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TETRAHEDRON

Isolation and Structure Determination of TMC-151s: Novel Polyketide Antibiotics from *Gliocladium catenulatum* Gilman & Abbott TC 1280

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Abstract: Six new antibiotics, TMC-151 A-F (**2-7**) have been isolated from organic extracts of the fermentation broth of *Gliocladium catenulatum* Gilman & Abbott TC 1280, isolated from a soil sample. Their structures including the absolute stereochemistries, were determined by extensive analyses of NMR and X-ray crystallography, and degradation studies. TMC-151s are novel polyketides containing D-mannopyranoside and D-mannitol or D-arabitol. These antibiotics showed moderate cytotoxicity to several tumor cell lines.

Introduction

Hypocreacean fungi and their anamorph such as *Trichoderma* and *Gliocladium* have played an important role in the field of biocontrol and biomolecular screening. Species belonging to the genus *Gliocladium* are in fact known to produce a variety of secondary metabolites, such as tetracycline,[1] piperazines,[2] peptides,[3] sesquiterpene,[4] and polyprenols.[5-6] These findings promoted us to investigate the fungal metabolites for discovery of novel chemotherapeutic agents.

During the course of our screening of fungal extracts for cytotoxicity, we have discovered new substances, designated by TMC-151 A-F (**2-7**). These antibiotics are structurally characterized by novel polyketides containing sugars and hexitol or pentitol moieties.

We report herein the isolation, structure elucidation and biological activity of these six new antibiotics, produced by a fungus *Gliocladium catenulatum* Gilman & Abbott TC 1280.

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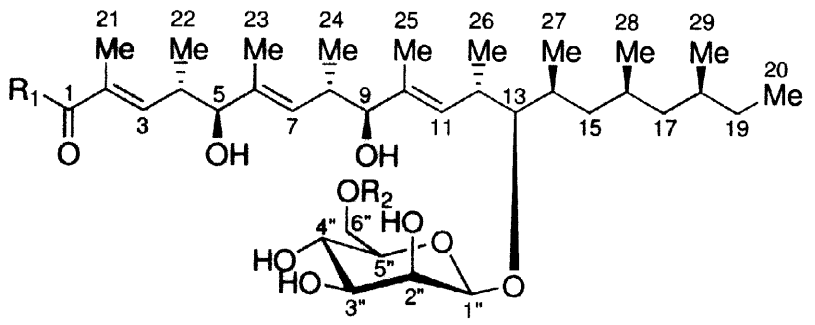
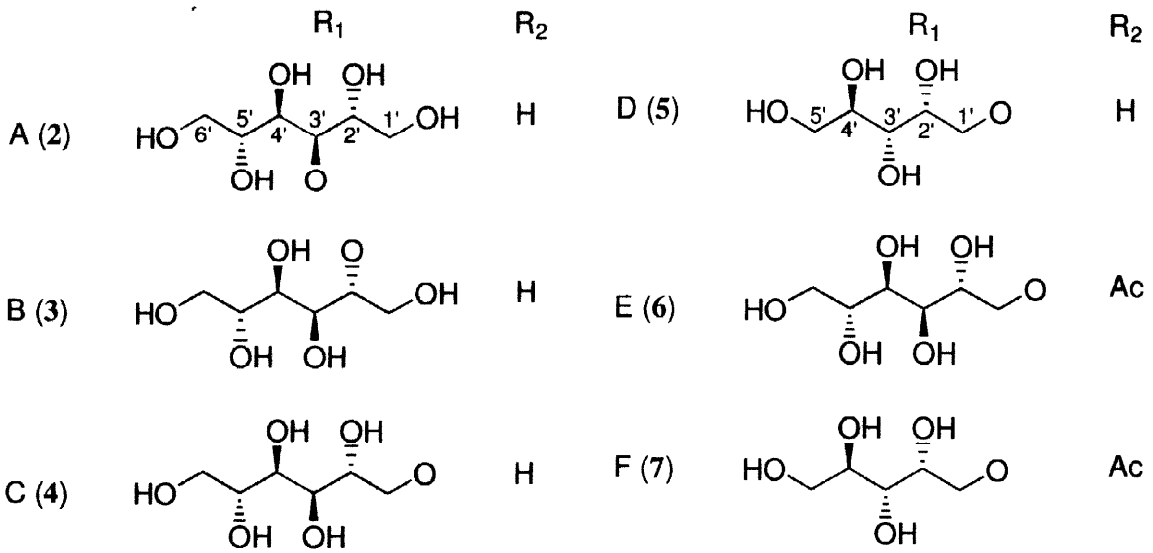




Results and Discussion

Isolation

Gliocladium catenulatum TC 1280 was fermented under the static condition, and the resultant solid was extracted with 1-butanol. The extract was purified by solvent partition, followed by column chromatography on a Sephadex LH-20 to afford TMC-151 complex (**1**). This complex was further purified by reverse phase silica gel column chromatography and preparative HPLC to give pure six components, designated as TMC-151 A-F (**2-7**).

Structure Elucidation

TMC-151 C (4)

The molecular formula of **4** was identified as $C_{41}H_{74}O_{15}$ on the basis of its HRESI-MS [*m/z* found 805.4962 $(M-H)^-$, calcd 805.4949] and ^1H and ^{13}C NMR spectral data. The IR spectra of **4** indicated the presence of hydroxyl (3400 cm^{-1}), ester (1700 cm^{-1}) and ether or hydroxyl (1075 cm^{-1}) groups. The ^{13}C and ^1H

NMR data are shown in Table 1 and 2. The ^{13}C NMR spectrum of **4** displayed 41 signals composed of 10 methyls, 3 methylenes, 3 oxygenated methylenes, 6 methines, 11 oxygenated methines, an anomeric methine, 3 olefinic methines, 3 olefinic quaternary carbons and an ester carbonyl carbons. The ^1H NMR spectrum of **4** showed D_2O exchangeable 11 protons at δ 3.97 - 4.78, which were assigned to hydroxyl groups. The extensive 1D and 2D-NMR spectra such as DQF-COSY, ROESY, HMQC and HMBC revealed the following three partial structures (A-C) of **4**, as shown in Figure 1.

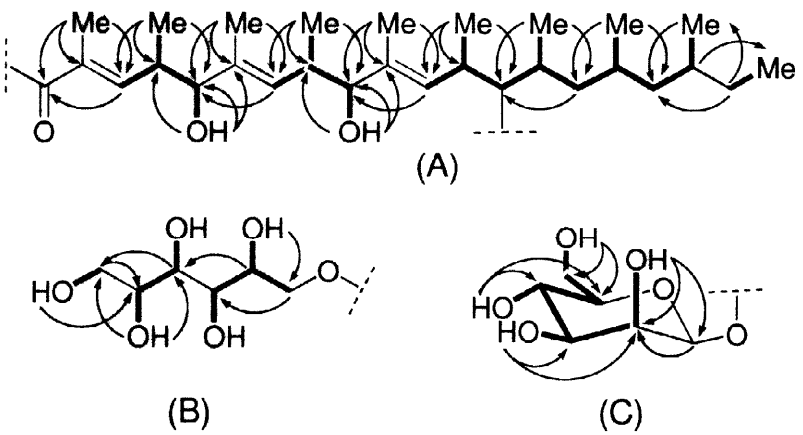


Figure 1. COSY (bold line) and HMBC ($^nJ_{CH}$ = 8.5 Hz) correlations of TMC-151 C (**4**).

(A): In the DQF-COSY spectrum, a sequential proton network of H11-H12(H26)-H13-H14(H27)-H15-H16(H28)-H17-H18(H29)-H19-H20 and two fragments, =CH-CH(CH_3)-CH(OH)- were observed. Low-field chemical shifts of three methyl groups (H-21: δ 1.80, H-23: δ 1.56 and H-25: δ 1.54) suggested that these methyl groups attached to the olefinic carbons. Full spin network of (A) was accomplished based on the observation of the ^1H-^{13}C correlations from methyl groups as follows: from H-21 to carbonyl carbon C-1 (δ 167.6), C-2 (δ 126.5) and C-3 (δ 146.4); from H-23 to C-5 (δ 80.9), C-6 (δ 136.0) and C-7 (δ 131.5); from H-25 to C-9 (δ 81.2), C-10 (δ 134.6) and C-11 (δ 130.1).

(B): the presence of one hydroxy methylene and four hydroxy methines was revealed by the following ^1H-^1H correlations: from δ 4.32 (m) to δ 3.63 and 3.40, from δ 4.78 (d) to δ 3.72, from δ 4.28 (d) to δ 3.59, from δ 4.14 (d) to δ 3.58 and from δ 4.42 (d) to δ 3.48. The connectivity of C-1' to C-6' was accomplished by ^1H-^1H and ^1H-^{13}C correlations. The ^{13}C chemical shifts at C-3', C-4', C-5' and C-6' were in good agreement with those of mannitol in literature,[7] with downfield shift at C-1' (δ 67.2) and upfield shift at C-2' (δ 68.4) due to 1'-substitution. (B) was thus concluded to be a 1'-substituted mannitol.

(C): Hydroxyl protons at δ 4.05 (d), 4.53 (d) and 4.68 (d) had correlations with methine protons H-2" (δ 3.70), H-3" (δ 3.20) and H-4" (δ 3.32), respectively, One hydroxyl proton at δ 4.20 (dd) coupled with methylene protons H-6" (δ 3.49 and 3.70). In the DQF-COSY spectrum, a sequential network of H2"(OH)-H3"(OH)-H4"(OH)-H5"(O-)-H6"(OH) was clarified. An anomeric proton H-1" at δ 4.32 (brs) was coupled to methine carbon C-2" (δ 70.7), and hydroxyl proton 2"-OH (δ 4.05) coupled to an anomeric carbon C-1" (δ 101.3).

Table 1. ^{13}C NMR data for TMC-151 A-F (2-7) in DMSO-d_6.

C no.	2	3	4	5	6	7
1	167.2 s	166.8 s	167.6 s	167.3 s	167.6 s	167.3 s
2	126.5 s	126.8 s	126.5 s	126.4 s	126.6 s	126.4 s
3	146.8 d	146.4 d	146.4 d	146.6 d	146.4 d	146.6 d
4	36.9 d	36.8 d	36.8 d	36.9 d	36.8 d	36.8 d
5	80.9 d	80.9 d	80.9 d	80.8 d	80.9 d	80.8 d
6	136.0 s	136.0 s	136.0 s	135.9 s	136.1 s	136.0 s
7	131.6 d	131.6 d	131.5 d	131.4 d	131.5 d	131.4 d
8	36.1 d	36.1 d	36.2 d	36.2 d	36.2 d	36.2 d
9	81.2 d	81.2 d	81.2 d	81.2 d	81.2 d	81.2 d
10	134.5 s	134.5 s	134.6 s	134.6 s	134.7 s	134.7 s
11	130.1 d	130.1 d	130.1 d	130.0 d	129.7 d	129.7 d
12	34.5 d	34.5 d	34.5 d	34.5 d	34.5 d	34.5 d
13	85.3 d	85.2 d	85.2 d	85.3 d	86.1 d	86.1 d
14	33.0 d	33.0 d	33.0 d	33.0 d	33.3 d	33.3 d
15	42.1 t	42.1 t	42.1 t	42.1 t	42.1 t	42.1 t
16	27.2 d	27.2 d	27.2 d	27.2 d	27.2 d	27.2 d
17	43.8 t	43.8 t	43.8 t	43.8 t	43.7 t	43.7 t
18	30.9 d	30.9 d	30.9 d	30.9 d	30.9 d	30.9 d
19	28.0 t	28.0 t	28.0 t	28.0 t	27.9 t	27.9 t
20	10.8 q	10.8 q	10.8 q	10.7 q	10.7 q	10.7 q
21	12.6 q	12.6 q	12.5 q	12.5 q	12.5 q	12.5 q
22	16.4 q	16.4 q	16.5 q	16.4 q	16.5 q	16.4 q
23	11.0 q	11.0 q	11.1 q	11.1 q	11.1 q	11.1 q
24	17.4 q	17.4 q	17.4 q	17.4 q	17.4 q	17.4 q
25	11.3 q	11.2 q	11.3 q	11.3 q	11.2 q	11.2 q
26	18.2 q	18.2 q	18.2 q	18.2 q	18.2 q	18.2 q
27	15.8 q	15.8 q	15.8 q	15.8 q	15.9 q	15.9 q
28	20.9 q	20.9 q	20.9 q	20.9 q	20.9 q	20.9 q
29	20.0 q	20.0 q	20.0 q	20.0 q	19.9 q	19.9 q
1'	63.0 t	59.8 t	67.2 t	66.0 t	67.2 t	66.0 t
2'	70.3 d	74.4 d	68.4 d	67.4 d	68.5 d	67.4 d
3'	72.5 d	67.1 d	69.3 d[a]	70.7 d[b]	69.3 d[c]	70.8 d[d]
4'	69.3 d	69.9 d	69.5 d[a]	71.0 d[b]	69.5 d[c]	71.0 d[d]
5'	70.7 d	70.9 d	71.1 d	63.5 t	71.1 d	63.5 t
6'	63.5 t	63.6 t	63.8 t		63.8 t	
1"	101.3 d	101.3 d	101.3 d	101.3 d	101.4 d	101.4 d
2"	70.7 d	70.7 d	70.7 d	70.7 d	70.6 d	70.6 d
3"	73.9 d	73.9 d	73.9 d	73.9 d	73.6 d	73.6 d
4"	67.0 d	67.0 d	67.0 d	67.0 d	67.0 d	67.0 d
5"	77.3 d	77.3 d	77.2 d	77.3 d	74.1 d	74.1 d
6"	61.4 t	61.4 t	61.4 t	61.4 t	64.0 t	64.0 t
CH_3CO					20.5 q	20.5 q
CH_3CO					170.1 s	170.1 s

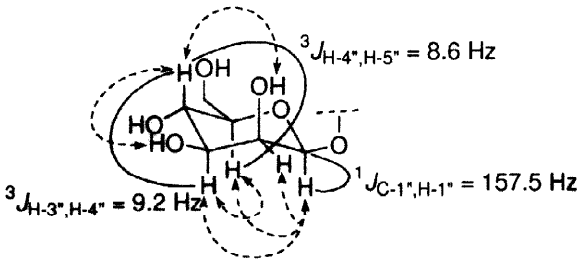
[a-d] May be exchangeable.

Table 2-1. ^{1}H NMR data for TMC-151 A, B and C (2-4) in DMSO-d_6.

no.	2	3	4
3	6.70 (1H, dd, 10, 1)	6.63 (1H, dd, 9.8, 1.2)	6.68 (1H, dd, 9.7, 1.2)
4	2.56 (1H, m)	2.56 (1H, m)	2.58 (1H, m)
5	3.67 (1H, m)	3.66 (1H, m)	3.67 (1H, m)
7	5.19 (1H, brd, 9)	5.19 (1H, brd, 9)	5.19 (1H, brd, 9)
8	2.45 (1H, m)	2.45 (1H, m)	2.46 (1H, m)
9	3.55 (1H, dd, 8.5, 3.4)	3.55 (1H, dd, 8.5, 3.4)	3.55 (1H, m)
11	5.39 (1H, brd, 9)	5.39 (1H, brd, 9)	5.39 (1H, brd, 9)
12	2.67 (1H, m)	2.67 (1H, m)	2.67 (1H, m)
13	3.30 (1H, m)	3.30 (1H, m)	3.30 (1H, m)
14	1.70 (1H, m)	1.71 (1H, m)	1.69 (1H, m)
15	1.30 (1H, m)	1.30 (1H, m)	1.30 (1H, m)
	0.86 (1H, m)	0.86 (1H, m)	0.86 (1H, m)
16	1.53 (1H, m)	1.53 (1H, m)	1.53 (1H, m)
17	1.20 (1H, m)	1.20 (1H, m)	1.20 (1H, m)
	0.82 (1H, m)	0.82 (1H, m)	0.82 (1H, m)
18	1.40 (1H, m)	1.40 (1H, m)	1.40 (1H, m)
19	1.34 (1H, m)	1.34 (1H, m)	1.34 (1H, m)
	1.03 (1H, m)	1.03 (1H, m)	1.03 (1H, m)
20	0.82 (1H, t, 7)	0.82 (1H, t, 7)	0.82 (1H, t, 7)
21	1.80 (3H, d, 1)	1.79 (3H, d, 1.2)	1.80 (3H, d, 1.2)
22	0.76 (3H, d, 6.8)	0.75 (3H, d, 6.8)	0.77 (3H, d, 6.9)
23	1.56 (3H, brs)	1.56 (3H, brs)	1.56 (3H, brs)
24	0.70 (3H, 6.8)	0.70 (3H, 6.8)	0.70 (3H, 6.7)
25	1.55 (3H, brs)	1.55 (3H, brs)	1.54 (3H, brs)
26	0.95 (3H, d, 6.8)	0.95 (3H, d, 6.8)	0.95 (3H, d, 6.9)
27	0.87 (3H, d, 6.8)	0.87 (3H, d, 6.8)	0.87 (3H, d, 6.7)
28	0.83 (3H, d, 7)	0.83 (3H, d, 7)	0.83 (3H, d, 7)
29	0.83 (3H, d, 7)	0.83 (3H, d, 7)	0.83 (3H, d, 7)
1'	3.28 (1H, m)	3.61 (1H, m)	4.34 (1H, dd, 11.3, 2.4)
	3.39 (1H, m)	3.76 (1H, m)	3.99 (1H, dd, 11.3, 7.0)
2'	3.78 (1H, m)	4.79 (1H, m)	3.72 (1H, m)
3'	5.02 (1H, dd, 8, 1)	3.97 (1H, t, 8)	3.59 (1H, m)
4'	3.73 (1H, m)	3.27 (1H, m)	3.58 (1H, m)
5'	3.21 (1H, m)	3.47 (1H, m)	3.48 (1H, m)
6'	3.39 (1H, m)	3.38 (1H, m)	3.40 (1H, ddd, 15.6, 10.2, 4.6)
	3.60 (1H, m)	3.59 (1H, m)	3.63 (1H, m)
1"	4.32 (1H, brs)	4.32 (1H, brs)	4.32 (1H, brs)
2"	3.70 (1H, m)	3.70 (1H, m)	3.70 (1H, m)
3"	3.20 (1H, m)	3.20 (1H, ddd, 9.1, 6.1, 3.1)	3.20 (1H, ddd, 9.2, 6.2, 3.1)
4"	3.30 (1H, m)	3.31 (1H, m)	3.32 (1H, m)
5"	2.99 (1H, ddd, 9, 6, 2)	2.99 (1H, ddd, 8.8, 5.9, 2.3)	2.99 (1H, ddd, 8.6, 5.7, 2.1)
6"	3.70 (1H, m)	3.70 (1H, m)	3.70 (1H, m)
	3.49 (1H, m)	3.49 (1H, m)	3.49 (1H, m)
5-OH	4.58 (1H, d, 3.9)	4.57 (1H, d, 3.7)	4.58 (1H, d, 3.8)
9-OH	3.97 (1H, d, 3.4)	3.97 (1H, d, 3.4)	3.97 (1H, d, 3.6)
1'-OH	4.43 (1H, t, 5.6)	4.53 (1H, t, 5.4)	
2'-OH	4.85 (1H, d, 5.9)		4.78 (1H, d, 6.0)
3'-OH		4.45 (1H, d, 7.6)	4.28 (1H, d, 7.5)
4'-OH	4.64 (1H, d, 6.3)	4.25 (1H, d, 7.6)	4.14 (1H, d, 7.5)
5'-OH	4.28 (1H, d, 5.4)	4.44 (1H, d, 5.6)	4.42 (1H, d, 5.5)
6'-OH	4.29 (1H, t, 5.6)	4.31 (1H, t, 5.6)	4.32 (1H, m)
2"-OH	4.05 (1H, d, 5.1)	4.05 (1H, d, 4.9)	4.05 (1H, d, 5.0)
3"-OH	4.53 (1H, d, 6.1)	4.53 (1H, d, 6.1)	4.53 (1H, d, 6.2)
4"-OH	4.68 (1H, d, 5.1)	4.68 (1H, d, 5.4)	4.68 (1H, d, 5.2)
6"-OH	4.20 (1H, t, 6)	4.20 (1H, t, 5.9)	4.20 (1H, dd, 5.9, 5.2)

Table 2-2. 1H NMR data for TMC-151 D, E and F (**5-7**) in DMSO-*d6*.

no.	5	6	7
3	6.65 (1H, dd, 9.7, 1.2)	6.67 (1H, dd, 9.7, 1.2)	6.65 (1H, dd, 9.7, 1.2)
4	2.58 (1H, m)	2.58 (1H, m)	2.58 (1H, m)
5	3.67 (1H, m)	3.67 (1H, dd, 8.3, 3.6)	3.67 (1H, dd, 8.3, 3.8)
7	5.19 (1H, brd, 9)	5.18 (1H, brd, 9)	5.18 (1H, brd, 9)
8	2.46 (1H, m)	2.45 (1H, m)	2.46 (1H, m)
9	3.55 (1H, dd, 8.3, 3.5)	3.53 (1H, dd, 8.5, 3.5)	3.53 (1H, dd, 8.5, 3.6)
11	5.39 (1H, brd, 9)	5.37 (1H, brd, 9)	5.37 (1H, brd, 9)
12	2.67 (1H, m)	2.67 (1H, m)	2.66 (1H, m)
13	3.30 (1H, m)	3.26 (1H, m)	3.25 (1H, m)
14	1.69 (1H, m)	1.67 (1H, m)	1.67 (1H, m)
15	1.30 (1H, m)	1.30 (1H, m)	1.30 (1H, m)
	0.86 (1H, m)	0.86 (1H, m)	0.86 (1H, m)
16	1.53 (1H, m)	1.53 (1H, m)	1.53 (1H, m)
17	1.20 (1H, m)	1.20 (1H, m)	1.20 (1H, m)
	0.82 (1H, m)	0.82 (1H, m)	0.82 (1H, m)
18	1.40 (1H, m)	1.40 (1H, m)	1.40 (1H, m)
19	1.34 (1H, m)	1.34 (1H, m)	1.34 (1H, m)
	1.03 (1H, m)	1.03 (1H, m)	1.03 (1H, m)
20	0.82 (1H, t, 7)	0.82 (1H, t, 7)	0.82 (1H, t, 7)
21	1.79 (3H, d, 1.2)	1.80 (3H, d, 1.2)	1.79 (3H, d, 1.2)
22	0.77 (3H, d, 6.7)	0.76 (3H, d, 6.9)	0.77 (3H, d, 6.9)
23	1.56 (3H, brs)	1.56 (3H, brs)	1.56 (3H, brs)
24	0.70 (3H, 6.7)	0.69 (3H, 6.9)	0.69 (3H, 6.7)
25	1.54 (3H, brs)	1.54 (3H, brs)	1.54 (3H, brs)
26	0.95 (3H, d, 6.9)	0.94 (3H, d, 6.9)	0.94 (3H, d, 7.0)
27	0.87 (3H, d, 6.9)	0.87 (3H, d, 6.7)	0.87 (3H, d, 6.7)
28	0.83 (3H, d, 7)	0.83 (3H, d, 7)	0.83 (3H, d, 7)
29	0.83 (3H, d, 7)	0.83 (3H, d, 7)	0.83 (3H, d, 7)
1'	4.06 (2H, m)	4.34 (1H, dd, 11.1, 2.2)	4.05 (2H, m)
		4.00 (1H, dd, 11.1, 6.8)	
2'	3.94 (1H, m)	3.72 (1H, m)	3.94 (1H, m)
3'	3.29 (1H, m)	3.59 (1H, m)	3.29 (1H, m)
4'	3.49 (1H, m)	3.58 (1H, m)	3.49 (1H, m)
5'	3.40 (1H, m)	3.48 (1H, m)	3.40 (1H, m)
	3.60 (1H, m)		3.60 (1H, m)
6'		3.40 (1H, m)	
		3.63 (1H, m)	
1"	4.32 (1H, brs)	4.34 (1H, brs)	4.34 (1H, brs)
2"	3.69 (1H, m)	3.72 (1H, m)	3.71 (1H, t, 3.7)
3"	3.20 (1H, ddd, 9.3, 6.3, 3.4)	3.23 (1H, m)	3.23 (1H, m)
4"	3.32 (1H, m)	3.31 (1H, m)	3.31 (1H, m)
5"	2.99 (1H, ddd, 8.7, 5.9, 2.2)	3.22 (1H, m)	3.22 (1H, m)
6"	3.68 (1H, m)	4.27 (1H, dd)	4.26 (1H, dd)
	3.48 (1H, m)	4.08 (1H, dd, 11.5, 7.4)	4.08 (1H, dd)
5-OH	4.59 (1H, d, 3.8)	4.57 (1H, d, 3.6)	4.59 (1H, d, 3.8)
9-OH	3.97 (1H, d, 3.5)	3.92 (1H, d, 3.5)	3.92 (1H, d, 3.6)
1'-OH			
2'-OH	4.55 (1H, d, ~7)	4.78 (1H, d, 6.2)	4.56 (1H, d, 6.6)
3'-OH	4.43 (1H, d, 7.4)	4.28 (1H, d, 7.5)	4.44 (1H, d, 7.5)
4'-OH	4.48 (1H, d, 5.5)	4.14 (1H, d, 7.5)	4.49 (1H, d, 5.6)
5'-OH		4.41 (1H, d, 5.5)	
6'-OH	4.32 (1H, m)	4.32 (1H, m)	4.33 (1H, t, 5.4)
2"-OH	4.05 (1H, d, 5.0)	4.24 (1H, d, 4.8)	4.24 (1H, d, 4.7)
3"-OH	4.53 (1H, d, 6.3)	4.65 (1H, d, 5.9)	4.67 (1H, d, 5.9)
4"-OH	4.68 (1H, d, 5.2)	4.95 (1H, d, 5.2)	4.95 (1H, d, 5.2)
6"-OH	4.20 (1H, dd, 5.8, 5.1)		



The large vicinal coupling constants, $^3J_{H\text{-}3",H\text{-}4"} = 9.2$ Hz and $^3J_{H\text{-}4",H\text{-}5"} = 8.6$ Hz, and the NOE between H-3" and H-5" indicated that H-3", H-4" and H-5" protons were located at axial position. The observation of NOE between H-4" and 2"-OH proton suggested that 2"-OH was oriented at axial position. From these data above mentioned, (C) was deduced to be a mannopyranoside. β configuration was determined by an NOE correlation from H-1" to H-3" and H-5", and by a small coupling constant, $^1J_{C\text{-}1",H\text{-}1"} = 157.5$ Hz (<< 166 Hz).[8]

Finally, the connectivity of each fragment, (A)-(C) was accomplished from the ^1H-^{13}C correlations as follows: the long range couplings from anomeric proton H-1" to oxygenated methine carbon C-13 (δ 85.2); H-1' (δ 4.34 and 3.99) to carbonyl carbon C-1 (δ 167.6). Based on the results of the NMR studies described above, the gross structure of TMC-151 C was determined as shown.

TMC-151 A (2) and B (3)

The molecular formula of **2** and **3** was both elucidated to be $C_{41}H_{74}O_{15}$ from its HRESI-MS [*m/z* found **2**: 805.4962, **3**: 805.4916 $(M\text{-}H)^-$, calcd 805.4949], which was the same as that of **4**. The IR and UV spectra of **2** and **3** were quite similar to those of **4**. The ^1H and ^{13}C NMR data of **2** and **3** corresponded well to those of **4** except for the signals of the hexitol moiety. The oxygenated methine carbon C-3' (δ 72.5) in **2** were shifted lower field, and methylene carbon C-1' (δ 63.0) were shifted higher field relative to those of **4**. In addition, triplet hydroxyl proton '1'-OH, δ 4.43) in **2** was observed in place of the corresponding doublet hydroxyl proton (3'-OH) in **4**. On the other hand, the oxygenated methine carbon C-2' (δ 74.4) in **3** were shifted lower field relative to those of **4**. Consequently, the NMR data of **2** and **3** led to the structures of TMC-151 A and B.

TMC-151 E (6)

The HRESI-MS spectral analysis of **6** gave a molecular formula of $C_{43}H_{76}O_{16}$ [*m/z* found 847.5057 $(M\text{-}H)^-$, calcd 847.5055]. The molecular formula of **6** differs from that of **4** by C_2H_2O unit. The ^1H and ^{13}C NMR spectra of **6** were generally similar to those of **4** except for the additional signals of one methyl (^1H: δ 1.98; ^{13}C: δ 20.5) and one carbonyl (^{13}C: δ 170.1), and the absence of one hydroxyl proton. In addition, the oxygenated methylene carbon C-6" (δ 64.0) in **6** was shifted lower field relative to that of **4**. The structure of **6** was thus determined to be a 6"-acetyl analog of **4**.

TMC-151 D (5)

The molecular formula of **5**, determined as $C_{40}H_{72}O_{14}$ from its HRESI-MS [*m/z* found 775.4877 $(M\text{-}H)^-$, calcd 775.4844] differs from that of **4** by CH_2O unit. The ^1H and ^{13}C NMR data of **5** corresponded to those of **4** except for the absence of one –CHOH unit in the hexitol moiety, indicating the presence of a pentitol. Additional evidence for the pentitol structure was provided by DQF-COSY experiment.

TMC-151 F (7)

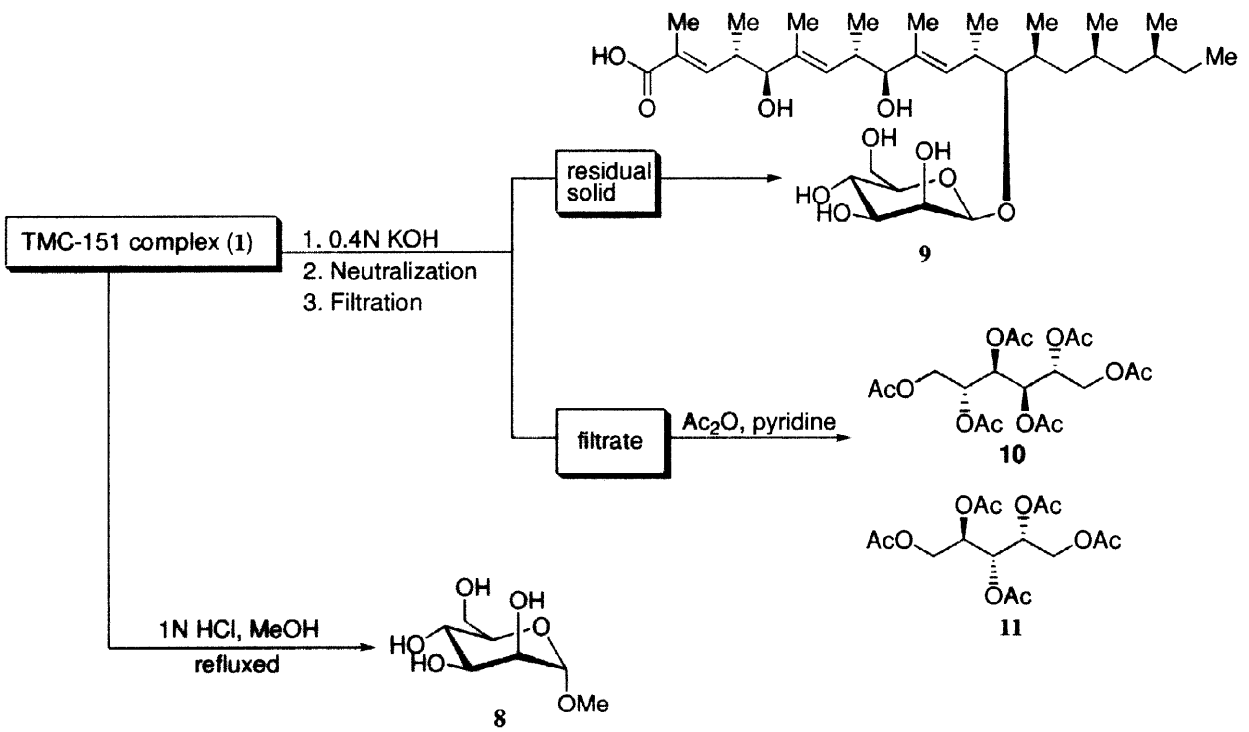
The molecular formula $C_{42}H_{74}O_{15}$ assigned for **7** from its HRESI-MS [*m/z* found 817.4948 $(M-H)^-$, calcd 817.4949]. This molecular formula suggested that it has C_2H_2O unit larger than **5**. The ^1H and ^{13}C NMR spectra of **7** were quite similar to those of **5** except for the additional signals derived from an acetyl group. On the other hand, the NMR signals in the sugar moiety of **7** were identical with those of **6**. Thus, the structure of **7** was determined as 6"-acetyl analog of **5**.

Absolute Stereochemistry of TMC-151 A-F

The absolute stereochemistries of **2-7** were determined by degradation studies and X-ray structure analysis.

Acid methanolysis of TMC-151 complex (**1**) with 1 N methanolic hydrogen chloride gave methyl α-mannopyranoside (**8**), which was confirmed by direct comparison of the ^1H and ^{13}C NMR spectra of **8** with those of the authentic sample. The configuration of **8** was determined to be ***D*** form by comparison of the optical rotational value of **8** with that of the authentic sample (**8**: $[\alpha]_D^{23}$ + 64° (*c* 0.53, MeOH), methyl α-D-mannopyranoside: $[\alpha]_D^{23}$ + 80° (*c* 0.56, MeOH)).

TMC-151 complex (**1**) was alkaline hydrolyzed with 0.4 N KOH at room temperature for 2 hours. After neutralization of the reaction mixture, the resulting precipitate was purified by silica gel column chromatography followed by crystallization from methanol to afford **9** as colorless columns. Single crystal X-ray structure analysis of **9** unequivocally confirmed the substructure of TMC-151 determined by the NMR analysis described above. Since the absolute configuration of the mannoside moiety was determined to *D*, the absolute stereochemistry of **9** was established to be 4*S*, 5*S*, 8*S*, 9*S*, 12*S*, 13*R*, 14*S*, 16*S*, and 18*S* (Figure 2).



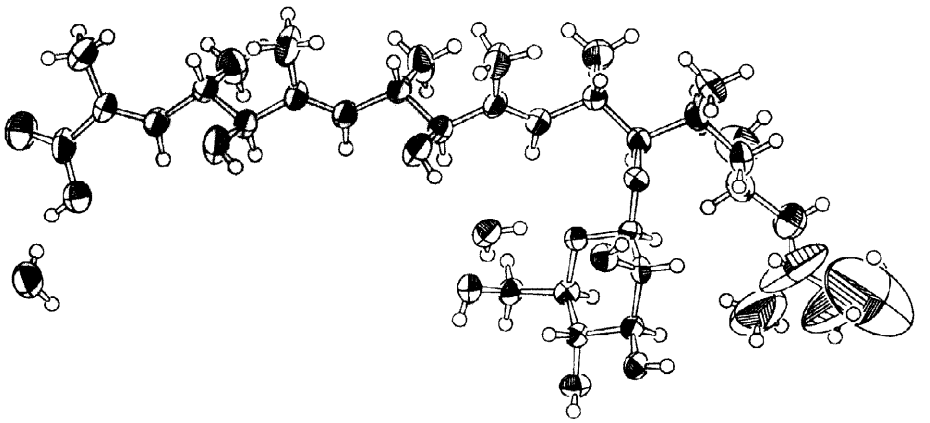
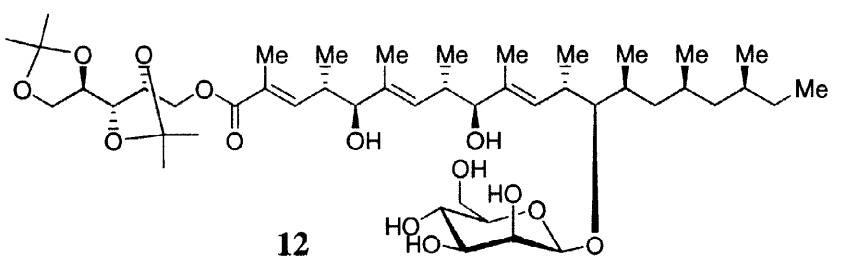


Figure 2. *ORTEP* II diagram of **9**.

The filtrate of the reaction solution was concentrated and acetylated with acetic anhydride in dry pyridine. The product was purified by Sephadex LH-20 column chromatography followed by a combination of crystallization and silica gel column chromatography to yield hexa-*O*-acetylmannitol, **10** and penta-*O*-acetylarabitol, **11**, respectively. Direct comparison of optical rotation values of **10** ($[\alpha]_D^{25}$ + 24° (*c* 0.264, $CHCl_3$)) and **11** ($[\alpha]_D^{25}$ + 29° (*c* 0.258, $CHCl_3$)) with those of authentic hexa-*O*-acetyl-D-mannitol ($[\alpha]_D^{25}$ + 26° (*c* 0.693, $CHCl_3$)) and penta-*O*-acetyl-D-arabitol ($[\alpha]_D^{25}$ + 36° (*c* 0.307, $CHCl_3$)) suggested that **10** and **11** were both ***D*** form.

Since D-mannitol is a meso compound, acylation at 1'/6' or 2'/5' or 3'/4' provides a same product, respectively. Consequently, the absolute structures of **2**, **3**, **4**, and **6** were determined as shown. On the other hand, in order to determine the absolute stereochemistry of 5 and **7**, the acylated position of D-arabitol (1'/5') was determined as follows. Compound **12** (1'-acylation of D-arabitol) was synthesized by two steps from **9** and 2,3:4,5-di-*O*-isopropyridene-D-arabitol, and deprotection of **12** with THF-H_2O-TFA gave **5**.



12

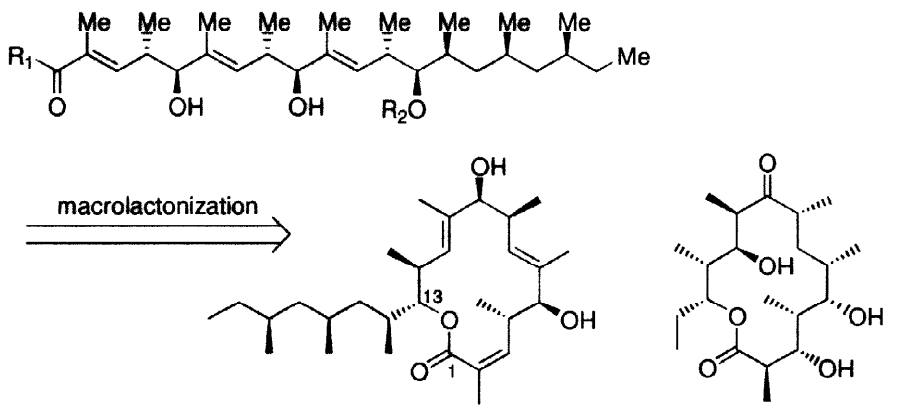
The ^1H and ^{13}C NMR data of the synthetic compound was identical with those of the authentic sample of **5**. The absolute stereochemistry of **5** and **7**, therefore, were unequivocally assigned as shown.

Biological Activity

TMC-151s **A-F** showed moderate cytotoxicity against several tumor cell lines human colon carcinoma HCT-116 (IC_{50}: 2.6 ~ 11.5 μM), human promyelocytic leukemia HL-60 (IC_{50}: 13 ~ 25 μM) and murine lymphoid neoplasm $P388D_1$ (IC_{50}: 3.1 ~ 7.9 μM).

Discussion

In this study, we isolated six new antibiotics, TMC-151 A-F (2-7), from *Gliocladium catenulatum* Gilman & Abbott TC 1280, and our structural study demonstrated that these antibiotics belonged to novel polyketides family. If the macrolactonization between C-1 and C-13 of TMC-151s occurred, the carbon skeleton of the product would have the same core polyketide structure of erythromycins, 14-membered macrolide antibiotics. All of the known 14-membered macrolide antibiotics represented by albocycline,[9] erythromycins,[10, 11] megalomycin[12] and oleandomycin,[13] have been isolated from actinomycete strains including the genus *Streptomyces* and *Micromonospora*. The core structure of TMC-151s, erythromycin-type polyketide, were isolated for the first time as fungal metabolites, although a large number of polyketides such as tetraketides and reduced polyketide, have been isolated from fungal origins.[14]



6-Deoxyerythronolide B,
precurcor of erythromycin A

The structures of TMC-151s possesses all of four functional groups formed in the polyketide synthases (PKSs) reaction,[15] that is, keto, hydroxy, alkane and alken groups. These fact suggests that six esseitial PKSs, acyl transferase (AT), acyl carrier protein (ACP), β-ketoacyl synthase (KS), β-ketoacyl reductase (KR), enoyl reductase (ER) and dehydrase (DH)[15] are utilized for assembly and elaboration of the polyketide chain in TMC-151s. In the biosynthesis of erythromycin family, the 14-membered macrolide core is derived from one propionyl-CoA and six methylmalonyl-CoA units. The chain elongation is initiated by condensation of a starter unit (propionate) with an extender unit (methylmalonate) and chain expansion carries out by mainly three essential PKSs (AT, ACP and KS). Once the chain has reached the required length (thirteen carbon units), it is cyclized by the thioesterase (TE) to give 14-membered macrolide.[15] On the basis of the above explanation, the biothynthetic pathway of TMC-151s could be expected as follows: 1) The chain elongation is initiated from acetate as a starter unit in a similar manner in erythromycin, and the resulting β-ketoester is then processed (reduced, dehydrated and reduced) repeatedly by fatty acid synthases (FASs)-type enzymes (ER, DH and KR) in the early step. 2) Subsequently, chain extension cycle takes place in a similar manner in the case of erythromycins. 3) Finally, ester-exchange of ACP at C-1 with D-mannitol or D-arabitol, and glycosidation at C-13 with D-mannopyranoside takes place instead of macrolactonization. The early stage of the expected biosynthesis pathway in TMC-151s closely resembles that of mavioquinone, isolated from *Mycobacterium avium*[16]. The expectation about biothynthesis of TMC-151s will be confirmed experimentally in future.

Experimental Section

General Methods

Preparative HPLC separations were carried out using Gilson HPLC system and YMC D-ODS-5B; column size: 30 x 250 mm; flow rate: 25 mL/minute; detection: UV light at 254 nm. *Rf* values were determined with Kieselgel 60 F_{254} TLC glas plates (E. Merck, Darmstadt, Germany) in EtOAc/MeOH/H_2O (15:3:1). TLC spots were visualized by exposure to a ammonium molibdate/H_2SO_4 spray reagent. Optical rotations were determined using the sodium D line on a Horiba model SEPA-200 high sensitive polarimeter in methanol at 23°C. UV spectra were measured on a Shimadzu model UV-2200A spectrophotometer in methanol. IR spectra were recorded on a JASCO model 100 infrared spectrophotometer. The samples were prepared and mounted as KBr micropellets. All mass spectra were obtained using MStation 700 tandem type mass spectrometer (JEOL, Japan) equipped with an electrospray ionization source. Analytical HPLC were obtained using a HP1100 system (Hewlett Packard, USA). ^1H and ^{13}C NMR spectra were recorded on a JEOL GSX-400 NMR spectrometer at 30°C. The chemical shifts are given in ppm (δ) relative to tetramethylsilane (TMS) as an internal standard. DQF-COSY, ROESY, HMQC and HMBC spectra were obtained using standard pulse sequences. DQF-COSY and ROESY spectra were recorded in phase-sensitive mode. The ROESY mixing times were 200 msec. The HMQC and HMBC experiments were optimized for $^1J_{CH}$ = 145 Hz and $^nJ_{CH}$ = 8.2 Hz, respectively. Typically 2048 x 128 data points were acquired and zerofilling was used in the t1 domain to 2048 points.

Fermentation of Gliocladium catenulatum TC 1280

Gliocladium catenulatum TC 1280 was inoculated into 500-ml Erlenmeyer flasks containing pressed barley 10 g, yeast extract 0.02 g, Na tartrate 0.01 g, KH_2PO_4 0.01 g, and deionized water 20 ml. The fermentation was conducted under the static condition at 25°C for 12 days.

Isolation of TMC-151 A-F (2-7)

The resultant solid was combined from the above fermentation and was extracted with 1-butanol (1.8 L) by shaking on a shaker at room temperature for 30 min. The solvent layer was separated and concentrated *in vacuo* to dryness. The residue (12.5 g) was suspended in water (200 mL), washed with EtOAc (200 mL) twice to remove liophilic impurities, and then extracted with 1-butanol (100 mL) twice to afford a crude solid (6.34 g). This solid was applied onto a column of Sephadex LH-20 (4.0 x 680 mm) and eluted with CH_2Cl_2-MeOH (1:1). The appropriate fractions, determined by TLC analysis, were combined and evaporated under reduced pressure to yield 4.55 g of the TMC-151 complex (**1**). This complex (1.55 g) was subjected to medium-pressure liquid chromatography (MPLC, 4.0 x 420 mm) on a reversed-phase silica gel and eluted with 30% aqueous acetonitrile (0.8 L) and then 55% aqueous acetonitrile (4.5 L). The eluates were collected in size of 90 mL, and the presence of TMC-151s was monitored by HPLC analysis (column: YMC ODS-Pack AM 301-3, 4.6 x 100 mm; eluent: 55% aqueous acetonitrile; flow rate 1.2 mL/min.; detection: UV at 230 nm; t_R: **2** 5.9 min, **3** 6.0

min, **4** 7.4 min, **5** 8.6 min, **6** 12.1 min, **7** 14.2 min).

First fraction (130 mg) obtained from 800-1160 mL of the MPLC eluate, was chromatographed by a reverse phase preparative HPLC (column: YMC D-ODS-5-B, 30 x 250 mm ; eluent: 50% aqueous acetonitrile, flow rate 25 mL/min). Active fractions were concentrated and lyophilized to afford **2** (13.2 mg, t_R 33 min) and **3** (10.7 mg, t_R 35 min) as colorless amorphous powders.

Second fraction (112 mg) obtained from 1160–1430 mL of the MPLC eluate, was subjected to the preparative HPLC and eluted with 55% aqueous acetonitrile to yield **2** (11.9 mg, t_R 20 min), **3** (9.8 mg, t_R 21 min) and **4** (19.9 mg, t_R 27 min) as colorless amorphous powder, respectively.

Third fraction (158 mg) obtained from 3400-4430 mL of the MPLC eluate, was applied to the preparative HPLC and eluted with 60% aqueous acetonitrile to give **4** (28.4 mg, t_R 19 min), **5** (33.4 mg, t_R 22 min), **6** (29.5 mg, t_R 30 min) and **7** (8.7 mg, t_R 36 min) as colorless amorphous powder, respectively. Total yield: **2** (25.1 mg), **3** (20.5 mg), **4** (48.3 mg), **5** (33.4 mg), **6** (29.5 mg) and **7** (8.7 mg).

TMC-151 A (2): colorless powder; $[\alpha]_D^{24.5}$ +4° (*c* 0.210, MeOH); *Rf* value 0.33; UV (MeOH) λ_{max} 220 sh (4.16) and 207 (4.21) nm (log ε); IR (KBr) ν_{max} 3400, 2950, 2920, 2870, 1695, 1640, 1450, 1370, 1265, 1225, 1120, 1075 and 1020 cm^{-1}; ESIMS *m/z* 829 $(M+Na)^+$, 805 $(M-H)^-$; HRESI-MS *m/z* 805.4962 $(M-H)^-$, (calcd for $C_{41}H_{73}O_{15}$ 805.4949); The ^1H and ^{13}C NMR data are given in Table 1 and 2.

TMC-151 B (3): colorless powder; $[\alpha]_D^{24.5}$ +43° (*c* 0.047, MeOH); *Rf* value 0.38; UV (MeOH) λ_{max} 220 sh (4.17) and 206 (4.24) nm (log ε); IR (KBr) ν_{max} 3400, 2950, 2920, 2870, 1695, 1640, 1455, 1375, 1265, 1225, 1120, 1075 and 1020 cm^{-1}; ESIMS *m/z* 829 $(M+Na)^+$, 805 $(M-H)^-$; HRESI-MS *m/z* 805.4916 $(M-H)^-$, (calcd for $C_{41}H_{73}O_{15}$ 805.4949); The ^1H and ^{13}C NMR data are given in Table 1 and 2.

TMC-151 C (4): colorless powder; $[\alpha]_D^{24.5}$ +27° (*c* 0.103, MeOH); *Rf* value 0.36; UV (MeOH) λ_{max} 220 sh (4.20) and 205 (4.26) nm (log ε); IR (KBr) ν_{max} 3400, 2950, 2920, 2870, 1695, 1640, 1455, 1370, 1270, 1225, 1120, 1070 and 1020 cm^{-1}; elemental analysis C 58.58, H 9.11 (calcd for $C_{41}H_{74}O_{15}\cdot 2H_2O$ C 58.41, H 9.32); ESIMS *m/z* 829 $(M+Na)^+$, 805 $(M-H)^-$; HRESI-MS *m/z* 805.4962 $(M-H)^-$, (calcd for $C_{41}H_{73}O_{15}$ 805.4949); The ^1H and ^{13}C NMR data are given in Table 1 and 2.

TMC-151 D (5): colorless powder; $[\alpha]_D^{24.5}$ +78° (*c* 0.069, MeOH); *Rf* value 0.40; UV (MeOH) λ_{max} 220 sh (4.15) and 205 (4.27) nm (log ε); IR (KBr) ν_{max} 3400, 2950, 2920, 2870, 1700, 1640, 1455, 1370, 1265, 1225, 1165, 1120, 1070 and 1020 cm^{-1}; ESIMS *m/z* 799 $(M+Na)^+$, 775 $(M-H)^-$; HRESI-MS *m/z* 775.4877 $(M-H)^-$, (calcd for $C_{40}H_{71}O_{14}$ 775.4844); The ^1H and ^{13}C NMR data are given in Table 1 and 2.

TMC-151 E (6): colorless powder; $[\alpha]_D^{24.5}$ +21° (*c* 0.244, MeOH); *Rf* value 0.42; UV (MeOH) λ_{max} 220 sh (4.13) and 206 (4.24) nm (log ε); IR (KBr) ν_{max} 3400, 2950, 2920, 2870, 1730, 1700, 1640, 1450, 1370, 1265, 1235, 1175, 1120, 1075 and 1020 cm^{-1}; ESIMS *m/z* 871 $(M+Na)^+$, 847 $(M-H)^-$; HRESI-MS *m/z* 847.5057 $(M-H)^-$, (calcd for $C_{43}H_{75}O_{16}$ 847.5055); The ^1H and ^{13}C NMR data are given in Table 1 and 2.

TMC-151 F (7): colorless powder; $[\alpha]_D^{24.5}$ +57° (*c* 0.077, MeOH); *Rf* value 0.50; UV (MeOH) λ_{max} 220

sh (4.19) and 207 (4.24) nm (log ε); IR (KBr) ν_{max} 3400, 2950, 2920, 2870, 1740, 1700, 1645, 1595, 1450, 1370, 1265, 1235, 1175, 1120, 1075 and 1025 cm^{-1}; ESIMS *m/z* 841 $(M+Na)^+$, 817 $(M-H)^-$; HRESI-MS *m/z* 817.4948 $(M-H)^-$, (calcd for $C_{42}H_{73}O_{15}$ 817.4949); The ^1H and ^{13}C NMR data are given in Table 1 and 2.

Acid Methanolysis of TMC-151 Complex (1)

A solution of 300 mg of **1** in 0.88 N methanolic hydrogen chloride (30 mL) was refluxed for 1.5 hours. After evaporation of the solvent *in vacuo*, the residue was dissolved in H_2O (15 mL), washed with diethyl ether (15 mL, twice), neutralized with 1 N NaOH. and then the solution was concentrated to dryness to give crude solid (147.5 mg). This solid was subjected to a silica gel column (2.2 x 67 cm) developed with EtOAc-MeOH-H_2O (20:3:1). The active eluate was concentrated and chromatographed on a Sephadex LH-20 column (2.2 x 44 cm) with 50% aqueous MeOH as an eluent to yield amorphous powder of **8** (50.4 mg). $[\alpha]_D^{23}$ +63.8° (*c* 0.53, MeOH); ESIMS *m/z* 195 $(M+H)^+$; elemental analysis C 41.50%, H 7.53%, (calcd for $C_7H_{14}O_6 \cdot 1/2\ H_2O$ C 41.37%, H 7.44%); ^{13}C NMR (DMSO-d_6) δ 100.9 (C-1), 73.7 (C-5), 70.9 (C-3), 70.1 (C-2), 67.0 (C-4), 61.2 (C-6), 53.8 (CH_3).

Alkaline Hydrolysis of TMC-151 Complex (1)

Fifteen milliliters of 0.4 N KOH was added to **1** (400 mg) and stand at room temperature for 2 hours. The reaction mixture was then neutralized with 1 N HCl. The resulting suspension was filtered and the residual solid was dried under reduced pressure to give 308 mg of the crude product. This product was purified by column chromatography on a silica gel (EtOAc-MeOH-H_2O = 30:3:1 as an eluent) to give **9** (181 mg). Compound 9 was recrystallized from MeOH to afford colorless column. $[\alpha]_D^{24.5}$ +6° (*c* 0.420, MeOH); *Rf* value 0.53; UV (MeOH) λ_{max} 211 (4.21) nm (log ε); IR (KBr) ν_{max} 3400, 2955, 2920, 2870, 1680, 1640, 1455, 1370, 1305, 1260, 1225, 1170, 1105, 1070 and 1025 cm^{-1}; ESIMS *m/z* 665 $(M+Na)^+$, 641 $(M-H)^-$; HRESI-MS *m/z* 641.4290 $(M-H)^-$, (calcd for $C_{35}H_{61}O_{10}$ 641.4265); ^1H NMR (DMSO-d_6) δ 6.60 (H-3, 1H, 9.8, 1 Hz), 5.39 (H-11, 1H, d, 9.3 Hz), 5.18 (H-7, 1H, d, 9.0), 4.58 (5-OH, 1H, d, 3.7 Hz), 4.32 (H-1", 1H, brs), 3.94 (9-OH, 1H, d, 3.4), 3.69 (H-2", 1H, m), 3.69 (H-6"a, 1H, m), 3.66 (H-5, 1H, m), 3.55 (H-9, 1H, brd, 8 Hz), 3.48 (H-6"b, 1H, dd, 11.4, 6.0 Hz), 3.32 (H-4", 1H, t, 9.5 Hz), 3.30 (H-13, 1H, m), 3.20 (H-3", 1H, dd, 9.3, 3.2 Hz), 2.99 (H-5", 1H, ddd, 8.7, 5.7, 2.1 Hz), 2.67 (H-12, 1H, m), 2.55 (H-4, 1H, m), 2.45 (H-8, 1H, m), 1.74 (H-21, 3H, brs), 1.69 (H-14, 1H, m), 1.55 (H-23, 3H, brs), 1.54 (H-25, 3H, brs), 1.52 (H-16, 1H, m), 1.38 (H-18, 1H, m), 1.33 (H-19a, 1H, m), 1.30 (H-15a, 1H, m), 1.20 (H-17a, 1H, m), 1.03 (H-19b, 1H, m), 0.95 (H-26, 3H, d, 6.8 Hz), 0.87 (H-27, 3H, d, 6.8 Hz), 0.86 (H-15b, 1H, m), 0.83 (H-28, 3H, d, 7 Hz), 0.83 (H-29, 3H, d, 7 Hz), 0.82 (H-17b, 1H, m), 0.82 (H-20, 3H, t, 7 Hz), 0.77 (H-22, 3H, d, 6.8 Hz), 0.70 (H-24, 3H, d, 6.6 Hz); ^{13}C NMR (DMSO-d_6) δ 169.0 (C-1), 145.8 (C-3), 136.1 (C-6), 134.6 (C-10), 131.1 (C-7), 130.0 (C-11), 126.8 (C-2), 101.3 (C-1"), 85.3 (C-13), 81.1 (C-9), 80.6 (C-5), 77.3 (C-5"), 73.9 (C-3"), 70.7 (C-2"), 67.0 (C-4"), 61.4 (C-6"), 43.8 (C-17), 42.1 (C-15), 36.9 (C-4), 36.2 (C-8), 34.5 (C-12), 33.0 (C-14), 30.9 (C-18), 28.0 (C-19), 27.2 (C-16), 20.9 (C-28), 20.0 (C-29), 18.2 (C-26), 17.4 (C-24), 16.5 (C-22), 15.8 (C-27), 12.4 (C-21), 11.3 and 11.2 (C-23 and 25), 10.8 (C-20).

The filtrate of the reaction mixture described above was concentrated and the product was acetylated in the following manner.

Acetylation of the Alkaline Hydrolysis Product of ***1***

To a solution of the product in dry pyridine (8.0 mL) was added acetic anhydride (2.0 mL), and the mixture was stand at room temperature for 2 hours. After completion of the reaction, EtOAc (100 mL) was added, and the mixture was washed with H_2O (50 mL, three times). The organic phase was evaporated under reduced pressure and was chromatographed over a Sephadex LH-20 (2.2 x 42 cm) column in CH_2Cl_2-MeOH (1:1). Elution with the same solvent system afforded 103 mg of colorless powder. This powder was recrystallized from hot methanol to afford 26.7 mg of **10** as colorless plates. On the other hand, the filtrate was concentrated and was chromatographed on a silica gel (2.2 x 68 cm) column. Elution with hexane-EtOAc (2:1) gave **11** (6.5 mg).

10: colorless plates; $[\alpha]_D^{25}$ +24° (*c* 0.264, $CHCl_3$); ESIMS *m/z* 452 $(M+NH_4)^+$, 457 $(M+Na)^+$, 473 $(M+K)^+$; ^1H and ^{13}C NMR data were identical with those of hexa-*O*-acetyl-D-mannitol.

11: colorless powder; $[\alpha]_D^{25}$ +29° (*c* 0.258, $CHCl_3$); ESIMS *m/z* 380 $(M+NH_4)^+$, 385 $(M+Na)^+$, 401 $(M+K)^+$; ^1H and ^{13}C NMR data were identical with those of penta-*O*-acetyl-D-arabitol.

X-ray Crystallography of ***9***

A colorless column of **9** with dimensions 0.30 x 0.10 x 0.10 mm was used for X-ray analysis. The intensity data were collected on a Rigaku AFC5R difractometer by using graphite-monochromated Cu-Kα (λ=1.5418 Å) radiation by $2\theta/\omega$ scan technique. Unit cell dimensions were determined by a least squares refinement by using the setting of 25 reflections in the range of $70°<2\theta<90°$. The crystallographic data are summarized as follows: $C_{35}H_{66}O_{12}$, Mr=678.9, column, $P2_1$, *a*=16.971(2)Å, *b*=6.908(1)Å, *c*=18.204(1)Å, β=112.04(1)°, *V*=1978.0(5)Å^3, Z=2, D_{calc}=1.14 g/cm^3, Mu=0.691 mm^{-1}. The intensities of 3675 reflections with $2.6°<2\theta<65.1°$, $0<h<19$, $0<k<8$, $-21<l<19$ were measured. Three standard reflections were monitored every 200 reflection intervals and showed insignificant fluctuations. The data were corrected for Lorentz and polarization effects, but not for absorption.

The structure was solved by a direct method by using SHELXS-97 and the subsequent difference Fourier method. The structure refinement on F^2 was carried out by a SHELXL-97 with anisotropic thermal parameters for all of non-hydrogen atoms. The hydrogen atoms were refined by riding with the atoms to which they were bonded. The full matrix least squares refinement varied 425 parameters and used all 3675 independent reflections weighted by $\omega=1/[\sigma^2(Fo^2)+(0.1000P)^2+0.0000P]$ where $P=(Fo^2+2Fc^2)/3$. Final *R1*=0.075, *wR2*=0.167 and *Goodness of Fit*(*S*)=1.08 for all data; *R1*=0.055 for 2952 reflections with $I>2\sigma(I)$. The final difference Fourier map showed maximum and minimum values of 0.21 and -0.24*e*/Å^3, respectively.

Preparation of ***12***

The mixture of 2, 3: 4, 5-di-*O*-isopropylidene-D-arabitol (obtained from SIGMA-ALDRICH JAPAN K.K., 128 mg, 0.55 mmol), triphenylphosphine (290 mg, 1.10 mmol) and imidazole (37.5 mg, 0.55 mmol) in

toluene (3.0 mL) was stirred at 90°C for 3 hours. After addition of tribromoimidazole (252 mg, 0.83 mmol), the resulting mixture was stirred at the same temperature for 2 hours. To the reaction mixture was added saturated aqueous $NaHCO_3$, I_2 and $Na_2S_2O_3$. The toluene layer was separated and the aqueous layer was extracted with chloroform, three times. The combined organic extract was dried over $MgSO_4$, filtered, and concentrated to give a oil. This oil was purified by silica gel column chromatography eluted with EtOAc-hexane (2:8) to give a colorless oil (144 mg).

The mixture of **9** (200 mg, 0.31 mmol) and Cs_2CO_3 (116 mg, 0.36 mmol) in HMPA (2.0 mL) was stirred at room temperature overnight. To the reaction mixture was added NaI (12 mg) and the oil (134 mg, 0.45 mmol) described above in HMPA (2.0 mL), and the resulting mixture was stirred at 70°C for 5 hours. To the mixture was added saturated $NaHCO_3$, I_2 and $Na_2S_2O_3$. The aqueous layer was extracted with EtOAc three times. The combined extract was washed with H_2O and saturated NaCl, dried over $MgSO_4$, filtered, and then concentrated to give 416 mg of oil. This oil was subjected to a silica gel column (20g) and eluted with MeOH-$CHCl_3$ (3:97) to give 111 mg of **12** as a colorless powder. ESIMS *m/z* 879 $(M+Na)^+$, 855 $(M-H)^-$; ^1H NMR (DMSO-d_6) δ 6.68 (H-3, 1H, 9.8, 1.5 Hz), 5.39 (H-11, 1H, brd, 9 Hz), 5.19 (H-7, 1H, brd, 9), 4.67 (4"-OH, 1H, d, 5.4 Hz), 4.62 (5-OH, 1H, d, 3.7 Hz), 4.51 (3"-OH, 1H, d, 6.1 Hz), 4.33 (1H, m), 4.32 (H-1", 1H, brs), 4.18 (6"-OH, 1H, dd, 6.1, 5.1 Hz), 4.13-4.05 (4H, m), 4.03 (2"-OH, 1H, 4.9 Hz), 3.94 (9-OH, 1H, d, 3.4), 3.83 (1H, dd, 8.1, 4.4), 3.79 (1H, t, 7.3 Hz), 3.69 (H-2", 1H, m), 3.68 (H-6"a, 1H, m), 3.67 (H-5, 1H, m), 3.55 (H-9, 1H, dd, 8.1, 3.4 Hz), 3.48 (H-6"b, 1H, m), 3.32 (H-4", 1H, m), 3.30 (H-13, 1H, m), 3.20 (H-3", 1H, ddd, 9.3, 6.1, 3.2 Hz), 2.98 (H-5", 1H, ddd, 8.9, 6.0, 2.3 Hz), 2.67 (H-12, 1H, m), 2.59 (H-4, 1H, m), 2.46 (H-8, 1H, m), 1.80 (H-21, 3H, d, 1.2), 1.69 (H-14, 1H, m), 1.56 (H-23, 3H, d, 1), 1.54 (H-25, 3H, d, 1), 1.52 (H-16, 1H, m), 1.40 (H-18, 1H, m), 1.34 (H-19a, 1H, m), 1.34 (CH_3, 3H, s), 1.33 (CH_3, 3H, s), 1.31 (CH_3, 3H, s), 1.30 (H-15a, 1H, m), 1.27 (CH_3, 3H, s), 1.20 (H-17a, 1H, m), 1.03 (H-19b, 1H, m), 0.95 (H-26, 3H, d, 6.8 Hz), 0.87 (H-27, 3H, d, 6.8 Hz), 0.86 (H-15b, 1H, m), 0.83 (H-28, 3H, d, 7 Hz), 0.83 (H-29, 3H, d, 7 Hz), 0.82 (H-17b, 1H, m), 0.82 (H-20, 3H, m), 0.77 (H-22, 3H, d, 6.8 Hz), 0.70 (H-24, 3H, d, 6.8 Hz); ^{13}C NMR (DMSO-d_6) δ 167.0 (C-1), 147.1 (C-3), 136.0 (C-6), 134.6 (C-10), 131.3 (C-7), 130.0 (C-11), 125.9 (C-2), 109.1 (>C<), 108.8 (>C<), 101.3 (C-1"), 85.3 (C-13), 81.1 (C-9), 80.6 (C-5), 77.4, 76.8 and 76.0 (C-2', 3' and 4'), 77.2 (C-5"), 74.0 (C-3"), 70.7 (C-2"), 67.0 (C-4"), 66.4 (C-1'), 63.8 (C-5'), 61.4 (C-6"), 43.8 (C-17), 42.1 (C-15), 37.0 (C-4), 36.2 (C-8), 34.5 (C-12), 33.0 (C-14), 30.9 (C-18), 28.0 (C-19), 27.2 (C-16), 26.9 (CH_3), 26.8 (CH_3), 26.4 (CH_3), 25.1 (CH_3), 20.9 (C-28), 20.0 (C-29), 18.2 (C-26), 17.3 (C-24), 16.4 (C-22), 15.8 (C-27), 12.4 (C-21), 11.3 and 11.2 (C-23 and 25), 10.8 (C-20).

*Deprotection of **12***

The solution of **12** (31.8 mg, 0.037 mmol) in THF-H_2O-TFA (2.0 mL-0.5 mL-1.5 mL) was stand at room temperature overnight, and the resulting solution was concentrated to give 12 mg of solid. This solid was applied onto a silica gel column (5 g) and eluted with EtOAc- MeOH-H_2O (30-3-1) to yield 5.5 mg of **5**. The MS and ^1H and ^{13}C NMR data of the product were identical with those of **5**.

In Vitro Cytotoxic Activity

The cells used for assay were cultured in following medium; HCT-116: complete McCoy's 5A supplemented with 10% fetal bovine serum, HL-60: complete RPMI-1640 supplemented with 20% fetal bovine serum, $P388D_1$: complete RPMI-1640 supplemented with 5% fetal bovine serum.

In vitro cytotoxic activity was tested in 96-well microtiter plates of which well containing 1 x 10^4 each cell lines in 135 μL medium. The test samples were dissolved in 10% DMSO. The serially diluted DMSO solution (15 μL) was added to each well of plates. After addition, the cells were incubated at 37°C for 72 hours in a humidified 5% CO_2 atmosphere. *In vitro* cytotoxic activity was evaluated by the microculture tetrazolium assay (MTT assay) method for each cell and by the colorimetrical determination method at 540 nm.

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