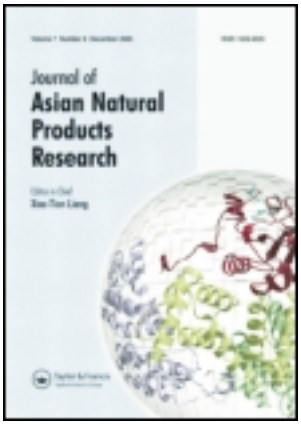

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Regioselective *O*-demethylation of two C_{19}-diterpenoid alkaloids

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The regioselective demethylations of two C_{19}-diterpenoid alkaloids, **2** and **3**, have been achieved with HBr–HOAc, trimethylsilyl iodide, or BBr_3. It was observed that HBr–HOAc is an optimal demethylating agent for these two C_{19}-diterpenoid alkaloids because it could provide different *O*-demethylation products by using different reaction temperature and reaction time. Especially, 1-*O*-methyl group in **2** and **3**, one of the most difficult ones to be demethylated, could be removed by the treatment with HBr–HOAc at an elevated temperature and a prolonged reaction time.

Keywords: C_{19}-diterpenoid alkaloids; *O*-demethylation; lycoconitine; talatisamine

1. Introduction

The C_{19}-diterpenoid alkaloids are a group of highly oxygenated and complex natural compounds. By the end of July 2008, approximately 672 C_{19}-diterpenoid alkaloids were isolated from about 315 species of plants, most of which belong to the two genera *Aconitum* and *Delphinium* in the family Ranunculaceae [1–4]. The C_{19}-diterpenoid alkaloids have been studied for over 100 years, and the prime and lasting attention of researchers to them is due to their interesting chemical reactions [1,3,4,5] and varied pharmacological activities [2–4,6]. Methoxyl groups are one type of the most common oxygenated groups for the C_{19}-diterpenoid alkaloids, and in most cases, the methoxyl groups are located at C-1, C-6, C-14, C-16, and C-18.

Regioselective *O*-demethylation of C_{19}-diterpenoid alkaloids is very important for their chemical transformations and for structure–activity relationship studies. Following the first investigation carried out by Jacobs and Craig [7] on the partial *O*-demethylation of alkaloids with HCl and with HNO_3, various reagents such as $ZnCl_2$-5% HCl [7–9], HI–phosphorus [10], 50% H_2SO_4 [11, 12], HBr–HOAc [13–17], $AlCl_3$/NaI [15], and Me_3SiI [17,18] were also applied to the *O*-demethylation of C_{19}-diterpenoid alkaloids. However, these *O*-demethylations are not actually very practical due to lower yields or limited substrates. Especially, it has been demonstrated that 1-*O*-demethylation is extremely difficult [16]. Indeed, 1-*O*-demethylation is currently a bottleneck reaction during our ongoing project to modify the C-1 position of the C_{19}-diterpenoid alkaloids, which prompted us to search for new methods for 1-*O*-demethylation. Herein, we describe some useful methods for *O*-demethylations of two C_{19}-diterpenoid alkaloids, **2** and **3**, together with the selectivity of the

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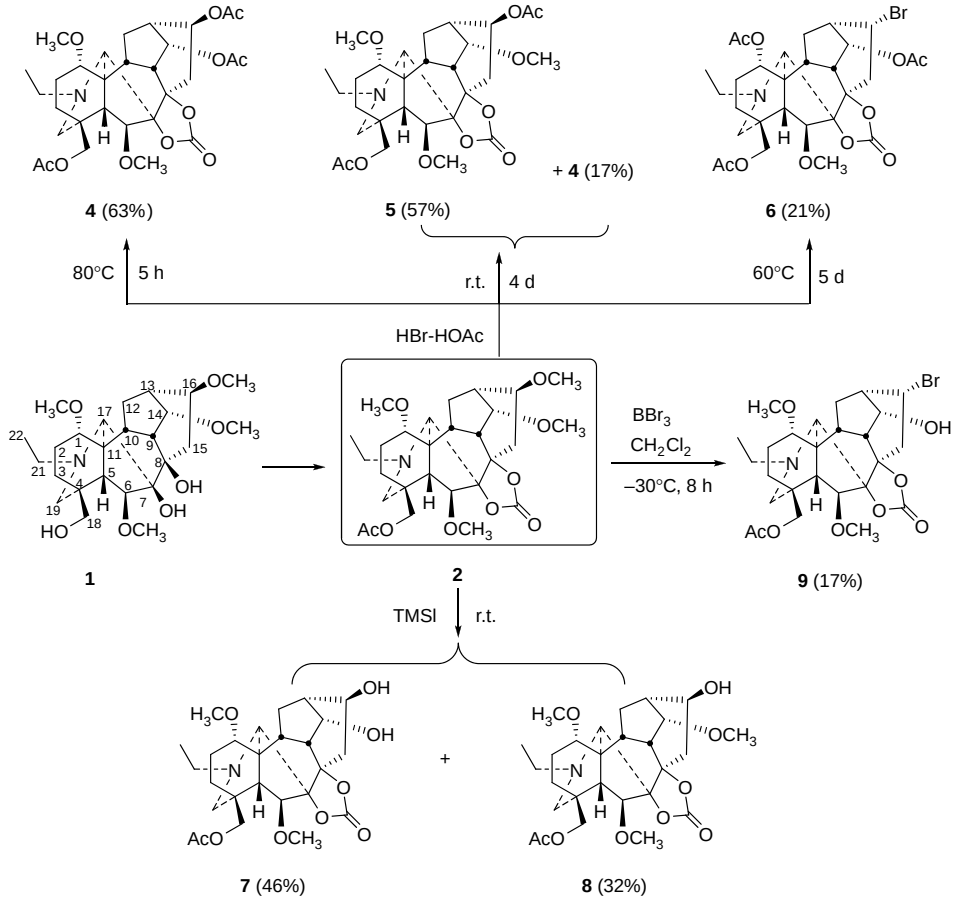
O-demethylation under different reaction conditions.

2. Results and discussion

Recently, we have reported that the methoxyl groups at C-14, C-16, and/or C-18 [16] of the C_{19}-diterpenoid alkaloids could be demethylated using HBr–HOAc (20 equiv.) at 50–80°C for 7–20 h in good yields [16]. However, the methoxyl group at C-1, which should be removed during our ongoing project to make the C-1 modified analogs, survived throughout these procedures. This spurred us to search for a new method for 1-*O*-demethylation of the C_{19}-diterpenoid alkaloids. Considering that the above-mentioned *O*-demethylation of the C_{19}-diterpenoid alkaloids with HBr–HOAc could tolerate the relative higher temperature and the longer reaction time [16], we envisioned that it might be possible to obtain 1-*O*-demethylated products by further prolonging reaction time or elevating the reaction temperature.

Accordingly, we further explored the *O*-demethylation with HBr–HOAc employing **2** as a model compound, which was prepared from lycoconitine **1** by protecting all of its hydroxyl groups. It was observed that regioselective *O*-demethylation of compound **2** was achieved by treating with 6.5% HBr–HOAc by tuning reaction temperature and reaction time (Scheme 1). The corresponding 14,16-di-*O*-demethylated product **4** was obtained in 63% yield by treating **2** with HBr–HOAc at



Scheme 1. *O*-demethylations of compound **2**.

80°C for 5 h, which is consistent with our reported results [16]. The lower reaction temperature (room temperature) provided the 16-*O*-demethylated product **5** (57%) as a major product, even though the reaction time was prolonged to 4 days. Interestingly, an elevated reaction temperature (60°C) in combination with a longer reaction time (5 days) did provide 1,14,16-tri-*O*-demethylation product **6**, in which 1,14-methoxyl groups were replaced with acetoxyl groups, and 16-methoxyl group was substituted by a bromine atom (Scheme 1). It is worthy of note that we obtained the 1-*O*-demethylation product **6**, even though the yield (21%) is not good enough. The structures of **4** and **5** were readily established by comparison of their NMR data with those of **2** (Table 1). Specifically, the NMR data of **4** showed that the methoxyl groups at C-14 and C-16 in **2** were replaced with two acetoxyl groups. Similarly, the NMR data of **5** showed the presence of an acetoxyl group at C-16 instead of the methoxyl group in **2**.

The molecular formula $C_{29}H_{38}NO_{10}Br$ of **6** was inferred from its HR-ESI-MS (*m/z* 640.1749 $[M + H]^+$, calcd for 640.1757) and ^{13}C NMR. As compared with **2**, its

Table 1. ^{13}C NMR spectral data for compounds **2**–**5**, **7**, **8**, and **11** (50 MHz, $CDCl_3$).

No.	**2**	**3**	**4**	**5**	**7**	**8**	**11**
1	82.6	85.5	80.7	80.8	82.4	82.4	86.0
2	26.0	26.0	25.9	26.1	25.4	25.4	28.7
3	31.1	26.7	31.2	31.0	31.9	31.3	29.3
4	36.7	38.9	36.8	36.9	37.2	37.2	38.8
5	39.4	41.9	39.3	39.3	41.9	39.3	41.6
6	87.4	37.1	87.1	87.3	87.2	87.4	42.7
7	96.2	126.4	96.5	96.8	97.5	97.5	127.6
8	88.8	126.1	87.3	87.0	86.0	86.0	128.2
9	50.9	77.6	49.9	50.0	50.0	50.3	81.2
10	38.7	38.6	38.4	39.1	39.3	37.7	38.3
11	50.8	42.9	50.1	49.5	49.2	49.1	43.0
12	27.5	25.8	26.5	26.8	26.8	26.6	25.8
13	48.1	45.8	46.9	47.1	47.6	47.5	45.8
14	80.1	174.2	72.2	82.1	71.3	82.4	173.8
15	34.2	34.1	33.9	35.1	37.4	37.6	33.8
16	80.3	85.5	73.6	73.6	73.6	71.2	54.2
17	62.5	53.7	62.3	62.6	62.6	62.5	53.7
18	68.5	78.1	68.4	68.3	68.5	68.5	67.8
19	52.1	53.1	52.2	52.2	52.8	52.6	52.5
21	50.2	50.6	50.1	50.1	50.2	50.2	51.7
22	13.6	12.0	13.6	13.6	13.7	13.7	12.3
1-OCH_3	54.9	57.2	55.1	55.1	55.7	55.7	57.3
6-OCH_3	59.2	–	59.5	59.5	59.4	59.6	–
14-OCH_3	57.8	–	–	58.1	–	58.2	–
16-OCH_3	56.3	56.3	–	–	–	–	–
18-OCH_3	–	59.1	–	–	–	–	–
O=C	170.7	–	170.8	170.8	170.8	170.8	–
CH_3	20.7	–	21.6	21.6	20.8	20.7	–
O=C	–	–	170.7	170.7	–	–	–
CH_3	–	–	21.6	21.6	–	–	–
O=C	–	–	170.2	–	–	–	–
CH_3	–	–	20.7	–	–	–	–
OCOO	155.3	–	155.1	155.1	155.4	155.4	–

NMR data showed that the methoxyl groups at C-1 and C-14 in **2** were replaced with two acetoxyl groups in **6**. The chemical shift of C-16 in **6** upshifted from δ_C 80.3 in **2** to δ_C 48.2, indicating that the methoxyl group at C-16 in **2** was substituted by a bromine atom in **6**. The structure of **6** was supported by its 2D NMR ($^1H-^1H$ COSY, HMQC, and HMBC) data (Table 2). The β-orientation of H-16 in compound **6** was deduced from the correlation between H-16 and H-9β in the NOEds spectrum.

We also explored the demethylations of compound **2** using other Lewis acids, such as trimethylsilyl iodide (TMSI) and BBr_3. It has been reported by Pelletier *et al.* [18] that demethylation of delphinine with TMSI afforded 18-*O*-desmethyl delphinine and 16,18-di-*O*-desmethyl delphinine.

Table 2. 1D and 2D NMR spectral data of compound **6** (400 MHz for ^1H, 100 MHz for ^{13}C, $CDCl_3$).

No.	δ_C	δ_H mult (J = Hz)	HMBC (H → C)	NOEds
1	75.3	4.72 hidden	C-10, C-17, 1-OCOCH$_3$	–
2	26.8	2.02 m	C-4, C-11	H-1
3	31.1	1.68 m	C-5	H-18, H-19
		1.45 m	C-19	
4	37.1	–	–	–
5	49.7	1.76 s	C-7, C-10, C-17, C-18, C-19	H-2, H-18, 6-OC*H*$_3$
6	86.5	3.88 s	C-4, C-8, 6-O*C*H$_3$	–
7	96.0	–	–	–
8	86.4	–	–	–
9	38.3	4.07 t (8.4)	C-12, C-15	H-16
10	45.9	2.17 m	C-8, C-14	–
11	48.5	–	–	–
12	23.7	1.78 m	C-16	H-9, H-16
		2.19 hidden	C-14	H-9, H-13, H-14
13	41.5	2.61 t (7.6)	C-9, C-10	H-16
14	73.6	4.76 hidden	C-8, C-10, C-12, C-16, 14-O*C*OCH$_3$	–
15	39.1	2.34 dd (16.4, 7.6)	C-13	H-9
		2.76 hidden	C-7	
16	48.2	4.78 hidden	C-12	–
17	62.6	4.05 s	C-19	–
18	67.9	2.88 ABq (17.2, 12.0)	C-19	–
19	52.7	2.47 d (15.6)	C-3, C-18, C-21	H-18
		2.76 hidden	C-3, C-7, C-21	
21	49.9	2.71 hidden	–	–
		2.87 m		
22	13.6	1.12 t (7.2)	C-21	–
6-OCH$_3$	59.4	s	C-6	–
O=C	170.7	–	–	–
CH$_3$	21.6	s	–	–
O=C	170.3	–	–	–
CH$_3$	21.0	s	–	–
O=C	170.3	–	–	–
CH$_3$	20.7	s	–	–
OCOO	154.6	–	–	–

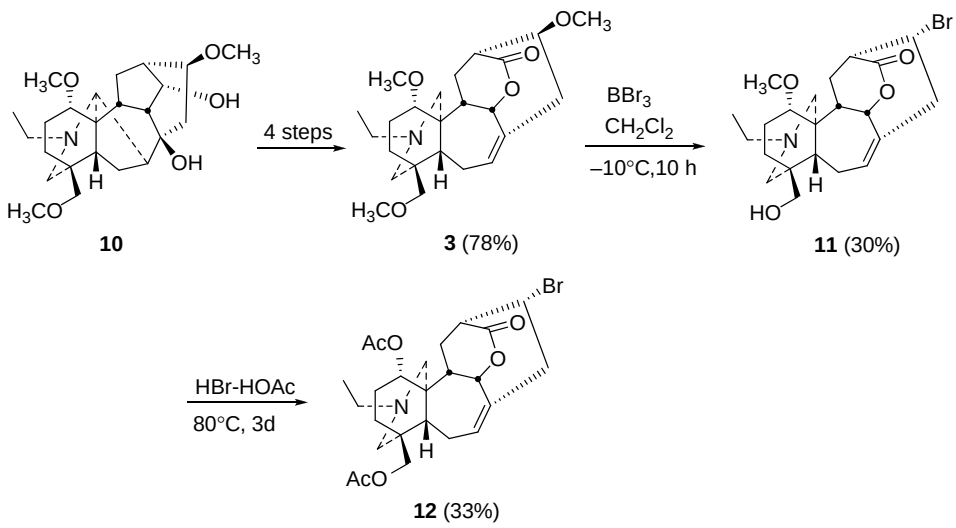
When compound **2** was treated with TMSI at room temperature, 14,16-*O*-desmethyl analog **7** (46%) and 16-*O*-desmethyl analog **8** (32%) were obtained (Scheme 1). Both compounds exhibited NMR spectral data consistent with the proposed structures (Table 1).

Blagbrough *et al.* [15] pointed out that demethylation of aconitine with BBr_3 was unsuccessful due to the decomposition during the reaction. In our experiment, 14,16-di-*O*-demethylation product **9** was obtained in 17% yield by the reaction of compound **2** with BBr_3 in CH_2Cl_2 at $-30^{\circ}C$ for 8 h (Scheme 1). Compound **9** has the molecular formula $C_{26}H_{36}NO_8Br$ based on its HR-ESI-MS and ^{13}C NMR data. The NMR spectra of **9** showed that the methoxyl group at C-14 in **2** was replaced with a hydroxyl group. The chemical shift of C-16 was upshifted from δ_C 82.6 in **2** to δ_C 48.8, implying that the methoxyl group at C-16 in **2** was substituted by a bromine atom. The proposed structure of **9** was confirmed by its 2D NMR ($^1H-^1H$ COSY, HMQC, and HMBC) (Table 3). The β-orientation of H-16 in **9** was established by the signal enhancement at H-9 (δ_H 2.54) when irradiating at H-16 (δ_H 5.07) in the NOEds spectrum.

Encouraged by the successful 1-*O*-demethylation of **2**, we explored the *O*-demethylation of 7,17-*seco* C_{19}-diterpenoid alkaloid **3**, which was prepared from talatisamine **10** in four steps with 61% overall yield (Scheme 2). Compound **3**

Table 3. 1D and 2D NMR spectral data of compound **9** (400 MHz for ^1H, 100 MHz for ^{13}C, $CDCl_3$).

No.	δ_C	δ_H mult (J = Hz)	HMBC (H → C)
1	82.6	3.05 t (10.0)	C-5, C-17,1-OCH_3
2	24.6	2.58 m	C-11
3	31.2	1.72 dt (12.8, 3.2) 1.34 dd (14.0, 5.2)	C-5, C-19
4	37.3	–	–
5	50.1	1.61 s	C-1, C-7, C-17, C-18, C-19
6	87.0	3.86 hidden	C-4, C-8, 6-O*C*H_3
7	96.7	–	–
8	87.0	–	–
9	44.0	2.54 d (7.2)	C-15
10	41.2	3.81 t, 4.8	C-8, C-13, C-14
11	49.8	–	–
12	25.1	2.04 hidden	C-11
13	47.3	2.03 hidden	C-10
14	73.2	4.07 t (4.8)	C-8, C-16
15	39.0	2.27 dd (16.0, 6.8) 2.69 m	C-9, C-13
16	48.8	5.07 d (9.2)	C-12
17	63.5	4.02 s	C-5, C-6, C-19
18	68.4	3.83 m	C-3, C-19, 18-O*C*OCH_3
19	52.5	2.40 d (11.6) 2.74 hidden	C-3, C-5, C-17, C-18, C-21
21	50.3	2.72 hidden 2.81 hidden	C-17, C-19
22	13.7	1.03 t (7.2)	–
6-OCH_3	59.5	3.40 s	C-6
16-OCH_3	56.0	3.33 s	C-16
O=C	170.8	–	–
CH_3	20.7	s	–
OCOO	154.8	–	–



Scheme 2. *O*-demethylations of compound **3**.

was firstly treated with BBr_3 in CH_2Cl_2 at $-10°C$ for 6 h to generate 16-demethoxyl-18-*O*-demethyl analog **11** in 30% yield. The structure assignment of **11** was consistent with the detailed NMR spectral studies (Table 1). The further *O*-demethylation of **11** with 6.5% HBr–HOAc at 80°C for 3 days afforded 1-*O*-demethylation product **12** in 33% yield. Compound **12** has the molecular formula $C_{25}H_{34}NO_6Br$ (HR-ESI-MS and ^{13}C NMR). Its NMR spectra showed that the methoxyl groups at C-18 and C-1 in **3** were substituted by acetoxyl groups. The upshifted signal of C-16 from δ_C 85.5 in **3** to δ_C 52.0 implied the replacement of the 16-OMe in **3** with a bromine atom. Its proposed structure was confirmed by the 2D NMR ($^1H-^1H$ COSY, HMQC, and HMBC) data (Table 4). The absolute configuration at C-16 was established by the observation that irradiating the signal at H-16 (δ_H 3.93) led to the signal enhancement at H-9β (δ_H 4.92) in its NOEds spectrum.

In conclusion, the regioselective *O*-demethylations of two C_{19}-diterpenoid alkaloids, **2** and **3**, were achieved with HBr–HOAc, TMSI, and BBr_3. Treatment with TMSI at room temperature is a mild condition, which selectively demethylated first the 16-methoxyl group to afford **8** and 14,16-di-*O*-desmethyl analog **7** in good yields. In contrast, the treatment with intense Lewis acid BBr_3 led to the much more complicated products, even at much lower reaction temperature. After careful separations, 14-*O*-desmethyl-16-demethoxyl-16-bromo analog **9** (17%) of **2** and 16-demethoxyl-16-bromo analog **11** (30%) of **3** were obtained. It is worth noting that HBr–HOAc is an optimal demethylating agent for the C_{19}-diterpenoid alkaloids because it could provide different *O*-demethylation products by using different reaction temperature and reaction time. Especially, 1-*O*-methyl group in **2** and **3** could be removed by the treatment with HBr–HOAc at a somewhat higher temperature for 3 to 5 days.

3. Experimental

3.1 General experimental procedures

Melting points were determined on a Kofler block (uncorrected). Optical rotations were measured on a PerkinElmer 341 polarimeter. IR spectra were obtained on a Nicolet FT-IR 200 SXV spectrophotometer. ^1H and ^{13}C NMR spectra were taken on a Varian

Table 4. 1D and 2D NMR spectral data of compound **12** (400 MHz for ^{1}H, 100 MHz for ^{13}C, $CDCl_3$).

No.	δ_C	δ_H mult (J = Hz)	HMBC (H → C)
1	80.4	4.61 dd (10.8, 6.8)	C-17, 1-O*C*OCH$_3$
2	26.9	2.09 hidden	C-4
		2.73 hidden	C-4
3	33.7	1.63 dd (13.6, 6.4)	C-1, C-5, C-18, C-19
		1.73 dd (13.6, 6.0)	C-5, C-18, C-19
4	37.5	–	–
5	38.4	2.15 hidden	C-1, C-3, C-10, C-18, C-19
6	26.9	1.98 m	C-7, C-8, C-15
		2.33 hidden	
7	126.6	5.49 brs	C-5, C-15
8	128.6	–	–
9	80.8	4.92 d (9.6)	C-12, C-14, C-15
10	45.9	4.04 s	C-7, C-8, C-14
11	41.6	–	–
12	28.7	2.00 m	C-13, C-16
		2.28 m	
13	41.8	2.08 hidden	C-10, C-15
14	172.4	–	–
15	42.1	2.47 hidden	C-7
		2.76 dd (13.6, 7.2)	C-9
16	53.7	3.93 dd (10.4, 7.2)	C-12
17	52.0	2.33 hidden	C-5
18	68.4	3.71 ABq (18.6, 11.2)	C-3, C-5, C-19, 18-O*C*OCH$_3$
19	53.4	2.13 d (8.8)	C-3, C-5, C-18, C-21
		2.47 hidden	
21	50.7	2.15 m	C-19, C-22
		2.82 m	
22	12.2	1.04 t (7.2)	–
O=C	170.1	–	–
CH$_3$	21.2	2.06 s	–
O=C	170.7	–	–
CH$_3$	21.2	2.08 s	–

Unity INOVA 400/54 NMR spectrometer in $CDCl_3$ with TMS as the internal standard. The ESI-MS and HR-ESI-MS were recorded on a VG Auto Spec-3000 or a Finnigan MAT 90 instrument. Silica gel H (Qingdao Marine Chemical Factory, Qingdao, China) was used for column chromatography. Zones on TLC (silica gel G) plates were detected with the modified Dragendorff's reagent.

3.2 *Preparation of compounds 2 and 3*

3.2.1 *Compound* **2**

This compound was prepared by treating lycoconitine (**1**) with triphosgene in CH_2Cl_2–pyridine at −10°C overnight. Compound **2**: mp 140–142°C; $[\alpha]$ + 33.0 (c = 1.0, $CHCl_3$); IR (KBr) ν_{max}: 2937, 2889, 1796, 1224 cm^{-1}; ^{1}H NMR (400 MHz) δ 1.05 (3H, t, J = 6.8 Hz, *N*CH_2*C*H_3), 2.07 (3H, s, OAc), 3.24, 3.33, 3.36, 3.39 (each 3H, s, 4 × OCH$_3$), 3.63 (1H, t, J = 4.4 Hz, H-14β); ^{13}C NMR spectral data, see Table 1; HR-ESI-MS: m/z 536.2854 $[M + H]^+$ (calcd for $C_{28}H_{42}O_9N$, 536.2860).

3.2.2 *Compound* **3**

Compound **3** was prepared from talatisamine (**10**). To a solution of **10** (500 mg,

1.19 mmol) in acetone, Jone's reagent (2 ml) was added, and the reaction was allowed to proceed for 3 h. After basifying with concentrated solution of ammonium hydroxide, the mixture was extracted with $CHCl_3$, and the combined extracts were dried and concentrated to give a residue. This residue was dissolved in H_2O_2–HCOOH (1:1, 5 ml), and the solution was kept standing at 25°C overnight prior to being basified with concentrated solution of ammonium hydroxide. The subsequent mixture was extracted with chloroform, and the combined extracts were dried and concentrated to afford a residue. To a solution of this residue in THF $SOCl_2$ (3 ml) was added, and the reaction was allowed to proceed at 25°C for 4 h prior to the addition of $NaBH_4$ (150 mg, 3.9 mmol). The subsequent mixture was stirred for 1 h prior to the removal of the solvent, the residue obtained was purified by column chromatography (silica gel H, $CHCl_3$–MeOH; 97:3) to afford compound **3** (white amorphous powder, 300 mg, 61%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.27, 3.31, 3.34 (each 3H, s, 3 × OCH_3), 4.73 (1H, d, $J = 10.0$ Hz, H-9), 5.43 (1H, s, H-7); ^{13}C NMR spectral data, see Table 1, ESI-MS *m/z* (%): 420 ($[M + H]^+$, 100).

3.3 *O-demethylation of compounds 2 and 3*

3.3.1 *General procedure for the demethylations with HBr–AcOH*

The substrates are dissolved in 6.5% HBr–HOAc, and the reaction solution was stirred at the specified temperature and for a specified time section shown in the sections below prior to being poured into ice water. The mixture was extracted with chloroform after basifying with concentrated NH_4OH. The chloroform extracts were dried over anhydrous sodium sulfate and concentrated to give a residue, which was subjected to column chromatography (silica gel H, $CHCl_3$–MeOH) to generate the pure products.

3.3.2 *General procedure for the demethylations with BBr_3*

To a solution of substrates in CH_2Cl_2 BBr_3 was added, and the solution was kept stirring at the specified temperature and for a specified time section shown in the sections below. And then the reaction was quenched by the addition of saturated solution of Na_2CO_3, and the subsequent mixture was extracted with chloroform after basifying with concentrated NH_4OH. The chloroform extracts were dried over anhydrous sodium sulfate and concentrated to give a residue, which was then subjected to column chromatography (silica gel H, $CHCl_3$–MeOH) to provide the pure products.

3.3.3 *Compound **4***

This compound (white amorphous powder, 70 mg, 63%) was prepared by demethylation of **2** (100 mg, 0.18 mmol) with 6.5% HBr–HOAc (2 ml) at 80°C for 5 h. Compound **4**: mp 120–122°C; $[\alpha]_D^{20} + 37.1$ ($c = 1.0$, $CHCl_3$); IR (KBr) ν_{max}: 2940, 1807, 1739, 1242 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.08 (3H, t, $J = 7.2$ Hz, NCH_2CH_3), 2.02, 2.08, 2.12 (each 3H, s, 3 × OAc), 3.26, 3.37 (each 3H, s, 2 × OCH_3), 3.51 (1H, s), 3.93 (1H, dd, $J = 7.2$, 5.6 Hz, 1-H), 3.90, 3.94 (each 1H, ABq, $J = 11.2$ Hz, H_2-18), 4.82 (1H, t, $J = 8.4$ Hz, H-16), 4.87 (1H, t, $J = 5.2$ Hz, H-14β); ^{13}C NMR spectral data, see Table 1; HR-ESI-MS: *m/z* 592.2763 $[M + H]^+$ (calcd for $C_{30}H_{42}NO_{11}$, 592.2758).

3.3.4 *Compound **5***

This compound (white amorphous powder, 60 mg, 57%), together with compound **4** (white amorphous powder, 10 mg, 9.1%), was prepared by treating **2** (100 mg, 0.18 mmol) with 6.5% HBr–HOAc (2 ml) at 25°C for 4 days. Compound **5**: mp 118–120°C; $[\alpha]_D^{20} + 41.0$ ($c = 1.0$, $CHCl_3$); IR

(KBr) ν_{max}: 2941, 1805, 1738, 1245 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.07 (3H, t, $J = 7.2$ Hz, NCH_2CH_3), 2.06, 2.07 (3H, s, 2 × OAc), 3.25, 3.37, 3.43 (each 3H, s, 3 × OCH_3), 3.66 (1H, t, $J = 4.4$ Hz, H-14β), 3.89, 3.93 (each 1H, ABq, $J = 10.8$ Hz, H_2-18), 4.75 (1H, t, $J = 8.4$ Hz, H-16); HR-ESI-MS: *m/z* 564.2799 $[M + H]^+$ (calcd for $C_{29}H_{42}NO_{10}$, 564.2809).

3.3.5 *Compound* **6**

This compound (white amorphous powder, 30 mg, 21%) was prepared by treating **2** (200 mg, 0.37 mmol) with 6.5% HBr–HOAc (3 ml) at 60°C for 5 days. Compound **6**: mp 111–113 °C; $[\alpha]_D^{20}$ −25.4 ($c = 1.0$, $CHCl_3$); IR (KBr) ν_{max}: 2933, 1809, 1741, 1235 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.12 (3H, t, $J = 7.2$ Hz, NCH_2CH_3), 2.06, 2.14, 2.14 (each 3H, s, 3 × OAc), 3.37 (3H, s, OCH_3), 3.87 (3H, m), 4.05 (1H, s), 4.08 (1H, t, $J = 4.8$ Hz, H-14β), 4.82 (3H, m); ^{13}C NMR spectral data, see Table 2; ESI-MS *m/z* (%): 640 ($[M_1 + H]^+$, 100), 642 ($[M_2 + H]^+$, 92.5); HR-ESI-MS: *m/z* 640.1749 $[M_1 + H]^+$ (calcd for $C_{29}H_{39}NO_{10}Br$, 640.1757).

3.3.6 *Compounds* **7** *and* **8**

To a solution of **2** (100 mg, 0.18 mmol) in CH_2Cl_2 (2 ml), TMSI (0.1 ml) was added, and the reaction was allowed to proceed at 25°C for 20 h. Methanol (3 ml) was added dropwise to quench the reaction, and the mixture was then extracted with chloroform after basifying with concentrated NH_4OH. The chloroform extracts were dried over anhydrous sodium sulfate and concentrated to give a residue, which was purified by column chromatography (silica gel H, $CHCl_3$–MeOH; 99:1) to yield compound **7** (white amorphous powder, 45 mg, 46%) and compound **8** (white amorphous powder, 30 mg, 32%). Compound **7**: mp 85–87 °C; $[\alpha]_D^{20}$ +2.8 ($c = 1.0$, $CHCl_3$); IR (KBr) ν_{max}: 3465, 2936, 1802, 1740, 1237, 1094 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.08 (3H, t, $J = 6.8$ Hz, NCH_2CH_3), 2.09 (3H, s, OAc), 3.26, 3.39, 3.49 (each 3H, s, 3 × OCH_3), 4.05 (1H, t, $J = 4.4$ Hz, H-14β), 3.88, 4.29 (each 1H, Abq, $J = 10.4$ Hz, H_2-18); ^{13}C NMR spectral data, see Table 1; HR-ESI-MS: *m/z* 478.2434 $[M + H]^+$ (calculated for $C_{25}H_{36}NO_8$, 478.2441). Compound **8**: mp 73–75°C; $[\alpha]_D^{20}$ + 8.4 ($c = 1.0$, $CHCl_3$); IR (KBr) ν_{max}: 3425, 2930, 1737, 1239, 1094 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.07 (3H, t, $J = 7.2$ Hz, NCH_2CH_3), 2.08 (3H, s, Oac), 3.25, 3.40 (each 3H, s, 2 × OCH_3), 3.77 (1H, s), 3.82 (1H, t, $J = 5.2$ Hz), 3.89–3.92 (2H, hidden, H_2-18), 4.22 (1H, t, $J = 4.8$ Hz, H-14β); ^{13}C NMR spectral data, see Table 1; HR-ESI-MS: *m/z* 522.2745 $[M + H]^+$ (calculated for $C_{27}H_{40}NO_9$, 522.2703).

3.3.7 *Compound* **9**

This compound (white amorphous powder, 18 mg, 17%) was prepared by treating **2** (100 mg, 0.18 mmol) with BBr_3 (0.1 ml) in CH_2Cl_2 at −15°C for 16 h. Compound **9**: mp 134–136°C; $[\alpha]_D^{20}$ + 33.0 ($c = 1.0$, $CHCl_3$); IR (KBr) ν_{max}: 2937, 1802, 1740, 1236 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.08 (3H, t, $J = 6.8$ Hz, NCH_2CH_3), 2.08 (3H, s, OAc), 3.33, 3.40 (each 3H, s, 2 × OCH_3), 3.88–3.91 (2H, hidden, H_2-18), 4.02 (1H, d, $J = 1.2$ Hz), 4.07 (1H, t, $J = 4.8$ Hz, H-14β), 5.07 (1H, t, $J = 9.2$ Hz, H-1); ^{13}C NMR spectral data, see Table 3; ESI-MS *m/z* (%): 570 ($[M_1 + H]^+$, 100), 572 ($[M_2 + H]^+$, 93.6); HR-ESI-MS: *m/z* 570.1693 $[M_1 + H]^+$ (calcd for $C_{26}H_{37}NO_8Br$, 570.1702).

3.3.8 *Compound* **11**

This compound (white amorphous powder, 60 mg, 30%) was prepared by reacting **3** (200 mg, 0.48 mmol) with BBr_3 (0.1 ml) in CH_2Cl_2 at −10°C for 6 h. Compound **11**: mp 96–98°C; $[\alpha]_D^{20}$ −10.3 ($c = 1.0$, $CHCl_3$); IR (KBr) ν_{max}: 3439, 2944, 1740, 1233 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ

1.00 (3H, t, $J = 7.2$ Hz, NCH_2CH_3), 3.35 (3H, s, OCH_3), 3.94 (1H, dd, $J_1 = 10.0$, $J_2 = 7.2$ Hz, H-16), 3.99 (1H, s), 4.90 (1H, t, $J = 10.0$ Hz, H-9β), 5.50 (1H, s, H-7); ^{13}C NMR spectral data, see Table 1; ESI-MS *m/z* (%): 454 ($[M_1]^+$, 100), 456 ($[M_2]^+$, 92.8).

3.3.9 Compound **12**

This compound (white amorphous powder, 32 mg, 33%) was prepared by reacting **11** (80 mg, 0.17 mmol) with 6.5% HBr–HOAc (3 ml) at 80°C for 3 days. Compound **12**: mp 115–117°C; $[\alpha]_D^{20}$ −19.5 ($c = 1.0$, $CHCl_3$); IR (KBr) ν_{max}: 2927, 1741, 1240 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.04 (3H, t, $J = 7.2$ Hz, NCH_2CH_3), 2.06, 2.08 (each 3H, s, 2 × OAc), 3.71, 3.75 (each 1H, $J = 11.6$ Hz, H_2-18), 3.93 (1H, dd, $J = 10.4$, 7.2 Hz, H-1), 4.61(1H, dd, $J = 10.8$, 6.8 Hz, H-16), 4.92 (1H, d, $J = 9.6$ Hz, H-9), 5.49 (1H, s, H-7); ^{13}C NMR spectral data, see Table 4; ESI-MS *m/z* (%): 546 ($[M_1 + Na]^+$, 100), 548 ($[M_2 + Na]^+$, 92.1); HR-ESI-MS: *m/z* 524.1638 $[M_1 + H]^+$ (calc for $C_{25}H_{35}NO_6Br$, 524.1648).

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