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## Saccharothriolides A–C, novel phenyl-substituted 10-membered macrolides isolated from a rare actinomycete *Saccharothrix* sp.†

Shan Lu,<sup>a</sup> Shinichi Nishimura,<sup>a</sup> Go Hirai,<sup>b</sup> Masashi Ito,<sup>c</sup> Teppei Kawahara,<sup>d</sup> Miho Izumikawa,<sup>d</sup> Mikiko Sodeoka,<sup>b</sup> Kazuo Shin-ya,<sup>e</sup> Toshio Tsuchida<sup>c</sup> and Hideaki Kakeya<sup>\*a</sup>

Three new 10-membered macrolides, saccharothriolides A–C (1–3), were discovered from a rare actinomycete *Saccharothrix* sp. A1506. All of the sp<sup>3</sup> carbons in the 10-membered ring had chirality, which was determined by extensive spectroscopic analysis and TDDFT-calculation of ECD spectra. Saccharothriolide B (2) exhibited cytotoxicity against human tumor cell lines HeLa and HT1080.

Rare actinomycetes (or non-streptomycete actinomycetes) are regarded as the actinomycete strains whose isolation frequency determined by conventional methods is much lower than that of the streptomycete strains. These under-explored microorganisms are expected to be a rich source of natural products with novel chemical structures and biological activities.<sup>1,2</sup> *Saccharothrix* sp., one of the rare actinomycetes, was first obtained from a soil sample collected in Australia in 1984.<sup>3</sup> Dozens of bioactive natural products with a structural and biological diversity have been isolated from this genus: for example, a naphthoquinone derivative sacchathridine A which was reported as a prostaglandin release inhibitor,<sup>4</sup> 16-membered macrolides tianchimycins A and B,<sup>5</sup> cytotoxic 20-membered macrolides ammocidins A–D,<sup>6,7</sup> antibacterial galacardins A and B,<sup>8</sup> and antitumor rebeccamycin.<sup>9</sup> In the course of our chemical screening for novel microbe metabolites, we discovered new 10-membered macrolides from a rare actinomycete *Saccharothrix* sp. A1506, designated as saccharothriolides A–C (1–3) (Fig. 1). Here we report their isolation, structure elucidation, and biological activities.

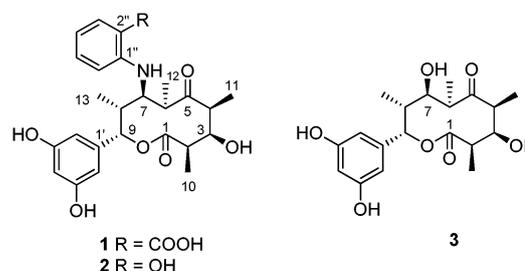


Fig. 1 Chemical structures of saccharothriolides A–C (1–3).

We surveyed more than 30 000 microbe cultures by LC-MS analysis to identify a novel metabolite from a culture broth of a rare actinomycete *Saccharothrix* sp. A1506. This metabolite, named saccharothriolide A (1), exhibited an MS signal ( $m/z$  486.2134  $[M + H]^+$ ) which was not found in metabolites database and a UV absorption at 345 nm characteristic to anthranilic acid. Further analysis of the LC-MS data revealed the presence of two congeners, saccharothriolides B (2) and C (3). By LC-MS-guided isolation, we purified metabolites 1 (24.7 mg), 2 (5.4 mg), and 3 (17.8 mg) from a 6 L-culture.

Saccharothriolide A (1) was obtained as a light yellow oil with  $[\alpha]_D^{20} + 18.0$  ( $c = 0.74$ , MeOH). The molecular formula was determined to be C<sub>26</sub>H<sub>31</sub>NO<sub>8</sub> by HR-ESI-MS ( $m/z$  486.2134  $[M + H]^+$ , calcd 486.2128), indicating the presence of 12 degrees of unsaturation. The IR spectrum showed absorptions corresponding to hydroxyl (3327 cm<sup>-1</sup>), ester carbonyl (1732 cm<sup>-1</sup>), and ketone (1679 cm<sup>-1</sup>) groups. The <sup>1</sup>H NMR spectrum of 1 displayed seven aromatic protons ( $\delta_H$  7.95, 7.31, 6.66, 6.57, 6.06, 5.87  $\times$  2 ppm) and two oxymethine protons ( $\delta_H$  5.50, 3.79 ppm) in addition to four aliphatic methyl signals ( $\delta_H$  1.09, 1.20, 1.35, 1.46 ppm) and five methine protons ( $\delta_H$  2.19, 2.88, 3.28, 3.43, 3.66 ppm) (Table 1). The <sup>13</sup>C NMR spectrum included 26 carbon signals, corresponding to four aliphatic methyls ( $\delta_C$  10.7, 14.1, 18.4, 20.3), seven aliphatic methines including two carbons adjacent to an oxygen atom ( $\delta_C$  74.3 and 81.4) and one carbon adjacent to a nitrogen atom ( $\delta_C$  62.2), twelve aromatic carbons and three carbonyl carbons ( $\delta_C$  173.8  $\times$  2, 224.6).

<sup>a</sup> Department of System Chemotherapy and Molecular Sciences, Division of Bioinformatics and Chemical Genomics, Graduate School of Pharmaceutical Sciences, Kyoto University, Sakyo-ku, Kyoto 606-8501, Japan.  
E-mail: scseigyo-hisyo@pharm.kyoto-u.ac.jp

<sup>b</sup> Synthetic Organic Chemistry Laboratory, RIKEN, Wako, Saitama 351-0198, Japan

<sup>c</sup> Research & Development Division, MicroBioPharm Japan Co., Ltd. (MBJ), Iwata, Shizuoka 438-0078, Japan

<sup>d</sup> Japan Biological Informatics Consortium (JBIC), Koto-ku, Tokyo 135-0064, Japan

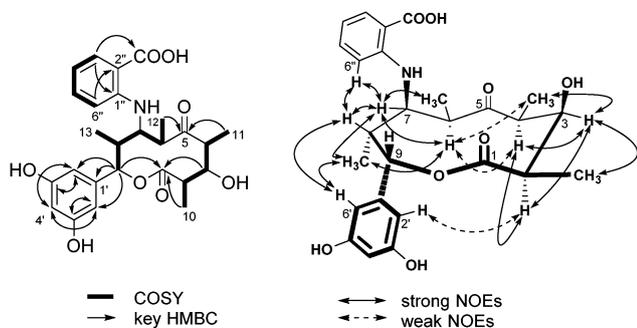
<sup>e</sup> National Institute of Advanced Industrial Science and Technology (AIST), Koto-ku, Tokyo 135-0064, Japan

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Table 1  $^1\text{H}$  and  $^{13}\text{C}$  NMR data of saccharothriolide A (**1**) in methanol- $d_4$ 

No.	$\delta_{\text{H}}$ , m, J (Hz)	$\delta_{\text{C}}$
1	—	173.8, C
2	2.88, qd, 6.9, 3.4	46.3, CH
3	3.79, brs	81.4, CH
4	3.28, q, 7.5	50.8, CH
5	—	224.6, C
6	3.43, q, 6.9	43.7, CH
7	3.66, brs	62.2, CH
8	2.19, qd, 6.9, 5.2	43.1, CH
9	5.50, brs	74.3, CH
10	1.20, d, 6.9	14.1, $\text{CH}_3$
11	1.46, d, 7.5	18.4, $\text{CH}_3$
12	1.35, d, 6.9	20.3, $\text{CH}_3$
13	1.09, d, 6.9	10.7, $\text{CH}_3$
1'	—	144.9, C
2',6'	5.87, s	104.7, CH
3',5'	—	159.5, C
4'	6.06, s	102.3, CH
1''	—	151.7, C
2''	—	113.7, C
3''	7.95, brs	134.1, CH
4''	6.57, t, 5.7	115.6, CH
5''	7.31, t, 7.5	135.6, CH
6''	6.66, d, 8.6	111.8, CH
2''-COOH	—	173.8, C

Fig. 2  $^1\text{H}$ - $^1\text{H}$  COSY (left, bold) correlations and selected HMBC (left, arrow) and NOESY (right) correlations in saccharothriolide A (**1**).

The  $^1\text{H}$ - $^1\text{H}$  COSY experiment revealed the presence of three spin systems:  $\text{CH}_3$ -10/H-2/H-3/H-4/ $\text{CH}_3$ -11,  $\text{CH}_3$ -12/H-6/H-7/H-8/ $\text{CH}_3$ -13/H-9, and H-3''/H-4''/H-5''/H-6'' (Fig. 2). HMBC correlations from  $\text{CH}_3$ -11 to C-5, and from  $\text{CH}_3$ -12 to C-5 connected C-4 and C-6 through a ketone group. HMBC correlations from both H-3 and H-9 to carbonyl C-1 connected C-2 and C-9 through an ester bond, leading to the formation of the 10-membered lactone ring (Fig. 2). The formation of the lactone ring was further supported by the observation of the down-field shifted chemical shift for H-9 ( $\delta_{\text{H}}$  5.50). The *meta*-disubstituted benzene ring was determined on the basis of the HMBC correlations from H-4' to C-2'/C-6', and from H-2'/H-6' to C-3'/C-5'. This benzene ring was connected to C-9 due to the HMBC correlations from H-9 to aromatic carbons C-1' and C-2'/6'. The presence of anthranilic acid suggested by the characteristic UV absorption (Fig. S6, ESI $^\dagger$ )<sup>10</sup> was confirmed by HMBC correlations from H-6'' ( $\delta_{\text{H}}$  6.66) to C-2'' ( $\delta_{\text{C}}$  113.7), and from H-3'' ( $\delta_{\text{H}}$  7.95) to C-1'' ( $\delta_{\text{C}}$  151.7) and 2''-COOH ( $\delta_{\text{C}}$  173.8), along with the  $^1\text{H}$ - $^1\text{H}$  COSY

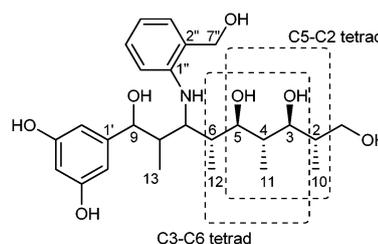
correlations from H-3'' to H-6'' (Fig. 2). The anthranilic acid was connected to the lactone ring at C-7 through an NH group, which was deduced by the up-field shifted chemical shift for C-7 ( $\delta_{\text{C}}$  62.2) and the presence of a free carboxylic acid ( $\delta_{\text{C}}$  173.8). This connection was further confirmed by the mutual NOESY correlations among H-6'', H-7, and H-8 (Fig. 2).

The relative stereochemistry of metabolite **1** was deduced from the NOESY data (Fig. 2). NOESY cross peaks between H-2 and H-4 indicated that they are placed in the same  $\alpha$  face, whereas NOESY correlations between H-3 and H-2, H-4,  $\text{CH}_3$ -10, and  $\text{CH}_3$ -11 suggested  $\alpha$  configuration for H-3. NOESY correlations between H-6 and H-4(weak),  $\text{CH}_3$ -11(weak), and  $\text{CH}_3$ -13 indicated an  $\alpha$  configuration for these protons. A  $\beta$  configuration for H-8 was then determined. Correlations between H-7 and H-6, H-8,  $\text{CH}_3$ -12, and  $\text{CH}_3$ -13 suggested that H-7 has an  $\alpha$  configuration. Finally, NOESY correlations between the aromatic proton H-6' and H-8, and  $\text{CH}_3$ -13, together with a weak correlation from the aromatic proton H-2' to H-2, revealed the  $\beta$  orientation of H-9. Thus, the relative configurations were deduced to be 2*R*\*, 3*R*\*, 4*S*\*, 6*R*\*, 7*R*\*, 8*R*\*, 9*S*\*.

Macrolides can exist in several conformations, which can be a potential cause of mis-interpretation of the NOESY data. We next analyzed the relative stereochemistry of **1** by the advanced statistical Universal NMR Database (UDB) approach, originally developed by Kishi and co-workers.<sup>11–13</sup> Reductive opening of the lactone ring of **1** using  $\text{LiAlH}_4$  furnished a linear product **4** (Fig. 3). The  $^{13}\text{C}$  NMR data of the two tetrad segments, C3–C6 and C5–C2, were subjected to the statistical UDB analysis. The difference between the adjusted NMR data of the tetrad segments in **4** and Kishi's database was calculated to reveal that both sequences have an *anti-anti-anti* configuration (Table S2–S5, ESI $^\dagger$ ), which was in good agreement with the NOESY data in **1**.

The absolute stereochemistry was determined by the modified Mosher's method (Fig. 4).<sup>14</sup> Phenolic hydroxy groups and carboxylic acid of **1** were first protected by methylation using  $\text{CH}_3\text{I}$  to yield a tri-methyl derivative **5**. The methylated derivative **5** was treated with (*R*)- and (*S*)-MTPA chloride to afford (*S*)- and (*R*)-MTPA esters of **5**, respectively. The  $\Delta\delta$  ( $\delta_{\text{S}} - \delta_{\text{R}}$ ) values of the protons flanking the C-3 chiral center revealed the 3*R* absolute configuration, which in turn concluded the absolute configurations of **1** as 2*R*, 3*R*, 4*S*, 6*R*, 7*R*, 8*R*, 9*S*.

The modified Mosher's method is sometimes not applicable to axial hydroxyl groups.<sup>15</sup> In order to confirm the above results,

Fig. 3 Analysis of the stereochemistry of compound **4** (C3–C6 and C5–C2 tetrads) by the statistical UDB approach.

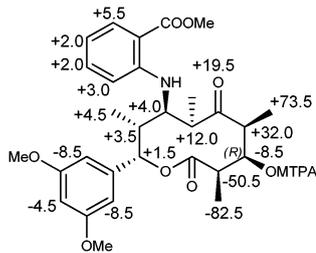


Fig. 4  $\Delta\delta$  ( $\delta_S - \delta_R$ ) values (in Hz) for the MTPA esters of **5**.

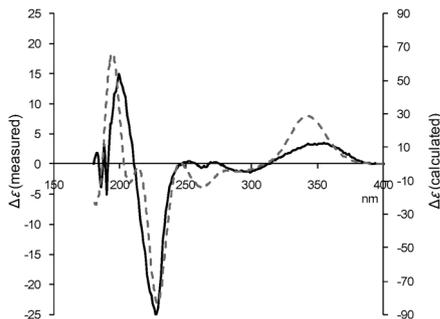


Fig. 5 Experimental CD (solid line) and calculated ECD (dotted line) spectra of saccharothriolide A (**1**). ECD was calculated for  $2R$ ,  $3R$ ,  $4S$ ,  $6R$ ,  $7R$ ,  $8R$ ,  $9S$ .

we finally measured the CD spectrum of **1**, which was compared with the electronic circular dichroism (ECD) spectrum calculated by the time-dependent density functional theory (TDDFT).<sup>16</sup> As shown in Fig. 5, the experimental CD spectrum showed a Cotton effect at 199 ( $\Delta\epsilon$ , +15.0), 227 ( $\Delta\epsilon$ , -25.0) and 355 ( $\Delta\epsilon$ , +3.46) nm, all of which were observed in the ECD spectrum calculated for the stereochemistry of  $2R$ ,  $3R$ ,  $4S$ ,  $6R$ ,  $7R$ ,  $8R$ ,  $9S$ . Thus, the absolute configuration of **1** was unambiguously established by two independent methods.

To find congeners of saccharothriolide A (**1**), we investigated the LC-MS data of the crude broth extract. We detected a metabolite whose ion peak was observed at  $m/z$  458.2162 ( $[M + H]^+$ ), revealing the molecular formula of  $C_{25}H_{31}NO_7$ . This metabolite, designated as saccharothriolide B (**2**), contained one nitrogen atom similar to **1**, but its molecular size was 28 Da smaller than **1**. Metabolite **2** was obtained as a light yellow oil with  $[\alpha]_D^{20}$  -81.2 ( $c$  = 0.36, MeOH).

The  $^1H$  and  $^{13}C$  NMR data of **2** were very similar to those of **1** (Table S1, ESI<sup>†</sup>), with the exception of the disappearance of the carboxylic acid. The obvious downfield shift of C-2'' ( $\delta_C$  113.7 in **1**,  $\delta_C$  146.0 in **2**) and an upfield shift of C-1'' and C-3'' ( $\delta_C$  151.7 in **1**,  $\delta_C$  138.2 in **2** and  $\delta_C$  134.1 in **1**,  $\delta_C$  115.2 in **2**, respectively) were observed, accompanied by a marked upfield shift of H-3'' and H-5'' ( $\delta_H$  7.95 in **1**,  $\delta_H$  6.73 in **2** and  $\delta_H$  7.31 in **1**,  $\delta_H$  6.70 in **2**, respectively), which suggested that **2** possessed a phenolic hydroxyl group instead of a carboxylic acid at C-2''. This was in agreement with the fact that **2** was 28 Da smaller than **1**. The planar structure was deduced by the COSY, HMQC and HMBC data (Fig. S1, ESI<sup>†</sup>). Detailed analysis of the NOESY spectrum of **2** revealed that the relative stereochemistry was the same as that of **1** (Fig. S1, ESI<sup>†</sup>):  $2R^*$ ,  $3R^*$ ,  $4S^*$ ,  $6R^*$ ,  $7R^*$ ,  $8R^*$ ,  $9S^*$ .

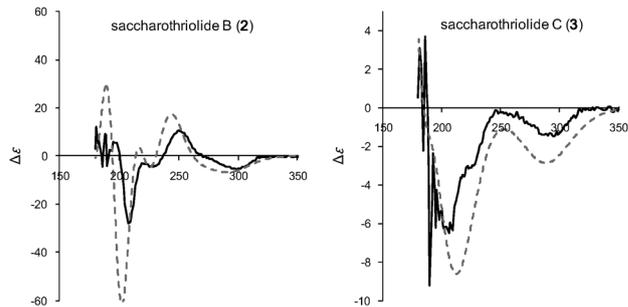


Fig. 6 Experimental CD (solid line) and calculated ECD (dotted line) spectra for saccharothriolides B (**2**) and C (**3**). Both ECD spectra were calculated for  $2R$ ,  $3R$ ,  $4S$ ,  $6R$ ,  $7R$ ,  $8R$ ,  $9S$ .

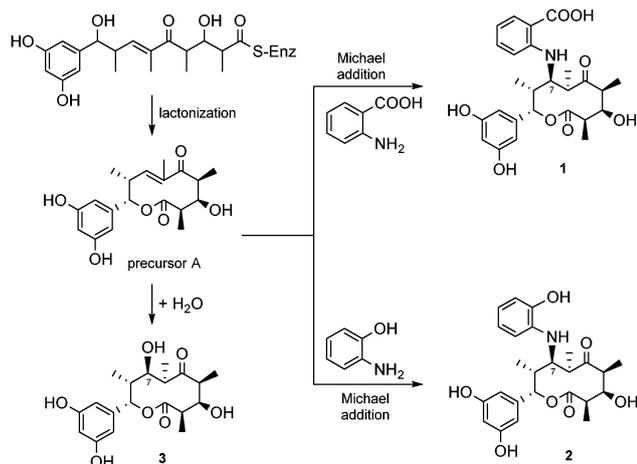
The absolute stereochemistry of **2** was determined by measurement and calculation of ECD. The ECD calculation of **2** revealed that the absolute stereochemistry was the same as that of **1**. The calculated ECD spectrum overlapped well with the experimental CD spectrum (Fig. 6), concluding that the absolute stereochemistry of **2** was  $2R$ ,  $3R$ ,  $4S$ ,  $6R$ ,  $7R$ ,  $8R$ ,  $9S$ .

Saccharothriolides A (**1**) and B (**2**) have an aminoaryl substitution at C-7, suggesting the presence of a common precursor for them, *i.e.*, Michael addition of nucleophilic amines to the corresponding precursor can afford metabolites **1** and **2**. For investigating this possibility, we surveyed the LC-MS data of the culture broth to identify saccharothriolide C (**3**). Metabolite C (**3**) was obtained as a light yellow oil with  $[\alpha]_D^{20}$  -111.8 ( $c$  = 0.58, MeOH). The HR-ESI-MS data indicated an  $[M + Na]^+$  ion peak at  $m/z$  389.1586 ( $[M + Na]^+$ , calcd 389.1576), revealing a molecular formula of  $C_{19}H_{26}O_7$ . The  $^1H$  and  $^{13}C$  NMR data of **3** were similar to those of **1** and **2** (Table S1, ESI<sup>†</sup>), except for the absence of a set of aromatic protons and carbon signals corresponding to the amino aryl groups substituted at C-7. Instead, metabolite **3** possessed a hydroxyl group at C-7, supported by the downfield shift of the carbon signal of C-7 ( $\delta_C$  80.1). The planar structure was elucidated by detailed analysis of the 2D NMR data, while the NOESY data indicated the same relative configuration as those of **1** and **2** (Fig. S2, ESI<sup>†</sup>). As expected, the absolute stereochemistry of **3** was the same as those of **1** and **2**, because the calculated ECD spectrum for the stereochemistry of  $2R$ ,  $3R$ ,  $4S$ ,  $6R$ ,  $7R$ ,  $8R$ ,  $9S$  showed a high similarity to that of the measured one (Fig. 6).

Metabolite **2** exhibited moderate cytotoxicity against cancer cells including HeLa and HT1080 cell lines with  $IC_{50}$  values of 17.9 and 13.9  $\mu M$ , respectively. Other saccharothriolides **1** and **3** were inactive even at 100  $\mu M$ . Additionally, only metabolite **2** showed weak antibacterial activity at 50  $\mu g$  per disc against *Staphylococcus aureus* in a paper disc assay. These results indicate that saccharothriolides are capable of regulating their biological activities by modifying the functional group at the C-7 position.

Saccharothriolides are 10-membered macrolides. As in the case of other macrolides, saccharothriolides seem to be synthesized *via* the polyketide biosynthetic pathway (Scheme 1). An aryl starter unit and four units of methyl-malonyl-CoA seem to be conjugated followed by cyclization to yield the precursor





Scheme 1 Plausible biosynthetic pathway of saccharothriolides A–C (**1–3**).

metabolite A. The aromatic unit-priming polyketide system has been found only in a limited number of metabolites, *i.e.* soraphen A from *Sorangium cellulosum*,<sup>17</sup> rifamycins from *Amycolatopsis rifamycinica*<sup>18</sup> and enterocin from *Streptomyces maritimus*.<sup>19</sup> The precursor A is likely attacked by an aminoaryl group to furnish metabolites **1** and **2**, or by a water molecule to furnish metabolite **3** (Scheme 1). In fact, the culture broth included a significant amount of anthranilic acid (23.5 mg) and 2-hydroxyacetanilide (120.9 mg).<sup>20</sup> Although we explored the presence of precursor A using the LC-MS data, ion peaks corresponding to the precursor were not found, probably due to the high reactivity of the  $\alpha,\beta$ -unsaturated ketone.

In conclusion, we discovered three novel 10-membered macrocyclics from a rare actinomycete *Saccharothrix* sp. A1506. Structural analysis implied their common biosynthetic origin, whereas we predict the presence of precursor A possessing  $\alpha,\beta$ -unsaturated ketone. It should be noted that only saccharothriolide B (**2**) showed moderate biological activities. Detailed studies on the biosynthetic mechanism and modes of action are currently being undertaken in our laboratory.

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## Notes and references

- 1 J. Bérdy, *J. Antibiot.*, 2005, **58**, 1–26.
- 2 K. Tiwari and R. K. Gupta, *Crit. Rev. Biotechnol.*, 2012, **32**, 108–132.
- 3 D. P. Labeda, R. T. Testa, M. P. Lechevalier and H. A. Lechevalier, *Int. J. Syst. Bacteriol.*, 1984, **34**, 426–431.
- 4 K. Nakae, I. Kurata, F. Kojima, M. Igarashi, M. Hatano, R. Sawa, Y. Kubota, H. Adachi and A. Nomoto, *J. Nat. Prod.*, 2013, **76**, 720–722.
- 5 X. L. Wang, J. Tabudravu, M. Jaspars and H. Deng, *Tetrahedron*, 2013, **69**, 6060–6064.
- 6 R. Murakami, T. Tomikawa, K. Shin-ya, J. Shinozaki, T. Kajiura, T. Kinoshita, A. Miyajima, H. Seto and Y. Hayakawa, *J. Antibiot.*, 2001, **54**, 710–713.
- 7 R. Murakami, J. Shinozaki, T. Kajiura, I. Kozono, M. Takagi, K. Shin-ya, H. Seto and Y. Hayakawa, *J. Antibiot.*, 2009, **62**, 123–127.
- 8 M. Takeuchi, S. Takahashi, R. Enokita, Y. Sakaida, H. Haruyama, T. Nakamura, T. Katayama and M. Inukai, *J. Antibiot.*, 1992, **45**, 297–305.
- 9 D. E. Nettleton, T. W. Doyle, B. Krishnan, G. K. Matsumoto and J. Clardy, *Tetrahedron Lett.*, 1985, **26**, 4011–4014.
- 10 T. Stalin and N. Rajendiran, *J. Photochem. Photobiol., A*, 2006, **182**, 137–150.
- 11 E. Fleury, M. I. Lannou, O. Bistri, F. Sautel, G. Massiot, A. Pancrazi and J. Ardisson, *Eur. J. Org. Chem.*, 2009, 4992–5001.
- 12 Y. Kobayashi, J. Lee, K. Tezuka and Y. Kishi, *Org. Lett.*, 1999, **1**, 2177–2180.
- 13 J. Lee, Y. Kobayashi, K. Tezuka and Y. Kishi, *Org. Lett.*, 1999, **1**, 2181–2184.
- 14 I. Ohtani, T. Kusumi, Y. Kashman and H. Kakisawa, *J. Am. Chem. Soc.*, 1991, **113**, 4092–4096.
- 15 J. M. Seco, E. Quinoá and R. Rigueru, *Chem. Rev.*, 2004, **104**, 17–117.
- 16 We also calculated  $^3J_{\text{H-H}}$  values for the stable conformer, which were almost the same as those of the measured ones (Table S6, ESI†).
- 17 J. Ligon, S. Hill, J. Beck, R. Zirkle, I. Molnár, J. Zawodny, S. Money and T. Schupp, *Gene.*, 2002, **285**, 257–267.
- 18 P. R. August, L. Tang, Y. J. Yoon, S. Ning, R. Müller, T. W. Yu, M. Taylor, D. Hoffmann, C. G. Kim, X. H. Zhang, C. R. Hutchinson and H. G. Floss, *Chem. Biol.*, 1998, **5**, 69–79.
- 19 J. Piel, C. Hertweck, P. R. Shipley, D. M. Hunt, M. S. Newman and B. S. Moore, *Chem. Biol.*, 2000, **7**, 943–955.
- 20 Anthranilic acid and 2-hydroxyacetanilide were purified from a part of the 6 L-culture.

