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Stereochemistry of iejimalide B

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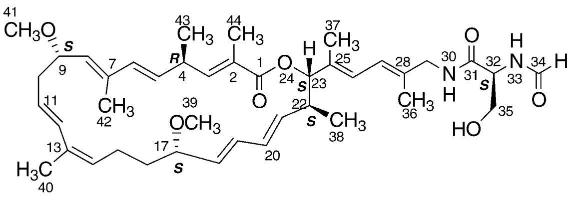
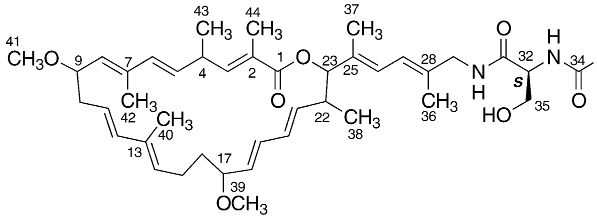
Abstract—The absolute configurations at five chiral centers, except for C-32(*S*) reported previously, in iejimalide B (**1**), a potent cytotoxic 24-membered macrolide isolated from a tunicate *Eudistoma* cf. *rigida*, were assigned as 4*R*, 9*S*, 17*S*, 22*S*, and 23*S* on the basis of detailed analysis of NMR data and chemical means. Furthermore, the structure of iejimalide B was revised from **2** (original: 13*E*) to its 13*Z*-isomer (**1**). © 2003 Elsevier Science Ltd. All rights reserved.

Iejimalides obtained from a marine tunicate *Eudistoma* cf. *rigida* are unique 24-membered macrolides having two methoxy groups, four dienes units, and an *N*-formyl-L-serine terminus, and exhibit potent cytotoxic activity in vitro.[1,2] The gross structures have been elucidated by means of 2D NMR data, and the absolute configuration of the serine residue (C-32) has been determined to be L-form (32*S*) by chiral HPLC analysis of their hydrolysates. Potent cytotoxic activity of iejimalides have attracted great interest as one of the challenging targets for total synthesis[3,4] or as a candidate of new antitumor drugs.[5] Since of a limited amount of samples isolated from the tunicate, the stereochemistry of five chiral centers (C-4, C-9, C-17, C-22, and C-23) remains unsolved, and detail of its antitumor activity has not been examined. Recently, we have isolated a relatively large amount of iejimalides from a tunicate *Cystodytes* sp. collected off Ie Island, Okinawa,[6,7] and reexamined the structure and stereochemistry of iejimalide B (**1**). Here we describe the revised structure of iejimalide B (from **2** to **1**) and the assignment of relative and absolute stereochemistry of **1** on the basis of NMR data, synthesis of a degradation product, and distance geometry calculation.

The stereochemistry of five chiral centers in iejimalide B (**1**) was examined by the following experiments: (a) evaluation of dihedral angles on basis of ^{1}H–^{1}H and long-range ^{1}H–^{13}C coupling constants and ROESY data, (b) comparison of NMR data of a segment (C-6–C-11) obtained by oxidative degradation with those of synthetic segments, and (c) distance geometry calculation carried out using SYBYL program.



1 (revised structure)



2 (original structure)

The relative stereochemistry of five chiral centers of iejimalide B (**1**) was elucidated by detailed analysis of *J*(H,H),[8] long-range *J*(C,H), and ROESY correlations.[9] The geminal and vicinal coupling constants were obtained by the resolution-enhanced spectra, decoupling difference experiments, and the DQF COSY spectra. To elucidate the total stereochemistry of the macrocyclic moiety except for the side chain (C-25–C-34) of **1**, rotational conformations of all bonds of three portions including a diene part, C-1–C-11, C-8–C-18, and C-15–C-27, were analyzed.

Keywords: macrolide; tunicate; absolute configuration.

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For the C-8–C-18 portion (Fig. 1), both 1,3-*syn*-relations for H-9–H-11 and H-10b–H-12 were inferred by ROESY correlations for H-9/H-11 and H-10b/H-12 and the *J*(H-10b/H-11) value (10.2 Hz). The *J*(H-14/H-15a) and *J*(H-14/H-15b) values (11.1 and 4.6 Hz, respectively) suggested that relationships for H-14–H-15a and H-14–H-15b were *anti* and *gauche*, respectively. On the other hand, the *gauche*- and *anti*-relations for H-15a–H-16a and H-15a–H-16b, respectively, were deduced from the *J* values (H-15a/H-16a: 2.3 Hz, H-15a/H-16b: 11.2 Hz), while the *J* values for H-15b/H-16a (2.0 Hz) and H-15b/H-16b (3.0 Hz) were suggestive of both *gauche*-relations for H-15b–H-16a and H-15b–H-16b. The 1,3-*syn*-relation for H-14–H-16b was implied by ROESY correlations of H-14/H-16b. The *J*(H-16a/H-17) (10.0 Hz) and *J*(H-16b/H-17) values (3.0 Hz) were typical for *anti*- and *gauche*-relations, respectively. Transannular-type ROESY correlations were observed for H-6/H$_3$-39, H-8/H$_3$-39, and H-12/H$_3$-39 (Fig. 4), indicating that the methoxy group (C-39) at C-17 was oriented to the inside of the macrocyclic ring. The proton chemical shift of H$_3$-39 showed a remarkable higher-field shift (δ_H 2.89) probably due to shielding by the two diene chromophore at C-5–C-8 and C-11–C-14, whereas the other methoxy (H$_3$-41) at C-9 seemed to be oriented to the outside of the ring (Fig. 4). Although the trisubstituted double bond at C-13–C-14 has been previously assigned as *E* by the ROESY correlation for H-15a/H$_3$-40,[1] detailed analysis of the ROESY spectrum revealed the absence of this correlation. The carbon signal of C-40 resonated at relatively lower field (δ_C 20.2) than those of other olefinic methyls (δ_C 15.2–16.3), and the *J*(H-14/C-40) value (7.0 Hz) was smaller than the *J*(H-14/C-12) one (10.1 Hz), suggesting that the C-13–C-14 double bond possessed *Z*-geometry. This was also supported by ROESY correlations observed for H-12/H-15 and H-14/H$_3$-40. The diene at C-11–C-14 was indicated as *S*-*trans* by the ROESY correlation for H-11/H$_3$-40. Thus, the conformation of the C-8–C-18 portion and the relative configuration at C-9 and C-17 were assigned as shown in Figure 1.

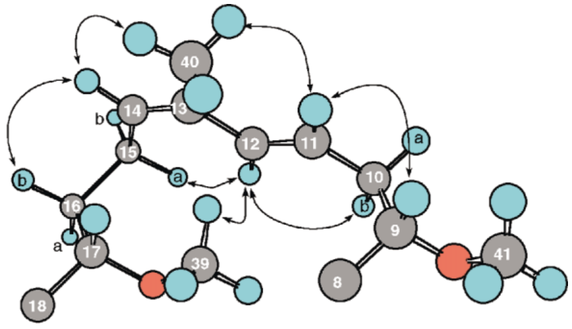


Figure 1. NOESY correlations and relative stereochemistry for C-8–C-18 part of iejimalide B (**1**). NOESY correlations are illustrated by arrows. *J* in Hz (H/H): H-10a/H-11: 4.7, H-10b/H-11: 10.2, H-11/H-12: 15.8, H-14/H-15a: 11.1, H-14/H-15b: 4.6, H-15a/H-16a: 2.3, H-15a/H-16b: 11.2, H-15b/H-16a: 2.0, H-15b/H-16b: 3.0, H-16a/H-17: 10.0, H-16b/H-17: 3.0.

For the C-1–C-11 portion (Fig. 2), both *anti*-relations for H-3–H-4 and H-4–H-5 were suggested by relatively large *J* values (H-3/H-4: 10.3 Hz, H-4/H-5: 9.0 Hz) and ROESY correlations for H-3/H-5, H-4/H-6, and H-4/H$_3$-44. ROESY correlations were observed for H-5/H$_3$-42 and H-6/H-8, indicating the presence of *S*-*trans*-diene at C-5–C-8. Orientation of the methyl group (C-43) at C-4 to the outside of the ring was indicated by ROESY correlations for H-3/H$_3$-43 and H-5/H$_3$-43. The *J*(H-8/H-9) (9.4 Hz) and *J*(H-9/H-10b) (10.2 Hz) values were typical for both *anti*-relations, while the relatively small *J*(H-9/H-10a) value (2.7 Hz) was attributed to a *gauche*-relation for H-9/H-10a. ROESY correlations for H-8/H-10b, H-8/H$_3$-41, and H-10a/H$_3$-41 also supported that the methoxyl group at C-9 was oriented to the outside of the macrolactone ring. Therefore, the conformation of the C-1–C-11 portion and the relative configuration at C-4 and C-9 were assigned as shown in Figure 2.

For the C-15–C-27 portion (Fig. 3), an *erythro*-relation for the C-22–C-23 bond was deduced from relatively large *J*(H-22/H-23) and *J*(H-22/C-23) values (10.0 Hz and 8.0 Hz, respectively) and ROESY correlations for H-26/H$_3$-38 and H$_3$-37/H$_3$-38.[10] These ROESY correlations suggested that the methyl group (C-38) at C-22 and the long side chain at C-23 were both oriented to the outside of the macrolactone ring. Relationships for H-17–H-18, H-20–H-21, and H-21–H-22 were assigned as all *anti* by relatively large *J*(H,H) values (H-17/H-18:

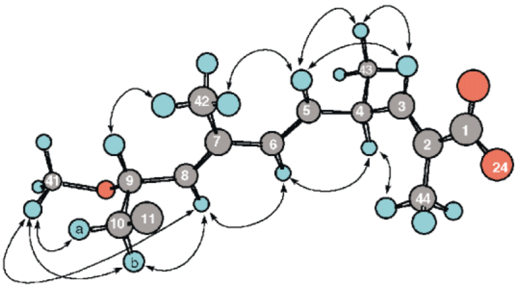


Figure 2. NOESY correlations and relative stereochemistry for C-1–C-11 part of iejimalide B (**1**). NOESY correlations are illustrated by arrows. *J* in Hz (H/H): H-3/H-4: 10.3, H-4/H-5: 9.0, H-5/H-6: 15.5, H-8/H-9: 9.4, H-9/H-10a: 2.7, H-9/H-10b: 10.2.

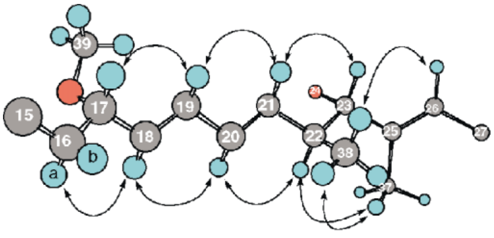
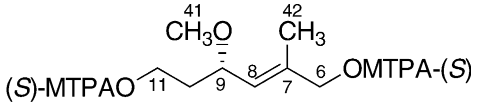


Figure 3. NOESY correlations and relative stereochemistry for C-15–C-27 part of iejimalide B (**1**). NOESY correlations are illustrated by arrows. *J* in Hz (H/H): H-17/H-18: 8.2, H-18/H-19: 14.7, H-19/H-20: 9.8, H-20/H-21: 14.5, H-21/H-22: 9.6, H-22/H-23: 10.0.

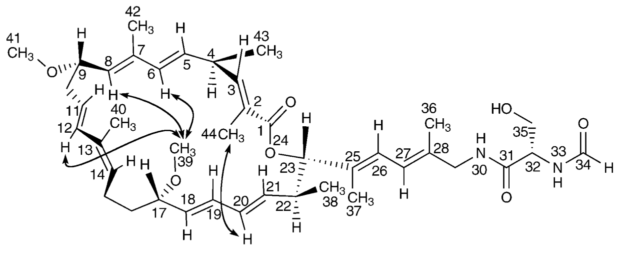
8.2 Hz, H-19/H-20: 9.8 Hz, H-21/H-22: 9.6 Hz). ROESY correlations for H-17/H-19, H-18/H-20, H-19/H-21, and H-20/H-22 indicated an *S-trans* relation between the two *E*-olefins at C-18–C-19 and C-20–C-21. Thus, the conformation of the C-15–C-27 portion and the relative configurations at C-17, C-22, and C-23 were assigned as shown in Figure 3. Therefore, the relative stereochemistry of iejimalide B (**1**) was elucidated to be **1** or *ent*-**1** as shown in Figure 4.

The distance geometry calculation was carried out using the SYBYL program.[11] Conformational analysis was performed using distance constraints based on intensity of the 69 cross-peaks obtained from the ROESY spectrum in $CDCl_3$. 100 conformers were generated by the sequential calculation including distance geometry, simulated annealing, and molecular mechanics.[12] In the 10 stable conformers of **1** obtained by the calculation, two major clusters **A** (three conformers, total energy: 32.53–32.75 kcal/mol) and **B** (seven conformers, total energy: 34.04–34.55 kcal/mol) were found due to difference of the torsion angle for C-3–C-2–C-1–O-24 (**A**, ca. −30°; **B**, ca. +33°) as shown in Figure 5.[13] The Boltzmann distribution of the obtained conformers suggested that the clusters **A** and **B** existed in the ratio of 87:13. Inspection of the major cluster **A** indicated that **1** (or *ent*-**1**) adopted the preferred conformation consistent with the ROESY data.[9]



3a

Oxidative degradation of iejimalide B (**1**) was performed as follows. Compound **1** was subjected to ozonolysis, reduction with $NaBH_4$, and esterification with (*R*)-(−)-2-methoxy-2-trifluoromethyl-2-phenylacetyl chloride (MTPACl). HPLC separation of the reaction mixture afforded a segment with a methoxy and two MTPA groups, whose structure was assigned as the 6,11-bis-(*S*)-MTPA ester of C-6–C-11 segment (**3a**) by the ^1H–^1H COSY and FABMS spectra.[14] The absolute configuration at C-9 in the segment **3a** was examined by comparison of ^1H NMR data of **3a** derived from natural specimen with those of (*S*)- and (*R*)-MTPA esters of the synthetic C-6–C-11 segment. The C-6–C-11 segment with 9*S*-configuration was synthesized from (2*S*)-1,2-isopropylidenedioxybutan-4-ol (**4**), which was derived from (*S*)-(−)-malic acid by literature procedure.[15] Compound **4** was converted into the *tert*-butyldiphenylsilyl (TBDPS) ether, and then deprotection of its isopropylidene group afforded a 1,2-diol **5** (Scheme 1). Selective protection of the primary hydroxyl group of **5** with 4,4′-dimethoxytrityl chloride (DMTrCl) followed by methylation of the secondary hydroxyl group gave **6**. Removal of the DMTr group in **6** was carried out by treatment with 80% AcOH to yield a primary alcohol **7**. Three-carbon elongation of **7** with Wittig reaction afforded an unsaturated ester **8** in two steps. The ester carbonyl in **8** was reduced with DIBAL-H, and then the TBDPS group was removed with tetrabutylammonium fluoride (TBAF) to give the (9*S*)-C-6–C-11 segment (**9**), which was finally transformed into the bis-(*S*)- and bis-(*R*)-MTPA esters (**3a** and **3b**, respectively).[16,17] Though **3a** and **3b** showed similar ^1H NMR profiles, significant differences were observed for the signal patterns of H_2-11 and H-9 (Fig. 6b and c). In the ^1H NMR spectrum of the synthetic bis-(*S*)-MTPA ester (**3a**), the methylene protons at C-11 resonated at δ_H 4.48 and 4.22, while those of C-11 of the synthetic bis-(*R*)-MTPA ester (**3b**) were observed at δ_H 4.41 and 4.30. Furthermore, the methine protons at C-9 of **3a** resonated at higher-field (δ_H 3.87) than that of **3b** (δ_H 3.93). The ^1H NMR spectrum of the bis-(*S*)-MTPA ester derived from natural specimen was identical with that of **3a** (Fig. 6a and b). Thus, the absolute configuration at C-9 was determined to be *S*, and the absolute configurations at the remaining four chiral centers in iejimalide B (**1**) were assigned as 4*R*, 17*S*, 22*S*, and 23*S*.



1

Figure 4. Relative stereochemistry of iejimalide B (**1**). ROESY correlations from H_3-39 and H_3-44 are illustrated by arrows.

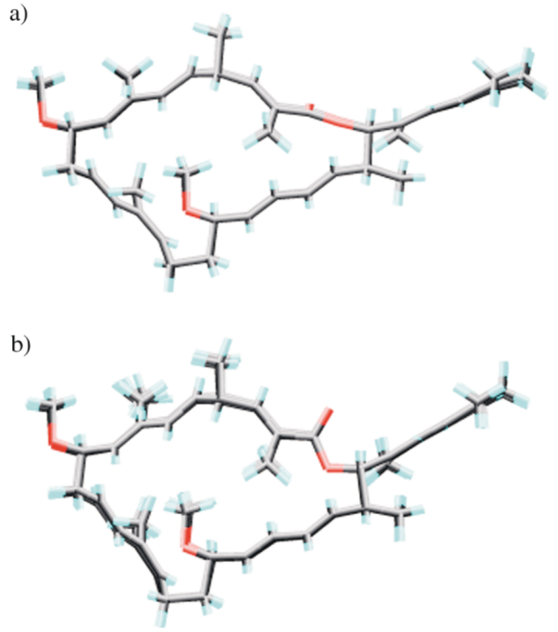
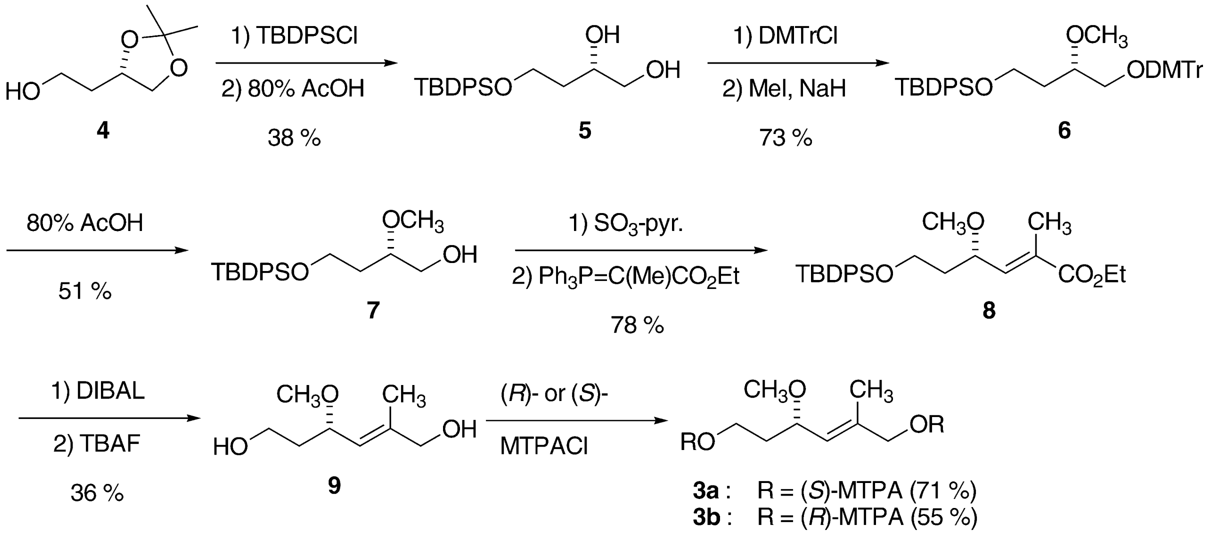


Figure 5. Overlay of the stable conformers [clusters a) **A** and b) **B**] of iejimalide B (**1**) calculated by distance geometry calculation.



Scheme 1.

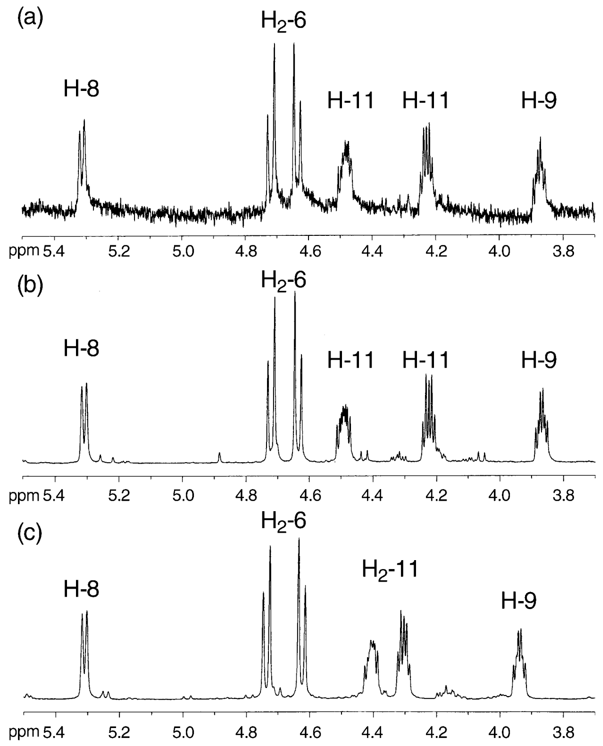


Figure 6. Proton signal patterns of H$_2$-11 of (a) 6,11-bis-(*S*)-MTPA ester of C-6–C-11 segment (**3a**) derived from iejimalide B (**1**), (b) 6,11-bis-(*S*)- and (c) 6,11-bis-(*R*)-MTPA esters (**3a** and **3b**, respectively) of the synthetic C-6–C-11 segment.

Acknowledgements

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8. ^{1}H–^{1}H coupling constants for **1** (H/H): 3/4: 10.3, 4/5: 9.0, 4/43: 6.6, 5/6: 15.5, 8/9: 9.4, 9/10a: 2.7, 9/10b: 10.2, 10a/11: 4.7, 10b/11: 10.2, 11/12: 15.8, 14/15a: 11.1, 14/15b: 4.6, 15a/15b: 15.3, 15a/16a: 2.3, 15a/16b: 11.2, 15b/16a: 2.0, 15b/16b: 3.0, 16a/16b: 14.2, 16a/17: 10.0, 16b/17: 3.0, 17/18: 8.2, 18/19: 14.7, 19/20: 9.8, 20/21: 14.5, 21/22: 9.7, 22/23: 10.0, 22/38: 6.6, 26/27: 11.1, 29a and 29b/30: 5.8, 29a/29b: not determined, 32/35a: 3.0, 32/35b: 10.3, 35a/35b: 11.6.
9. ROESY correlations for macrocyclic ring portion of **1** [H/H (s: strong, m: medium, w: weak)]: 3/4 (s), 3/5 (m), 3/6 (w), 3/43 (w), 4/6 (s), 4/44 (s), 5/42 (s), 5/43 (m); 6/8 (s), 6/39 (m), 8/10b (s), 8/39 (m), 8/41 (w), 9/10a (s), 9/10b (w), 9/11 (m), 9/42 (s), 10a/11 (m), 10a/41 (w), 10b/11 (w), 10b/12 (m), 10b/39 (w), 10b/41 (w), 11/40 (s), 12/15a (s), 12/17 (m), 12/39 (w), 14/15a (w), 14/15b (m), 14/16b (m), 14/17 (m), 14/40 (s), 15a/16a (w), 15a/15b (m), 15a/17 (w), 15a/18 (m), 15b/16a (m), 15b/16b (m), 16a/17 (m), 16a/18 (w), 16b/17 (m), 16b/18 (s), 16b/39 (w), 17/19 (s), 18/20 (s), 18/39 (w), 19/21 (s), 19/39 (w),

20/22 (s), 20/38 (w), 20/44 (w), 21/22 (w), 21/23 (m), 21/38 (m), 22/26 (w), 22/37 (s), 23/26 (s), 23/27 (w), 23/38 (m), 26/29 (s), 26/36 (w), 26/38 (w), 27/29 (m), 27/37 (s), 29/36 (s), 37/38 (w), 39/44 (m), 41/42 (s), and 42/43 (w).

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12. Computer model and all calculations were carried out using the molecular-modeling software package SYBYL ver.6.5 (Tripos, Inc.,) on a Silicon Graphics O2 computer. Distance geometry, simulated annealing, and molecular mechanics calculations were performed with Tripos 5.2 force field. In the distance geometry calculation, interatomic distances were calculated from the integrated volumes of the 69 NOESY cross-peaks[8] for iejimalide B (**1**). For **1**, 100 conformers were generated. In simulated annealing simulation, each conformation was equilibrated for 5000 fs in a thermal bath at 1000 K, and thereafter successively for 1450 fs, the temperature was deceased until a final structure of 300 K was reached, and each conformation was finally minimized.
13. Summary of distance geometry culculation of **1**; cluster **A**: E_{total}, 32.60±0.13 kcal/mol, RMSD, 0.68±0.17 Å; distance RMS violation, 0.081±0.00; cluster **B**: E_{total}, 34.28±0.24 kcal/mol, RMSD, 0.62±0.09 Å; distance RMS violation, 1.080±0.01.
14. 6,11-Bis-(*S*)-MTPA ester of C-6–C-11 segment (**3a**) derived from natural specimen: ^{1}H NMR ($CDCl_3$): δ 1.53 (3H, s, H_3-42), 1.71 (1H, m, H-10b), 1.85 (1H, m, H-10a), 3.12 (3H, s, H_3-41), 3.50 (6H, s, OCH_3 for MTPA), 3.87 (1H, m, H-9), 4.22 (1H, m, H-11b), 4.48 (1H, m, H-11a), 4.63 (1H, d, $J=12.3$ Hz, H-6b), 4.72 (1H, d, $J=12.3$ Hz, H-6a), 5.31 (1H, d, $J=8.9$ Hz, H-8), 7.37 (6H, Ph for MTPA), and 7.46 (4H, m, Ph for MTPA); ^{1}H–^{1}H COSY correlations (H/H): 6a/6b, 8/9, 9/10a, 9/10b, 10a/11a, 10a/11b, 10b/11a, and 10b/11b; FABMS *m*/*z* 615 $(M+Na)^+$; HRESIMS *m*/*z* 615.1783 $[(M+Na)^+$, calcd. for $C_{28}H_{30}O_7F_6Na$, 615.1793].
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16. 6,11-Bis-(*S*)-MTPA ester of synthetic C-6–C-11 segment (**3a**): colorless oil; ^{1}H NMR ($CDCl_3$): δ 1.53 (3H, s, H_3-42), 1.71 (1H, m, H-10), 1.85 (1H, m, H-10), 3.12 (3H, s, H_3-41), 3.50 (6H, s, OCH_3 for MTPA), 3.87 (1H, m, H-9), 4.22 (1H, m, H-11), 4.48 (1H, m, H-11), 4.63 (1H, d, $J=12.3$ Hz, H-6), 4.72 (1H, d, $J=12.3$ Hz, H-6), 5.31 (1H, d, $J=8.9$ Hz, H-8), 7.37 (6H, Ph for MTPA), and 7.46 (4H, m, Ph for MTPA); FABMS *m*/*z* 615 $(M+Na)^+$; HRFABMS *m*/*z* 615.1771 $[(M+Na)^+$, calcd. for $C_{28}H_{30}O_7F_6Na$, 615.1793].
17. 6,11-Bis-(*R*)-MTPA ester of synthetic C-6–C-11 segment (**3b**): colorless oil; ^{1}H NMR ($CDCl_3$): δ 1.54 (3H, s, H_3-42), 1.72 (1H, m, H-10), 1.88 (1H, m, H-10), 3.15 (3H, s, H_3-41), 3.49 (3H, s, OCH_3 for MTPA), 3.50 (3H, s, OCH_3 for MTPA), 3.93 (1H, m, H-9), 4.30 (1H, m, H-11), 4.41 (1H, m, H-11), 4.62 (1H, d, $J=12.3$ Hz, H-6), 4.73 (1H, d, $J=12.3$ Hz, H-6), 5.31 (1H, d, $J=8.9$ Hz, H-8), 7.37 (6H, Ph for MTPA), and 7.46 (4H, m, Ph for MTPA); FABMS *m*/*z* 615 $(M+Na)^+$; HRFABMS *m*/*z* 615.1794 $[(M+Na)^+$, calcd. for $C_{28}H_{30}O_7F_6Na$, 615.1793].