Mol Divers (2014) 18:13–23
DOI 10.1007/s11030-013-9480-8

FULL-LENGTH PAPER

Synthesis and anti-cancer activity of a glycosyl library of *N*-acetylglucosamine-bearing oleanolic acid

Yi-Bing Zeng · Hsin-Min Hsiao · She-Hung Chan · Ying-Hsin Wang · You-Yu Lin · Yu-Hsuan Kuo · Jih-Hwa Guh · Pi-Hui Liang

Received: 7 May 2013 / Accepted: 1 September 2013 / Published online: 25 September 2013

Abstract *N*-Acetylglucosamine-bearing triterpenoid saponins (GNTS) were reported to be a unique type of saponins with potent anti-tumor activity. In order to study the structure–activity relationship of GNTS, 24 oleanolic acid saponins with (1 → 3)-linked, (1 → 4)-linked, (1 → 6)-linked *N*-acetylglucosamine oligosaccharide residues were synthesized in a combinatorial and concise method. The cytotoxicity of these compounds toward the leukemia cell line HL-60 and the colorectal cancer cell line HT-29 could not be improved. Half maximal inhibition below 10 μM was achieved in one single case. The study revealed that the activity decreased following the order of 3′ > 4′ > 6′ glycosyl modifications. GNTS that incorporated (D/L)-xylose and L-arabinose at positions 3′ and 4′ were more potent than those bearing other sugars.

Keywords Oleanolic acid · Glycosylation · Cytotoxicity · Glycoside

Introduction

Saponins, glycosylated secondary metabolites in plants, are routinely synthesized during their normal growth and development. It is believed that these molecules act as natural surfactants—chemical barriers which protect the plant against fungal attack [1]. Structurally, these natural plant surfactants are glycosides of steroids and triterpenes [2]. Oleanolic acid (OA) and its glycoside are among the most important triterpenes, and are found widely distributed in nature [3]. OA saponins impart a multitude of biological effects [3,4], including antitumor [5–14], antiviral [15–17], anti-inflammatory [18], and hepatoprotective activities [19]. Structure–activity relationship studies of OA derivatives revealed that the glycosidic portion of OA saponins was important for their bioactivities [4].

Oleanolic acid (OA) derivatives bearing an *N*-acetylglucosamine (such as compound **1**, Fig. 1) moiety are rarely found in natural products, but have attracted a lot of attention recently due to their remarkable cytotoxicity [3,20–24]. It was noticed that glycans attached with specific linkages of *N*-acetylglucosamine of **1** might have important roles in boosting the antiproliferative activity of triterpenoid. For example, lotoidoside D (**2**) and lotosidoside E (**3**) (Fig. 1) were found to have antiproliferative activity against the Hela cell line with IC_{50} values of 2.74 and >10 μM, respectively [20]. Saponin **4** (6′-α-L-arabinoyl of **1**) was reported to be cytotoxic against human head and neck squamous cells and melanoma cells with IC_{50} in the range 4.7–12.4 μM [24]. Recently, (1 → 4)-linked and (1 → 6)-linked *N*-acetyl-β-D-glucosamine oligosaccharide residues were synthesized and none of the newly synthesized saponins displayed equal potency comparing to compound **1** [23].

Isolation of sufficient samples of saponins from natural sources is generally not possible, a problem which can be solved by chemical synthesis. The development of glycosylation procedures and sophisticated protecting group strategies has enabled the syntheses of a number of glycoconjugates. There are two general strategies to construct saponins [25]: a convergent approach, in which the aglycon is directly glycosylated with a prefabricated sugar donor; or linear synthesis,

Electronic supplementary material The online version of this article (doi:10.1007/s11030-013-9480-8) contains supplementary material, which is available to authorized users.

Y.-B. Zeng · H.-M. Hsiao · S.-H. Chan · Y.-H. Wang · Y.-Y. Lin · Y.-H. Kuo · J.-H. Guh · P.-H. Liang (✉)
School of Pharmacy, College of Medicine, National Taiwan University, 1 Jen-Ai Road, Section 1, Taipei 100, Taiwan
e-mail: phliang@ntu.edu.tw

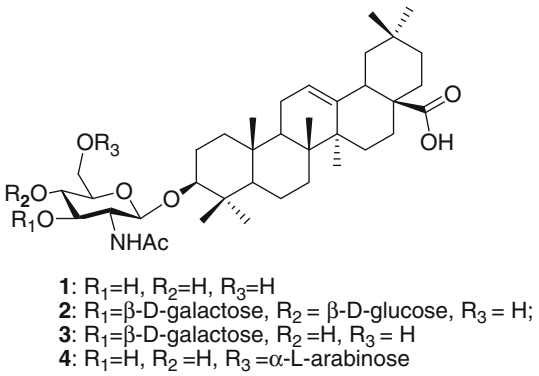


Fig. 1 The structures of **1**, lotoidoside D (**2**), lotoidoside E (**3**), and **4**

in which the aglycon is glycosylated with a monosaccharide, which is subsequently elongated through the protective group manipulations. Both strategies have been applied in the synthesis of *N*-acetylglucosamine-bearing saponins [20,22,23], but each has some limitations. The first is limited to specific glycoconjugates and glycosylation of oligosaccharide with sapogenin is relatively low yielding due to the reduced reactivity of the oligosaccharide. The second is inefficient, due to the laborious protecting group manipulations required between each glycosylation step. To alleviate these problems, orthogonal-protecting group strategies [26–28] have been developed.

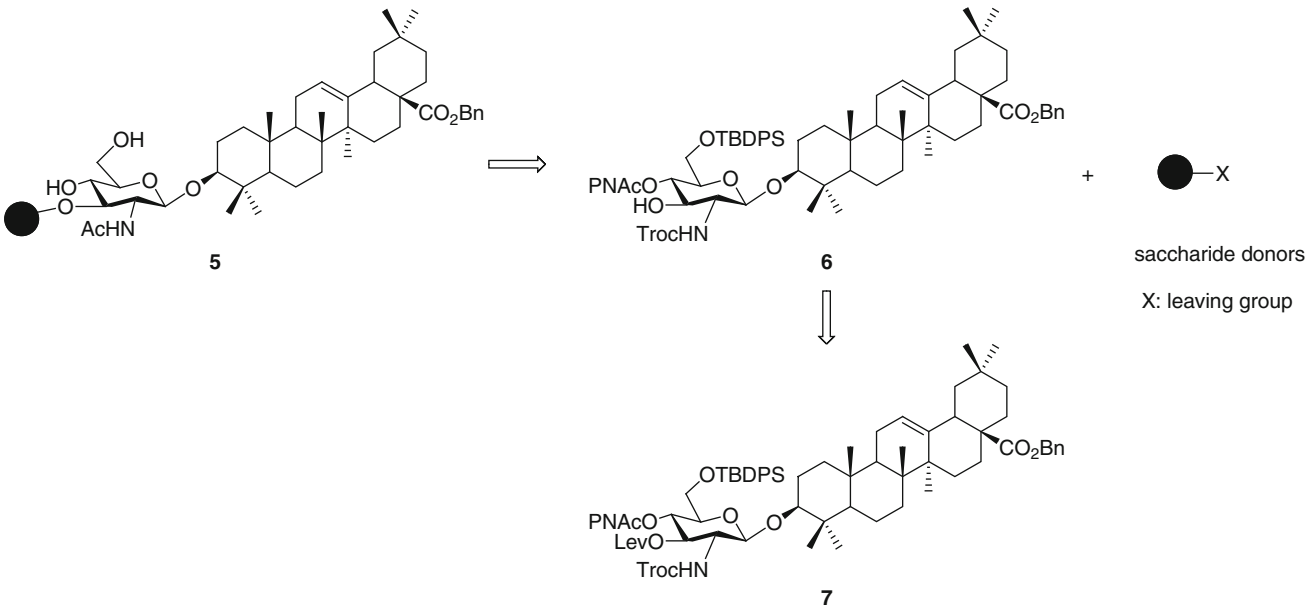
Herein, the development of a combinatorial and highly regioselective method to construct the saccharide library of *N*-acetylglucosamine-bearing OA (**1**) is described. A structure–activity relationship (SAR) study of these compounds was conducted by their submission to two cancer cell lines.

Results and discussion

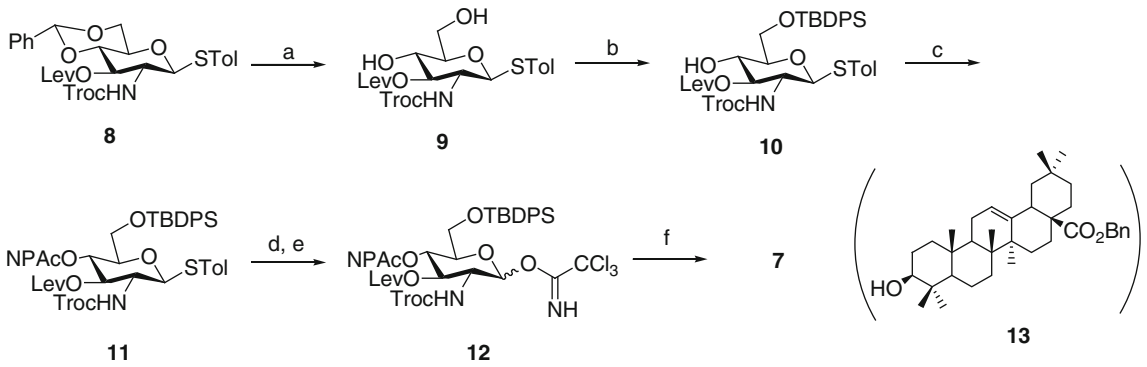
Chemistry

Construction of the saccharide library of *N*-acetylglucosamine-bearing OA (**1**) relies on the acquisition of a designed *N*-acetylglucosamine as a glycan donor with orthogonal protection groups at different positions, and high yielding coupling with different donors. As illustrated in Scheme 1, the retrosynthesis of 3′-glycosylated derivatives (**5**) can be accomplished by selective removal of 3′-*O*-Lev of **7** to give **6** which can be glycosylated with different saccharide donors and then fully deprotected to afford **5**. Compound **7**, wherein the amino group of glucosamine is protected by the Troc group, turned out to be a key intermediate for the synthesis of the library. The Troc group was chosen because it was reported to be able to increase the reactivity in the glycosylation step comparing to acetyl group [29]. The other three protecting groups chosen were levulinoyl (Lev), 2-nitrophenylacetyl (NPAc), and *tert*-butyldiphenylsilyl (TBDPS) at the 3′-, 4′-, and 6′-OH of glucosamine. These protecting groups can be selectively removed using the conditions reported previously [30] with slight modifications to insure that deprotection of each group in the presence of the others is highly selective.

The synthesis of key intermediate **7** is illustrated in Scheme 2. Compound **8** [31] was subjected to acid hydrolysis to remove the benzylidene group. Use of DL-camphorsulfonic acid (CSA) in CH_2Cl_2/MeOH led to the removal of the benzylidene and Lev groups. Use of AcOH/H_2O/CH_2Cl_2 (10:1:10), however, gave the desired compound **9**, which was selectively silylated at the primary hydroxy group using



Scheme 1 Retrosynthesis analysis is exemplified by 3′-glycosylated derivatives **5**. *Black dot* means saccharide donors

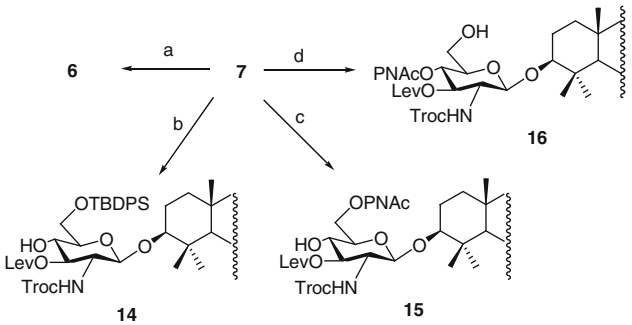


Scheme 2 Reagents and conditions: *a* HOAc/H_2O, CH_2Cl_2, 60 °C; *b* TBDPSCl, imidazole, DMF, rt, 93 % (2 steps); *c* 2-NPAcOH, DCC, DMAP, CH_2Cl_2, rt, 95 %; *d* NBS, acetone/H_2O, 0 °C-rt, 93 %; *e* CCl_3CN, DBU, CH_2Cl_2, rt, 89 %; *f* **13**, TMSOTf, CH_2Cl_2, 4 Å M.S., −12 to 0 °C, 76 % (β only). DCC: 1,3-dicyclohexylcarbodiimide, DMAP: 4-(dimethylamino)pyridine, TMSOTf: trimethylsilyltrifluoromethane sulfonate

tert-butyldiphenylsilyl chloride (TBDPSCl) and imidazole in DMF to afford **10** in 93 % yield (2 steps). Ester bond formation between 4-OH of compound **10** and 2-nitrophenylacetic acid was achieved using the coupling reagents-DCC and DMAP in CH_2Cl_2 to give compound **11** in 95 % yield. The more reactive glycosyl donor **12** was also synthesized via removal of 1-thiotoluene by *N*-bromosuccinimde (NBS) followed by trichoroimidate formation using CCl_3CN and DBU. Glycosylation of **12** with 3-OH of OA benzyl ester (**13**) [23,32] in the presence of TMSOTf at −15 to 0 °C afforded **7** in 76 % yield. Straightforward glycosylation of compound **11** with **13** in the presence of catalysts of *N*-iodosuccinimide (NIS)/trifluoromethanosulfonic acid (TfOH) was also investigated, however, little desired glycosylation product was obtained. The corresponding hydrolyzed compound **11** became the major product and the migration of double bond between C(12) and C(13) was also detected.

Orthogonal deprotection of key intermediate **7** was next conducted (Scheme 3). Use of hydrazine-acetic acid to remove the Lev group gave compound **6** in 87 % yield. Use of activated Zn in ammonium chloride to remove the 2-nitrophenylacetyl group provided compound **14** in 43 % yield. Deprotection of 6′-*O*-TBDPS of **7** with TBAF/AcOH gave 2-nitrophenylacetyl migrated **15**, not the desired **16**, presumably due to the basic property of TBAF. To avoid this, treatment of **7** with 3 % AcCl–MeOH gave compound **16** in 54 % yield.

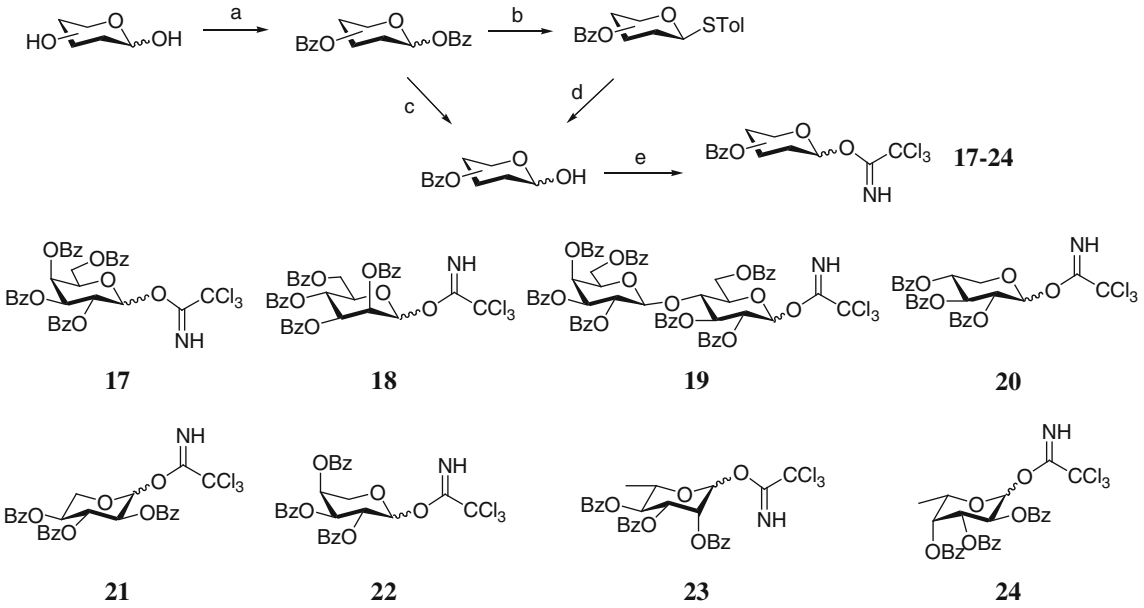
Eight common sugars (D-galactose, D-mannose, D-lactose, D-xylose, L-xylose, L-arabinose, L-rhamnose, L-fucose) [25,33] were selected for attachment at the 3′, or 4′, and 6′ positions of *N*-acetylglucosaminoyl OA. The subsequent Schmidt donors were prepared through per-benzoylation of commercial saccharides [34], selective deprotection of the anomeric hydroxyl groups [35,36], and conversion to the desired glycosyl trichloroacetimidates **17–19** and **23–24** (Scheme 4). Unfortunately, per-benzoylated-xylose and arabinose were not tolerant of the hydrazine-acetate conditions used to get **17–19** and **23–24**, and complete benzoyl deprotection was observed. An alternative route to **20–22** was therefore sought. Hydrolysis of the thiotoluene group with NBS gave 2,3,4-tribenzoyl -xylose and -arabinose, which were subsequently subjected to DBU and CCl_3CN conditions to afford glycosyl trichloroacetimidates (**20–22**).



Scheme 3 *a* NH_2NH_2-HOAc, CH_2Cl_2/MeOH, rt, 87 %; *b* Zn, NH_4Cl, THF/MeOH, rt, 43 %; *c* TBAF/HOAc, THF, rt, 95 %; *d* 3 % AcCl–MeOH, CH_2Cl_2, rt, 54 %

With Schmidt donors **17–24** at hand, glycosylation with compound **6** using TMSOTf in CH_2Cl_2 at 0 °C to room temperature smoothly afforded compounds **25–32** (Table 1). However, attempts to glycosylate compound **14** with **17–24** were extremely low yielding (<10 %), presumably due to the stereo-hindrance of TBDPS group at 6′ position of **14**. Using compound **15** instead, compounds **33–40** were obtained in moderate to good yield (43–73 %). Glycosylation between compound **16** with donors **17–24** proceeded smoothly to afford compounds **41–48** in 41–96 % yield.

Followed by global deprotection to give fully deprotected products **3–4**, **49–70** (Scheme 5), compounds **25–32** were treated with (1) 3 % AcCl/MeOH to remove the TBDPS group at the 6′ position [37]; (2) activated Zn powder in acetic anhydride to remove the Troc group and immediately acetylated at the corresponding amine with Ac_2O; (3) 1.0 N



Scheme 4 *a* BzCl, DMAP, CH_2Cl_2, 0 °C-rt, 87–100 %; *b* *p*-thiocresol, $BF_3 \cdot OEt_2$, CH_2Cl_2, 0 °C -rt, 53–71 %; *c* NH_2NH_2-HOAc, DMF, 60 °C, 40–50 % (for **17**–**19**, **23**–**24**); *d* NBS, acetone/H_2O, 0 °C -rt, 76–94 %; *e* CCl_3CN, DBU, CH_2Cl_2, rt, 92–98 % (for **20**–**22**)

Table 1 Combinatorial glycosylation of compounds **6**, **15**, and **16** with glycosyl doners **17**–**24** to afford glcyosylated **25**–**48**

6, **15**, **16** —(**17-24**; TMSOTf, CH_2Cl_2, 4A M.S., 0 °C to rt.)→ **25-48**

Donors	D-Gal (yield %)	D-Man	D-Lac	D-Xyl	L-Xyl	L-Ara	L-Rha	L-Fuc
6 (3-OH)	**25**[a]	**26**	**27**	**28**	**29**	**30**	**31**	**32**
	(32)	(51)	(46)	(59)	(95)	(58)	(49)	(80)
15 (4-OH)	**33**	**34**	**35**	**36**	**37**	**38**	**39**	**40**
	(51)	(66)	(43)	(73)	(68)	(65)	(71)	(69)
16 (6-OH)	**41**	**42**	**43**	**44**	**45**	**46**	**47**	**48**
	(41)	(48)	(41)	(72)	(96)	(57)	(95)	(42)

[a] At −20 °C

NaOH in CH_2Cl_2/MeOH to hydrolyze all ester bonds; and (4) hydrogenolysis to remove the benzyl group at C(28) position. The desired products **3** and **49**–**55** were obtained. Compounds **4**, and **56**–**70** were also obtained by similar deprotecting methods (Scheme 5).

Biological activity and SAR

The effects of these derivatives on human acute promyelocytic leukemia cell line HL-60 and colorectal cancer cell line HT-29 were studied via MTT and sulforhodamine B (SRB) assays, respectively. As shown in Table 2, compounds **1**, **51**, **52**, and **53** showed more than 50 % of growth inhibition of leukemia cell line HL-60 at 30 μM. Compound **66** displayed 55 % growth inhibition in the HT-29 cell line. The IC_{50} of **1**, **51**, **52**, and **53** for the HL-60 cell line were 4.8, 13, 10, and 7.0 μM, respectively. Although no compound displayed stronger growth inhibition than 1 in the HL-60 and HT-29 cell lines, the preliminary SAR results indicated that derivatives bearing sugar units at 3′ position of *N*-acetylglucosamine had similar or slightly lower cytotoxicity than **1**, except for D-lactose and L-fucose modifications; modification at the 4′ position resulted in weak cytotoxicity and modification at the 6′ position completely eliminated cytotoxic activity, except for compound **66**. Of all the sugar modifications, derivatives bearing D-xylose, L-xylose, and L-arabinose attached at the 3′ or 4′ positions were found to be more active than other derivatives bearing other sugars at the same position for all cell lines tested. It is noteworthy that compound **4** has been reported to be potent in several cancer cell lines with IC_{50} ranging from 4.7 to 12.4 μM [24] whereas we found it to be non-cytotoxic in HL-60 and HT-29 cell lines. This might

Table 2 Growth inhibition (in percentage) of synthetic compounds at 30 μM on HL-60, and HT-29 cell lines and the IC_{50} values of active compounds are shown in parentheses

Compd	HL-60 (%) (IC_{50})	HT-29 (%)	Compd	HL-60 (%)	HT-29 (%)
OA	36	28	**58**	0	0
1	93 (4.8 μM)	90	**59**	33	16
3	37	1	**60**	25	5
4	−5	4	**61**	18	9
49	29	5	**62**	−5	0
50	−16	0	**63**	41	0
51	61 (13 μM)	10	**64**	−2	8
52	73 (10 μM)	20	**65**	−3	3
53	85 (7.0 μM)	25	**66**	−1	55
54	43	6	**67**	1	0
55	2	7	**68**	−10	0
56	2	10	**69**	−7	0
57	9	6	**70**	4	3

be due to the difference in the cell-lines tested. These compounds displayed cell-specific cytotoxic effect rather than non-specific saponin-mediated membrane permeability.

Conclusion

N-Acetylglucosamine-bearing triterpenoid saponins (GNTS) were reported to be a unique type of saponins with potent anti-tumor activity, but isolation of sufficient amounts for biological assay and SAR studies is probelmatic. One solution to this problem is chemical synthesis, and herein the development of a combinatorial and concise method to synthesize 24 OA saponins with (1 → 3)-linked, (1 → 4)-linked, (1 → 6)-linked *N*-acetylglucosamine oligosaccharide residues is described. This method is suitable for preparing OA glycosides with structure diversity for extensive biological evaluation and SAR studies. This method is also thought adaptable to the synthesis of glycoside derivatives of other saponins.

The cytotoxic activity of the compounds synthesized against the leukemia cell line HL-60 revealed the glycosylated, OAs bearing *N*-acetylglucosamine at the 3′-position, such as compounds **51**–**53**, to have IC_{50} values ranging from 7.0 to 12.8 μM. The ranking of cytotoxicity in different positions was as follows: 3′ → 4′ → 6′-positions. Among these modification, D-xylose, L-xylose, and L-arabinose attached at 3′ or 4′ positions were found to be more active than other sugars at the same position.

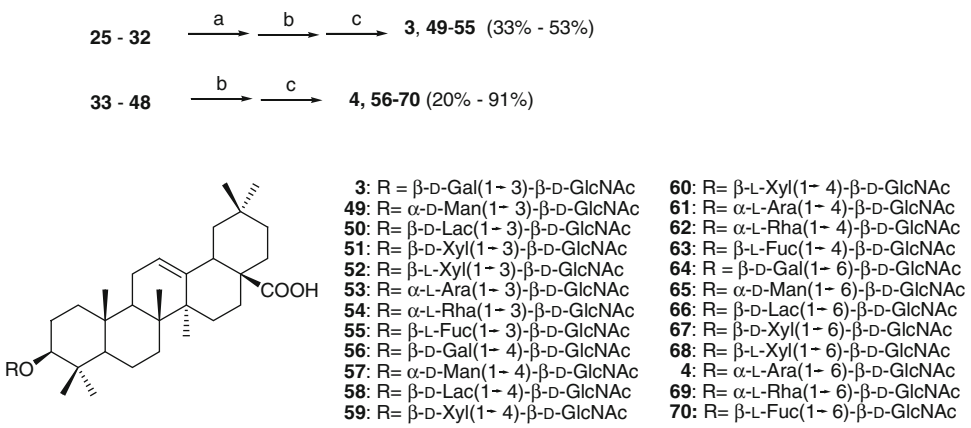
Experimental section

Cell lines and cell culture

Human acute promyelocytic leukemia cell line HL-60 and colorectal cancer cell line HT-29 were obtained from American Type Culture Collection (Rockville, MD). Cells were cultured in RPMI 1640 medium with 10 % FBS (v/v) and penicillin (100 U/mL)/streptomycin (100 μg/mL). Cultures were maintained in a humidified incubator at 37 °C in 5 % CO_2/95 % air.

Mitochondrial MTT reduction activity assay

Cells were incubated in the absence or presence of the compound for the indicated concentrations and times, and then the mitochondrial MTT reduction activity was assessed. MTT was dissolved in PBS at a concentration of 5 mg/mL and filtered. From the stock solution, 10 μL per 100 μL of medium was added to each well, and plates were gently shaken and incubated at 37 °C for 1 h. After the loading of MTT, the medium was replaced with 100 μL acidified β-isopropanol and was left for 5–10 min at room temperature for color development, and then the plate was read by enzyme-linked immunosorbent assay reader (570 nm) to get the absorbance density values.



Scheme 5 Global deprotection to give the target compounds **3**–**4**, **49**–**70**. Reagent and conditions: *a* 3 % AcCl/MeOH, CH_2Cl_2, rt; *b* (i) Zn, Ac_2O, CH_2Cl_2, rt, (ii) 1.0 N NaOH, CH_2Cl_2/MeOH, rt; *c* H_2, 10 % Pd(C), MeOH, rt

Sulforhodamine B (SRB) assay

Cells were seeded in 96-well plates in medium with 5 % FBS. After 24 h, cells were fixed with 10 % trichloroacetic acid (TCA) to represent cell population at the time of compound addition (T_0). After additional incubation of 0.1 % DMSO or indicated compound for 48 h, cells were fixed with 10 % TCA and SRB at 0.4 % (w/v) in 1 % acetic acid was added to stain cells. Unbound SRB was washed out by 1 % acetic acid and SRB bound cells were solubilized with 10 mM Trizma base. The absorbance was read at a wavelength of 515 nm. Using the following absorbance measurements, such as time zero (T_0), control growth (C), and cell growth in the presence of the compound (Tx), the percentage growth was calculated at each of the compound concentrations levels. Percentage growth inhibition was calculated as: $[(1-Tx-T_0)/(C-T_0)] \times 100$ %. Growth inhibition of 50 % (IC_{50}) is defined as the compound concentration which results in 50 % reduction of total protein increase in control cells during the compound incubation.

Chemistry

Reactions were monitored by thin-layer chromatography (Merck, silica gel 60F-254) using *p*-anisaldehyde or cerium molybdate as the stain reagent. Silica gel used for flash column chromatography was Mallinckrodt type 60 (230–400 mesh). Unless otherwise noted, reagents and materials were obtained from commercial sources and used as provided without further purification. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AV-400 or AVIII-600-Cry spectrometer and referenced to residual solvent signals ($CDCl_3$:^{1} H δ = 7.24, ^{13}C δ = 77.0; CD_3OD :^{1} H δ = 3.30,^{13} C δ = 49). The following abbreviations were used to designate the multiplicities: s = single, d = doublet, t = triplet, q = quaterlet, m = multiplet, and dd = double of doublets. Exact mass measurements were performed on VG plateform electrospray ESI/MS or BioTOF II (Taiwan).

The synthesis of the target compounds were shown below. A typical procedure for the preparation of **26**–**48** was exemplified by the synthesis compound **25**. For preparation and structure characterization data for compounds **3**–**4**,**26**–**70**, see Supplemental Material.

p-Methylphenyl-3-O-levulinoyl-6-O-tert-butyldiphenylsilyl-2-deoxy-2-(2,2,2-trichloroethoxylcarbonylamino)-1-thio-β-D-glucopyranoside **(10)**

To a solution of compound **8** [31] (19.0 g, 29.37 mmol) in CH_2Cl_2 (100 mL) was added acetic acid/water (vol/vol = 10:1, 220 mL) and stirred overnight at reflux. The mixture was diluted with EtOAc, washed with $NaHCO_3$(aq) and brine. The organic layers were collected, dried over Na_2SO_4, filtered, and concentrated under reduced pressure to afford the intermediate **9**. The residue **9** and imidazole (5.9 g, 88.1 mmol) were dissolved in DMF (100 mL), followed by addition of TBDPSCl (11.5 mL, 44.05 mmol). The mixture was stirred for 4 h, and then the mixture was concentrated under reduced pressure. The residue was diluted with CH_2Cl_2, washed with 1.0 N HCl, $NaHCO_3$(aq) and brine. The organic layers were collected, dried over Na_2SO_4, filtered, concentrated, column chromatographied (silica gel; EtOAc/ Hex 1:4 to 1:2) to yield compound **10** (21.88 g, 93 %), as white foam. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.70 (d, J = 7.2 Hz, 2H, Ar-H), 7.69 (d, J = 7.2 Hz, 2H, Ar-H), 7.36–7.40 (m, 8H, Ar-H), 7.00 (d,J = 7.8 Hz, 2H, Ar-H), 5.35 (d, J = 9.0 Hz, 1H, *N*-H), 5.07 (t, J = 7.2 Hz, 1H, H-3), 4.73–4.75 (m, 2H, Troc-C$\underline{H}_2$), 4.70 (d, J = 10.8 Hz, 1H, H-1), 3.96 (d, J = 10.8Hz, 1H, H-6a), 3.92 (dd, J = 4.2, 10.8 Hz, 1H, H-6b), 3.77 (t, J = 9.0 Hz, 1H, H-4), 3.65 (q, J = 10.0 Hz, 1H, H-2), 3.47–3.49 (m, 1H, H-5), 3.23 (s, 1H, C-4-OH), 2.75 (d, J = 5.4 Hz, 2H, Lev-C$\underline{H}_2$), 2.46–2.58 (m, 2H, Lev-C$\underline{H}_2$), 2.28 (s, 3H, STol-CH_3), 2.12 (s, 3H, Lev-C$\underline{H}_3$), 1.05 ppm (s, 9H, TBDPS-(C$\underline{H}_3$)$_3$); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 207.6 (Lev-C=O), 173.3 (Lev-OC=O), 154.1 (Troc-C=O), 137.9, 135.7, 135.6, 133.1, 132.8, 129.7, 129.0, 127.7, 95.6 (Troc-$\underline{C}Cl_3$), 87.0 (C-1), 79.6 (C-5), 77.0 (C-3), 74.5 (Troc-$\underline{C}H_2$), 69.6 (C-4), 63.8 (C-6), 54.6 (C-2), 38.3 (Lev-$\underline{C}H_2$), 29.7 (Lev-$\underline{C}H_3$), 28.2 (Lev-$\underline{C}H_2$), 26.8 (TBDPS-$\underline{C}H_3$), 21.1 (STol-$\underline{C}H_3$), 19.2 ppm (TBDPS-$\underline{C}(CH_3)_3$); HRMS (ESI) calcd. for $C_{37}H_{44}Cl_3NO_8SSi$+Na $[M+Na]^+$: 818.1515, found m/z 818.1470.

p-Methylphenyl-3-O-levulinoyl-4-O-(2-nitro)phenylacetyl-6-O-tert-butyldiphenylsilyl-2-deoxy-2-(2,2,2-trichloroethoxylcarbonylamino)-1-thio-β-D-glucopyranoside **(11)**

To a solution of compound **10** (13.75 g, 17.24 mmol) in CH_2Cl_2 (200 mL) was added NPAcOH (6.25 g, 34.48 mmol), DCC (7.12 g, 34.48 mmol) and DMAP (21 mg, 0.17 mmol). The mixture was stirred at rt for 4 h, and washed with 1.0 N HCl, $NaHCO_3$(aq) and brine. The collected organic layers were dried over Na_2SO_4, filtered, concentrated, and column chromatographied (silica gel; EtOAc/Hex/Tol 1:1:1) to afford compound **11** (15.7 g, 95 %), as brown syrup. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.06 (d, J = 8.4 Hz, 1H, Ar-H), 7.71 (d, J = 6.6 Hz, 2H, Ar-H), 7.67 (d, J = 7.2 Hz, 2H, Ar-H), 7.52 (t, J = 7.2 Hz, 1H,Ar-H), 7.33–7.44 (m, 8H, Ar-H), 7.25 (d, J = 9.6 Hz, 2H, Ar-H), 6.98 (d, J = 7.2 Hz, 2H, Ar-H), 5.27–5.32 (m, 2H, *N*-H, H-4), 5.13 (t, J = 9.6 Hz, 1H, H-3), 4.87 (d, J = 10.8 Hz, 1H, H-1), 4.76 (d, J = 12.6 Hz, 1H, Troc-C$\underline{H}_2$), 4.73 (d, J = 12.6 Hz, 1H, Troc-C$\underline{H}_2$), 3.97 (d, J = 17.4 Hz, 1H, NPAc-C$\underline{H}_2$), 3.80 (d, J = 11.4 Hz, 1H, H-6a), 3.76 (dd,

J = 4.8, 11.4 Hz, 1H, H-6b), 3.71 (d, J = 17.4 Hz, 1H, NPAc-C$\underline{H}_2$), 3.50–3.60 (m, 2H, H-2, H-5), 2.65–2.75 (m, 2H, Lev-C$\underline{H}_2$), 2.45–2.55 (m, 2H, Lev-C$\underline{H}_2$), 2.26 (s, 3H, STol-C$\underline{H}_3$), 2.12 (s, 3H, Lev-C$\underline{H}_3$), 1.02 ppm (s, 9H, TBDPS-(C$\underline{H}_3$)$_3$); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 206.7 (Lev-$\underline{C}$=O), 172.5 (Lev-O$\underline{C}$=O), 168.6 (NPAc-$\underline{C}$=O), 153.9 (Troc-$\underline{C}$=O), 148.4, 138.2, 135.8, 135.7, 133.6, 133.2, 133.1, 129.7, 129.6, 129.5, 128.7, 127.7, 125.2, 95.5 (Troc-$\underline{C}Cl_3$), 86.6 (C-1), 78.8 (C-5), 74.6 (Troc-$\underline{C}H_2$), 73.4 (C-4), 68.7 (C-3), 62.8 (C-6), 55.4 (C-2), 39.4 (NPAc-$\underline{C}H_2$), 37.7 (Lev-$\underline{C}H_2$), 29.7 (Lev-$\underline{C}H_3$), 27.9 (Lev-$\underline{C}H_2$), 26.7 (TBDPS-$\underline{C}H_3$), 21.1 (STol-$\underline{C}H_3$), 19.2 ppm (TBDPS-$\underline{C}(CH_3)_3$); HRMS (ESI) calcd. for $C_{45}H_{49}Cl_3N_2O_{11}$ SSi+Na[M+Na]$^+$: 981.1784, found m/z 981.1722.

28-O-Benzyl-3-O-[6-O-tert-butyldiphenylsilyl-3-O-levulinoyl-4-O-(2-nitro)phenylacetyl-2-(2,2,2-trichloroethoxylcarbonylamino)-2-deoxy-β-D-glucopyranosyl]oleanolic ester **(7)**

Compound **11** (16.53 g, 17.21 mmol) in acetone/H_2O (10:1, 220 mL) was added NBS (24.4 g, 137.92 mmol) and stirred at 0 °C for 30 min. The mixture was quenched by $Na_2S_2O_3$(aq) and then evaporated to remove acetone. The residue was dissolved in CH_2Cl_2, washed with $NaHCO_3$(aq), $Na_2S_2O_3$(aq) and brine. The organic layers were collected, dried over Na_2SO_4, filtered and concentrated under reduced pressure, and column chromatographied (silica gel; EtOAc/Hex 1:4 to 1:3) to yield 6-*O*-*tert*-butyldiphenylsilyl-3-*O*-levulinoyl-4-*O*-(2-nitro)phenylacetyl-2- (2,2,2-trichloroethoxylcarbonylamino)-2-deoxy-D-glucopyranose (13.6 g, 93 %). ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.08 (d, J = 7.8 Hz, 1H, Ar-H), 7.68 (d, J = 7.2 Hz, 2H, Ar-H), 7.65 (d, J = 7.2 Hz, 2H, Ar-H), 7.52 (t, J = 7.2 Hz, 1H, Ar-H), 7.30–7.45 (m, 8H, Ar-H), 5.45 (d, J = 9.6 Hz, 1H, *N*-H), 5.33 (t, J = 10.2 Hz, 1H, H-3), 5.20–5.25 (m, 2H, H-1, H-4), 4.75 (d, J = 12.6 Hz, 1H, Troc-C$\underline{H}_2$), 4.71 (d, J = 12.6 Hz, 1H, Troc-C$\underline{H}_2$), 4.05–4.07 (m, 1H, H-5), 4.01 (d, J = 17.4 Hz, 1H, NPAc-C$\underline{H}_2$), 3.96 (t, J = 12.0 Hz, 1H, H-2), 3.75–3.80 (m, 3H, H-6a, H-6b, NPAc-C$\underline{H}_2$), 3.23 (s, 1H, C-1-O$\underline{H}$), 2.69–2.71 (m, 2H, Lev-CH_2), 2.45–2.60 (m, 2H, Lev-CH_2), 2.13 (s, 3H, Lev-CH_3), 1.02 ppm (s, 9H, TBDPS-$(CH_3)_3$); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 206.7 (Lev-$\underline{C}$=O), 172.9 (Lev-O$\underline{C}$=O), 168.6 (NPAc-C=O), 154.2 (Troc-$\underline{C}$=O), 148.4, 135.8, 133.7, 133.4, 129.6, 128.6, 127.6, 125.2, 95.5 (Troc-CCl_3), 91.6 (C-1), 74.6 (Troc-$\underline{C}H_2$), 71.1 (C-3), 70.3 (C-5), 68.8 (C-4), 62.7 (C-6), 54.3 (C-2), 39.4 (NPAc-CH_2), 37.6 (Lev-CH_2), 29.7 (Lev-$\underline{C}H_3$), 27.9 (Lev-$\underline{C}H_2$), 26.8 (TBDPS-$\underline{C}H_3$), 19.3 ppm (TBDPS-$\underline{C}(CH_3)_3$); HRMS (ESI) calcd. for $C_{38}H_{43}Cl_3N_2O_{12}Si$+Na [M+Na]$^+$: 875.1543, found m/z 875.1501. DBU (25.0 μL, 0.22 mmol) was added to a stirring solution of 6-*O*-*tert*-butyldiphenylsilyl-3 - *O*-levulinoyl - 4 - *O*-(2-nitro) phenylacetyl-2 - (2,2,2- trichloroethoxylcarbonylamino)-2-deoxy-D-glucopyranose (925 mg, 1.083 mmol) and trichloroacetonitrile (0.33 mL, 3.3 mmol) in CH_2Cl_2 (10 mL) at 0 °C, and the mixture was warmed to rt and stirred for another 2 h. The mixture was concentrated to give a residue, which was subjected to column chromatography (silica gel; EtOAc/Hex/triethylamine = 1:1:0.1) to give intermediate **12** (970 mg, 89 %). A mixture of compound **12** (970 mg, 0.97 mmol), benzyl ester of oleanolate (**13**, 583 mg, 1.07 mmol), and 4 Å M.S. in dry CH_2Cl_2 (5 mL) was stirred at −12 °C for 10 min. Then TMSOTf (10 μL, 0.05 mmol) was added, stirred for 30 min at 0 °C and then Et_3N (1 mL) was added. The mixture was filtered and concentrated to give a residue, which was column chromatographied (silica gel; EtOAc/Hex = 1:4) to give compound **7** (1.138 g, 76 %). ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.05 (d, J = 8.4 Hz, 1H, Ar-H), 7.66 (d, J = 7.8 Hz, 2H, Ar-H), 7.64 (d, J = 7.8 Hz, 2H, Ar-H), 7.52 (t, J = 7.2 Hz, 1H, Ar-H), 7.23–7.41 (m, 13H, Ar-H), 5.26–5.31 (m, 2H, H-12, H-3′), 5.19 (d, J = 8.4 Hz, 1H, *N*-H), 5.07 (d, J = 12.6 Hz, 1H, $CO_2C\underline{H}_2$Ph), 5.02–5.04 (m, 1H, H-4′), 5.02 (d, J = 12.6 Hz, 1H, $CO_2C\underline{H}_2$Ph), 4.68–4.69 (m, 3H, H-1′, Troc-CH_2), 3.97 (d, J = 17.4 Hz, 1H, NPAc-C$\underline{H}_2$), 3.70–3.76 (m, 2H, H-6a′, H-6b′), 3.67 (d, J = 17.4 Hz, 1H, NPAc-C$\underline{H}_2$), 3.54–3.60 (m, 2H, H-2′, H-5′), 3.09 (dd, J = 7.2, 11.4 Hz, 1H, H-3), 2.88 (d, J = 11.4 Hz, 1H, H-18), 2.70–2.72 (m, 2H, Lev-CH_2), 2.47–2.60 (m, 2H, Lev-C$\underline{H}_2$), 2.13 (s, 3H, Lev-C$\underline{H}_3$), 1.93–1.98 (m, 2H), 1.77–1.81 (m, 2H), 1.58–1.70 (m, 4H), 1.43–1.55 (m, 5H), 1.12–1.40 (m, 8H), 1.10 (s, 3H), 1.01 (s, 9H, TBDPS-$(CH_3)_3$), 0.90 (s, 6H), 0.88 (s, 3H), 0.85 (s, 3H), 0.76 (s, 3H), 0.66 (d, J = 11.4 Hz, 1H), 0.57 ppm (s, 3H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 206.7 (Lev-$\underline{C}$ = O), 177.4 ($\underline{C}O_2CH_2$-Ph), 172.6 (Lev-O$\underline{C}$=O), 168.7 (NPAc-C=O), 153.9 (Troc-$\underline{C}$=O), 148.4, 143.7 (C-13), 136.4, 135.7, 133.6, 133.3, 129.6, 129.4, 128.7, 128.4, 128.0, 127.9, 127.7, 125.2, 122.5 (C-12), 102.8 (C-1′), 95.3 (Troc-CCl_3), 90.5 (C-3), 74.72 (Troc-CH_2), 74.68 (C-5′), 72.1 (C-3′), 69.3 (C-4′), 65.9 ($CO_2\underline{C}H_2$Ph), 62.9 (C-6′), 57.3 (C-2′), 55.5, 47.6, 46.7, 45.9, 41.7, 41.4 (C-18), 39.4 (NPAc-$\underline{C}H_2$), 39.3, 38.9, 38.4, 37.9 (Lev-$\underline{C}H_2$), 36.7, 33.9, 33.1, 32.7, 32.4, 30.7, 29.7 (Lev-CH_3), 28.1 (Lev-CH_2), 28.0, 27.6, 26.7, 25.8, 23.6, 23.4, 23.0, 19.2 (TBDPS-$\underline{C}(CH_3)_3$), 18.2, 16.9, 16.5, 15.3 ppm; HRMS (ESI) calcd. for $C_{75}H_{95}Cl_3N_2O_{14}Si$+Na [M+Na]$^+$: 1403.5510, found m/z 1403.5397.

*28-O-Benzyl3-O-[6-O-tert-butyldiphenylsilyl-4-O-(2-nitro)phenylacetyl 2-(2,2,2-trichloroethoxylcarbony lamino)-2-deoxy-β-D-glucopyranosyl]oleanolic ester (***6***)*

Compound **7** (145 mg, 0.105 mmol) in CH_2Cl_2/MeOH (1:1, 4 mL) was added hydrazine acetate (14.5 mg, 0.157 mmol) and stirred for 4 h. The mixture was concentrated under

reduced pressure. The residue was diluted with CH_2Cl_2, and washed with water. The organic layers were collected, dried with Na_2SO_4, filtered, concentrated, column chromatographied (silica gel; EtOAc/hexane = 1:3) to give compound **6** (117 mg, 87 %), as yellow foam. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.04 (d, J = 8.4 Hz, 1H, Ar-H), 7.65 (t, J = 7.2 Hz, 4H, Ar-H), 7.53 (t, J = 7.8 Hz, 1H, Ar-H), 7.21–7.54 (m, 13H, Ar-H), 5.26 (t, J = 3.6 Hz, 1H, H-12), 5.21–5.23 (m, 1H, *N*-H), 5.07 (d, J = 12.6 Hz, 1H, CO_2CH_2Ph), 5.02 (d, J = 12.0 Hz, 1H, CO_2CH_2Ph), 4.88 (t, J = 9.0 Hz, 1H, H-4′), 4.66–4.68 (m, 3H, Troc-CH_2, H-1′), 4.00–4.02 (m, 1H, H-3′), 3.92 (d, J = 16.8 Hz, 1H, NPAc-CH_2), 3.79 (d, J = 16.8 Hz, 1H, NPAc-CH_2), 3.70–3.72 (m, 2H, H-6a′, H-6b′), 3.51–3.53 (m, 1H, H-5′), 3.34–3.36 (m, 1H, H-2′), 3.10 (dd, J = 3.0, 11.4 Hz, 1H, H-3), 2.87–2.91 (m, 2H, H-18, C-3′-OH), 1.94 (t, J = 13.2 Hz, 2H), 1.79–1.81 (m, 2H), 1.58–1.72 (m, 4H), 1.43–1.50 (m, 3H), 1.15–1.40 (m, 6H), 1.10 (s, 3H), 1.01(s, 9H, TBDPS-(CH_3)$_3$), 0.92 (s, 3H), 0.90 (s, 3H), 0.88 (s, 3H), 0.86 (s, 3H), 0.81–0.83 (m, 4H), 0.79 (s, 3H), 0.66–0.68 (m, 1H), 0.58 ppm (s, 3H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 177.5 (CO_2CH_2-Ph), 169.7 (NPAc-C=O), 154.3 (Troc-C=O), 148.4, 143.7 (C-13), 136.3, 135.7, 133.8, 133.3, 133.1, 129.6, 129.2, 128.8, 128.4, 128.0, 127.9, 127.7, 125.3, 122.4 (C-12), 102.2 (C-1′), 95.1 (Troc-CCl_3), 90.2 (C-3), 74.7 (Troc-CH_2), 74.4 (C-5′), 72.8 (C-4′), 72.1 (C-3′), 65.9 (CO_2CH_2-Ph), 62.9 (C-6′), 59.2 (C-2′), 55.4, 47.5, 46.7, 45.8, 41.6, 41.3 (C-18), 39.7 (NPAc-CH_2), 39.2, 38.9, 38.3, 36.6, 33.8, 33.1, 32.6, 32.3, 30.7, 28.0, 27.5, 26.6, 25.8, 23.6, 23.3, 23.0, 19.2 (TBDPS-$C(CH_3)_3$), 18.1, 16.8, 16.5, 15.3 ppm; HRMS (ESI) calcd. for $C_{70}H_{89}Cl_3N_2O_{12}Si$+Na $[M+Na]^+$: 1305.5143, found m/z 1305.5029.

28-O-Benzyl 3-O-[6-O-tert-butyldiphenylsilyl-3-O-levulinoyl-2-(2,2,2-trichloroethoxylcarbonylamino)-2-deoxy-β-D-glucopyranosyl]oleanolic ester **(14)**

Compound **12** (85.7 mg, 0.062 mmol) in THF/MeOH (4:1, 5 mL) was added fresh prepared Zn powder (20.3 mg, 0.310 mmol) and NH_4Cl (9.8 mg, 0.186 mmol), and then stirred overnight at rt. The mixture was filtered through a pad of Celite, the filter cake was washed with MeOH, and then the filtrate was concentrated under reduced pressure. The residue was subjected to column chromatography (silica gel; EtOAc/Hex = 1:2) to give compound **14** (29 mg, 43 %), as yellow foam. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.59 (d, J = 7.2 Hz, 4H, Ar-H), 7.05–7.35 (m, 11H, Ar-H), 5.18 (t, J = 3.6 Hz, 1H, H-12), 5.13 (d, J = 9.0 Hz, 1H, N-H), 4.94–5.05 (m, 3H, CO_2CH_2Ph, H-3′), 4.63 (d, J = 12.0 Hz, 1H, Troc-CH_2), 4.57 (d, J = 12.0 Hz, 1H, Troc-CH_2), 4.45 (d, J = 8.4 Hz, 1H, H-1′), 3.86 (dd, J = 3.0, 10.8 Hz, 1H, H-6a′), 3.79 (dd, J = 5.4, 10.8 Hz, 1H, H-6b′), 3.53–3.63 (m, 2H, H-4′, H-2′), 3.37–3.40 (m, 1H, H-5′), 3.16 (br s, 1H, C-4′-OH), 3.00 (dd, J = 4.2, 11.4 Hz, 1H, H-3), 2.81 (dd, J = 4.2, 13.8 Hz, 1H, H-18), 2.68–2.70 (m, 2H, Lev-CH_2), 2.42–2.52 (m, 2H, Lev-CH_2), 2.07 (s, 3H, Lev-CH_3), 1.86–1.90 (m, 1H), 1.80–1.83 (m, 1H), 1.71–1.73 (m, 2H), 1.36–1.61 (m, 9H), 1.17–1.32 (m, 3H), 1.05–1.13 (m, 3H), 1.01 (s, 3H), 0.95 (s, 9H, TBDPS-(CH_3)$_3$), 0.92–0.94 (m, 2H), 0.82 (s, 6H), 0.80 (s, 3H), 0.77 (s, 3H), 0.67 (s, 3H), 0.58 (d, J = 10.8 Hz, 1H), 0.49 ppm (s, 3H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 207.5 (Lev-C = O), 177.4(CO_2CH_2-Ph), 173.4 (Lev-OC=O), 154.1 (Troc-C=O), 143.7 (C-13), 136.4, 135.6, 133.0, 129.8, 129.0, 127.9, 127.7, 125.3, 122.5 (C-12), 103.4 (C-1′), 95.4 (Troc-CCl_3), 90.3 (C-3), 75.7 (C-3′), 75.2 (C-5′), 74.7 (Troc-CH_2), 70.4 (C-4′), 65.9 (CO_2CH_2-Ph), 64.2 (C-6′), 56.4 (C-2′), 55.5, 47.6, 46.7, 45.9, 41.6, 41.4 (C-18), 39.3, 38.9, 38.4, 38.3 (Lev-CH_2), 36.7, 33.8, 33.1, 32.7, 32.4, 30.7, 29.7 (Lev-CH_3), 28.2 (Lev-CH_2), 28.1, 27.6, 26.6, 25.8, 23.6, 23.4, 23.0, 19.2 (TBDPS-$C(CH_3)_3$), 18.2, 16.8, 16.4, 15.2 ppm; HRMS (ESI) calcd. for $C_{67}H_{90}Cl_3NO_{11}Si$+Na $[M+Na]^+$: 1240.5241, found m/z 1240.5152.

28-O-Benzyl 3-O-[3-O-levulinoyl-6-O-(2-nitro)phenylacetyl-2-(2,2,2- trichloroethoxylcarbonylamino)-2-deoxy-β-D-glucopyranosyl]oleanolic ester **(15)**

TBAF/AcOH (40 μL, 0.041 mmol/2.5 μL, 0.028 mmol) were added dropwisely to a solution of compound **7** (35 mg, 0.025 mmol) in THF (1 mL) and stirred at rt overnight. The mixture was concentrated and the residue was diluted with CH_2Cl_2, and washed with $NaHCO_3$(aq) and brine. The organic layers were collected, dried with Na_2SO_4, filtered, concentrated, and column chromatographied (silica gel; EtOAc/Hex 1:2) to give compound **15** (27.2 mg, 95 %), as white foam. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.09 (d, J = 7.8 Hz, 1H, Ar-H), 7.59 (t, J = 7.2 Hz, 1H, Ar-H), 7.47 (t, J = 7.8 Hz, 1H, Ar-H), 7.36 (d, J = 7.2 Hz, 1H, Ar-H), 7.30–7.32 (m, 5H, Ar-H), 5.26 (t, J = 3.6 Hz, 1H, H-12), 5.22 (d, J = 9.0 Hz, 1H, N-H), 5.07 (d, J = 12.0 Hz, 1H, CO_2CH_2Ph), 5.02 (d, J = 12.6 Hz, 1H, CO_2CH_2Ph), 5.01–5.03 (m, 1H, H-3′), 4.70 (d, J = 12.0 Hz, 1H, Troc-CH_2), 4.64 (d, J = 11.4 Hz, 1H, Troc-CH_2), 4.53 (d, J = 8.4 Hz, 1H, H-1′), 4.43 (d, J = 11.4 Hz, 1H, H-6a′), 4.39 (dd, J = 5.4, 12.0 Hz, 1H, H-6b′), 4.05 (d, J = 17.4 Hz, 1H, NPAc-CH_2), 4.02 (d, J = 16.8 Hz, 1H, NPAc-CH_2), 3.62–3.64 (m, 1H, H-2′), 3.58 (t, J = 9.6 Hz, 1H, H-4′), 3.51–3.53 (m, 1H, H-5′), 3.31 (br s, 1H, C-4′-OH), 3.10 (dd, J = 3.0, 10.8 Hz, 1H, H-3), 2.87 (dd, J = 3.0, 13.2 Hz, 1H, H-18), 2.75–2.79 (m, 2H, Lev-CH_2), 2.49–2.59 (m, 2H, Lev-CH_2), 2.16 (s, 3H, Lev-CH_3), 1.93–1.98 (m, 1H), 1.81–1.82 (m, 2H), 1.13–1.51 ppm (m, 21H), 1.09 (s, 3H), 0.90 (s, 3H), 0.86 (s,

3H), 0.85 (s, 3H), 0.74 (s, 3H), 0.69 (d, J = 12.0 Hz, 1H), 0.57 ppm (s, 3H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 207.6 (Lev-C=O), 177.4 (CO_2CH_2-Ph), 173.4 (Lev-OC=O), 170.1 (NPAc-C=O), 154.1 (Troc-C=O), 148.8, 143.7 (C-13), 136.4, 134.5, 133.6, 129.5, 128.7, 128.0, 126.0, 125.6, 125.3, 122.9, 122.5 (C-12), 103.4 (C-1′), 95.3 (Troc-CCl_3), 90.5 (C-3), 75.3 (C-3′), 74.7 (Troc-CH_2), 73.3 (C-5′), 69.2 (C-4′), 65.9 (CO_2CH_2-Ph), 63.9 (C-6′), 56.3 (C-2′), 55.4, 47.6, 46.7, 45.9, 41.6, 41.4 (C-18), 39.6 (NPAc-CH_2), 39.3, 38.9, 38.4, 37.5 (Lev-CH_2), 36.7, 33.8, 33.1, 32.7, 32.1, 30.7, 29.7 (Lev-CH_3), 28.2 (Lev-CH_2), 28.0, 27.6, 26.7, 25.8, 23.6, 23.4, 23.0, 18.2, 16.9, 16.4, 15.2 ppm; HRMS (ESI) calcd. for $C_{59}H_{77}Cl_3N_2O_{14}$+Na $[M+Na]^+$: 1165.4333, found m/z 1165.4453.

28-O-Benzyl 3-O-[3-O-levulinoyl-4-O-(2-nitro) phenylacetyl- 2-(2,2,2-trichloroethoxylcarbonylamino)-2-deoxy-β-D-glucopyranosyl]oleanolic ester **(16)**

Acetyl chloride/MeOH (1:25, 2.6 mL) was added dropwise at 0°C to a stirring solution of compound **7** (100 mg, 0.072 mmol) in CH_2Cl_2 (10 mL) and stirred at rt overnight. The mixture was quenched with $NaHCO_3$(aq), washed with brine. The organic layers were collected, dried over Na_2SO_4, filtered, and concentrated. The residue was subjected to column chromatography (silica gel; EtOAc/Hex = 1:3) to give compound **16** (44.7 mg, 54 %), as yellow foam. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.11 (d, J = 8.4 Hz, 1H, Ar-H), 7.44–7.49 (m, 2H, Ar-H), 7.28–7.32 (m, 1H, Ar-H), 7.28–7.30 (m, 5H, Ar-H), 5.35 (t, J = 10.2 Hz, 1H, H-3′), 5.26 (t, J = 3.6 Hz, 1H, H-12), 5.17 (d, J = 8.4 Hz, 1H, N-H), 5.01–5.08 (m, 3H, CO_2CH_2Ph, H-4′), 4.71 (d, J = 7.8 Hz, 1H, H-1′), 4.68 (d, J = 12.0 Hz, 1H,Troc-CH_2), 4.65 (d, J = 12.0 Hz, 1H, Troc-CH_2), 4.11 (d, J = 16.8 Hz, 1H, NPAc-CH_2), 3.99 (d, J = 16.8 Hz, 1H, NPAc-CH_2), 3.74–3.76 (m, 1H, H-6a′), 3.66–3.68 (m, 1H, H-6b′), 3.56–3.58 (m, 1H, H-2′), 3.50–3.51 (m, 1H, H-5′), 3.09 (dd, J = 4.2, 11.4 Hz, 1H, H-3), 2.88 (dd, J = 4.2, 13.8 Hz, 1H, H-18), 2.71–2.74 (m, 2H, Lev-CH_2), 2.57–2.58 (m, 1H, Lev-CH_2), 2.50–2.52 (m, 1H, Lev-CH_2), 2.13 (s, 3H, Lev-CH_3), 1.93–1.98 (m, 1H), 1.75–1.82 (m, 2H), 1.56–1.71 (m, 3H), 1.50–1.51 (m, 1H), 1.43–1.47 (m, 2H), 1.24–1.40 (m, 2H), 1.12–1.21 (m, 2H), 1.09 (s, 3H), 1.00 (d, J = 14.4 Hz, 1H), 0.90 (s, 3H), 0.89 (s, 3H), 0.88 (s, 3H), 0.84 (s, 3H), 0.74 (s, 3H), 0.67 (d, J = 11.4 Hz, 1H), 0.57 ppm; ^{13}C NMR ($CDCl_3$, 150 MHz) δ 206.1 (Lev-C=O), 177.4 (CO_2CH_2-Ph), 172.4 (Lev-OC=O), 169.8 (NPAc-C=O), 153.9 (Troc-C=O), 148.4, 143.7 (C-13), 136.4, 133.9, 129.5, 128.9, 128.4, 128.0, 127.9, 125.3, 122.5 (C-12), 102.7 (C-1′), 95.3 (Troc-CCl_3), 90.5 (C-3), 74.7 (Troc-CH_2), 73.8 (C-5′), 71.7 (C-3′), 69.4 (C-4′), 65.9 (CO_2CH_2-Ph), 61.5 (C-6′), 57.2 (C-2′), 55.5, 47.6, 46.7, 45.9, 41.7, 41.4 (C-18), 39.6 (NPAc-CH_2), 39.3, 38.9, 38.4, 37.8 (Lev-CH_2), 36.7, 33.9, 33.1, 32.7, 32.4, 30.7, 29.7 (Lev-CH_3), 28.1 (Lev-CH_2), 27.9, 27.6, 26.7, 25.8, 23.6, 23.4, 23.1, 18.2, 16.9, 16.5, 15.2 ppm; HRMS (ESI) calcd. for $C_{59}H_{77}Cl_3N_2O_{14}$ + Na $[M+Na]^+$: 1165.4333, found m/z 1165.4243.

*28-O-Benzyl 3-O-[2,3,4,6-terta-O-benzoyl-β-*D *-galacto pyranosyl(1 → 3)- 6-O-tert-butyldiphenylsilyl-4-O-(2-nitro)phenylacetyl-2-(2,2,2-trichloroethoxyl carbonylamino)-2-deoxy-β-D-glucopyranosyl]oleanolic ester* **(25)**

2,3,4,6-*Tetra-O*-benzoyl-D-galactopyranose (100 mg, 0.168 mmol) was dissolved in dry CH_2Cl_2 (1 mL), followed by addition of trichloroacetonitrile (50 μL, 0.50 mmol) and DBU (5.0 μL, 0.034 mmol). After stirring 2h at rt, the mixture was evaporated then column chromatographied (silica gel; EtOAc/Hex/Et_3N = 1 : 1 : 0.01) to give imidate intermediate **17**. A mixture of imidate **17**, compound **6** (215 mg, 0.168 mmol) and 4 Å M.S. in dry CH_2Cl_2 (1 mL) was stirred at −20 °C for 10 min, and then TMSOTf (1.5 μL, 0.008 mmol) was added. After stirring 30 min at −20°C, Et_3N (0.5 mL) was added. The mixture was filtered and concentrated to give a residue. The residue was purified by column chromatography (silica gel; EtOAc/Hex = 1:5) to obtain compound **25** (100 mg, 32 %) as white foam. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.06 (d, J = 7.8 Hz, 3H, Ar-H), 8.02 (d, J = 7.8 Hz, 2H, Ar-H), 7.97 (d, J = 8.4 Hz, 1H, Ar-H), 7.74 (d, J = 7.2 Hz, 2H, Ar-H), 7.63 (d, J = 6.6 Hz, 2H, Ar-H), 7.61 (d, J = 6.6 Hz, 2H, Ar-H), 7.27–7.54 (m, 23H, Ar-H), 7.19–7.22 (m, 2H, Ar-H), 7.15 (t, J = 7.2 Hz, 1H, Ar-H), 7.01 (d, J = 7.2 Hz, 1H, Ar-H), 5.94–5.95 (m, 1H, H-4″), 5.77 (t, J = 9.0 Hz, 1H, H-2″), 5.45 (d, J = 10.2 Hz, 1H, H-3″), 5.25 (t, J = 3.6 Hz, 1H, H-12), 5.07 (d, J = 13.2 Hz, 1H, CO_2CH_2Ph), 5.01–5.03 (m, 2H, N-H, CO_2CH_2Ph), 4.97 (d, J = 12.0 Hz, 1H, Troc-CH_2), 4.90 (d, J = 7.8 Hz, 1H, H-1′), 4.86 (d, J = 8.4Hz, 1H, H-1″), 4.85 (t, J = 9.6 Hz, 1H, H-4′), 4.63–4.66 (m, 1H, H-6a″), 4.54 (t, J = 10.2 Hz, 1H, H-3′), 4.50 (d, J = 12.0 Hz, 1H, Troc-CH_2), 4.43–4.46 (m, 1H, H-6b″), 4.26 (t, J = 5.4Hz, 1H, H-5″), 3.88 (d, J = 16.8 Hz, 1H, NPAc-CH_2), 3.83 (d, J = 17.4 Hz, 1H, NPAc-CH_2), 3.76 (d, J = 11.4 Hz, 1H, H-6a′), 3.68–3.71 (m, 1H, H-6b′), 3.54 (t, J = 8.4 Hz, 1H, H-5′), 3.05 (d, J = 12.0 Hz, 1H, H-3), 2.81–2.89 (m, 2H, H-18, H-2′), 1.96 (t, J = 13.2 Hz, 1H), 1.86 (d, J = 13.2 Hz, 1H), 1.51–1.77 (m, 5H), 1.13–1.48 (m, 12H), 1.10 (s, 3H), 0.99–1.01 (m, 1H), 0.99 (s, 9H, TBDPS-(CH_3)$_3$), 0.88 (s, 3H), 0.90 (s, 3H), 0.80 (s, 6H), 0.74 (t, J = 12.6 Hz, 1H), 0.67 (s, 3H), 0.63 (d, J = 6.0 Hz, 1H), 0.55 ppm (s, 3H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 177.5 (CO_2CH_2-Ph), 168.4 (NPAc-C=O), 166.1, 165.5, 164.6, 153.9 (Troc-C=O), 148.7, 143.7 (C-13), 136.8, 136.4, 135.7, 135.6, 133.4, 133.2, 129.8, 129.5, 129.3, 129.0, 128.8, 128.6, 128.4, 128.0, 127.6, 125.1, 122.5 (C-12), 102.1 (C-1″), 100.3 (C-1′),

95.3 (Troc-CCl_3), 89.7 (C-3), 78.0 (C-3′), 74.9 (C-5′), 74.7 (Troc-CH_2), 71.41(C-3″), 71.40 (C-5″), 70.2 (C-4′), 69.9 (C-2″), 68.0 (C-4′), 65.9 ($CO_2\underline{C}H_2$-Ph), 63.2 (C-6′), 62.0 (C-6″), 59.6 (C-2′), 55.4, 47.6, 46.7, 41.7, 41.4 (C-18), 39.6 (NPAc-$\underline{C}H_2$), 39.3, 38.7, 36.6, 33.9, 33.1, 32.7, 32.4, 30.7, 27.9, 27.6, 26.7, 25.8, 23.6, 23.4, 23.1, 19.3 (TBDPS-$\underline{C}(CH_3)_3$), 18.2, 16.9, 16.5, 15.2 ppm; HRMS (ESI) calcd. for $C_{104}H_{115}Cl_3\ N_2O_{21}Si$+Na $[M+Na]^+$: 1883.6719, found m/z 1883.6761.

General procedures for deprotection reactions

Method 1: To a solution of compound in CH_2Cl_2 (10–15 mL) was added AcCl/MeOH (vol/vol = 1:25, 2.6 mL) dropwise at 0 °C. The mixture was stirred at rt overnight, and concentrated under reduced pressure to give the de-silyl product. Method 2: The de-silyl product or glycosylation product (1 mmol = 1 eq) in CH_2Cl_2(5–10 mL), followed by addition of Zn powder (5 mmol = 5 eq), Ac_2O (10 mmol = 10 eq) and stirred 3 days at rt. The mixture was filtered and concentrated under reduced pressure to obtain a crude intermediate. Then, 1.0 N NaOH was added to a stirring solution of the intermediate, the pH value was to adjusted to around 10, and stirred at rt for 4 h. The mixture was filtered and concentrated under reduced pressure. The residue was subjected to silica gel column chromatography (CH_2Cl_2/MeOH = 20 : 1) to get a de-benzoylated product. The de-bezoylated product dissolved in MeOH and Pd(C) (5 mg, 10 %) was added. The mixture was stirred under H_2 for 12 h. The residue was filtered, concentrated and purified by column chromatography (silica gel; CH_2Cl_2/MeOH = 10 : 1).

Acknowledgments We gratefully acknowledge the National Science Council of Taiwan ROC (NSC 98-2320-B-002-017- MY3) and Academia Sinica for financial support.

References

1. Papadopoulou K, Melton RE, Legget M, Daniels MJ, Osbourn AE (1999) Compromised disease resistance in saponin-deficient plants. Proc Natl Acad Sci USA 96:12923–12928. doi:10.1073/pnas.96.22.12923
2. Haralampidis K, Trojanowska M, Osbourn AE (2002) Biosynthesis of triterpenoid saponins in plants. Adv Biochem Eng Biotechnol 75:31–49. doi:10.1007/3-540-44604-4_2
3. Liu J (1995) Pharmacology of oleanolic acid and ursolic acid. J Ethnopharmacol 49:57–68
4. Liby KT, Sporn MB (2012) Synthetic oleanane triterpenoids: multifunctional drugs with a broad range of applications for prevention and treatment of chronic disease. Pharmacol Rev 64:972–1003. doi:10.1124/pr.111.004846
5. Lin CN, Lu CM, Cheng MK, Gan KH, Won SJ (1990) The cytotoxic principles of *Solanum incanum*. J Nat Prod 53:513–516. doi:10.1021/np50068a041
6. Jung HJ, Lee CO, Lee KT, Choi J, Park HJ (2004) Structure-activity relationship of oleanane disaccharides isolated from *Akebia quinata* versus cytotoxicity against cancer cells and NO inhibition. Bio Pharm Bull 27:744–747. doi:10.1248/bpb.27.744
7. Barthomeuf C, Debiton E, Mshvildadze V, Kemertelidze E, Balansard G (2002) In vitro activity of hederacolchisid A1 compared with other saponins from *Hedera colchica* against proliferation of human carcinoma and melanoma cells. Planta Med 68:672–675. doi:10.1055/s-2002-33807
8. Tani C, Ogihara Y, Mutuga M, Nakamura T, Tekeda T (1996) Studies on the constituents of *Calliandra anomala* (Kunth) Macbr. III. Structure elucidation of six acylated triterpenoidal saponins. Chem Pharm Bull 44:816–822. doi:10.1248/cpb.44.816
9. Abdel-Kader M, Hoch J, Berger JM, Evans R, Miller JS, Wisse JH, Mamber SW, Dalton JM, Kingston DG (2001) Two bioactive saponins from *Albizia subdimidiata* from the Suriname rainforest. J Nat Prod 64:536–539. doi:10.1021/np000295u
10. Seo Y, Hoch J, Abdel-Kader M (2002) Bioactive saponins from *Acacia tenuifolia* from the suriname rainforest. J Nat Prod 65:170–174. doi:10.1021/np0103620
11. Krief S, Thoison O, Sevenet T, Wrangham RW, Lavaud C (2005) Triterpenoid saponin anthranilates from *Albizia grandibracteata* leaves ingested by primates in Uganda. J Nat Prod 68:897–903. doi:10.1021/np049576i
12. Ohtsuki T, Miyagawa T, Koyano T, Kowithayakorn T, Kawahara N, Goda Y, Ishibashi M (2008) Acylated triterpenoid saponins from *Schima noronhae* and their cell growth inhibitory activity. J Nat Prod 71:918–921. doi:10.1021/np800036t
13. Ma SG, Hu YC, Yu SS, Zhang Y, Chen XG, Liu J, Liu YX (2008) Cytotoxic triterpenoid saponins acylated with monoterpenic acid from *Pithecellobium lucidum*. J Nat Prod 71:41–46. doi:10.1021/np0703794
14. Cioffi G, Dal Piaz F (2008) Antiproliferative oleanane saponins from *Meryta denhamii*. J Nat Prod 71:1000–1004. doi:10.1021/np8000464
15. Konoshima T, Yasuda I, Kashiwada Y, Cosentino LM, Lee KH (1995) Anti-AIDS agents, 21. Triterpenoid saponins as anti-HIV principles from fruits of *Gleditsia japonica* and *Gymnocladus chinensis*, and a structure-activity correlation. J Nat Prod 58:1372–1377. doi:10.1021/np50123a006
16. Yu D, Sakurai Y, Chen C, Chang FR, Huang L, Kashiwada Y, Lee KH (2006) Anti-AIDS agents 69. Moronic acid and other triterpene derivatives as novel potent anti-HIV agents. J Med Chem 49:5462–5469. doi:10.1021/jm0601912
17. Dat NT, Bae K, Wamiru A, Mcmahon GB, Grice S, Bona M, Beutler JA, Kim YH (2007) A dimeric lactone from *Ardisia japonica* with inhibitory activity for HIV-1 and HIV-2 ribonuclease H. J Nat Prod 70:839–841. doi:10.1021/np060359m
18. Fu HZ, Li CJ, Yang JZ, Shen ZF, Zhang DM (2011) Potential anti-inflammatory constituents of the stems of *Gordonia chrysandra*. J Nat Prod 74:1066–1072. doi:10.1021/np200021f
19. Pollier J, Goossens A (2012) Oleanolic acid. Phytochemistry 77:10–15. doi:10.1016/j.phytochem.2011.12.022.20
20. Yan MC, Liu Y, Chen H, Ke Y, Xu QC, Cheng MS (2006) Synthesis and antitumor activity of two natural *N*-acetylglucosamine-bearing triterpenoid saponins: lotoidoside D and E. Bioorg Med Chem Lett 16:4200–4204. doi:10.1016/j.bmcl.2006.05.086
21. Abdel-Kader M, Hoch J, Berger JM, Evans R, Miller JS, Wisse JH, Mamber SW, Dalton JM, Kingston JDI (2001) Two bioactive saponins from *Albizia subdimidiata* from the Suriname rainforest. J Nat Prod 64:536–539. doi:10.1021/np000295u
22. Sun J, Han X, Yu B (2003) Synthesis of a typical *N*-acetylglucosamine-containing saponin, oleanolic acid 3-yl α-l-arabinopyranosyl-(1 → 2)-α-l-arabinopyranosyl-(1 → 6)-2-acetamido-2-deoxy-β-d-glucopyranoside. Carbohydr Res 338:827–883. doi:10.1016/S0008-6215(03)00047-8

23. Wang P, Wang J, Guo T, Li Y (2010) Synthesis and cytotoxic activity of the *N*-acetylglucosamine-bearing triterpenoid saponins. Carbohy Res 345:607–620. doi:10.1016/j.carres.2010.01.002
24. Zhang H, Samadi AK, Rao KV, Cohen MS, Timmermann BN (2011) Cytotoxic oleanane-type saponin from *Albizia inundata*. J Nat Prod 74:477–482. doi:10.1021/np100702p
25. Yu B, Sun J (2009) Current synthesis of triterpene saponins. Chem Asian J 4:642–654. doi:10.1002/asia.200800462
26. Wong CH, Ye XS, Zhang Z (1998) Assembly of oligosaccharide libraries with a designed building block and an efficient orthogonal protection-deprotection strategy. J Am Chem Soc 120:7137–7138. doi:10.1021/ja9813616
27. Wunberg T, Kallus C, Opatz T, Henke S, Schmidt W, Kunz H (1998) Carbohydrates as multifunctional chiral scaffolds in combinatorial synthesis. Angew Chem Int Ed 37:2503–2505. doi:10.1016/S0008-6215(02)00301-4
28. Wang YH, Yeh HW, Wang HW, Yu CW, Guh JH, Liou DR, Liang PH (2013) Synthesis of a chlorogenin glycoside library using an orthogonal protecting group strategy. Carbohydr Res 375:118–135. doi:10.1016/j.carres.2013.04.022
29. Mong TK, Lee HK, Duron SG, Wong CH (2003) Reactivity-based one-pot total synthesis of fucose GM1 oligosaccharide: a sialylated antigenic epitope of small-cell lung cancer. Proc Natl Acad Sci USA 100:797–802. doi:10.1073/pnas.0337590100
30. Wuts PGM, Greene TW (2006) Greene's protective groups in organic synthesis. Wiley, New Jersey
31. Yan MC, Chen YN, Wu HT, Lin CC, Chen CT, Lin CC (2007) Removal of acid-labile protecting groups on carbohydrates using water-tolerant and recoverable vanadyl triflate catalyst. J Org Chem 72:299–302. doi:10.1021/jo061881g
32. Sha Y, Yan MC, Liu J, Liu Y, Cheng MS (2008) Facile synthesis of oleanolic acid monoglycosides and diglycosides. Molecules 13:1472–1486. doi:10.3390/molecules13071472
33. Hostettmann KA, Marston A (1995) Saponins. Chemistry and pharmacology of natural products. Cambridge University Press, Cambridge, UK
34. Lindhorst TK (2007) Essential of carbohydrate chemistry and biochemistry, chap 3. Wiley-VCH, Weinheim
35. Excoffier G, Gagnaire D, Utille JP (1975) Coupure sélective par l'hydrazine des groupements acétyles anomères de résidus glycosyles acétylés. Carbohydr Res 39:368–373. doi10.1016/S0008-6215(00)86150-9
36. Ren T, Liu D (1999) Synthesis of targetable cationic amphiphiles. Tetrahedron Lett 40:7621–7625. doi10.1016/S0040-4039(99)01558-0
37. Khan AT, Mondal E (2003) A highly efficient and useful synthetic protocol for the cleavage of *tert*-butyldimethylsilyl (TBS) ethers using a catalytic amount of acetyl chloride in dry methanol. Synlett 5:694–696. doi:10.1055/s-2003-38360