Synthesis of (–)-pericosine B, the antipode of the cytotoxic marine natural product†

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The stereoselective synthesis of (–)-pericosine B, which is the antipode of the cytotoxic metabolite of the fungus *Periconia byssoides* OUPS-N133 separated from the sea hare, was accomplished in 9 steps in 12% total yield from (–)-quinic acid, together with the synthesis of its epimer. Every crucial step of this total synthesis, including ring opening of a β-epoxide and $NaBH_4$ reduction of an unstable β,γ-unsaturated enone, proceeded with excellent stereoselectivity.

Introduction

The isolation and structure determination of highly functionalized C-7 cyclohexenoid natural products pericosines A–E **1–5** (Fig. 1), which are cytotoxic metabolites of the fungus *Periconia byssoides* OUPS-N133 originally separated from the sea hare *Aplysia kurodai*, were reported in 1997 and 2007 by Numata and co-workers.[1,2] The absolute configuration of pericosines A–D **1–4** was elucidated by total syntheses.[3–9] Compound **1** was reported to exhibit significant inhibitory activity against protein kinase EGFR and human topoisomerase II, but similar biological tests on **2–4** were not reported. We were therefore interested in the biological activity of **2** against human cancer cell lines. In addition to their significant bioactivity, it is noteworthy that pericosines C **3** and E **5** exist as a mixture of enantiomers. X-ray analysis by Numata and co-workers[2] established the relative stereochemistry of pericosine E **5** to originate from two monomeric pericosines **1** and **2** having different chirality. As we pointed out previously, their finding meant that the presence of the antipode of monomeric **1** or **2** in nature is possible.[10] Therefore, synthesis of the antipode of **1–3** is significant. We have already reported the synthesis of the antipode of **1**[6,7] and **3**[3] but not that of **2**.

The total synthesis of (+)-**2**, a naturally occurring enantiomer, was reported only once in 1998 by Donohoe and co-workers[9] in spite of extensive effort by other groups including ours to date.[11–13] However, the only successful synthesis had problems in terms of the use of expensive starting materials and a stoichiometric amount of toxic osmium tetroxide. Recently, we reported the determination of the absolute configuration of pericosine D by a synthetic approach.[8] Unfortunately, the total yield of desired product **4** was quite low when relatively inexpensive (–)-quinic acid was used. Nevertheless, that work gave us a hint for a short synthesis of **2**. Following several years of failed attempts at synthesizing **2**,[11] we describe herein a short synthesis of the antipode of **2** and its epimer.

Results and discussion

From the results of our previous work that dealt with the determination of pericosine D **4**, we suspected that the introduction of a 6α-methoxy group into the pericosine core 6-membered ring is possible when MeOH is used as solvent in the stereoselective ring opening of intermediate β-epoxide **8**.[8]

The synthesis of (–)-pericosine B is summarized in Scheme 1 followed by Scheme 2. Methyl quinate derivative **6** was prepared from commercially available (–)-quinic acid in 78% according to the literature.[14] Compound **6** was converted into unstable diene **7** in 2 steps. Then, without purification, crude **7** was oxidized with mCPBA at 40 °C to afford an inseparable mixture of epoxides **8** and **9** in 40% yield in 3 steps.

Ring opening of a mixture of **8** and **9** with a catalytic amount of HCl in MeOH gave the desired 6α-methoxypericosine derivative **10** in 54% yield, with small amount of **11**[8] (1%) and 1-methoxylated alcohol **12** (1%) (whose configuration at C-1 could not be determined), with the recovery of **9** (32%). The relative

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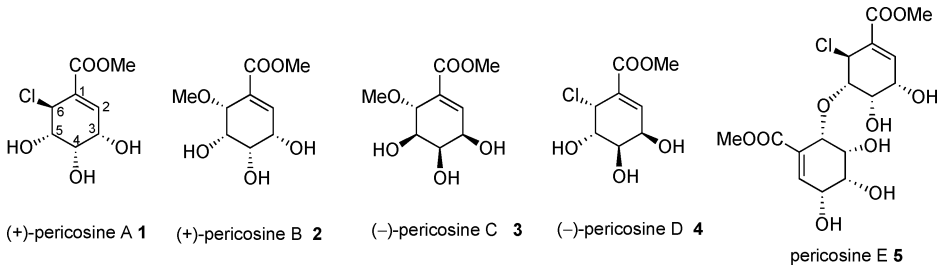
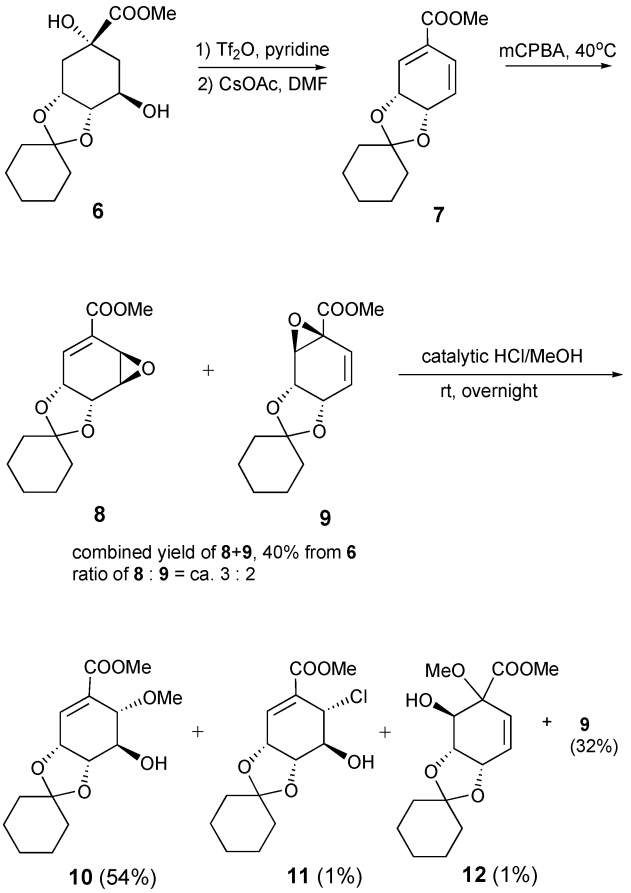
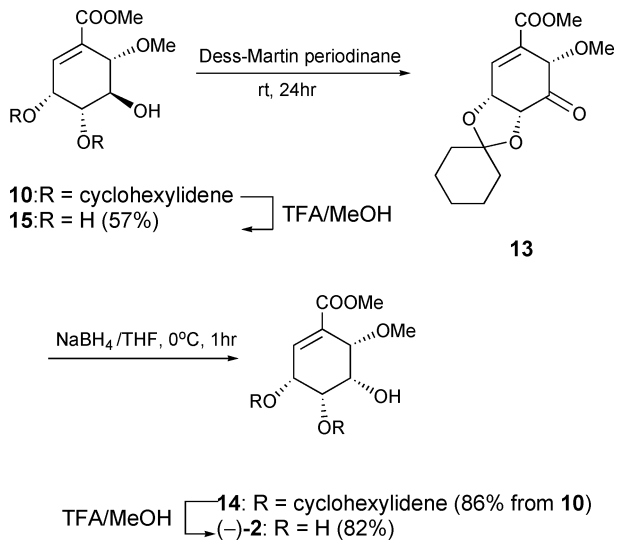


Fig. 1 Structures of naturally occurring pericosines.



Scheme 1 Stereoselective synthesis of 6α-methoxyalchohol **10**.



Scheme 2 Completion of total synthesis of (–)-pericosine B **2**.

stereochemistry of major product **10** was confirmed by NOESY analysis as shown in Scheme 1. Cross-peaks of H-4/H-6 and H-5/6-methoxy group were observed.

In next step, it was difficult to promote the S_N2-type Walden inversion by Mitsunobu reaction at C-5 in **10**. Close inspection of the ^{1}H-NMR spectra of **10**, which had relatively large coupling constants of $J_{4,5} = 7.3$ Hz and $J_{5,6} = 6.6$ Hz, suggested a half-chair conformation, as illustrated in Fig. 2, that inhibits S_N2-type attack of the nucleophile from inside the pericosine core 6-membered ring, which is in a fixed conformation due to the cyclohexylidene bridge.

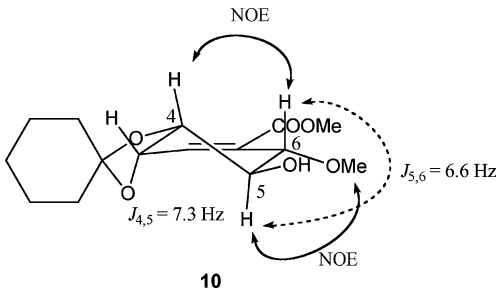


Fig. 2 Plausible conformation of **10**.

Then, the inversion of stereochemistry of C-5 in **10** was attempted by means of Dess–Martin oxidation followed by stereoselective reduction with $NaBH_4$. Methoxy alcohol **10** was oxidized with Dess–Martin periodinane, albeit very slowly, to give crude β,γ-unsaturated enone **13**. Without purification, **13**[15] was reduced with $NaBH_4$ at 0 °C to give the desired diastereomer **14** as the sole product in 86% yield in 2 steps.

This total synthesis was completed by deprotection of the cyclohexylidene moiety in **14** with TFA in MeOH to afford (–)-pericosine B **2** in 82% yield. All spectral data except specific rotation agreed with those of reported natural pericosine B. The specific rotation ($[\alpha]_D^{25}$ –32.6) of synthesized compound showed almost the same value as previously synthesized (+)-**2** ($[\alpha]_D^{21}$ +30.6 (*c* 0.8 in EtOH)) but with the opposite sign.

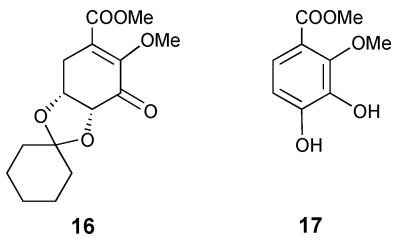


Fig. 3 Structures of undesired compounds.[15]

The overall yield of this total synthesis of **2** was 12% in 9 steps starting from (–)-quinic acid. Similarly, epimer **15** was prepared in 57% yield from **10**.

Conclusions

We have accomplished the stereoselective total synthesis of (–)-pericosine B **2**, which has opposite chirality to the natural product, in 9 steps in 12% total yield. Its epimer **15** was also prepared. The second synthesis of pericosine B described herein is a toxic-reagent-free method and is also applicable to the synthesis of (+)-pericosine B, which was obtained as a minor component in nature and has significant biological activity, since either enantiomer of unstable diene **7**[16] could be prepared from (–)-quinic acid. This synthetic route toward **2** is also a divergent one, as the common intermediate yielded pericosine D **4**.[8]

Experimental section

General information

IR spectra were obtained with a JEOL FT/IR-680 Plus spectrometer. HRMS was determined with a JEOL JMS-700 (2) mass spectrometer. NMR spectra were recorded at 27 °C on Varian UNITY INOVA-500 and Mercury-300 spectrometers in $CDCl_3$ with tetramethylsilane (TMS) as internal standard. Melting points were determined on a Yanagimoto micromelting point apparatus and are uncorrected. Specific rotations were measured on a JASCO DIP-1000 polarimeter and $[\alpha]_D$ values are given in 10^{-1} deg cm^2 g^{-1}. Liquid column chromatography was conducted over silica gel (Nacalai, silica gel 60, mesh 70–230 or 230–400). Analytical TLC was performed on precoated Merck glass plates (silica gel 60 F_{254}), and compounds were detected by dipping an ethanol solution of phosphomolybdic acid, followed by heating. Dry THF was distilled over sodium benzophenone ketyl under argon atmosphere.

Synthesis of a mixture of epoxides 8 and 9 from 6

To a solution of diol **6** (552 mg, 1.93 mmol) in CH_2Cl_2 (25 mL) were added pyridine (780.5 μL, 9.65 mmol) and catalytic DMAP (30.0 mg). A solution of Tf_2O (729 μL, 4.25 mmol) in CH_2Cl_2 (25 mL) was added dropwise to the mixture at 0 °C with stirring. After stirring overnight at rt, the reaction mixture was treated with aqueous $NaHCO_3$ and then extracted with CH_2Cl_2. The organic layer was dried over $MgSO_4$, filtered, and evaporated to give a crude residue containing triflates and diene **7**. The mixture was dissolved in DMF (3 mL), and CsOAc (1.99 mmol) was added to the solution with stirring at rt. After 3 hr, the reaction mixture was extracted with *t*-butylmethylether and H_2O. The organic layer was washed with brine twice, dried over $MgSO_4$, filtered, and evaporated to afford crude diene **7**. To a solution of crude diene **7** in CH_2Cl_2 (10 mL), mCPBA (518.8 mg, max 77%, calcd *ca.* 2.3 mmol) was added and the reaction mixture was kept at 40 °C overnight. The reaction mixture was treated with aqueous $NaHCO_3$ and then extracted with CH_2Cl_2. The organic layer was separated, dried over $MgSO_4$, filtered, and evaporated to afford a crude residue that was purified by column chromatography (eluent: hexane:EtOAc = 4:1) to give a mixture of **8** and **9** (204.9 mg, 40% from **6**, ratio: **8**:**9** = 3:2 from ^{1}H-NMR spectrum).

Methyl (3*R*,4*R*,5*S*,6*S*)-3,4-*O*-cyclohexylidene-3,4,5-trihydroxy-6-methoxy-1-cyclohexene-1-carboxylate 10

Methyl (4*S*,5*R*,6*R*)-4,5-*O*-cyclohexylidene-4,5,6-trihydroxy-1-methoxy-2-cyclohexene-1-carboxylate 12

To a mixture of **8** and **9** (*ca.* 3:2) (73.9 mg, combined amount) in MeOH (5 mL) was added 1 drop of 1.0 M HCl in Et_2O with a microsyringe. After stirring overnight at rt, the reaction mixture was condensed under reduced pressure to afford a crude residue that was purified by column chromatography (eluent: hexane:EtOAc = 5:3) to give **10** (44.9 mg, 54%), **11** (0.8 mg, 1%), and **12** (0.9 mg, 1%) with recovery of **9** (23.6 mg, 32%). **10**: Colorless crystals (CH_2Cl_2); mp 132–135 °C; $[\alpha]_D^{25}$ −55.2 (*c* 0.165 in $CHCl_3$); IR ν_{max} (KBr)/cm^{-1} 3395 (OH), 1719 (C=O), 1660 (C=C); ^{1}H-NMR (500 MHz; $CDCl_3$; Me_4Si) δ 1.25–1.70 (10H, m), 3.60 (3H, s, 6-OMe), 3.80 (3H, s, COOMe), 3.94 (1H, dd, J = 7.3, 6.6 Hz, H-5), 4.00 (1H, dt, J = 6.6, 1.4 Hz, H-6), 4.17 (1H, dd, J = 7.3, 6.4 Hz, H-4), 4.66 (1H, ddd, J = 6.4, 3.9, 1.4 Hz, H-3), 6.68 (1H, dd, J = 3.9, 1.4 Hz, H-2); ^{13}C-NMR (125.6 MHz; $CDCl_3$; Me_4Si) δ 23.6 (t), 24.0 (t), 25.0 (t), 35.3 (t), 37.8 (t), 52.0 (q), 60.5 (q), 70.7 (d), 72.6 (d), 75.9 (d), 78.0 (d), 111.7 (s), 132.3 (d), 134.7 (s), 166.4 (s); EIMS *m*/*z* 298 (M$^+$, 76%); HREIMS *m*/*z* calcd for $C_{15}H_{22}O_6$ (M)$^+$ 298.1416, found 298.1415.

12 : Colorless oil; $[\alpha]_D^{25}$ +146.8 (*c* 0.5 in $CHCl_3$); IR ν_{max} (liquid film)/cm^{-1} 3553 (OH), 1724 (C=O), 1660 (C=C); ^{1}H-NMR (500 MHz; $CDCl_3$; Me_4Si) δ 1.35–1.77 (10H, m), 2.63 (1H, d, J = 3.4 Hz, 6-OH), 3.45 (3H, s, –OMe), 3.78 (3H, s, COOMe), 3.91 (1H, dd, J = 8.4, 3.4 Hz, H-4), 4.45 (1H, dd, J = 8.4, 7.1 Hz, H-5), 4.78 (1H, ddd, J = 7.1, 3.4, 1.1 Hz, H-6), 5.86 (1H, dd, J = 10.1, 1.1 Hz, H-1), 6.17 (1H, dd, J = 10.1, 3.4 Hz, H-2); ^{13}C-NMR (125.6 MHz; $CDCl_3$; Me_4Si) δ 23.5 (t), 24.0 (t), 25.1 (t), 34.5 (t), 37.6 (t), 52.2 (q), 52.8 (q), 71.9 (d), 73.1 (d), 76.5 (d), 83.3 (s), 111.2 (s), 127.7 (d), 129.9 (d), 170.0 (s); EIMS *m*/*z* 298 (M$^+$, 85%); HREIMS *m*/*z* calcd for $C_{15}H_{22}O_6$ (M)$^+$ 298.1416, found 298.1416.

Methyl (3*R*,4*R*,5*R*,6*S*)-3,4-*O*-cyclohexylidene-3,4,5-trihydroxy-6-methoxy-1-cyclohexene-1-carboxylate 14

To a solution of **10** (13.8 mg, 0.046 mmol) in CH_2Cl_2 (2 mL) was added Dess–Martin periodinane (99.1 mg, 0.23 mmol) at rt with stirring. After 24 hr, the reaction mixture was diluted with *tert*-butylmethylether and treated with aq. $Na_2S_2O_4$ and aq. $NaHCO_3$. The organic layer was separated, dried over $MgSO_4$, and filtered and the solvent was removed under reduced pressure to afford crude ketone **13**. Data of crude **13**: IR ν_{max} (liquid film)/cm^{-1} 1726 (C=O), 1612 (C=C); ^{1}H-NMR (300 MHz; $CDCl_3$; Me_4Si) δ 1.25–1.70 (10H, m), 3.60 (3H, s, 6-OMe), 3.80 (3H, s, COOMe), 3.94 (1H, dd, J = 7.3, 6.6 Hz, H-5), 4.00 (1H, dt, J = 6.6, 1.4 Hz, H-6), 4.17 (1H, dd, J = 7.3, 6.4 Hz, H-4), 4.66 (1H, ddd, J = 6.4, 3.9, 1.4 Hz, H-3), 6.68 (1H, dd, J = 3.9, 1.4 Hz, H-2); ^{13}C-NMR (75.5 MHz; $CDCl_3$; Me_4Si) δ 24.1 (t), 25.2 (t), 30.0 (t), 35.8 (t), 37.2 (t), 52.6 (q), 59.6 (q), 74.5 (d), 77.1 (d), 113.3 (s), 133.0 (d), 134.3 (s), 164.6 (s), 200.9 (s); EIMS *m*/*z* 296 (M$^+$, 94%); HREIMS *m*/*z* calcd for $C_{15}H_{20}O_6$ (M)$^+$ 296.1260, found 296.1262

To a suspension of $NaBH_4$ in MeOH (0.5 mL) was added crude ketone **13** dissolved in CH_2Cl_2 (2 mL) at 0 °C with stirring. After 1 hr, the reaction mixture was treated with aq. NH_4Cl and extracted with CH_2Cl_2. The organic layer was dried over $MgSO_4$ and filtered. The solvent was removed under reduced pressure to afford a crude residue that was purified by column chromatography (eluent: EtOAc: hexane = 1: 1) to give **14** (11.9 mg, 86% in 2 steps). **14**: Colorless oil; $[\alpha]_D^{25}$ +25.1 (*c* 0.12 in $CHCl_3$); IR ν_{max} (liquid film)/cm^{-1} 3497 (OH), 1720 (C=O), 1656 (C=C); ^{1}H-NMR (300 MHz; $CDCl_3$; Me_4Si) δ 1.25–1.70 (10H, m), 3.17 (1H, *d*, J = 11.5 Hz, 5-OH), 3.58 (3H, s, 6-OMe), 3.81 (3H, s, COOMe), 3.83 (1H, m, H-5), 4.28 (1H, dd, J = 4.9, 0.6 Hz, H-6), 4.48 (1H, ddd, J = 5.7, 3.4, 0.7 Hz, H-3), 4.66 (1H, dd, J = 5.7, 3.2 Hz, H-4), 6.82 (1H, br d, J = 3.4 Hz, H-2); ^{13}C-NMR (75.5 MHz; $CDCl_3$; Me_4Si) δ 24.1(t), 24.3 (t), 25.3 (t), 36.0 (t), 37.5 (t), 52.3 (q), 61.3 (q), 68.1 (d), 72.1 (d), 73.0 (d), 74.2 (d), 111.5 (s), 129.7 (s), 137.3 (d), 166.0 (s); EIMS *m*/*z* 298 (M$^+$, 69%); HREIMS *m*/*z* calcd for $C_{15}H_{22}O_6$ (M)$^+$ 298.1416, found 298.1419.

(–)-Pericosine B: Methyl (3*R*,4*R*,5*R*,6*R*)-6-methoxy-3,4,5-trihydroxy-1-cyclohexene-1-carboxylate 2

To a solution of alcohol **14** (13.2 mg) in MeOH (0.5 mL) was added TFA (0.5 mL, excess) at 0 °C and the reaction mixture was stirred for 1 hr. After stirring for another 4 hr at rt, the reaction mixture was condensed under reduced pressure to afford a crude residue that was purified by silica gel chromatography (eluent: 3–5% MeOH in CH_2Cl_2) to give (–)-**2** (7.9 mg, 82%). (–)-**2**: Colorless crystals (hexane-EtOAc); mp 69–71 °C; $[\alpha]_D^{25}$ –32.6 (*c* 0.35 in EtOH); IR ν_{max} (liquid film)/cm^{-1}3433 (OH), 1713 (C=O), 1651 (C=C); ^{1}H-NMR (500 MHz; acetone-d_6; Me_4Si) δ 3.60 (3H, s, 6-OMe), 3.78 (3H, s, COOMe), 3.85 (1H, dd, J = 4.1, 2.0 Hz, H-5), 3.98 (1H, m, H-4), 4.20 (1H, m, H-3), 4.26 (1H, ddd, J = 4.1, 1.1, 0.9 Hz, H-6), 6.74 (1H, dd, J = 2.5, 1.1 Hz, H-2); ^{13}C-NMR (125.6 MHz; acetone-d_6; Me_4Si) δ 52.2 (q), 61.5 (q), 69.5 (d), 70.0 (d), 72.8 (d), 77.0 (d), 130.5 (s), 141.9 (d), 166.9 (s); EIMS *m/z* 219 (M^+, 0.8%), 186 (M^+–MeOH, 8%); HREIMS *m/z* calcd for $C_9H_{15}O_6$ $(M + H)^+$ 219.0868, found 219.0860.

Methyl (3*R*,4*R*,5*S*,6*S*)-6-methoxy-3,4,5-trihydroxy-1-cyclohexene-1-carboxylate 15

Alcohol **10** (11.4 mg) was converted to (–)-**15** (4.7 mg, 57%) by the same process as above. (–)-**15**: Colorless crystals (hexane-EtOAc); mp 94–97 °C; $[\alpha]_D^{25}$ –75.6 (*c* 0.23 in EtOH); IR ν_{max} (KBr)/cm^{-1} 3418 (OH), 1716 (C=O), 1651 (C=C); ^{1}H-NMR (500 MHz; acetone-d_6; Me_4Si) δ 3.51 (3H, s, 6-OMe), 3.676 (1H, dd, J = 7.3, 4.1 Hz, H-4), 3.76 (3H, s, COOMe), 3.98 (1H, ddd, J = 4.8, 0.9, 0.7 Hz, H-6), 4.11 (1H, dd, J = 7.3, 4.8 Hz, H-5), 4.32 (1H, br dd, J = 4.1, 3.9 Hz, H-3), 6.69 (1H, ddd, J = 3.9, 0.9, 0.5 Hz, H-2); ^{13}C-NMR (125.6 MHz; acetone-d_6; Me_4Si) δ 52.0 (q), 59.7 (q), 66.7 (d), 70.5 (d), 71.2 (d), 79.3 (d), 132.3 (s), 139.2 (d), 167.2 (s); EIMS *m/z* 219 (M^+, 0.4%), 186 (M^+–MeOH, 6%); HREIMS *m/z* calcd for $C_9H_{15}O_6$ $(M + H)^+$ 219.0868, found 219.0861.

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15 β,γ-Unsaturated enone **13** was so unstable that purification by silica gel chromatography afforded a complex mixture including more stable **16** shown in Fig. 3 formed by double bond migration. Therefore, other oxidizing agents could not be used in the preparation of **13**. Dess–Martin oxidation at 40 °C to accelerate the reaction resulted in the formation of **16** and aromatized product **17**. **Methyl (4*R*,5*R*)-4,5-cyclohexylidene-2-methoxy-3-oxo-1-cyclohexene-1-carboxylate 16**: Colorless crystals (CH_2Cl_2); mp 60–62 °C; $[\alpha]_D^{25}$ –1.8 (*c* 0.085 in $CHCl_3$); IR ν_{max} (KBr)/cm^{-1}1732 (C=O), 1693 (C=O), 1617 (C=C);^{1}H-NMR (500 MHz; $CDCl_3$; Me_4Si) δ 1.35–1.70 (10H, m), 2.91 (1H, dd, J = 9.2, 4.8 Hz, H-6_A), 3.09 (1H, dd, J = 9.2, 2.1 Hz, H-6_B), 3.79 (3H, s, -OMe), 3.85 (3H, s, COOMe), 4.36 (1H, d, J = 5.3 Hz, H-4), 4.62 (1H, ddd, J = 5.3, 4.8, 2.1 Hz, H-5);^{13}C-NMR (125.6 MHz; $CDCl_3$; Me_4Si) δ 23.7 (t), 23.8 (t), 24.9 (t), 26.4(t), 35.2 (t), 37.1 (t), 52.4 (q), 60.6 (q), 71.4 (d), 76.3 (d), 76.5 (d), 110.5 (s), 127.4 (s), 151.2 (s), 166.4 (s), 193.1 (s); EIMS *m/z* 296 (M^+, 76%); HREIMS *m/z* calcd for $C_{15}H_{20}O_6$ $(M)^+$ 296.1260, found 296.1259. **Methyl 3,4-dihydroxy-2-methoxybenzoate 17**: Yellow oil; IR ν_{max} (liquid film)/cm^{-1} 3538 (OH), 1714 (C=O), 1604 (C=C); ^{1}H-NMR ($CDCl_3$) δ 3.89 (3H, s, -OMe), 3.93 (3H, s, COOMe), 5.87 (2H, br s, -OH), 6.74 (1H, d, J = 8.9 Hz), 7.46 (1H, d, J = 8.9 Hz); ^{13}C-NMR ($CDCl_3$) δ 51.9 (q), 62.3 (q), 110.9 (d), 115.2 (s), 123.9 (d), 136.7 (s), 148.1 (s), 148.6 (s), 165.4 (s); EIMS *m/z* 198 (M^+, 76%), 166 (M^+–MeOH, 88%); HREIMS *m/z* calcd for $C_9H_{10}O_5$ $(M)^+$ 198.0520, found 198.0524.
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