushed activated 4 Å molecular sieves (~0.5 mg) in CH_3CN and the resulting suspension was stirred at room temperature for 24 hours. The reaction was filtered and the filtrate was concentrated to dryness. The residue was purified by flash chromatography (gradient elution: 100% $CHCl_3$→90% $CHCl_3$/MeOH) to yield Et 875 (180 μg, 20%, R_f 0.53 in 90% $CHCl_3$/MeOH); HRFABMS, Calcd for $C_{44}H_{50}N_3O_{14}S$ $[M+H]^+$ m/z 876.3013, Found 876.2986, see FIGS. **10** and **11**.

EXAMPLE 4

Semi-synthesis of Et 1560 (Et 729 dimer)



Et 729
$C_{36}H_{41}N_3O_{11}S$
Mol. Wt.: 747.82



Et 1560
$C_{86}H_{88}N_6O_{22}S_2$
Mol. Wt.: 1596

To a reaction flask containing Et 729 (2.4 mg, 0.0032 mmol, 2 eq), diisopropylamine (2 μL) and CH_3CN (75 μL) and a magnetic stirrer was added α,α'-dibromo-p-xylene (34 μL of a 12.5 μg/μL α,α'-dibromo-p-xylene/CH_3CN solution, 0.0016 mmol, 1 eq) and the resulting solution was stirred at 60° C. for 1 hour. The reaction mixture was concentrated to dryness under a nitrogen stream. The residue purified by flash chromatography (gradient elution: 100% $CHCl_3$→90% $CHCl_3$/MeOH) to yield Et 1560 (300 μg, 12%, R_f 0.53 in 90% $CHCl_3$/MeOH); HRFABMS, Calcd for $C_{84}H_{85}N_6O_{20}S_2$ $[M+H-2H_2O]^+$ m/z 1561.5260, Found 1561.5221, see FIG. **12**.

BIOLOGICAL ACTIVITIES

As described above, the ecteinascidins are highly functionalized bis- or tris-(tetrahydroisoquinoline) alkaloids that exhibit potent in vivo antitumor activity. These compounds have chiefly been isolated as natural products from the mangrove tunicate *Ecteinascidia turbinata*, which grows throughout the Caribbean and the Gulf of Mexico. The major product of most extractions, Et 743, is currently undergoing Phase I clinical trials for treatment of human solid tumors. See for example, Kuffel et al., *Proceedings of the American Association for Cancer Research,* 38: 596 (1997); Moore et al., *Proceedings of the American Association for Cancer Research,* 38: 314 (1997); Mirsalis et al., *Proceedings of the American Association for Cancer Research,* 38: 309 (1997); Reid et al., *Cancer Chemotherapy and Pharmacology,* 38: 329–334 (1996); Faircloth et al., *European Journal of Cancer,* 32A, Supp. 1, pp. S5 (1996); Garcia-Rocha et al., *British Journal of Cancer,* 73: 875–883 (1996); Eckhardt et al., *Proceedings of the American Association for Cancer Research,* 37: 409 (1996); and Hendriks et al., *Proceedings of the American Association for Cancer Research,* 37: 389 (1996).

In view of the exceptional antitumor properties of the natural ecteinascidins, the present invention has studied the antitumor activities of the semi-synthetic analogs prepared herein. Table I shows the in vitro cytotoxic activities of the new Et compounds compared to the activity of two natural products, Et 743 and Et 729:

TABLE I

	Cytotoxicity to L1210 murine leukemia	
Compound Name	IC_{50}	IC_{50}(Et 743)/IC_{50}
Et 729	0.05	10
Et 743	0.5	1
Et 757	0.01	50

TABLE I-continued

Compound Name	Cytotoxicity to L1210 murine leukemia IC_{50}	IC_{50}(Et 743)/IC_{50}
Iso-Et 743	0.03	17
Boc-Et 729	5.0	0.1
Et 1560	2.0	0.25
Et 875	0.5	1

As shown by the in vitro data presented in Table I, the new compounds of the present invention possess cytotoxic activities levels up to 10 times better than those of two natural ecteinascidin compounds. Accordingly, it is expected that these compounds will also prove useful as pharmaceutical compositions for the treatment of mammalian, and particularly, human tumors in vivo.

REFERENCES

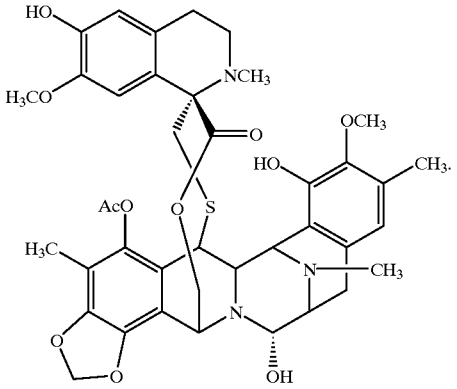
The following publications are cited as additional background information. To the extent necessary to allow a complete understanding of this invention, each is hereby incorporated herein by reference:

1. Rinehart, K. L. et al., *J. Nat. Prod.,* 53: 771–791 (1990).
2. Wright, A. E. et al., *J. Org. Chem.,* 55: 4508–4512 (1990).
3. Sakai et al., *Proc. Nat. Acad. Sci. U.S.A.,* 89: 11456–11460 (1992).
4. Rinehart et al., *J. Org. Chem.,* 55: 4512–4515 (1990).

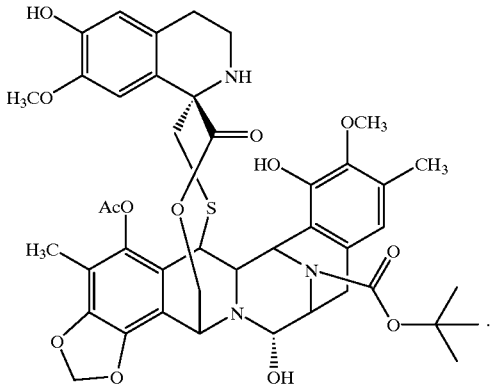
The present invention has been described in detail, including the preferred embodiments thereof. However, it will be appreciated that those skilled in the art, upon consideration of the present disclosure, may make modifications and/or improvements on this invention and still be within the scope and spirit of this invention.

What is claimed is:

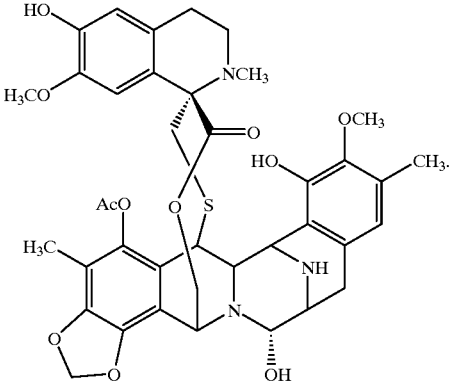
1. The compound Et 757, which has the following structure:



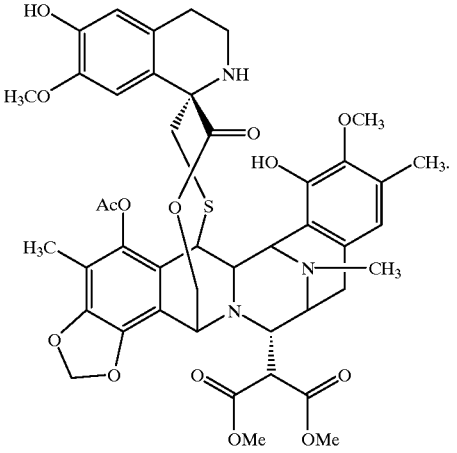
2. The compound Boc-Et 729, which has the following structure:



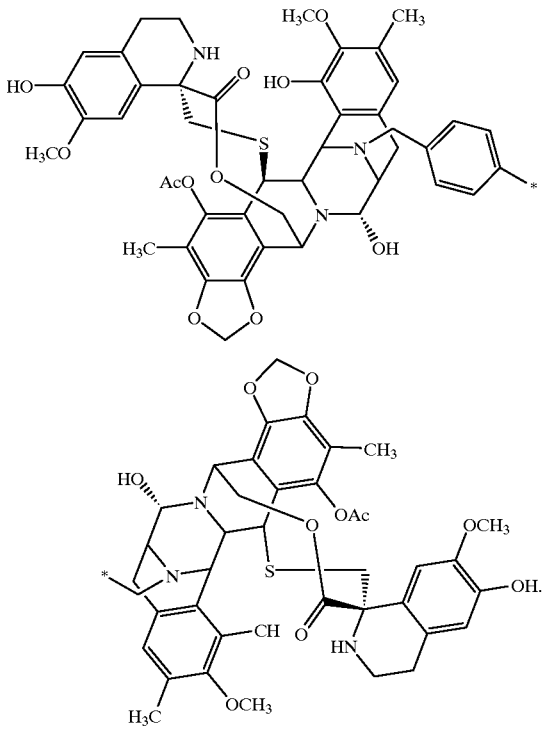
3. The compound Iso-Et 743, which has the following structure:



4. The compound Et 875, which has the following structure:



5. The compound Et 1560, which has the following structure:



6. A pharmaceutical composition comprising the compound Et 757 and a pharmaceutically acceptable diluent, carrier, or excipient.

7. A pharmaceutical composition comprising the compound Boc-Et 729 and a pharmaceutically acceptable diluent, carrier, or excipient.

8. A pharmaceutical composition comprising the compound Iso-Et 743 and a pharmaceutically acceptable diluent, carrier, or excipient.

9. A pharmaceutical composition comprising the compound Et 875 and a pharmaceutically acceptable diluent, carrier, or excipient.

10. A pharmaceutical composition comprising the compound Et 1560 and a pharmaceutically acceptable diluent, carrier, or excipient.

11. A method of treating a patient suffering from a mammalian tumor selected from the group consisting of mammalian leukemia, mammalian melanoma and mammalian lung carcinoma, comprising administering to said patient, an effective antitumor amount of carcinoma, comprising administering to said patient, an effective antitumor amount of the substantially pure compound designated herein as Et 757 and a pharmaceutically acceptable carrier, diluent or excipient.

12. A method of treating a patient suffering from a mammalian tumor selected from the group consisting of mammalian leukemia, mammalian melanoma and mammalian lung carcinoma, comprising administering to said patient, an effective antitumor amount of the substantially pure compound designated herein as Boc-Et 729 and a pharmaceutically acceptable carrier, diluent or excipient.

13. A method of treating a patient suffering from a mammalian tumor selected from the group consisting of mammalian leukemia, mammalian melanoma and mammalian lung carcinoma, comprising administering to said patient, an effective antitumor amount of the substantially pure compound designated herein as Iso-Et 743 and a pharmaceutically acceptable carrier, diluent or excipient.

14. A method of treating a patient suffering from a mammalian tumor selected from the group consisting of mammalian leukemia, mammalian melanoma and mammalian lung carcinoma, comprising administering to said patient, an effective antitumor amount of the substantially pure compound designated herein as Et 875 and a pharmaceutically acceptable carrier, diluent or excipient.

15. A method of treating a patient suffering from a mammalian tumor selected from the group consisting of mammalian leukemia, mammalian melanoma and mammalian lung carcinoma, comprising administering to said patient, an effective antitumor amount of the substantially pure compound designated herein as Et 1560 and a pharmaceutically acceptable carrier, diluent or excipient.

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