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Synthesis and antitumor activity of two natural *N*-acetylglucosamine-bearing triterpenoid saponins: Lotoidoside D and E

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Abstract—Two natural triterpenoid saponins bearing *N*-acetylglucosamine, lotoidoside D and lotoidoside E, which had been available only from *Glinus lotoides* growing in Egyptian desert, were facilely synthesized from readily available oleanolic acid. Preliminary pharmacological research showed their antitumor activity against HeLa cell.
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Triterpenoid saponins are a large family of natural products with great variety of structures and bioactivities.[1] Some of them have *N*-acetylglucosamine in the carbohydrate moiety, namely *N*-acetylglucosamine-bearing triterpenoid saponins. These rarely reported natural products have been drawing more and more attention of phytochemists and pharmacologists in recent years. Quite a number of these saponins were found to have remarkable cytotoxicity or antiproliferative activities.[2] For instance, lotoidoside D (**1**) and lotoidoside E (**2**) (Fig. 1) are two *N*-acetylglucosamine-bearing triterpenoid saponins isolated from the roots of *Glinus lotoides* (a medical plant growing in Egyptian desert) by Hamed et al.[3] in 2005. These triterpenoid saponins show prominent antiproliferative activity against several murine and human cell lines.[3] For example, IC_{50} values of **1** and **2** against murine fibrosarcoma cell line WEHI-164 are 0.36 and 0.03 μM, respectively.

However, **1** and **2** are only available from the tedious isolation procedure from the roots of *Glinus lotoides* in low isolated yields (only 25 mg of **1** and 20 mg of **2** from 800 g roots).[3] The scarceness of **1** and **2** in the nature turns out to be a great obstacle for further pharmacological research. Considering the rapid discovery of *N*-acetylglucosamine-bearing triterpenoid saponins with attractive bioactivities in recent years, it is valuable to develop a convenient way to prepare this kind of saponins to promote further studies, such as thorough pharmacological research and further structure–activity relationship (SAR) investigation. Herein, we report the chemical synthesis of **1** and **2** from commercially available oleanolic acid (**3**). The preliminary antitumor activity of these synthetic saponins was then investigated.

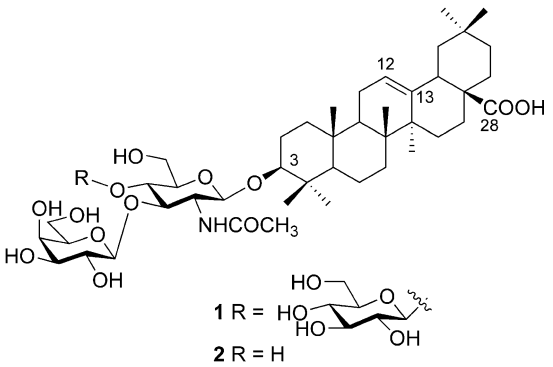

Figure 1. Structures of lotoidosides D (**1**) and E (**2**).

Keywords: Lotoidoside D; Lotoidoside E; Triterpenoid saponin; Glycosylation; Antitumor.
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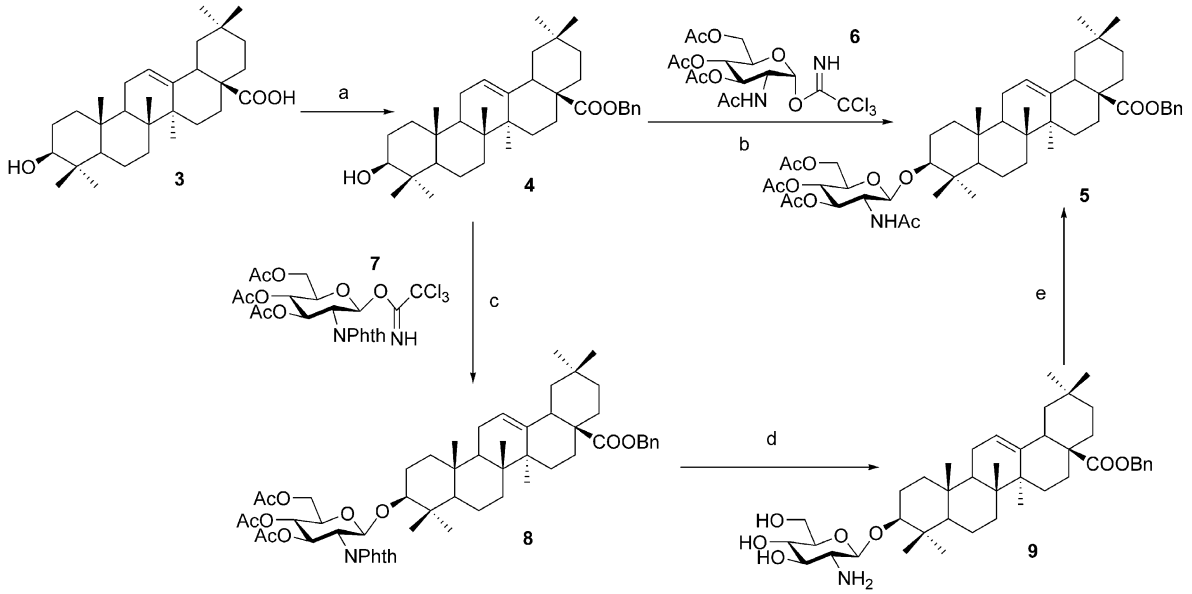
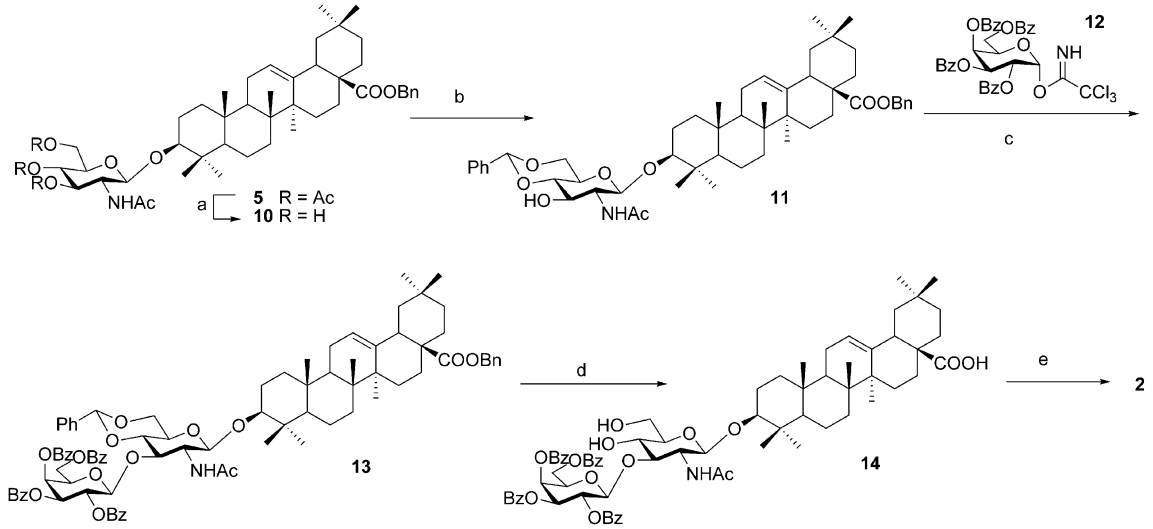
Stepwise glycosylation was adopted to construct the oligosaccharide moiety because it is a preferable method of preparing glycoside analogues for SAR research by altering monosaccharide donors.[4] It is known that ester linkage at C-28 carboxyl of pentacyclic triterpenoid is extremely resistant to basic hydrolysis due to its high steric hindrance.[5] Therefore, it is difficult to deprotect general alkyl protective groups from C-28 alkyl ester. Based on our experience of triterpenoid saponin synthesis,[6] benzyl was adopted as a permanent protective group for it can be removed conveniently through catalytic hydrogenolysis.[7] Especially in this work, the benzyl ester would be cleaved together with benzylidene acetal or benzyl ether in carbohydrate moiety during catalytic hydrogenolysis, which would make the synthetic route more concise and efficient.

The synthetic route of **2** is shown in Schemes 1 and 2. Oleanolic acid (**3**) was first converted to its benzyl ester **4** by treating **3** with BnBr and K_2CO_3 in aqueous THF in high yield (98%).[8] Next, a straightforward preparation of intermediate **5** using readily available 2-acetamino-2-deoxyglucose donor **6**[9] was investigated. However, little desired product was obtained while the corresponding oxazoline derivative became the major product. Therefore, 2-phthalimido-2-deoxyglucose donor **7**[10] was employed to be coupled with **4** under the promotion of TMSOTf to give **8** in a high yield (96%), which was then treated with hot ethylenediamine/butanol and Ac_2O/pyridine afterward to afford **5**. The overall yield for 3 steps from **4** to **5** was 84%.

Then, the *O*-acetyl groups of **5** were removed by NaOMe/MeOH to give **10**, and benzylidene was



Scheme 1. Synthesis of intermediate **5**. Reagents and conditions: (a) BnBr, K_2CO_3, THF–H_2O, rt, 98%; (b) TMSOTf, 4 Å MS, CH_2Cl_2, 0 °C to rt, 4%; (c) TMSOTf, 4 Å MS, CH_2Cl_2, 0 °C to rt, 96%; (d) $H_2NCH_2CH_2NH_2$, *n*-BuOH, 90 °C; (e) Ac_2O, pyridine, rt, 88% for two steps.



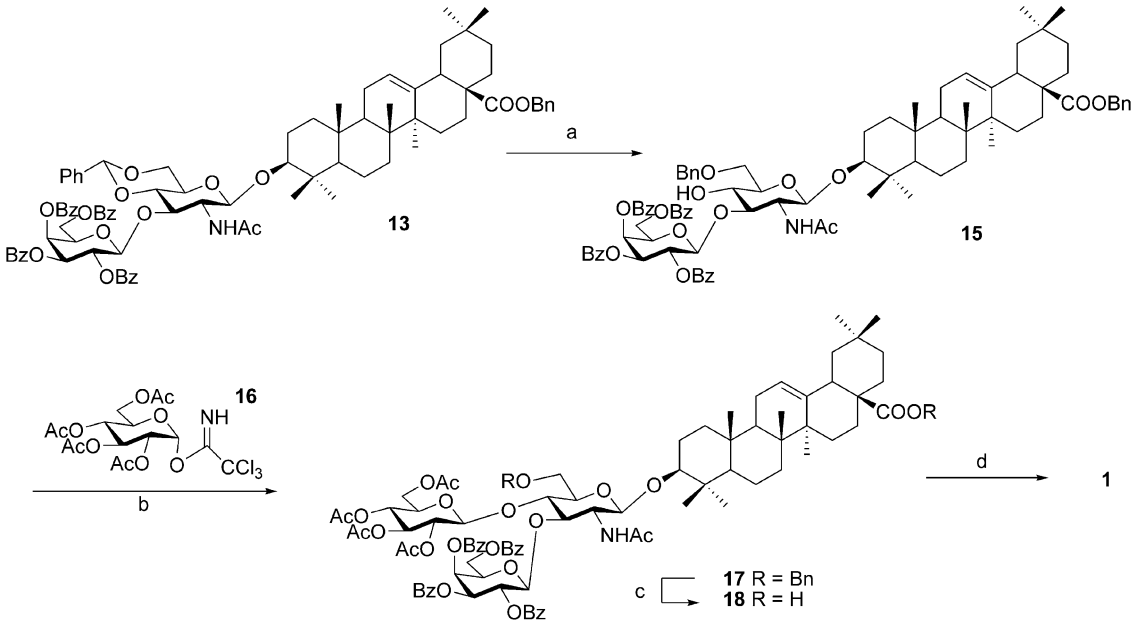
Scheme 2. Synthesis of lotoidoside E (**2**). Reagents and conditions: (a) NaOMe, CH_2Cl_2–MeOH, rt; (b) $PhCH(OMe)_2$, CSA, DMF, 70 °C, 87% for two steps; (c) TMSOTf, CH_2Cl_2, 0 °C to rt, 69%; (d) H_2, Pd–C (10%), HOAc–THF, 40 °C; (e) NaOMe, CH_2Cl_2–MeOH, rt, 82% for two steps.

introduced to protect 4′,6′-OH by the reaction of **10** with $PhCH(OMe)_2$ and DL-camphorsulfonic acid (CSA) in dry DMF (Scheme 2). 3-OH of 4,6-*O*-benzylidene-2-acetamido-2-deoxyglucopyranosides have been found to be unreactive sugar acceptors in glycosylation.[11] In current study, no reaction product was detected from TLC (petroleum ether/EtOAc = 4:1) when a catalytic amount of TMSOTf (0.05–0.20 equiv) was used in the glycosylation of **11** with **12**. Furthermore, adding excess galactose donor **12**[12] (1.60 equiv) and TMSOTf (1.20 equiv) gave a yield of merely 22%. Therefore, the 'inverse procedure,'[13] which was developed by Schmidt et al. originally to prevent sensitive sugar donors from decomposing and later was employed in glycosylation of unreactive acceptors, was applied here and worked well to give the desired product **13** in a yield of 69%. Catalytic hydrogenolysis of **13** was performed to remove the benzyl and benzylidene cleanly. Finally, the resulted **14** was treated with NaOMe in CH_2Cl_2–MeOH to give the target product **2**. The overall yield from oleanolic acid was 41%.

The key intermediate **13** was also utilized for the facile synthesis of **1** (Scheme 3). Regioselective cleavage of 4,6-*O*-benzylidene acetal of carbohydrate derivatives is proved to be an outstanding strategy to furnish 4-OH-6-benzyl ether or 6-OH-4-benzyl ether.[14] Considering the compatibility to the glycosidic linkages and various protecting groups on the substrate, mild reducing condition Et_3SiH–$BF_3{\cdot}Et_2O$[15] was applied to **13** to give benzyl ether **15** in a yield of 79%. Coupling of **15** and **16**[16] afforded fully protected saponin **17** in a yield of 66%. Deprotection of **17** through catalytic hydrogenolysis and ester interchange gave the final product **1**. The overall yield from oleanolic acid to **1** was 19%. The analytical data of synthetic **1** and **2** were identical with those reported for the natural products.[3,17]

The in vitro antiproliferative activity of these triterpenoid saponins against HeLa tumor cell was investigated. HeLa cell in RPMI1640 medium with 10% fetal bovine serum was plated on 96-well microtiter plates (4.0×10^4 cells per well), and allowed to adhere at 37 °C with 5% CO_2 for 4 h. Then the test compound was added, and the cells were incubated at 37 °C with 5% CO_2 for 72 h. The cell viability was assessed through standard MTT assay.[18]

As shown in Table 1, **1** displayed prominent inhibition activity to HeLa cell even at very low concentrations. The inhibition at 10 nM was 34.44%. On the other hand, saponin **2**, which was more potent than **1** in the previous research,[3] showed relatively lower inhibition against HeLa cell. Despite the sensitivity difference between these cell lines, it may be indicated that **1** and **2** possibly have different inhibition mechanisms against various tumor cells. Extensive antitumor screening and intensive mechanism investigation are demanded in this area to



Scheme 3. Synthesis of lotoidoside D (**1**). Reagents and conditions: (a) Et_3SiH, $BF_3{\cdot}Et_2O$, 4 Å MS, CH_2Cl_2, 0 °C, 79%; (b) TMSOTf, 4 Å MS, CH_2Cl_2, 0 °C to rt, 66%; (c) H_2, Pd–C (10%), HOAc–THF, reflux; (d) NaOMe, CH_2Cl_2–MeOH, rt, 74% for two steps.

Table 1. Antitumor activity of synthetic saponins against HeLa tumor cell line

Saponin	Inhibition rate at different concentration (%)				IC_{50} (μM)
	10^{-5} M	10^{-6} M	10^{-7} M	10^{-8} M	
1	56.43	35.33	32.84	34.44	2.74
2	42.02	10.04	1.71	0.79	>10

elucidate the mechanism and develop more desirable bioactive agents.

In summary, lotoidosides D (**1**) and E (**2**) were efficiently synthesized from oleanolic acid in high yields. This synthesis may provide a convenient way for the preparation of other *N*-acetylglucosamine-bearing triterpenoid saponins. Preliminary biological evaluation was performed and showed significant antitumor activity. Further biological studies and SAR research of their derivatives are in progress and will be reported in due course.

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13. 'Inverse procedure' here means mixing the sugar acceptor and promoter (herein TMSOTf) before the sugar donor is added, which is different to common glycosylation procedure (adding the promoter to the mixture of sugar acceptor and donor). See: Schmidt, R. R.; Toepfer, A. *Tetrahedron Lett.* **1991**, *32*, 3353.
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17. Selected data for products and key intermediates: Compound **5**: $[\alpha]_D$ +24.8 (*c* 0.62, acetone); ^1H NMR (300 MHz, $CDCl_3$) δ 7.35–7.30 (m, 5H, Ar-H), 5.52 (br t, $J = 8.3$, NH), 5.31 (br s, 1H), 5.25 (t, 1H, $J = 10.2$), 5.06 (dd, 2H, $J = 18.6$, 12.5), 5.02 (t, 1H, $J = 9.6$), 4.68 (d, 1H, $J = 8.3$, H-1′), 4.24–4.21 (m, 1H), 4.09 (dd, 1H, $J = 12.0$, 2.6), 3.86 (dd, 1H, $J = 14.6$, 8.3), 3.69–3.66 (m, 1H), 3.07 (dd, 1H, $J = 11.3$, 4.6), 2.84 (dd, 1H, $J = 13.1$, 3.4), 2.07 (s, 3H), 2.03 (s, 3H), 2.02 (s, 3H), 1.92 (s, 3H), 1.11 (s, 3H), 0.92 (s, 3H), 0.90 (s, 3H), 0.89 (s, 3H), 0.87 (s, 3H), 0.77 (s, 3H), 0.59 (s, 3H); ESI-MS *m/z*: 898.6 $(M+Na)^+$. Compound **11**: $[\alpha]_D$ +13.2 (*c* 1.30, acetone); ^1H NMR (600 MHz, $CDCl_3$) δ 7.50–7.30 (m, 10H, Ar-H), 5.78 (d, 1H, $J = 6.5$), 5.53 (s, 1H), 5.28 (br s, 1H), 5.07 (dd, 1H, $J = 30.2$, 12.5), 4.80 (d, 1H, $J = 8.2$, H-1′), 4.29 (dd, 1H, $J = 10.4$, 4.9), 4.19 (t, 1H, $J = 9.4$), 3.78 (t, 1H, $J = 10.3$), 3.74 (br s, 1H), 3.53 (t, 1H, $J = 9.3$), 3.46–3.43 (m, 2H), 3.13 (dd, 1H, $J = 11.6$, 4.3), 2.90 (dd, 1H, $J = 13.6$, 3.8), 2.01 (s, 3H), 1.11 (s, 3H), 0.96 (s, 3H), 0.92 (s, 3H), 0.90 (s, 3H), 0.87 (s, 3H), 0.80 (s, 3H), 0.60 (s, 3H); ESI-HRMS *m/z* 860.5069 $(M+Na)^+$ ($C_{52}H_{71}NNaO_8$ requires 860.5072). Compound **13**: $[\alpha]_D$ +45.2 (*c* 0.30, acetone); ^1H NMR (600 MHz, $CDCl_3$) δ 8.06–7.20 (m, 30H, Ar-H), 5.88 (d, 1H, $J = 3.3$), 5.72 (dd, 1H, $J = 10.2$, 8.1), 5.55 (s, 1H), 5.53–5.51 (m, 2H), 5.28 (br s, 1H), 5.18 (d, 1H, $J = 8.1$, H-1′), 5.06 (dd, 2H, $J = 31.2$, 12.5), 4.98 (d, 1H, $J = 8.1$, H-1″), 4.76 (t, 1H, $J = 9.0$), 4.31–4.28 (m, 3H), 3.89 (dd, 1H, $J = 7.3$, 6.3), 3.78 (t, 1H, $J = 10.3$), 3.73 (t, 1H, $J = 9.1$), 3.57 (dd, 1H, $J = 9.8$, 5.0), 3.05 (dd, 2H, $J = 11.2$, 4.1), 2.89 (dd, 1H, $J = 13.7$, 3.6), 1.60 (s, 3H), 1.09 (s, 3H), 0.91 (s, 3H), 0.89 (s, 3H), 0.85 (s, 3H), 0.80 (s, 3H), 0.72 (s, 3H), 0.58 (s, 3H); ESI-HRMS *m/z* 1438.6655 $(M+Na)^+$ ($C_{86}H_{97}NNaO_{17}$ requires 1438.6649). Compound **15**: $[\alpha]_D$ +84.8 (*c* 1.20, acetone); ^1H NMR (600 MHz, $CDCl_3$) δ 8.09–7.22 (m, 30H, Ar-H), 5.96 (d, 1H, $J = 3.4$), 5.81 (dd, 1H, $J = 10.4$, 8.1), 5.67 (dd, 1H, $J = 10.4$, 3.4), 5.29 (br s, 1H), 5.26 (d, 1H, $J = 6.7$, NH), 5.06 (dd, 2H, $J = 31.8$, 12.6), 5.01 (d, 1H, $J = 8.7$, H-1′), 4.90 (d, 1H, $J = 8.0$, H-1″), 4.63 (d, 1H, $J = 8.0$), 4.61 (dd, 2H, $J = 31.4$, 7.9) 4.60–4.58 (m, 1H), 4.46–4.43 (m, 2H), 3.85 (d, 1H, $J = 10.6$), 3.64 (dd, 1H, $J = 10.7$, 6.0), 3.55–3.52 (m, 2H), 3.07 (dd, 1H, $J = 11.8$, 4.4), 2.90 (dd, 1H, $J = 10.6$, 2.3), 2.89–2.86 (m, 1H), 1.34 (s, 3H), 1.09 (s, 3H), 0.92 (s, 3H), 0.90 (s, 3H), 0.85 (s, 3H), 0.79 (s, 3H), 0.71 (s, 3H), 0.58 (s, 3H); HRMS *m/z* 1418.7001 $(M+H)^+$ ($C_{86}H_{100}NO_{17}$ requires 1418.6986). Compound **17**: $[\alpha]_D$ +31.4 (*c* 0.35, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$) δ 8.10–7.24 (m, 30H, Ar-H), 6.11 (d, 1H, $J = 9.3$), 6.04 (d, 1H, $J = 3.2$), 5.81 (dd, 1H, $J = 10.6$, 8.1), 5.70 (dd, 1H, $J = 10.6$, 3.3), 5.34 (d, 1H, $J = 8.0$, H-1″), 5.30 (br s, 1H), 5.19 (t, 1H, $J = 9.6$), 5.11 (dd, 2H, $J = 25.2$, 12.6), 5.05 (t, 1H, $J = 9.6$), 4.93 (t, 1H, $J = 9.4$), 4.65 (dd, 1H, $J = 11.0$, 6.0), 4.59 (d, 1H, $J = 11.0$, H-1‴), 4.50 (dd, 2H, $J = 20.6$, 8.8), 4.47–4.46 (m, 1H), 4.44 (t, 1H, $J = 6.4$), 4.31 (d, 1H, $J = 5.3$, H-1′), 4.25–4.22 (m, 2H), 4.18 (br s, 1H), 4.11 (m, 1H), 4.04 (br d, 1H, $J = 12.2$), 3.76–3.73 (m, 2H), 3.64 (dd, 1H, $J = 12.7$, 8.9), 3.43 (br d, 1H, $J = 10.0$), 2.93 (dd, 1H, $J = 13.7$, 2.8), 2.78 (dd, 1H, $J = 11.7$, 3.8, H-3), 2.06 (s, 3H), 2.05 (s, 3H), 2.04 (s, 3H), 2.03 (s, 3H), 2.00 (s, 3H), 1.11 (s, 3H), 0.94 (s, 3H), 0.92 (s, 3H), 0.79 (s, 3H), 0.79 (s, 3H), 0.63 (s, 3H), 0.61 (s, 3H); ESI-HRMS *m/z* 1770.7772 $(M+Na)^+$ ($C_{100}H_{117}NNaO_{26}$ requires 1770.7756). Compound **1**: $[\alpha]_D$ +22.1 (*c* 0.50, MeOH); ^1H NMR (600 MHz, MeOH-d_4) δ 5.29 (t, 1H, $J = 3.3$), 4.96–4.94 (m, 1H), 4.67 (d, 1H, $J = 7.6$, H-1‴), 4.57 (d, 1H, $J = 7.8$, H-1″), 4.51 (d, 1H, $J = 7.5$, H-1′), 4.15 (t, 1H, $J = 9.2$), 4.00 (t, 1H, $J = 11.4$), 3.98 (dd, 1H, $J = 8.7$, 7.4), 3.93–3.90 (m, 2H), 3.87 (d, 1H, $J = 2.8$), 3.85–3.83 (dd, 1H, $J = 11.8$, 2.6), 3.77–3.75 (dd, 1H, $J = 11.4$, 4.5), 3.73 (dd, 1H, $J = 11.7$, 4.3), 3.65 (dd, 1H, $J = 9.0$, 7.9), 3.59–3.57 (m, 1H), 3.52 (dd, 1H, $J = 9.0$, 3.3), 3.47–3.45 (m, 1H), 3.42–3.41 (m, 1H), 3.39 (dd, 1H, $J = 9.3$, 7.6), 3.37–3.35

(m, 2H), 3.15 (dd, 1H, $J = 11.5$, 4.2), 2.90 (dd, 1H, $J = 13.8$, 4.0), 2.02 (s, 3H), 1.20 (s, 3H), 1.02 (s, 3H), 1.00 (s, 3H), 0.99 (s, 3H), 0.96 (s, 3H), 0.86 (s, 3H), 0.81 (s, 3H); ESI-HRMS *m/z* 1006.5339 $(M+Na)^+$ ($C_{50}H_{81}NNaO_{18}$ requires1006.5346). Compound **2**: $[\alpha]_D$ +18.6 (*c* 0.50, MeOH); ^{1}H NMR (600 MHz, MeOH-d_4) δ 5.29 (br s, 1H), 4.55 (d, 1H, $J = 8.1$, H-1′), 4.33 (d, 1H, $J = 7.6$, H-1″), 3.91 (dd, 1H, $J = 11.4$, 1.4), 3.85 (d, 1H, $J = 3.0$), 3.81–3.79 (m, 2H), 3.76–3.74 (m, 2H), 3.72 (t, 1H, $J = 8.6$), 3.61–3.58 (m, 2H), 3.51 (dd, 1H, $J = 8.5$, 3.0), 3.47 (t, 1H, $J = 9.0$), 3.35–3.34 (m, 1H, H-5′), 3.17 (dd, 1H, $J = 11.6$, 4.2), 2.89 (dd, 1H, $J = 13.7$, 3.7,), 2.00 (s, 3H), 1.21 (s, 3H), 1.02 (s, 3H), 1.01 (s, 3H), 0.99 (s, 3H), 0.96 (s, 3H), 0.86 (s, 3H), 0.82 (s, 3H); ESI-HRMS *m/z* 844.4824 $(M+Na)^+$ ($C_{44}H_{71}NNaO_{13}$ requires 844.4818).

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