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Synthesis of Lanostane-Type Triterpenoid *N*-Glycosides and Their Cytotoxicity against Human Cancer Cell Lines

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Seventeen lanostane-type triterpenoid derivatives (**2**–**18**), including 11 *N*-glycosides (**8**–**18**), were synthesized from the natural triterpenoid, lanosterol (**1**), and were evaluated for their cytotoxicity against the human cancer cell lines, HL-60, A549, and MKN45, as well as the normal human lung cells, WI-38. Among them, *N*-β-D-2-acetamido-2-deoxyglucoside (**10**) showed cytotoxicity against HL-60, A549, MKN45, and WI-38 cells (IC_{50} 0.0078–2.8 μM). However, *N*-β-D-galactoside (**12**) showed cytotoxicity against HL-60 and MKN45 cells (IC_{50} 0.0021–4.0 μM), but not the normal WI-38 cells. Furthermore, western blot analysis suggested that **12** induces apoptosis by activation of caspases-3, 8, and 9. These results will be useful for the synthesis of other tetracyclic triterpenoids or steroid *N*-glycosides to increase their cytotoxicity and apoptosis-inducing activities.

Keywords: terpenoid • glycosylation • *N*-glycoside • cytotoxicity • apoptosis

Introduction

Triterpenoids are naturally occurring lipids and some of them are known to be precursors of steroids. Triterpenoid glycosides have been reported to possess various biological activities, including anti-inflammatory, antitumor promoter, antiviral, and cytotoxic activities.[1-4] Naturally occurring triterpenoid glycosides usually have an *O*-bond between the aglycone (triterpenoids) and sugar moieties. Recently, *N*-glycosides were synthesized from steroids and reported to have cytotoxicity against human cancer cell lines. On the basis of this information, we aimed to synthesize lanostane-type triterpenoid *N*-glycosides and evaluate their cytotoxicity against human normal and cancer cell lines.[5-8] Lanostane-type triterpenoids are considered to be promising agents for the development of anticancer drugs[9] and their derivatives have been shown to have cytotoxic and apoptosis-inducing

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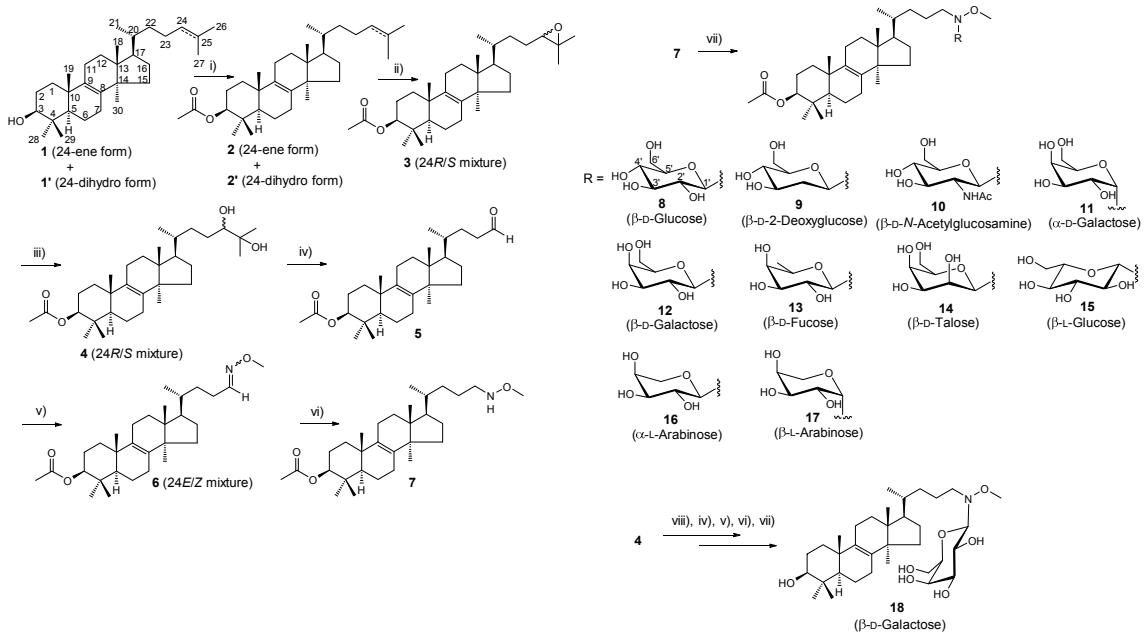
effects.[10] To the best of our knowledge, this is the first report on the synthesis of lanostane-type triterpenoid *N*-glycosides, and the evaluation of their cytotoxicity and proapoptotic effects.

Results and Discussion

Synthesis

In this study, 17 lanostane-type triterpenoid derivatives (**2**–**18**) were synthesized from the natural triterpenoid, lanosterol (**1**). As shown in *Scheme 1*, lanostane-type triterpenoid amine (**7**) was synthesized from **1**, and the amine (**7**) was glycosylated with six D-type monosaccharides, D-glucose (D-Glc), D-2-deoxyglucose (2-deoxy-D-Glc), D-*N*-acetylglucosamine (D-GlcNAc), D-galactose (D-Gal), D-fucose (D-Fuc; 6-deoxy-D-Gal), and D-talose (D-Tal; 2'-epimer of D-Gal), and two L-type monosaccharides, L-glucose (L-Glc) and L-arabinose (L-Ara; 6'-nor-D-Gal). Furthermore, in order to evaluate the influence of the acetoxy group at C-3, 3β-hydroxy-25,26,27-trinorlanost-8-en-24-al methoxy amine *N*-β-D-galactoside (**18**) was synthesized.

Starting from commercially available lanosterol (a mixture of lanosterol (**1**) and dihydrolanosterol (**1'**)), the hydroxyl group was protected by acetylation to yield a mixture of lanosterol 3-*O*-acetate (**2**) and dihydrolanosterol 3-*O*-acetate (**2'**). This mixture was oxidized with meta-chloroperoxybenzoic acid (*m*-CPBA) and separated from unreacted **2'** to give a mixture of (24*S*)- and (24*R*)-lanosterol epoxide 3-*O*-acetate (**3**). The mixture was treated with perchloric acid to afford a mixture of (24*S*)- and (24*R*)-3β-acetoxylanost-8-ene-24,25-diol (**4**), which was then treated with sodium periodate to yield 3β-acetoxy-25,26,27-trinorlanost-8-en-24-al (**5**). The aldehyde (**5**) was treated with methoxylamine hydrochloride to give 3β-acetoxy-24-methoxyimino-25,26,27-trinorlanost-8-ene (**6**) as a mixture of (24*E*) and (24*Z*) isomers. Compound **6** was reduced with borane *tert*-butylamine complex to give 3β-acetoxy-24-methoxyamino-25,26,27-trinorlanost-8-ene (**7**). The glycosylation of **7** with D-Glc, 2-deoxy-D-Glc, D-GlcNAc, D-Fuc, D-Tal, and L-Glc produced the corresponding one *N*-glycosides (**8**–**10**, **13**, **14**, and **15**, respectively). In contrast, glycosylation of **7** with D-Gal and L-Ara gave the two corresponding *N*-glycoside anomers (**11** and **12** for D-Gal, **16** and **17** for L-Ara). Compound **18**, which is the C-3 deacetoxy derivative of **12** (β-D-Gal), was also synthesized from **4** after hydrolysis.



Scheme 1. Synthetic schemes of **2**–**18**.

The stereochemistry at anomeric position (C(1')) of **8**–**17** was assigned as follows: the anomeric proton of **8** (D-Glc), **9** (2-deoxy-D-Glc), **10** (D-GlcNAc), and **13** (D-Fuc) appeared at δ(H) 4.71 (*d*, $^3J_{HH}$ = 8.2), 4.68 (*dd*, $^3J_{HH}$ = 1.4, 10.5), 4.91 (*d*, $^3J_{HH}$ = 9.6), and 4.60 (*d*, $^3J_{HH}$ = 8.7), respectively. Their coupling constants were in good agreement with the reference data for β-glycosides[11-14] and the anomeric configurations of **8**, **9**, **10**, and **13** were assigned as β. The anomeric proton signals of the L-arabinoside isomers (**16** and **17**) appeared at δ(H) 4.52 (*d*, $^3J_{HH}$ = 8.7) and 5.20 (*d*, $^3J_{HH}$ = 5.5), respectively. Comparing these data with reference data[14, 15], we determined that **16** and **17** were α- and β-L-arabinosides, respectively. However, it was difficult to assign the anomeric stereochemistry of compound **12** (D-Gal) by its anomeric proton signal in ^{1}H-NMR because the signal overlapped with that of three other protons. Thus, ^{1}H-, and ^{13}C-NMR were measured at 40 °C and 2D-NMR experiments (heteronuclear multiple quantum coherence (HMQC), heteronuclear multiple bond correlation (HMBC), ^{1}H-^{1}H correlation spectroscopy (COSY), and nuclear Overhauser effect spectroscopy (NOESY)) were performed. As a result, the anomeric proton was assigned at δ(H) 4.61 (*d*, $^3J_{HH}$ = 8.9), which indicated that **12** was β-D-galactoside. In addition, the H-C(1') signal showed correlation peaks with H-C(3') and H-C(5') in the NOESY experiment, which indicated that they were oriented on the same face as the cyclohexane ring (*Figure 1*). Based on these data, the anomeric configuration of **12** was determined to be β. Another galactoside (**11**) was determined to be α-D-galactoside by comparison of the anomeric proton signal at δ(H) 5.23 (*d*, $^3J_{HH}$ = 5.5) with reference data.[14] Because the assignment of the anomeric configuration of **14** by $^3J_{HH}$ coupling constant was difficult, the ^{13}C-^{1}H coupling constant ($^1J_{CH}$) of the ^{13}C-NMR signal at C(1') was measured and compared with that of α- and β-D-talose. Specifically, the coupling constant $^1J_{CH}$ at δ(C) 100.0 (C(1')) of **14** was 155.3 Hz, as measured by the non-decoupling ^{13}C-NMR experiment. The $^1J_{CH}$ of α- and β-D-talose were 168.7 Hz (δ(C) 96.0) and 159.1 Hz (δ(C) 96.5) in non-decoupling ^{13}C-NMR, respectively. Since the $^1J_{CH}$ of **14** at C-1' was in good agreement with that of β-D-talose, **14** was

determined to be β-D-taloside. To determine the anomeric configuration of **15**, ^{1}H-, and ^{13}C-NMR, and 2D-NMR experiments were performed. The anomeric proton signal (δ(H) 4.71–4.73) showed correlation peaks with H-C(3') and H-C(5') in the NOESY experiment, which indicated that they were oriented on the same face as the cyclohexane ring (*Figure 1*). Based on these results, **15** was determined to be β-L-glucoside. The C(3) deacetoxy D-galactoside (**18**) was determined to be β-D-galactoside by comparing its ^{1}H-NMR data with that of **12**.

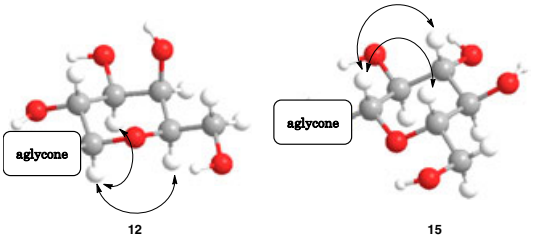


Figure 1. Sugar moiety of **12** and **15** and representative nuclear Overhauser effect correlation (↷)

*Cytotoxic Activity of Compounds **1–18***

Among the glycosides (**8**–**18**), β-D-2-deoxyglucoside (**9**), β-D-2-acetamido-2-deoxyglucoside (**10**), and β-D-galactoside (**12**) showed cytotoxicity at IC_{50} values of 9.4, 2.8, and 0.0021 μM, respectively, against leukemia cell lines (HL-60), which were higher than that of amine (**7**; >100 μM). In contrast with the reference compound, cisplatin (IC_{50} = 1.3 μM), **12** (IC_{50} = 0.0021 μM) showed potent activity. In addition, **10** showed cytotoxicity against lung cancer (A549; IC_{50} = 0.67 μM) and stomach cancer cell lines (MKN45; IC_{50} = 0.0078 μM), which were more potent than that of cisplatin (IC_{50} of 18.4 and 7.0 μM, respectively) ($p < 0.01$). With respect to structure-activity relationships, β-D-2-deoxyglucoside (**9**) and β-D-2-acetamido-2-deoxyglucoside (**10**) showed more potent cytotoxicity than did β-D-glucoside (**8**), which indicated that the hydroxyl group at C(2') may decrease the activity. In comparison with β-D-galactoside (**12**), α-D-galactoside (**11**; C(1') epimer of **12**), β-D-glucoside (**8**; C(4') epimer of **12**), β-D-taloside (**14**; C(2') epimer of **12**), β-D-fucoside (**13**; 6'-deoxy derivative of **12**), and α-L-arabinoside (**16**; 6' nor derivative of **12**) showed no cytotoxicity against HL-60. These results showed that, in the case of the lanostane side chain *N*-glycosides, the stereochemistry at C(1'), C(2'), and C(4') and the presence of the 6'-hydroxymethylene group were essential for cytotoxicity against HL-60 cells.

Table 1. Cytotoxic Activities (IC_{50} ± S.D. [μM]) of Lanostane-type Triterpenoid Derivatives **1**–**18** against One Human Normal and Three Human Cancer Cell Lines[a]

Compounds	HL-60 (Leukemia)	A549 (Lung)	MKN45 (Stomach)	WI-38 (Normal Lung)
1	>100	>100	>100	>100
2	>100	>100	>100	>100
3[b]	>100	>100	>100	>100
4[b]	30.8 ± 4.8	>100	>100	>100
5	38.7 ± 6.9	46.7 ± 4.4	98.9 ± 1.8	>100
6[c]	>100	>100	>100	>100
7	>100	>100	>100	>100
8	>100	>100	>100	>100
9	9. 4± 0.2	>100	>100	>100
10	2.8 ± 0.8	0.67 ±0.07	0.0078 ± 0.0033	0.62 ± 0.03
11	>100	>100	>100	>100
12	0.0021 ± 0.0004	>100	4.0 ± 2.1	>100
13	>100	>100	>100	>100
14	>100	>100	>100	>100
15	>100	>100	>100	>100
16	>100	>100	>100	>100
17	>100	>100	>100	>100
18	>100	>100	>100	>100
Cisplatin[d]	1.3 ± 0.1	18.4 ± 1.9	7.0 ± 0.1	20.1 ± 2.4

[a] Cells were treated with compounds (1×10^{-4} –1×10^{-6} M) for 48 h, and cell viability was analyzed by the MTT assay. IC_{50} Values were calculated from five point triplicates. [b] (24*R*) and (24*S*) mixture. [c] (24*E*) and (24*Z*) mixture. [d] Reference compound.

Western Blot Analysis for Apoptosis-related Proteins.

In this study, β-D-galactoside (**12**), which showed the most potent cytotoxicity against HL-60 cells, was selected and its proapoptotic mode of action was evaluated by western blot for the apoptosis-related proteins, caspases-3, 8, and 9. After treatment of HL-60 cells with **12** (1 μM), the expression

levels of procaspases-3, 8, and 9 were decreased, and cleaved caspases-3, 8, and 9 were detected after 48 h (*Figure 2*). These results suggestedthat compound **12**-induced cell death occurs through the activation of caspases-3, 8, and 9.

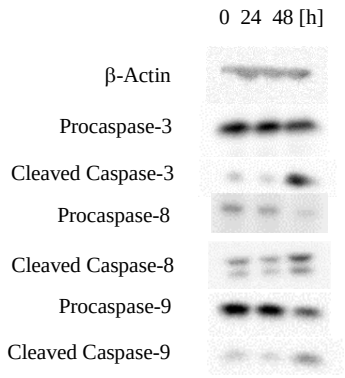


Figure 2. Western Blot Analysis for Apoptosis-related Proteins

HL-60 cells were treated with **12** (1 μM) for 0–48 h. The expression levels of the apoptosis-related proteins were assessed by western blotting; β-actin was used as the loading control. The results are shown from one representative experiment among three independent runs.

Conclusions

In this study, we synthesized 17 lanostane-type triterpenoid derivatives including 11 *N*-glycosides and evaluated their cytotoxicity against one normal human lung and three human cancer cell lines. Among them, β-D-2-acetamido-2-deoxyglucoside (**10**) not only showed potent cytotoxicity against the three cancer cell lines but also showed cytotoxicity against the normal cells. In contrast, β-D-galactoside (**12**) showed potent cytotoxicity only against HL-60 cells and its mode of action was suggested to be apoptosis by western blotting. To the best of our knowledge, this is the first report of the evaluation of lanostane-type *N*-glycosides and their proapoptotic effects. We believe that these results will be useful for the synthesis of other tetracyclic triterpenoids or steroid *N*-glycosides to increase their cytotoxicity and apoptosis-inducing activities.

Experimental Section

General

Silica gel 60 (SiO_2, 230–400 mesh; *Merck KGaA*, Darmstadt, Germany) was used for open column chromatography. Reversed-phase (RP) preparative high-performance liquid chromatography (HPLC) was conducted using a refractive index detector with an octadecyl silica (ODS) column (*Superiorex ODS* column, 25 cm × 4.6 cm i.d.; *Shiseido Co., Ltd.*, Tokyo, Japan) with a mobile phase of MeOH (1 ml/min; HPLC system I), or with an ODS column (*Pegasil ODS SP100* column, 25 cm × 10 cm i.d.; *Senshu Scientific Co., Ltd.*, Tokyo, Japan) with a mobile phase of MeOH (4 ml/min; HPLC system II) or EtOH/H_2O 75:25 (2 ml/min; HPLC system III) at 25 ºC. The infrared (IR) spectra were recorded using a *Perkin-Elmer Spectrum One* Fourier-transform IR (FTIR) spectrophotometer and potassium bromide (KBr) disks, with ν in cm^{-1}. The nuclear magnetic resonance (NMR) spectra were recorded using a *JEOL ECX-400 spectrometer* (^{1}H, 400 MHz; ^{13}C, 100 MHz) or *JEOL ECA-500* (^{1}H, 500 MHz; ^{13}C, 125 MHz) at room temperature (r.t.; 20 °C) or 40 °C in deuterated chloroform ($CDCl_3$), $CDCl_3$/deuterated methanol (CD_3OD), or deuterated pyridine (C_5D_5N) with tetramethylsilane as an

internal standard. High-resolution electrospray ionization mass spectrometry (HR-ESI-MS) was performed using an *Agilent 1100 LC/MSD* time-of-flight (TOF) system (ionization mode: positive or negative, capillary voltage: 3000 V, and fragmentor voltage: 225 V); HR-atmospheric-pressure chemical ionization (APCI)-MS was performed using the *Agilent 1100 LC/MSD TOF* system (ionization mode: positive, capillary voltage: 3000 V, fragmentor voltage: 40 V, and corona current: 2.0 μA).

Chemicals and Materials

The chemicals used were purchased from the following sources: methoxyamine hydrochloride, borane *tert*-butylamine complex, D-galactose, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT), and DMSO from *Sigma-Aldrich Japan Co.* (Tokyo, Japan); lanosterol (63 : 37 mixture of lanosterol and dihydrolanosterol based on HPLC analysis), *N*-acetyl-D-glucosamine, D-fucose, D-talose, L-glucose, and L-arabinose from *Tokyo Chemical Industry Co., Ltd.* (Tokyo, Japan); Roswell Park Memorial Institute (RPMI)-1640 medium, fetal bovine serum (FBS), and penicillin-streptomycin from *Invitrogen Co.* (Auckland, NZ); anti-procaspase-3, anti-cleaved caspase-3, anti-procaspase-8, anti-cleaved caspase-8, anti-procaspase-9, anti-cleaved caspase-9, and anti-β-actin primary antibodies from *Cell Signaling Technology* (Beverly, MA, U.S.A.); enhanced chemiluminescence (ECL) Plus Western Blotting Detection System from *GE Healthcare* (Buckinghamshire, U.K.); sodium periodate, 2-deoxy-D-glucose, and cisplatin from *Wako Pure Chemical Industries Ltd.* (Osaka, Japan); and perchloric acid and D-glucose from *Kanto Chemical Co. Inc.* (Tokyo, Japan). All other chemicals and reagents were of analytical grade.

Preparation of Compounds

Purification of **lanosterol** (**1**). For the evaluation of cytotoxicity, a mixture of lanosterol (**1**) and dihydrolanosterol (**1'**) (20.2 mg) was purified by HPLC (System I) to give purified **1** (t_R = 19.3 min, 8.0 mg), which was identified by spectral comparison with literature data.[16]

Synthesis of **lanosterol 3-*O*-acetate** (**2**). (i) Starting from purified **1**: To a solution of purified lanosterol (**1**) (5.1 mg, 0.01 mmol) in dried pyridine (1 ml), acetic anhydride (1 ml, 9.9 mmol) was added and stirred at r.t. overnight. The reaction mixture was diluted with H_2O and extracted with diethyl ether. The organic layer was washed sequentially with 1 M HCl, saturated $NaHCO_3$ aq, and H_2O, and was then dried over Na_2SO_4. The dried organic layer was evaporated under reduced pressure to yield a mixture of lanosterol 3-*O*-acetate (**2**) (5.2 mg, 100%), which was identified by spectral comparison with literature data.[16] (ii) Starting from crude **1**: To a solution of a crude lanosterol (**1** : **1'** = 63 : 37) (2.1 g, 4.9 mmol) in dried pyridine (30 ml), acetic anhydride (30 ml, 32 mmol) was added and stirred at r.t. overnight. The reaction mixture was diluted with H_2O and extracted with diethyl ether. The organic layer was washed sequentially with 1 M HCl, saturated $NaHCO_3$ aq, and H_2O, and was then dried over Na_2SO_4. The dried organic layer was evaporated under reduced pressure to yield a mixture of lanosterol 3-*O*-acetate (**2**) and dihydrolanosterol 3-*O*-acetate (**2'**) (2.3 g, 99%). The products were used for the next reaction without purification.

Synthesis of **(24*R*)-** and **(24*S*)-lanosterol epoxide 3-*O*-acetate** (**3**). To a solution of a mixture of **2** and **2'** (2.3 g, 4.9 mmol) in dried $CHCl_3$ (35 ml), *m*-CPBA (0.88 g, 5.1 mmol) was added at 0 °C and stirred for 10 min. The mixture was warmed to r.t. and stirred for 12 h. Afterward, the reaction mixture was diluted with $CHCl_3$, washed sequentially with 1 M NaOH aq. and H_2O, and was then dried over Na_2SO_4. The dried solution was evaporated under reduced pressure to yield crude products (3.3 g). The products were purified with Si-gel column chromatography (hexane/AcOEt = 1 : 0 to 9 : 1) to give **3** (0.86 g, 36%) as a 24*R*/24*S* mixture, which was identified by spectral comparison with literature data.[10]

Synthesis of **(24*R*)-** and **(24*S*)- 3β-acetoxylanost-8-ene-24,25-diol** (**4**). To a solution of **3** (0.86 g, 1.8 mmol) in 1,2-dimethoxyethane (85 ml), a solution (21 ml) formed by adding perchloric acid (0.2 ml) to H_2O (21 ml) was added and the reaction mixture was stirred at r.t. overnight. Afterward, the reaction mixture was diluted with H_2O and extracted with diethyl ether. The organic layer was washed sequentially with 2 M Na_2CO_3 aq. and H_2O, and was then dried over Na_2SO_4. The dried solution was evaporated under reduced pressure to yield **4** (0.73 g, 81%) as a mixture of (24*R*)- and (24*S*)-stereoisomers, which was identified by spectral comparison with literature data.[17]

Synthesis of **3β-acetoxy-25,26,27-trinorlanost-8-en-24-al** (**5**). To a solution of **4** (0.60 g, 1.2 mmol) in THF (22 ml) and H_2O (7 ml), sodium periodate (1.1 g, 5.2 mmol) was added and stirred at r.t. for 1.5 h. Afterward, 2 M HCl was added and the mixture was extracted with $CHCl_3$. The organic layer was washed with H_2O, dried over Na_2SO_4, and then evaporated under reduced pressure to yield **5** (0.51 g, 96%), which was identified by spectral comparison with literature data.[10]

Synthesis of **(*E*)-** and **(*Z*)-3β-acetoxy-24-methoxyimino-25,26,27-trinorlanost-8-ene** (**6**) To a solution of **5** (0.49 g, 1.1 mmol) in pyridine (12 ml) and methanol (0.5 ml), methoxyamine hydrochloride (0.15 g, 1.8 mmol) was added and stirred at r.t. for 1 h. Afterward, the reaction mixture was diluted with H_2O and extracted with $CHCl_3$. The organic layer was washed sequentially with 1 M HCl and H_2O, and was then dried over Na_2SO_4. The solution was evaporated under reduced pressure to yield **6** (0.51 g, 98%) as a mixture of (24*E*)- and (24*Z*)-stereoisomers. Amorphous solid. IR (KBr): 873, 1033, 1054, 1079, 1251, 1371, 1455, 1632, 1727, 2951, 3434. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.69 (*s*, 3H, Me); 0.88 (*s*, 9H, Me); 0.94 (*d*, *J* = 6.4, 3H, H-C(21)); 1.00 (*s*, 3H, Me); 2.05 (*s*, 3H, MeCO); 3.81 (*s*, 1.5H, OMe); 3.88 (*s*, 1.0H, OMe); 4.50 (*dd*, *J* = 4.6, 11.5, 1H, H-C(3)); 6.63 (*t*, *J* = 5.3, 0.3H, H-C(24)); 7.37 (*t*, *J* = 6.2, 0.5H, H-C(24)). HR-ESI-MS (pos.): 472.3782 ($[M + H]^+$, $C_{30}H_{49}NO_3^+$; calc. 472.3791).

Synthesis of **3β-acetoxy-24-methoxyamino-25,26,27-trinorlanost-8-ene** (**7**). To a solution of **6** (0.50 g, 1.1 mmol) in 1,4-dioxane (12 ml) and ethanol (3 ml), borane *tert*-butylamine complex (0.30 g, 3.4 mmol) was added. The reaction mixture was cooled to 0 °C, 3 M HCl (1 ml) was added dropwise, and the mixture was stirred at 0 °C for 2.5 h. Afterward, solid Na_2CO_3 and saturated $NaHCO_3$ aq. were added until gas evolution ceased, and the reaction mixture was extracted with $CHCl_3$. The organic layer was dried over Na_2SO_4 and evaporated under reduced pressure to yield crude product (0.63 g). A portion of the product (14 mg) was purified with HPLC (System II) to give purified **7** (t_R 16.4 min, 6.4 mg). The other portion was used for synthesis of *N*-glycosides (**8**–**17**) without purification. Amorphous solid. IR (KBr): 983, 1010, 1032, 1250, 1371, 1466, 1736, 2950, 3439. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.69 (*s*, 3H, Me); 0.87 (*s*, 3H, Me); 0.88 (*s*, 6H, Me); 0.92 (*d*, *J* = 6.0, 3H, H-C(21)); 1.00 (*s*, 3H, Me); 2.05 (*s*, 3H, MeCO); 2.85–2.92 (*m*, 2H, H-C(24)); 3.54 (*s*, 3H, OMe); 4.50 (*dd*, *J* = 4.4, 11.7, 1H, H-C(3)). HR-ESI-MS (pos.): 474.3970 ($[M + H]^+$, $C_{30}H_{51}NO_3^+$; calc. 474.3947).

Synthesis of **3β-acetoxy-24-methoxyamino-25,26,27-trinorlanost-8-ene *N*-β-D-glucoside** (**8**). The amine **7** (77.0 mg, 0.16 mmol) and D-glucose (97.2 mg, 0.54 mmol) were dissolved in DMF (1.5 ml) and acetic acid (0.5 ml) and stirred at 40 °C for 28 h. The reaction mixture was concentrated under reduced pressure to give crude product (182.2 mg). The product was subjected to Si-gel column chromatography ($CHCl_3$: acetone, 5 : 5 to 4 : 6) to give crude **8** (34.8 mg), which was further purified by HPLC (system III) to give purified **8** (t_R 19.0 min, 20.8 mg, 20%). Amorphous solid. IR (KBr): 900, 1031, 1083, 1247, 1372, 1451, 1734, 2948, 3401. ^{1}H-NMR (400 MHz, C_5D_5N): 0.74 (*s*, 3H, H-C(18)); 0.94 (*s*, 3H, H-C(28)); 0.96 (*s*, 6H, H-C(29), H-C(30)); 0.98 (*d*, *J* = 6.4, 3H, H-C(21)); 1.02 (*s*, 3H, H-C(19)); 2.09 (*s*, 3H, MeCO); 3.11–3.14 (*m*, 1H, H-C(24)); 3.37–3.41 (*m*, 1H, H-C(24)); 3.90 (*s*, 3H, OMe); 3.96–3.98 (*m*, 1H, H-C(Glc)); 4.22-4.37 (*m*, 3H, H-C(Glc)); 4.42 (*dd*, *J* = 5.5, 11.9, 1H, H-C(6')); 4.60 (*d*, *J* = 11.4, 1H, H-C(6')); 4.71 (*d*, *J* = 8.2, 1H, H-C(1')); 4.70–4.73 (*m,* 1H, H-C(3)). ^{13}C-NMR (100 MHz , C_5D_5N): 16.4 (C(18)); 17.1 (C(29)); 18.7 (C(6)); 19.3 (C(21)); 19.6 (C(19)); 21.5 (MeCO); 21.6 (C(11)); 24.7 (C(30)); 24.9 (C(2)); 25.5 (C(23)); 26.9 (C(7)); 28.3 (C(28)); 28.8 (C(16)); 31.4 (C(15)); 31.6 (C(12)); 34.7 (C(22)); 35.7 (C(1)); 37.2 (C(20)); 37.4 (C(10)); 38.4 (C(4)); 45.0 (C(13)); 50.4 (C(14)); 51.0 (C(5)); 51.1 (C(17)); 54.3 (C(24)); 62.9 (NOMe); 63.4 (C(6')); 72.1, 72.2 (C(2') or C(4')); 80.5, 80.7 (C(3') or C(5')); 81.0 (C(3)); 95.4 (C(1')); 134.9 (C(8), C(9)); 171.0 (MeCO). HR-ESI-MS (pos.): 658.4324 ($[M + Na]^+$, $C_{36}H_{61}NO_8Na^+$; calc. 658.4295).

Synthesis of **3β-acetoxy-24-methoxyamino-25,26,27-trinorlanost-8-ene *N*-β-D-2-deoxyglycoside** (**9**). The amine **7** (85.1 mg, 0.18 mmol) and D-2-deoxyglucose (88.6 mg, 0.54 mmol) were reacted by the same method as used for **8** to give crude product (214.7 mg). The product was subjected to Si-gel column chromatography ($CHCl_3$: acetone, 8 : 2 to 6 : 4) to give crude **9** (34.8 mg), which was further purified by HPLC (system III) to give purified **9** (t_R 39.5 min, 3.6 mg, 3%). Amorphous solid. IR (KBr): 824, 875, 1050, 1075, 1266, 1384, 1451, 1494, 1714, 2924, 3435. ^{1}H-NMR (400 MHz , C_5D_5N): 0.76 (*s*, 3H, Me); 0.94 (*s*, 3H, Me); 0.96 (*s*, 3H, Me); 0.97 (*s*, 3H, Me); 1.00 (*d*, *J* = 6.9, 3H, Me); 1.02 (*s*, 3H, Me); 2.09 (*s*, 3H, MeCO); 2.37–2.46 (*m*, 1H, H-C(2')); 2.59–2.63 (*m*, 1H, H-C(2')); 2.99–3.06 (*m*, 1H, H-C(24)); 3.25–3.32 (*m*, 1H, H-C(24)); 3.73 (*s*, 3H, OMe); 3.85–3.88 (*m*, 1H, H-C(2-DeoxyGlc)); 4.10 (*t*, *J* = 9.1, 1H, H-C(2-DeoxyGlc)); 4.26–4.32 (*m*, 1H, H-C(2-DeoxyGlc)); 4.43 (*dd*, *J* = 5.1, 11.5, 1H, H-C(2-DeoxyGlc)); 4.59 (*dd*, *J* = 2.3, 11.9, 1H, H-C(2-DeoxyGlc)); 4.68 (*dd*, *J* = 1.4, 10.5, 1H, H-C(1')); 4.72 (*dd*, *J* = 4.1, 11.5, 1H, H-C(3)). HR-APCI-MS (pos.): 620.4499 ($[M + H]^+$, $C_{36}H_{62}NO_7^+$; calc. 620.4526).

Synthesis of **3β-acetoxy-24-methoxyamino-25,26,27-trinorlanost-8-ene *N*-β-D-2-acetamido-2-deoxyglucoside** (**10**). The amine **7** (101.1 mg, 0.21 mmol) and D-*N*-acetylglucosamine (139.4 mg, 0.63 mmol) were reacted by the same method as used for **8** to give crude product (286.5 mg). The product was subjected to Si-gel column chromatography ($CHCl_3$: acetone, 9 : 1 to 5 : 5) to give crude **10** (17.6 mg), which was further purified by HPLC (system III) to give purified **10** (t_R 33.0 min, 4.6 mg, 3%). Amorphous solid. IR (KBr): 1030, 1082, 1246, 1372, 1455, 1558, 1735, 2948, 3399 cm^{-1}. ^{1}H-NMR (400 MHz , C_5D_5N): 0.75 (*s*, 3H, Me); 0.94 (*s*, 3H, Me); 0.96 (*s*, 6H, Me); 1.01 (*d*, *J* = 5.0 Hz, 3H, H-C(21)); 1.02 (*s*, 3H, Me); 2.09 (*s*, 3H, MeCO); 2.17 (*s*, 3H, MeCO); 3.14–3.20 (*m*, 1H, H-C(24)); 3.38–3.45 (*m*, 1H, H-C(24)); 3.80 (*s*, 3H, OMe); 3.93–3.98 (*m*, 1H, H-C(GlcNAc)); 4.23 (*t*, *J* = 9.2, 1H, H-C(GlcNAc)); 4.34–4.41 (*m*, 1H, H-C(GlcNAc)); 4.37 (*t*, *J* = 9.6, 1H, H-C(GlcNAc)); 4.60 (br.*d*, *J* = 11.5, 1H, H-C(GlcNAc)); 4.72 (*dd*, *J* = 4.1, 11.5, 1H, H-C(3)); 4.76 (*t*, *J* = 9.6, 1H, H-C(GlcNAc)); 4.91 (*d*, *J* = 9.6, 1H, H-C(1')); 8.91 (*d*, *J* = 9.2, 1H, NH). HR-APCI-MS (pos.): 677.4738 ($[M + H]^+$, $C_{38}H_{65}N_2O_8^+$; calc. 677.4741).

Synthesis of **3β-acetoxy-24-methoxyamino-25,26,27-trinorlanost-8-ene *N*-α-D-galactoside** (**11**) and **3β-acetoxy-24-methoxyamino-25,26,27-trinorlanost-8-ene *N*-β-D-galactoside** (**12**). The amine **7** (78.3 mg, 0.17 mmol) and D-galactose (100 mg, 0.56 mmol) were reacted by the same method as used for **8** to give crude product (243.2 mg). The product was diluted with H_2O and extracted with ethyl acetate to give a mixture of **11** and **12** (39.2 mg), which was further purified by HPLC (system III) to give purified **11** (t_R 35.1 min, 3.6 mg, 3%) and **12** (t_R 32.9 min, 7.1 mg, 7%).

Data for **11**: Amorphous solid. IR (KBr): 1035, 1247, 1371, 1733, 2950, 3500. ^{1}H-NMR (400 MHz , C_5D_5N): 0.74 (*s*, 3H, Me); 0.94 (*s*, 3H, Me); 0.96 (*s*, 6H, Me); 0.97 (*d*, *J* = 8.7, 3H, H-C(21)); 1.01 (*s*, 3H, Me); 2.08 (*s*, 3H, MeCO); 3.09–3.16 (*m*, 1H, H-C(24)); 3.24–3.31 (*m*, 1H, H-C(24)); 3.79 (*s*, 3H, OMe); 4.39–4.44 (*m*, 2H, H-C(Gal)); 4.54–4.60 (*m*, 1H, H-C(Gal)); 4.71 (*dd*, *J* = 4.5, 11.4, 1H, H-C(3)); 4.86 (*dd*, *J* = 2.8, 8.3, 1H, H-C(Gal)); 5.04–5.09 (*m*, 1H, H-C(Gal)); 5.17–5.22 (*m*, 1H, H-C(Gal)); 5.23 (*d*, *J* = 5.5, 1H, H-C(Gal)). HR-ESI-MS (pos.) 658.4293 ($[M + Na]^+$, $C_{36}H_{61}NO_8Na^+$; calc. 658.4295).

Data for **12**: Amorphous solid. IR (KBr) 1035, 1248, 1372, 1458, 1736, 2927, 3431. ^{1}H-NMR (400 MHz, C_5D_5N): 0.73 (*s*, 3H, H-C(18)); 0.94 (*s*, 3H, H-C(28)); 0.96 (*s*, 6H, H-C(29), H-C(30)); 0.96 (*d*, *J* = 6.4, 3H, H-C(21)); 1.02 (*s*, 3H, H-C(19)); 2.09 (*s*, 3H, MeCO); 3.18–3.24 (*m*, 1H, H-C(24)); 3.33–3.40 (*m*, 1H, H-C(24)); 3.83 (*s*, 3H, OMe); 4.11 (*t*, *J* = 5.9, 1H, H-C(5')); 4.24 (*dd*, *J* = 2.8, 8.3, 1H, H-C(3')); 4.50 (*d*, *J* = 6.0, 2H, H-C(6')); 4.64–4.68 (*m*, 3H, H-C(Gal)); 4.72 (*dd*, *J* = 4.1, 11.5, 1H, H-C(3)). ^{1}H-NMR (500 MHz , C_5D_5N, 40 °C): 0.74 (*s*, 3H, H-C(18)); 0.94 (*s*, 3H, H-C(28)); 0.96 (*s*, 6H, H-C(29), H-C(30)); 0.97 (*d*, *J* = 6.6, 3H, H-C(21)); 1.02 (*s*, 3H, H-C(19)); 2.06 (*s*, 3H, MeCO); 3.17–3.23 (*m*, 1H, H-C(24)); 3.31–3.36 (*m*, 1H , H-C(24)); 3.80 (*s*, 3H, OMe); 4.03 (*t*, *J* = 5.7, 1H, H-C(5')); 4.17 (*dd*, *J* = 3.0, 8.5, 1H, H-C(3')); 4.43 (br.*d*, *J* = 5.7, 2H, H-C(6')); 4.55–4.59 (*m*, 2H, H-C(4'), H-C(2')); 4.61 (*d*, *J* = 8.9, 1H, H-C(1')); 4.69–4.71 (*m*, overlapped with H_2O, 1H, H-C(3)). ^{13}C-NMR (125 MHz , C_5D_5N, 40 °C): 16.5 (C(18)); 17.2 (C(29)); 18.8 (C(6)); 19.4 (C(21)); 19.7 (C(19)); 21.5 (MeCO); 21.7 (C(11)); 24.8 (C(30)); 25.0 (C(2)); 25.7 (C(23)); 27.1 (C(7)); 28.4 (C(28)); 28.8 (C(16)); 31.5 (C(15)); 31.7 (C(12)); 34.8 (C(22)); 35.9 (C(1)); 37.2 (C(20)); 37.6 (C(10)); 38.5 (C(4)); 45.2 (C(13)); 50.5 (C(14)); 51.2 (C(5), C(17)); 53.8 (C(24)); 62.6 (NOMe); 63.0 (C(6')); 69.8 (C(2')); 70.8 (C(4')); 77.1 (C(3')); 79.0 (C(5')); 81.2 (C(3)); 95.9 (C(1')); 135.1 (C(8), C(9)); 171.0 (MeCO). HR-APCI-MS (pos.): 636. 4517 ($[M + H]^+$, $C_{36}H_{62}NO_8^+$; calc. 636.4475).

Synthesis of **3β-acetoxy-24-methoxyamino-25,26,27-trinorlanost-8-ene *N*-β-D-fucoside** (**13**). The amine **7** (62.5 mg, 0.13 mmol) and D-fucose (64.0 mg, 0.39 mmol) were reacted by the same method as used for **8** to give crude product (109.0 mg). The product was subjected to Si-gel column chromatography ($CHCl_3$: acetone, 8 : 2 to 5 : 5) to give crude **13** (39.9 mg), which was further purified by HPLC (system III) to give purified **13** (t_R 30.9 min, 3.9 mg, 5%). Amorphous solid. IR (KBr): 1030, 1247, 1371, 1453, 1735, 2948, 3418. ^{1}H-NMR (400 MHz , C_5D_5N): 0.74 (*s*, 3H, Me); 0.95 (*s*, 3H, Me); 0.96 (*s*, 6H, Me); 0.97 (*d*, *J* = 5.0, 3H, H-C(21)); 1.02 (*s*, 3H, Me); 1.60 (*d*, *J* = 6.4, 3H, H-C(6')); 2.09 (*s*, 3H, MeCO); 3.19–3.26 (*m*, 1H, H-C(24)); 3.31–3.38 (*m*, 1H, H-C(24)); 3.82 (*s*, 3H, OMe); 3.80–3.88 (*m*, 1H, H-C(Fuc)); 4.10 (*d*, *J* = 2.8, 1H, H-C(Fuc)); 4.17 (*dd*, *J* = 3.6, 8.7, 1H, H-C(Fuc)); 4.54 (*t*, *J* = 9.4, 1H, H-C(Fuc)); 4.60 (*d*, *J* = 8.7, 1H, H-C(1')); 4.72 (*dd*, *J* = 4.1, 11.4, 1H, H-C(3)). HR-APCI-MS (pos.): 620.4474 ($[M + H]^+$, $C_{36}H_{62}NO_7^+$; calc. 620.4526).

Synthesis of **3β-acetoxy-24-methoxyamino-25,26,27-trinorlanost-8-ene *N*-β-D-taloside** (**14**). The amine **7** (81.8 mg, 0.17 mmol) and D-talose (89.5 mg, 0.50 mmol) were reacted by the same method as used for **8** to give crude product (174.6 mg). The product was purified by HPLC (system II) to give purified **14** (t_R 7.5 min, 15.5 mg, 14%). Amorphous solid. IR (KBr): 1033, 1267, 1371, 1712, 2916, 3381. ^{1}H-NMR (400 MHz , CD_3OD-$CDCl_3$ = 5 : 3): 0.72 (*s*, 3H, H-C(18)); 0.88 (*s*, 3H,

H-C(28)); 0.90 (*s*, 6H, H(29), H-C(30)); 0.94 (*d*, *J* = 5.9, 3H, H-C(21)); 1.03 (*s*, 3H, H-C(19)); 2.04 (*s*, 3H, MeCO); 2.75–2.82 (*m*, 1H, H-C(24)); 2.87–2.94 (*m*, 1H, H-C(24)); 3.59 (*s*, 3H, OMe); 3.59–3.63 (*m*, 2H, H-C(6')); 3.66–3.69 (*m*, 1H, H-C(5')); 3.89 (*dd*, *J* = 3.7, 5.5, 1H, H-C(4')); 4.14 (*t*, *J* = 5.0, 1H, H-C(3')); 4.18 (*dd*, *J* = 3.7, 5.5, 1H, H-C(2')); 4.46 (*dd*, *J* = 7.3, 9.6, 1H, H-C(3)); 4.55 (*d*, *J* = 3.2, 1H, H-C(1')). ^{13}C-NMR(100 MHz, CD_3OD-$CDCl_3$ = 5 : 3): 16.3 (C(18)); 17.0 (C(29)); 18.9 (C(6)); 19.2 (C(21)); 19.7 (C(19)); 21.6 (MeCO); 21.8 (C(11)); 24.7 (C(30)); 24.8 (C(2)); 24.9 (C(23)); 27.1 (C(7)); 28.4 (C(28)); 29.0 (C(16)); 31.6 (C(15)); 31.8 (C(12)); 34.6 (C(22)); 36.1 (C(1)); 37.2 (C(20)); 37.7 (C(10)); 38.5 (C(4)); 45.3 (C(13)); 50.6 (C(14)); 51.3 (C(5)); 51.4 (C(17)); 55.5 (C(24)); 63.1 (NOMe); 64.5 (C(6')); 72.2, 72.9, 73.3 (C(2'), C(3'), or C(5')); 82.3 (C(3)); 83.5 (C(4')); 100.0 (C(1')); 135.1, 135.4 (C(8) or C(9)); 172.7 (MeCO). HR-ESI-MS (pos.): 658.4283 ([M + Na]$^+$, $C_{36}H_{61}NO_8Na^+$; calc. 658.4295).

Synthesis of **3β-acetoxy-24-methoxyamino-25,26,27-trinorlanost-8-ene *N*-β-L-glucoside** (**15**). The amine **7** (52.5 mg, 0.11 mmol) and L-glucose (59.4 mg, 0.33 mmol) were reacted by the same method as used for **8** to give crude product (124.9 mg). The product was purified by HPLC (system III) to give purified **15** (t_R 39.0 min, 8.8 mg, 12%). Amorphous solid. IR (KBr) 1031, 1078, 1265, 1371, 1710, 2910, 3076. ^{1}H-NMR (400 MHz , C_5D_5N): 0.74 (*s*, 3H, H-C(18)); 0.94 (*s*, 3H, H-C(28)); 0.95 (*s*, 3H, H-C(30)); 0.96 (*s*, 3H, H-C(29)); 0.98 (*d*, *J* = 6.5, 3H, H-C(21)); 1.01 (*s*, 3H, H-C(19)); 2.09 (*s*, 3H, MeCO); 3.19–3.26 (*m*, 1H, H-C(24)); 3.29–3.35 (*m*, 1H, H-C(24)); 3.88 (*s*, 3H, OMe); 3.95–3.99 (*m*, 1H, H-C(5')); 4.24–4.29 (*m*, 1H, H-C(4')); 4.31–4.33 (*m*, 2H, H-C(2'), H-C(3')); 4.42 (*dd*, *J* = 5.5, 11.9, 1H, H-C(6')); 4.60 (*dd*, *J* = 2.3, 11.9, 1H, H-C(6')); 4.69–4.71 (*m*, 1H, H-C(3)); 4.71–4.73 (*m*, 1H, H-C(1')). ^{13}C-NMR (100 MHz , C_5D_5N): 16.4 (C(18)); 17.1 (C(29)); 18.7 (C(6)); 19.3 (C(21)); 19.6 (C(19)); 21.5 (MeCO); 21.6 (C(11)); 24.7 (C(30)); 24.9 (C(2)); 25.4 (C(23)); 26.9 (C(7)); 28.3 (C(28)); 28.8 (C(16)); 31.4 (C(15)); 31.6 (C(12)); 34.7 (C(22)); 35.7 (C(1)); 37.1 (C(20)); 37.4 (C(10)); 38.4 (C(4)); 45.0 (C(13)); 50.4 (C(14)); 51.0 (C(5)); 51.2 (C(17)); 53.7 (C(24)); 62.7 (NOMe); 63.3 (C(6')); 72.1, 72.2 (C(2') or C(4')), 80.5 (C(3')); 80.7 (C(5')); 81.0 (C(3)); 95.1 (C(1')); 134.9 (C(8), C(9)); 171.0 (MeCO). HR-ESI-MS (pos.): 658.4311 ([M + Na]$^+$, $C_{36}H_{61}NO_8Na^+$; calc. 658.4295).

Synthesis of **3β-acetoxy-24-methoxyamino-25,26,27-trinorlanost-8-ene *N*-α-L-arabinoside** (**16**) and **3β-acetoxy-24-methoxyamino-25,26,27-trinorlanost-8-en-24-methoxy amine *N*-β-L-arabinoside** (**17**). The amine **7** (69.2 mg, 0.15 mmol) and L-arabinose (82.1 mg, 0.54 mmol) were reacted by the same method as used for **8** to give crude product (161.5 mg). The product was purified by HPLC (system II) to give crude **16** (t_R 8.5 min, 33.8 mg) and **17** (t_R 7.8 min, 11.2 mg), which was further purified with HPLC (system III) to give purified **16** (t_R 42.4 min, 7.1 mg, 8%) and **17** (t_R 41.7 min, 6.3 mg, 7%).

Data for **16**: Amorphous solid. IR (KBr): 1035, 1267, 1714, 2985, 3521. ^{1}H-NMR (400 MHz , C_5D_5N): 0.74 (*s*, 3H, Me); 0.94 (*s*, 3H, Me); 0.95 (*s*, 3H, Me); 0.96 (*s*, 3H, Me); 0.96 (*d*, *J* = 6.0, 3H, H-C(21)); 1.01 (*s*, 3H, Me); 2.08 (*s*, 3H, MeCO); 3.18–3.25 (*m*, 1H, H-C(24)); 3.32–3.39 (*m*, 1H, H-C(24)); 3.75–3.76 (*m*, 1H, H-C(Ara)); 3.83 (*s*, 3H, OMe); 4.17–4.19 (*m*, 1H, H-C(Ara)); 4.29–4.31 (*m*, 1H, H-C(Ara)); 4.36 (*dd*, *J* = 1.4, 11.9, 1H, H-C(Ara)); 4.52 (*d*, *J* = 8.7, 1H, H-C(1')); 4.61 (*dt*, *J* = 3.2, 9.2, 1H, H-C(Ara)); 4.71 (*dd*, *J* = 4.1, 11.4, 1H, H-C(3)). HR-ESI-MS (pos.): 628.4196 ([M + Na]$^+$, $C_{35}H_{59}NO_7Na^+$; calc. 628.4189).

Data for **17**: Amorphous solid. IR (KBr) 1033, 1267, 1712, 2923, 3527. ^{1}H-NMR (400 MHz , C_5D_5N): 0.75 (*s*, 3H, Me); 0.94 (*s*, 3H, Me); 0.96 (*s*, 6H, Me); 0.98 (*d*, *J* = 6.4, 3H, H-C(21)); 1.01 (*s*, 3H, Me); 2.09 (*s*, 3H, MeCO); 3.12–3.18 (*m*, 1H, H-C(24)); 3.28–3.35 (*m*, 1H, H-C(24)); 3.79 (*s*, 3H, OMe); 4.22–4.27 (*m*, 1H, H-C(Ara)); 4.40–4.43 (*m*, 1H, H-C(Ara)); 4.67–4.70 (*m*, 1H, H-C(Ara));

4.71 (*dd*, *J* = 4.6, 11.5, 1H, H-C(3)); 4.95–5.00 (*m*, 1H, H-C(Ara)); 5.07 (*dd*, *J* = 5.5, 12.0, 1H, H-C(Ara)); 5.20 (*d*, *J* = 5.5, 1H, H-C(1')). HR-ESI-MS (pos.): 628.4187 ($[M + Na]^+$, $C_{35}H_{59}NO_7Na^+$; calc. 628.4189).

Synthesis of **3β-hydroxy-24-methoxyamino-25,26,27-trinorlanost-8-ene *N*-β-D-galactoside** (**18**): Compound **4** (107.4 mg, 0.21 mmol) was dissolved in 5% KOH-MeOH (10 mL) and extracted with diethyl ether to give lanost-8-ene-3β,24,25-triol (84.6 mg, 0.18 mmol), which was used under the same reaction conditions as those used to synthesize **5**, **6**, and **7** to give 3β-hydroxy-25,26,27-trinorlanost-8-ene-24-methoxyamine (89.6 mg). The amine (89.6 mg, 0.21 mmol) and D-galactose (100.0 mg, 0.56 mmol) were then reacted by the same method as used for **8** to give crude product (29.1 mg). The product was purified by HPLC (system III) to give purified **18** (t_R 12.1 min, 3.8 mg, 3%). Amorphous solid. IR (KBr): 1031, 2945, 3197, 3677. ^{1}H-NMR (400 MHz , C_5D_5N): 0.77 (*s*, 3H, Me); 0.97 (*s*, 3H, Me); 0.97 (*d*, *J* = 6.0, 3H, H-C(21)); 1.09 (*s*, 3H, Me); 1.11 (*s*, 3H, Me); 1.28 (*s*, 3H, Me); 3.17–3.24 (*m*, 1H, H-C(24)); 3.33–3.40 (*m*, 1H, H-C(24)); 3.46–3.52 (*m*, 1H, H-C(3)); 3.83 (*s*, 3H, OMe); 4.10 (*t*, *J* = 6.0, 1H, H-C(Gal)); 4.24 (*dd*, *J* = 2.7, 7.8, 1H, H-C(Gal)); 4.50 (*d*, *J* = 5.5, 2H, H-C(6')); 4.63–4.64 (*m*, 2H, H-C(Gal)); 4.68 (*d*, *J* = 8.7, 1H, H-C(1')). HR-ESI-MS (pos.): 616.4177 ($[M + Na]^+$, $C_{34}H_{59}NO_7Na^+$; calc. 616.4189).

Cytotoxicity Assay

Cytotoxicity assays were performed as previously described.[18] Briefly, 3×10^3 cells/well of HL-60 (leukemia), A549 (lung), MKN45 (stomach), or WI-38 (normal lung) cell lines were treated with the indicated compounds for 48 h prior to adding MTT solution to each well. After incubation for 3 h, the generated blue formazan was solubilized using 0.04 M HCl in isopropanol. The absorbance at 570 nm (top) and 630 nm (bottom) were measured by a microplate reader.

Western Blot Analysis

Western blot analysis was performed according to a previously reported method.[18] Briefly, HL-60 cell (from 1×10^6 cells/well) lysates containing 20 μg of total protein were separated by 15% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene difluoride (PVDF) membranes. After blocking, the membranes were incubated with anti-caspase-3, anti-cleaved caspase-3, anti-caspase-8, anti-cleaved caspase-8, anti-caspase-9, anti-cleaved caspase-9, or anti-β-actin primary antibodies at 4 °C overnight. The signals were detected with the ECL Plus Western Blotting Detection System and imaged using the cooled CCD camera system *Atto Light-Capture II*.

Supplementary Material

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/MS-number.

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Author Contribution Statement

T. H., *K. O.*, *M. H.*, *H. A.*, *W. L.*, and *K. K.* participated in the acquisition and interpretation of the data. *M. F.* contributed to the polishing of the manuscript. *M. U.* participated in the conception and design of the research, in the acquisition and interpretation of the data, and in the drafting of the manuscript.

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