Absolute Stereochemistry Determination of Tetrin B

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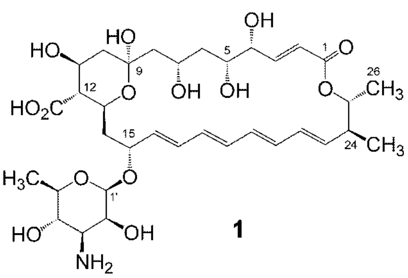
The absolute stereochemistry of tetrin B (**1**), an antifungal antibiotic isolated from a soil actinomycete was determined by applying Rychnovsky analysis, the modified Mosher method, and CD exciton chirality to be 4*R*, 5*R*, 7*S*, 9*R*, 11*S*, 12*R*, 13*S*, 15*R*, 24*S*, and 25*R*.

Key Words : Tetrin B, Absolute stereochemistry

A novel antifungal antibiotic tetrin B (**1**) was isolated from a soil actinomycete in 1963 and its structure was finally established after a number of erroneous proposals.[1-3] Although tetrin B was ascertained to belong to the 26-membered tetraene macrolide, its stereochemistry has remained undefined. Spectroscopic and synthetic investigations of the stereochemistry of tetrin-type macrocycles have been carried out mainly for pimaricin.[3-5] A controlled degradation protocol designed for stereostructural studies was developed to lead a C3-C16 segment, a possible target for its synthetic work by Fraser-Reid's group.[6] Duplantier and Masamune reported stereochemical studies based on the reagent controlled asymmetric synthesis of the diastereomers, which also defined the stereochemistry of pimarolide, thus representing the first synthetic access to this type of macrocycles.[7] Recently, Beau's group determined the complete configuration of pimaricin on the basis of coupling constants (J) derived from nuclear magnetic resonance and qualitative NOEs.[8] Nevertheless, such efforts have not given the absolute configuration of tetrin B. More recently, we isolated tetrin-related compounds from *Streptomyces* sp. GK9244.[9] In this paper we describe the complete stereochemistry of its major metabolite tetrin B.



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A method to determine the absolute configuration of **1** was developed (Scheme 1). First, compound **1** was treated with 1.0 N NaOH to afford sodium dicarboxylate and pentaenal **4**. The dicarboxylate was immediately methylated with diazomethane to give a methyl diester **3**, which was subsequently converted to two acetonides (**5**, **6**). The gross structure of **5** was readily identified by the mass and NMR spectral data. The chemical shifts of the acetonide ketal (δ 99.3) and two methyl carbons (30.1, 19.9)[10] and the coupling constants between the methine protons (H5 and H7) in the six membered dioxane ring of **5** suggested that the acetonide of a *syn*-1,3-diol had been produced (Figure 1). The oxymethine proton (H5) signal at 3.87 ppm showed 2.9 Hz and 10.1 Hz couplings to the $H6_{eq}$ and $H6_{ax}$ signal at 1.12 ppm and 1.36 ppm, respectively, and another oxymethine proton (H7) at 3.74 ppm was coupled to $H6_{eq}$ and $H6_{ax}$ to 2.0 Hz and 11.8 Hz values, respectively. The magnitude of these values, which reflects the chair conformation of the acetonide ring, indicates that both H5 and H7 are *anti* to $H6_{ax}$. As expected in this conformation, H5 and H7 signals exhibited strong NOEs to the acetonide methyl (*syn* to H5 and H7) signal at 0.81 ppm, whereas the $H6_{ax}$ signal displayed no NOE correlation with other protons in the dioxane ring. We concluded that the relative configurations at C5 and C7 in **5** are 5*R** and 7*S**. The relative stereochemistry of C4 and C5 in **1** was established by NMR data for acetonide **6** (Fig. 1); a coupling constant ($J_{H4\text{-}H5}$ = 8.1 Hz) and the NOE correlations between H5 and H3 and between H6a and H4 in **6** indicated a syn relationship between two hydroxyl groups at C4 and C5. This was consistent with an empirical rule for assigning the stereochemistry of 1,2-acetonides by chemical shifts of methyl groups: both methyls of the isopropylidene unit in **6** resonated at 1.38-1.39 ppm,[11] thereby, resulting in 4*R*,* 5*R**-configuration.

The absolute stereochemistry determination of two acetonides (**5**, **6**) was achieved by the modified Mosher's method.[12] The (*R*)- and (*S*)-MTPA [2-methoxy-2-(trifluoromethyl)-2-phenyl acetyl] derivatives (**7a** and **7b**, and **8a** and **8b**) were prepared, and $\Delta\delta$ (δ_S-δ_R) values for all the assignable protons were determined with 400 MHz NMR (Fig. 2). The absolute configurations of C4 in **5** and C11 in **6** were indicated as *R* and *S*, respectively. Furthermore, CD analysis of di-*p*-bromobenzoate **11** afforded more evident absolute configurations of C4 and C5 in **6**. A series of hydrogenation (H_2/Pd), acetylation (Ac_2O/pyr), acidic hydrolysis (80% aqueous AcOH), and *p*-bromobenzoylation (*p*-BrC_6H_4COCl/

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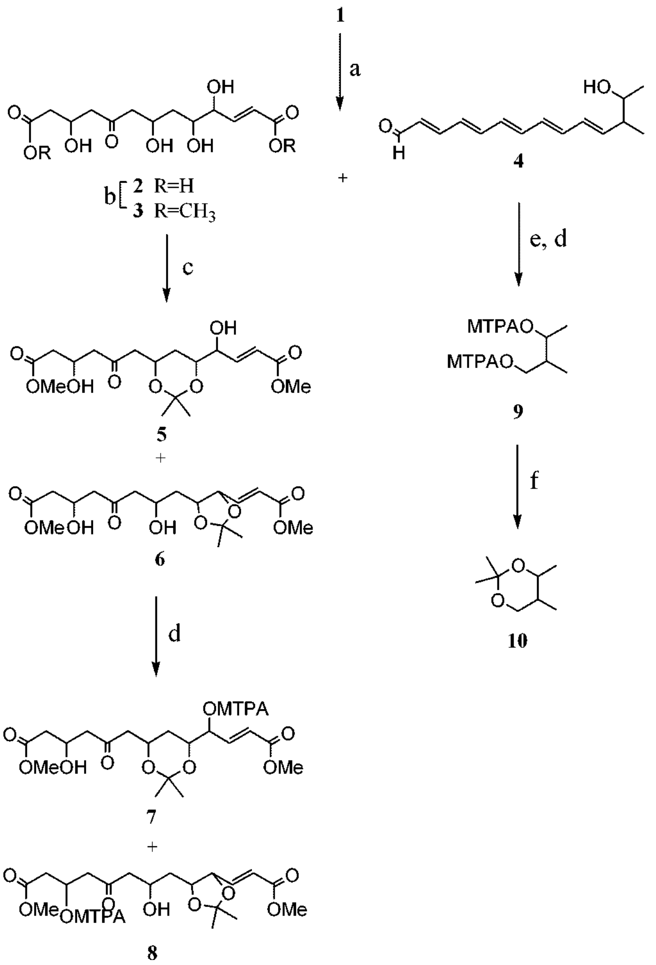


Scheme 1. a: 1.0 N NaOH; b: CH_2N_2/Et_2O; c: 2,2-DMP, CSA, acetone; d: MTPA-Cl, DMAP, CH_2Cl_2; e: i) O_3/MeOH-CH_2Cl_2, $NaBH_4$ ii) $LiAlH_4$; f: i) LiOH, MeOH-H_2O; ii) 2,2-DMP, CSA, acetone.

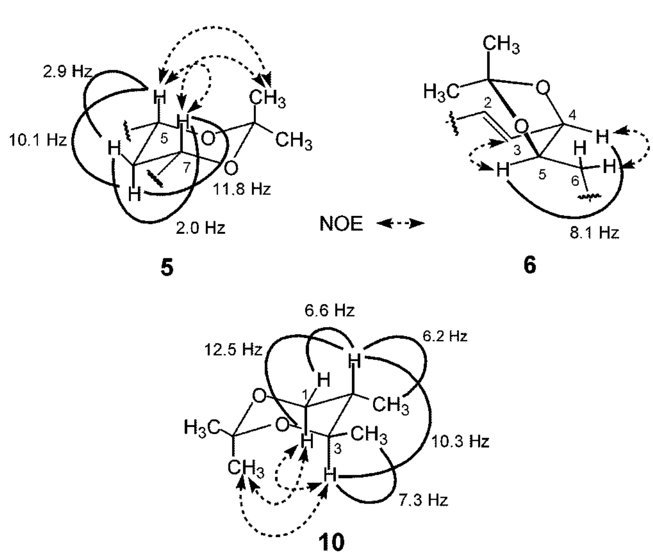
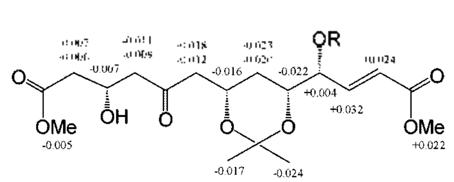


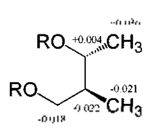
Figure 1. Coupling constants and NOE correlations providing evidence for relative configuration and conformation of the acetonide in **5**, **6**, and **10**.

pyr) of **6** produced a saturated di-*p*-bromobenzoate derivative **11** (Scheme 2). The CD spectrum of **11** showed a clear negative exciton split [first Cotton at 255 nm ($\Delta\varepsilon$ = -6.5); second Cotton at 239 nm ($\Delta\varepsilon$ = +7.1)], and thus the 4*R* and 5*R* configurations were confirmed unambiguously. On the other hand, the relative stereochemistry of the carboxyl group-substituted tetrahydropyran ring of **1** was assigned by NOESY experiments in the preceding paper.[9] The absolute stereochemistry of the tetrahydropyran ring in **1** was determined as 9*R*, 11*S*, 12*R*, and 13*S*.

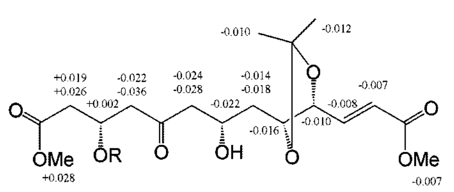
Determination of the absolute configurations at C24 and C25 in **1** was achieved by NMR experiments for a degraded fragment of **4**. Sequential ozonolysis (O_3/MeOH-CH_2Cl_2, $NaBH_4$) of **4** and reduction ($LiAlH_4$) efficiently produced a



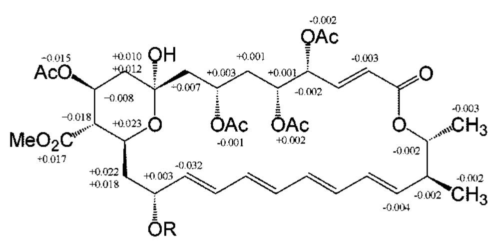
7a R=(−)-(*R*)-MTPA
7b R=(-)-(*S*)-MTPA



9a R=(+)-(*R*)-MTPA
9b R=(-)-(*S*)-MTPA

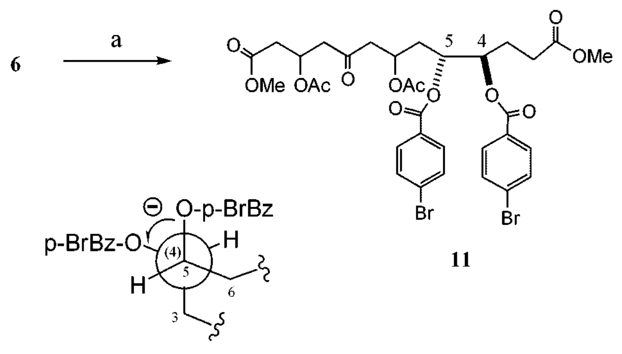


8a R=(+)-(*R*)-MTPA
8b R=(-)-(*S*)-MTPA



12 R=H
13a R=(+)-(*R*)-MTPA
13b R=(-)-(*S*)-MTPA

Figure 2. $\Delta\delta = \Delta_S - \Delta_R$ values in ppm obtained at 400 Hz for the MTPA esters **7a**, **7b**, **8a**, **8b**, **9a**, **9b**, **13a**, and **13b**.



Scheme 2. a: i) H_2, Pd/C, MeOH; ii) Ac_2O, py, r.t. iii) 80% AcOH, 60 °C; iv) p-BrC_6H_4COCl, py, 40 °C.

1,3-diol, which was treated with MTPA-Cl to give MTPA diesters (**9a** and **9b**). The modified Mosher analysis established the R-configuration at C3 (Fig. 2). To determine the configuration of C2 in **9**, the combined MTPA diesters (**9a** and **9b**) were treated with LiOH to afford the 1,3-diol, which was finally converted to acetonide **10**. The coupling constant and NOE data of **10** provided the chair conformation and configuration of the acetonide ring (Fig. 1). The relative configurations at C2 and C3 in **10** were determined as 2S* and 3R.* At this stage, the stereochemistry of C15 in **1** remained uncertain. A series of methylation (CH_2N_2/MeOH), acetylation (Ac_2O/pyr), and acidic hydrolysis (5% HCl/MeOH) of **1** gave a deglycosylated tetraacetyl derivative **12**. Using the same steps, the (R)- and (S)-MTPA esters (**13a** and **13b**) were produced, and the $\Delta\delta$ (δ_S-δ_R) values indicated that the absolute configuration at C15 is R (Fig. 2). Therefore, the absolute stereochemistry of **1** was determined as 4R, 5R, 7S, 9R, 11S, 12R, 13S, 15R, 24S, and 25R.

The aminosugar mycosamine, as shown in the previous paper,[9] was established as D-series in comparison with the literature data.[4]

Experimental Section

Moisture and air sensitive reactions were performed in a flame-dried glassware equipped with rubber septa under positive nitrogen pressure. Et_2O and THF were distilled from sodium benzophenone ketyl. CH_2Cl_2 and pyridine were also distilled from CaH_2 before use. Kieselgel 60 (0.063-0.2 mm) was used for column chromatography and Merck Kieselgel 60 F_{254} for TLC. HPLC was carried out on a Waters 510 apparatus equipped with a reversed-phase column (Cosmosil ODS, 5 μm, 10 × 250 mm). FAB mass spectra were measured on a JEOL JMX-SX 102 mass spectrometer. CD spectrum was measured on a JASCO J-20 Automatic Recording Spectropolarimeter. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Brucker ARX-400 spectrometer and chemical shifts are given in ppm relative to the solvent peaks [$CDCl_3$ (δ_H=7.26 and δ_C=77.1); CD_3OD (δ_H=3.30 and δ_C=49.0)] as internal standards.

Organisms and fermentation. *Streptomyces* sp. GK9244 from a soil sample collected in Taejon, Korea, was cultured in the seed medium consisting of glucose 2%, starch 1%, soybean flour 2.5%, yeast extract 0.4%, NaCl 0.2%, K_2HPO_4 0.005%, and beef extract 0.1% (adjusted to pH 7.3 before sterilization). The seed culture was carried out on a rotary shaker (250 rpm) at 28 °C for 24 hours in 500-mL Erlenmeyer flasks containing 100 mL of the seed medium. Then, the seed culture (100 mL) was inoculated to a 50-L jar fermenter containing 10 L of the production medium (antifoam 0.08%). Fermentation was carried out at 27 °C for 4 days with aeration (10 L/min) under constant agitation (250 rpm).

Extraction and isolation. The culture broth (80 L) was centrifuged to separate the mycelial cake. The mycelial cake was stirred overnight in 70% aqueous acetone and filtered. The filtrate was concentrated *in vacuo* to remove the organic solvent, resulting in an aqueous solution. The combined filtrates were passed through a Diaion HP-20 column, and washed with H_2O followed by MeOH. The MeOH eluate was partitioned between CH_2Cl_2 and 60% aqueous MeOH, and then the 60% aqueous MeOH was re-partitioned between n-BuOH and H_2O. The n-BuOH fraction having antifungal activity was fractionated by ODS flash chromatography with aqueous MeOH. The 70% aqueous MeOH fraction was further purified on Sephadex LH-20 with MeOH followed by reversed-phase HPLC with 63% aqueous MeOH to yield tetrin B (**1**, 152 mg) as a major metabolite together with other tetrin-related compounds.

Methyl dicarboxylate 3 and pentaenal 4. Tetrin B (150 mg) in 5 mL of 1.0 N sodium hydroxide was stirred at room temperature overnight and extracted with ether several times. The water phase was neutralized with 1 N HCl and worked up with ethyl acetate to give a diacid residue (**2**). This residue was dried in a vacuum oven overnight and then immediately esterified with diazomethane. The reaction mixture was purified through a small silica gel column to afford a methyl dicarboxylate (**3**, 63 mg). **3**: FABMS (pos) m/z 349 $(M+H)^+$; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.77 (1H, dd, J = 15.4, 5.6 Hz, H-3), 5.51 (1H, d, J = 15.4 Hz, H-2), 4.01 (1H, dd, J = 7.2, 5.6 Hz, H-4), 3.66 (1H, m, H-11), 3.47 (1H, m, H-5), 3.41 (1H, m, H-7), 3.21 (3H, s, 13-OCH_3), 3.12 (3H, s, 1-OCH_3), 2.38 (1H, dd, J = 12.6, 3.8 Hz, H-8), 2.72 (1H, dd, J = 12.6, 8.6 Hz, H-8), 2.40 (1H, dd, J = 12.3, 8.2 Hz, H-10), 2.18 (1H, dd, J = 13.2, 6.9 Hz H-12), 2.08 (1H, dd, J = 13.2, 4.2 Hz, H-12), 1.91 (1H, dd, J = 12.3, 4.1 Hz, H-10), 1.47 (1H, m, H-6), 1.26 (1H, m, H-6).

The ether phase was worked up to give a yellow residue (56 mg), which was purified by preparative TLC [20% AcOEt/hexane]. Pentaenal (**4**, 42 mg) was recrystallized from cyclohexane as a major product. **4**: FABMS (pos) m/z 255 $(M+Na)^+$; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.42 (1H, d, J = 8.7 Hz, H-1), 7.17 (1H, dd, J = 15.2, 7.8 Hz, H-3), 6.2-6.9 (7H, complex), 6.14 (1H, dd, J = 15.2, 8.7 Hz, H-2), 5.67 (1H, dd, J = 15.8, 8.4 Hz, H-11), 3.80 (1H, m, H-13), 2.12 (H, m, H-12), 1.28 (3H, d, J = 6.1 Hz, H-14), 1.19 (3H, d, J = 6.0 Hz, 12-CH_3).

Acetonides 5 and 6. A solution of the methyl dicarboxylate **3** (58 mg) dissolved in a 4 : 1 mixture (3 mL) of acetone and 2,2-dimethoxypropane (DMP) was treated with camphor sulfonic acid (CSA, 5 mg). The reaction was stirred under N_2 at room temperature for 1 h. The reaction was then quenched with Et_3N (0.5 mL) and concentrated under a stream of N_2. Silica gel chromatography (15% AcOEt/hexane) gave an acetonide mixture (46 mg), which was purified by reversed-phase HPLC (94% aqueous MeOH) to give **5** (18 mg) and **6** (22 mg), respectively. **5**: FABMS (pos) *m/z* 389 $(M+H)^+$; ^1H NMR ($CDCl_3$, 400 MHz) δ 6.74 (1H, dd, J = 15.6, 7.4 Hz, H-3), 5.68 (1H, d, J = 15.6 Hz, H-2), 4.10 (1H, dd, J = 7.4, 4.6 Hz, H-4), 3.87 (1H, ddd, J = 10.1, 4.6, 2.9 Hz, H-5), 3.74 (1H, m, H-7), 3.63 (1H, m, H-11), 3.18 (3H, s, 13-OCH_3), 3.14 (3H, s, 1-OCH_3), 2.38 (1H, dd, J = 13.8, 8.6 Hz, H-10), 2.22 (1H, dd, J = 12.8, 6.8 Hz, H-12), 2.12 (1H, dd, J = 13.8, 2.8 Hz, H-10), 2.01 (1H, dd, J = 14.2, 3.4 Hz, H-8), 1.92 (1H, dd, J = 12.8, 4.2 Hz, H-12), 1.78 (1H, dd, J = 14.2, 8.2 Hz, H-8), 1.41 (3H, s, acetonide CH_3), 1.36 (1H, ddd, J = 14.6, 11.8, 10.1 Hz, H-6_{ax}), 1.12 (1H, ddd, J = 14.6, 2.9, 2.0 Hz, H-6_{eq}), 0.81 (3H, s, acetonide CH_3); ^{13}C NMR NMR ($CDCl_3$, 400 MHz) δ 206.3 (s, C-9), 168.4 (s, C-1), 164.2 (s, C-13), 144.3 (d, C-3), 123.6 (d, C-2), 99.3 (s, ketal C), 68.4 (d, C-4), 66.9 (d, C-11), 62.6 (d, C-5), 61.9 (d, C-7), 52.6 (q, 13-OCH_3), 51.8 (q, 1-OCH_3), 44.2 (t, C-10), 42.3 (t, C-8), 39.3 (t, C-6), 37.4 (t, C-12), 30.1 (q, acetonide CH_3), 19.9 (q, acetonide CH_3). **6**: FABMS (pos) *m/z* 411 $(M+Na)^+$, 389 $(M+H)^+$; ^1H NMR ($CDCl_3$, 400 MHz) δ 6.81 (1H, dd, J = 15.4, 8.1 Hz, H-3), 5.52 (1H, d, J = 15.4 Hz, H-2), 4.18 (1H, t, J = 8.1 Hz, H-4), 3.92 (1H, m, H-5), 3.48 (1H, m, H-11), 3.34 (1H, m, H-7), 3.15 (3H, s, 1-OCH_3), 3.12 (3H, s, 13-OCH_3), 2.13 (1H, dd, J = 13.8, 6.6 Hz, H-8), 2.01 (1H, dd, J = 13.2, 6.8 Hz, H-12), 1.92 (1H, dd, J = 12.6, 6.2 Hz, H-10), 1.83 (1H, dd, J = 13.2, 4.1 Hz, H-12), 1.74 (1H, dd, J = 13.8, 4.2 Hz, H-8), 1.70 (1H, m, H-10), 1.62 (1H, ddd, J = 13.2, 7.9, 6.4 Hz, H-6), 1.39 (3H, s, acetonide CH_3), 1.38 (3H, s, acetonide CH_3), 1.30 (1H, ddd, J = 13.2, 4.9, 2.2 Hz, H-6).

MTPA esters 7a and 7b. A small amount of acetonide **5** (3 mg) dissolved in 2 mL of CH_2Cl_2 was treated with DMAP (5 mg) and (*R*)-MTPA-Cl (0.1 mL). After stirring overnight, the reaction was quenched with saturated $NaHCO_3$ solution and extracted with CH_2Cl_2. The organic layer was washed with H_2O and brine. The residue was dried (Na_2SO_4) and concentrated under reduced pressure. Silica gel chromatography (10% AcOEt/hexane) gave MTPA ester mixtures, of which the major component was isolated by reveresd-phase HPLC (95% aqueous MeOH) to afford an (*R*)-MTPA ester **7a** (2.8 mg). **7a**: FABMS (pos) *m/z* 605 $(M+H)^+$; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.405 (5H, complex, MTPA-phenyl), 6.801 (1H, dd, J = 15.6, 7.1 Hz, H-3), 5.722 (1H, d, J = 15.6 Hz, H-2), 4.834 (1H, dd, J = 7.1, 4.6 Hz, H-4), 3.957 (1H, ddd, J = 10.8, 4.6, 2.9 Hz, H-5), 3.895 (1H, m, H-7), 3.712 (1H, m, H-11), 3.529 (3H, s, MTPA-OCH_3), 3.152 (3H, s, 13-OCH_3), 3.065 (3H, s, 1-OCH_3), 2.382 (1H, dd, J = 13.8, 8.6 Hz, H-10), 2.261 (1H, dd, J = 13.4, 4.2 Hz, H-12), 2.101 (1H, dd, J = 13.8, 2.8 Hz, H-10), 2.043 (1H, dd, J = 14.2, 3.4 Hz, H-8), 1.934 (1H, dd, J = 13.4, 6.8 Hz, H-12), 1.821 (1H, dd, J = 14.2, 8.1 Hz, H-8), 1.392 (1H, ddd, J = 14.6, 10.8, 8.2 Hz, H-6), 1.185 (1H, ddd, J = 14.6, 2.9, 2.0 Hz, H-6), 1.036 (3H, s, acetonide CH_3), 0.837 (3H, s, acetonide CH_3).

On the other hand, the (*S*)-MTPA ester was prepared from acetonide **5** (3 mg) by the same procedure used to prepare the (*R*)-MTPA ester **7a** described above. The reaction mixture was purified by a series of silica gel chromatography (10% AcOEt/hexane) and ODS HPLC (95% MeOH) to yield an (*S*)-MTPA ester **7b** (2.3 mg). **7b**: FABMS (pos) *m/z* 605 $(M+H)^+$; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.408 (5H, complex, MTPA-phenyl), 6.833 (1H, dd, J = 15.6, 7.1 Hz, H-3), 5.746 (1H, d, J = 15.6 Hz, H-2), 4.838 (1H, dd, J = 7.1, 4.6 Hz, H-4), 3.935 (1H, ddd, J = 10.8, 4.6 Hz, 2.9, H-5), 3.879 (1H, m, H-7), 3.705 (1H, m, H-11), 3.529 (3H, s, MTPA-OCH_3), 3.147 (3H, s, 13-OCH_3), 3.087 (3H, s, 1-OCH_3), 2.371 (1H, dd, J = 13.8, 8.6 Hz, H-10), 2.254 (1H, dd, J = 13.4, 4.2 Hz, H-12), 2.092 (1H, dd, J = 13.8, 2.8 Hz, H-10), 2.025 (1H, dd, J = 14.2, 3.4 Hz, H-8), 1.928 (1H, dd, J = 13.4, 6.8 Hz, H-12), 1.809 (1H, dd, J = 14.2, 8.1 Hz, H-8), 1.369 (1H, ddd, J = 14.6, 10.8, 8.2 Hz, H-6), 1.165 (1H, ddd, J = 14.6, 2.9, 2.0 Hz, H-6), 1.012 (3H, s, acetonide CH_3), 0.820 (3H, s, acetonide CH_3).

MTPA esters 8a and 8b. Preparation of (*R*)-MTPA ester **8a** (2.2 mg) from acetonide **6** (3 mg) as a starting material was achieved in the same procedure as **7a**. **8a**: FABMS (pos) *m/z* 627 $(M+Na)^+$, 605 $(M+H)^+$; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.384 (5H, complex, MTPA-phenyl), 6.822 (1H, dd, J = 15.4, 8.1 Hz, H-3), 5.541 (1H, d, J = 15.4 Hz, H-2), 4.623 (1H, m, H-11), 4.177 (1H, t, J = 8.1 Hz, H-4), 3.942 (1H, t, J = 8.1 Hz, H-5), 3.548 (3H, s, MTPA-OCH_3), 3.204 (1H, m, H-7), 3.164 (3H, s, 13-OCH_3), 3.082 (3H, s, 1-OCH_3), 2.115 (1H, dd, J = 13.8, 6.7 Hz, H-8), 2.104 (1H, dd, J = 13.2, 6.9 Hz, H-12), 1.996 (1H, dd, J = 12.6, 6.3 Hz, H-10), 1.908 (1H, dd, J = 13.2, 4.2 Hz, H-12), 1.841 (1H, m, H-10), 1.806 (1H, dd, J = 13.8, 4.2 Hz, H-8), 1.714 (1H, ddd, J = 13.2, 8.0, 6.4 Hz, H-6), 1.396 (3H, s, acetonide CH_3), 1.301 (1H, dd, J = 13.2, 5.0 Hz, H-6), 1.383 (3H, s, acetonide CH_3).

Preparation of (*R*)-MTPA ester **8b** (1.8 mg) from acetonide **6** (3 mg) as a starting material was also achieved in the same procedure as **7b**. **8b**: FABMS (pos) *m/z* 627 $(M+Na)^+$; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.384 (5H, complex, MTPA-phenyl), 6.814 (1H, dd, J = 15.4, 8.1 Hz, H-3), 5.534 (1H, d, J = 15.4 Hz, H-2), 4.625 (1H, m, H-11), 4.167 (1H, t, J = 8.1 Hz, H-4), 3.926 (1H, t, J = 8.1 Hz, H-5), 3.548 (3H, s, MTPA-OCH_3), 3.384 (1H, m, H-7), 3.192 (3H, s, 13-OCH_3), 3.075 (3H, s, 1-OCH_3), 2.091 (1H, dd, J = 13.8, 6.7 Hz, H-8), 2.123 (1H, dd, J = 13.2, 6.9 Hz, H-12), 1.974 (1H, dd, J = 12.6, 6.3 Hz, H-10), 1.954 (1H, dd, J = 13.2, 4.2 Hz, H-12), 1.805 (1H, m, H-10), 1.778 (1H, dd, J = 13.8, 4.2 Hz, H-8), 1.750 (1H, ddd, J = 13.2, 8.0, 6.4 Hz, H-6), 1.376 (3H, s, acetonide CH_3), 1.347 (1H, dd, J = 13.2, 4.9 Hz, H-6), 1.326 (3H, s, acetonide CH_3).

MTPA diesters 9a and 9b. The yellow pentaenal **4** (40 mg) was dissolved in a 2 : 1 mixture (2 mL) of MeOH and CH_2Cl_2 and cooled to 78 °C in a dry ice-acetone bath. Ozone was bubbled through the solution until a blue color persisted. Nitrogen was then bubbled through the solution until it turned to colorless and $NaBH_4$ (5 mg) was added. The reaction mixture was allowed to warm slowly to room temperature. After 1 h, the solution was diluted with AcOEt (2 mL) and quenched with saturated $NaHCO_3$ solution (1 mL). The organic portion was decanted and the aqueous portion was washed with Et_2O (3 × 3 mL). The combined organic portions were then washed with brine. The residue was dried ($MgSO_4$) and concentrated under reduced pressure to yield a colorless residue (13 mg). This residue dissolved in 3 mL of THF was treated with $LiAlH_4$ (5 mg). After stirring for 10 h, the reaction was quenched with $Na_2SO_4 \cdot 10H_2O$. The reaction mixture was filtered through a small column of Na_2SO_4, and concentrated under reduced pressure to give a diol as colorless oil (10 mg).

A small amount (6 mg) of the diol was dissolved in CH_2Cl_2 (2 mL) and treated with DMAP (5 mg) and (*R*)-MTPA-Cl (0.1 mL). After stirring overnight, the reaction was quenched with saturated $NaHCO_3$ solution and extracted with CH_2Cl_2. The organic layer was washed with H_2O and brine. The residue was dried (Na_2SO_4) and concentrated under reduced pressure. Purification by reversed-phase HPLC (92% aqueous MeOH) gave an (*R*)-MTPA ester **9a** (4.4 mg). **9a**: FABMS (pos) *m/z* 559 $(M+Na)^+$; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.683 (4H, d, *J* = 7.7 Hz, MTPA-phenyl), 7.12-7.05 (6H, m, MTPA-phenyl), 4.265 (1H, m, H-3), 3.990 (2H, d, *J* = 6.1 Hz, H-1), 3.584 (3H, MTPA-OCH_3), 3.529 (3H, MTPA-OCH_3), 1.785 (1H, m, H-2), 1.067 (3H, d, *J* = 6.8 Hz, 2-CH_3), 0.928 (3H, d, *J* = 6.9 Hz, H-4).

On the other hand, the (*S*)-MTPA ester was prepared from the diol (4 mg) by the same procedure to prepare the (*R*)-MTPA ester **9a** described above. The reaction mixture was purified by reversed-phase HPLC (92% aqueous MeOH) to give an (*S*)-MTPA ester **9b** (2.1 mg). **9b**: FABMS (pos) *m/z* 559 $(M+Na)^+$; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.683 (4H, d, *J* = 7.7 Hz, MTPA-phenyl), 7.12-7.05 (6H, m, MTPA-phenyl), 4.269 (1H, m, H-3), 3.972 (2H, d, *J* = 6.1 Hz, H-1), 3.584 (3H, MTPA-OCH_3), 3.529 (3H, MTPA-OCH_3), 1.763 (1H, m, H-2), 1.089 (3H, d, *J* = 6.8 Hz, 2-CH_3), 0.964 (3H, d, *J* = 6.9 Hz, H-4).

Acetonide 10. To a solution of the combined MTPA esters (**9a** and **9b**, 5.1 mg) in 4 : 1 mixture of MeOH and H_2O (3 mL) was added LiOH (5 mg). The mixture was stirred at room temperature for 1 day and concentrated under reduced pressure. The residue was diluted with saturated NaCl solution (2 mL). The aqueous mixture was acidified to pH 4 with 1 N HCl and extracted with AcOEt (3 × 2 mL). The combined organic layers were washed, dried, and concentrated *in vacuo*. The oily residue was purified by column chromatography on silica gel (12% AcOEt/hexane) to afford colorless oil (3.6 mg). The resulting oil was treated with CSA (4 mg) and a 3 : 1 mixture (2 mL) of acetone and DMP. After stirring for 2 h, the reaction was quenched with Et_3N and concentrated under reduced pressure. The reaction mixture was purified by reversed-phase HPLC (94% aqueous MeOH) to afford acetonide **10** (1.8 mg). **10**: FABMS (pos) *m/z* 167 $(M+Na)^+$; ^1H NMR ($CDCl_3$, 400 MHz) δ 3.66 (1H, dd, *J* = 13.0, 6.6 Hz, H-1), 3.48 (1H, dd, *J* = 13.0, 12.5 Hz, H-1), 3.38 (1H, dd, *J* = 10.3, 7.3 Hz, H-3), 1.64 (1H, m, H-2), 1.41 (3H, s, acetonide CH_3), 1.36 (3H, s, acetonide CH_3), 1.13 (3H, d, *J* = 7.3 Hz, H-4), 1.02 (3H, d, *J* = 6.2 Hz, 2-CH_3).

***p*-Bromodibenzoate 11**. A suspension of **6** (10 mg) in methanol was treated with hydrogen (50 psi) in the presence of 10% palladium on carbon. After 1 h, the catalyst was filtered and thoroughly washed with methanol. The solution was concentrated completely under reduced pressure. The resulting residue (14 mg) was continuously treated with acetic anhydride (2 mL) in 2 mL of pyridine at room temperature overnight. The reaction mixture was cooled to 0 °C and methanol (2 mL) was added dropwise for 30 min. After evaporation, the mixture was treated with 80% aqueous AcOH (1 mL) at 60 °C for 4 h. After stirring at room temperature for 1 h, the reaction mixture was partitioned between Et_2O and saturated $NaHCO_3$. The organic layer was washed with H_2O and brine, and dried (Na_2SO_4). The residue was concentrated under reduced pressure to afford a white amorphous powder. To a solution of this powder (17 mg) and DMAP (5 mg) in 2 mL of dry pyridine was added *p*-bromobenzoyl chloride (5 mg). The solution was stirred at 40 °C for 2 days, poured into ice water, and extracted with EtOAc. The organic layer was washed with 5% HCl, 10% $NaHCO_3$ and brine, dried (Na_2SO_4), and concentrated *in vacuo*. The residue was purified by a series of preparative TLC (10% AcOEt/hexane) and reversed-phase HPLC (96% aqueous MeOH) to give a major di-*p*-bromobenzoate (**11**, 2.6 mg) as an amorphous solid. **11**: FABMS (pos) *m/z* 799 $(M+H)^+$; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.93 (2H, d, *J* = 8.7 Hz, 4-bromobenzoate), 7.86 (2H, d, *J* = 8.7 Hz, 5-bromobenzoate), 7.70 (2H, d, *J* = 8.7 Hz, 4-bromobenzoate), 7.66 (2H, d, *J* = 8.7 Hz, 5-bromobenzoate), 4.42 (1H, m, H-5), 4.27 (1H, m, H-4), 3.68 (1H, m, H-11), 3.54 (1H, m, H-7), 3.16 (3H, s, 13-OCH_3), 3.02 (3H, s, 1-OCH_3), 2.40 (1H, dd, *J* = 13.6, 8.5 Hz, H-10), 2.29 (1H, dd, *J* = 13.5, 4.1 Hz, H-12), 2.20 (1H, dd, *J* = 14.2, 3.3 Hz, H-8), 2.14 (3H, s, 7-OAc), 2.10 (1H, dd, *J* = 13.6, 3.0 Hz, H-10), 2.06 (3H, s, 11-OAc), 1.99 (2H, t, *J* = 7.2 Hz, H-2), 1.92 (1H, dd, *J* = 13.5, 6.5 Hz, H-12), 1.80 (1H, dd, *J* = 14.2, 8.0 Hz, H-8), 1.61 (2H, m, H-3), 1.38 (1H, ddd, *J* = 14.8, 11.6, 9.9 Hz, H-6), 1.20 (1H, ddd, *J* = 14.8, 2.9, 2.0 Hz, H-6).

Tetraacetyl derivative 12. Tetrin B (**1**, 20 mg) dissolved in 2 mL of MeOH was treated with diazomethane at room temperature for 1 h. The solution was evaporated under reduced pressure to give a reaction mixture. This mixture was then diluted with ethyl acetate (2 mL) and washed with H_2O (2 mL) and brine (2 mL). A yellow solid residue (14 mg) was obtained by silica gel column chromatography (15% AcOEt/hexane). The yellow residue in 2 mL of pyridine was continuously treated with acetic anhydride (2 mL) at room temperature overnight. After evaparation the reaction residue was dissolved in 5% HCl-MeOH (1 mL) and heated under reflux for 4 h. The reaction mixture was adjusted to neutral with $AgNO_3$ and then filtered. The filtrate was evaporated under reduced pressure and the residue was adsorbed on a silica column (15% AcOEt/hexane) to yield deglycosylated tetraacyl tetrin B **12** (9 mg). **12**: FABMS (pos) *m/z* 757 (M+Na)$^+$, 735 (M+H)$^+$; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.87 (1H, dd, *J* = 15.5, 3.2 Hz, H-3), 6.46-6.05 (6H, complex), 5.94 (1H, dd, *J* = 15.6, 8.9 Hz, H-16), 5.82 (1H, d, *J* = 15.5 Hz, H-2), 5.70 (1H, dd, *J* = 15.0, 6.8 Hz, H-23), 4.86 (1H, ddd, *J* = 10.8, 10.2, 4.8 Hz, H-11), 4.72 (1H, m, H-25), 4.60 (1H, dd, *J* = 11.2, 3.2 Hz, H-4), 4.49 (1H, ddd, *J* = 11.2, 9.6, 2.0 Hz, H-5), 4.37 (1H, m, H-7), 4.26 (1H, ddd, *J* = 10.2, 8.5, 1.5 Hz, H-13), 3.54 (1H, ddd, *J* = 8.9, 4.0, 2.5 Hz, H-15), 3.46 (3H, s, 12-CO_2CH_3), 2.39 (1H, m, H-24), 2.28 (1H, t, *J* = 10.2 Hz, H-12), 2.20 (1H, ddd, *J* = 15.3, 8.5, 4.0 Hz, H-14), 2.14 (3H, s, 5-OAc), 2.11 (3H, s, 7-OAc), 2.09 (3H, s, 11-OAc), 2.07 (3H, s, 4-OAc), 1.91 (1H, dd, *J* = 12.6, 4.8 Hz, H-10), 1.80 (1H, dd, *J* = 13.6, 2.1 Hz, H-8), 1.74 (1H, dd, *J* = 12.6, 10.8 Hz, H-10), 1.64 (1H, dd, *J* = 13.6, 11.0 Hz, H-8), 1.55 (1H, ddd, *J* = 15.3, 2.5, 1.5 Hz, H-14), 1.47 (1H, m, H-6), 1.26 (1H, m, H-6), 1.12 (3H, d, *J* = 6.4 Hz, H-26), 1.04 (3H, d, *J* = 6.1 Hz, 24-CH_3).

MTPA esters 13a and 13b. To a stirred solution of compound **12** (4 mg) in 0.5 mL of CH_2Cl_2 were added DMAP (6 mg) and (*R*)-MTPA-Cl (0.1 mL). After 18 h the reaction mixture was concentrated under reduced pressure. The residue was dissolved in ethyl acetate and the organic solution was washed successively with water, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was subjected to reversed-phase HPLC (95% MeOH) to give (*R*)-MTPA ester **13a** (2.6 mg). **13a**: FABMS (pos) *m/z* 951 (M+H)$^+$; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.326 (5H, complex, MTPA-phenyl), 6.783 (H, dd, *J* = 15.5, 3.2 Hz, H-3), 6.540-6.220 (6H, complex), 6.083 (1H, dd, *J* = 15.6, 8.9 Hz, H-16), 5.864 (1H, d, *J* = 15.5 Hz, H-2), 5.686 (1H, dd, *J* = 15.0, 6.8 Hz, H-23), 4.743 (1H, ddd, *J* = 10.8, 10.2, 4.8 Hz, H-11), 4.549 (1H, m, H-25), 4.638 (1H, dd, *J* = 11.2, 3.2 Hz, H-4), 4.552 (3H, MTPA-OCH_3), 4.482 (1H, ddd, *J* = 11.2, 9.6, 2.0 Hz, H-5), 4.381 (1H, m, H-7), 4.254 (1H, dd, *J* = 10.2, 1.6 Hz, H-13), 4.146 (1H, ddd, *J* = 8.9, 4.0, 2.5 Hz, H-15), 3.443 (3H, s, 12-CO_2CH_3), 2.386 (1H, m, H-24), 2.168 (1H, t, *J* = 10.2 Hz, H-12), 2.252 (1H, ddd, *J* = 15.3, 8.5, 4.0 Hz, H-14), 2.101 (3H, s, 5-OAc), 2.087 (3H, s, 7-OAc), 2.084 (3H, s, 11-OAc), 2.062 (3H, s, 4-OAc), 1.944 (1H, dd, *J* = 12.6, 4.8 Hz, H-10), 1.862 (1H, dd, *J* = 13.6, 2.1 Hz, H-8), 1.756 (1H, dd, *J* = 12.6, 10.8 Hz, H-10), 1.634 (1H, dd, *J* = 13.6, 11.0 Hz, H-8), 1.501 (1H, ddd, *J* = 15.3, 2.5, 1.6 Hz, H-14), 1.398 (1H, m, H-6), 1.247 (1H, m, H-6), 1.166 (3H, d, *J* = 6.4 Hz, H-26), 1.062 (3H, d, *J* = 6.1 Hz, 24-CH_3).

The (*S*)-MTPA ester was prepared from acetonide **12** (4 mg) by the same procedure used to prepare the (*R*)-MTPA ester **13a** described above. The reaction mixture was purified by reversed-phase HPLC (95% MeOH) to afford an (*S*)-MTPA ester **13b** (2.2 mg). **13b**: FABMS (pos) *m/z* 951 (M + H)$^+$; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.326 (5H, complex, MTPA-phenyl), 6.783 (1H, dd, *J* = 15.5 Hz, 3.2, H-3), 6.560-6.210 (6H, complex), 6.051 (1H, dd, *J* = 15.6, 8.9 Hz, H-16), 5.861 (1H, d, *J* = 15.5 Hz, H-2), 5.682 (1H, dd, *J* = 15.0, 6.8 Hz, H-23), 4.751 (1H, ddd, *J* = 10.8, 10.2 Hz, 4.8, H-11), 4.547 (1H, m, H-25), 4.639 (1H, dd, *J* = 11.2, 3.2 Hz, H-4), 4.552 (3H, MTPA-OCH_3), 4.483 (1H, ddd, *J* = 11.2, 9.6, 2.0 Hz, H-5), 4.379 (1H, m, H-7), 4.272 (1H, dd, *J* = 10.2, 1.6 Hz, H-13), 4.149 (1H, ddd, *J* = 8.9, 4.0, 2.5 Hz, H-15), 3.460 (3H, s, 12-CO_2CH_3), 2.384 (1H, m, H-24), 2.191 (1H, t, *J* = 10.2 Hz, H-12), 2.274 (1H, ddd, *J* = 15.3, 8.5, 4.0 Hz, H-14), 2.103 (3H, s, 5-OAc), 2.086 (3H, s, 7-OAc), 2.099 (3H, s, 11-OAc), 2.060 (3H, s, 4-OAc), 1.954 (1H, dd, *J* = 12.6, 4.8 Hz, H-10), 1.869 (1H, dd, *J* = 13.6, 2.1 Hz, H-8), 1.768 (1H, dd, *J* = 12.6, 10.8 Hz, H-10), 1.641 (1H, dd, *J* = 13.6, 11.0 Hz, H-8), 1.519 (1H, ddd, *J* = 15.3, 2.5, 1.6 Hz, H-14), 1.400 (1H, m, H-6), 1.248 (1H, m, H-6), 1.163 (3H, d, *J* = 6.4 Hz, H-26), 1.060 (3H, d, *J* = 6.1 Hz, 24-CH_3).

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