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New lycorine-type alkaloid from *Lycoris traubii* and evaluation of antitrypanosomal and antimalarial activities of lycorine derivatives

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ABSTRACT

A new lycorine derivative LT1 (**4**) was isolated from the aerial part and bulbs of *Lycoris traubii* Hayward (Amaryllidaceae). Its structure including absolute configuration was established by spectroscopic analysis and semi-synthesis to be 1-*O*-(3′*S*)-hydroxybutanoyllycorine. Some lycorine ester derivatives including LT1 were examined for their inhibitory activity against *Trypanosoma brucei brucei*, the parasite associated with sleeping sickness, and against *Plasmodium falciparum*, the causative agent of malaria. Among them, 2-*O*-acetyllycorine (**6**) showed the most potent activity against parasitic *T. b. brucei*, and LT1 (**4**), 1-*O*-(3′*R*)-hydroxybutanoyllycorine (**8**), 1,2-di-*O*-butanoyllycorine (**11**), and 1-*O*-propanoyllycorine (**12**) showed significant activity against *P. falciparum* in an in vitro experiment.

1. Introduction

Extracts of plants belonging to the Amaryllidaceae family have long been used as herbal remedies. The alkaloids from their extracts are known to have various chemical structures and a wide range of biological activities. The alkaloids affect the central nervous system and have analgesic, antiviral, antimalarial, or antineoplastic activity. Pancratistatin, an alkaloid isolated from the spider lily *Pancratium littorale* (re-identified as *Hymenocallis littorale*), exhibits potent antineoplastic activity. Galanthamine is a long-acting, selective, reversible, and competitive acetylcholine esterase (AChE) inhibitor that has been approved for use in the European Union and the United States for the treatment of Alzheimer's disease (AD) (marketed as a hydrobromide salt under the name Razadyne®, formerly Reminyl®). Lycorine (**1**), the first alkaloid to be discovered in this group, is an antiviral agent and a powerful inhibitor of cell division and growth in higher plants (Fig. 1).[1] Recent reports indicate that it has antimalarial (against chloroquine-sensitive and chloroquine-resistant strains of cultured *Plasmodium falciparum*),[2,3] anti-inflammatory,[4] and antitumor[5] activities. 1-*O*-acetyllycorine (**2**)[6] has twofold stronger AChE inhibitory effect than galanthamine.[7] Recent in vitro bioactivity tests on parasitic protozoa were performed using some isolated alkaloids and 1,2-di-*O*-acetyllycorine (**3**)[2,8,9] showed activity against *Trypanosoma brucei rhodesiense*,[10] the causative agent of human African trypanosomiasis[11] (HAT) or sleeping sickness. Today, millions of people in sub-Saharan Africa still suffer from this dreaded disease.

To discover new biologically active alkaloids, we investigated alkaloidal constituents in the aerial part and bulbs of *Lycoris traubii* Hayward and isolated one new lycorine-type alkaloid named LT1 (**4**). In this paper, we describe the structure elucidation and semi-syntheses of LT1, as well as the biological evaluation of some lycorine ester derivatives, including this new alkaloid, focusing on their antitrypanosomal and antimalarial activities.

2. Results and discussion

2.1. Isolation and structure elucidation of LT1 (4)

The aerial part and bulbs of *L. traubii* Hayward (3.54 kg, wet weight) were extracted with MeOH to give the MeOH extract (176.4 g). The MeOH extract was dissolved in H_2O containing a small volume of MeOH and extracted successively with *n*-hexane, EtOAc, 5% MeOH/$CHCl_3$, and *n*-BuOH to give the *n*-hexane extract (7.53 g), the EtOAc extract (3.17 g), the 5% MeOH/$CHCl_3$ extract (0.33 g), and the *n*-BuOH extract (21.10 g). The 5% MeOH/$CHCl_3$ extract was purified by repeated chromatography to afford new

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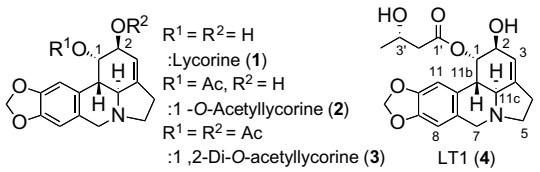


Figure 1. Known and new lycorine-type alkaloids.

lycorine-type alkaloid LT1 (**4**, 2.4 mg), together with four known alkaloids, galanthamine,[12,13] lycoramine,[14–16] sternbergine,[17] and ungiminorine.[18] From the EtOAc extract and the *n*-BuOH extract, four known alkaloids, ungiminorine, lycorine (**1**),[16,19,20] narciclasine[21,22] and lycoricidine,[23,24] were isolated.

New alkaloid LT1 (**4**) has the molecular formula $C_{20}H_{23}NO_6$ as determined from the FABHRMS spectrum (*m/z* 374.1619 $[MH]^+$), and it has four more carbons compared to common Amaryllidaceae alkaloids. The ^{1}H NMR spectrum (in CD_3OD) revealed some readily assignable signals due to the lycorine (**1**) skeleton, including signals assigned to two *p*-oriented aromatic protons [δ 6.74 (s, H-11), 6.63 (s, H-8)], one olefinic proton [δ 5.54 (m, H-3)], two protons of the methylenedioxy group [δ 5.89 (2H, s)], one oxymethine proton [δ 4.16 (m, H-2)], and two benzylic methylene protons bearing a nitrogen atom [δ 4.12 and 3.53 (each d, J = 13.6 Hz, H_2-7)]. In addition, signals assignable to two oxymethine protons [δ 5.73 (br s, H-1), δ 4.02 (br sex, J = 6.3 Hz, H-3′)], the former of which was observed in a lower field than usual, methylene protons [δ 3.33 (1H, overlapped) and 2.46 (1H, ddd) (H_2-5), δ 2.67 and 2.62 (each 1H, m, H_2-4)], and methyl protons [δ 1.02 (3H, d, J = 6.3 Hz, H_3-4′)] were observed. The ^{13}C NMR spectrum (in CD_3OD) revealed twenty carbons, including one ester carbonyl carbon at δ 172.5, six aromatic carbons, two olefinic carbons, one methylenedioxy carbon at δ 102.4, three oxygenated methine carbons at δ 73.5, 70.3, and 65.6, and one benzylic methylene carbon bearing a nitrogen atom at δ 57.7. ^{1}H–^{1}H COSY and HMQC analyses indicated a partial structure, –CH_2CH(OH)CH_3 (C-2′–C-4′) (Fig. 2). HMBC correlations from H-2′ and H-3′ to the carbonyl carbon signal at δ 172.5 (C-1′) revealed the presence of a 3-hydroxybutanoyl unit. Furthermore, HMBC correlations from the oxymethine proton at δ 5.73 to the carbonyl carbon as well as the carbons at C-3 and C-11c confirmed that a 3-hydroxybutanoyl unit was connected to the hydroxyl group on C-1 of lycorine (**1**). Relative stereochemistry among C-1, C-2, C-11b, and C-11c was deduced to be the same as that of lycorine (**1**) from the similarity of the coupling constants of these protons in the ^{1}H-NMR spectrum.

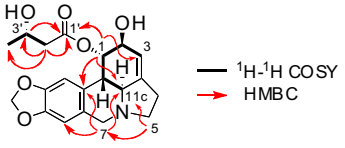


Figure 2. Selected ^{1}H–^{1}H COSY and HMBC correlations of **4**.

2.2. Semi-synthesis of LT1 (4) from lycorine (1)

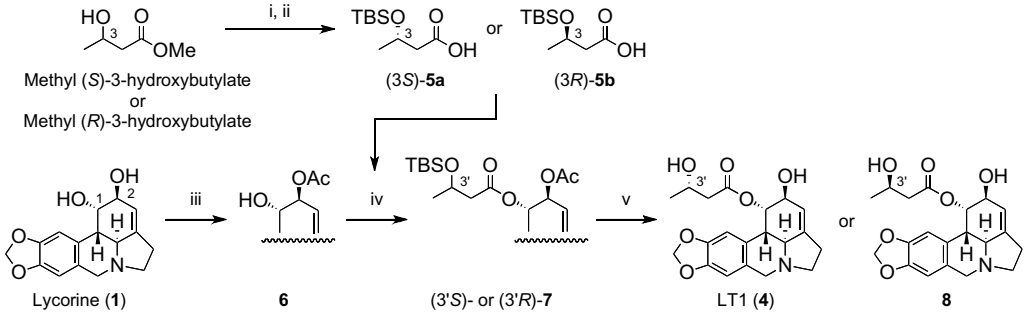
To confirm the structure of LT1 (**4**), including the undetermined stereochemistry at C-3′ in the side chain, the chemical transformation of lycorine (**1**) into **4** was attempted.

First, *O*-silyl protected units **5a** and **5b**,[25] which correspond to the 3-hydroxybutanoyl units in **4**, were respectively synthesized from commercially available methyl 3-hydroxybutylate by TBDMS protection of the hydroxyl group, followed by acid hydrolysis of the ester group (Scheme 1). Selective acetylation of the secondary allylic hydroxyl group on C-2 in lycorine (**1**) with acetic anhydride and 4-dimethylaminopyridine (DMAP) in pyridine gave 2-*O*-acetyllycorine (**6**) in 44% yield.[2,8–10,26] Introduction of (*S*)- or (*R*)-silyl protected unit **5a** or **5b** to the C-1 hydroxyl group by DCC-mediated esterification[27] afforded (3′*S*)- or (3′*R*)-**7**, respectively [(3′*S*)-**7** in 70% yield, (3′*R*)-**7** in 73% yield]. Finally, acid hydrolysis of the acetyl group in (3′*S*)- or (3′*R*)-**7** was carried out by heating with concd HCl in MeOH to give **4** or **8** in quantitative yield, respectively. Comparison of the ^{1}H NMR spectra and the optical rotation of natural LT1 with those of the semi-synthetic compounds led to the unambiguous determination of the structure of LT1 including the absolute configuration, as 1-*O*-(3′*S*)-hydroxybutanoyllycorine (**4**).

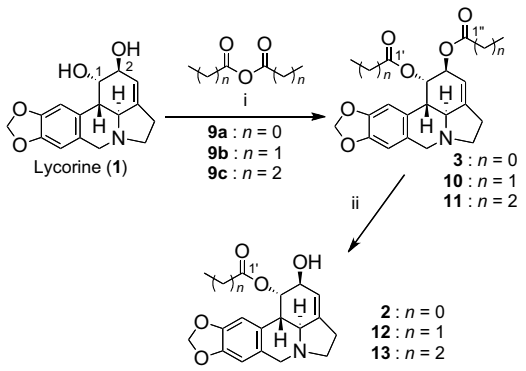
2.3. Semi-syntheses and antitrypanosomal and antimalarial activities of ester derivatives of lycorine analogue

2.3.1. Semi-synthesis of lycorine derivatives (2, 3, 10, 11, 12, 13) from lycorine (1)

As described in Introduction, the diacetyl derivative of lycorine (**3**) was reported to exhibit antitrypanosomal activity (IC_{50} 1.0 μg/mL, *T. b. rhodesiense* strain STIB 900, stage trypomastigotes, std Melarsoprol)[10] as well as antimalarial activity (IC_{50} 1.0 μg/mL and 1.0 μg/mL, *P. falciparum* strain D10 and strain FAC8, respectively).[2] We have been interested in the SAR of ester derivatives of lycorine, including new ester alkaloid **4**, specifically their antitrypanosomal and antimalarial activities. We prepared some lycorine derivatives with an acyl group on C-1 and/or a C-2 hydroxyl group. First, lycorine (**1**) was treated with anhydride (**9a**, **9b** or **9c**) and DMAP in pyridine to give 1,2-di-*O*-acetyllycorine (**3**, y. 99%), 1,2-di-*O*-propanoyllycorine (**10**, y. 92%) or 1,2-di-*O*-butanoyllycorine (**11**, y. 95%), respectively (Scheme 2). Then, acid hydrolysis of the ester group at C-2 position in **3**, **10** or **11** was carried out by heating with concd HCl in MeOH[6] to give 1-*O*-acetyllycorine (**2**, y. 57%), 1-*O*-propanoyllycorine (**12**, y. 87%) or 1-*O*-butanoyllycorine (**13**, y. 73%), respectively.



Scheme 1. Reagents and conditions: (i) TBDMSCl, imidazole, DMF, rt; (ii) LiOH aq. (1.03 M), THF, rt; (iii) Ac_2O, DMAP, pyridine, rt, 44%; (iv) (3*S*)-**5a** or (3*R*)-**5b**, DCC, DMAP, toluene, rt, 70% (3′*S*), 73% (3′*R*); (v) concd HCl, MeOH, 55 °C, quant (**4**: 3′*S*), quant (**8**: 3′*R*).



Scheme 2. Reagents and conditions: (i) **9a** or **9b** or **9c**, DMAP, pyridine, rt, 99% for **3**, 92% for **10**, 95% for **11**; (ii) concd HCl, MeOH, 55 °C, 1 h, 57% for **2**, 87% for **12**, 73% for **13**.

2.3.2. In vitro antitrypanosomal activity and cytotoxicity of lycorine derivatives[28]

In vitro bioactivity test using *T. b. brucei* was performed on isolated alkaloid **4** and lycorine derivatives (**2**, **3**, **6**, **8**, **12**, and **13**). Pentamidine, suramin, and eflornithine were used as standards.

These compounds were also tested for their cytotoxicity to MRC-5, a human diploid embryonic cell line (Table 1). *T. b. brucei* is a causative factor of the cattle disease, N'gana, although it cannot infect humans. 2-*O*-Acetyllycorine (**6**) showed significant inhibitory activity against *T. b. brucei* (strain GUTat 3.1) with 50% inhibitory concentration (IC_{50}) of 0.15 μg/mL (approved drugs pentamidine, suramin, and eflornithine had IC_{50} values of 0.00158, 1.58, and 2.27 μg/mL, respectively), and showed low cytotoxicity of 6.11 and high selectivity index (SI) of 40.7. 1-*O*-Acetyllycorine (**2**), 1,2-di-*O*-acetyllycorine (**3**), and 1-*O*-(3′*R*)-hydroxybutanoyllycorine (**8**) showed low antitrypanosomal activities while 1-*O*-propanoyllycorine (**12**), 1-*O*-butanoyllycorine (**13**), and the natural compound 1-*O*-(3′*S*)-hydroxybutanoyllycorine (**4**) showed moderate activities. Although 1,2-di-*O*-acetyllycorine (**3**) was reported[10] to exhibit potent activity against *T. b. rhodesiense*, our result showed that it had low inhibitory activity against *T. b. brucei*, probably because of the difference in strain.

2.3.3. In vitro antimalarial activities and cytotoxicities of lycorine derivatives[29]

Malaria is one of the most common vector-borne infectious diseases. This disease is caused by parasites of the genus *Plasmodium* and causes such symptoms as anemia, fever, chills, nausea, and in severe cases, coma and death.

The effects of lycorine derivatives (**2**, **3**, **6**, **8**, **10**, **11**, **12**, and **13**) including the natural compound LT1 (**4**) on in vitro antimalarial activity were evaluated by using the drug-resistant K1 strain and the drug-sensitive FCR3 strain of *P. falciparum*. Their IC_{50} values are listed in Table 2.

Among the tested compounds, **11**, **12**, LT1 (**4**), and **8** showed high antimalarial activities with IC_{50} values of 0.67, 0.37, 0.60, and 0.62 μg/mL for the K1 strain and of 0.53, 0.30, 0.45, and 0.49 μg/mL for the FCR3 strain, respectively. We then investigated the cytotoxicities of these compounds to the human diploid embryonic cell line MRC-5 to assess cytotoxicity to host cells. Their IC_{50} values and SI are also listed in Table 2. Among the tested compounds, **11**, **12**, LT1 (**4**), and **8** showed higher SIs with ratios of 21.7, 13.5, 13.5, and 10.2 for the K1 strain, respectively. The other compounds showed SIs lower than 10.

3. Conclusion

In conclusion, we have isolated and identified new lycorine alkaloid LT1 (**4**) from the aerial part and bulbs of *L. traubii* Hayward by means of spectroscopic analysis and semi-synthesis.

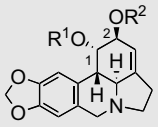
Our preliminary biological evaluation of lycorine ester derivatives demonstrated that 2-*O*-acetyllycorine (**6**) has significant inhibitory activity against *T. b. brucei* with an IC_{50} of 0.15 μg/mL in in vitro experiments and that 1,2-di-*O*-butanoyllycorine (**11**), 1-*O*-propanoyllycorine (**12**), 1-*O*-(3′*R*)-hydroxybutanoyllycorine (**8**), and new lycorine alkaloid LT1 (**4**) showed high inhibitory activity against *P. falciparum* with IC_{50} of 0.67, 0.37, 0.62 and 0.60 μg/mL in in vitro experiments, respectively.

4. Experimental

4.1. General

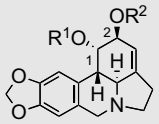
UV: recorded in MeOH on a JASCO V-560 instrument. IR: recorded on a JASCO FT/IR-230 spectrophotometer. ^{1}H and ^{13}C

Table 1
In vitro antitrypanosomal activity against *Trypanosoma brucei brucei* GUTat 3.1 and cytotoxicity in MRC-5 cells.

Compound			IC_{50} (μg/ml)		Selectivity index (SI)[b]
	R^1	R^2	Antitrypanosomal activity	Cytotoxicity	
3	Acetyl	Acetyl	>12.5	18.11	<1.4
2	Acetyl	H	5.22	1.29	0.2
6	H	Acetyl	0.15	6.11	40.7
12	Propanoyl	H	1.34	4.99	3.7
13	Butanoyl	H	1.38	3.6	2.6
4	(3′*S*)-Hydroxybutanoyl	H	1.9	8.11	4.3
8	(3′*R*)-Hydroxybutanoyl	H	2.45	6.35	2.6
Pentamidine[a]			0.00158	5.71	3,614
Suramin[a]			1.58	>100	>63
Eflornithine[a]			2.27	>100	>44

[a] Existing antitrypanosomal drugs.
[b] SI: cytotoxicity/antitrypanosomal activity.

Table 2
In vitro antimalarial activity against *Plasmodium falciparum* K1 and FCR3 strains and cytotoxicity in MRC-5 cells.

Compound			IC_{50} (μg/ml)			Selectivity index (SI)	
	R^1	R^2	Antimalarial activity		Cytotoxicity		
			K1	FCR3	MRC-5	M/K1	M/F
3	Acetyl	Acetyl	7.6	ND	18.11	2.4	–
10	Propanoyl	Propanoyl	4.2	ND	>100	>23.8	–
11	Butanoyl	Butanoyl	0.67	0.53	14.56	21.7	27.5
2	Acetyl	H	0.36	0.3	1.29	3.6	4.3
6	H	Acetyl	8.21	ND	6.11	0.7	–
12	Propanoyl	H	0.37	0.3	4.99	13.5	16.6
13	Butanoyl	H	0.41	0.32	3.6	8.8	11.3
4	(3′*S*)-Hydroxybutanoyl	H	0.6	0.45	8.11	13.5	18
8	(3′*R*)-Hydroxybutanoyl	H	0.62	0.49	6.35	10.2	13
Artemisinin[a]			0.0057	0.006	45.17	7,924.5	7,528.3

[a] Existing antimalarial drug.

NMR spectra: recorded on JEOL JNM A-400, JNM ECP-400, JNM A-500, and JNM ECP-600 at 400, 500 or 600 MHz (^{1}H NMR) and at 100, 125 or 150 MHz (^{13}C NMR), respectively. *J* values are given in Hz. EIMS and EIHRMS: direct probe insertion at 70 eV recorded on a JEOL JMS GC-mate spectrometer. FABMS: recorded on JEOL JMS-AX500 or JEOL JMS-AX500 mass spectrometer. FABHRMS: recorded on a JEOL JMS-HX110 mass spectrometer. CD: recorded on a JASCO J-720WI spectrometer. TLC: precoated silica gel 60 F254 plates (Merck, 0.25 mm thick), NH TLC (Fuji Silysia Chemical, amino-silica gel). Column chromatography: Silica gel 60 [Merck, 70–230 mesh (for open chromatography)], Silica gel 60N [Kanto Chemical, 40–50 mm (for flash chromatography)], Chromatorex NH [Fuji Silysia Chemical, 100–200 mesh (for amino-silica gel column chromatography)]. Medium pressure liquid column chromatography (MPLC): C.I.G. prepacked column CPS-HS-22-05 (Kusano Kagakukikai, SiO_2), Ultra Pack NH-40A (Yamazen, amino-silica gel). Optical rotation: measured on a JASCO P-1020 polarimeter.

4.2. Plant material

Lycoris traubii Hayward (Amaryllidaceae) was harvested from the medicinal plant garden of Chiba University in June 2006 (aerial part 0.87 kg, and bulbs 2.67 kg), and identified by Dr. F. Ikegami, Chiba University. A voucher specimen was deposited at the Faculty of Pharmaceutical Sciences, Chiba University.

4.3. Extraction and isolation

The aerial part and bulbs of *L. traubii* (3.54 kg, wet weight) were extracted with MeOH (19.7 L, two times at room temperature and two times under reflux) to give the extract (176.4 g). The MeOH extract was dissolved in MeOH/H_2O (1.0 L) and extracted with *n*-hexane (2.5 L) to give the *n*-hexane extract (7.53 g). The aqueous layer was successively extracted with EtOAc (2.6 L), 5% MeOH/$CHCl_3$ (3.0 L), and *n*-BuOH (2.6 L) to give the EtOAc extract (3.17 g), the 5% MeOH/$CHCl_3$ extract (0.33 g), and the *n*-BuOH extract (21.10 g), respectively. The 5% MeOH/$CHCl_3$ extract was roughly separated by silica gel flash column chromatography using $CHCl_3$/MeOH gradient to give seven fractions: fr. A ($CHCl_3$, 0–5% MeOH/$CHCl_3$, 37.2 mg), fr. B (10%, 35.1 mg), fr. C (10%, 52.2 mg), fr. D (15%, 30.7 mg), fr. E (15–30%, 34.5 mg), fr. F (30%, 10.6 mg), and fr. G (MeOH, 46.3 mg). Fr. D was purified by using a combination of silica gel flash column chromatography (20–30% MeOH/EtOAc), MPLC (amino-silica gel, 5% MeOH/EtOAc), and preparative TLC (amino-silica gel, developed with 5% MeOH/EtOAc and eluted with 20% MeOH/EtOAc) to afford LT1 (**4**, 2.4 mg). Seven known alkaloids were isolated as follows. Galanthamine (28.1 mg), lycoramine (9.9 mg), sternbergine (2.7 mg), and ungiminorine (2.6 mg) were isolated from the 5% MeOH/$CHCl_3$ extract. Ungiminorine (3.2 mg), lycorine (**1**, 17.0 mg), and narciclasine (12.8 mg) were obtained from the EtOAc extract. From the *n*-BuOH extract, lycoricidine (19.3 mg) and narciclasine (9.1 mg) were isolated.

4.4. LT1 (4)

$[\alpha]_D^{25}$ −58.5 (*c* 0.10, MeOH); UV (MeOH) λ_{max} nm: 290.5, 260.5, 238.5, 204.5; IR ($CHCl_3$) ν_{max} cm^{-1}: 2960, 2927, 2855, 1730, 1261, 1096, 1028, 806; EIMS *m/z*: 373 (M^+, 46), 268 (45), 227 (99), 226 (91), 83 (100); FABMS (NBA) *m/z*: 374 $[MH]^+$; FABHRMS (NBA) *m/z*: 374.1619 $[MH]^+$ (Calcd for $C_{20}H_{24}NO_6$: 374.1604); ^{1}H NMR (600 MHz, CD_3OD) δ: 6.74 (1H, s, H-11), 6.63 (1H, s, H-8), 5.89 (2H, s, $-OCH_2O-$), 5.73 (1H, br s, H-1), 5.54 (1H, m, H-3), 4.16 (1H, m, H-2), 4.12 (1H, d, *J* = 13.6 Hz, H-7), 4.02 (1H, br sex, *J* = 6.3 Hz, H-3′), 3.53 (1H, d, *J* = 13.6 Hz, H-7), 3.33 (1H, overlapped with CD_3OD signal, H-5), 2.88 (1H, br d, *J* = 10.6 Hz, H-11b), 2.84 (1H, br d, *J* = 10.6 Hz, H-11c), 2.67 (1H, m, H-4), 2.62 (1H, m, H-4), 2.46 (1H, ddd, *J* = 8.9, 8.9, 8.9 Hz, H-5), 2.33 (1H, dd, *J* = 15.0, 7.4 Hz, H-2′), 2.23 (1H, dd, *J* = 15.0, 5.8 Hz, H-2′), 1.02 (3H, d, *J* = 6.3 Hz, H_3-4′); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 6.71 (1H, s, H-11), 6.58 (1H, s, H-8), 5.922 (1H, d, *J* = 1.5 Hz, $-OCH_2O-$), 5.918 (1H, d, *J* = 1.5 Hz, $-OCH_2O-$), 5.69 (1H, br s, H-1), 5.55 (1H, m, H-3), 4.24 (1H, m, H-2), 4.15 (1H, d, *J* = 14.0 Hz, H-7), 4.08 (1H, m, H-3′), 3.52 (1H, br d, *J* = 12.5 Hz, H-7), 3.37 (1H, ddd, *J* = 9.1, 5.9, 3.4 Hz, H-5), 2.88 (1H, br d, *J* = 10.4 Hz, H-11b), 2.71 (1H, br d, *J* = 10.4 Hz, H-11c), 2.64 (2H, m, H_2-4), 2.42–2.30 (3H, overlapped, H-5, H_2-2′), 1.12 (3H, d, *J* = 6.1 Hz, H_3-4′); ^{13}C NMR (150 MHz, CD_3OD) δ: 172.5 (C-1′), 148.1 and 148.0 (C-9, C-10), 143.8 (C-3a), 130.5 (C-7a), 128.3 (C-11a), 119.1 (C-3), 108.3 (C-8), 105.9 (C-11), 102.4 ($-OCH_2O-$), 73.5 (C-1), 70.3 (C-2), 65.6 (C-3′), 63.0 (C-11c), 57.7 (C-7), 54.6 (C-5), 44.6 (C-2′), 40.3 (C-11b), 29.3 (C-4), 23.0 (C-4′); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 172.3 (C-1′), 146.4 and 146.3 (C-10, C-9), 144.6 (C-3a), 129.6 (C-7a), 126.7 (C-11a), 116.9 (C-3), 107.4 (C-8), 104.8 (C-11), 101.0 ($-OCH_2O-$), 73.0 (C-1), 69.8 (C-2), 64.2 (C-3′), 61.6 (C-11c), 56.9 (C-7), 53.7 (C-5), 43.0 (C-2′), 39.5 (C-11b), 28.6 (C-4), 22.2 (C-4′); CD (*c* = 0.290 mmol/L, MeOH, 25 °C) $\Delta\varepsilon$ (λ nm): 0 (310), −1.8 (292), 0 (260), +1.8 (244), 0 (233), −1.3 (211).

4.5. 3-(*S*)-(*t*-Butyldimethylsilyloxy)butanoic acid (5a)

To a solution of methyl (*S*)-3-hydroxybutylate (371 mg, 3.1 mmol) in dry DMF (1.6 mL) were added *t*-butyldimethylsilyl chloride (568.1 mg, 3.8 mmol) and imidazole (256.6 mg, 3.8 mmol), and the reaction mixture was stirred at room temperature for 19.5 h. The reaction mixture was then diluted with hexane, washed with 1 M HCl/H_2O (1:1), and dried over anhydrous magnesium sulfate. After evaporation, the crude product (colorless oil, 802.5 mg, quant.) was used in the next reaction without further purification. To a mixture of methyl (*S*)-3-(*t*-butyldimethylsilyloxy)butylate (184.1 mg, 0.79 mmol) in THF (2.0 mL) was added aqueous LiOH solution (1.03 M, 1.2 mL, 1.2 mmol). The reaction mixture was stirred at room temperature for 17 h. After removal of THF in vacuo, the residue was extracted with chloroform. The aqueous layers were cautiously acidified with 1 N HCl and extracted with chloroform. The combined organic layers were dried over anhydrous magnesium sulfate. After concentration in vacuo, **5a** (159.3 mg, 92%) was obtained as a colorless oil; $[\alpha]_D^{25}$ +25.9 (*c* 0.99, MeOH); IR (ATR) ν_{max} cm^{-1}: 2955, 2929, 2892, 2857, 1710, 1253, 1082, 1000, 827, 773; ^1H NMR (400 MHz, $CDCl_3$) δ: 4.28 (1H, m, H-3), 2.50 (1H, dd, *J* = 14.8, 7.2 Hz, H-2), 2.43 (1H, dd, *J* = 14.8, 5.6 Hz, H-2), 1.22 (3H, d, *J* = 6.0 Hz, H_3-4), 0.86 (9H, s, –Si–C–$(CH_3)_3$), 0.07 and 0.05 (each 3H, s, –Si–$(CH_3)_2$); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 177.6 (C-1), 65.6 (C-3), 44.5 (C-2), 25.7 (–Si–C–*C*$H_3)_3$), 23.7 (C-4), 17.9 (–Si–*C*–$(CH_3)_3$), −4.6 and −5.1 (–Si–$(CH_3)_2$).

4.6. 3-(*R*)-(*t*-Butyldimethylsilyloxy)butanoic acid (5b)

To a solution of methyl (*R*)-3-hydroxybutylate (371 mg, 3.1 mmol) in dry DMF (1.6 mL) were added *t*-butyldimethylsilyl chloride (568.1 mg, 3.8 mmol) and imidazole (256.6 mg, 3.8 mmol) and the reaction mixture was stirred at room temperature for 22.5 h. The mixture was then diluted with hexane, washed with 1 M HCl/H_2O (1:1), and dried over anhydrous magnesium sulfate. After evaporation, the crude product (colorless oil, 834.3 mg, quant.) was used in the next reaction without further purification. To a mixture of methyl (*R*)-3-(*t*-butyldimethylsilyloxy)butylate (197.8 mg, 0.85 mmol) in THF (2.1 mL) was added aqueous LiOH solution (1.03 M, 1.3 mL, 1.3 mmol). The reaction mixture was stirred at room temperature for 17 h. After removal of THF in vacuo, the residue was extracted with chloroform. The aqueous layers were cautiously acidified with 1 N HCl and extracted with chloroform. The combined organic layers were dried over anhydrous magnesium sulfate. After concentration in vacuo, **5b** (168.2 mg, 91%) was obtained as a pale yellow oil; $[\alpha]_D^{25}$ −33.2 (*c* 0.97, MeOH); IR (ATR) ν_{max} cm^{-1}: 2955, 2929, 2892, 2857, 1711, 1254, 1082, 1001, 827, 773; ^1H NMR (400 MHz, $CDCl_3$) δ: 4.27 (1H, m, H-3), 2.49 (1H, dd, *J* = 14.8, 7.2 Hz, H-2), 2.43 (1H, dd, *J* = 14.8, 5.2 Hz, H-2), 1.21 (3H, d, *J* = 6.0 Hz, H_3-4), 0.86 (9H, s, –Si–C–$(CH_3)_3$), 0.06 and 0.05 (each 3H, s, –Si–$(CH_3)_2$); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 177.9 (C-1), 65.6 (C-3), 44.5 (C-2), 25.6 (–Si–C–*C*$H_3)_3$), 23.7 (C-4), 17.9 (–Si–*C*–$(CH_3)_3$), −4.6 and −5.1 (–Si–$(CH_3)_2$).

4.7. 2-*O*-Acetyllycorine (6)

To a solution of lycorine (**1**, 100.5 mg, 0.35 mmol) in dry pyridine (5 mL) were added acetic anhydride (36.4 μL, 0.39 mmol) and DMAP (4.3 mg, 0.04 mmol) and the reaction mixture was stirred at room temperature for 12 h. It was then quenched with cold water and extracted with chloroform. The combined organic layers were washed with brine and dried over anhydrous magnesium sulfate. After removal of the volatiles, the crude product was purified by SiO_2 open column chromatography (EtOAc/$CHCl_3$/MeOH, 40:40:20) to give **6** (50.5 mg, 44%) as a colorless solid; $[\alpha]_D^{25}$ +41.3 (*c* 0.05, MeOH); UV (MeOH) λ_{max} nm: 289.5, 203.5; IR (ATR) ν_{max} cm^{-1}: 3065, 2960, 2922, 2850, 2769, 1714, 1485, 1258, 1234, 1031, 1010, 930, 795; FABMS (NBA) *m*/*z*: 330 (MH^+); ^1H NMR (400 MHz, $CDCl_3$) δ: 6.81 (1H, s, H-11), 6.60 (1H, s, H-8), 5.94 (1H, d, *J* = 1.6 Hz, –O*C*H_2O–), 5.92 (1H, d, *J* = 1.2 Hz, –O*C*H_2O–), 5.47 (1H, m, H-3), 5.32 (1H, m, H-2), 4.52 (1H, s, H-1), 4.14 (1H, d, *J* = 14.0 Hz, H-7β), 3.53 (1H, d, *J*=14.0 Hz, H-7α), 3.37 (1H, ddd, *J* = 9.0, 4.5, 4.5 Hz, H-5β), 2.79 (1H, br d, *J* = 10.4 Hz, H-11c), 2.71 (1H, br d, *J* = 10.4 Hz, H-11b), 2.65 (2H, overlapped, H_2-4), 2.38 (1H, ddd, *J* = 8.9, 8.9, 8.9 Hz, H-5α), 2.09 (3H, s, –$COCH_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 170.6 (–O*C*OCH_3), 146.6 (C-10), 146.4 (C-9), 146.0 (C-3a), 130.2 (C-11a), 127.0 (C-7a), 113.6 (C-3), 107.7 (C-8), 104.6 (C-11), 101.0 (–OCH_2O–), 73.7 (C-2), 69.4 (C-1), 60.7 (C-11c), 58.3 (C-7), 57.0 (C-5), 41.8 (C-11b), 29.4 (C-4), 21.3 (–OCO*C*H_3).

4.8. 1-*O*-(3′*S*)-[*t*-Butyldimethylsilyloxybutanoyl]-2-*O*-acetyllycorine [(3′*S*)-7]

To a stirred solution of carboxylic acid **5a** (91.2 mg, 0.42 mmol) in dry toluene (1.8 mL) was added DCC (86.3 mg, 0.42 mmol) at room temperature. After 5 min, a solution of **6** (68.8 mg, 0.21 mmol) and DMAP (76.6 mg, 0.63 mmol) in dry toluene (6.9 mL) was added and the reaction mixture was stirred at room temperature for 3 h. After dilution with EtOAc, the whole was washed with saturated sodium bicarbonate solution and brine and dried over anhydrous magnesium sulfate. After removal of the volatiles, the residue was purified by SiO_2 open column chromatography (50% EtOAc/$CHCl_3$) to give 1-*O*-(3′*S*)-[*t*-butyldimethylsilyloxybutanoyl]-2-*O*-acetyllycorine [(3′*S*)-**7**, 54.5 mg, 70%] as a pale yellow solid; $[\alpha]_D^{24}$ +13.2 (*c* 0.11, MeOH); UV (MeOH) λ_{max} nm: 291.5, 237.0, 207.5; IR (ATR) ν_{max} cm^{-1}: 2952, 2928, 2892, 2856, 1730, 1486, 1235, 1173, 1004, 835, 776; EIMS *m*/*z*: 529 (M^+, 11), 312 (24), 252 (100); HREIMS *m*/*z*: 529.2512 [M^+] (Calcd for $C_{28}H_{39}NO_7Si$: 529.2496); ^1H NMR (400 MHz, $CDCl_3$) δ: 6.72 (1H, s, H-11), 6.56 (1H, s, H-8), 5.91 (1H, d, *J* = 1.4 Hz, –O*C*H_2O–), 5.89 (1H, d, *J* = 1.4 Hz, –O*C*H_2O–), 5.73 (1H, s, H-1), 5.52 (1H, br s, H-3), 5.24 (1H, m, H-2), 4.16 (2H, overlapped, H-7, H-3′), 3.51 (1H, d, *J* = 13.6 Hz, H-7), 3.37 (1H, m, H-5), 2.88 (1H, br d, *J* = 10.4 Hz, H-11b), 2.77 (1H, br d, *J* = 10.8 Hz, H-11c), 2.64 (2H, m, H_2-4), 2.39 (2H, overlapped, H-5, H-2′), 2.23 (1H, dd, *J* = 15.2, 6.4 Hz, H-2′), 2.07 (3H, s, –$COCH_3$), 1.02 (3H, d, *J* = 6.0 Hz, H_3-4′), 0.83 (9H, s, –Si–C$(CH_3)_3$), 0.02 (6H, s, –Si–$(CH_3)_2$); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 170.6 (C-1′),* 169.6 (–*C*OCH_3),* 146.4 and 146.2 (C-10, C-9), 145.9 (C-3a), 129.4 (C-11a),** 126.5 (C-7a),** 113.8 (C-3), 107.3 (C-8), 105.1 (C-11), 100.9 (–OCH_2O–), 70.8 (C-1), 69.1 (C-2), 65.5 (C-3′), 61.3 (C-11c), 56.9 (C-7), 53.6 (C-5), 44.5 (C-2′), 40.5 (C-11b), 28.6 (C-4), 25.7 (–Si–C(*C*$H_3)_3$), 23.4 (–$COCH_3$),*** 21.1 (C-4′),*** 17.9 (–Si–*C*$(CH_3)_3$), −4.7 and −5.0 (–Si–$(CH_3)_2$) (*, **, ***: interchangeable).

4.9. 1-*O*-(3′*R*)-[*t*-Butyldimethylsilyloxybutanoyl]-2-*O*-acetyllycorine [(3′*R*)-7]

To a stirred solution of carboxylic acid **5b** (58.8 mg, 0.27 mmol) in dry toluene (1.1 mL) was added DCC (55.7 mg, 0.27 mmol) at room temperature. After 5 min, a solution of **6** (44.4 mg, 0.13 mmol) and DMAP (49.5 mg, 0.40 mmol) in dry toluene (4.3 mL) was added and the reaction mixture was stirred at room temperature for 2.5 h. After dilution with EtOAc, the whole was washed with saturated sodium bicarbonate solution and brine and dried over anhydrous magnesium sulfate. After removal of the volatiles, the residue was purified by SiO_2 open column chromatography (50% EtOAc/$CHCl_3$) to give 1-*O*-(3′*R*)-[*t*-butyldimethylsilyloxybutanoyl]-2-*O*-acetyllycorine [(3′*R*)-**7**, 38 mg, 73%] as a

pale yellow oil; $[\alpha]_D^{24}$ −3.2 (*c* 0.09, MeOH); UV (MeOH) λ_{max} nm: 291.0, 261.0, 238.0, 205.5; IR (ATR) ν_{max} cm^{-1}: 2954, 2928, 2892, 2856, 1736, 1486, 1227, 1000, 832, 775; EIMS *m*/*z*: 529 (M^+, 11), 312 (15), 252 (100); HREIMS *m*/*z*: 529.2492 [M^+] (Calcd for $C_{28}H_{39}NO_7Si$: 529.2496); ^1H NMR (400 MHz, $CDCl_3$) δ: 6.72 (1H, s, H-11), 6.56 (1H, s, H-8), 5.92 (1H, br s, –OCH_2O–), 5.88 (1H, br s, –OCH_2O–), 5.71 (1H, s, H-1), 5.53 (1H, br s, H-3), 5.24 (1H, br s, H-2), 4.16 (1H, d, *J* = 14.0 Hz, H-7), 4.02 (1H, sex, *J* = 6.0 Hz, H-3′), 3.50 (1H, br d, *J* = 14.0 Hz, H-7), 3.38 (1H, ddd, *J* = 9.0, 4.5, 4.5 Hz, H-5), 2.88 (1H, br d, *J* = 10.4 Hz, H-11b), 2.76 (1H, br d, *J* = 10.4 Hz, H-11c), 2.64 (2H, m, H_2-4), 2.39 (2H, overlapped, H-5, H-2′), 2.27 (1H, dd, *J* = 14.8, 6.8 Hz, H-2′), 2.08 (3H, s, –$COCH_3$), 1.06 (3H, d, *J* = 6.4 Hz, H_3-4′), 0.81 (9H, s, –Si–C$(CH_3)_3$), −0.01 and −0.03 (each 3H, s, –Si–$(CH_3)_2$); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 170.3 (C-1′), 169.7 (–*C*OCH_3) 146.4 and 146.3 (C-10, C-9), 146.0 (C-3a), 129.4 (C-11a), 126.5 (C-7a), 113.8 (C-3), 107.3 (C-8), 105.1 (C-11), 100.9 (–OCH_2O–), 70.9 (C-1), 69.3 (C-2), 65.5 (C-3′), 61.4 (C-11c), 57.0 (C-7), 53.7 (C-5), 44.7 (C-2′), 40.5 (C-11b), 28.6 (C-4), 25.7 (–Si–C(*C*H_3)$_3$), 23.4 (–*C*OCH_3),* 21.1 (C-4′),* 18.0 (–Si–*C*-$(CH_3)_3$), −4.8 and −5.0 (–Si–$(CH_3)_2$) (*: interchangeable).

4.10. 1-*O*-(3′*S*)-Hydroxybutanoyllycorine (4, LT1)

A solution of 1-*O*-(3′*S*)-[*t*-butyldimethylsilyloxybutanoyl]-2-*O*-acetyllycorine [(3′*S*)-**7**, 20.1 mg, 0.04 mmol] and concd hydrochloric acid (0.38 mL) in methanol (2.0 mL) was heated on an oil-bath for 1 h. After cooling, the mixture was cautiously basified (pH 8) with aqueous ammonia solution and diluted with water, and the whole was extracted with chloroform. The combined organic layers were washed with brine and dried over anhydrous magnesium sulfate. After evaporation, the residue was chromatographed over silica gel (EtOAc/$CHCl_3$/MeOH, 40:40:20) to give **4** (20.8 mg, quant.) as a pale yellow solid. Synthetic **4** was completely identical in all respects (chromatographic behavior, MS, IR, UV, ^1H and ^{13}C NMR, $[\alpha]_D$) with natural LT1.

4.11. 1-*O*-(3′*R*)-Hydroxybutanoyllycorine (8)

A solution of 1-*O*-(3′*R*)-[*t*-butyldimethylsilyloxybutanoyl]-2-*O*-acetyllycorine [(3′*R*)-**7**, 23.3 mg, 0.04 mmol] and concd hydrochloric acid (0.38 mL) in methanol (2.0 mL) was heated on an oil-bath for 1 h. After cooling, the mixture was cautiously basified (pH 8) with aqueous ammonia solution, diluted with water, and extracted with chloroform. The combined organic layers were washed with brine and dried over anhydrous magnesium sulfate. After evaporation, the residue was chromatographed over silica gel (EtOAc/$CHCl_3$/MeOH, 40:40:20) to give **8** (18.4 mg, quant.) as a yellow solid; $[\alpha]_D^{25}$ −63.7 (*c* 0.10, MeOH); UV (MeOH) λ_{max} nm: 291.5, 261.0, 237.5, 208.0; IR (ATR) ν_{max} cm^{-1}: 3362, 2921, 2850, 1730, 1486, 1237, 1035, 999, 932; EIMS *m*/*z*: 373 (M^+, 51), 268 (41), 227 (95), 226 (86), 85 (65), 83 (100); EIHRMS *m*/*z*: 373.1524 [M^+] (Calcd for $C_{20}H_{23}NO_6$: 373.1525); ^1H NMR (500 MHz, $CDCl_3$) δ: 6.70 (1H, s, H-11), 6.57 (1H, s, H-8), 5.92 (2H, s, –OCH_2O–), 5.70 (1H, br s, H-1), 5.56 (1H, s like, H-3), 4.24 (1H, m, H-2), 4.15 (1H, d, *J* = 14.0 Hz, H-7), 4.07 (1H, m, H-3′), 3.53 (1H, d, *J* = 15.0 Hz, H-7), 3.37 (1H, m, H-5), 2.90 (1H, d, *J* = 10.0 Hz, H-11b), 2.74 (1H, br d, *J* = 10.5 Hz, H-11c), 2.64 (2H, m, H_2-4), 2.40 (1H, overlapped, H-5), 2.38 (1H, dd, *J* = 16.0, 3.5 Hz, H-2′), 2.30 (1H, dd, *J* = 16.0, 8.5 Hz, H-2′), 1.12 (3H, d, *J* = 6.5 Hz, H_3-4′); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 172.4 (C-1′), 146.5 and 146.3 (C-10, C-9), 144.5 (C-3a), 129.4 (C-11a),126.7 (C-7a), 117.0 (C-3), 107.4 (C-8), 104.8 (C-11), 101.0 (–OCH_2O–), 72.8 (C-1), 69.8 (C-2), 64.3 (C-3′), 61.6 (C-11c), 56.8 (C-7), 53.6 (C-5), 42.9 (C-2′), 39.4 (C-11b), 28.6 (C-4), 22.3 (C-4′); CD (*c* = 0.280 mmol/L, MeOH, 23 °C) $\Delta\varepsilon$ (λ nm): 0 (310), −1.9 (292), 0 (259), +1.7 (244), 0 (233), −2.2 (216), −1.5 (209).

4.12. 1,2-Di-*O*-acetyllycorine (3)

To a solution of lycorine (50 mg, 0.17 mmol) in dry pyridine (6.8 mL) were added acetic anhydride (82.3 μL, 0.87 mmol) and DMAP (2.1 mg, 0.02 mmol) and the reaction mixture was stirred at room temperature for 18.5 h. The reaction mixture was then quenched with cold water and extracted with chloroform. The combined organic layers were washed with brine and dried over anhydrous magnesium sulfate. After removal of the volatiles, the crude product was purified by SiO_2 open column chromatography (EtOAc/$CHCl_3$/MeOH, 40:40:20) to give **3** (63.7 mg, 99%) as pale yellow needles; $[\alpha]_D^{24}$ +11.1 (*c* 0.84, MeOH); UV (MeOH) λ_{max} nm: 290.5, 240.0, 207.0; IR (ATR) ν_{max} cm^{-1}: 3463, 2952, 2925, 2883, 2777, 1726, 1487, 1367, 1218, 1032, 965, 932, 757; EIMS *m*/*z*: 371 (M^+, 31), 311 (20), 252 (100), 250 (36), 226 (24); ^1H NMR (400 MHz, $CDCl_3$) δ: 6.75 (1H, s, H-11), 6.57 (1H, s, H-8), 5.92 (2H, s, –OCH_2O–), 5.73 (1H, br s, H-1), 5.53 (1H, m, H-3), 5.25 (1H, m, H-2), 4.16 (1H, d, *J* = 14.1 Hz, H-7β), 3.53 (1H, d, *J* = 14.5 Hz, H-7α), 3.37 (1H, ddd, *J* = 9.2, 4.6, 4.6 Hz, H-5β), 2.87 (1H, br d, *J* = 10.4 Hz, H-11b), 2.76 (1H, br d, *J* = 10.4 Hz, H-11c), 2.65 (2H, m, H_2-4), 2.40 (1H, ddd, *J* = 8.8, 8.8, 8.8 Hz, H-5α), 2.08 (3H, s, H_3-2″), 1.95 (3H, s, H_3-2′); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 170.0 and 169.8 (C-1′, C-1″), 146.4 and 146.3 (C-10, C-9), 146.1 (C-3a), 129.4 (C-7a), 126.5 (C-11a), 113.8 (C-3), 107.3 (C-8), 105.0 (C-11), 101.0 (–OCH_2O–), 70.9 (C-2), 69.2 (C-1), 61.2 (C-11c), 56.8 (C-7), 53.6 (C-5), 40.4 (C-11b), 28.7 (C-4), 21.1 and 20.9 (C-2′, C-2″); CD (*c* = 0.337 mM, MeOH, 24 °C) $\Delta\varepsilon$ (λ nm): 0 (309), −1.6 (294), −0.1 (264), +1.7 (246), 0 (231), −0.7 (228), 0 (219), +7.5 (206).

4.13. 1,2-Di-*O*-propanoyllycorine (10)

To a solution of lycorine (**1**, 19.8 mg, 0.07 mmol) in dry pyridine (2.8 mL) were added propionic anhydride (44.2 μL, 0.34 mmol) and DMAP (0.84 mg, 0.01 mmol) and the reaction mixture was stirred at room temperature for 17 h. It was then quenched with cold water and extracted with chloroform. The combined organic layers were washed with brine and dried over anhydrous magnesium sulfate. After removal of the volatiles, the crude product was purified by SiO_2 open column chromatography (50% EtOAc/$CHCl_3$) to give **10** (25.2 mg, 92%) as a colorless solid; $[\alpha]_D^{25}$ +13.5 (*c* 0.52, MeOH); UV (MeOH) λ_{max} nm: 292.0, 235.5, 207.5; IR (ATR) ν_{max} cm^{-1}: 2925, 2799, 1732, 1487, 1156, 1028, 931, 808; EIMS *m*/*z*: 399 (M^+, 20), 325 (21), 252 (100), 250 (40); EIHRMS *m*/*z*: 399.1700 [M^+] (Calcd for $C_{22}H_{25}NO_6$: 399.1682); ^1H NMR (400 MHz, $CDCl_3$) δ: 6.74 (1H, s like, H-11), 6.56 (1H, s, H-8), 5.91 (2H, s like, –OCH_2O–), 5.73 (1H, s, H-1), 5.52 (1H, s like, H-3), 5.25 (1H, s like, H-2), 4.16 (1H, d, *J* = 14.0 Hz, H-7β), 3.52 (1H, d, *J* = 14.0 Hz, H-7α), 3.37 (1H, ddd, *J* = 9.2, 4.6, 4.6 Hz, H-5β), 2.88 (1H, br d, *J* = 10.4 Hz, H-11b), 2.77 (1H, br d, *J* = 10.4 Hz, H-11c), 2.64 (2H, m, H_2-4), 2.43-2.29 (3H, overlapped, H-5α, H_2-2″), 2.20 (1H, dq, *J* = 16.5, 7.6 Hz, H-2′), 2.19 (1H, dq, *J* = 16.5, 7.5 Hz, H-2′), 1.15 (3H, t, *J* = 7.6 Hz, H_3-3″), 1.01 (3H, t, *J* = 7.6 Hz, H_3-3′); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 173.4 and 173.2 (C-1′, C-1″), 146.4 and 146.3 (C-10, C-9), 145.9 (C-3a), 129.4 (C-11a), 126.7 (C-7a), 113.9 (C-3), 107.3 (C-8), 105.1 (C-11), 100.9 (–OCH_2O–), 70.8 (C-1), 69.1 (C-2), 61.3 (C-11c), 56.9 (C-7), 53.6 (C-5), 40.6 (C-11b), 28.7 (C-4), 27.6 and 27.5 (C-2′, C-2″), 9.0 (C-3′, C-3″).

4.14. 1,2-Di-*O*-butanoyllycorine (11)

To a solution of lycorine (**1**, 100.4 mg, 0.35 mmol) in dry pyridine (5 mL) were added butyric anhydride (286.1 μL, 1.7 mmol) and DMAP (4.3 mg, 0.03 mmol) and the reaction mixture was stirred at room temperature for 23 h. It was then quenched with cold water and extracted with chloroform. The combined organic layers

were washed with brine and dried over anhydrous magnesium sulfate. After removal of the volatiles, the crude product was purified by SiO_2 open column chromatography (EtOAc/$CHCl_3$/MeOH, 40:40:20) to give **11** (142.1 mg, 95%) as a pale yellow solid; $[\alpha]_D^{25}$ +11.5 (*c* 0.98, MeOH); UV (MeOH) λ_{max} nm: 292.5, 236.0, 205.0; IR (ATR) ν_{max} cm^{-1}: 2961, 2935, 2874, 1730, 1504, 1485, 1155, 1026, 966, 935; EIMS *m*/*z*: 427 (M^+, 25), 339 (31), 268 (22), 253 (40), 252 (100), 250 (61); EIHRMS *m*/*z*: 427.2004 [M^+] (Calcd for $C_{24}H_{29}NO_6$: 427.1995); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 6.74 (1H, s, H-11), 6.56 (1H, s, H-8), 5.91 (2H, s, –OCH_2O–), 5.74 (1H, s, H-1), 5.52 (1H, s, H-3), 5.25 (1H, s like, H-2), 4.16 (1H, d, *J* = 14.4 Hz, H-7β), 3.52 (1H, d, *J* = 14.4 Hz, H-7α), 3.37 (1H, ddd, *J* = 9.2, 4.6, 4.6 Hz, H-5β), 2.88 (1H, br d, *J* = 10.4 Hz, H-11b), 2.77 (1H, br d, *J* = 10.4 Hz, H-11c), 2.64 (2H, m, H_2-4), 2.40 (1H, ddd, *J* = 8.8, 8.8, 8.8 Hz, H-5α), 2.31 (1H, dt, *J* = 15.6, 7.5 Hz, H-2"), 2.30 (1H, dt, *J* = 15.6, 7.3 Hz, H-2"), 2.16 (2H, t, *J* = 7.6 Hz, H_2-2′), 1.66 (2H, sex, *J* = 7.4 Hz, H_2-3"), 1.50 (2H, sex, *J* = 7.4 Hz, H_2-3′), 0.95 (3H, t, *J* = 7.6 Hz, H_3-4"), 0.79 (3H, t, *J* = 7.6 Hz, H_3-4′); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 172.5 and 172.3 (C-1′, C-1″), 146.4 and 146.2 (C-10, C-9), 145.9 (C-3a), 129.4 (C-11a), 126.6 (C-7a), 113.9 (C-3), 107.2 (C-8), 105.2 (C-11), 100.9 (–OCH_2O–), 70.8 (C-1), 69.0 (C-2), 61.3 (C-11c), 56.9 (C-7), 53.6 (C-5), 40.6 (C-11b), 36.2 and 36.0 (C-2′, C-2″), 28.7 (C-4), 18.4 and 18.3 (C-3′, C-3″), 13.6 and 13.3 (C-4′, C-4″).

4.15. 1-*O*-Acetyllycorine (2)

1,2-Di-*O*-acetyllycorine (**3**, 21.1 mg, 0.06 mmol) in methanol (3.0 mL) and concd hydrochloric acid (0.7 mL) was heated on an oil-bath for 1 h. After cooling, the mixture was cautiously basified (pH 8) with aqueous ammonia solution and diluted with water, and the whole was extracted with chloroform. The combined organic layers were washed with brine and dried over anhydrous magnesium sulfate. After evaporation, the residue was chromatographed over silica gel (EtOAc/$CHCl_3$/MeOH, 40:40:20) to give **2** (10.6 mg, 57%) as a colorless solid; $[\alpha]_D^{24}$ −62.5 (*c* 0.17, MeOH); UV (MeOH) λ_{max} nm: 291.5, 234.5, 206.0; IR (ATR) ν_{max} cm^{-1}: 3090, 2918, 2877, 2823, 1732, 1482, 1368, 1238, 1030, 996; EIMS *m*/*z*: 329 (M^+, 63), 268 (56), 227 (100), 226 (100); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 6.71 (1H, s, H-11), 6.58 (1H, s, H-8), 5.92 (2H, s like, –OCH_2O–), 5.64 (1H, br s, H-1), 5.56 (1H, s like, H-3), 4.24 (1H, m, H-2), 4.15 (1H, d, *J* = 14.0 Hz, H-7β), 3.53 (1H, d, *J* = 14.4 Hz, H-7α), 3.37 (1H, m, H-5β), 2.87 (1H, br d, *J* = 10.4 Hz, H-11b), 2.75 (1H, br d, *J* = 10.8 Hz, H-11c), 2.65 (2H, overlapped, H_2-4), 2.40 (1H, ddd, *J* = 8.7, 8.7, 8.7 Hz, H-5α), 1.95 (3H, s, H_3-2′); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 170.8 (C-1′), 146.5 and 146.2 (C-10, C-9), 144.1 (C-3a), 129.3 (C-11a),* 127.0 (C-7a),* 117.2 (C-3), 107.3 (C-8), 104.9 (C-11), 100.9 (–OCH_2O–), 72.6 (C-1), 69.6 (C-2), 61.5 (C-11c), 56.8 (C-7), 53.6 (C-5), 39.3 (C-11b), 28.6 (C-4), 21.0 (C-2′) (*: interchangeable).

4.16. 1-*O*-Propanoyllycorine (12)

1,2-Di-*O*-propanoyllycorine (**10**, 31.0 mg, 0.08 mmol) in methanol (4.0 mL) and concd hydrochloric acid (0.8 mL) was heated on an oil-bath for 1 h. After cooling, the mixture was cautiously basified (pH 8) with aqueous ammonia solution and diluted with water, and the whole was extracted with chloroform. The combined organic layers were washed with brine and dried over anhydrous magnesium sulfate. After evaporation, the residue was chromatographed over amino-silica gel (5% MeOH/EtOAc) to give **12** (23.1 mg, 87%) as a colorless amorphous solid; $[\alpha]_D^{25}$ −76.4 (*c* 0.05, MeOH); UV (MeOH) λ_{max} nm: 290.5, 261.5, 237.5, 205.5; IR (ATR) ν_{max} cm^{-1}: 2921, 2780, 1731, 1486, 1235, 1175, 1034, 994, 932; EIMS *m*/*z*: 343 (M^+, 38), 268 (34), 251 (36), 250 (83), 227 (98), 226 (100); EIHRMS *m*/*z*: 343.1409 [M^+] (Calcd for $C_{19}H_{21}NO_5$: 343.1419); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 6.71 (1H, s, H-11), 6.57 (1H, s, H-8), 5.92 (1H, d, *J* = 1.6 Hz, –OCH_2O–), 5.91 (1H, d, *J* = 1.6 Hz, –OCH_2O–), 5.64 (1H, s, H-1), 5.56 (1H, s like, H-3), 4.22 (1H, br s, H-2), 4.15 (1H, d, *J* = 14.0 Hz, H-7β), 3.52 (1H, d, *J* = 14.4 Hz, H-7α), 3.36 (1H, m, H-5β), 2.86 (1H, br d, *J* = 10.4 Hz, H-11b), 2.74 (1H, br d, *J* = 10.4 Hz, H-11c), 2.64 (2H, m, H_2-4), 2.40 (1H, dd, *J* = 8.8, 8.8, 8.8 Hz, H-5α), 2.20 (2H, q, *J* = 7.6 Hz, H_2-2′), 1.02 (3H, t, *J* = 7.6 Hz, H_3-3′); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 174.2 (C-1′), 146.4 and 146.2 (C-10, C-9), 143.7 (C-3a), 129.1 (C-11a), 127.1 (C-7a), 117.4 (C-3), 107.2 (C-8), 104.9 (C-11), 100.9 (–OCH_2O–), 72.4 (C-1), 69.5 (C-2), 61.5 (C-11c), 56.7 (C-7), 53.6 (C-5), 39.2 (C-11b), 28.6 (C-4), 27.5 (C-2′), 9.1 (C-3′).

4.17. 1-*O*-Butanoyllycorine (13)

1,2-Di-*O*-butanoyllycorine (**11**, 20.1 mg, 0.05 mmol) in methanol (2.5 mL) and concd hydrochloric acid (0.4 mL) was heated on an oil-bath for 1 h. After cooling, the mixture was cautiously basified (pH 8) with aqueous ammonia solution and diluted with water, and the whole was extracted with chloroform. The combined organic layers were washed with brine and dried over anhydrous magnesium sulfate. After evaporation, the residue was chromatographed over silica gel (EtOAc/$CHCl_3$/MeOH, 40:40:20) and amino-silica gel (5% MeOH/EtOAc) to give **13** (12.2 mg, 73%) as a colorless solid; $[\alpha]_D^{25}$ −87.6 (*c* 0.05, MeOH); UV (MeOH) λ_{max} nm: 291.5, 235.5, 206.0; IR (ATR) ν_{max} cm^{-1}: 3324, 2964, 2923, 2875, 2806, 1727, 1504, 1486, 1237, 1174, 1034, 997, 933; EIMS *m*/*z*: 357 (M^+, 22), 250 (29), 227 (61), 226 (52), 149 (54), 85 (64), 83 (100); EIHRMS *m*/*z*: 357.1570 [M^+] (Calcd for $C_{20}H_{23}NO_5$: 357.1576); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 6.72 (1H, s, H-11), 6.57 (1H, s, H-8), 5.91 (2H, s, –OCH_2O–), 5.66 (1H, s, H-1), 5.55 (1H, s like, H-3), 4.22 (1H, s like, H-2), 4.15 (1H, d, *J* = 14.4 Hz, H-7β), 3.52 (1H, d, *J* = 13.2 Hz, H-7α), 3.36 (1H, m, H-5β), 2.86 (1H, br d, *J* = 10.4 Hz, H-11b), 2.74 (1H, br d, *J* = 10.8 Hz, H-11c), 2.64 (2H, m, H_2-4), 2.40 (1H, dd, *J* = 8.7, 8.7, 8.7 Hz, H-5α), 2.17 (2H, t, *J* = 7.4 Hz, H_2-2′), 1.50 (2H, sex, *J* = 7.4 Hz, H_2-3′), 0.80 (3H, t, *J* = 7.6 Hz, H_3-4′); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 173.3 (C-1′), 146.4 and 146.1 (C-10, C-9), 144.3 (C-3a), 129.4 (C-11a), 127.0 (C-7a), 117.1 (C-3), 107.2 (C-8), 105.0 (C-11), 100.9 (–OCH_2O–), 72.4 (C-1), 69.8 (C-2), 61.6 (C-11c), 56.9 (C-7), 53.7 (C-5), 39.5 (C-11b), 36.2 (C-2′), 28.6 (C-4), 18.4 (C-3′), 13.4 (C-4′).

4.18. Assay for in vitro antitrypanosomal activity against *T. brucei* species

Trypanosoma brucei brucei strain GUTat 3.1 (Glasgow University, *Trypanozoon* antigenic type 3.1) was a generous gift from Dr. Y. Yabu (Nagoya City University). *T. b. brucei* GUTat 3.1 strain was cultured in IMDM with 3.024 g/L $NaHCO_3$, 100 μM hypoxanthine, 30 μM thymidine, 40 μM adenosine, 1.0 mM sodium pyruvate, 50 μM L-glutamine, 100 μM 2-mercaptoethanol, 50 U/mL of penicillin, and 50 μg/mL of streptomycin containing 10% heat-inactivated FBS at 37 °C, under 5.0% CO_2–95% air, according to the method of Yabu et al.[30] In vitro antitrypanosomal activity of the test compounds was determined from a dose–response curve using the fluorescent dye, Alamar Blue, according to the method of Räz et al. and Tasdemir et al.[31,32] with some modifications. Ninety-five microliters of trypanosome suspension ($2.0–2.5 \times 10^4$ trypanosomes/mL for GUTat 3.1 strain) of bloodstream forms was seeded into a 96-well microplate and 5.0 μL of test compound solution (dissolved in 5.0% dimethylsulfoxide: DMSO) was added. After incubating for 72 h at 37 °C under 5.0% CO_2–95% air, 10 μL of Alamar Blue was added to each well. After further incubation for 3–6 h at 37 °C under 5.0% CO_2–95% air, the microplate was read at 528/20 nm excitation wavelength and 590/35 nm emission wavelength with an FLx800 fluorescent plate reader (Bio-Tek Instrument, Inc.

Vermont, USA). Data were transferred into a graphic program (Excel) and IC_{50} values were determined with fluorescent plate reader software (KC-4, Bio-Tek).

4.19. Assay for in vitro antimalarial activity against *P. falciparum*

Type A^+ human plasma and erythrocytes were obtained from healthy volunteers at the Research Center for Clinical Pharmacology, Kitasato University. *P. falciparum* strains K1 (drug-resistant) and FCR3 (drug-sensitive) were generous gifts from Professor K. Kita (The University of Tokyo). The parasites were grown according to the method described by Trager and Jensen.[33] *P. falciparum* strains were cultured in human erythrocytes in RPMI medium (RPMI-1640 with 25 mM HEPES buffer, 24 mM $NaHCO_3$, 0.2% glucose, 0.05% L-glutamine, 50 μg/mL hypoxanthine, and 25 μg/mL gentamicin) supplemented with 10% human plasma at 37 °C, under 93% N_2, 4% CO_2, and 3% O_2. Antimalarial activity of the test compound was determined from the dose–response curve using the parasite lactate dehydrogenase (pLDH) assay according to the method of Makler et al.[34] One hundred and ninety microliters of asynchronous parasites (2.0% hematocrit and 0.5 or 1% parasitemia) was seeded into a 96-well microplate and 10 μL of test compound solution (dissolved in 25% ethanol or 5% DMSO) was added. After incubating at 37 °C for 72 h under 93% N_2, 4% CO_2, and 3% O_2, the microplate was immediately frozen at −20 °C for 18 h. Then, the microplate was thawed at 37 °C and 20 μL of the hemolyzed parasite suspension was transferred to another microplate containing 100 μL of Malstat reagent. The plate was further incubated for 15 min at room temperature, and 20 μL of a 1:1 mixture of nitroblue tetrazolium and phenazine ethosulfate (2 mg and 0.1 mg/mL, respectively) was added to each well. After incubation for 2 h at room temperature in the dark, the blue formazan product was measured at 655 nm with iEMS microplate reader MF (Labsystems, Helsinki, Finland). The 50% inhibitory concentration (IC_{50}) value was estimated from the dose–response curve.

4.20. Cytotoxicity tests on MRC-5 cells

The human diploid embryonic cell line, MRC-5, was a generous gift from Dr. L. Maes (Tibotec NV, Mechelen, Belgium). Cytotoxicity of the test compound was measured by the colorimetric MTT assay[35,36] in 96-well microplates. In brief, 100 μL of MRC-5 cell suspension was added to 96-well microplates at 1×10^3 cells/well and the cells were cultured for 24 h. Then, 90 μL of standard culture medium (MEM +10% FCS) with or without 10 μL of test compound solution that was dissolved in 25% ethanol or 5% DMSO was added to each well. Further incubation was conducted at 37 °C under 5% CO_2–95% air for 7 days and then 20 μL of MTT–PBS solution (5 mg/mL) was added to each well. The microplate was incubated at 37 °C for 4 h under 5% CO_2–95% air. Then, the incubation medium was aspirated and 100 μL of DMSO was added to solubilize the MTT formazan product. After mixing, absorbance at 540 nm was measured with iEMS microplate reader MF. The 50% inhibitory concentration (IC_{50}) value was estimated from the dose–response curve.

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