VOL. 53 NO. 6, JUNE 2000 THE JOURNAL OF ANTIBIOTICS pp. 615 — 622

A New Antimitotic Substance, FR182877

III. Structure Determination

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(Received for publication February 17, 2000)

During the course of screening for novel cell cycle inhibitors, FR182877†† was isolated from the fermentation broth of *Streptomyces* sp. No.9885. During the NMR measurements, FR182877 decomposed so much that the structure elucidation of FR182877 itself was difficult. Then, combinations of chemical correlations and spectroscopic methods clarified that FR182877 possesses an unprecedented multi-ring system including the strained double bond, which was unexpectedly epoxidized by molecular oxygen. FR182877 showed broad antitumor activities *in vitro* and promoted assemblies of tublins *in vitro* as well as taxol. It is noteworthy that epoxidation of the distorted double bond resulted in significant decrease in antitumor activities.

Antimitotic drugs, such as *vinca* alkaloids or taxol, are now those of the most important agents for the chemotherapy of human cancers[1,2]. These drugs inhibit microtubule assembly or disassembly in eukaryotic cells and arrest the cell cycle at G2/M phase[3,4].

In the course of screening new antimitotic substances, we isolated a novel antimitotic agent FR182877 (**1**) from fermentation broth of a *Streptomyces* sp. No. 9885, and **1** was shown to induce tublin polymerization[5,6]. The chemical structure of **1** was found to be a unique multicyclic one, characterized by its highly strained conjugated olefin as shown in Fig. 1. This paper describes how the structure of FR182877 was elucidated using spectroscopic methods and chemical reactions.

Results and Discussion

Substructures of FR182877 (**1**)

The molecular formula of **1** was determined to be $C_{24}H_{32}O_5$ by HRFAB-MS (calcd. for $M+H^+$: 401.2328, found: 401.2328). IR spectrum indicated the presence of a hydroxy (3450 cm^{-1}) and an unsaturated ester group (1700 cm^{-1}). DEPT data showed that there are 30 carbon-bound protons, and hence the remaining two protons must be exchangeable ones. These two protons constitute two hydroxy groups since **1** is neutral. The unsaturated degree of **1** is nine, and one carbonyl and four olefinic carbons were observed in the ^{13}C NMR spectrum. Thus, the compound **1** is hexacyclic. The extensively polarized tetra-substituted olefinic carbon pair of δ_C 169.2 (s) and 115.9 (s) was expected to form β-oxyunsaturated ester.

^1H-^1H COSY and HMQC data clarified substructures shown as thick lines in Fig. 3. The apparent HMBC data shown as arrows allowed to build up two substructures. The HMBC correlations from 3-H were not sure because of signal overlapping of 3-H/6-H. Further analysis was impossible because ^1H signals (3-H/6-H, 5-H/7-H/20-H_2) were overlapped and above all **1** gradually decomposed. All efforts to isolate the degraded product were in vain. After *ca.* 30% of **1** decomposed, the resulting mixture was acetylated in order to isolate the product degraded. The

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†† FR182877 was originally designated WS9885B.

Fig. 1. Structure of FR182877 (**1**).

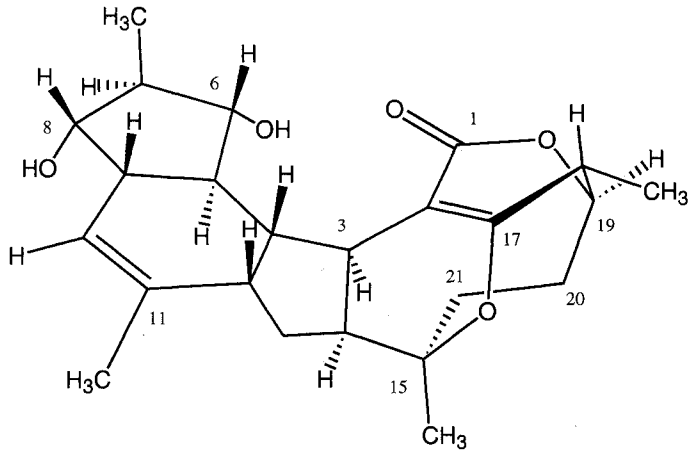
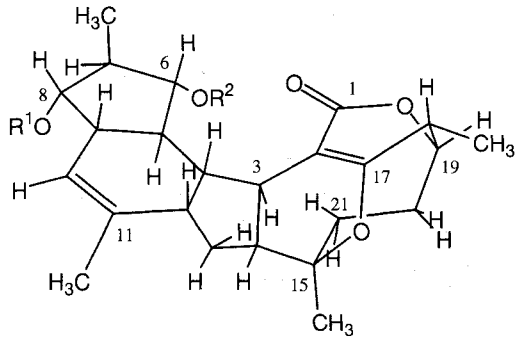
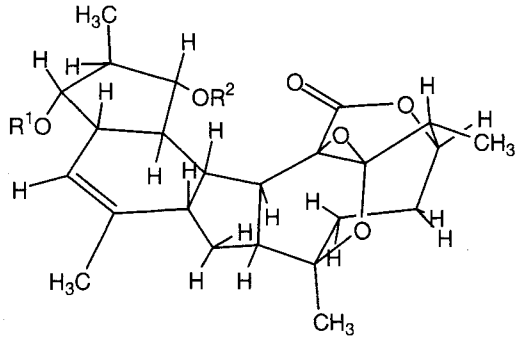


Fig. 2. Structures of FR182877's derivatives.



(**2**): R^1=R^2=CH_3CO-
(**4**): R^1=R^2=Bz
(**6**): R^1=(*S*)-MTPA, R^2=H
(**7**): R^1=(*R*)-MTPA, R^2=H



(**3**): R^1=R^2=CH_3CO-
(**5**): R^1=R^2=Bz

acetylation gave two products, **2** (see Fig. 2, Rf 0.3, $CHCl_3$-MeOH=100:1) and **3** (Rf 0.4), which were easily separated on silica gel chromatography. In the FAB-MS spectra, pseudomolecular ion of **2** was detected at *m/z* 485 ($M+H^+$), and **3** at 501 ($M+H^+$). In the next step, we decided to elucidate the structure of **2** as it appeared a diacetate of **1**.

Planar Structure of **2**

The FAB-MS, ^1H, ^{13}C NMR and ^{13}C-^1H COSY data of **2** concluded that the molecular formula of **2** was $C_{28}H_{36}O_7$. In the ^1H and ^{13}C NMR spectra, two acetyl groups were clearly observed and δ_H of two oxy-methine protons were shifted to lower field at 4.70 and 4.61 ppm than those of **1** (δ_H 3.61, 3.46). The extensively polarized tetra-substituted olefin exists like **1**. ^1H-^1H spin networks were clarified by ^1H-^1H COSY as bold lines in Fig. 4. Pivotal HMBC correlations were obtained from 3-H clearly separated from the other proton signals. HMBC data from 3-H to C-1, C-2 and C-17 joined C-2 and C-3. The connection of C-3 and C-4 was deduced from the long-range coupling of 4-H/C-2

Fig. 3. Substructures of FR182877 (**1**).

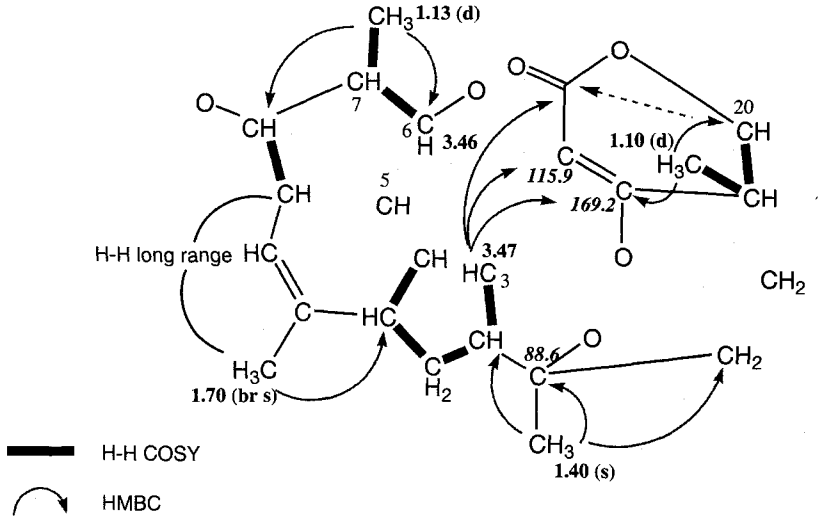
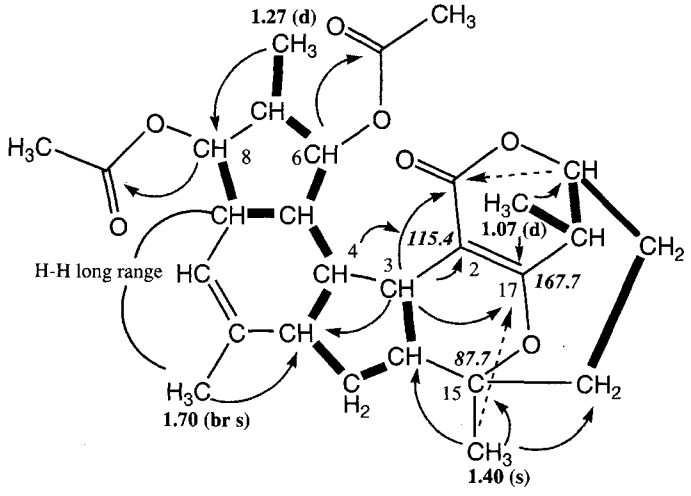


Fig. 4. COSY and HMBC experiments of **2** in CD_2Cl_2.

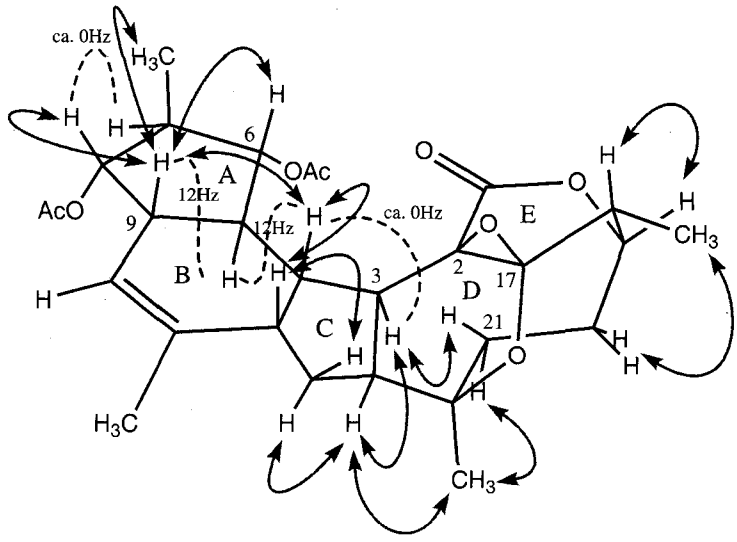


although the *J* value of 3-H/4-H is nearly zero. Two protons at 6-H (δ_H 4.61) and 8-H (δ_H 4.70) were assigned to be those of acetyloxymethines by the coupling of 6-H/6-$COCH_3$ and 8-H/8-$COCH_3$. Finally, the assumption of an ether linkage between C-15 and C-17 would complete the structure. This linkage was supported by weak ^4J-coupling of 15-CH_3/C-17. However, decomposition of **2** during NMR measurement precluded further analysis as in the case of **1**. The structure of **2**, including a highly distorted olefin, seemed unprecedented and needed further chemical evidence for confirmation. Therefore, analysis of **3**, a related compound to **2**, was set about for confirming the structure of **2**.

Planar Structure of 3

In contrast to **1** and **2**, **3** was stable and lacked UV absorption at 254 nm. The molecular formula of **3** was established as $C_{28}H_{36}O_8$, one more oxygen than that of **2** by FAB-MS, ^1H, ^{13}C NMR and HMQC. In the ^{13}C NMR

Fig. 5. NOESY Experiment of **3** in C_6D_6.



spectrum, signals at δ_C 83.8 (s) and 55.0 (s) were observed in stead of polarized olefin signals of **2** (δ_C 169.6, 115.4).

All the planar structure of **3** was elucidated by ^1H-^1H COSY, HMQC and HMBC data except for the epoxide portion. The presence of ether linkage between C-15 and C-17 was supported by weak HMBC correlations from 15-CH_3 to C-17. The existence of epoxide between C-2 (δ_C 55.0) and C-17 (δ_C 83.8) was indicated from the ^{13}C chemical shifts and requirement of the molecular formula.

Relative Stereochemistry of 3

The relative configuration of **3** was inferred by NOESY spectrum and $^3J_{HH}$ values as follows (see Fig. 5). NOE (9-H/6-H, 7-CH3 and 8-H) showed these protons are in the same face of the A-ring. Both 4-H/5-H and 5-H/9-H are *trans*-diaxial as their $^3J_{HH}$ are 12 Hz. The relative configuration of C-ring was supposed by $^3J_{3\text{-H},4\text{-H}}$ (*ca.* 0 Hz) and NOE (4-H/12-H, 12-H/13a-H, 13b-H/14-H and 14-H/3-H) that 3-H/4-H is *trans*, 4-H/12-H is *cis* and 3-H/14-H is *cis*. The stereochemistry of C-15, C-18 and C-19 was deduced as Fig. 5 from NOE (3-H/21a-H and 18-CH_3/20-H). For example, NOE (3-H/21a-H) enforced C-21 axially downward from the D-ring plane. C-20 should be placed axially under the E-ring since the epimer on C-19 can never be constructed with the Dreiding molecular model. For the similar reason, epoxide must be *cis* and oriented toward β-face.

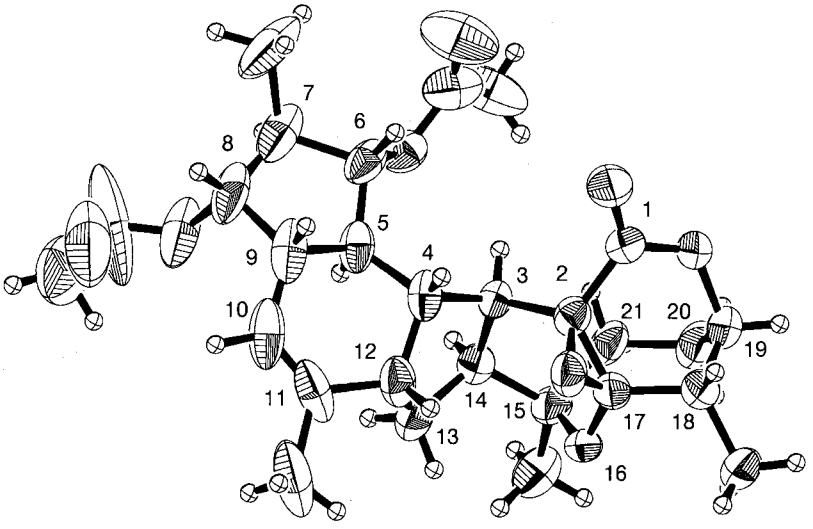
Although the structure of **3** including the relative stereochemistry was speculated, its structural validity was not clear as it had a quite novel ring system. Finally, the exact structure of **3** was confirmed by X-ray crystallographic analysis. The molecular structure of **3** is illustrated in Fig. 6.

Chemical Correlations of 1, 2 and 3

To establish the structure of **1**, the chemical correlations were carried out *vide infra*. The compound **2** gradually decomposed in CD_2Cl_2 as mentioned above and ^1H signals newly generated were superimposable with those of **3**. This finding showed that **2** decomposed to yield **3**. The molecular formula of **3** was independently determined to be one more oxygen than that of **2**. Compounds **2** and **3** resemble each other in proton spin networks, HMBC correlations to quaternary carbons and ^{13}C chemical shifts except near epoxide. These results show that **2** was epoxidized to **3** without migration or backbone rearrangement.

Time course observation of ^1H NMR spectra showed that **1** and **2** similarly decomposed. After decomposition of **1**, *m/z* 417 was observed in addition to 401 ($M+H^+$), the pseudomolecular ion of **1** itself in the FAB-MS spectrum. Acetylation of this mixture gave **2** and **3**, which indicated that the product gradually occurring in CD_3OD solution of **1** must be an epoxidized one at the distorted double bond of

Fig. 6. ORTEP drawing of **3**.



1. In addition, δ_C of **1** (CD_3OD) and **2** (CD_2Cl_2) resemble each other except near acetylated parts. Therefore, **2** was concluded to be the diacetate of **1**.

Besides these facts, other chemical correlations were studied for ensuring the structure of **1** and **2**. Benzoylation of **1** under nitrogen atmosphere gave di-benzoate (**4**, see Fig. 2) in moderate yield (72%). Vigorous stirring of chloroform solution of **4** under oxygen resulted in the formation of **5** whose spectra of ^1H and ^{13}C NMR were well-resolved and supported the structure of **5**. This reaction never occurred under the atmosphere of argon instead of oxygen. Therefore, the formation of **5** can be most reasonably explained by the epoxidation of the distorted α,β-unsaturated ester of **4** with molecular oxygen[7].

From these discussions, not only the structure of **2** but also that of **1** were determined as Figs. 1 and 2 including the relative stereochemistry. The ^1H and ^{13}C NMR assignment was shown in Table 1.

Absolute Stereochemistry of FR182877 (1)

The only remaining structural problem was the absolute stereochemistry. First, we tried to apply the exciton coupling method[8,9] for the compound **4**. However, the CD spectrum of **4** was not significantly different from that of **1**, which unabled us to use the exciton coupling method. Though the characteristic positive Cotton effect ($\Delta\varepsilon$+5.7 at 240 nm, in methanol) in the CD spectrum of **1** must derive from the distorted α,β-unsaturated ester, it seemed difficult to estimate the absolute configurations of **1** from this effect since the CD spectra of *s-trans* conjugated systems are affected by their surrounding stereochemistry as well as "diene chirality"[10].

Thus, we turned to use a modified Mosher's method[11]. (*R*)- and (*S*)-MTPA chloride reacted regioselectively with **1** to yield mono acylated products **6** and **7**, respectively (see Fig. 2). The difference of δ_H between **6** and **7** is distributed as Fig. 7, which indicated the absolute configuration of C-8 is (*S*).

As consequence, the absolute stereochemistry of **1** was determined as Fig. 1.

Conclusion

We determined the structure of FR182877 including the absolute stereochemistry on the basis of spectroscopic methods and chemical reactions. The structure of FR182877, including the intriguing condensed ring system and the bridgehead olefin, is remarkably different from the known tublin polymerization promoters such as taxol[3,12], epothilones[13~15] and discodermolide[16,17]. It is also worth noticing that the stable epoxide **3** was devoid of antitumor activities. FR182877 is not only a drug candidate for cancer chemotherapy but also a possible probe for analyzing tublin polymerization in molecular level.

Fig. 7. $\Delta\delta_H$ between (*S*)-MTPA and (*R*)-MTPA esters of FR182877.

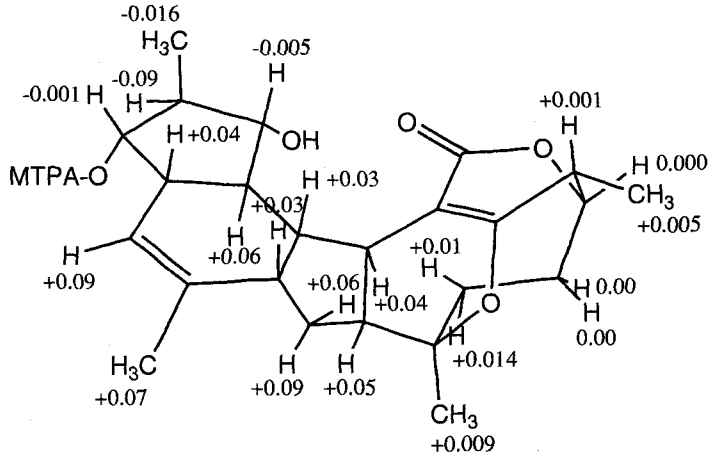


Table 1. ^1H and ^{13}C NMR assignment of FR182877 and its derivatives.

position	1		2		3			4	5		6	7
	$\delta_H(CD_3OD)$	$\delta_C(CD_3OD)$	$\delta_H(CD_2Cl_2)$	$\delta_C(CD_2Cl_2)$	$\delta_C(CD_2Cl_2)$	$\delta_H(C_6D_6)$	$\delta_C(C_6D_6)$	$\delta_H(CD_2Cl_2)$	$\delta_H(CD_2Cl_2)$	$\delta_C(CD_2Cl_2)$	$\delta_H(CD_2Cl_2)$	$\delta_H(CD_2Cl_2)$
1		172.9		169.6	169.3		168.5			168.9		
2		115.9		115.4	55.0		55.0			54.8		
3	3.47	43.4	3.10	42.3	42.1	3.36	42.3	3.10	3.12	42.1	3.41	3.37
4	2.04	53.2	2.12	52.1	48.3	2.75	48.0	2.26	2.66	48.3	2.05	2.02
5	1.80	46.2	2.07	44.16	43.5	2.16	43.3	2.37	2.32	44.2	1.68	1.62
6	3.46	84.6	4.61	84.3	84.6	4.83	84.9	4.93	4.93	85.4	3.56	3.57
7	1.74	54.6	1.87	49.8	49.7	2.06	50.0	2.18	2.20	49.7	1.96	2.05
7-CH_3	1.13	18.6	1.27	18.6	18.6	1.40	18.6	1.44	1.45	18.5	1.24	1.26
8	3.61	78.4	4.70	80.6	80.5	4.80	80.6	4.97	4.98	81.6	5.01	5.01
9	1.94	46.9	2.21	44.19	43.5	1.92	42.9	2.45	2.51	43.8	2.11	2.07
10	5.41	121.2	5.31	118.9	118.3	5.34	118.8	5.43	5.42	118.2	5.35	5.24
11		140.5		140.1	141.0		140.3			141.4		
11-CH_3	1.70	22.9	1.70	22.7	22.5	1.51	22.5	1.73	1.66	22.5	1.68	1.61
12	2.43	47.4	2.52	46.5	47.8	2.93	47.5	2.56	2.81	48.0	2.58	2.54
13 H13a	2.28	33.7	2.33	33.3	31.0	2.02	31.1	2.39	2.12	31.1	2.31	2.25
H13b	1.58		1.55			1.23		1.63	1.50		1.43	1.34
14	2.64	52.5	2.58	52.5	46.1	1.88	45.7	2.66	2.47	46.3	2.52	2.47
15		88.6		87.7	81.0		80.3			80.9		
15-CH_3	1.40	24.1	1.40	23.9	25.8	0.88	25.5	1.41	1.30	25.8	1.43	1.42
17		169.2		167.7	83.9		83.8			83.9		
18	2.76	42.5	2.77	41.5	43.3	1.58	42.9	2.72	1.90	43.2	2.86	2.86
18-CH_3	1.10	9.3	1.07	9.3	10.6	0.75	10.2	1.05	1.17	10.5	1.10	1.10
19	4.43	79.4	4.35	77.8	82.3	3.71	81.0	4.27	4.31	82.1	4.43	4.43
20 H20a	1.78	25.2	1.80	24.4	24.3	1.37	23.9	1.68	1.94	24.2	1.80	1.80
H20b	1.73		1.66			1.37		1.56	1.82		1.70	1.70
21 H21a	1.67	36.2	1.60	36.0	35.5	1.64	35.0	1.56	1.70	35.2	1.64	1.63
H21b	1.61		1.50			1.16		1.34	1.57		1.52	1.50
6-*C*O				171.0	170.6		169.4			165.5		
6-CO*C*H_3			2.02	21.5	21.3	1.82	20.8					
8-*C*O				171.0	171.0		169.9			165.8		
8-CO*C*H_3			2.00	21.3	21.3	1.68	20.7					

Experimental

^1H 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 (TLC) was carried out on a Merck Silica gel F254 pre-coated plate, Art 5744.

Isolation of **2** and **3**

Compound **1** (10 mg) was dissolved in CD_3OD and the solution was used to acquire a series of NMR spectra. After overnight measurement, ^1H NMR of the resultant solution showed that a third of **1** decomposed. The solution was concentrated *in vacuo* and dissolved in a mixture of acetic anhydride (200 μl) and pyridine (300 μl). After 10 hours at room temperature, the mixture was evaporated and chromatographed on preparative TLC (1% methanol in chloroform) to give **2** (4 mg, 33%): FAB-MS *m/z* 485 $(M+H)^+$ and **3** (4 mg, 32%): FAB-MS *m/z* 501 $(M+H)^+$, $[\alpha]_D^{25}$ −139° (*c* 0.50, $CHCl_3$).

X-ray Crystallography of **3**

Crystal Data: Orthorhombic, $a=13.46$ (1)Å, $b=15.76$ (1)Å, $c=12.827$ (8)Å, $V=2720$ (2)Å^3, $Z=4$, space group $P2_12_12_1$, $Dcalc=1.222\,Mgm^{-3}$, $\mu=0.733\,mm^{-1}$. Data collection: A total of 2486 reflections were measured by Rigaku AFC5R diffractometer with graphite monochromated Cu-Kα radiation ($\lambda=1.54178$Å) using a colorless prismatic crystal, 0.15×0.1×0.1 mm at room temperature. Structure analysis and refinement: The crystal structure was solved by the direct methods with the program SHELX-86[18], and refined by full-matrix least squares. Non-hydrogen atoms were refined with anisotropic temperature factors, and hydrogen atoms were included at calculated positions and not refined. The final *R* and *Rw* for 1525 reflections with $I>2\sigma$ (*I*) were 0.076 and 0.069, respectively. The maximum and minimum peaks in final difference Fourier map were 0.51 eÅ^{-3} and −0.28 eÅ^{-3}, respectively.

Di-benzoation of **1** (**4**)

p-Bromobenzoyl chloride (28 mg, 3 eq.) was added to an ice-cooled solution of **1** (10 mg) in pyridine (200 μl), and the mixture was vigorously stirred at room temperature for 20 minutes. The resulting clear solution was quenched with *N,N*-dimethyl-1,3-propanediamine (11 mg) with ice-cooling and stirred for more 30 minutes. Then, the solution was evaporated with nitrogen flow and the residue was purified on preparative TLC (1% methanol in chloroform) to give **4** (14 mg, 72%): ESI-MS *m/z* 765 $(M+H)^+$.

Epoxidation of **4** to **5**

A solution of **4** (4 mg) in chloroform (0.5 ml) was vigorously stirred under the atmosphere of oxygen for 3 days. The solution was purified on preparative TLC (1% methanol in chloroform) to yield **5** (1.7 mg, 42%): ESI-MS *m/z* 894 $(M+TFA-H)^-$.

MTPA Esterification of **1**

(*R*)-MTPA chloride (3.8 mg, 3 eq.) was mixed with **1** (2 mg) in pyridine and stood at room temperature for 1 hour. Then, the solution was evaporated with nitrogen flow and the residue was purified on preparative TLC (50% ethyl acetate in *n*-hexane) to give **6** ((*S*)-MTPA ester, 2 mg, 65%): ESI-MS *m/z* 617 $(M+H)^+$.

(*R*)-MTPA ester **7** (1 mg, 33%) was prepared from **1** (2 mg) and (*S*)-MTPA chloride (3.8 mg, 3 eq.) by using similar methods: ESI-MS *m/z* 617 $(M+H)^+$.

Acknowledgements

The authors thank Dr. ERICK J. SORENSEN for his kind advice on the mechanism of the oxidation of FR182877.

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