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FR182876, a New Microtubule Modulator with High Water-solubility, from a *Streptomyces*

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In the course of seeking new anti-tumor drugs, a new microtubule modulator with high water-solubility, FR182876, was isolated from a *Streptomyces* which also produces FR182877. Even modern spectroscopic methods could not solve the structure of FR182876 due to its structural complexity and chemical instability. Thus, we have combined chemical correlations with spectroscopic methods and determined its structure, which features a highly fused ring system and 3-methylhistidine. The latter is believed to contribute to both solubility in water and activity in promoting tubulin polymerization. FR182876 showed potent cytotoxicity against a panel of cancer cells at concentrations of 28～75 ng/ml.

Microtubule modulators have been eliciting much interest from chemists and biologists of both academics and industrial companies, because those agents have been proved clinically effective against slow-growing tumors and provided many attractive challenges in chemistry and biology. Vinblastine is known as an inhibitor of microtubule assembly and exhibits prominent anti-tumor activities.[1] Taxol,[2] isolated in 1967 and was found by HORWITZ *et al.*[3] to accelerate microtubule polymerization to arrest cell cycle at the G2/M phase, was approved for treatment of ovarian cancer in 1992 and is now widely used in clinical. However, taxol has serious drawbacks, *i.e.* lack of water solubility and emergence of multiple drug resistance. In the past decade, many efforts have been made to discover compounds acting in the same manner as taxol in order to overcome these drawbacks and taxol-like microtubule modulators, such as epothilones,[4] discodermolide,[5] eleutherobins,[6] and laulimalide,[7] have been reported. While clinical trials of epothilones are ongoing, both types of microtubule modulators should be attractive targets for anti-cancer drugs.

Since microtubules not only form mitotic spindles but are also important components of the cell skeleton, modulation of microtubules causes characteristic morphological changes of cells.[8] We applied this morphological change as an indicator to screen microtubule-acting agents from microbial products and identified FR182877 (**1**) from *Streptomyces* sp. No.9885.[8,9] This compound showed potent cytotoxicity against a panel of cancer cells, and promoted tubulin polymerization. In terms of chemistry, the structure of **1** was intriguing enough to encourage its total synthesis.[10] The structure features a highly fused ring system and a strained olefin, and **1** showed unique reactivity at the olefin. Another active compound (FR182876, **2**) was isolated from water extracts of the same source as **1**, however, its structural complexity and chemical instability precluded its structure determination even by the use of modern NMR techniques. We have thus applied a combination of spectroscopic methods and chemical correlations, and have finally elucidated the structure of **2**, including its absolute configuration (Figure 1). In this article, the isolation, biological activities, and structure determination of **2** will be discussed.

Results and Discussion

Isolation

Fractions including FR182876 (**2**) were originally observed in water partition of a fermentation broth of *Streptomyces* sp. No.9885 concurrently with FR182877, isolated from the organic fraction. As production of **2** was quite low under these conditions, fermentation conditions (additives, temperature, time, pH) were modified to optimize the yield of **2**. The broth was diluted with acetone

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Fig. 1. Structures of FR182877 (**1**) and FR182876 (**2**).

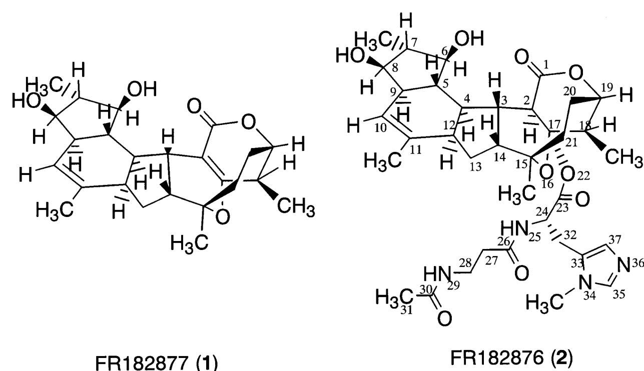
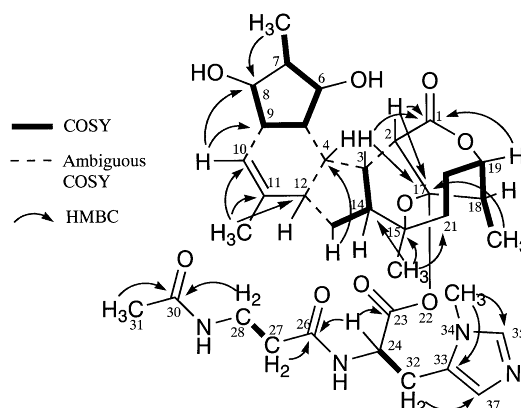


Fig. 2. Structure elucidation of **2** by NMR.



and filtered, and the filtrate was then evaporated, adjusted to pH 4, and partitioned between water and ethyl acetate. The aqueous phase was purified successively by Diaion HP-20, anion exchange resin, silica gel column chromatography and HPLC to give 270 mg of **2** as a colorless amorphous solid (mp 179~183°C (dec.), $[\alpha]_D$ −52.8 (*c* 0.5, 0.01 N HCl in H_2O, 23°C). It was soluble in water to a concentration of 10 mg/ml.

Structure Elucidation

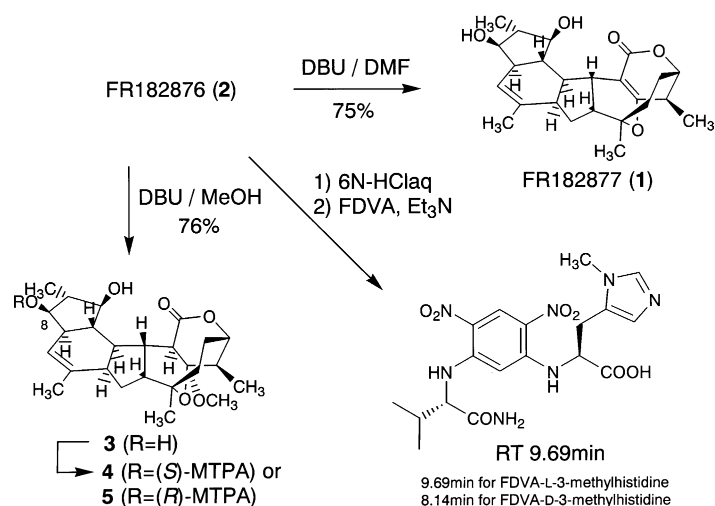
ESI-MS analysis detected a protonated molecular ion at *m/z* 683. Analysis of the HRFAB-MS and ^{13}C NMR data of **2** established its molecular formula to be $C_{36}H_{50}N_4O_9$ (obsd. 683.3692; M+H requires 683.3656). The IR spectrum indicated the presence of hydroxyl ($3400\,cm^{-1}$), ester ($1720\,cm^{-1}$) and amide ($1660\,cm^{-1}$) functios, suggesting a peptidyl structure in view of its molecular formula. The compound was unstable in the usual deuterated solvents such as DMSO-d_6, CD_3OD and D_2O. Additionally, all NMR signals appeared broad in these solvents, and some signals were apparently missing. Line broadening caused a poor S/N ratio in NMR spectra, requiring much longer times for NMR measurement than usual. Thus, we tested various solvent systems where **2** could be stable during a series of NMR measurement, and found 0.01 N-DCl in D_2O to be suitable for this purpose although signals were still broad. DMSO-d_6 was used as a supportive solvent to observe exchangeable protons.

The ^1H and ^{13}C NMR data showed the existence of five carbonyl, one *N*-methyl (δ_C 34.4, δ_H 3.92) and one ketal (δ_C 90.4) groups. Three ^{13}C NMR signals resonating at δ_C 136.2(d), 134.8(s) and 116.9(d) and their large $^1J_{CH}$ values (225 Hz for 35-CH and 201 Hz for 37-CH) were indicative of an imidazole ring.[11] A combination of 1D and 2D NMR spectra clearly showed the presence of acetyl and β-alanyl groups.

COSY data clarified H–H spin networks shown in Figure 2 as bold lines. The existence of a 3-methylhistidine residue was revealed by HMBC data of 34-CH_3/C-33 and C-35 and 32-H_2/C-37 and confirmed by chemical degradation (*vide infra*). The peptide sequence was determined to be acetyl-β-alanine-3-methylhistidine from HMBC data of 24-H/C-23 and C-26, 27-H_2/C-26, 28-H_2/C-30 and 31-H_3/C-30. Because several ambiguous H–H correlations, shown in Figure 2 as dotted lines, were observed due to small coupling constants, line broadening or signal overlap, elaborate analysis was carried out with HMBC correlations from methyl protons as follows. HMBC correlation of 7-CH_3/C-8 links C-7 to C-8 while the vicinal coupling constant of H-7/H-8 is small. HMBC correlations of 11-CH_3/C-10, C-11 and C-12 showed a tri-substituted olefin linked to 11-CH_3 and C-12. Junctions of C-14/C-15 and C-15/C-21 were concluded by HMBC data from 15-CH_3 to C-14, C-15 and C-21. HMBC correlation of 18-CH_3/C-17 allowed C-17/C-18 linkage.

Further HMBC analysis was performed as shown below. An inferred bond of C-9/C-10 was confirmed by HMBC correlations of 10-H/C-8 and C-9. HMBC correlations from 2-H and 3-H to C-1 and C-17 suggested C-1/C-2 and C-2/C-17 linkages. A δ-lactone structure was shown by HMBC correlation of 19-H/C-1 to unveil a highly fused ring system as that of **1**. NOESY of **2** showed spatial proximity of 2-H/37-H, which suggested an ester linkage

Scheme 1. Chemical correlations of **2**.



between C-17 and C-23. Finally, location of a hydroxyl function at C-6 was in agreement with the chemical shift of 6-H (3.44), and indicated the tentative planar structure of **2** (Figure 2).

Chemical Correlations

Spectroscopic evidence, especially around C-4, was not conclusive due to line broadening and signal overlapping. As a close structural resemblance was inferred between **1** and **2**, we expected that **2** could be transformed into **1** by β-elimination of the peptide moiety. Thus, we treated **2** with 1,8-diazabicyclo[5,4,0]undec-7-ene (DBU) in methanol, and unexpectedly obtained a stable product **3**. Since shorter reaction time gave an unseparable mixture of **1** and **3**, **3** was probably formed by β-elimination followed by Michael addition of methanol. On using DMF instead of methanol, **1** was successfully obtained in a moderate yield (see Scheme 1). The identification was made based on ^{13}C NMR data shown in Table 1. This result shows not only that the inferred planar structure of **2** is valid, but also that the relative configurations of its polyketide core are the same as those of **1** except at C-2 and C-17. With the proven structure in hand, further stereochemical analysis was performed using NOESY data of **2**. NOE of H-2/H-18 indicates that these protons are on the same side of the δ-lactone ring. The peptide moiety must be oriented on the same face as H-2, as NOEs of H-2/H-37 and H-18/H-37 were observed. The modified Mosher method[12] was applied to **3** to determine its absolute configuration since direct esterification of **2** gave a complex mixture. (*S*)- or (*R*)-MTPA ester was selectively introduced at the 8-postion to give **4** and **5**. NMR data of **4** and **5** are depicted in Table 1, which allowed the assignment of an 8*R* configuration according to the reported method. The only remaining problem for the structure of **2** is the absolute stereochemistry at C-24, and it was solved by chemical degradation as follows (see Scheme 1). Acid hydrolysis of **2** followed by FDVA (*N*-(3-fluoro-4,6-dinitrophenyl)-L-valinamide) derivatization[13] gave FDVA-3-methylhistidine detected by LC-MS at 9.69 minutes of retention time. As the derivatives of (*S*)- or (*R*)-3-methylhistidine were observed at 9.69 minutes or 8.14 minutes respectively, the configuration of C-24 was determined to be *S*. Therefore, the gross structure of **2** was determined as shown in Fig. 1. With the structure of **2** in hand, the complete ^{1}H/^{13}C assignment of **3** was made unequivocally and the data are shown in Table 1.

Biological Activities

The cytotoxicity of **1** and **2** are shown in Table 2. FR182876 (**2**) was as effective as **1** against a panel of human tumor cell lines, while it was less toxic against mouse bone marrow cells (IC_{50} 600 ng/ml). Compound **3** has only weak cytotoxicity (*i.e.* IC_{50} 8.9 μg/ml against HT-29). Interestingly, **3** lacks an ability to induce multi-nucleation to BHK (baby hamster's kidney) cells at the concentration of its IC_{50}, while multi-nuclear cells are formed on treatment of BHK cells with the IC_{50}

Table 1. ^{1}H and ^{13}C NMR assignment of FR182876 (**2**) and its derivatives.[a]

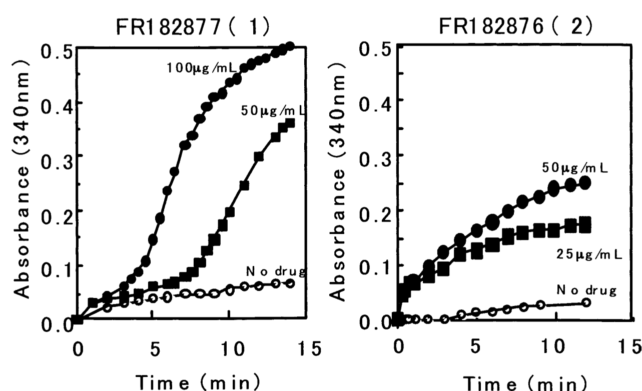
position	**2**		**1 converted from 2**	**1**[b]	**3**		**4**	**5**
	δ_H(0.01N DCl)	δ_C(0.01N DCl)	δ_C(CD_3OD)	δ_C(CD_3OD)	δ_H(CD_3OD)	δ_C(CD_3OD)	δ_H($CDCl_3$)	δ_H($CDCl_3$)
1		176.3	172.9	172.9		178.1		
2	3.27	49.6	115.9	115.9	2.80	48.3	2.74	2.73
3	3.74	43.5	43.4	43.4	3.67	43.9	3.64	3.60
4	1.63	54.6	53.2	53.2	2.10	56.5	2.08	2.04
5	1.70	45.4	46.2	46.2	1.78	46.8	1.71	1.67
6	3.44	83.3	84.6	84.6	3.42	84.7	3.59	3.60
7	1.73	53.2	54.6	54.6	1.75	54.6	1.96	2.06
7-CH_3	1.10	17.7	18.6	18.6	1.13	18.5	1.25	1.26
8	3.71	77.3	78.4	78.4	3.62	78.4	5.02	5.01
9	1.91	44.8	46.9	46.9	1.95	46.6	2.15	2.12
10	5.40	120.2	121.2	121.2	5.44	121.0	5.34	5.23
11		140.4	140.5	140.5		140.7		
11-CH_3	1.59	22.1	22.9	22.9	1.69	22.7	1.66	1.59
12	1.29	45.6	47.4	47.4	2.74	45.8	2.80	2.75
13 H13a	2.22	33.1	33.7	33.7	2.24	34.0	2.28	2.22
H13b	1.64				1.52		1.41	1.33
14	2.55	43.3	52.4	52.5	2.31	44.8	2.30	2.26
15		78.9	88.6	88.6		75.5		
15-CH_3	1.42	26.8	24.1	24.1	1.22	27.9	1.22	1.23
17		90.4	169.2	169.2		99.1		
17-OCH_3					3.30	50.9	3.28	3.27
18	2.68	44.8	42.5	42.5	2.39	42.5	2.35	2.34
18-CH_3	0.74	8.2	9.3	9.3	1.09	9.9	1.11	1.11
19	4.82	79.9	79.3	79.4	4.46	80.1	4.44	4.44
20 H20a	2.15	23.1	25.2	25.2	2.01	24.2	1.93	1.94
H20b	2.01				1.82		1.86	1.85
21 H21a	1.95	34.7	36.2	36.2	1.71	35.9	1.68	1.67
H21b	1.61				1.55		1.53	1.53
23		176.3						
24	4.72	51.9						
25-NH								
26		174.3						
27	2.46 2.38	35.6						
28	3.37	36.5						
29-NH								
30		174.7						
31	1.96	22.4						
32a	3.39	26.3						
32b	3.13							
33		134.8						
34-CH_3	3.92	34.4						
35	9.24	136.2						
37	7.71	116.9						

a ^{1}H NMR, 500MHz; ^{13}C NMR, 125MHz. b see ref. 10.

Table 2. Cytotoxicity of **1** and **2** against cancer cells (IC_{50} ng/ml).

	MCF-7	A549	HT-29	Jurkat	P388	B16
1	27	73	73	33	21	67
2	28	57	44	48	39	75

Fig. 3. Effects of **1** and **2** on tubulin polymerization.



concentration of **1** or **2** (data are not shown).[8] These results indicate that the peptide moiety of **2** as well as the highly reactive olefin of **1** plays an important role for their inhibition of cell cycle.

Effects of **1** and **2** on tubulin polymerization are shown in Figure 3. In this experiment, taxol induced tubulin polymerization while vincristine and rhizoxin inhibited microtubule assembly as known in the literatures (data are not shown).[3,8b,14,15] Though both **1** and **2** exhibited concentration-dependent tubulin-polymerizing activities as potent as taxol, there was a difference in the kinetics of polymerization between **1** and **2**. In the case of **1**, a time lag of about 4 minutes preceded acceleration of the tubulin-polymerization. On the contrary, the polymerization started without delay on adding **2** to tubulin solution. This kinetics could be the result of the peptide structure of **2** as opposed to the highly reactive olefin of **1**. The possibility that this biological difference might be due to the difference in the solubilities of **1** or **2** was ruled out, because this experiment was performed with **1** or **2** predissolved in the medium. We postulate that a nucleophilic addition to the olefin of **1** initiates its activity whereas **2** acts on tubulins directly, although more detail mechanistic study is needed to confirm this postulate.

Conclusion

In conclusion, the structure of FR182876 (**2**), isolated from a *Streptomyces*, was determined as depicted in Fig. 1 including its absolute stereochemistry using spectroscopic methods and chemical correlations. This natural product has the advantage of its high solubility in water. We believe that 3-methylhistidine residue contributes to the solubility, and also, the ability to induce microtubule assembly, which indicates the potential of natural product chemistry. The fact that its kinetics on tubulin polymerization differ from that of FR182877 (**1**) indicates their unique features in the mechanism of action. The postulated mechanism, that a Michael addition to the olefin of **1** gives an active form, was inferred from the high reactivity of the olefin, and led us to another postulate on its biosynthesis that FR187876 might derive from FR182877, although Michael additions of carboxylic acids are very rare. Thus, FR182876, joins a structurally diverse family of microtubule modulators, should shed light on both chemistry and biology in the near future.

Experimental

^{1}H and ^{13}C NMR were measured on a Bruker DRX500 NMR spectrometer. Mass spectra were recorded on a VG ZAB-SE mass spectrometer or Micromass Platform. Preparative thin layer chromatography was carried out on a Merck Silica gel F254 pre-coated plate, Art 5744. L-(CAS No. 368-16-1) and D-3-Methylhistidine (CAS No. 163750-76-3) was purchased from Calbiochem-Novabiochem Corporation and Bachem AG, respectively.

Fermentation and Isolation

Taxonomy of the producing organism was reported previously.[8] A loopful of slant culture of *Streptomyces* sp. No.9885 was inoculated into one 500 ml flask containing 160 ml of the first stage seed medium consisting of glucose (1%), yeast extract (0.5%), defatted soybean meal (1%) and bouillon (0.5%). The flask was incubated in a rotary shaker (220 rpm) at 30°C for 5 days. The seed culture (3.2 ml×9) was transferred into nine 500 ml flasks each containing 160 ml of the second seed medium consisting of glucose (1%), soluble starch (2%), dried yeast (1%), wheat germ (1%), soybean meal (1%) and $CaCO_3$ (0.2%) adjusted to pH 7. The inoculated flasks were incubated on a rotary shaker (220 rpm) at 30°C for 3 days. The entire second stage seed culture was transferred into three 30-liter jar fermentors containing 60-liter of the production medium consisting of glucose (1.4%), soluble starch (4.2%), chicken meat bone meal (1.5%), meat extract (0.5%), Adekanol LG-109 (Asahi Denka, 0.05%), and Silicone KM-70 (Shin-Etsu Kagaku, 0.05%) adjusted to pH 6.8. Cultivation was carried out at 30°C for 6 days at 200 rpm, under atmospheric pressure and 20 liters/minute of aeration.

Acetone (50 liters) was then added to the fermentation broth and the mixture was filtered through diatomaceous earth. The filtrate was concentrated *in vacuo* and adjusted to pH 4.0 with 6 N HCl. The aqueous concentrate was washed twice with ethyl acetate, and the resulting aqueous solution was purified with Diaion HP-20 (10 liters, 30～50% methanol in water). The eluate was concentrated, passed through Dowex-1 (Cl^-, 2 liters), and purified with 2 liters of YMC ODS-AM 120-S50 (10～15% acetonitrile in water). Active fractions were collected and further purified with two successive silica gel chromatographies (10 g, 0～30% water in 2-propanol, then 100 g, butanol - acetic acid - water (4 : 1 : 2)). The active fractions were combined and concentrated *in vacuo* to a volume of 10 ml. The concentrate was purified on HPLC (ODS-AM 120-S50, 25% aqueous acetonitrile containing acetic acid (0.1%)) and lyophilized to give white powder of FR182876 (**2**, 270 mg).

Conversion of **2** to **1**

To a solution of FR182876 (**2**, 5.2 mg) in DMF (0.2 ml) was added a solution of DBU (2.6 mg) in DMF (26 μl). The resulting solution was allowed to stand for 40 minutes, evaporated under reduced pressure, and purified by preparative TLC using ethyl acetate as an eluant to give **1** (2.3 mg, 75%): ESI-MS *m*/*z* 401 (M+H).

Conversion of **2** to **3**

To a solution of FR182876 (**2**, 5.0 mg) in methanol (0.2 ml) was added a solution of DBU (2.5 mg) in methanol (25 μl). The resulting solution was allowed to stand for 1 hour, evaporated under reduced pressure, and purified on preparative TLC using 5% methanol in chloroform as an eluant to give **3** (2.4 mg, 76%): ESI-MS *m*/*z* 433 (M+H).

MTPA Esterification of **3**

A solution of (*R*)-MTPA chloride (2.3 mg) in pyridine (20 μl) was added to a solution of **2** (1.1 mg) in pyridine (30 μl). (*R*)-MTPA chloride (4 mg×3) was added with an interval of 30 minutes, and the reaction mixture was quenched after 2 hours with *N*,*N*-dimethylamino-propylamine (10 μl). The resultant mixture was evaporated and diluted with ethyl acetate. The organic solution was washed with 0.1 N hydrochloric acid, saturated aqueous sodium bicarbonate, water and brine, dried over $MgSO_4$, and evaporated. The residue was purified by preparative TLC to give **4** (1.1 mg, 67%): ESI-MS *m*/*z* 649 (M+H).

(*R*)-MTPA ester (**5**) was synthesized by a similar method using **3** (1.3 mg) and (*S*)-MTPA chloride instead of (*R*)-MTPA chloride to give **5** (1.3 mg, 67%): ESI-MS *m*/*z* 649 (M+H).

Marfey's Analysis

A sample of **2** (1 mg), recovered from a 0.01 N DCl/D_2O solution was dissolved in aqueous 6 N HCl, and heated at 110°C for 16 hours. The mixture was evaporated, and dissolved in water (0.25 ml). To a portion of this solution (20 μl) was added a 6% solution of triethylamine in acetone (10 μl) and a 10% solution of FDVA in acetone (20 μl). The mixture was heated to 40°C for an hour, diluted with water (50 μl) and subjected to LC-MS analysis (YMC-Pack ODS-AM (150×2.0 mm)) with a 20 minutes gradient from 20 to 40% of 0.04% formic acid/acetonitrile in 0.05% formic acid/H_2O. The FDVA derivative was detected at Rt 9.69 minutes, while the derivatives of (*S*)- or (*R*)-3-methylhistidine were observed at 9.69 minutes or 8.14 minutes respectively.

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References

1) HIMES, R. H.; R. N. KERSEY, I. HELER-BETTINGER & F. E. SAMSON: Action of the *vinca* alkaloids vincristine, vinblastine, and desacetyl vinblastine amide on microtubules *in vitro*. Cancer Res. 36: 3798～3802, 1976
2) WANI, H. C.; H. L. TAYLOR, M. E. WALL, P. COGGON & A. T. MCPHAIL: Plant antitumor agents. VI. The isolation and structure of taxol, a novel antileukemic and antitumor agent from *Taxus brevifolia*. J. Am. Chem. Soc. 93: 2325～2327, 1971
3) SCHIFF, P. B.; J. FANT & S. B. HORWITZ: Promotion of microtubule assembly *in vitro* by taxol. Nature 277: 665～667, 1979
4) HOFLE, G.; N. BEDORF, H. STEINMETZ, D. SCHOMBURG, K. GERTH & H. REICHENBACH: Epothilone A and B—Novel 16-membered macrolides with cytotoxic activity: Isolation, crystal structure, and conformation in solution. Angew. Chem. Int. Ed. Engl. 35: 1567～1569, 1996
5) GUNASEKERA, S. P.; M. GUNASEKERA & R. E. LONGLEY: Discodermolide: A new bioactive polyhydroxylated lactone from the marine sponge *Discodermia dissoluta*. J. Org. Chem. 55: 4912～4915, 1990. Correction: J. Org. Chem. 56: 1346, 1991
6) LINDEL, T.; P. R. JENSEN, W. FENICAL, B. H. LONG, A. M. CASAZZA, J. CARBONI & C. R. FAIRCHILD: Eleutherobin, a new cytotoxin that mimics paclitaxel (Taxol) by stabilizing microtubules. J. Am. Chem. Soc. 119: 8744～8745, 1997
7) CORLEY, D. G.; R. HERB, R. E. MOORE, P. J. SCHEUER & V. J. PAUL: Laulimalides: new potent cytotoxic macrolides from a marine sponge and a nudibranch predator. J. Org. Chem. 53: 3644～3646, 1988

8) SATO, B.; H. MURAMATSU, M. MIYAUCHI, S. TAKASE, M. HINO & H. TERANO: New antimitotic substance, FR182877. I. Taxonomy, fermentation, isolation, physico-chemical properties and biological activities. J. Antibiotics 53: 123～130, 2000
9) SATO, B.; H. NAKAJIMA, M. HINO & H. TERANO: New antimitotic substance, FR182877. II. The mechanism of action. J. Antibiotics 53: 204～206, 2000
10) YOSHIMURA, S.; B. SATO, T. KINOSHITA, S. TAKASE & H. TERANO: New antimitotic substance, FR182877. III. Structure determination. J. Antibiotics 53: 615～622, 2000. Correction: J. Antibiotics 55: C-1, 2002
11) Excellent total syntheses of FR182877 based on biogenetic consideration were accomplished by Sorensen and Evans group independently.
a) VOSBURG, D. A.; C. D. VANDERWAL & E. J. SORENSEN: A synthesis of (+)-FR182877, featuring tandem transannular Diels-Alder reactions inspired by a postulated biogenesis. J. Am. Chem. Soc. 124: 4552～4553, 2002
b) VANDERWAL, C. D.; D. A. VOSBURG, S. WEILER & E. J. SORENSEN: An enantioselective synthesis of FR182877 provides a chemical rationalization of its structure and affords multigram quantities of its direct precursor. J. Am. Chem. Soc. 125: 5393～5407, 2003
c) EVANS, D. A. & J. T. STARR: A cascade cycloaddition strategy leading to the total synthesis of (−)-FR182877. Angew. Chem., Int. Ed. Engl. 41: 1787～1790, 2002
d) EVANS, D. A. & J. T. STARR: A cycloaddition cascade approach to the total synthesis of (−)-FR182877. J. Am. Chem. Soc. 125: 13531～13540, 2003
12) BREITMAIER, E. & W. VOELTER: Chapter 4 and 5, *In* Carbon-13 NMR Spectroscopy, 3rd Ed., pp. 183～467, VCH Verlagsgesellshaft, Weinheim, 1987
13) OHTANI, I.; T. KUSUMI, Y. KASHMAN & H. KAKISAWA: High-field FT NMR application of Mosher's method. The absolute configurations of marine terpenoids. J. Am. Chem. SOC. 113: 4092～4096, 1991
14) FUJII, K.; Y. IKAI, H. OKA, M. SUZUKI & K.-I. HARADA: A nonempirical method using LC/MS for determination of the absolute configuration of constituent amino acids in a peptide: combination of Marfey's method with mass spectrometry and its practical application. Anal. Chem. 69: 5146～5151, 1997
15) KUMAR, N.; Taxol-induced polymerization of purified tubulin. J. Biol. Chem. 256: 10435～10441, 1981
16) a) IWASAKI, S.; H. KOBAYASHI, J. FURUKAWA, M. NAMIKOSHI, S. OKUDA, Z. SATO, I. MATSUDA & T. NODA: Studies on macrocyclic lactone antibiotics. VII. Structure of a phytotoxin "rhizoxin" produced by *Rhizopus chinensis*. J. Antibiotics 37: 354～362, 1984
b) KIYOTO, S.; Y. KAWAI, T. KAWAKITA, E. KINO, M. OKUHARA, I. UCHIDA, H. TANAKA, M. HASHIMOTO, H. TERANO, M. KOHSAKA, H. AOKI & H. IMANAKA: A new antitumor complex, WF-1360, WF-1360A, B, C, D, E and F. J. Antibiotics 39: 762～772, 1986