Conversional synthesis and cytotoxic evaluation of novel taxoid analogs

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Available online: 10 Aug 2011

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Four novel taxoid analogs were conversionally synthesized from the C_{19}-diterpenoid alkaloid deltaline, and their cytotoxic activities were evaluated against a small panel of cancer cell lines.

Keywords: deltaline; conversional synthesis; taxoids; cytotoxicity

1. Introduction

The breakthrough discovery of the anticancer drug paclitaxel (Taxol®) and docetaxel (Taxotere®; Figure 1) is one of the major milestones in natural product-based drug discovery and development [1]. It has spurred extensive and intensive research on both paclitaxel and related taxoids from numerous research groups across many countries. One of the attractive sub-areas is to search for alternative sources of starting materials. On the basis of our extensive research experience on the chemistry of the diterpenoid alkaloids and careful skeletal and functional group analysis, we have sequentially envisioned the five different strategies toward the conversional synthesis of taxoids from two abundant C_{19}-diterpenoid alkaloids, yunaconitine and deltaline (Figure 2) [2–4]. After a 15-year, persistent, and challenging journey in this program, we finally completed the conversional synthesis of the desired taxane-like compound **1**, which possesses the $[6 + 8 + 6]$ tricyclic A/B/C ring system of paclitaxel and

docetaxel, using deltaline as starting material [4]. In addition to the characteristic tricyclic core structure of taxoids, compound **1** also features an *N*-containing heterocycle of the diterpenoid alkaloids. These structural features of compound **1** encouraged us to incorporate the side chain of paclitaxel or docetaxel into **1** and to evaluate the *in vitro* antiproliferative activities of the corresponding taxoid analogs. In this paper, we report the conversional synthesis and biological evaluation of four structurally novel taxoid analogs.

2. Results and discussion

Initially, we synthesized compound **1** employing the synthetic procedure developed by us [4]. However, attempt to remove the methoxyl group from the C-16 (corresponding to the C-13 based on the taxane numbering system) of **1** failed, which prevented us from introducing the β-phenylisoserine side chain. At this point, we reconsidered to protect the hydroxyl

Paclitaxel R_1 = Ac; R_2 = Bz
Docetaxel R_1 = H; R_2 = Boc
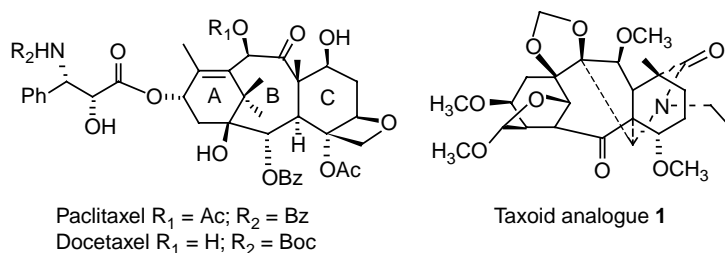
Taxoid analogue **1**

Figure 1.   Structures of paclitaxel, docetaxel, and taxoid **1**.

groups at C-6 and C-16 as MOM ethers instead of the methoxyl groups. Consequently, as described in the literature [4], the key intermediates **2a** and **2b** were prepared from deltaline, and **2a** was transformed into ketone **3** via Grob fragmentation. As illustrated in Scheme 1, protection of the hydroxyl groups in **3** furnished the MOM ether **5**, which could also be obtained from **2b** through protection of the hydroxyl groups as MOM ethers, conversion of the benzoate in C-14 to mesylate, and Grob fragmentation. It is worth noting that **2b** was only recycled to prepare **2a** in our previous report [4]. Afterwards, the transformations from **5** to acetal **7** were straightforward and proceeded via a one-pot procedure consisting of three reactions (ozone oxidation, aldol condensation–acetalation, and methylation) in 70% yield.

Having made taxoid **7** with the [6 + 8 + 6] tricyclic ring system, its C-2

(from now on, the taxane numbering system is adapted) ketone was reduced with B_2H_6 to yield a pair of epimers **8a** and **8b** (Scheme 2). The paclitaxel has a benzoate located at C-2α, which was considered as an essential moiety for its anticancer activity. Consequently, **8a** that possesses a hydroxyl group at C-2α was selected for further transformations. Incorporation of a benzoate at C-2α followed by selective deprotection of the MOM ether from C-13 generated the advanced intermediate **10**, from which the paclitaxel analog **12** was synthesized by the Holton-Ojima β-lactam synthon method [5]. In brief, the docetaxel side chain was attached to the C-13 hydroxyl in **10** through coupling with commercially available β-lactam **11** in the presence of excess sodium hydride. Considering the fact that paclitaxel has an α-oriented side chain at the C-13 position, the C-13β hydroxyl group in **10** was converted to α orientation in **13**

Yunaconitine
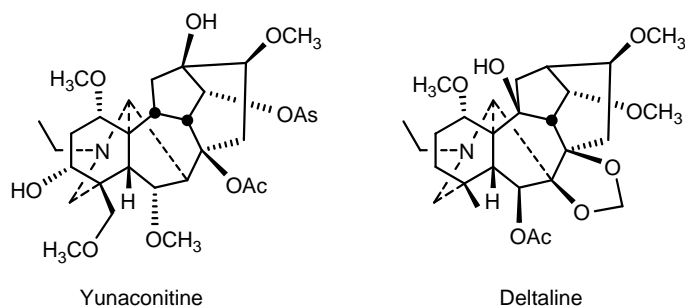
Deltaline

Figure 2.   Representatives of the abundant C_{19}-diterpenoid alkaloids.
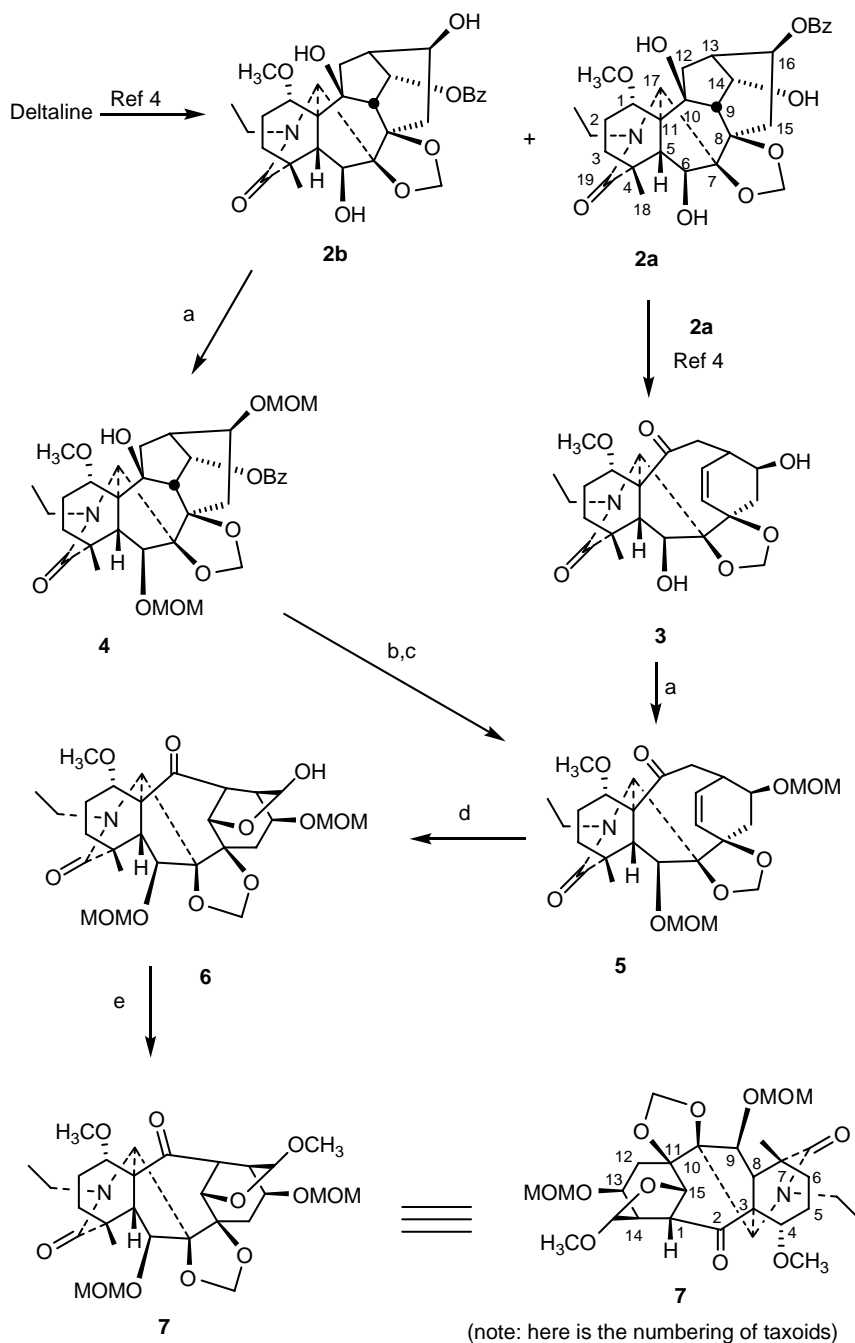
Scheme 1. Conversional synthesis of tricyclic core of taxoids from deltaline. Reagents and conditions: (a) MOMCl, DIPEA, tBu$_4$N$^+$I$^-$, CH$_2$Cl$_2$, rt, 18 h, 81% for **5**, 60% for **4**; (b) (i) NaOH, CH$_3$OH, 60°C, 1 h; (ii) MsCl, Pyr, rt, 2 h, 95%; (c) 5% NaOH in MeOH, reflux 3 h, 88%; (d) (i) O$_3$, CH$_2$Cl$_2$, −78°C, 5 min; (ii) Me$_2$S, rt, 1 h; (iii) Na$_2$SO$_3$, THF−H$_2$O, rt, 5 h; (e) CH$_3$I, NaH, THF, 70% from **5**.
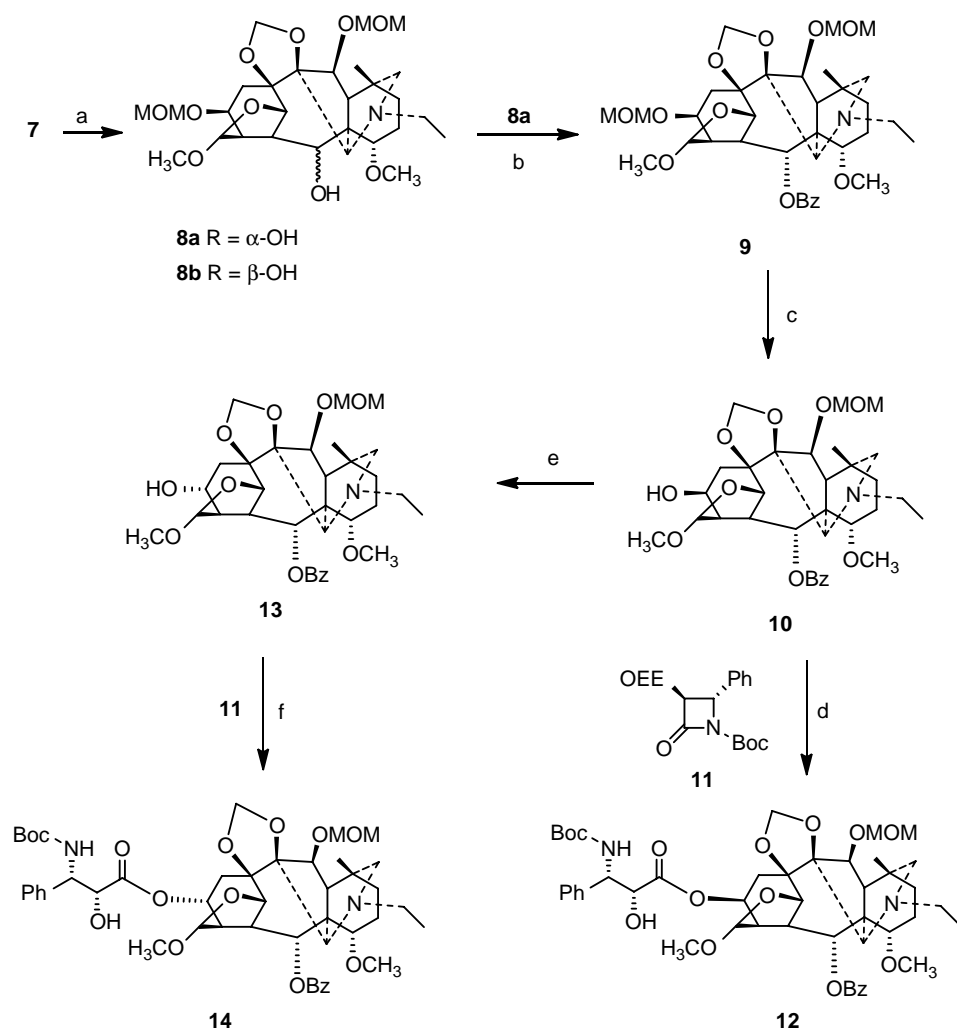
Scheme 2. Synthesis of taxoid analogs **12** and **14**. Reagents and conditions: (a) BH$_3$.Me$_2$S, THF, reflux, **8a** (40%), **8b** (46%); (b) BzCl, DMAP, CH$_2$Cl$_2$, 40°C, 8 h, 85% based on the recovery of 30% **8a**; (c) 4N HCl, 40°C, 5 h, 85% based on the recovery of 30% **9**; (d) (i) NaH, THF, 35°C, 4 h; (ii) ethanol, 0.5% HCl, 0°C, 16 h, 47%; (e) (i) Jone's reagent, acetone, 0°C, 10 min; (ii) excess NaBH$_4$, THF, rt, 80%; (f) (i) NaH, THF, 35°C, 3 h; (ii) ethanol, 0.5% HCl, 0°C, 10 h, 60%.

through the oxidation of the hydroxyl group followed by reduction of the corresponding ketone. The synthesis of another paclitaxel analog **14** was also accomplished using the same procedure described for the synthesis of **12** (Scheme 2).

Two more paclitaxel analogs **16** and **18**, without benzoate at C-2, were also synthesized for the biological comparisons. The paclitaxel analog **16** was directly

prepared from **7** by selective deprotection at C-13 followed by attachment of the side chain at C-13. Similar to the preparation of **14**, the conversion of β hydroxyl group at C-13 in **15** to the α-orientation in **17**, followed by side chain attachment, yielded the paclitaxel analog **18** (Scheme 3).

The *in-vitro* cytotoxicities of these four palcitaxel analogs were evaluated toward HCT8, MCF7, A2780, A549,
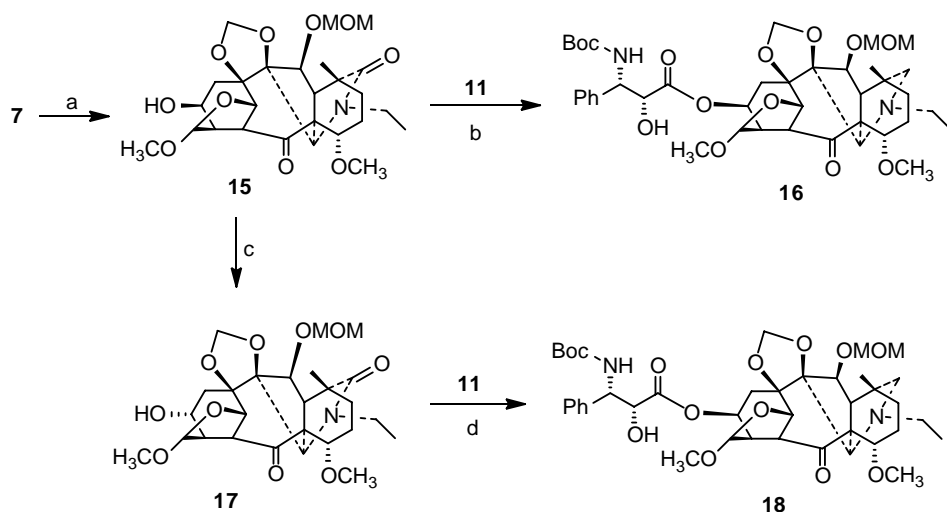
Scheme 3.　Synthesis of taxoid analogs **16** and **18**. Reagents and conditions: (a) Methanol, conc. HCl, reflux, 1.5 h, 95% based on the recovery of 45% **7**; (b) (i) NaH, THF, 35°C, 2 h; (ii) ethanol, 0.5% HCl, 0°C, 18 h, 60%; (c) (i) Jone's reagent, acetone, 0°C, 30 min; (ii) excess NaBH₄, THF, 90%; (d) NaH, THF, 35°C, 3 h; (ii) ethanol, 0.5% HCl, 0°C, 2d, 7%.

BGC823, and SKOV3 cancer cell lines by MTT assay [6] using paclitaxel as a positive control. As shown in Table 1, all of these analogs did not show significant activity. The loss of cytotoxicity might be explained by the following differences between their tricyclic core system and that of paclitaxel: (i) these paclitaxel analogs do not possess the critical oxetane ring D of paclitaxel; (ii) they have an additional *N*-heterocycle relative to paclitaxel; and (iii) they possess an extra five-membered acetal ring.

In conclusion, four paclitaxel analogs were synthesized from the naturally abundant C_{19}-diterpenoid alkaloid delta-line. Structurally, all these compounds are characteristic both of tricyclic core structure of taxoids and of an *N*-containing heterocycle of the diterpenoid alkaloids. Even though they did not show significant cytotoxicities against HCT8, MCF7, A2780, A549, BGC823, and SKOV3 cancer cell lines by MTT assay, this investigation can still enrich the structure–activity relationship of paclitaxel, as well as the chemical properties of the diterpenoid alkaloids.

Table 1.　Cytotoxicity of taxoids **12**, **14**, **16**, and **18**.

Compounds	IC$_{50}$ (μM)					
	HCT8	MCF7	A2780	A549	BGC823	SKOV3
Paclitaxel	0.67	0.0071	0.65	0.1	0.0063	0.005
12	>10	>10	>10	>10	>10	>10
14	>10	>10	>10	>10	>10	>10
16	>100	>100	>100	>100	>100	>100
18	>100	>100	>100	>100	>100	>100

3. Experimental

3.1 General experimental procedures

Melting points were determined on a Kofler block (uncorrected); optical rotations were measured in a 1.0 dm cell with a PE-314 polarimeter at $20 \pm 1°C$; IR spectra were recorded on a Nicolet 200 SXV spectrometer; MS spectra were obtained from Finnigan LCQ DECA mass spectrometer; ^{1}H and ^{13}C NMR spectra were acquired from a Bruker AC-E 200 or a Varian INOVA-400/54 spectrometer, with TMS as an internal standard; silica gel GF254 and H (10–40 mm, Qingdao Sea Chemical Factory, China) were used for TLC and column chromatography. Zones on TLC (silica gel G) were detected using the modified Dragendorff's reagent, 10% sulfuric acid in ethanol spray, or iodine. THF was distilled from sodium–benzophenone, and benzene and pyridine were distilled from calcium hydride. Other reagents and solvents were purchased from commercial sources and used without further purification. The solution of 6.5% hydrobromic acid in acetic acid was prepared by adding dropwise 40% solution of HBr in water (5 ml) to 25.73 ml of acetic acid with stirring under ice bath.

3.2 Preparation of compounds

3.2.1 Compound 4

To a solution of **2b** (5.3 g, 9.54 mmol) in dried dichloromethane (80 ml) were sequentially added diisopropylethyl amine (DIPEA; 10 ml, 57.2 mmol, 6 equiv.), methyl chloromethyl ether (MOMCl; 4.3 ml, 57.2 mmol, 6 equiv.), and $t\text{Bu}_4\text{N}^+\text{I}^-$ (3.52 g, 9.54 mmol, 1 equiv.) under argon, and the mixture was stirred at room temperature for 24 h. The reaction was quenched by the addition of 25% (v/v) ammonium hydroxide solution and extracted with dichloromethane (50 ml × 3). The combined extracts were dried over anhydrous Na_2SO_4, and the dichloromethane was removed in vacuum.

The residue obtained was chromatographed over silica gel H eluting with cyclohexane:acetone (10:1) to give compound **4** (white amorphous powder, 3.5 g, 60%): mp 100–102°C; $[\alpha]_D^{25}$ + 17.6 (*c* 0.5, acetone); IR (KBr) ν_{max} 3450, 1702, 1450, 1590, 700 cm^{-1}; ^{1}H NMR (CDCl$_3$, 400 MHz) δ 1.16 (3H, t, $J = 7.2$ Hz), 1.33 (3H, s), 2.95, 4.05 (each 1H, m), 3.22, 3.24, 3.33 (each 3H, s), 3.61 (1H, d, $J = 3.2$ Hz), 4.42–4.66 (4H, m), 5.11, 5.16 (each 1H, s), 5.60 (1H, t, $J = 5.2$ Hz), 7.42 (2H, t, $J = 7.6$ Hz), 7.54 (1 H, t, $J = 7.2$ Hz), 8.12 (2H, d, $J = 7.6$ Hz); ESI-MS: m/z 644 [M + H]$^+$; HR-ESI-MS: m/z 644.3033 [M + H]$^+$ (calcd for C$_{34}$H$_{46}$NO$_{11}$, 644.3071).

3.2.2 Compound 5

To a solution of **3** (1 g, 2.3 mmol) in dried dichloromethane (15 ml) were added DIPEA (5 ml, 28.6 mmol, 12 equiv.), MOMCl (2.1 ml, 27.6 mmol, 12 equiv.), and $t\text{Bu}_4\text{N}^+\text{I}^-$ (1 g, 2.7 mmol, 1.1 equiv.) under argon, and the mixture was stirred at room temperature for 18 h before being quenched by the addition of 25% (v/v) ammonium hydroxide solution. The subsequent mixture was extracted with dichloromethane (25 ml × 3), the combined extracts were dried over anhydrous Na$_2$SO$_4$, and the dichloromethane was removed. The residue obtained was purified by column chromatography (silica gel H) eluting with cyclohexane:acetone (10:1) to give compound **5** (white amorphous powder, 980 mg, 81%): mp 132–134°C; $[\alpha]_D^{25}$ + 20.8 (*c* 0.5, acetone); IR (KBr) ν_{max}: 3045, 1715, 1690, 1640, 1200 cm^{-1}; ^{1}H NMR(CDCl$_3$, 400 MHz) δ 1.22 (3H, t, $J = 7.2$ Hz), 1.33 (3H, s), 2.48 (1H, d, $J = 6.4$ Hz), 2.90 (1H, m), 3.30, 3.38, 3.42 (each 3H, s), 3.64 (1H, d, $J = 12.4$ Hz), 4.01 (1H, s), 4.22 (1H, d, $J = 2.8$ Hz), 4.30 (1H, m), 4.57 (2H, m), 4.70 (2H, m), 4.92 (1H, s), 5.06 (1H, s), 5.92 (1H, dd, $J = 9.6$, 6.8 Hz), 6.12 (1H, d, $J = 10.0$ Hz); ESI-MS: m/z 522 [M + H]$^+$; HR-ESI-MS: m/z

522.2699 [M + H]$^+$ (calcd for C$_{27}$H$_{40}$NO$_9$, 522.2703).

3.2.3   Compound 6

The solution of **5** (370 mg, 0.70 mmol) in dichloromethane (10 ml) was flushed with ozone for 15 min at −78°C before the addition of SMe$_2$ (1 ml). The reaction mixture was then warmed to room temperature and kept stirring for additional 1 h. After removal of the dichloromethane, THF (10 ml) and saturated aqueous solution of Na$_2$SO$_3$ (10 ml) were added to the residue. After vigorously stirring for 5 h and removing the volatile fractions, the mixture was extracted with dichloromethane (20 ml × 3). The combined extracts were dried over Na$_2$SO$_4$ and concentrated. The residue obtained was purified by column chromatography over silica gel H eluting with cyclohexane: acetone (10:1) to furnish compound **6** (white amorphous powder, 280 mg, 70%): mp 106–108°C; $[\alpha]_D^{25}$ + 25.2 (c 0.5, acetone); IR (KBr) ν_{max}: 3378, 2939, 1632 cm^{-1}; ^{1}H NMR (CDCl$_3$, 400 MHz) δ 1.17 (3H, t, J = 7.2 Hz, NCH$_2$CH$_3$), 1.24 (3H, s), 1.42 (1H, m), 1.51 (1H, m), 1.87 (1H, d, J = 3.2 Hz), 2.09 (1H, m), 1.92 (1H, m), 1.90, 2.90 (each 1H, m), 2.40 (1H, dd, J = 7.2, 1.4 Hz), 2.74 (1H, t, J = 4.0 Hz), 3.27, 3.30, 3.38 (each 3H, s), 3.93 (1H, dd, J = 6.4, 4.8 Hz), 4.26 (1H, s), 4.51 (2H, s), 4.65 (2H, s), 5.02, 5.13 (each 1H, s), 5.51(1H, d, J = 6.8 Hz), 5.66 (1H, d, J = 3.6 Hz); ^{13}C NMR (CDCl$_3$, 100 MHz) δ 12.6 q, 20.4 q, 25.0 t, 35.6 t, 38.7 t, 42.6 t, 43.8 s, 50.5 d, 55.3 q, 55.8 q, 56.4 q, 58.3 d, 62.6 d, 64.1 s, 68.6 d, 75.5 d, 81.5 d, 87.3 d, 92.9 t, 95.2 t, 95.2 t, 96.4 d, 96.2 s, 171.5 s, 211.0 s; ESI-MS m/z: 554 [M + H]$^+$; HR-ESI-MS: m/z 554.2617 [M + H]$^+$ (calcd for C$_{27}$H$_{40}$NO$_{11}$, 554.2601).

3.2.4   Compound 7

To a solution of **6** (55 mg, 0.1 mmol) in anhydrous THF (2 ml) were added NaH (20 mg) and CH$_3$I (0.03 ml, 0.5 mmol), and the mixture was stirred under argon for 1 h before being quenched by the addition of ice water. The subsequent mixture was extracted with dichloromethane (5 ml × 3), the combined extracts were dried over Na$_2$SO$_4$ and concentrated. The residue obtained was chromatographed (silica gel H, cyclohexane:acetone 5:1) to give **7** (white amorphous powder, 53 mg, 95%): mp 118–120°C; $[\alpha]_D^{25}$ + 15.8 (c 0.5, acetone); IR (KBr) ν_{max}: 2845, 1650 cm^{-1}; ^{1}H NMR (CDCl$_3$, 400 MHz) δ 1.17 (3H, t, J = 7.2 Hz), 1.24 (3H, s), 1.43 (1H, m), 1.53 (1H, m), 1.86 (1H, d, J = 2.8 Hz), 1.93 (1H, dd, J = 11.2, 14 Hz), 2.08 (1H, m), 2.41 (1H, dd, J = 14.0, 6.8 Hz), 1.73 (1H, t, J = 4.0 Hz), 2.90, 4.36 (each 1H, m), 3.26, 3.30, 3.38, 3.40 (each 3H, s), 3.86 (1H, dd, J = 6.4, 4.8 Hz), 4.26 (1H, s), 4.51 (2H, S), 4.65 (2H, S), 5.05, 5.15 (each 1H, s), 5.18 (1H, s), 5.48 (1H, d, J = 6.8 Hz); ESI-MS m/z: 568 [M + H]$^+$; HR-ESI-MS: m/z 568.2772 [M + H]$^+$ (calcd for C$_{28}$H$_{42}$NO$_{11}$, 568.2758).

3.2.5   Compound 8a

To a solution of compound **7** (960 mg, 1.70 mmol) in dried THF (25 ml) was added BH$_3$.Me$_2$S (0.5 ml, 8.5 mmol, 5 equiv.) under argon, and the mixture was refluxed for 4 h. The reaction mixture was cooled down to room temperature and then quenched by the addition of ice water. The subsequent mixture was extracted with dichloromethane (30 ml × 3), the extracts were dried over Na$_2$SO$_4$ and concentrated. The residue obtained was chromatographed over silica gel H eluting with cyclohexane:acetone (5:1) to give compound **8a** as a white amorphous powder (380 mg, 40%): mp 130–132°C; $[\alpha]_D^{25}$ + 12 (c 0.5, acetone); IR (KBr) ν_{max}: 3462, 1715, 1152 cm^{-1}; ^{1}H NMR (CDCl$_3$, 400 MHz) δ 0.86 (3H, s), 1.16 (3H, t, J = 7.2 Hz), 1.40 (1H, d, J = 2.4 Hz), 1.63 (1H, m), 1.70 (1H, dd, J = 13.2, 10.4 Hz),

1.90 (1H, m), 2.26 (1H, dd, $J = 13.2$, 6.8 Hz), 2.41 (1H, s), 2.68 (1H, t, $J = 4.0$ Hz), 2.84, 3.22 (each 1H, m), 3.32, 3.33, 3.34, 3.38 (each 3H, s), 3.83 (1H, d, $J = 2.0$ Hz), 4.02 (1H, dd, $J = 10.4$, 7.2 Hz), 4.10, 4.40 (each 1H, s), 4.50 (2H, m), 4.92 (1H, d, $J = 1.2$ Hz), 5.03 (2H, s), 5.48 (1H, d, $J = 6.4$ Hz); ^{13}C NMR (CDCl$_3$, 100 MHz) δ 13.6 q, 25.0 q, 25.5 t, 33.3 s, 37.2 t, 8.4 t, 44.4 d, 47.6 d, 49.8 t, 3.9 q, 54.0 s, 54.8 q, 55.2 q, 55.7 q, 56.2 t, 60.4 d, 65.1 d, 69.7 d, 74.3 d, 78.6 d, 79.1 d, 86.1 d, 87.1 s, 92.4 t, 94.0 s, 94.8 t, 95.0 t, 102.7 d; ESI-MS m/z: 578 [M + Na]$^+$; HR-ESI-MS: m/z 556.3129 [M + H]$^+$ (calcd for C$_{28}$H$_{46}$NO$_{10}$, 556.3122).

3.2.6 Compound **9**

To a solution of compound **8a** (110 mg, 0.2 mmol) in dry dichloromethane (2 ml) were sequentially added (DMAP; 120 mg, 1 mmol, 5 equiv.) and benzoyl chloride (0.06 ml, 1.0 mmol, 5 equiv.), and the mixture was stirred at 40°C for 8 h. After quenching the reaction with 25% (v/v) ammonium hydroxide solution, the sub-sequent mixture was extracted with dichloromethane (10 ml × 3), the organic layers were combined and dried over Na$_2$SO$_4$, and the organic solvents were removed under reduced pressure. The residue was purified by column chroma-tography over silica gel H eluting with cyclohexane:acetone (20:1) to furnish compound **9** as white amorphous powder (85 mg, 85%), as well as starting material **8a** (35 mg, 32%). Compound **9**: mp 130–132°C; $[\alpha]_D^{25}$ + 30 (*c* 0.5, acetone); IR (KBr) ν_{max}: 2933, 1715, 1656, 1602, 1452, 1151, 1101 cm^{-1}; ^{1}H NMR (CDCl$_3$, 400 MHz) δ 0.91 (3H, s), 1.06 (3H, t, $J = 7.2$ Hz), 1.69 (1H, d, $J = 2.0$ Hz), 1.58 (1H, s), 2.45 (1H, s), 2.70 (1H, t, $J = 4.0$ Hz), 2.63, 2.90 (each 1H, m), 3.09 (3H, s), 3.14 (1H, m), 3.32 (3H, s), 3.41 (3H, s), 3.43 (3H, s), 4.06 (1H, d, $J = 2.4$ Hz), 4.26 (1H, s), 4.58 (1H, m), 4.62 (2H, m), 4.77 (2H, m), 4.99 (1H, s),

5.07, 5.09 (each 1H, s), 5.03 (1H, s), 5.54 (1H, d, $J = 6.4$ Hz), 5.83 (1H, d, $J = 7.6$ Hz), 7.44 (2H, m), 7.58 (1H, m), 8.15 (2H, m); ESI-MS m/z: 682 [M + Na]$^+$; HR-ESI-MS: m/z 660.3380 [M + H]$^+$ (calcd for C$_{35}$H$_{50}$NO$_{11}$, 660.3384).

3.2.7 Compound **10**

To a solution of compound **9** (110 mg, 0.17 mmol) in THF (1.5 ml) was added 4N HCl solution (0.9 ml), and the mixture was stirred at 40°C for 5 h before being quenched with 25% (v/v) ammonium hydroxide solution. The mixture was partitioned between water and dichloro-methane. The combined organic phases were dried over Na$_2$SO$_4$ and concentrated. The residue was subjected to column chromatography over silica gel H eluting with cyclohexane:acetone (3:1) to yield **10** as a white amorphous powder (40 mg, 85%): mp 116–118°C; $[\alpha]_D^{25}$ + 18 (*c* 0.5, acetone); IR (KBr) ν_{max}: 3400, 2936, 1699, 1056 cm^{-1}; ^{1}H NMR (CDCl$_3$, 400 MHz) δ 0.87 (3H, s), 1.03 (3H, d, $J = 7.2$ Hz), 2.59 (1H, t, $J = 4.0$ Hz), 2.68, 6.85 (each 1H, m), 3.08, 3.33, 3.41 (each 3H, s), 3.13 (1H, m), 4.24 (1H, s), 4.65 (2H, m), 4.99 (1H, s), 5.09 (1H, s), 5.12 (1H, s), 5.56 (1H, d, $J = 6.4$ Hz), 5.85 (1H, d, $J = 7.2$ Hz); ESI-MS m/z: 638 [M + Na] $^+$; HR-ESI-MS: m/z 616.3116 [M + H]$^+$ (calcd for C$_{33}$H$_{46}$NO$_{10}$, 616.3122).

3.2.8 Compound **12**

The solution of **10** (30 mg, 0.05 mmol) and **11** (25 mg, 0.10 mmol) in dried THF was added via syringe into the suspension of NaH (60 mg) in 1 ml of THF at 0°C, and the mixture was stirred at 35°C under argon for 4 h when TLC indicated the completion of the reaction. The reaction was quenched with brine at 0°C, the subsequent mixture was extracted with dichloromethane, and the organic solvent

was removed from the extracts. The residue was dissolved in ethanol (1 ml) and 0.5% HCl (0.5 ml), and the reaction was allowed to proceed at 0°C for 16 h before being quenched with saturated NaHCO$_3$ solution. The mixture was extracted with dichloromethane, the extracts were dried over Na$_2$SO$_4$, and the organic solvent was removed under reduced pressure to give a crude product. Chromatography of this crude product over silica gel eluting with cyclohexane:acetone (25:1) generated **12** (white amorphous powder, 20 mg, 47%): mp 160–162°C; $[\alpha]_D^{25} + 5.6$ (*c* 0.5, acetone); IR (KBr) ν_{max}: 3439, 2972, 2928, 1714, 1608, 1101 cm^{-1}; ^{1}H NMR (CDCl$_3$, 400 MHz) δ 0.89 (3H, s), 1.09 (3H, t, *J* = 7.2 Hz), 1.43 (9H, s), 2.70 (1H, t, *J* = 4.0 Hz), 3.12, 3.34, 3.41 (each 3H, s), 3.15 (1H, m), 4.06 (1H, d, *J* = 2.0 Hz), 4.27 (1H, s), 4.55 (1H, s), 4.65 (2H, m), 4.98, 5.05 (each 1H, s), 5.07 (1H, s), 5.35 (1H, m), 5.58 (1H, d, *J* = 6.4 Hz), 5.77 (1H, m), 5.86 (1H, d, *J* = 7.2 Hz), 7.28–8.15 (10H, m); ^{13}C NMR (CDCl$_3$, 100 MHz) δ 13.8 q, 24.9 q, 26.3 t, 28.16 q, 33.0 s, 36.8 t, 39.6 t, 39.6 s, 39.7 t, 45.2 d, 45.7 d, 50.3 t, 54.4 q, 55.3 q, 55.8 q, 60.1 d, 67.2 d, 69.9 d, 73.4 d, 74.5 d, 77.3 d, 78.0 d, 80.0 s, 86.2 d, 86.9 s, 92.6 t, 93.6 s, 94.8 t, 102.2 d, 126.7 d, 127.6 d, 128.3 d, 128.5 d, 130.0 d, 133.1 d, 140.0 s, 133.9 s, 154.9 s, 165.8 s, 171.8 s; ESI-MS *m/z*: 901 [M + Na]$^+$; HR-ESI-MS: *m/z* 879.4296 [M + H]$^+$ (calcd for C$_{47}$H$_{63}$N$_2$O$_{14}$, 879.4279).

3.2.9 *Compound* **13**

To a solution of **10** (10 mg, 0.015 mmol) in acetone (0.5 ml) was added Jones' reagent (0.01 ml) at 0°C, and the mixture was vigorously stirred for 10 min before being quenched with ammonium hydroxide to pH 10. The subsequent mixture was extracted with dichloromethane, and the combined extracts were dried over Na$_2$SO$_4$ and concentrated to give a residue. To a solution of this residue in THF (1 ml) was

added excess NaBH$_4$, and the mixture was kept stirring for 24 h before being quenched with water. The mixture was extracted with dichloromethane, and the combined extracts were dried over Na$_2$SO$_4$ and concentrated to give a crude product. This crude product was subjected to column chromatography over silica gel eluting with cyclohexane: acetone (5:1) to yield **13** (white amorphous powder, 7.2 mg, 80%): mp 105–107°C; $[\alpha]_D^{20} + 25.4$ (*c* 0.5, acetone); IR (KBr) ν_{max}: 3452, 2925, 1714, 1100 cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$) δ round-bottomed flask 0.89 (3H, s), 1.04 (3H, t, *J* = 7.2 Hz), 1.76 (1H, d, *J* = 2.4 Hz), 2.06 (1H, dd, *J* = 15.6, 8.0 Hz), 2.58 (1H, m), 2.66 (1H, dd, *J* = 13.2, 6.8 Hz), 2.88 (1H, m), 3.14, 3.28, 3.42 (each 3H, s), 4.17 (1H, t, *J* = 9.2 Hz), 4.34 (1H, s), 4.67 (2H, m), 4.71 (1H, d, *J* = 2.4 Hz), 4.72 (1H, s), 5.01, 5.08 (each 1H, s), 5.62 (1H, d, *J* = 6.8 Hz), 5.96 (1H, d, *J* = 7.6 Hz), 7.48 (2H, m), 7.60 (1H, m), 8.13 (2H, m); ^{13}C-NMR (CDCl$_3$, 100 MHz) δ 11.5 q, 22.4 q, 22.5 q, 27.6 d, 33.2 s, 34.3 t, 38.2 t, 43.0 d, 47.0 d, 47.7 t, 51.9 q, 52.5 t, 52.6 q, 53.36 q, 54.4 s, 57.9 d, 64.2 d, 65.1 d, 72.8 d, 75.4 d, 75.8 d, 84.3 d, 86.3 s, 90.1 t, 92.5 t, 92.9 s, 103.2 d, 126.1 d, 129.7 s, 131.0 d, 165.7 s; ESI-MS *m/z*: 638 [M + Na]$^+$; HR-ESI-MS: *m/z* 616.3126 [M + H]$^+$ (calcd for C$_{33}$H$_{46}$NO$_{10}$, 616.3122).

3.2.10 *Compound* **14**

The solution of **13** (6 mg, 0.01 mmol) and **11** (4.8 mg, 0.02 mmol) in THF (1 ml) was added dropwise by a syringe to the suspension of NaH (11 mg) in THF (1 ml) at 0°C, and the mixture was stirred at 35°C under argon for 3 h. The reaction was quenched by the addition of brine (1 ml) at 0°C, and the was extracted with dichloromethane (5 ml × 3). The combined extracts were dried over sodium sulfate and concentrated to give a residue, which was dissolved in ethanol (1 ml) and 0.5% HCl (0.5 ml) at 0°C. The reaction

was kept to proceed for an additional 10 h before quenching with saturated $NaHCO_3$ solution (3 ml). The mixture was extracted with dichloromethane (5 ml × 3), and the combined extracts were dried over anhydrous sodium sulfate and concentrated to a crude product. Purification of the crude product through column chromatography (silica gel, cyclohexane:acetone /25:1) furnished **14** as a white amorphous powder (5 mg, 60%): mp 140–142°C; $[\alpha]_D^{25}$ − 6.2 (*c* 0.25, acetone); IR (KBr) ν_{max}: 3354, 2965, 2928, 1714, 1102 cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$) δ 0.94 (3H, s), 1.06 (3H, t, *J* = 6.8 Hz), 1.43 (9H, s), 3.27 (3H, s), 3.42 (3H, s), 4,23 (1H, s), 4.65 (2H, m), 4.80 (1H, s), 4.88 (1H, s), 4.98 (1H, s), 5.07 (1H, m), 5.59 (1H, d, *J* = 6.0 Hz), 5.82 (1H, s), 5.91 (1H, d, *J* = 6.8 Hz); ^{13}C NMR (CDCl$_3$, 100 MHz) δ 13.8 q, 25.3 q, 26.1 t, 28.3 q, 33.4 s, 37.1 t, 37.1 t, 46.0 d, 46.0 d, 51.2 t, 53.8 t, 54.2 q, 55.8 d, 55.9 q, 56.1 s, 56.3 q, 60.3 d, 67.1 d, 69.5 d, 74.1 d, 74.3 d, 78.2 d, 79.4 s, 80.6 d, 84.7 s, 85.9 d, 92.3 t, 94.9 t, 94.3 s, 105.2 d, 126.9 d, 127.4 d, 128.0 d, 128.6 d, 129.8 d, 133.3 d, 133.4 s, 139.6 s, 154.9 s, 166.2 s, 172.1 s; ESI-MS *m/z*: 901 [M + Na]$^+$; HR-ESI-MS: *m/z* 879.4296 [M + H]$^+$ (calcd for C$_{47}$H$_{63}$N$_2$O$_{14}$, 879.4279).

3.2.11 *Compound* **15**

The solution of **7** (560 mg, 1 mmol) in methanol (20 ml) and concentrated hydrochloric acid (0.2 ml) was refluxed for 1.5 h. After cooling to room temperature, the reaction mixture was basified with ammonia hydroxide to pH 10 and extracted with dichloromethane. The extracts were dried over Na$_2$SO$_4$ and concentrated to give a residue, which was chromatographed over silica gel H (cyclohexane:acetone /5:1) to yield **15** (white amorphous powder, 270 mg, 95% based on the recovery of the starting material) and recover starting material (250 mg, 45%). Compound **15**: mp 84–86°C; $[\alpha]_D^{25}$ + 14.8(*c* 0.5, acetone); IR (KBr) ν_{max}: 3420, 1633,

1262, 1031, 1154 cm^{-1}; ^{1}H NMR (CDCl$_3$, 400 MHz) δ 1.16 (3H, t, *J* = 7.2 Hz), 1.24 (3H, s), 3.87 (1H, dd, *J* = 4.8, 6.4 Hz), 4.37 (1H, d, *J* = 3.2 Hz), 4.29, 2.87 (each 1H, m), 4.25 (1H, s), 4.45 (1H, dd, *J* = 10.8, 7.6 Hz), 4.51 (2H, m), 5.03, 5.13 (each 1H, s), 5.20 (1H, s), 5.50 (1H, d, *J* = 6.4 Hz), 2.41 (1H, dd, *J* = 13.6, 6.4 Hz), 2.65 (1H, t, *J* = 4.8 Hz), 3.26, 3.30, 3.41 (each 3H, s); ^{13}C NMR (CDCl$_3$, 100 MHz) δ 12.5 q, 20.4 q, 24.9 t, 35.6 t, 40.6 t, 43.5 t, 43.8 s, 52.0 d, 54.8 q, 55.8 q, 56.7 q, 57.0 d, 63.4 d, 63.6 d, 63.7 s, 74.7 d, 81.5 d, 86.2 s, 87.2 d, 92.8 t, 92.9 s, 95.2 t, 102.4 d, 171.5 s, 210.6 s; ESI-MS *m/z*: 524 [M + H]$^+$; HR-ESI-MS: *m/z* 524.2479 [M + H]$^+$ (calcd for C$_{26}$H$_{38}$NO$_{10}$, 524.2496).

3.2.12 *Compound* **16**

The solution of **15** (52 mg, 0.1 mmol) and **11** (38 mg, 0.15 mmol) in THF (4.5 ml) was added dropwise into the suspension of NaH (100 mg) in THF (4.5 ml) at 0°C, and the reaction mixture was warmed to 35°C and stirred for 2 h before being quenched with brine (1 ml) at 0°C. The subsequent mixture was extracted with dichloromethane (10 ml × 3), and the combined extracts were dried over anhydrous Na$_2$SO$_4$ and concentrated to give a residue. The solution of the residue in ethanol (10 ml) and 0.5% hydrochloric acid (7 ml) was stirred as room temperature for 18 h before the addition of saturated NaHCO$_3$ (2 ml). The consequent mixture was extracted with dichloromethane, and the dichloromethane fractions were dried over Na$_2$SO$_4$ and concentrated. The residue obtained was purified by column chromatography (silica gel H, cyclohexane:acetone /5:1) to furnish **16** (white 42 mg, 60%): mp 152–154°C; $[\alpha]_D^{25}$ + 34.2 (*c* 0.5, acetone); IR (KBr) ν_{max}: 3438, 1747, 1458, 1160 cm^{-1}; ^{1}H NMR (CDCl$_3$, 400 MHz) δ 1.19 (3H, t, *J* = 7.2 Hz), 1.21 (3H, s), 1.41 (9H, s), 1.86 (1H, d, *J* = 2.8Hz), 2.53 (1H, dd,

$J = 13.6, 6.4\,\text{Hz}$), 3.23, 3.28, 3.42 (each 3H, s), 3.92 (1H, t, $J = 6.0\,\text{Hz}$), 4.25 (1H, s), 4.39 (1H, dd, $J = 11.2, 8.0\,\text{Hz}$), 4.47 (2H, m), 4.55 (1H, m), 5.00, 5.06 (each 1H, s), 5.18 (1H, s), 5.49 (1H, d, $J = 6.8\,\text{Hz}$), 5.60 (1H, m), 5.30 (1H, m); ^{13}C NMR (CDCl$_3$, 100 MHz) δ 12.1 q, 20.3 q, 24.8 t, 28.1 q, 30.7 d, 35.6 t, 36.8 t, 42.9 t, 43.5 s, 48.7 d, 54.7 q, 55.8 q, 56.7 q, 58.2 d, 58.9 d, 62.8 d, 64.0 s, 68.4 d, 73.4 d, 75.0 d, 80.0 s, 81.5 d, 86.8 d, 86.0 s, 92.9 t, 95.0 t, 102.5 d, 126.7 d, 126.7 d, 127.7 d, 128.0 d, 128.0 d, 138.8 s, 155.0 s, 171.1 s, 171.7 s, 210.3 s; ESI-MS *m/z*: 809 [M + Na]$^+$; HR-ESI-MS: *m/z* 787.3623 [M + H]$^+$ (calcd for C$_{40}$H$_{55}$N$_2$O$_{14}$, 787.3653).

3.2.13 Compound **17**

To a solution of **15** (120 mg, 0.23 mmol) in acetone (2 ml) was added dropwise Jones' reagent (0.12 ml) at 0°C, and the mixture was vigorously stirred for 0.5 h. The reaction was quenched by ammonium hydroxide to pH 10, and the mixture was extracted with dichloromethane. The extracts were dried over Na$_2$SO$_4$, and the dichloromethane was removed in vacuum. The residue obtained was chromatographed over silica gel H, eluting with cyclohexane:acetone (4:1), to give 115 mg of pure compound. To the solution of this pure compound (10 mg, 0.016 mmol) in THF (2 ml) was added excess sodium borohydride at 0°C, and the mixture was stirred for 1 h. The reaction was quenched by the addition of water, the mixture was extracted with dichloromethane, and the combined extracts were dried over sodium sulfate and concentrated. The residue obtained was subjected to column chromatography eluting with cyclohexane:acetone (4:1) to furnish **17**: mp 90–92°C; $[\alpha]_{\text{D}}^{25} - 4.2$ (*c* 0.5, acetone); IR (KBr) ν_{max}: 3432, 1657, 1103 cm^{-1}; ^1H NMR (CDCl$_3$, 400 MHz) δ 1.15 (3H, t, $J = 7.2\,\text{Hz}$), 1.26 (3H, s), 1.44, 1.60 (each 1H, m), 1.94 (1H, d, $J = 3.6\,\text{Hz}$), 1.97 (1H,

d, $J = 2.8\,\text{Hz}$), 2.08 (1H, m), 2.73 (1H, t, $J = 2.0\,\text{Hz}$), 2.88, 4.31 (each 1H, m), 3.31, 3.72, 3.37 (each 3H, s), 4.0 (1H, dd, $J = 3.6, 6.4\,\text{Hz}$), 4.20 (1H, t, $J = 3.6\,\text{Hz}$), 4.29 (1H, s), 4.48 (1H, dd, $J = 7.2, 11.6\,\text{Hz}$), 4.52 (2H, m), 5.04, 5.12 (each 1H, s), 5.46 (1H, d, $J = 2.8\,\text{Hz}$), 5.53 (1H, d, $J = 6.8\,\text{Hz}$); ^{13}C NMR (CDCl$_3$, 100 MHz) δ 12.4 q, 20.4 q, 24.2 t, 35.6 t, 40.4 t, 42.6 t, 43.7 s, 53.2 d, 54.6 q, 55.8 q, 56.3 q, 57.8 d, 58.5 d, 62.2 d, 63.9 s, 66.1 d, 75.4 d, 82.4 d, 85.4 s, 87.2 s, 97.2 t, 95.2 t, 105.5 d, 171.1 s, 210.5 s; ESI-MS *m/z*: 524 [M + H]$^+$; HR-ESI-MS: *m/z* 524.2484 [M + H]$^+$ (calcd for C$_{26}$H$_{38}$NO$_{10}$, 524.2496).

3.2.14 Compound **18**

The solution of **17** (20 mg, 0.038 mmol) and **11** (13.5 mg, 0.054 mmol) in THF (1.5 ml) was added dropwise into the suspension of NaH (20 mg) in THF (1.5 ml) at 0°C, and the reaction mixture was warmed to 35°C and stirred for 2 h before being quenched with brine (3 ml) at 0°C. The subsequent mixture was extracted with dichloromethane (10 ml × 3), and the combined extracts were dried over anhydrous Na$_2$SO$_4$ and concentrated to give a residue (40 mg). The solution of the residue (10 mg) in ethanol (1.5 ml) and 0.5% hydrochloric acid (1.5 ml) was stirred at 0°C for two days before the addition of saturated NaHCO$_3$ (1 ml). The consequent mixture was extracted with dichloromethane (5 ml × 3), and the dichloromethane fractions were dried over Na$_2$SO$_4$ and concentrated. The residue obtained was purified by column chromatography (silica gel H, cyclohexane:acetone/ 10:1) to furnish **18** (2 mg, 7%): mp 140–142°C; $[\alpha]_{\text{D}}^{25} - 24.2$ (*c* 0.1, acetone); IR (KBr) ν_{max}: 3444, 1653, 1714, 1455 cm^{-1}; ^1H NMR (CDCl$_3$, 400 MHz) δ 1.34 (3H, t, $J = 7.2\,\text{Hz}$), 1.24 (3H, s), 1.40 (9H, s), 1.67 (1H, m), 2.82, 3.98 (each 1H, m), 2.81 (1H, m), 3.22, 3.30, 3.37 (each 3H, s), 3.46 (1H, m), 4.00 (1H, m), 4.20 (1H, s), 4.49 (1H, d, $J = 6.4\,\text{Hz}$), 4.51 (2H, s), 4.86, 5.00 (each

1H, s), 5.05 (1H, s), 5.24 (1H, s), 5.49 (1H, s), 5.60 (1H, d, $J = 6.8$ Hz), 7.24–7.38 (5H, m); ^{13}C NMR (CDCl$_3$, 100 MHz) δ 12.5 q, 20.7 q, 24.2 t, 28.3 q, 30.9 d, 35.6 t, 36.7 t, 44.2 s, 46.9 t, 49.9 d, 54.7 q, 56.0 q, 58.4 q, 75.5 d, 56.4 d, 63.5 s, 64.4 d, 68.4 d, 74.1 d, 74.2 d, 79.8 s, 83.6 s, 83.9 d, 86.6 d, 92.5 t, 94.2 s, 95.2 t, 104.7 d, 126.8 d, 126.8 d, 127.7 d, 128.3 d, 128.3 d, 139.1 s, 154.7 s, 171.8 s, 171.8 s, 208.9 s; ESI-MS m/z: 809 [M + Na]$^+$; HR-ESI-MS: m/z 787.3658 [M + H]$^+$ (calcd for C$_{40}$H$_{55}$N$_2$O$_{14}$, 787.3653).

Acknowledgement

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