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NOTE

# Absolute configuration of NFAT-133, an aromatic polyketide with immunosuppressive and antidiabetic activity from actinomycetes

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NFAT-133 (**1**) is a relatively small, aromatic polyketide isolated from actinomycete strains of the genera *Dactylosporangium*[1] and *Streptomyces* (Figure 1).[2] This trialkyl-substituted benzene derivative bears three contiguous stereocenters comprising hydroxy and methyl substitutions on an alkyl side chain but the relative and absolute configurations remain unknown. NFAT-133 (**1**) is a pharmaceutically interesting molecule with immunosuppressive and antidiabetic activity. It inhibits transcription mediated by NFAT (nuclear factor of activated T cells), leading to the suppression of IL-2 (interleukin-2) expression and the following T cell proliferation.[1] In addition, this compound stimulates glucose uptake in skeletal muscle cells. *In vivo* experiments with *db*/*db* mice demonstrated its efficacy of lowering plasma glucose and insulin, without body weight gaining,[2] which is a side effect of currently used thiazolidine-class antidiabetic drugs (In Kulkarni-Almeida *et al.*,[2] the structure of NFAT-133 is incorrectly presented with respect to the C2/C3 double bond). In order to explore the biological potency of chemically modified derivatives of NFAT-133 (**1**), it became necessary to assign its absolute configuration.

In our ongoing program on chemical investigation of secondary metabolite diversity in *Streptomyces* species,[3–5] production of **1** was noticed in the culture extract of *Streptomyces karnatakensis* NBRC 13051. This strain was grown in liquid medium and several steps of purification from the culture extract afforded **1** in a pure form. $^{1}$H and $^{13}$C NMR spectral data of **1** from strain NBRC 13051 were identical with those previously reported for NFAT-133 (Table 1).[1,2]

Stereochemical analysis was started with the application of chiral anisotropy method[6] to the secondary hydroxy group at C11. Esterification of **1** by treatment with (*S*)- and (*R*)-MTPACl gave bis-(*R*)- and (*S*)-MTPA esters (**2a** and **2b**), respectively. The $\Delta\delta_{S-R}$ values were calculated by subtracting the $^{1}$H NMR chemical shifts $\delta_H$ of **2a** from that of **2b**. Although the $\Delta\delta_{S-R}$ values were zero for H12 and H17, a positive value was observed for H14 and negative values were observed for H2, H3, H10 and H16.[6] The absolute configuration at C11 was thus determined as *R*. The relative configuration at C10 and C12 was deduced from the analysis of the coupling constants. The large $^{3}J_{H10,H11}$ (7.0 Hz) and the small $^{3}J_{H11,H12}$ values (3.9 Hz) indicated the *anti* and *syn* relationships for H10/H11 and H11/H12, respectively (Figure 2). This assignment was in good agreement with the NOEs observed between H10 and H17 and between H12 and H16 in the NOESY spectrum (Figure 3). Furthermore, NOEs between H3 and H10, H8 and H11, and H2 and H5 suggested that the side chains at C4 and C9 were extending from the aromatic core to set apart from each other (Figure 3). To further validate the stereochemical assignment, *J*-based configuration analysis[7] was employed for the rotamers around C10–C11 and C11–C12 axes. Heteronuclear long-range coupling constants $^{2}J_{CH}$ were determined to be 5.6 Hz for H10/C11 and 2.3 Hz for H12/C11 by *J*-HMBC experiment.[8] These values established the *gauche* relationship for H10 and 11-OH and the *anti* relationship for H12 and 11-OH (Figure 4), consistent with the results from the NOESY experiment and configuration analysis based on $^{3}J_{HH}$ coupling values.

On the basis of these spectroscopic evidences, the asymmetric centers present in **1** were determined to have 10*R*, 11*R* and 12*S* configurations. This stereochemical information will facilitate the structure-activity relationship study for evaluation of **1** as a lead scaffold for the immunosuppressive or antidiabetic agent.

## EXPERIMENTAL SECTION

### General

Optical rotation was measured using a JASCO DIP-3000 polarimeter. UV spectrum was recorded on a Hitachi U-3210 spectrophotometer (Hitachi-High-Technologies Co., Tokyo, Japan). IR spectrum was measured on a PerkinElmer Spectrum 100. NMR spectra were obtained on a Bruker AVANCE 500 spectrometer (Bruker Biospin K. K., Yokohama, Japan). High resolution ESITOFMS were recorded on a Bruker micrOTOF focus (Bruker Daltonics K. K., Yokohama, Japan).

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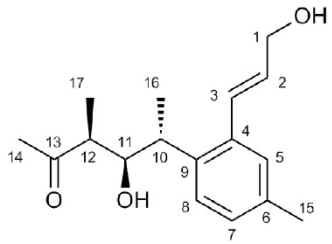
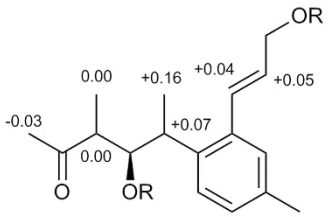



**Figure 1** Absolute configuration of NFAT-133 (**1**).




**2a** : R = (*R*)-MTPA
**2b** : R = (*S*)-MTPA

**Figure 2** $\Delta\delta_{S-R}$ values for MTPA esters (**2a**) and (**2b**) of **1**.

**Table 1 NMR data for NFAT-133 (1)**

| Position | $CD_3OD$[a] | | $CDCl_3$[b] | |
|---|---|---|---|---|
| | $\delta_H$ mult (J in Hz) | $\delta_C$ | $\delta_H$ (mult, J in Hz) | $\delta_C$ |
| 1 | 4.28 dd (5.4, 1.4) | 63.7 | 4.35 d (5.4) | 63.7 |
| 2 | 6.18 dt (15.6, 5.4) | 132.8 | 6.19 dt (15.6, 5.4) | 131.5 |
| 3 | 6.97 d (15.6) | 129.3 | 6.93 d (15.6) | 128.8 |
| 4 | | 137.1 | | 136.0 |
| 5 | 7.27s | 128.6 | 7.24s | 127.9 |
| 6 | | 137.0 | | 135.8 |
| 7 | 7.09 d (8.0) | 129.7 | 7.05 d (8.0) | 128.6 |
| 8 | 7.17 d (8.0) | 128.0 | 7.07 d (8.0) | 126.5 |
| 9 | | 140.2 | | 138.5 |
| 10 | 3.11 dq (9.0, 6.9) | 39.7 | 3.11 dq (7.8, 6.9) | 37.3 |
| 11 | 4.24 dd (9.0, 3.6) | 76.6 | 4.11 dd (7.8, 4.0) | 74.6 |
| 12 | 2.38 dq (3.6, 7.0) | 51.2 | 2.48 dq (4.0, 7.2) | 48.5 |
| 13 | | 214.1 | | 214.2 |
| 14 | 2.07s | 28.4 | 2.13s | 29.3 |
| 15 | 2.30s | 21.0 | 2.33s | 21.0 |
| 16 | 1.32 d (6.9) | 19.3 | 1.32 d (6.9) | 17.5 |
| 17 | 0.97 d (7.0) | 9.6 | 1.08 d (7.2) | 10.7 |

[a]Referenced to $\delta_H$ 3.31 and $\delta_C$ 49.0.
[b]Referenced to $\delta_H$ 7.27 and $\delta_C$ 77.0.

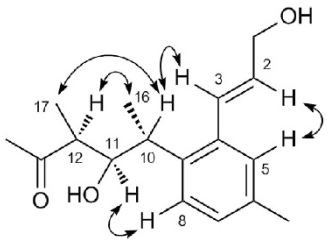



**Figure 3** Key NOESY correlations for **1**.

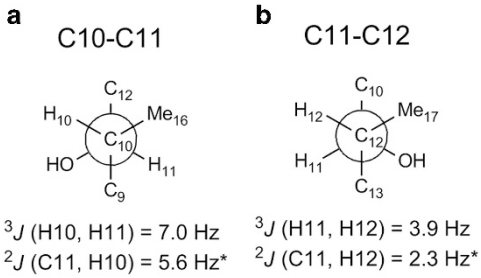



**Figure 4** Configuration analysis for C10-C12 based on the coupling constants for **1**. *Indicates absolute values.

### Fermentation and isolation

*Streptomyces karnatakensis* NBRC 13051 was obtained from Biological Resource Center, National Institute of Technology and Evaluation, Chiba, Japan. Strain NBRC 13051 cultured on a slant agar medium was inoculated into 500-ml K-1 flasks each containing 100 ml of the V-22 seed medium consisting of soluble starch 1%, glucose 0.5%, NZ-case 0.3%, yeast extract 0.2%, Tryptone (Difco Laboratories, Sparks, MD, USA) 0.5%, $K_2HPO_4$ 0.1%, $MgSO_4{\cdot}7H_2O$ 0.05%, and $CaCO_3$ 0.3% (pH 7.0). The flasks were placed on a rotary shaker (200 r.p.m.) at 30 °C for 4 days. The seed culture (3 ml) was transferred into 500-ml K-1 flasks each containing 100 ml of the A-3M production medium consisting of glucose 0.5%, Pharmamedia (Traders Protein, Memphis, TN, USA) 1.5%, $CaCO_3$ 0.5%, and Diaion HP-20 (Mitsubishi Chemical, Kanagawa, Japan) 1.0%. The pH of the medium was adjusted to 7.0 before sterilization. The inoculated flasks were cultured on a rotary shaker (200 r.p.m.) at 30 °C for 6 days.

At the end of the fermentation period, 50 ml of 1-butanol were added to each flask, and they were allowed to shake for 1 h. The mixture was centrifuged at 6000 r.p.m. for 10 min and the organic layer was separated from the aqueous layer containing the mycelium. Evaporation of the solvent gave 5.55 g of extract from 2 l of culture. The crude extract (5.55 g) was subjected to silica gel column chromatography with a step gradient of $CHCl_3$-MeOH (1:0, 20:1, 10:1, 4:1, 2:1, 1:1 and 0:1 v/v). The fourth fraction (4:1) was concentrated to provide 2.25 g of brownish oil, a portion (374 mg) of which was further separated by reversed-phase ODS column chromatography with a gradient of MeCN-0.1% $HCO_2H$ solution (2:8, 3:7, 4:6, 5:5, 6:4, 7:3 and 8:2 v/v). The fourth fraction (5:5) was evaporated and extracted with EtOAc. The organic layer was concentrated to afford **1** (61 mg).

**NFAT-133 (1)**: pale yellow oil; $[\alpha]^{22}_D$ +24 (*c* 0.45, MeOH); UV (MeOH) $\lambda_{max}$ (log ε) 213 (4.95), 247 (4.59) nm; IR (ATR) $\nu_{max}$ 3364, 2969, 2932, 1699 $cm^{-1}$; HR-ESITOFMS $[M+Na]^+$ 299.1610 (calcd for $C_{17}H_{24}O_3Na$, 299.1618).

**Bis-(*R*)-MTPA ester of 1 (2a).** To a solution of **1** (0.5 mg, 1.8 μmol) in dry pyridine (100 μl) was added (*S*)-MTPA chrolide (7 μl, 37 μmol) at room temperature. After standing for 1 h, the reaction mixture was concentrated under reduced pressure, and the residue was purified by silica gel column chromatography (*n*-hexane/EtOAc = 8:1 ~ 1:1) to give bis-(*R*)-MTPA ester **2a** (0.6 mg, 46% yield): $^1H$ NMR (500 MHz, $CDCl_3$) δ 0.93 (3H, d, *J* = 7.0 Hz, H-17), 1.06 (3H, d, *J* = 6.9 Hz, H-16), 2.09 (3H, s, H-14), 2.34 (3H, s, H-15), 2.44 (1H, dq, *J* = 2.6, 7.0 Hz, H-12), 3.25 (1H, dq, *J* = 9.7, 6.9 Hz, H-10), 4.99 (2H, m, H-1),

5.83 (1H, dd, $J$=9.7, 2.6 Hz, H-11), 6.12 (1H, dt, $J$= 15.6, 6.3 Hz, H-2), 7.00 (1H, d, $J$=15.6 Hz, H-3); HR-ESITOFMS $m/z$ 731.2414 $[M+Na]^+$ (calcd for $C_{37}H_{38}F_6O_7Na$ 731.2414).

**Bis-(*S*)-MTPA ester of 1 (2b).** In the same manner as described for **2a**, **2b** (0.4 mg, 32%) was prepared from **1** (0.5 mg, 1.8 μmol) and (*R*)-MTPA chrolide: $^1H$ NMR (500 MHz, $CDCl_3$) δ 0.93 (3H, d, $J$=7.0 Hz, H-17), 1.23 (3H, d, $J$=6.9 Hz, H-16), 2.06 (3H, s, H-14), 2.35 (3H, s, H-15), 2.44 (1H, dq, $J$=2.9, 7.0 Hz, H-12), 3.32 (1H, dq, $J$=9.4, 6.9 Hz, H-10), 5.02 (2H, m, H-1), 5.83 (1H, dd, $J$=9.4, 2.9 Hz, H-11), 6.15 (1H, dt, $J$= 15.6, 6.2 Hz, H-2), 7.05 (1H, d, $J$=15.6 Hz, H-3); HR-ESITOFMS $m/z$ 731.2414 $[M+Na]^+$ (calcd for $C_{37}H_{38}F_6O_7Na$ 731.2414).

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

1 Burres, N. S. *et al.* Simple aromatics identified with a NFAT-*lacZ* transcription assay for the detection of immunosuppressants. *J. Antibiot.* **48**, 380–386 (1995).
2 Kulkarni-Almeida, A. A. *et al.* Fermentation, isolation, structure, and antidiabetic activity of NFAT-133 produced by *Streptomyces* strain PM0324667. *AMB Express* **1**, 42 (2011).
3 Igarashi, Y. *et al.* Alchivemycin A, a bioactive polycyclic polyketide with an unprecedented skeleton from *Streptomyces* sp. *Org. Lett.* **12**, 3402–3405 (2010).
4 Igarashi, Y. *et al.* Akaeolide, a carbocyclic polyketide from marine-derived *Streptomyces*. *Org. Lett.* **15**, 5678–5681 (2013).
5 Igarashi, Y. *et al.* Jomthonic acid A, a modified amino acid from a soil-derived *Streptomyces*. *J. Nat. Prod.* **75**, 986–990 (2012).
6 Seco, J. M., Quiñoá, E. & Riguera, R. The assignment of absolute configuration by NMR. *Chem. Rev.* **104**, 17–117 (2004).
7 Matsumori, N., Kaneno, D., Murata, M., Nakamura, H. & Tachibana, K. Stereochemical determination of acyclic structures based on carbon-proton spin-coupling constants. A method of configuration analysis for natural products. *J. Org. Chem.* **64**, 866–876 (1999).
8 Meissner, A. & Sørensen, W. Measurement of *J*(H,H) and long-range *J*(X,H) coupling constants in small molecules. Broadband XLOC and J-HMBC. *Magn. Reson. Chem.* **39**, 49–52 (2001).

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