Accepted Manuscript

Design, synthesis and cytotoxic activity of *N*-Modified oleanolic saponins bearing A glucosamine

You-Yu Lin, She-Hung Chan, Yu-Pu Juang, Hsin-Min Hsiao, Jih-Hwa Guh, Pi-Hui Liang

PII: S0223-5234(17)30890-5

DOI: 10.1016/j.ejmech.2017.11.004

Reference: EJMECH 9879

To appear in: *European Journal of Medicinal Chemistry*

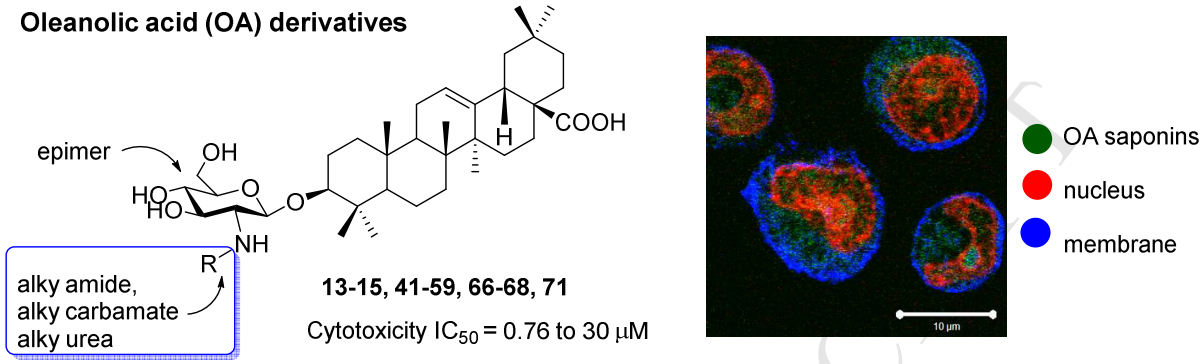
Received Date: 14 August 2017

Revised Date: 1 November 2017

Accepted Date: 2 November 2017

Please cite this article as: Y.-Y. Lin, S.-H. Chan, Y.-P. Juang, H.-M. Hsiao, J.-H. Guh, P.-H. Liang, Design, synthesis and cytotoxic activity of *N*-Modified oleanolic saponins bearing A glucosamine, *European Journal of Medicinal Chemistry* (2017), doi: 10.1016/j.ejmech.2017.11.004.

Graphical Abstract



Design, Synthesis and Cytotoxic Activity of *N*-Modified Oleanolic Saponins Bearing A Glucosamine

You-Yu Lin,[1] She-Hung Chan,[2] Yu-Pu Juang,[1] Hsin-Min Hsiao[1], Jih-Hwa Guh,[1] Pi-Hui Liang[*,1, 3]

Y.-Y. Lin,Y.-P. Juang, H.-M. Hsiao, Prof. Dr. J.H. Guh, Prof. Dr. P.-H. Liang

1. School of Pharmacy, College of Medicine, National Taiwan University, Taipei 100, Taiwan.

E-mail: phliang@ntu.edu.tw

Prof. Dr. S.-H. Chan

2. Department of Cosmetic Science, Providence University, Taichuang 433, Taiwan

Prof. Dr. P.-H. Liang

3. The Genomic Research Center, Academia Sinica, Taipei 128, Taiwan

AUTHOR INFORMATION

Corresponding Author

E-mail: phliang@ntu.edu.tw

Key words: Oleanolic acid; Glycosylation; Cytotoxicity; Glycoside

Abstract

A series of *N*-acyl, *N*-alkoxycarbonyl, and *N*-alkylcarbamoyl derivatives of 2′-deoxy-glucosyl bearing oleanolic saponins were synthesized and evaluated against HL-60, PC-3, and HT29 tumor cancer cells. The SAR studies revealed that the activity increased in order of conjugation of 2′ -amino group with carbamate > amide > urea derivatives. Lengthening the alkyl chain increased the cytotoxicity, the peak activity was found to around heptyl to nonyl substitutions. 2′-*N*-heptoxycarbonyl derivative **56** was found to be the most cytotoxic (IC_{50} = 0.76 μM) againt HL-60 cells. Due to the interesting SARs of alkyl substitutions, we hypothesized that their location in the cell was different, and pursued a location study using 2′-(4″-pentynoylamino) 2′-deoxy-glucosyl OA, which suggested that these compounds distributed mainly in the cytosol.

1. Introduction

Oleanolic acid (3β-hydroxyolean-12-en-28-oic acid, OA, Figure 1), a pentacyclic triterpenoid, can be found in more than 1600 plant species as either a free acid or as a saponin [1]. It is particularly abundant in the Oleaceae family, and named for the olive (*Olea europaea* L.) plant species that still serves as the main source of commercial OA. Due to its natural abundance and ready availability, it is a good natural starting material for synthetic modification and drug discovery[2].

Many biological activities have been reported for OA and its derivatives[3, 4]. The hepatoprotective effect of OA is particularly well known, and OA formulations are widely sold over-the-counter in China for this purpose [5]. Other studies found that OA imparts anti-inflammatory and moderate anti-cancer activity [6-8]. Attempts to improve the anti-cancer activity by the chemical synthesis of several OA derivatives have been described

[9], such as 2-cyano-3,12-dioxooleana-1,9(11)-dien-28-oic acid **2** (CDDO, Figure 1)[6, 10, 11], C ring oxidized OA[12], and 28-urea containing OA [13].

OA is poorly water soluble, which results in low absolute oral bioavailability. Modifications to the hydrophobic scaffold of OA alone failed to significantly improve its solubility, thus limiting opportunities to evaluate its activity [14]. In fact, most naturally occurring OA molecules are glycosylated, bearing a glycan at either C-3 or C-28 via an ether or ester linkage respectively, and are termed OA saponins. OA derivatives bearing an *N*-acetyl glucosamine (such as compound **3**, Figure 1) moiety have attracted a great deal of attention due to their remarkable cytotoxicity; however, few OA saponins containing *N*-acetyl-glucosamine moieties have been found in nature [15-18]. Albiziatrioside A (**4**, Figure 1**)**, is one example of an *N*-acetyl glucosamine bearing natural product; it was isolated from *Acacia tenuifolia*, and exhibited potent cytotoxic activity (IC_{50} = 0.9 μg/mL against A2780 ovarian cancer cells).[16, 17] Lotoidoside D (**5**) (Figure 1) was found to have anti-proliferative activity against the Hela cell line with IC_{50} value of 2.7 μM [18].

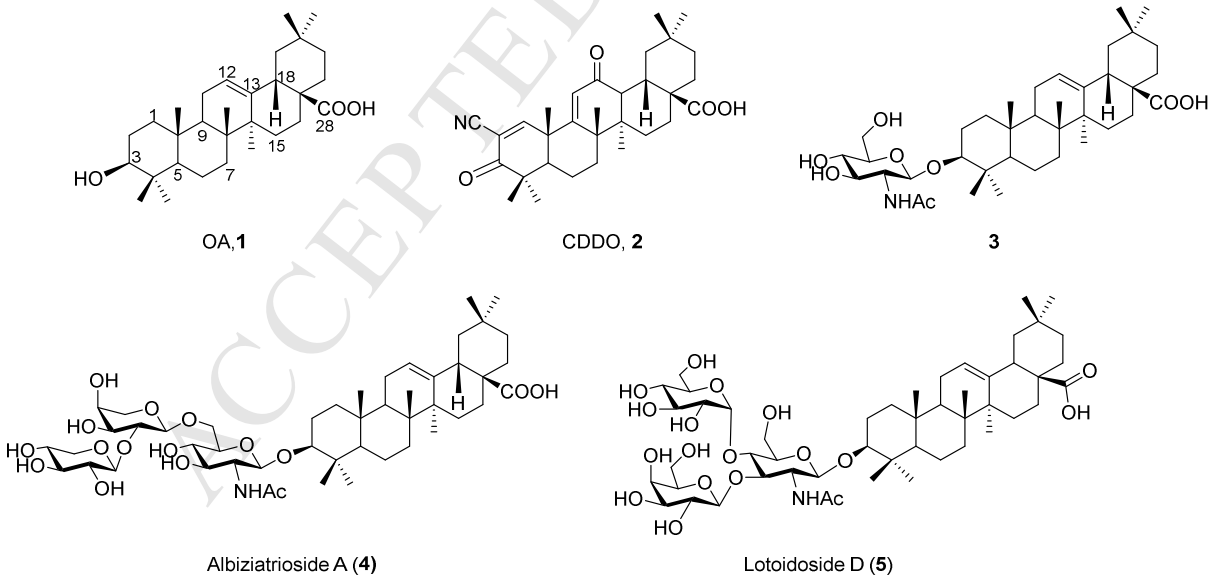


Figure 1. OA and OA saponin derivatives

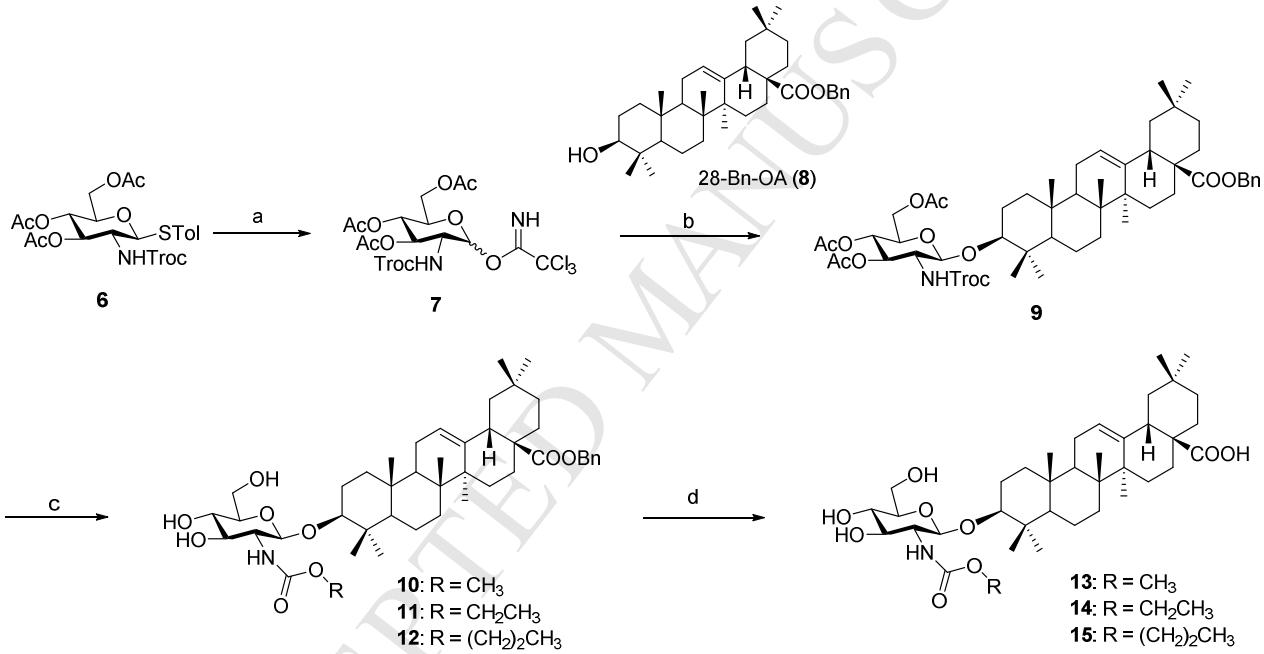
It was noticed that glycans attached with specific linkages of triterpenoid might have

important roles in boosting the cytotoxic activity [14, 18-20]. Synthesis of non-natural saponins bearing different monosaccharide substitutions is one way to probe the glycan linkages for their biological function and SARs [19], Li′s group attached additional *N*-acetyl glucosamine moieties to compound **3**, to give a series of mono- to tetra-(*N*-acetylglucosamine) OA saponins [21]; however, neither of them exhibited superior cytotoxicity against HL-60 cell lines than **3**. In previous work, we developed a concise method for synthesizing OA saponins with (1→3)-linked, (1→4)-linked, (1→6)-linked *N*-acetylglucosamine oligosaccharide residues, and found those bearing (D/L)-xylose and L-arabinose at positions 3′ and 4′ were more cytotoxic to HL-60 and HT-29 cell lines than those bearing other sugars; unfortunately, the magnitude of this improvement was not great, compared to compound **3** [20]. Accordingly, in order to clarify and investigate the SARs of the *N*-acetyl glucosamine moiety, we set out to synthesize a series of *N*-modified derivatives of glucosamine-bearing OA saponins containing *N*-acyl, *N*-alkoxycarbonyl, *N*-alkylcarbamoyl groups. *N*-modified derivatives of galactosamine-bearing OA saponins were also synthesized, to investigate the effect of the epimer of the glucose moiety on cytotoxic activity. We also sought to vary the length of the carbon chain on these derivatives, a common strategy to improve compound activity since a chain length within a particular range may permit penetration into the cell membranes, and interaction with the target organelle. However, compounds with excessively long carbon chains may exhibit poor solubility, and be prevented from interacting with the target receptor due to micelle formation [22]. OA saponins bearing a variety of different length of *N*-alkyl glucosamine moieties were found to have superior cytotoxicity against HL-60 than **3**. The dependence of alkyl chain substitutions on cytotoxicity prompted us to visualize their location using 2′-(4″-pentynoylamino) 2′-deoxy-glucosyl OA as a model compound.

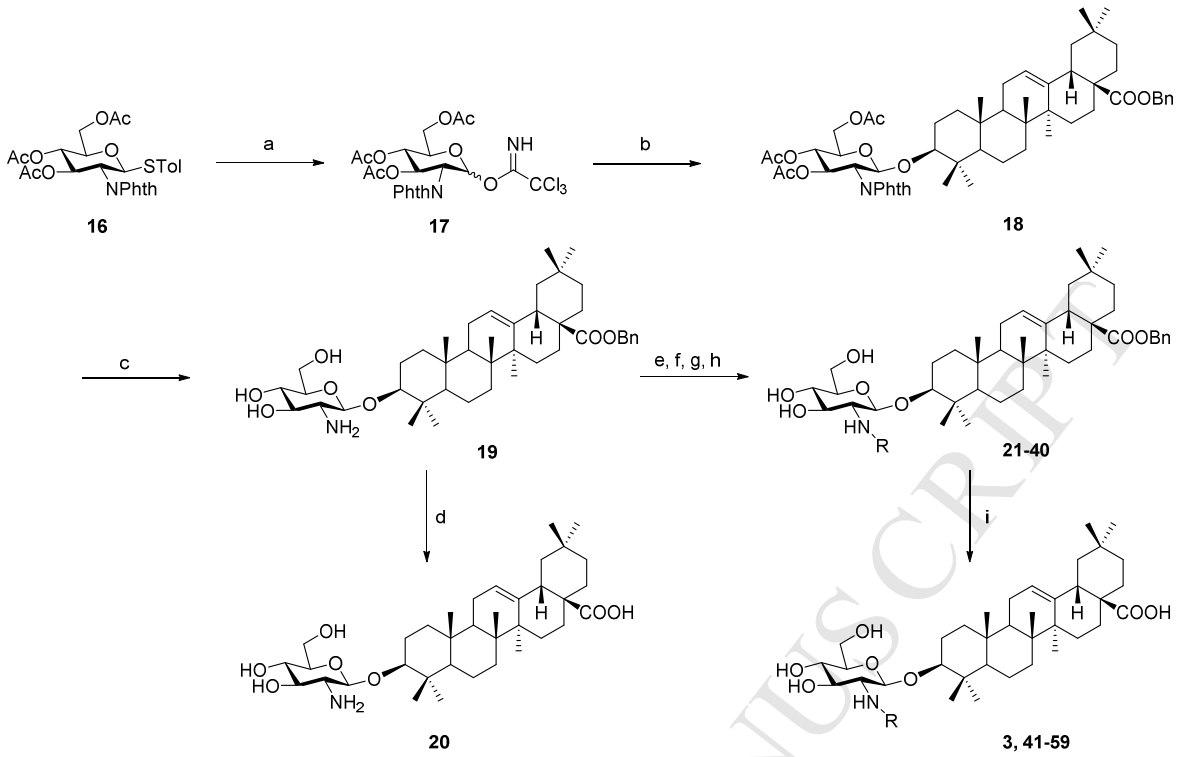
2. Results and discussion

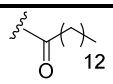
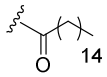
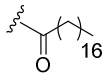
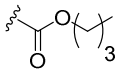
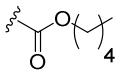
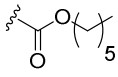
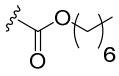
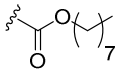
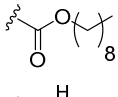
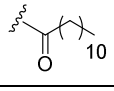
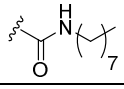
2.1. *Chemistry*

Our synthesis of compounds **13**–**15** is depicted in Scheme 1. With compound **6** in hand, removal of the 1-thiotoluene group using *N*-bromosuccinimide (NBS) followed by trichloroimidate formation using CCl_3CN and DBU gave trichloroacetimidate glycosyl donor **7**. Glycosylation of donor **7** with 28-Bn-OA (**8**)[20] in the presence of TMSOTf afforded compound **9**, which underwent hydrolysis in 4.0 N NaOH and a variety of different alcohols to give the carbamate derivatives **10**–**12**. Deprotection of the benzyl group with hydrogen in the presence of 10% Pd/C afforded the desired products **13**–**15**.



Scheme 1. Synthesis of saponins. (a) i. NBS, acetone/H_2O, -20 □, 54%; ii. CCl_3CN, DBU, CH_2Cl_2, r.t.; (b) 28-Bn-OA (**8**), TMSOTf, 4 Å MS, CH_2Cl_2, 0 □, 74% for 3 steps; (c) CH_2Cl_2, **10**: MeOH, **11**: EtOH and **12**: *n*-propanol, 4.0 N $NaOH_{(aq)}$, rt; (d) H_2, 10% Pd/C, MeOH, rt. 60% (**13**), 46% (**14**) and 39% (**15**) for two steps.



Group	Compound	R	Group	Compound	R
acyl	**21**, **3**		acyl	**31**, **50**	
acyl	**22**, **41**		acyl	**32**, **51**	
acyl	**23**, **42**		acyl	**33**, **52**	
acyl	**24**, **43**		alkoxycarbonyl	**34**, **53**	
acyl	**25**, **44**		alkoxycarbonyl	**35, 54**	
acyl	**26**, **45**		alkoxycarbonyl	**36, 55**	
acyl	**27**, **46**		alkoxycarbonyl	**37, 56**	
acyl	**28**, **47**		alkoxycarbonyl	**38**, **57**	
acyl	**29**, **48**		alkoxycarbonyl	**39**, **58**	
acyl	**30**, **49**		carbamoyl	**40**, **59**	

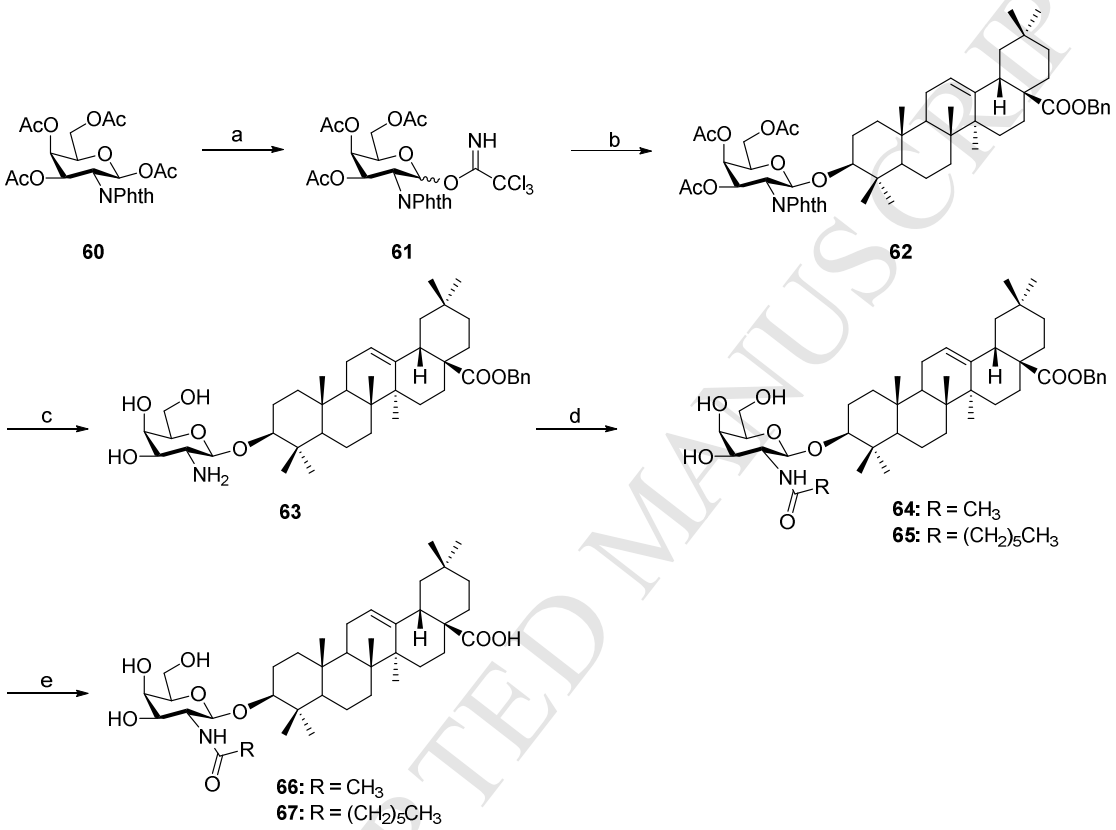
Scheme 2. Synthesis of saponin library. (a) i. NBS, acetone/H_2O, -20 □, 64%; ii. CCl_3CN, DBU, CH_2Cl_2, rt; (b) 28-Bn-OA (**8**), TMSOTf, 4 Å MS, CH_2Cl_2, 0 □, 55% for 3 steps; (c)

ethylenediamine, EtOH , 70–80 □, 72%; (d) H_2, 20% Pd/C, MeOH; (e) **21**–**26**: $(RCO)_2O$, pyridine, CH_2Cl_2, rt; (f) **27**–**33**: RCOOH, EDC, HOBt, DMF, rt; (g) **34**–**39**: CDI, TEA, ROH, rt; (h) **40**: DPPA, *n*-nonanoic acid, TEA, toluene, 80–90 □; (i) H_2, 10% Pd/C, MeOH, rt.

Reductive removal of the *N*-Troc group of compound **9** using $Zn/(RCO)_2O$ allowed the one-pot conversion of the *N*-Troc group into an *N*-acyl group, but generated a mixture of side products, including those arising from trans-esterification. Accordingly, the *N*-Troc group was replaced with an *N*-phthaloyl group (Phth), which could be efficiently removed using ethylenediamine [23]. The thiol group of compound **16** was removed by NBS, and then trichloroimidate formation with CCl_3CN and DBU resulted in glycosyl donor **17,** which was reacted with 28-Bn-OA (**8**) in catalytic TMSOTf to give compound **18** (Scheme 2). All the glycoside protecting groups were removed using ethylenediamine at 70–80 □ to give compound **19**. Hydrogenation of the benzyl ester group of **19** with 20% Pd/C under hydrogen gave compound **20**. The amide derivatives **21–33** were obtained by the reaction of compound **19** with various anhydrides in the presence of pyridine, or with various acids in the presence of coupling reagents (1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDCI), hydroxybenzotriazole (HOBt) and *N*,*N*-diisopropylethylamine (DIPEA)) [24]. The carbamate bearing derivatives were obtained by reacting compound **19** with various alcohols, 1′-carbonyldiimidazole (CDI), and trietheylamine (TEA) in DCM.[25] Ureido derivative **40** was synthesized by a Curtius rearrangement of **19** using diphenylphosphoryl azide (DPPA), n-nonanoic acid and TEA [26]. The final compounds **3** and **41–59** were obtained by hydrogenation using hydrogen and 10% Pd/C in methanol.

The 4-epimer derivatives (**66** and **67**), *N*-acetyl and *N*-heptanoyl galactosamine-bearing OA saponins, were also synthesized (Scheme 3). Hydrolysis of the anomeric acetate group of compound **60** followed by trichloroimidate formation with CCl_3CN and DBU gave glycosyl donor **61**. Glycosylation of donor **61** and 28-Bn-OA (**8**) in the presence of catalytic TMSOTf afforded **62** in a 46% yield over three steps. After treatment with ethylenediamine in EtOH at

70–80 □, compound **63** was obtained, which was subjected to amide formation using the corresponding anhydrides to afford compounds **64**-**65**. Hydrogenation of **64** and **65** in the presence of hydrogen and 10% Pd/C in methanol afforded **66** and **67**, respectively. Overall, twenty-five novel oleanolic saponins bearing *N*-acyl, *N*-alkoxycarbonyl, and *N*-alkylcarbamoyl modification on 2′-deoxy-glucosyl were synthesized



Scheme 3. Synthesis of the saponin library. (a) i. ethylenediamine, acetic acid, THF, 0 □, 61%; ii. CCl_3CN, DBU, CH_2Cl_2, rt; (b) 28-Bn-OA, TMSOTf, 4 Å MS, CH_2Cl_2, 0 □, 46% for 2 steps; (c) ethylenediamine, EtOH, 70–80 □, 85%; (d) i. $(RCO)_2O$, imidazole, CH_2Cl_2, rt; ii. NaOMe, MeOH; (e) H_2, 10% Pd/C, MeOH, rt.

2.2 Cytotoxic activity

The cytotoxic activities of the oleanolic acid saponins **3**, **13**–**15**, **41**–**59**, and **66**–**67** were evaluated against four human tumor cell lines (promyelocytic leukemia cells (HL-60), prostate cancer cells (PC-3), colon adenocarcinoma (HT29)) using the methyl-thiazol-tetrazolium (MTT) reduction test (Table 1) [27].

Amongst the three cancer cell lines tested, these compounds were more cytotoxic to HL-60 than the other two. Some of the compounds bearing an amide moiety displayed significant cytotoxicity against the HL-60 cell line at concentrations below 10 μM, such as compound **46** (IC_{50} 1.68 μM), **47** (IC_{50} 1.46 μM), **48** (IC_{50} 1.40 μM), and **49** (IC_{50} 1.57 μM). Likewise, some carbamate derivatives also exhibited significant cytotoxicity at concentrations of less than 10 μM, such as compound **53** (IC_{50} 2.23 μM), **54** (IC_{50} 1.46 μM), **55** (IC_{50} 1.44 μM), **56** (IC_{50} 0.76 μM), and **58** (IC_{50} 1.23 μM). Derivatives bearing longer carbon chains were more cytotoxic than compound **3** up until the chain length reached eleven carbons, after which cytotoxicity significantly decreased; for example, compound **51** (2′-*N*-hexadecanoyl moiety, IC_{50} >30 μM), and **52** (2′-*N*-octadecanoyl moiety, IC_{50} >30 μM). For the carbamate derivatives, the trend in cytotoxicity was similar to that of the amide derivatives; overall, all were more potent inhibitors. Of the derivatives bearing carbon chains of the same length, the ureido derivative **59** had approximately the same activity (IC_{50} 2.14 μM) as the amide (**48**, IC_{50} 1.40 μM) and carbamate (**57**, IC_{50} 1.42 μM). Comparing galactosamine compounds **66** (IC_{50} 11.6 μM) and **67** (IC_{50} 2.69 μM) with glucosamine compounds **3** (IC_{50} 10.3 μM) and **45** (IC_{50} 2.85 μM) showed that the activities were not significantly different, suggesting the C4-epimer of glucosamine moiety to be well tolerated.

Against prostate cancer cells PC-3, derivatives were found to less active to this cell lines, only those bearing longer carbon chains were found to be more active; for example, in amide derivatives **41**, **46-47** (IC_{50} 11.9–19.4 μM), and carbamate derivatives **53**, **55-58** (IC_{50} 13.9–16.1 μM). Against colon adenocarcinoma HT29, increasing the carbon length increased the potency, such as amide derivatives **41**, **43**, **46-47** (IC_{50} 16.4–22.8 μM) and carbamate derivatives **53**, **55-58** (IC_{50} 18.1–22.0 μM).

Table 1. *In vitro* cytotoxicity of OA and a series of newly synthesized compounds

Compound	$IC_{50}(\mu M)$[a]		
	HL-60	PC-3	HT29
OA	>30	>30	>30
3	10.3 ± 0.26	>30	>30
13	11.4 ± 0.04	>30	>30
14	12.5 ± 0.33	>30	>30
15	21.5 ± 0.06	>30	>30
20	2.65 ± 0.01	25.0 ± 1.59	>30
41	6.83 ± 0.02	11.9 ± 0.21	16.4 ± 8.51
42	2.48 ± 0.01	>30	>30
43	10.6 ± 0.07	24.0 ± 0.07	21.7 ± 1.22
44	5.82 ± 0.20	>30	>30
45	2.85 ± 0.01	>30	>30
46	1.68 ± 0.00	19.4 ± 2.22	21.9±0.76
47	1.46 ± 0.01	14.7 ± 1.84	22.8 ± 1.82
48	1.40 ± 0.01	>30	>30
49	1.57 ± 0.00	21.4 ± 0.67	>30
50	10.7 ± 0.01	>30	>30
51	>30	>30	>30
52	>30	>30	>30
53	2.23 ± 0.06	15.0 ± 1.58	18.1 ± 5.12
54	1.46 ± 0.01	>30	>30
55	1.44 ± 0.05	13.9 ± 1.15	21.4 ± 2.63
56	0.76 ± 0.01	15.4 ± 4.65	21.7 ± 6.09
57	1.42 ± 0.00	15.4 ± 3.20	20.3 ± 5.09
58	1.23 ± 0.06	16.1 ± 3.21	22.0 ± 1.82
59	2.14 ± 0.03	>30	>30
66	11.6 ± 0.74	23.4 ± 0.57	23.3 ± 2.57
67	2.69 ± 0.03	>30	>30
68	10.6 ± 0.06	>30	>30
71	9.13 ± 0.04	22.4 ± 0.55	23.1 ± 1.30
Vincristine	1.8 ± 0.19[b]	>25[b]	-
5-FU	> 30	>30	8.3 ± 0.65

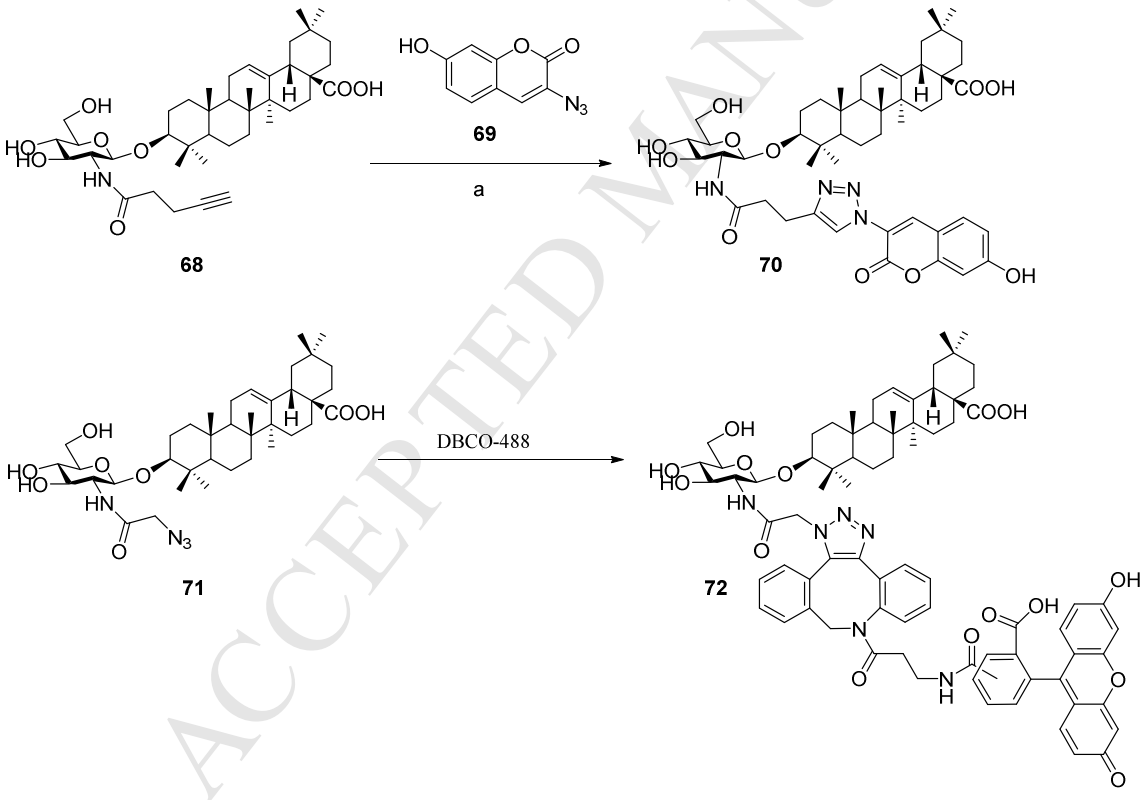
[a]IC_{50} values from MTT assays after 48 h of treatment. The value are mean ± SD of at least three independent experiments. [b]Unit is nM

2.3 *Determination of the location of the OA derivatives in the cell by fluorescent labeling*

Our study showed that the cytotoxicity of the glucosamine-bearing OA saponins tested depended in part on the length of the carbon chain at the glucosamine nitrogen. However, compound bearing a simple long-chain carbamate conjugation with the 3-hydroxyl group of OA was not active (data not shown), indicating the glucosamine moiety to be essential. One possible explanation for this is that the distribution of small molecules bearing carbon chains in cancer cells depends on the length of the carbon chain. For example, 3,6-bis(1-methyl-4-vinylpyridinium) carbazole diiodide (BMVC) derivatives bearing a linker chain longer than nine carbons were found to accumulate in mitochondria, while those bearing linker chain fewer than nine carbons long remained in the nucleus [28]. Apparently, the distribution of our OA derivatives is affected by the length of the carbon chains, but as none of them incorporate a fluorophore, quantification of their uptake by individual organelles is not possible. Further study of this effect was undertaken using compound **68** (synthesis of **68** is in the Supporting Information), the alkyne moiety of which was expected to be amenable to conjugation with a fluorogenic probe (**69)** using click chemistry to give the highly fluorescent triazole-compound **70** (Scheme 4) [29].

A cytotoxic assay of **68** against HL-60 determined it to have an IC_{50} of 10.6 μM, suggesting its activity to be similar to that of compound **43** (IC_{50} = 10.6 μM). HL-60 cells were treated with or without compound **68**, incubated for 1 day, fixed by alcohol, permeabilized, and mixed with fluorogenic probe (**69**) and the click reagents ($CuSO_4$, TBTA and Na ascorbate, Scheme 4). Fluorescence images were detected using a confocal microscope (Figure 2). Another way to introduce a fluorophore was conducted by using azido-bearing OA **71** which can be labelled with DBCO-488 in a copper-free procedure (Scheme 4, synthesis of **71** is in the Supporting Information) to treat with cells. For comparison, samples were also stained with CellBrite™ Blue (cell membrane marker) and RedDot™2 (nucleus marker). As shown in Figure 2, the control group of cells (no OA

compound treatment) did not fluoresce. The fluorescent signals corresponding to **70** were found evenly distributed in the HL-60 cells (green channel), while fluorescent signals of compound **72** were found mainly in the cytosol of the HL-60 cells. These results implied that alkynyl **68** and azido **71** were not solely located in the cell membrane, albeit they had subtle difference in the cell distributions. Since some of nature saponins were previously reported to cause hemolysis by interacting with sterols of erythrocyte membrane [30, 31], this effect caused concerns in the further development of saponins as pharmacological agents. Distribution of OA compounds in the intact cells suggested that their cytotoxic effects were from the interactions of compounds with the mitochondrion or other organelles, but not cell membrane.



Scheme 4. Synthesis of fluorogenic probe (a) TBTA, $CuSO_4$, Na ascorbate, rt, 6 h.

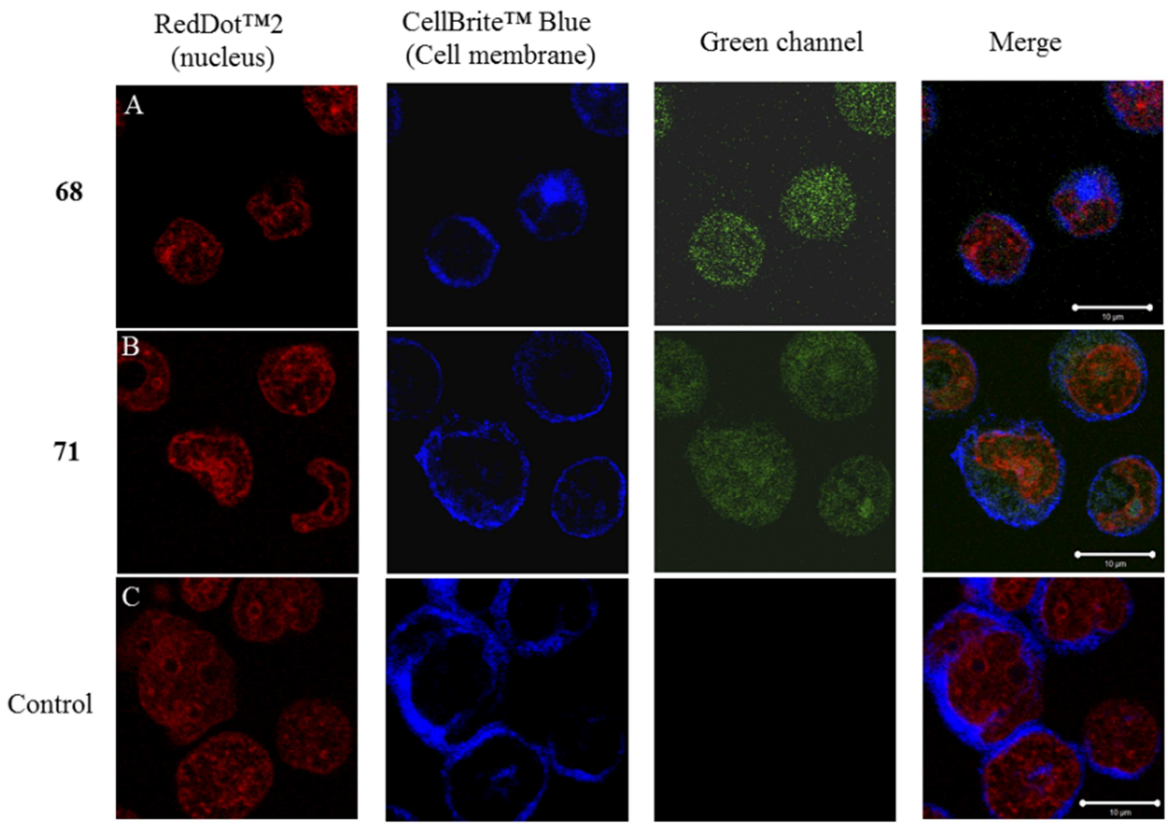


Figure 2. Click reaction to locate cellular uptake of (A) with compound **68** in the presence of fluorogenic probe **69**, TBTA and Cu^{+}, (B) Compound **71** in the presence of fluorogenic probe, DBCO-488. The confocal images of HL-60 cell fluorescence Green: [3 + 2] cycloaddition of the compounds **68** and **71**; Blue: CellBrite™ Blue (Cell membrane); Red: RedDot™2 (nucleus).

2.4 Effect on mitochondria

Since mitochondria are involved in wide variety of cellular responses and functions, including energy production, they are implicated in cell growth, survival, differentiation and apoptosis [32, 33]. Recently, saponins such as reevesioside F [34], triphenylphosphonium cation-containing betulin, and betulinic acid derivatives [35] were reported to cause the death of cancer cells by mitochondria-stress. As we have observed the apoptosis of these OA compounds to HL-60 cells, we then examined their effect on mitochondria using compound **45** as a represented compound. After compound **45** treatment, HL-60 cells were stained by

JC-1 to determine the $\Delta\Psi_m$ (mitochondria membrane potential) of the mitochondrial membrane, and analyzed by flow cytometry. As shown in Figure 3, compound **45** induced mitochondrial membrane depolarization with significant increase of JC-1 monomer (green color) in a dose dependent manner, indicating that compound **45** caused mitochondria damage.

The above mentioned locations of compounds and mitochondrial membrane stability study suggested that glucosamine bearing OA compounds with appropriate proper length of carbon chain conjugation might target mitochondria and cause cancer cell apoptosis. Further mechanistic studies of these OA compounds are ongoing in this laboratories, and results will be reported in due course.

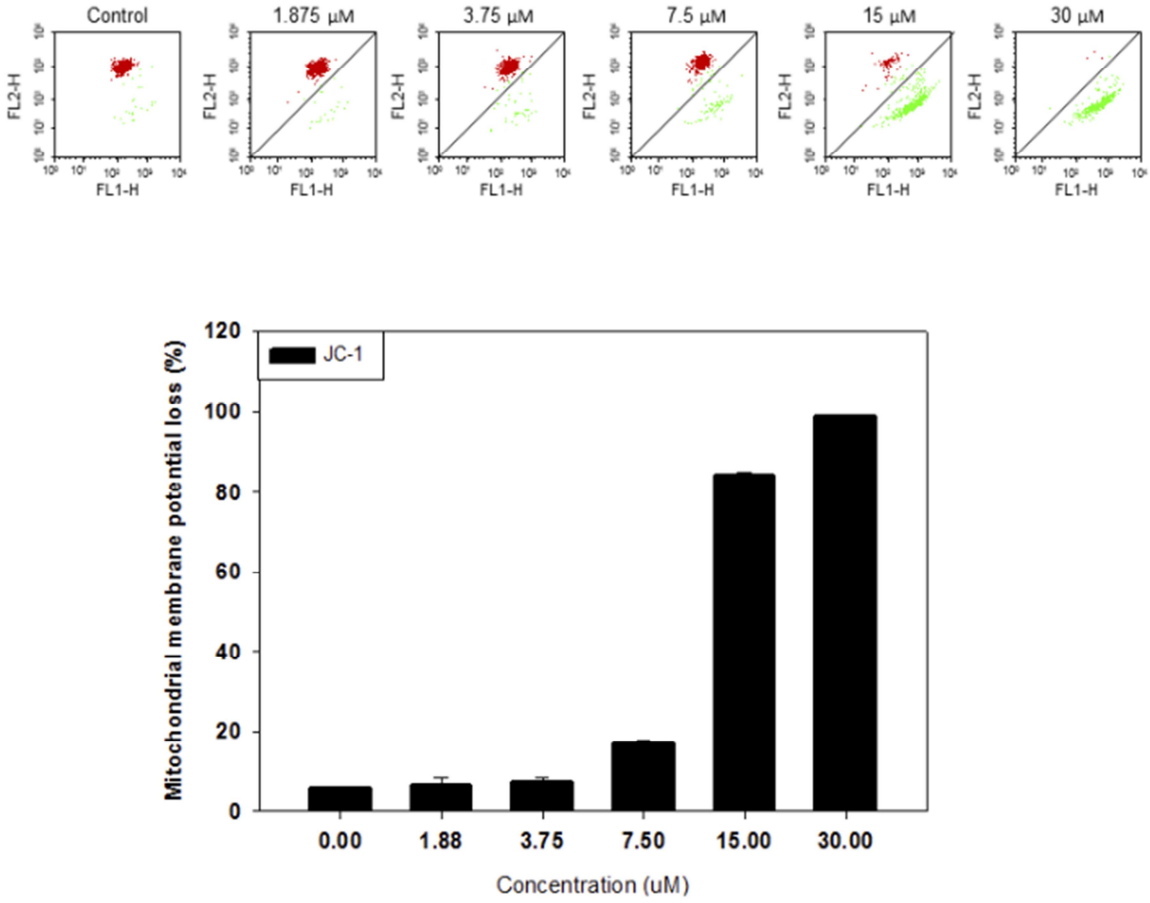


Figure 3. Effect of compound on $\Delta\Psi_m$ and related protein expression. HL-60 cells were incubated in the absence or dose-dependent concentration of compound **45** for the indicated

times. Cells were incubated with JC-1 for the detection of $\Delta\Psi m$ using FACScan flow cytometric analysis and mitochondria membrane potential loss (%).

3. Conclusion

In summary, a practical, concise and versatile synthesis of *N*-modified derivatives of 2′-amino-2′-deoxysugar-bearing oleanolic saponins has been developed and used to synthesize twenty-five novel derivatives (**13-15, 20, 41-59, 66-67**), the cytotoxicity of which was evaluated in an MTT assay. Of the twenty-five novel compounds synthesized, certain amide and carbamate-based derivatives (**41-42**, **44-49** and **53-59**) exhibited more potent growth inhibition than compound **3** (2′-*N*-acetyl-2′-deoxy-glucosyl OA), the first time the cytotoxicity of **3** has been surpassed.

Amide, carbamate, and urea derivatives of the same carbon chain length exhibited near-identical cytotoxicity against HL-60 cells. Increasing the carbon chain length increased the cytotoxicity of the derivative up until the chain reached eleven carbons in length for amide derivatives; and nine carbons in length for the carbamate derivatives. The different activities of these compounds may reflect their different lipophilicities, which may affect their uptake by cancer cells. Carbamate derivative **56** (2′-*N*-heptoxycarbonyl moiety) was found to be the most cytotoxic (IC_{50} = 0.76 μM). The cytotoxicities of compounds **66** and **67** bearing a galactosamine moiety were almost equal to that of gluocosamine compounds with the same carbon length modification, suggesting that the configuration of the C-4 in the sugar moiety does not significantly influence cytotoxicity. These SAR relationships should help inform the future development of anti-cancer drugs.

Preliminary studies into the mechanism of cytotoxicity imparted by the synthesized compounds were also undertaken. Images obtained in a cellular uptake assay clearly show that compounds **68** and **71** enter the cells and locate mainly the cytosol. Direct location measurement of OA derivatives using fluorogenic probe allowed us to detect the distribution

of compounds in the intact cells. A mitochondrial membrane potential assay found that compound **45** induced mitochondria damage and this result coincided on compound distribution on cytosol from cell image. These findings suggest that the apoptosis caused by the compounds synthesized proceeds via perturbation of the mitochondria; more detailed mechanistic studies, antiproliferative and apoptotic pathway analyses are ongoing.

4. Experimental

4.1 Chemistry part

All reagents and solvents were of reagent grade and used without further purification, unless otherwise stated. Reaction progress was monitored by analytical TLC on 0.25 mm E. Merck silica gel 60 F_{254}, stained with *p*-anisaldehyde, ninhydrin, and cerium ammonium molybdate, and visualized by heating. ^{1}H and ^{13}C NMR spectra were recorded on either a Bruker AV-400 or AVIII-600-Cry spectrometer. Chemical shifts are referenced to residual solvent peaks ($CDCl_3$: ^{1}H δ = 7.24, ^{13}C δ = 77.0; CD_3OD: ^{1}H δ = 3.30, ^{13}C δ = 49.0). Splitting patterns are reported as s (singlet), d (doublet), t (triplet), q (quartet), dd (double of doublets), m (multiplet). Coupling constants (*J*) are given in Hertz. The purities of all of the biologically tested compounds were confirmed to be higher than 95% using an analytical HPLC with a dual pump Shimadzu LC-20AT system. Mass spectra were recorded on a Bruker bioTOF III. Melting points were measured on a FARGO melting point apparatus MP-1D (Mandarin Scientific Co., Ltd.).

4.1.1. *p*-Methylphenyl 3,4,6-tri-*O*-acetyl-2-deoxy-1-thio-2-(2′,2′,2′-trichloroethyloxycarbonyl amino)-*β*-D-glucopyranoside (**6**)

Trichloroethoxycarbonyl chloride (38.4 mL, 279.0 mmol) was added dropwise to a solution of D-glucosamine hydrochloride (50 g, 231.9 mmol) and $NaHCO_3$ (58.6 g, 698 mmol) in water (500 mL), at 0 □, with stirring. The mixture was stirred at room temperature for 4 h, then filtered and washed with $CHCl_3$ to give a white solid (82.3 g, 232.1 mmol), which was

dissolved in pyridine (400 mL). The solution was cooled to 0 □; then, DMAP and Ac_2O were added, with stirring. The cooling bath was removed, and the mixture stirred for 5 h. The mixture was concentrated *in vacuo*; dissolved in CH_2Cl_2, and washed with 1.0 N HCl, $NaHCO_3$ solution, and brine. The organic layer was collected, dried with Na_2SO_4, filtered, and concentrated *in vacuo* to give a white syrup (120 g). The white syrup (120 g, 230 mmol) and *p*-toluenethiol (57 g, 459 mmol) were dissolved in dry CH_2Cl_2 (800 mL), and cooled to 0 ^oC. BF_3□OEt_2 (58.4 mL, 461 mmol) was added dropwise, the ice bath removed, and the mixture stirred for 8h. The mixture was washed with $NaHCO_3$ solution and brine. The organic layer was separated, dried with Na_2SO_4 and concentrated *in vacuo*. The residue was recrystallized from methanol to afford **7** (80 g, 59% over 3 steps) as a white solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 (d, *J* = 8.0 Hz, 2H, Ar-H), 7.09 (d, *J* = 8.0 Hz, 2H, Ar-H), 5.31 (d, *J* = 9.1 Hz, 1H, H-1), 5.25 (t, *J* = 9.8 Hz, 1H, H-3), 4.98 (t, *J* = 9.8 Hz, 1H, H-4), 4.80–4.74 (m, 2H, Troc-CH_2, H-2), 4.70 (d, *J* = 12.0, 1H, Troc-CH_2), 4.20 (dd, *J* = 12.2, 5.1 Hz, 1H, H-6a), 4.14 (dd, *J* = 12.2, 2.2 Hz, 1H, H-6b), 3.72–3.57 (m, 2H, H-5, N-H), 2.32 (s, 3H, STol-CH_3), 2.06 (s, 3H, Ac-CH_3), 1.97 ppm (s, 6H, Ac-CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.6, 169.4, 153.8, 138.7, 133.6, 129.7, 95.4 (Troc-CCl_3), 86.7 (C-1), 75.7 (C-5), 74.5 (C-3), 73.2 (Troc-CH_2), 68.5 (C-4), 62.3 (C-6), 55.0 (C-2), 21.2 (STol-CH_3), 20.7 (Ac-CH_3), 20.6 (Ac-CH_3), 20.6 ppm (Ac-CH_3); HRMS (ESI) calcd. for $C_{22}H_{26}Cl_3NO_9S$+Na $[M+Na]^+$: 608.0286, found: 608.0300.

4.1.2.

28-*O*-benzyl-3-*O*-[3′,4′,6′-tri-*O*-acetyl-2′-deoxy-2′-(2″,2″,2″-trichloroethyloxycarbonylamino)-*β*-D-glucopyranosyl]oleanolic ester (**9**)

Compound **6** (7.63 g, 13 mmol) was dissolved in a mixture of acetone/H_2O = 10/1 (165 mL) and cooled to -20 □. NBS (11.57 g, 65 mmol) was added slowly and the mixture stirred at -20 □ for 2 h. The cooling bath was removed and the mixture diluted with $Na_2S_2O_3$ solution

and $NaHCO_3$ solution and the acetone removed *in vacuo*. The residue was diluted with CH_2Cl_2, washed with water, and concentrated *in vacuo* to give an off-white solid, which was purified by flash column chromatography to give a white solid (3.4 g, 7.1 mmol, 54 %). DBU (31.08 μL, 0.208 mmol) was added to a solution of this white solid (500 mg, 1.04 mmol) and trichloroacetonitrile (312.9 μL, 3.12 mmol) in CH_2Cl_2 (5 mL). The mixture was stirred at room temperature for 2 h, then concentrated *in vacuo*, and the residue purified by flash column chromatography (silica gel; EtOAc/hexane/TEA = 1:1:0.1) to give trichloroimidate intermediate **7**. A mixture of trichloroimidate intermediate **7** (650 mg, 1.04 mmol), benzyl oleanolate (682 mg, 1.25 mmol), and 4 Å MS in dry CH_2Cl_2 (13 mL) was stirred at 0 □ for 30 minutes. TMSOTf (9.4 μL, 0.052 mmol) was added and the mixture was stirred for another 30 minutes at 0 □. The reaction was quenched by the addition of TEA (1 drop); then the mixture was filtered, concentrated *in vacuo*, and purified by flash column chromatography (silica gel; EtOAc/hexane/toluene = 1/4/2) to give compound **9**, as a white solid (780 mg, 74 %); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33–7.27 (m, 5H, Ar-H), 5.29–5.17 (m, 3H, H-1, H-3′, H-12), 5.09–4.96 (m, 3H, H-4′, $COCH_2Ph$), 4.70 (d, *J* = 12.0 Hz, 1H, Troc-CH_2), 4.64–4.58 (m, 2H, H-2′, Troc-CH_2), 4.23 (dd, *J* = 12.0, 5.4 Hz, 1H, H-6a′), 4.08 (d, *J* = 12.0 Hz, 1H, H-6b′), 3.70–3.60 (m, 2H, H-5′, N-H), 3.08 (dd, *J* = 11.4, 4.3 Hz, 1H, H-3), 2.87 (d, *J* = 10.1 Hz, 1H, H-18), 2.05 (s, 3H, Ac-CH_3), 2.00 (s, 6H, Ac-CH_3), 1.96 – 1.90 (m, 1H), 1.84–1.77 (m, 2H), 1.68 – 1.60 (m ,5H), 1.58–1.54 (m, 2H), 1.52 – 1.49 (m, 1H), 1.48–1.42 (m, 2H), 1.39–1.33 (m, 2H), 1.32–1.26 (m, 2H), 1.25–1.20 (m, 2H), 1.19–1.14 (m, 2H),1.08 (s, 3H), 1.0 –0.97 (m,1H), 0.89 (s, 6H), 0.87 (s, 3H), 0.85 (s, 3H), 0.74 (s, 3H), 0.56 ppm (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.42 ($\underline{C}O_2CH_2Ph$), 170.7 (Ac-C=O), 169.5 (Ac-C=O), 153.9 (Troc-C=O), 143.7 (C-13), 136.4 (Ar-C), 128.4 (Ar-C), 128.0 (Ar-C), 127.9 (Ar-C), 122.4 (C-12), 103.1 (C-1′), 95.2 (Troc-CCl_3), 90.8 (C-3), 74.7 (C-5′), 71.9 (C-3′), 71.5 (Troc-CH_2), 68.9 (C-4′), 65.9 (CO_2CH_2Ph), 62.4 (C-6′), 56.8 (C-2′), 55.5, 47.6, 46.7, 45.9, 41.6, 41.3, 39.3, 38.9, 38.4, 36.7, 33.8, 33.1, 32.7, 32.3, 30.7, 28.0, 27.6, 26.3, 25.8, 25.7, 23.6, 23.4, 23.0, 20.8,

20.6, 18.2, 16.83, 16.42, 15.2 ppm; HRMS (ESI) calcd. for $C_{52}H_{72}Cl_3NO_{12}$+Na $[M+Na]^+$: 1030.4012, found: 1030.4051.

4.1.3. General procedure A for the synthesis of carbamates **13**–**15**

Compound **9** was dissolved in an alcohol (3 mL, MeOH for **10** or EtOH for **11** or *n*-propanol/ CH_2Cl_2 = 1/2 for **12**) and the solution diluted with 4.0 N NaOH (0.5 mL) and stirred for 5 h. The mixture was concentrated *in vacuo*, dissolved in CH_2Cl_2, washed with NH_4Cl solution and brine, and purified by flash column chromatography (silica gel; CH_2Cl_2/MeOH = 20:1) to give deacylated product **10**–**12**. This was dissolved in MeOH and Pd/C (10%) was added. The mixture was stirred under $H_{2(g)}$ at room temperature for 8h. The residue was filtered, concentrated *in vacuo*, and purified by flash column chromatography (silica gel; CH_2Cl_2/MeOH = 14/1) to give final products **13**–**15**.

4.1.4. 3-*O*-(2′-*N*-methoxycarbonyl-2′-deoxy-*β*-D-glucopyranosyl)oleanolic acid (**13**)

According to the general procedure A, compound **13** (20 mg, 60%) was obtained from compound **9** (50 mg, 49.5 μmol) as a white solid; m.p. 250 □; ^{1}H NMR (600 MHz, $CD_3OD/CDCl_3$ = 4/1) δ 5.23 (t, *J* = 3.5 Hz, 1H, H-12), 4.41 (d, *J* = 7.9 Hz, 1H, H-1′), 3.82 (dd, *J* = 11.9, 2.7 Hz, 1H, H-6a′), 3.71 (dd, *J* = 11.9, 5.0 Hz, 1H, H-6b′), 3.61 (s, 3H, CO_2CH_3), 3.46 –3.33 (m, 3H, H-2′, H-3′, H-4′), 3.23 (ddd, *J* = 9.3, 5.0, 2.7 Hz, 1H, H-5′), 3.09 (dd, *J* = 11.7, 4.4 Hz, 1H, H-3), 2.81 (dd, *J* = 13.9, 3.9 Hz, 1H, H-18), 1.99–1.92 (m, 1H), 1.87–1.82 (m, 3H), 1.75–1.62 (m, 4H), 1.60–1.55 (m, 2H), 1.54–1.48 (m, 3H), 1.46–1.40 (m, 1H), 1.38–1.31 (m, 2H), 1.29–1.23 (m, 3H), 1.20–1.15 (m, 1H), 1.14–1.12 (m, 1H), 1.12 (s, 3H), 1.07–1.02 (m, 1H), 0.92 (s, 3H), 0.91 (s, 3H), 0.89 (s, 3H), 0.88 (s, 3H), 0.77 (s, 3H), 0.74 ppm (s, 3H); ^{13}C NMR (150 MHz, CD_3OD /$CDCl_3$ = 4/1) δ 181.5 (COOH), 158.8 (NC=O), 144.5 (C-13), 123.0 (C-12), 104.6 (C-1′), 90.9 (C-3), 76.5 (C-5′), 74.9 (C-3′), 71.5 (C-4′), 62.4 (C-6′), 58.8 (C-2′), 52.3 ($CO_2\underline{C}H_3$), 48.3, 47.1, 46.6, 42.3, 42.0, 39.9, 39.4, 39.1, 37.3, 34.5,

33.4, 33.2, 31.2, 30.2, 28.3, 28.1, 26.33, 26.28, 24.0, 23.9, 23.6, 23.5, 18.8, 17.3, 16.7, 15.7 ppm; HRMS (ESI) calcd. for $C_{38}H_{61}NO_9$+Na $[M+Na]^+$: 698.4239, found: 698.4253; HPLC purity 95.7% (t_R: 10.5 min, Mightysil, RP-18, 250 × 4.6 mm, 5 μm, H_2O/ACN = 40/60, 1 mL/min, 30 min).

4.1.5. 3-*O*-(2′-*N*-ethoxycarbonyl-2′-deoxy-*β*-D-glucopyranosyl)oleanolic acid (**14**)

According to general procedure A, compound **14** (15 mg, 46%) was obtained from compound **9** (48 mg, 47.5 μmol) as a white solid; m.p. 245 □; ^{1}H NMR (600 MHz, CD_3OD /$CDCl_3$ = 4/1) δ 5.23 (t, *J* = 3.5 Hz, 1H, H-12), 4.40 (d, *J* = 8.1 Hz, 1H, H-1′), 4.11–4.00 (m, 2H, H-1″), 3.83 (dd, *J* = 11.9, 2.5 Hz, 1H, H-6a′), 3.69 (dd, *J* = 11.9, 5.2 Hz, 1H, H-6b′), 3.44–3.39 (m, 1H, H-3′), 3.38–3.33 (m, 2H, H-2′, H-4′), 3.23 (ddd, *J* = 9.3, 5.2, 2.5 Hz, 1H, H-5′), 3.10 (dd, *J* = 11.7, 4.3 Hz, 1H, H-3), 2.82 (dd, *J* = 13.8, 4.0 Hz, 1H, H-18), 2.00–1.93 (m, 1H), 1.92–1.85 (m, 3H), 1.77–1.69 (m, 2H), 1.68–1.61 (m, 2H), 1.61–1.55 (m, 2H), 1.55–1.49 (m, 3H), 1.48–1.43 (m, 1H), 1.41–1.35 (m, 2H), 1.34–1.32 (m, 1H), 1.30–1.27 (m, 3H), 1.21 (t, *J* = 7.1 Hz, 3H, H-2″), 1.19–1.16 (m, 1H), 1.13 (s, 3H), 1.07–1.03 (m, 1H), 0.95(s, 3H), 0.92 (s, 3H), 0.91 (s, 3H), 0.89 (s, 3H), 0.79 (s, 3H), 0.76 ppm (s, 3H); ^{13}C NMR (150 MHz, CD_3OD /$CDCl_3$ = 4/1) δ 181.7 (COOH), 158.7 (NC=O), 144.8 (C-13), 123.3 (C-12), 105.0 (C-1′), 91.0 (C-3), 76.9 (C-5′), 75.3 (C-3′), 71.8 (C-4′), 62.6 (C-6′), 61.4 (C-1″), 58.9 (C-2′), 46.9, 42.6, 42.3, 40.2, 39.8, 39.65, 39.4, 37.6, 34.7, 33.7, 33.51, 33.47, 31.4, 30.4, 28.5, 28.4, 26.6, 26.4, 24.2, 23.9, 23.8, 19.0, 17.5, 16.9, 15.8, 15.0, 14.4 ppm; HRMS (ESI) calcd. for $C_{39}H_{63}NO_9$+Na $[M+Na]^+$: 712.4395, found: 712.4417; HPLC purity 96.2% (t_R: 12.8 min, Mightysil, RP-18, 250 × 4.6 mm, 5 μm, H_2O/ACN = 40/60, 1 mL/min, 30 min).

4.1.6. 3-*O*-(2′-*N*-propoxycarbonyl-2′-deoxy-*β*-D-glucopyranosyl)oleanolic acid (**15**)

According to general procedure A, compound **15** (15 mg, 39 %) was obtained from compound **9** (55 mg, 54.5 μmol) as a white solid; m.p. 226 □; ^{1}H NMR (600 MHz, CD_3OD

/$CDCl_3$ = 4/1) δ 5.23 (t, *J* = 3.5 Hz, 1H, H-12), 4.41 (d, *J* = 8.1 Hz, 1H, H-1′), 4.00–3.88 (m, 2H, H-1″), 3.82 (dd, *J* = 11.9, 2.7 Hz, 1H, H-6a′), 3.71 (dd, *J* = 11.9, 4.9 Hz, 1H, H-6b′), 3.45–3.33 (m, 3H, H-2′, H-3′, H-4′), 3.23 (ddd, *J* = 9.3, 4.9, 2.7 Hz, 1H, H-5′), 3.09 (dd, *J* = 11.7, 4.4 Hz, 1H, H-3), 2.81 (dd, *J* = 13.9, 3.9 Hz, 1H, H-18), 1.98–1.92 (m, 1H), 1.87–1.83 (m, 3H), 1.75–1.68 (m, 2H), 1.67–1.63 (m, 1H), 1.63–1.55 (m, 5H), 1.54–1.48 (m, 3H), 1.46–1.41 (m, 1H), 1.40–1.30 (m, 3H), 1.29–1.25 (m, 2H), 1.19–1.13 (m, 2H), 1.12 (s, 3H), 1.07–1.02 (m, 1H), 0.93 (s, 3H), 0.93–0.91 (m, 3H), 0.91 (s, 3H), 0.89 (s, 3H), 0.88 (s, 3H), 0.77 (s, 3H), 0.75 ppm (s, 3H); ^{13}C NMR (150 MHz, CD_3OD /$CDCl_3$ = 4/1) δ 181.5 (COOH), 158.4 (NC=O), 144.5 (C-13), 122.9 (C-12), 104.7 (C-1′), 90.8 (C-3), 76.4 (C-5′), 75.0 (C-3′), 71.5 (C-4′), 67.1 (C-1″), 62.4 (C-6′), 58.6 (C-2′), 48.3, 46.6, 42.3, 42.0, 39.9, 39.4, 39.1, 37.3, 34.5, 33.4, 33.2, 31.2, 30.2, 29.9, 28.3, 26.34, 26.27, 24.0, 23.9, 23.6, 23.2, 22.9, 18.8, 17.3, 16.8, 15.7, 14.3, 10.6 ppm; HRMS (ESI) calcd. for $C_{40}H_{65}NO_9$+Na $[M+Na]^+$: 726.4552, found: 726.4575; HPLC purity 96.4% (t_R: 16.0 min, Mightysil, RP-18, 250 × 4.6 mm, 5 μm, H_2O/ACN = 40/60, 1 mL/min, 30 min).

4.1.7. *p*-Methylphenyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-1-thio-*β*-D-glucopyranoside (**16**)

Phthalic anhydride (6.88 g, 46.4 mmol) was added to a stirring solution of D-glucosamine hydrochloride (10g, 46.4 mmol) and $NaHCO_3$ (7.8 g, 92.8 mmol) in H_2O/acetone = 1/1 (200 mL) at room temperature. The mixture was stirred for 4h, and then the mixture was concentrated under reduced pressure to give a light yellow solid. The solid was suspended in pyridine (200 mL), Ac_2O (20g, 196 mmol) and DMAP (114 mg, 0.92 mmol), and stirred 8 h. The mixture was concentrated *in vacuo*, diluted with CH_2Cl_2, washed with 1.0 N HCl, $NaHCO_3$ solution, and brine. The organic layers were collected, filtered and concentrated to get light yellow syrup. The syrup and *p*-toluenethiol (11.5 g, 92.8 mmol) were dissolved in CH_2Cl_2 (350 mL), and $BF_3 \cdot OEt_2$ (13.15 g, 92.8 mmol) was added dropwise at

0^{o}C. The mixture was stirred at room temperature for 24 h. The mixture was then washed with $NaHCO_3$ solution and brine. The organic layer was separated, dried with $MgSO_4$ and concentrated *in vacuo*. The residue was recrystallized from ethanol to afford compound **16** as a white solid (13.2 g, 53 % for three steps); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.84 (dd, *J* = 5.1, 3.1 Hz, 2H, Ar-H), 7.73 (dd, *J* = 5.1, 3.1 Hz, 2H, Ar-H), 7.27 (d, *J* = 7.9 Hz, 2H, Ar-H), 7.05 (d, *J* = 7.9 Hz, 2H, Ar-H), 5.75 (t, *J* = 9.7 Hz, 1H, H-3), 5.62 (d, *J* = 10.5 Hz, 1H, H-1), 5.09 (t, *J* = 9.7 Hz, 1H, H-4), 4.33–4.22 (m, 2H, H-2, H-6a), 4.17 (d, *J* = 12.2 Hz, 1H, H-6b), 3.85 (dd, *J* = 10.1, 2.5 Hz, 1H, H-5), 2.30 (s, 3H, STol-CH_3), 2.08 (s, 3H, Ac-CH_3), 1.99 (s, 3H, Ac-CH_3), 1.81 ppm (s, 3H, Ac-CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.6, 170.1, 169.4, 138.7, 134.4, 133.9, 129.6, 126.9, 123.7, 83.1 (C-1), 75.8 (C-5), 71.6 (C-3), 68.7 (C-4), 62.2 (C-6), 53.6 (C-2), 21.1 (STol-CH_3), 20.7 (Ac-CH_3), 20.6 (Ac-CH_3), 20.4 ppm (Ac-CH_3); HRMS (ESI) calcd. for $C_{27}H_{27}NO_9S$+Na $[M+Na]^+$: 564.1299, found: 564.1324.

4.1.8. 28-*O*-benzyl 3-*O*-[3′,4′,6′-tri-*O*-acetyl-2′-deoxy-2′-phthalimido-*β*-D-glucopyranosyl] oleanolic ester (**18**)

NBS (5.6 g, 31.5 mmol) was added slowly to a solution of compound **16** (4.09 g, 7.55 mmol) in acetone/H_2O = 10/1 (100 mL) at -20 □. After 2 h, the mixture was diluted with $Na_2S_2O_3$ solution, $NaHCO_3$ solution, then concentrated *in vacuo*. The residue was diluted with CH_2Cl_2, washed with water, concentrated *in vacuo*, and purified by column chromatography (silicagel; hexane/EtOAc = 1.5/1) to give anomer-OH intermediate as a white solid (2.1 g, 4.82 mmol, 64 %). DBU (12.9 μL, 0.086 mmol), was added into a solution of this intermediate (200 mg, 0.459 mmol) and trichloroacetonitrile (260 μL, 2.59 mmol) in CH_2Cl_2 (4 mL), and the mixture was stirred under room temperature for 2 h. The mixture was concentrated *in vauco*, then subjected to column chromatography (silica gel; EtOAc/hexane/TEA = 1: 1: 0.1) to give trichloroimidate **17** as an oil. A mixture of trichloroimidate intermediate **17** (200 mg, 0.345 mmol), benzyl oleanolate (200 mg, 0.366

mmol), and 4 Å MS in dry CH_2Cl_2 (3 mL) was stirred at 0 □ for 30 minutes. TMSOTf (4.3 μL, 0.024 mmol) was then added, stirred for 30 minutes at 0 □, and then TEA (1 drop) was added. The mixture was filtered, concentrated *in vacuo*, and purified by column chromatography (silica gel; EtOAc/hexane/toluene = 1/4/2) to give compound **18** (250 mg, 75 %); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.82 (dd, *J* = 5.4, 3.1 Hz, 2H, Ar-H), 7.70 (dd, *J* = 5.4, 3.1 Hz, 2H, Ar-H), 7.31–7.26 (m, 5H, Ar-H), 5.80 (dd, *J* = 10.6, 9.2 Hz, 1H, H-3′), 5.34 (d, *J* = 8.4 Hz, 1H, H-1′), 5.24 (s, 1H, H-12), 5.10 (dd, *J* = 10.0, 9.2 Hz, 1H, H-4′), 5.02 (q, *J* = 12.6 Hz, 2H, $CO_2C\underline{H}_2Ph$), 4.36–4.26 (m, 2H, H-2′, H-6a′), 4.14–4.09 (m, 1H, H-6b′), 3.89–3.81 (m, 1H, H-5′), 3.02 (dd, *J* = 11.6, 4.4 Hz, 1H, H-3), 2.86 (d, *J* = 9.8 Hz, 1H, H-18), 2.07 (s, 3H, Ac-CH_3), 2.01 (s, 3H, Ac-CH_3), 1.84 (s, 3H, Ac-CH_3), 1.97–1.88 (m, 1H), 1.81– 1.75 (m, 2H), 1.68–1.65 (m, 1H), 1.64–1.58 (m, 4H), 1.57–1.48 (m, 4H), 1.43–1.37 (m, 1H), 1.33–1.28 (m, 1H), 1.26–1.21 (m, 3H), 1.18–1.15 (1H), 1.13–1.07 (m, 3H), 1.04 (s, 3H), 0.97 –0.92 (m, 1H), 0.88 (s, 3H), 0.86 (s, 3H), 0.79 (s, 3H), 0.55 (s, 3H), 0.51 (s, 3H), 0.38 ppm (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.4($\underline{C}OCH_2Ph$), 170.7 170.23, 169.5, 143.6 (C-13), 136.4, 134.3 128.4, 127.9, 127.9, 123.5 (C-12), 99.9 (C-1′), 90.7 (C-3), 71.5 (C-5′), 70.7 (C-3 ′), 69.3 (C-4′), 65.9 ($CO\underline{C}H_2Ph$), 62.4 (C-6′), 55.1 (C-2′), 54.8, 47.5, 46.7, 45.8, 41.6, 41.3, 39.2, 38.3, 38.2, 36.6, 33.8, 33.1, 32.6, 32.3, 30.7, 27.5, 27.4, 25.8, 23.6, 23.34, 22.98, 20.77, 20.6, 20.5, 18.0, 16.8, 16.3, 15.2 ppm; HRMS (ESI) calcd. for $C_{57}H_{73}NO_{12}$+Na $[M+Na]^+$: 986.5025, found: 986.5039.

4.1.9. 3-*O*-(2′-amino-2′-deoxy-*β*-D-glucopyranosyl)oleanolic acid (**20**)

Starting material **18** (100 mg, 0.104 mmol) was dissolved in ethanol/ethylenediamine = 2/1 (3 mL) and the mixture was stirred at 80 □ for 6 h. The mixture was concentrated *in vacuo* and toluene was added twice to azeotropically remove ethanol and ethylenediamine. The residue was purified by column chromatography (silica gel; CH_2Cl_2/MeOH/TEA = 9/1/0.1) to afford amine containing intermediate **19** (69 mg, 93%). Intermediate **19** (15 mg,

0.021 mmol) was dissolved in MeOH and 20% Pd/C was added. The mixture was stirred under H_2 (balloon) at room temperature for 8h. The residue was filtered, concentrated *in vacuo*, and purified by column chromatography (silica gel; CH_2Cl_2/MeOH = 12/1) to compound **20** (10 mg, 77%) as a white solid. m.p. 234–237 □; ^{1}H NMR (600 MHz, $CD_3OD/CDCl_3$ = 4/1) δ 5.24 (t, *J* = 3.2, 1H, H-12), 4.33 (d, *J* = 7.9 Hz, 1H, H-1), 3.84 (dd, *J* = 12.0, 2.4 Hz, 1H, H-6a′), 3.69 (dd, *J* = 12.0, 5.2 Hz, 1H, H-6b′), 3.32 (t, *J* = 8.8 Hz, 1H, H-4′), 3.29 (t, *J* = 8.8 Hz, 1H, H-3′), 3.24-3.28 (m, 1H, H-5′), 3.22 (dd, *J* =11.7, 4.3 Hz, 1H, H-3), 2.84 (dd, *J* = 13.6, 3.5 Hz, 1H, H-18), 2.68 (t, *J* = 8.9 Hz, 1H, H-2′), 2.05–1.96 (m, 1H), 1.96–1.93 (m, 1H), 1.93–1.91 (m, 1H), 1.91–1.83 (m, 2H), 1.81–1.77 (m, 1H), 1.75–1.70 (m, 1H), 1.70–1.63 (m, 2H), 1.63–1.58 (m, 2H), 1.58–1.53 (m, 3H), 1.53–1.45 (m, 2H), 1.45–1.34 (m, 3H), 1.34–1.24 (m, 3H), 1.21–1.16 (m, 1H), 1.14 (s, 3H), 1.12–1.09 (m, 1H), 1.09–1.04 (m, 1H), 1.03 (s, 3H), 1.01–0.95 (m, 2H), 0.94 (s, 3H), 0.93 (s, 3H), 0.90 (s, 3H), 0.89–0.86 (m, 1H), 0.84 (s, 3H), 0.81 (s, 3H), 0.80–0.77 ppm (m, 1H); ^{13}C NMR(150MHz, $CD_3OD/CDCl_3$ = 4/1) δ 182.1 (COOH), 145.1 (C-13), 123.2 (C-12), 105.7 (C-1′), 90.2 (C-3), 77.6 (C-5′), 76.9 (C-3′), 71.6 (C-4′), 62.6 (C-6′), 58.7 (C-2′), 56.6, 47.5, 47.1, 42.7, 42.5, 40.3, 39.8, 39.5, 37.7, 34.8, 33.8, 33.6, 33.5, 31.5, 28.9, 28.7, 26.7, 26.4, 24.3, 24.0, 19.1, 17.6, 17.1, 15.9 ppm; HRMS (ESI TOF-MS) $[M+H]^+$ calcd. for $C_{36}H_{59}NO_7^+$: 618.4364; found: 618.4380.

4.1.10. General procedures B for amide bond coupling and deprotection to afford **41**-**45**

Compound **19** (22 mg) was diluted with CH_2Cl_2 (3 mL); then, pyridine (0.5 mL), and anhydrides (0.1 mL) were added. The reaction was stirred for 2 h to a mixture of partially acetylated products. The mixture was concentrated, then dissolved in CH_2Cl_2/MeOH = 1/1 (4 mL), diluted with 4.0 N NaOH (aq) (0.1 mL), and stirred for 6 h. The mixture was concentrated *in vacuo*, dissolved in CH_2Cl_2, washed with NH_4Cl and brine, concentrated *in vacuo*, and purified by flash column chromatography (silica gel; CH_2Cl_2/MeOH = 18/1) to get

deacylated products **22**-**26**. Then, the deacylated products were dissolved in MeOH and 10% Pd/C was added. The mixture was stirred under H_2 at room temperature for 8h. The residue was filtered, concentrated *in vacuo*, and purified by flash column chromatography (silica gel; CH_2Cl_2/MeOH = 14/1).

4.1.11. 3-*O*-(2′-propanoylamino-2′-deoxy-*β*-D-glucopyranosyl)oleanolic acid (**41**)

Compound **41** (50 mg, 60%), as a white solid, was prepared according to the general procedure B for amide bond coupling using **19** (88 mg, 0.124 mmol), propinoic anhydride (0.1 mL), pyridine (0.5 mL) and purified by column chromatography (silica gel; CH_2Cl_2/MeOH = 15/1) to give compound **22,** which was further deprotected using 10% Pd/C (6 mg) and purified by column chromatography (silica gel; CH_2Cl_2/MeOH = 15/1). m.p. 217 □; ^{1}H NMR (600 MHz, CD_3OD /$CDCl_3$ = 4/1) δ 5.23 (t, *J* = 3.5 Hz, 1H, H-12), 4.46 (d, *J* = 8.3 Hz, 1H, H-1′), 3.83 (dd, *J* = 11.9, 2.6 Hz, 1H, H-6a′), 3.71 (dd, *J* = 11.9, 5.0 Hz, 1H, H-6b′), 3.65 (dd, *J* = 10.3, 8.3 Hz, 1H, H-2′), 3.46 (dd, *J* = 10.3, 9.0 Hz, 1H, H-3′), 3.36 (t, *J* = 9.0 Hz, 1H, H-4′), 3.24 (ddd, *J* = 9.0, 5.0, 2.6 Hz, 1H, H-5′), 3.08 (dd, *J* = 11.7, 4.4 Hz, 1H, H-3), 2.81 (dd, *J* = 13.7, 4.2 Hz, 1H, H-18), 2.23–2.17 (m, 2H, H-2″), 2.00–1.92 (m, 1H), 1.89–1.83 (m, 3H), 1.76–1.69 (m, 2H), 1.67–1.60 (m, 2H), 1.60–1.56 (m, 2H), 1.54–1.50 (m, 2H), 1.49–1.46 (m, 1H), 1.45–1.41 (m, 2H), 1.37–1.34 (m, 2H), 1.31–1.29 (m, 2H), 1.20–1.16 (m, 2H), 1.14–1.09 (m, 6H), 1.07–1.02 (m, 2H), 0.93 (s, 3H), 0.91 (s, 3H), 0.90 (s, 3H), 0.88 (s, 3H), 0.77 (s, 3H), 0.74 ppm (s, 3H); ^{13}C NMR (150MHz, CD_3OD/$CDCl_3$ = 4/1) δ 181.6 (COOH), 176.6 (NC=O), 144.6 (C-13), 123.1 (C-12), 104.3 (C-1′), 90.6 (C-3), 76.6 (C-5′), 75.3 (C-3′), 71.7 (C-4′), 62.4 (C-6′), 57.1 (C-2′), 47.1, 46.7, 42.4, 42.1, 40.0, 39.6, 39.2, 37.4, 34.5, 33.5, 33.3, 32.6, 31.1, 30.1, 29.6, 28.5, 28.4, 26.3, 24.1, 23.9, 23.7, 23.3, 18.9, 17.4, 16.8, 15.7, 14.4, 11.3 ppm; HRMS (ESI) calcd. for $C_{39}H_{63}NO_8$+Na $[M+Na]^+$: 696.4446, found: 696.4464; HPLC purity 96.4% (t_R: 10.4 min, Mightysil, RP-18, 250 × 4.6 mm, 5 μm, H_2O/ACN = 40/60, 1 mL/min, 30 min).

4.1.12. 3-*O*-(2′-butanoylamino-2′-deoxy-*β*-D-glucopyranosyl)oleanolic acid (**42**)

Compound **42** (9.8 mg, 51%), as a white solid, was prepared according to the general procedure B for amide bond coupling using **19** (20 mg, 0.028 mmol), butyric anhydride (0.1 mL), and pyridine (0.5 mL); and purified by column chromatography (silica gel; CH_2Cl_2/MeOH = 15/1) to give compound 23 which was further deprotected using 10% Pd/C (3 mg) and purified by column chromatography (silica gel; CH_2Cl_2/MeOH = 15/1); m.p. 196 □; ^{1}H NMR (600 MHz, $CD_3OD/CDCl_3$ = 4/1) δ 5.23 (t, *J* = 3.5 Hz, 1H, H-12), 4.46 (d, *J* = 8.3 Hz, 1H, H-1′), 3.82 (dd, *J* = 11.9, 2.6 Hz, 1H, H-6a′), 3.71 (dd, *J* = 11.9, 5.0 Hz, 1H, H-6b′), 3.66–3.63 (m, 1H, H-2′), 3.44 (dd, *J* = 10.2, 9.0 Hz, 1H, H-3′), 3.39–3.34 (m, 1H, H-4′), 3.24 (ddd, *J* = 9.7, 5.0, 2.6 Hz, 1H, H-5′), 3.08 (dd, *J* = 11.7, 4.4 Hz, 1H, H-3), 2.81 (dd, *J* = 13.9, 3.7 Hz, 1H, H-18), 2.16 (dd, *J* = 8.2, 7.0 Hz, 2H, H-2″), 1.99–1.92 (m, 1H), 1.89–1.83 (m, 3H), 1.75–1.68 (m, 2H), 1.66–1.61 (m, 4H), 1.60–1.55 (m, 2H), 1.54–1.50 (m, 3H), 1.45–1.40 (m, 2H), 1.36–1.34 (m, 2H), 1.30–1.29 (m, 2H), 1.19–1.15 (m, 2H), 1.12 (s, 3H), 1.06–1.03 (m, 1H), 0.95–0.92 (m, 6H), 0.91 (s, 3H), 0.89 (s, 3H), 0.88 (s, 3H), 0.77 (s, 3H), 0.74 ppm (s, 3H); ^{13}C NMR (150 MHz, $CD_3OD/CDCl_3$ = 4/1) δ 181.5 (COOH), 175.8 (NC=O), 144.6 (C-13), 123.0 (C-12), 104.2 (C-1′), 90.5 (C-3), 76.6 (C-5′), 75.3 (C-3′), 71.7 (C-4′), 62.4 (C-6′), 57.1 (C-2′), 48.4, 47.1, 46.7, 42.4, 42.1, 40.0, 39.5, 39.1, 37.4, 34.5, 33.4, 33.3, 32.6, 31.2, 30.0, 29.60, 28.47, 28.3, 26.3, 24.1, 23.9, 23.3, 19.6, 18.9, 17.4, 16.8, 15.7, 14.3, 14.2 ppm; HRMS (ESI) calcd. for $C_{40}H_{65}NO_8$+Na $[M+Na]^+$: 710.4602, found: 710.4620; HPLC purity 95.8% (t_R: 11.7 min, Mightysil, RP-18, 250 × 4.6 mm, 5 μm, H_2O/ACN = 40/60, 1 mL/min, 30 min).

4.1.13. 3-*O*-(2′-pentanoylamino-2′-deoxy-*β*-D-glucopyranosyl)oleanolic acid (**43**)

Compound **43** (16.0 mg, 65%), as a white solid, was prepared according to the general procedure B for amide bond coupling using **19** (25 mg, 0.035 mmol), valeric anhydride (0.1

mL), and pyridine (0.5 mL); and purified by column chromatography (silica gel; CH_2Cl_2/MeOH = 15/1) to give compound **24** which was further deprotected using 10% Pd/C (3 mg) and purified by column chromatography (silica gel; CH_2Cl_2/MeOH = 15/1); m.p. 206 □; ^{1}H NMR (600 MHz, $CD_3OD/CDCl_3$ = 4/1) δ 5.24 (s, 1H, H-12), 4.45 (d, *J* = 8.3 Hz, 1H, H-1′), 3.84 (d, *J* = 11.8 Hz, 1H, H-6a′), 3.72-3.60 (m, 2H, H-6b′, H-2′), 3.44 (t, *J* = 9.5 Hz, 1H, H-3′), 3.35–3.30 (m, 1H, H-4′), 3.26–3.21 (m, 1H, H-5′), 3.12–3.07 (m, 1H, H-3), 2.83 (d, *J* = 13.6 Hz, 1H, H-18), 2.24–2.17 (m, 2H, H-2″), 2.02–1.94 (m, 1H), 1.90–1.85 (m, 2H), 1.78–1.70 (m, 2H), 1.68–1.63 (m, 2H), 1.62–1.57 (m, 5H), 1.56–1.48 (m, 4H), 1.48–1.44 (m, 1H), 1.39–1.32 (m, 6H), 1.21–1.16 (m, 2H), 1.14 (s, 3H), 1.08–1.04 (m, 1H), 0.95 (s, 3H), 0.93 (s, 3H), 0.93–0.90 (m, 6H), 0.90 (s, 3H), 0.80 (s, 3H), 0.76 ppm (s, 3H); ^{13}C NMR (150 MHz, CD_3OD /$CDCl_3$ = 4/1) δ 181.8 (COOH), 176.2 (NC=O), 144.9 (C-13), 123.4 (C-12), 104.6 (C-1′), 90.7 (C-3), 77.2 (C-5′), 75.7 (C-3′), 72.0 (C-4′), 62.6 (C-6′), 57.4 (C-2′), 49.9, 47.4, 47.0, 42.7, 42.4, 40.3, 39.8, 39.5, 37.7, 37.1, 34.8, 33.8, 33.6, 33.5, 31.5, 30.5, 28.7, 28.7, 28.6, 26.6, 26.4, 24.3, 24.0, 23.9, 23.4, 19.2, 17.6, 17.0, 15.9, 14.1 ppm; HRMS (ESI) calcd. for $C_{41}H_{67}NO_8$+Na $[M+Na]^+$: 724.4759, found: 724.4784; HPLC purity 95.1% (t_R: 9.8 min, Mightysil, RP-18, 250 × 4.6 mm, 5 μm, H_2O/ACN = 30/70, 1 mL/min, 30 min).

4.1.14. 3-*O*-(2′-hexanoylamino-2′-deoxy-*β*-D-glucopyranosyl)oleanolic acid (**44**)

Compound **44** (13.2 mg, 49%) was prepared as a white solid according to the general procedure B for amide bond coupling using **19** (27.0 mg, 0.038 mmol), hexanoic anhydride (0.1 mL), and pyridine (0.5 mL); and purified by column chromatography (silica gel; CH_2Cl_2/MeOH = 15/1) to give compound **25** which was further deprotected using 10% Pd/C (3 mg), and purified by column chromatography (silica gel; CH_2Cl_2/MeOH = 15/1); m.p. 204 □; ^{1}H NMR (600 MHz, $CD_3OD/CDCl_3$ = 4/1) δ 5.23 (t, *J* = 3.5 Hz, 1H, H-12), 4.46 (d, *J* = 8.3 Hz, 1H, H-1′), 3.82 (dd, *J* = 11.9, 2.6 Hz, 1H, H-6a′), 3.71 (dd, *J* = 11.9, 4.9 Hz, 1H, H-6b′), 3.63 (dd, *J* = 10.3, 8.3 Hz, 1H, H-2′), 3.44 (dd, *J* = 10.3, 9.0 Hz, 1H, H-3′), 3.36 (t, *J* =

9.0 Hz, 1H, H-4′), 3.23 (ddd, *J* = 9.0, 4.9, 2.6 Hz, 1H, H-5′), 3.08 (dd, *J* = 11.7, 4.4 Hz, 1H, H-3), 2.83–2.78 (m, 1H, H-18), 2.19–2.15 (m, 2H, H-2″), 1.99–1.91 (m, 1H), 1.88–1.82 (m, 3H), 1.75–1.68 (m, 3H), 1.66–1.62 (m, 2H), 1.61–1.56 (m, 6H), 1.54–1.48 (m, 4H), 1.44–1.41 (m, 1H), 1.35–1.33 (m, 2H), 1.31–1.29 (m, 3H), 1.18–1.16 (m, 2H), 1.11 (s, 3H), 1.07–1.02 (m, 1H), 0.93 (s, 3H), 0.91 (s, 3H), 0.89 (s, 3H), 0.88–0.86 (m, 6H), 0.77 (s, 3H), 0.74 ppm (s, 3H); ^{13}C NMR (150 MHz, $CD_3OD/CDCl_3$ = 4/1) δ 181.5 (COOH), 175.9 (NC=O), 144.5 (C-13), 123.0 (C-12), 104.1 (C-1′), 90.5 (C-3), 76.5 (C-5′), 75.2 (C-3′), 71.6 (C-4′), 62.4 (C-6′), 56.3 (C-2′), 48.3, 47.1, 46.6, 42.3, 42.0, 39.9, 39.5, 39.1, 37.3, 37.1, 34.5, 33.4, 33.2, 32.5, 32.2, 31.2, 30.2, 28.5, 28.3, 26.3, 26.3, 25.8, 24.0, 23.9, 23.6, 22.9, 18.8, 17.3, 16.8, 15.7, 14.2 ppm; HRMS (ESI) calcd. for $C_{42}H_{69}NO_8$+Na $[M+Na]^+$: 738.4915, found: 738.4940; HPLC purity 95.7% (t_R: 11.4 min, Mightysil, RP-18, 250 × 4.6 mm, 5 μm, H_2O/ACN = 30/70, 1 mL/min, 30 min).

4.1.15. 3-*O*-(2′-heptanoylamino-2′-deoxy-*β*-D-glucopyranosyl)oleanolic acid (**45**)

Compound **45** (16.2 mg, 58%) was prepared as a white solid according to the general procedure B for amide bond coupling using **19** (27.0 mg, 0.038 mmol), hexanoic anhydride (0.1 mL), pyridine (0.5 mL); and purified by column chromatography (silica gel; CH_2Cl_2/MeOH = 15/1) to give compound **26** which was further deprotected using 10% Pd/C (3 mg) and purified by column chromatography (silica gel; CH_2Cl_2/MeOH = 15/1); m.p. 204 □; ^{1}H NMR (600 MHz, $CD_3OD/CDCl_3$ = 4/1) δ 5.23 (t, *J* = 3.5 Hz, 1H, H-12), 4.46 (d, *J* = 8.3 Hz, 1H, H-1′), 3.82 (dd, *J* = 11.9, 2.7 Hz, 1H, H-6a′), 3.71 (dd, *J* = 11.9, 5.0 Hz, 1H, H-6b′), 3.63 (dd, *J* = 10.3, 8.3 Hz, 1H, H-2′), 3.44 (dd, *J* = 10.3, 9.2 Hz, 1H, H-3′), 3.36 (t, *J* = 9.2 Hz, 1H, H-4′), 3.23 (ddd, *J* = 9.2, 5.0, 2.7 Hz, 1H, H-5′), 3.08 (dd, *J* = 11.7, 4.4 Hz, 1H, H-3), 2.81 (dd, *J* = 13.8, 4.0 Hz, 1H, H-18), 2.17 (td, *J* = 7.3, 2.0 Hz, 2H, H-2″), 1.99–1.92 (m, 1H), 1.88–1.83 (m, 3H), 1.75–1.69 (m, 2H), 1.66–1.62 (m, 2H), 1.61–1.56 (m, 5H), 1.54–1.49 (m, 3H), 1.45–1.41 (m, 1H), 1.37–1.35 (m, 1H), 1.34–1.32 (m, 2H), 1.32–1.31 (m, 2H), 1.30–

1.29 (m, 2H), 1.28–1.27 (m, 3H), 1.20–1.15 (m, 2H), 1.11 (s, 3H), 1.06–1.03 (m, 1H), 0.93 (s, 3H), 0.91 (s, 3H), 0.89 (s, 3H), 0.88 (s, 3H), 0.87–0.85 (m, 3H), 0.77 (s, 3H), 0.74 ppm (s, 3H); ^{13}C NMR (150 MHz, CD_3OD /$CDCl_3$ = 4/1) δ 181.4 (COOH), 175.9 (NC=O), 144.5 (C-13), 123.0 (C-12), 104.1 (C-1′), 90.5 (C-3), 76.5 (C-5′), 75.3 (C-3′), 71.6 (C-4′), 62.4 (C-6′), 57.1 (C-2′), 48.3, 47.0, 46.6, 42.3, 42.0, 39.9, 39.5, 39.1, 37.3, 37.2, 34.5, 33.4, 33.2, 32.5, 32.2, 31.2, 29.9, 29.7, 28.5, 28.3, 26.31, 26.27, 26.1, 24.0, 23.9, 23.6, 23.0, 18.8, 17.3, 16.8, 15.7, 14.3 ppm; HRMS (ESI) calcd. for $C_{43}H_{71}NO_8$+Na $[M+Na]^+$: 752.5072, found: 752.5098; HPLC purity 95.2% (t_R: 17.0 min, Mightysil, RP-18, 250 × 4.6 mm, 5 μm, H_2O/ACN = 30/70, 1 mL/min, 30 min).

4.1.16. General procedure C for amide bond coupling and deprotection to afford **46-52**

Intermediate **19** (150 mg) was dissolved in CH_2Cl_2 (3 mL). EDC hydrochloride (1.2 equiv), HOBt (1 equiv), DIPEA (2 equiv), and carboxylic acids (0.1 mL) were added and the mixture stirred for 6 h. Amide products containing partial acylated hydroxyl groups were obtained. The mixture was concentrated *in vacuo*, then dissolved in CH_2Cl_2/MeOH = 1/1 (4 mL), diluted with 4.0 N $NaOH_{(aq)}$ (0.1 mL), and stirred for 6 h. The mixture was concentrated *in vacuo*, dissolved in CH_2Cl_2, washed with NH_4Cl and brine, concentrated, and purified by column chromatography (silica gel; CH_2Cl_2/MeOH = 20/1) to give deacylated products **27-33**. The deacylated products were dissolved in MeOH and 10% Pd/C was added. The mixture was stirred under H_2 at room temperature for 8h. The residue was filtered, concentrated *in vacuo*, and purified by column chromatography (CH_2Cl_2/MeOH = 16/1) to give the final compounds **46-52**.

4.1.17. 3-*O*-(2′-octanoylamino-2′-deoxy-*β*-D-glucopyranosyl)oleanolic acid (**46**)

Compound **46** (98 mg, 62%), as a white solid, was prepared according to the general procedure C for amide bond coupling using **19** (150 mg, 0.212 mmol), octanoic acid (0.1 mL),

HOBt (28.6 mg, 0.212 mmol), DIPEA (54.8 mg, 0.424 mmol) and EDC (39.5 mg, 0.254 mmol) and purified by column chromatography (silica gel; CH_2Cl_2/MeOH = 15/1) to give **27** which was further deprotected using 10% Pd/C (14 mg) and purified by column chromatography (silica gel; CH_2Cl_2/MeOH = 15/1); m.p. 196 □; ^{1}H NMR (600 MHz, $CD_3OD/CDCl_3$ = 4/1) δ 5.24 (t, *J* = 3.5 Hz, 1H, H-12), 4.45 (d, *J* = 8.3 Hz, 1H, H-1′), 3.84 (dd, *J* = 11.9, 2.4 Hz, 1H, H-6a′), 3.71–3.64 (m, 2H, H-6b′, H-2′), 3.44 (dd, *J* = 10.4, 8.8 Hz, 1H, H-3′), 3.35–3.34 (m, 1H, H-4′), 3.24 (ddd, *J* = 9.7, 5.4, 2.4 Hz, 1H, H-5′), 3.10 (dd, *J* = 11.7, 4.5 Hz, 1H, H-3), 2.83 (dd, *J* = 13.5, 4.4 Hz, 1H, H-18), 2.23–2.16 (m, 2H, H-2″), 2.01–1.95 (m, 1H), 1.92–1.86 (m, 3H), 1.79–1.71 (m, 2H), 1.71–1.63 (m, 3H), 1.63–1.55 (m, 6H), 1.55–1.49 (m, 3H), 1.48–1.44 (m, 1H), 1.40–1.35 (m, 3H), 1.3–1.29 (m, 7H), 1.2–1.17 (m, 2H), 1.14 (s, 3H), 1.08–1.04 (m, 1H), 0.96 (s, 3H), 0.93 (s, 3H), 0.92 (s, 3H), 0.90 (s, 3H), 0.90–0.87 (m, 3H), 0.79 (s, 3H), 0.76 ppm (s, 3H); ^{13}C NMR (150 MHz, $CD_3OD/CDCl_3$ = 4/1) δ 181.8 (COOH), 176.2 (NC=O), 144.9 (C-13), 123.4 (C-12), 104.6 (C-1′), 90.7 (C-3), 77.2 (C-5′), 75.8 (C-3′), 72.0 (C-4′), 62.7 (C-6′), 57.4 (C-2′), 49.9, 47.4, 47.0, 42.7, 42.5, 40.3, 39.8, 39.5, 37.7, 37.4, 34.8, 33.8, 33.6, 33.5, 32.9, 32.7, 31.5, 30.3, 30.0, 28.8, 28.6, 26.64, 26.6, 26.4, 24.4, 24.0, 23.9, 23.5, 19.2, 17.6, 17.0, 15.9, 14.4 ppm; HRMS (ESI) calcd. for $C_{44}H_{73}NO_8$+Na $[M+Na]^+$: 766.5228, found: 766.5254; HPLC purity 97.5% (t_R: 21.4 min, Mightysil, RP-18, 250 × 4.6 mm, 5 μm, H_2O/ACN = 30/70, 1 mL/min, 30 min).

4.1.18. 3-*O*-(2′-nonanoylamino-2′-deoxy-*β*-D-glucopyranosyl)oleanolic acid (**47**)

Compound **47** (157 mg, 73%), as a white solid, was prepared according to the general procedure C for amide bond coupling using **19** (200 mg, 0.282 mmol), *n*-nonanoic acid (0.1 mL), HOBt (38.1 mg, 0.282 mmol), DIPEA (72.9 mg, 0.564 mmol) and EDC (52.5 mg, 0.338 mmol); and purified by column chromatography (silica gel; CH_2Cl_2/MeOH = 15/1) to give **28** which was further de-benzylated using 10% Pd/C (20 mg) and purified by column chromatography (silica gel; CH_2Cl_2/MeOH = 15/1); m.p. 196 □; ^{1}H NMR (400 MHz,

$CD_3OD/CDCl_3$ = 4/1) δ 5.24 (s, 1H, H-12), 4.45 (d, *J* = 8.3 Hz, 1H, H-1′), 3.85 (dd, *J* = 11.9, 2.3 Hz, 1H, H-6a′), 3.73–3.64 (m, 2H, H-6b′, H-2′), 3.43 (dd, *J* = 10.2, 8.8 Hz, 1H, H-3′), 3.38–3.33 (m, 1H, H-4′), 3.27–3.21 (m, 1H, H-5′), 3.13–3.06 (m, 1H, H-3), 2.84 (d, *J* = 13.7 Hz, 1H, H-18), 2.20 (td, *J* = 7.4, 2.7 Hz, 2H, H-2″), 2.03–1.92 (m, 2H), 1.91–1.84 (m, 2H), 1.80–1.72 (m, 2H), 1.71–1.65 (m, 2H), 1.63–1.56 (m, 5H), 1.53–1.46 (m, 3H), 1.46–1.38 (m, 2H), 1.37–1.31 (m, 4H), 1.30–1.26 (m, 8H), 1.23–1.17 (m, 2H), 1.14 (s, 3H), 1.09–1.02 (m, 2H), 0.95 (s, 3H), 0.93 (s, 3H), 0.92 (s, 3H), 0.89 (s, 3H), 0.89–0.86 (m, 3H), 0.79 (s, 3H), 0.75 ppm (s, 3H); ^{13}C NMR (150 MHz, $CD_3OD/CDCl_3$ = 4/1) δ 181.1 (COOH), 176.2 (NC=O), 145.0 (C-13), 123.3 (C-12), 104.6 (C-1′), 90.7 (C-3), 77.2 (C-5′), 75.8 (C-3′), 72.0 (C-4′), 62.7 (C-6′), 57.4 (C-2′), 49.9, 47.1, 42.7, 42.5, 40.3, 39.9, 39.5, 37.7, 37.4, 34.8, 33.8, 33.7, 33.6, 32.9, 32.8, 31.5, 30.5, 30.4, 30.3, 30.1, 28.8, 28.7, 26.6, 26.5, 26.4, 24.4, 24.0, 23.6, 19.2, 17.6, 17.0, 15.9, 14.4 ppm; HRMS (ESI) calcd. for $C_{45}H_{75}NO_8$+Na $[M+Na]^+$: 780.5385, found: 780.5415; HPLC purity 97.2% (t_R: 7.5 min, Alltima RP-C8, 150 × 4.6 mm, 5 μm, H_2O/ACN = 30/70, 1 mL/min, 30 min).

4.1.19. 3-*O*-(2′-decanoylamino-2′-deoxy-*β*-D-glucopyranosyl)oleanolic acid (**48**)

Compound **48** (85 mg, 52%), as a white solid, was prepared according to the general procedure C for amide bond coupling using **19** (150 mg, 0.212 mmol), decanoic acid (0.1 mL), HOBt (28.6 mg, 0.212 mmol), DIPEA (54.8 mg, 0.424 mmol) and EDC (39.5 mg, 0.254 mmol) and purified by column chromatography (silica gel; CH_2Cl_2/MeOH = 15/1) to give **29** which was further de-benzylated using 10% Pd/C (14 mg) and purified by column chromatography (silica gel; CH_2Cl_2/MeOH = 15/1); m.p. 211 □; ^{1}H NMR (600 MHz, $CD_3OD/CDCl_3$ = 4/1) δ 5.23 (t, *J* = 3.6 Hz, 1H, H-12), 4.44 (d, *J* = 8.3 Hz, 1H, H-1′), 3.83 (dd, *J* = 11.9, 2.5 Hz, 1H, H-6a′), 3.70–3.63 (m, 2H, H-6b′, H-2′), 3.43 (dd, *J* = 10.4, 8.8 Hz, 1H, H-3′), 3.35–3.32 (m, 1H, H-4′), 3.23 (ddd, *J* = 9.7, 5.3, 2.5 Hz, 1H, H-5′), 3.09 (dd, *J* = 11.7, 4.5 Hz, 1H, H-3), 2.82 (dd, *J* = 13.6, 4.2 Hz, 1H, H-18), 2.21–2.15 (m, 2H, H-2″), 2.00–

1.94 (m, 1H), 1.91–1.85 (m, 3H), 1.77–1.71 (m, 2H), 1.71–1.64 (m, 2H), 1.64–1.55 (m, 6H), 1.55–1.47 (m, 4H), 1.47–1.42 (m, 1H), 1.40–1.35 (m, 3H), 1.34–1.32 (m, 2H), 1.31–1.29 (m, 3H), 1.29–1.27 (m, 6H), 1.20–1.16 (m, 2H), 1.13 (s, 3H), 1.07–1.03 (m, 1H), 0.95 (s, 3H), 0.92 (s, 3H), 0.91 (s, 3H), 0.88 (s, 3H), 0.88–0.85 (m, 3H), 0.78 (s, 3H), 0.75 ppm (s, 3H); ^{13}C NMR (150 MHz, CD_3OD /$CDCl_3$ = 4/1) δ 181.8 (COOH), 176.2 (NC=O), 144.9 (C-13), 123.4 (C-12), 104.6 (C-1′), 90.6 (C-3), 77.1 (C-5′), 75.7 (C-3′), 72.0 (C-4′), 62.6 (C-6′), 57.4 (C-2′), 49.9, 47.4, 47.0, 42.7, 42.4, 40.3, 39.8, 39.5, 37.7, 37.4, 34.7, 33.8, 33.5, 32.8, 31.4, 30.5, 30.31, 30.28, 30.2, 28.8, 28.6, 26.6, 26.5, 26.4, 24.3, 24.0, 23.9, 23.5, 19.1, 17.6, 17.0, 15.9, 14.4 ppm; HRMS (ESI TOF-MS) calcd. for $C_{46}H_{77}NO_8$+Na $[M+Na]^+$: 794.5541, found: 794.5570; HPLC purity 96.0% (t_R: 9.0 min, Alltima RP-C8, 150 × 4.6 mm, 5 μm, H_2O/ACN = 30/70, 1 mL/min, 30 min).

4.1.20. 3-*O*-(2′-dodecanoylamino-2′-deoxy-*β*-D-glucopyranosyl)oleanolic acid(**49**)

Compound **49** (101 mg, 84%), as a white solid, was prepared according to the general procedure C for amide bond coupling using **19** (107 mg, 0.151 mmol), lauric acid (33.3 mg, 0.166 mmol), HOBt (20.4 mg, 0.151 mmol), DIPEA (39.0 mg, 0.302 mmol) and EDC (28.1 mg, 0.181 mmol) and purified by column chromatography (silica gel; CH_2Cl_2/MeOH = 15/1) to give **30** which was further de-benzylated using 10% Pd/C (13 mg) and purified by column chromatography (silica gel; CH_2Cl_2/MeOH = 15/1); m.p. 218–219 □;^{1}H NMR (400 MHz, CD_3OD/$CDCl_3$ = 4/1):δ 5.23 (t, *J* = 3.5, 1H, H-12), 4.44 (d, *J* = 8.3 Hz, 1H, H-1′), 3.84 (dd, *J* = 12.0, 2.3 Hz, 1H, H-6a′), 3.71–3.65 (m, 2H, H-6b′, H-2′), 3.43 (dd, *J* = 10.2, 8.7 Hz, 1H, H-3′), 3.34 (dd, *J* = 9.5, 8.7Hz, 1H, H-4′) 3.24 (ddd, *J* = 9.5, 5.0, 2.3 Hz, 1H, H-5′), 3.10 (dd, *J* =11.6, 4.1 Hz, 1H, H-3), 2.84 (dd, *J* = 13.8, 3.7 Hz, 1H, H-18), 2.20 (td, *J* = 7.5, 3.0 Hz, 1H, H–2″), 2.01–1.97 (m, 1H), 1.93–1.85 (m, 3H), 1.82–1.76 (m, 1H), 1.73–1.65 (m, 2H), 1.64–1.56 (m, 5H), 1.55–1.52 (m, 2H), 1.52–1.49 (m, 1H), 1.47–1.43 (m, 1H), 1.42–1.34 (m, 2H), 1.34–1.32 (m, 2H), 1.32–1.30 (m, 3H), 1.30–1.24 (m, 13H), 1.23–1.15 (m, 2H), 1.14 (s, 1H),

1.12–1.01 (m, 2H), 0.96 (s, 3H), 0.93(s, 6H, 2 x CH_3), 0.90–0.86 (m, 6H), 0.80 (s, 3H), 0.77 ppm (s, 3H); ^{13}C NMR(100MHz, $CD_3OD/CDCl_3$ = 4/1): δ 181.1 (COOH), 175.1 (NC=O), 144.0 (C-13), 122.2 (C-12), 103.6 (C-1′), 89.5 (C-3), 76.1 (C-5′), 74.7 (C-3′), 70.8 (C-4′), 61.5 (C-6′), 56.2 (C-2′), 55.6, 46.4, 46.0, 41.6, 41.4, 39.2, 38.8, 38.4, 36.6, 36.3, 33.7, 32.7, 32.5, 31.8, 30.4, 29.6, 29.5, 29.3, 29.2, 27.7, 27.6, 25.5, 25.4, 25.3, 23.3., 22.9, 22.9, 22.5, 18.1, 16.5, 16.0, 14.8, 13.4 ppm; HRMS (ESI TOF-MS) calcd. For $C_{48}H_{82}NO_8^+$ $[M+H]^+$: 800.6035; found: 800.6043; HPLC purity 97.4% (t_R: 12.7 min, Alltima RP-C8, 150 × 4.6 mm, 5 μm, H_2O/ACN = 30/70, 1 mL/min, 30 min).

4.1.21. 3-*O*-(2′-tetradecanoylamino-2′-deoxy-*β*-D-glucopyranosyl)oleanolic acid(**50**)

Compound **50** (45 mg, 45%), as a white solid, was prepared according to the general procedure C for amide bond coupling using **19** (85 mg, 0.120 mmol), myristic acid (27.4 mg, 0.120 mmol), HOBt (16.2 mg, 0.120 mmol), DIPEA (31.0 mg, 0.240 mmol) and EDC (28.1 mg, 0.181 mmol) and purified by column chromatography (silica gel; CH_2Cl_2/MeOH = 15/1) to give **31** which was further de-benzylatedsing 10% Pd/C (5 mg) and purified by column chromatography (silica gel; CH_2Cl_2/MeOH = 15/1); m.p. 200 □; ^{1}H NMR (400 MHz, $CD_3OD/CDCl_3$ = 4/1): δ 5.24 (t, *J* = 3.6, 1H, H-12), 4.45 (d, *J* = 8.3 Hz, 1H, H-1′), 3.84 (dd, *J* = 11.9, 2.1 Hz, 1H, H-6a′), 3.71 – 3.65 (m, 2H, H-6b′, H-2′), 3.44 (dd, *J* =10.1, 9.2 Hz, 1H, H-3′), 3.34 (m, 1H, H-4′) 3.24 (ddd, *J* = 9.4, 5.0, 2.2 Hz, 1H, H-5′), 3.10 (dd, *J* =11.8, 4.3 Hz, 1H, H-3), 2.83 (dd, *J* = 13.5, 3.4 Hz, 1H, H-18), 2.20 (td, *J* = 7.4, 2.8 Hz, 1H, H-2″), 2.04–1.96 (m, 1H), 1.96–1.83 (m, 3H), 1.80–1.69 (m, 2H), 1.68–1.65 (m, 1H), 1.65–1.53 (m, 6H), 1.53–1.44 (m, 2H), 1.43–1.38 (m, 1H), 1.38–1.35 (m, 1H), 1.35–1.32 (m, 2H), 1.32–1.30 (m, 3H), 1.30–1.29 (m, 3H), 1.30–1.24 (m, 14H), 1.23–1.16 (m, 2H), 1.14 (s, 3H), 1.12–1.01 (m, 2H), 0.96 (s, 3H), 0.93(s, 6H, 2 x CH_3), 0.91–0.86 (m, 6H), 0.80 (s, 3H), 0.76 ppm (s, 3H); ^{13}C NMR(100MHz, $CD_3OD/CDCl_3$ = 4/1): δ 181.6 (COOH), 176.1 (NC=O), 144.9 (C-13), 123.4 (C-12), 104.6 (C-1′), 90.6 (C-3), 77.2 (C-5′), 75.7 (C-3′), 71.9 (C-4′), 62.6 (C-6′), 57.4

(C-2′), 56.7, 47.4, 47.0, 42.7, 42.4, 40.3, 39.8, 39.4, 37.7, 37.4, 34.7, 33.8, 33.6, 33.0, 31.5, 30.7, 30.7, 30.6, 30.4, 30.3, 28.8, 28.6, 26.6, 26.5, 26.4, 24.3, 24.0, 23.9, 23.6, 19.2, 17.6, 17.0, 15.9, 14.5 ppm; HRMS (ESI TOF-MS) calcd. for $C_{50}H_{86}NO_8^+$ $[M+H]^+$: 828.6348; found: 828.6373; HPLC purity 95.2% (t_R: 8.1 min, Alltima RP-C8, 150 × 4.6 mm, 5 μm, H_2O/ACN = 20/80, 1 mL/min, 30 min).

4.1.22. 3-*O*-(2′-hexadecanoylamino-2′-deoxy-*β*-D-glucopyranosyl)oleanolic acid(**51**)

Compound **51** (65 mg, 47%), as a white solid, was prepared according to the general procedure C for amide bond coupling using **19** (114 mg, 0.161 mmol), palmitic acid (41.3 mg, 0.161 mmol), HOBt (21.8 mg, 0.161 mmol), DIPEA (41.6 mg, 0.322 mmol) and EDC (30.0 mg, 0.193 mmol) and purified by column chromatography (silica gel; CH_2Cl_2/MeOH = 15/1) to give **32** which was further de-benzylated using 10% Pd/C (5 mg) and purified by column chromatography (silica gel; CH_2Cl_2/MeOH = 15/1); m.p. 218–219 □; ^{1}H NMR (400 MHz, $CD_3OD/CDCl_3$ = 4/1): δ 5.24 (t, *J* = 3.2, 1H, H-12), 4.45 (d, *J* = 8.3 Hz, 1H, H-1′), 3.84 (dd, *J* = 11.9, 2.1 Hz, 1H, H-6a′), 3.71–3.64 (m, 2H, H-6b′, H-2′), 3.43 (dd, *J* =10.3, 8.8 Hz, 1H, H-3′), 3.33 (m, 1H, H-4′) 3.24 (ddd, *J* = 10.4, 5.3, 2.4 Hz, 1H, H-5′), 3.10 (dd, *J* =11.5, 4.1 Hz, 1H, H-3), 2.83 (dd, *J* = 13.4, 3.0 Hz, 1H, H-18), 2.20 (td, *J* = 7.5, 2.6 Hz, 1H, H-2″), 2.08–1.97 (m, 1H), 1.97–1.84 (m, 3H), 1.82–1.72 (m, 2H), 1.72–1.67 (m, 2H), 1.66–1.60 (m, 3H), 1.60–1.55 (m, 3H), 1.55–1.47 (m, 3H), 1.46–1.42 (m, 1H), 1.42–1.38 (m, 1H), 1.38–1.35 (m, 1H), 1.35–1.31 (m, 5H), 1.31–1.23 (m, 24H), 1.23–1.16 (m, 2H), 1.14 (s, 3H), 1.13–1.02 (m, 3H), 1.02–0.97 (m, 1H), 0.96 (s, 3H), 0.93(s, 3H), 0.92 (s, 3H), 0.91–0.88 (m, 6H), 0.88–0.83 (m, 3H), 0.79 (s, 3H), 0.76 ppm (s, 3H); ^{13}C NMR(100MHz, $CD_3OD/CDCl_3$ = 4/1): δ 181.7 (COOH), 176.2 (NC=O), 144.9 (C-13), 123.4 (C-12), 104.6 (C-1′), 90.6 (C-3), 77.2 (C-5′), 75.8 (C-3′), 71.9 (C-4′), 62.6 (C-6′), 57.4 (C-2′), 56.7, 47.4, 47.0, 42.7, 42.4, 40.3, 39.9, 39.5, 37.7, 37.4, 34.8, 33.8, 33.6, 32.9, 31.5, 30.7, 30.7, 30.7, 30.6, 30.4, 30.3, 30.3, 28.8, 28.7, 26.6, 26.5, 26.5, 24.4, 24.0, 23.9, 23.6, 19.2, 17.6, 17.0, 15.9, 14.5 ppm; HRMS (ESI

TOF-MS) calcd. for $C_{52}H_{90}NO_8^+$[M +H]$^+$: 856.6661; found: 856.6690; HPLC purity 98.0% (t_R: 11.5 min, Alltima RP-C8, 150 × 4.6 mm, 5 μm, H_2O/ACN = 20/80, 1 mL/min, 30 min).

4.1.23. 3-*O*-(2′-octadecanoylamino-2′-deoxy-*β*-D-glucopyranosyl)oleanolic acid(**52**)

Compound **52** (63 mg, 61%), as a white solid, was prepared according to the general procedure C for amide bond coupling using **19** (75.0 mg, 0.106 mmol), stearic acid (45.0 mg, 0.158 mmol), HOBt (14.3 mg, 0.106 mmol), DIPEA (27.4 mg, 0.212 mmol) and EDC (19.7 mg, 0.127 mmol) and purified by column chromatography (silica gel; CH_2Cl_2/MeOH = 15/1) to give **33** which was further de-benzylated using 10% Pd/C (5 mg) and purified by column chromatography (silica gel; CH_2Cl_2/MeOH = 15/1); m.p. 203–205 □; ^{1}H NMR (400 MHz, $CD_3OD/CDCl_3$ = 4/1): δ 5.23 (t, *J* = 3.2, 1H, H-12), 4.45 (d, *J* = 8.3 Hz, 1H, H-1′), 3.84 (dd, *J* = 11.9, 2.3 Hz, 1H, H-6a′), 3.71–3.64 (m, 2H, H-6b′, H-2′), 3.43 (dd, *J* =10.3, 8.8 Hz, 1H, H-3′), 3.33 (m, 1H, H-4′) 3.24 (ddd, *J* = 10.4, 5.3, 2.4 Hz, 1H, H-5′), 3.10 (dd, *J* =11.5, 4.1 Hz, 1H, H-3), 2.83 (dd, *J* = 13.4, 3.0 Hz, 1H, H-18), 2.20 (td, *J* = 7.5, 2.6 Hz, 1H, H-2″), 2.08–1.97 (m, 1H), 1.97–1.84 (m, 3H), 1.82–1.72 (m, 2H), 1.72–1.67 (m, 2H), 1.66–1.60 (m, 3H), 1.60–1.55 (m, 3H), 1.55–1.47 (m, 3H), 1.46–1.42 (m, 1H), 1.42–1.38 (m, 1H), 1.38–1.35 (m, 1H), 1.35–1.31 (m, 5H), 1.31–1.23 (m, 24H), 1.23–1.16 (m, 2H), 1.14 (s, 3H), 1.13–1.02 (m, 3H), 1.02–0.97 (m, 1H), 0.96 (s, 3H), 0.93(s, 3H), 0.92 (s, 3H), 0.91–0.88 (m, 6H), 0.88–0.83 (m, 3H), 0.79 (s, 3H), 0.76 ppm (s, 3H); ^{13}C NMR(100MHz, $CD_3OD/CDCl_3$ = 4/1): δ 182.4 (COOH), 176.2 (NC=O), 145.1 (C-13), 123.2 (C-12), 104.6 (C-1′), 90.6 (C-3), 77.2 (C-5′), 75.8 (C-3′), 71.9 (C-4′), 62.6 (C-6′), 57.4 (C-2′), 56.7, 47.6, 47.2, 42.7, 42.6, 40.3, 39.8, 39.5, 37.7, 37.4, 34.8, 33.8, 33.7, 33.6, 32.9, 31.5, 30.6, 30.6, 30.6, 30.3, 30.3, 30.3, 28.8, 28.7, 26.6, 26.5, 26.4, 24.3, 24.0, 24.0, 23.6, 19.2, 17.6, 17.1, 15.9, 14.4 ppm; HRMS (ESI TOF-MS) calcd. for $C_{54}H_{94}NO_8^+$[M+H]$^+$: 884.6974; found: 884.7005; HPLC purity 96.5% (t_R: 16.6 min, Alltima RP-C8, 150 × 4.6 mm, 5 μm, H_2O/ACN = 20/80, 1 mL/min, 30 min)

4.1.24. General procedures D for the synthesis of carbamates and deprotection **53**–**58**

Compound **19** (35 mg) was dissolved in CH_2Cl_2 (1 mL), TEA (2 equiv), CDI (1.2 equiv), and alcohol (2 equiv), and stirred 6 h. The mixture was diluted with CH_2Cl_2, washed with $NaHCO_3$ solution, brine. The organic layers were collected, dried over $MgSO_4$, filtered and concentrated *in vacuo* and purified by flash column chromatography (silica gel; DCM/MeOH = 10/1) to give deacylated compounds **34**-**40**. Then, to a stirred solution of the deacylated product in MeOH and was added 10% Pd/C and stirred under H_2 (1 atm) for 12h. The mixture was filtered through a celite pad, and the filter cake was washed with MeOH. The filtrate was concentrated *in vacuo* and purified by the flash column chromatography (silica gel; CH_2Cl_2/MeOH = 10/1) to get final **53**-**58**.

4.1.25. 3-*O*-(2′-*N*-butoxycarbonyl-2′-deoxy-*β*-D-glucopyranosyl)oleanolic acid (**53**)

Compound **53** (20 mg, 51%), as a white solid, was prepared according to the general procedure C for amide bond coupling using **19** (35.0 mg, 0.049 mmol), n-butanol (9 μL, 0.098 mmol), CDI (14.3 mg, 0.059 mmol), and TEA (14 μL, 0.100 mmol) and purified by column chromatography (silica gel; CH_2Cl_2/MeOH = 10/1) to give **34** which was further de-benzylated using 10% Pd/C (4 mg) and purified by column chromatography (silica gel; CH_2Cl_2/MeOH = 10/1); m.p. 274 □; ^{1}H NMR (600 MHz, $CD_3OD/CDCl_3$ = 4/1) δ 5.26 (t, *J* = 3.4, 1H, H-12), 4.42 (d, *J* = 8.0 Hz, 1H, H-1′), 4.08–3.97 (m, 2H, H-1″), 3.86 (dd, *J* = 12.0, 2.3 Hz, 1H, H-6a′), 3.71 (dd, *J* = 12.0, 5.3 Hz, 1H, H-6b′), 3.45–3.35 (m, 3H, H-2′, H-3′, H-4′), 3.25 (ddd, *J* = 9.4, 5.3, 2.3 Hz, 1H, H-5′), 3.12 (dd, *J* = 11.6, 4.2 Hz, 1H, H-3), 2.86 (dd, *J* = 14.0, 4.1 Hz, 1H, H-18), 2.02–1.98 (m, 1H), 1.97–1.90 (m, 3H), 1.80–1.73 (m, 2H), 1.70–1.66 (m, 2H), 1.63–1.58 (m, 5H), 1.56–1.54 (m, 4H), 1.50–1.48 (m, 1H), 1.22–1.20 (m, 1H), 1.16 (s, 3H), 1.15–1.13 (m, 2H), 1.09–1.07 (m, 1H), 0.98 (s, 3H), 0.95 (s, 9H), 0.91 (s, 3H), 0.82 (s, 3H), 0.79 ppm (s, 3H); ^{13}C NMR(150MHz, $CD_3OD/CDCl_3$ = 4/1) δ 181.9 (COOH), 159.0 (NC=O), 145.0 (C-13), 123.4 (C-12), 105.2 (C-1′), 91.0 (C-3), 77.2 (C-5′), 75.6 (C-3′),

72.0 (C-4′), 65.5 (C-1″), 62.7 (C-6′), 59.2 (C-2′), 56.7,49.6, 47.5, 47.1, 42.7, 42.5, 40.4, 39.8, 39.5, 37.7, 34.8, 33.9, 33.7, 33.6, 32.2, 31.5, 28.7, 28.6, 26.7, 26.4, 24,4, 24.0, 24.0, 20.0, 19.2, 17.6, 17.0, 15.9, 14.1 ppm; HRMS(ESI TOF-MS) calcd. for $C_{41}H_{67}NO_9$ + Na $[M+Na]^+$: 740.4708; found: 740.4726. HPLC purity 99.3% (t_R: 12.7 min, Mightysil, RP-18, 250 × 4.6 mm, 5 μm, H_2O/ACN = 30/70, 1 mL/min, 30 min).

4.1.26. 3-*O*-(2′-*N*-pentoxycarbonyl-2-deoxy-*β*-D-glucopyranosyl)oleanolic acid (**54**)

Compound **54** (15.5 mg, 45%), as a white solid, was prepared according to the general procedure C for amide bond coupling using **19** (33.0 mg, 0.047 mmol), *n*-pentanol (10 μL, 0.092 mmol), CDI (9.1 mg, 0.056 mmol), and TEA (13 μL, 0.093 mmol) and purified by column chromatography (silica gel; CH_2Cl_2/MeOH = 10/1) to give **35** which was further de-benzylated using 10% Pd/C (3 mg) and purified by column chromatography (silica gel; CH_2Cl_2/MeOH = 10/1); m.p. 227 □; ^{1}H NMR (400 MHz, $CD_3OD/CDCl_3$ = 4/1) δ 5.26 (t, *J* = 3.0, 1H, H-12), 4.42 (d, *J* = 7.6 Hz, 1H, H-1′), 4.11–3.94 (m, 2H, H-1″), 3.86 (dd, *J* = 11.9, 2.3 Hz, 1H, H-6a′), 3.71 (dd, *J* = 11.9, 5.1 Hz, 1H, H-6b′), 3.46–3.36 (m, 3H, H-2′, H-3′, H-4′), 3.25 (ddd, *J* = 9.1, 5.1, 2.3 Hz, 1H, H-5′), 3.13 (dd, *J* = 11.4, 4.1 Hz, 1H, H-3), 2.86 (dd, *J* = 13.6, 3.6 Hz, 1H, H-18), 2.05–1.96 (m, 1H), 1.91–1.89 (m, 3H), 1.80–1.75 (m, 1H), 1.73–1.72 (m, 1H), 1.70– 1.67 (m, 1H), 1.64–1.60 (m, 4H), 1.58–1.53 (m, 3H), 1.50–1.47 (m, 1H), 1.42–1.34 (m, 6H), 1.30–1.20 (m, 4H), 1.17 (s, 3H), 1.13–1.02 (m, 2H), 0.99 (s, 3H), 0.95 (s, 6H, 2 x CH_3), 0.94 (s, 3H), 0.92 (s, 3H), 0.82 (s, 3H), 0.79 ppm (s, 3H); ^{13}C NMR(100MHz, $CD_3OD/CDCl_3$ = 4/1) δ 181.6 (COOH), 158.9 (NC=O), 144.8 (C-13), 123.4 (C-12), 105.2 (C-1′), 90.9 (C-3), 77.1 (C-5′), 75.5 (C-3′), 71.8 (C-4′), 65.6 (C-1″), 62.6 (C-6′), 59.0 (C-2′), 56.6, 47.3, 47.0, 42.6, 42.4, 40.2, 39.7, 39.4, 37.6, 34.7, 33.7, 33.5, 31.4, 29.7, 29.0, 28.6, 28.5, 26.6, 26.4, 24.3, 24.0, 23.8., 23.2, 19.1, 17.5, 17.0, 15.8, 14.3 ppm; HRMS(ESI TOF-MS) calcd. for $C_{42}H_{69}NO_9$ + Na $[M+Na]^+$: 754.4865; found: 754.4891; HPLC purity 95.5% (t_R: 14.0 min, Mightysil, RP-18, 250 × 4.6 mm, 5 μm, H_2O/ACN = 30/70, 1 mL/min, 30 min).

4.1.27. 3-*O*-(2′-*N*-hexoxycarbonyl-2′-deoxy-*β*-D-glucopyranosyl)oleanolic acid (**55**)

Compound **55** (10.6 mg, 41%), as a white solid, was prepared according to the general procedure C for amide bond coupling using **19** (25.0 mg, 0.035 mmol), *n*-hexanol (9 μL, 0.072 mmol), CDI (6.8 mg, 0.042 mmol), and TEA (10 μL, 0.071 mmol) and purified by column chromatography (silica gel; CH_2Cl_2/MeOH = 10/1) to give **36** which was further de-benzylated using 10% Pd/C (3 mg) and purified by column chromatography (silica gel; CH_2Cl_2/MeOH = 10/1); m.p. 207 □; ^{1}H NMR (400 MHz, $CD_3OD/CDCl_3$ = 4/1) δ 5.27 (t, *J* = 3.2, 1H, H-12), 4.43 (d, *J* = 7.6 Hz, 1H, H-1′), 4.10–3.93 (m, 2H, H-1″), 3.87 (dd, *J* = 11.9, 2.3 Hz, 1H, H-6a′), 3.72 (dd, *J* = 11.9, 5.3 Hz, 1H, H-6b′), 3.47–3.37 (m, 3H, H-2′, H-3′, H-4′), 3.26 (ddd, *J* = 8.8, 5.3, 2.3 Hz, 1H, H-5′), 3.14 (dd, *J* = 11.6, 4.2 Hz, 1H, H-3), 2.87 (dd, *J* = 13.7, 3.9 Hz, 1H, H-18), 2.06–2.02 (m, 1H), 2.00–1.90 (m, 3H), 1.80–1.73 (m, 3H), 1.70–1.68 (m, 1H), 1.66–1.63 (m, 3H), 1.61–1.54 (m, 4H), 1.52–1.48 (m, 1H), 1.46–1.37 (m, 4H), 1.37–1.30 (m, 6H), 1.24–1.21 (m, 1H), 1.18 (s, 3H), 1.14–1.02 (m, 2H), 1.00 (s, 3H), 0.96 (s, 6H, 2 x CH_3), 0.94 (s, 3H), 0.92 (s, 3H), 0.82 (s, 3H), 0.80 ppm (s, 3H); ^{13}C NMR(100MHz, $CD_3OD/CDCl_3$ = 4/1): δ 181.7 (COOH), 159.0 (NC=O), 144.9 (C-13), 123.4 (C-12), 105.2 (C-1′), 91.0 (C-3), 77.2 (C-5′), 75.5 (C-3′), 71.9 (C-4′), 65.7 (C-1″), 62.6 (C-6′), 59.1 (C-2′), 56.7, 47.4, 47.0, 42.7, 42.4, 40.3, 39.8, 39.5, 37.7, 34.8, 33.8, 33.6, 32.5, 31.5, 30.0, 28.6, 28.6, 26.7, 26.6, 26.4, 24.4, 24.0, 23.9., 23.5, 19.2, 17.6, 17.0, 15.9, 14.4 ppm; HRMS (ESI TOF-MS) calcd. for $C_{43}H_{71}NO_9$ + Na $[M+Na]^+$: 768.5021; found: 768.5051; HPLC purity 95.0% (t_R: 19.2 min, Mightysil, RP-18, 250 × 4.6 mm, 5 μm, H_2O/ACN = 30/70, 1 mL/min, 30 min).

4.1.28. 3-*O*-(2′-*N*-heptoxycarbonyl-2′-deoxy-*β*-D-glucopyranosyl)oleanolic acid (**56**)

Compound **56** (20.4 mg, 42%), as a white solid, was prepared according to the general procedure C for amide bond coupling using **19** (45.0 mg, 0.064 mmol), *n*-heptanol (18 μL,

0.127 mmol), CDI (12.5 mg, 0.077 mmol), and TEA (18 μL, 0.129 mmol) and purified by column chromatography (silica gel; CH_2Cl_2/MeOH = 10/1) to give **37** which was further de-benzylated using 10% Pd/C (3 mg) and purified by column chromatography (silica gel; CH_2Cl_2/MeOH = 10/1); m.p. 202 □; ^{1}H NMR (600 MHz, $CD_3OD/CDCl_3$ = 4/1) δ 5.24 (t, *J* = 3.4, 1H, H-12), 4.40 (d, *J* = 8.0 Hz, 1H, H-1′), 4.06–3.92 (m, 2H, H-1″), 3.84 (dd, *J* = 11.9, 2.3 Hz, 1H, H-6a′), 3.69 (dd, *J* = 11.9, 5.3 Hz, 1H, H-6b′), 3.43–3.33 (m, 3H, H-2′, H-3′, H-4′), 3.23 (ddd, *J* = 9.3, 5.3, 2.3 Hz, 1H, H-5′), 3.10 (dd, *J* = 11.6, 4.2 Hz, 1H, H-3), 2.83 (dd, *J* = 13.7, 3.8 Hz, 1H, H-18), 2.00–1.96 (m, 1H), 1.92–1.88 (m, 3H), 1.78–1.71 (m, 2H), 1.69–1.64 (m, 2H), 1.61–1.57 (m, 4H), 1.56–1.50 (m, 3H), 1.48–1.45 dd, 1H), 1.41–1.34 (m, 4H), 1.32–1.27 (m, 8H), 1.20–1.18 (m, 1H), 1.14 (s, 3H), 1.12–1.05 (m, 2H), 0.96 (s, 3H), 0.93 (s, 6H, 2 x CH_3), 0.90 (m, 6H), 0.80 (s, 3H), 0.77 ppm (s, 3H); ^{13}C NMR(150MHz, $CD_3OD/CDCl_3$ = 4/1): δ 181.8 (COOH), 158.9 (NC=O), 144.9 (C-13), 123.4 (C-12), 105.2 (C-1′), 91.0 (C-3), 77.2 (C-5′), 75.5 (C-3′), 71.9 (C-4′), 65.7 (C-1″), 62.7 (C-6′), 59.1 (C-2′), 56.7, 47.4, 47.0, 42.7, 42.5, 40.3, 39.8, 39.5, 37.7, 34.8, 33.8, 33.6, 33.5, 32.8, 31.5, 30.5, 30.1, 29.9, 28.6, 26.8, 26.6, 26.4, 24.3, 24.0, 23.9., 23.5, 19.2, 17.6, 17.0, 15.8, 14.4 ppm; HRMS (ESI TOF-MS) calcd. for $C_{44}H_{73}NO_9$+Na [M +Na]$^+$: 782.5178; found: 782.5203; HPLC purity 97.4% (t_R: 16.3 min, Alltima RP-C8, 150 × 4.6 mm, 5 μm, H_2O/ACN = 40/60, 1 mL/min, 30 min).

4.1.29. 3-*O*-(2′-*N*-octoxycarbonyl-2′-deoxy-*β*-D-glucopyranosyl)oleanolic acid (**57**)

Compound **57** (53.0 mg, 54%), as a white solid, was prepared according to the general procedure C for amide bond coupling using **19** (90.0 mg, 0.127 mmol), *n*-octanol (40 μL, 0.253 mmol), CDI (24.6 mg, 0.152 mmol), and TEA (35 μL, 0.251 mmol) and purified by column chromatography (silica gel; CH_2Cl_2/MeOH = 10/1) to give **38** which was further de-benzylation using 10% Pd/C (3 mg) and purified by column chromatography (silica gel; CH_2Cl_2/MeOH = 10/1); m.p. 204 □; ^{1}H NMR (400 MHz, $CD_3OD/CDCl_3$ = 4/1) δ 5.23 (t, *J* = 3.5, 1H, H-12), 4.40 (d, *J* = 7.8 Hz, 1H, H-1′), 4.08–3.90 (m, 2H, H-1″), 3.83 (dd, *J* = 11.9,

2.0 Hz, 1H, H-6a′), 3.69 (dd, *J* = 11.9, 5.2 Hz, 1H, H–6b′), 3.44–3.32 (m, 3H, H-2′, H-3′, H-4′), 3.23 (ddd, *J* = 9.1, 5.2, 2.0 Hz, 1H, H-5′), 3.10 (dd, *J* = 11.6, 4.1 Hz, 1H, H-3), 2.83 (dd, *J* = 13.6, 3.4 Hz, 1H, H-18), 2.01–1.98 (m, 1H), 1.94–1.87 (m, 3H), 1.81–1.63 (m, 4H), 1.62–1.55 (m, 6H), 1.51–1.44 (m, 2H), 1.39–1.36 (m, 3H), 1.33–1.26 (m, 10H), 1.20–1.17 (m, 1H), 1.14 (s, 3H), 1.07–1.04 (m, 2H), 0.96 (s, 3H), 0.93 (s, 6H, 2 x CH_3), 0.89–0.87 (m, 6H), 0.79 (s, 3H), 0.76 ppm (s, 3H); ^{13}C NMR(100MHz, $CD_3OD/CDCl_3$ = 4/1) δ 181.6 (COOH), 158.9 (NC=O), 144.8 (C-13), 123.4 (C-12), 105.2 (C-1′), 90.9 (C-3), 77.1 (C-5′), 75.5 (C-3′), 71.8 (C-4′), 65.6 (C-1″), 62.6 (C-6′), 59.1 (C-2′), 59.0, 56.6, 47.3, 47.0, 42.6, 42.4, 40.3, 39.7, 39.4, 37.6, 34.7, 33.8, 33.6, 32.8, 31.4, 30.3, 30.2, 30.1, 28.6, 26.9, 26.6, 26.4, 24.3, 24.0, 23.9., 23.6, 19.2, 17.6, 17.1, 15.9, 14.5 ppm; HRMS (ESI TOF-MS) calcd. for $C_{45}H_{75}NO_9$+Na $[M+Na]^+$: 796.5334; found: 796.5362; HPLC purity 95.1% (t_R: 19.3 min, Alltima RP-C8, 150 × 4.6 mm, 5 μm, H_2O/ACN = 40/60, 1 mL/min, 30 min).

4.1.30. 3-*O*-(2′-*N*-nonoxycarbonyl-2′-deoxy-*β*-D-glucopyranosyl)oleanolic acid (**58**)

Compound **58** (24.0 mg, 44%), as a white solid, was prepared according to the general procedure C for amide bond coupling using **19** (50.0 mg, 0.071 mmol), *n*-nonanol (25 μL, 0.144 mmol), CDI (13.8 mg, 0.085 mmol), and TEA (20 μL, 0.143 mmol) and purified by column chromatography (silica gel; CH_2Cl_2/MeOH = 10/1) to give **39** which was further de-benzylated using 10% Pd/C (4 mg) and purified by column chromatography (silica gel; CH_2Cl_2/MeOH = 10/1); m.p. 201 □; ^{1}H NMR (400 MHz, $CD_3OD/CDCl_3$ = 4/1) δ 5.24 (t, *J* = 3.4, 1H, H-12), 4.40 (d, *J* = 8.1 Hz, 1H, H-1′), 4.08–3.91 (m, 2H, H-1″), 3.84 (dd, *J* = 11.9, 2.3 Hz, 1H, H-6a′), 3.69 (dd, *J* = 11.9, 5.3 Hz, 1H, H-6b′), 3.43–3.33 (m, 3H, H-2′, H-3′, H-4′), 3.23 (ddd, *J* = 9.2, 5.3, 2.3 Hz, 1H, H-5′), 3.10 (dd, *J* = 11.6, 4.3 Hz, 1H, H-3), 2.83 (dd, *J* = 13.7, 4.0 Hz, 1H, H-18), 2.01–1.96 (m, 1H), 1.94–1.85 (m, 3H), 1.78–1.71 (m, 2H), 1.70–1.63 (m, 2H), 1.60–1.56 (m, 5H), 1.54–1.45 (m, 3H), 1.41–1.34 (m, 4H), 1.31–1.29 (m, 11H), 1.20–1.16 (m, 1H), 1.14 (s, 3H), 1.12–1.04 (m, 2H), 0.97 (s, 3H), 0.93 (s, 6H, 2 x CH_3), 0.90–

0.88 (m, 6H), 0.80 (s, 3H), 0.77 ppm (s, 3H); ^{13}C NMR(100MHz, $CD_3OD/CDCl_3$ = 4/1): δ 181.6 (COOH), 158.9 (NC=O), 144.8 (C-13), 123.4 (C-12), 105.2 (C-1′), 90.9 (C-3), 77.1 (C-5′), 75.5 (C-3′), 71.8 (C-4′), 65.6 (C-1″), 62.6 (C-6′), 59.1 (C-2′), 59.0, 56.6, 47.3, 47.0, 42.6, 42.4, 40.3, 39.7, 39.4, 37.6, 34.7, 33.8, 33.6, 32.8, 31.4, 30.3, 30.2, 30.1, 28.6, 26.9, 26.6, 26.4, 24.3, 24.0, 23.9., 23.6, 19.2, 17.6, 17.1, 15.9, 14.5 ppm; HRMS (ESI TOF-MS) calcd. for $C_{46}H_{77}NO_9$+Na $[M+Na]^+$: 810.5491; found: 810.5511; HPLC purity 98.7% (t_R: 11.5 min, Alltima RP-C8, 150 × 4.6 mm, 5 μm, H_2O/ACN = 30/70, 1 mL/min, 30 min).

4.1.31. 3-*O*-(2′-*N*-octylcarbamoyl-2′-deoxy-*β*-D-glucopyranosyl)oleanolic acid (**59**)

To a solution of *n*-nonanoic acid (50 μL, 0.284 mmol) in toluene (2 mL) was added diphenylphosphoryl azide (61 μL, 0.284 mmol) and TEA (50 μL, 0.359 mmol). The mixture was stirred at 50 □ for 2h, then warmed up to 80 □, and keep stirred for another 3h. The mixture was added compound **19** (100 mg, 0.141 mmol) at 50 □ for 8h. The mixture was concentrated and purified by column chromatography (silica gel; CH_2Cl_2/MeOH/TEA = 10/1/0.1) to afford urea containing intermediate **40** (51 mg, 42%). To a stirred solution of the residue in MeOH and was added 10% Pd/C and stirred under H_2 (1 atm) for 12h. The mixture was filtered through a Celite pad, and the filter cake was washed with MeOH. The filtrate was concentrated and purified by the column chromatography (silica gel; CH_2Cl_2/MeOH = 10/1) to compound **59** (45 mg, 98%) as a white solid; m.p. 193–194 □; ^{1}H NMR (600 MHz, $CD_3OD/CDCl_3$ = 4/1) δ 5.24 (t, *J* = 3.4, 1H, H-12), 4.41 (d, *J* = 8.0 Hz, 1H, H-1′), 3.84 (dd, *J* = 11.9, 2.5 Hz, 1H, H-6a′), 3.70 (dd, *J* = 11.9, 5.3 Hz, 1H, H-6b′), 3.44–3.33 (m, 3H, H-2′, H-3′, H-4′), 3.25 (ddd, *J* = 9.3, 5.3, 2.5 Hz, 1H, H-5′), 3.18–3.13 (m, 1H, H-1a″), 3.12 (dd, *J* = 8.5, 4.4 Hz, 1H, H-3), 3.05–3.01 (m, H-1b″), 2.84 (dd, *J* = 13.7, 4.0 Hz, 1H, H-18), 2.01–1.94 (m, 1H), 1.94–1.86 (m, 3H), 1.80–1.75 (m, 1H), 1.75–1.71 (m, 1H), 1.71–1.62 (m, 2H), 1.63–1.57 (m, 3H), 1.57–1.53 (m, 2H), 1.53–1.50 (m, 1H), 1.50–1.43 (m, 4H), 1.42–1.33 (m, 3H), 1.34–1.23 (m, 14H), 1.23–1.16 (m, 1H), 1.15–1.13 (m, 1H), 1.13–1.08 (m, 1H), 1.08–1.02 (m,

1H), 1.00–0.96 (m, 5H), 0.93 (s, 6H, 2 x CH_3), 0.91–0.87 (m, 7H), 0.80 (s, 3H), 0.78 (s, 3H), 0.77–0.74 ppm (m, 1H); ^{13}C NMR(150MHz, $CD_3OD/CDCl_3$ = 4/1) δ 182.0 (COOH), 160.9 (NC=O), 145.0 (C-13), 123.3 (C-12), 105.5 (C-1′), 90.8 (C-3), 77.1 (C-5′), 76.1 (C-3′), 72.0 (C-4′), 62.7 (C-6′), 56.7 (C-2′), 47.5, 47.1, 42.7, 42.5, 41.0, 40.3, 39.8, 39.5, 37.7, 34.8, 33.8, 33.6, 33.6, 33.6, 32.8, 31.5, 31.1, 30.4, 30.3, 28.7, 27.9, 26.7, 26.4, 24.4, 24.0, 23.9., 23.6, 19.2, 17.6, 17.1, 15.9, 14.4 ppm; HRMS (ESI TOF-MS) calcd. for $C_{45}H_{76}N_2O_8^+$ $[M+H]^+$: 773.5674; found: 773.5701; HPLC purity 95.1%(t_R: 12.4 min, Alltima RP-C8, 150 × 4.6 mm, 5 μm, H_2O/ACN = 40/60, 1 mL/min, 30 min).

4.1.32. 28-*O*-benzyl 3-*O*-[3′,4′,6′-tri-*O*-acetyl-2′-deoxy-2′-phthalimido-*β*-D-galactopyranosyl] oleanolic ester (**62**)

Ethylenediamine (27 μL, 0.403 mmol) and acetic acid (27 μL, 0.471 mmol) were added slowly at 0 □ to a solution of compound **60** (170 mg, 0.343 mmol) in THF (3 mL). After 2 h, the mixture was concentrated *in vacuo*, and purified by column chromatography (silicagel; hexane/ EtOAc = 1/1) to give anomer-OH intermediate as a white solid (91 mg, 0.209 mmol, 61 %). DBU (6 μL, 0.04 mmol) was added into a solution of intermediate (91 mg, 0.209 mmol) and trichloroacetonitrile (130 μL, 1.26 mmol) in CH_2Cl_2 (2 mL), the mixture was stirred under room temperature for 2 h. The mixture was concentrated, then subjected to column chromatography (silica gel; EtOAc/hexane = 1: 1) to give trichloroimidate **61**. A mixture of trichloroimidate intermediate **61** (96 mg, 0.166 mmol), benzyl oleanolate (98 mg, 0.183 mmol), and 4 Å MS in dry CH_2Cl_2 (3 mL) was stirred at 0 □ for 30 min. TMSOTf (3 μL, 0.017 mmol) was then added, stirred for 30 min at 0 □, and then TEA (1 drop) was added. The mixture was filtered, concentrated and purified by column chromatography (silica gel; EtOAc/hexane/toluene = 1/4/2) to give compound **62** (92 mg, 46 % for 2 steps); m.p. 216 □; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.86 (dd, *J* = 5.4, 2.9 Hz, 2H, Ar-H), 7.70 (dd, *J* = 5.4, 2.9 Hz, 2H, ArH), 7.35–7.25 (m, 5H), 5.79 (dd, *J* = 11.4, 3.5 Hz, 1H, H-3′), 5.45 (d, *J* = 3.5 Hz, 1H,

H-4′), 5.29 (d, *J* = 8.5 Hz, 1H, H-1′), 5.24 (t, *J* = 3.5, 1H, H-12), 5.02 (q, *J* = 12.5 Hz, 2H, $CO_2C\underline{H}_2Ph$), 4.56 (dd, *J* = 11.4, 8.5 Hz, 1H, H-2′), 4.23 (dd, *J* = 11.1, 6.8 Hz, 1H, H-6a′), 4.12 (dd, *J* = 11.1, 6.8 Hz, 1H, H-6b′), 4.05 (dt, *J* = 6.8, 0.8 Hz, 1H, H-5′), 3.01 (dd, *J* = 11.8, 4.4 Hz, 1H, H-3), 2.86 (dd, *J* = 13.9, 4.2 Hz, 1H, H-18), 2.17 (s, 3H, CH_3 of Ac), 2.03 (s, 3H, CH_3 of Ac), 1.95–1.88 (m, 1H), 1.83 (s, 3H, CH_3 of Ac), 1.81–1.76 (m, 3H), 1.71–1.63 (m, 2H), 1.61–1.56 (m, 5H), 1.55–1.50 (m, 2H), 1.42–1.37 (m, 1H), 1.33–1.26 (m, 1H), 1.25–1.20 (m, 2H), 1.19–1.16 (m, 2H), 1.13–1.07 (m, 2H), 1.05–1.03 (m, 3H), 0.89–0.85 (m, 7H), 0.79 (s, 3H), 0.58 (s, 3H), 0.51 (s, 3H), 0.37 (s, 3H); ^{13}C NMR(150MHz, $CDCl_3$) δ 177.6 ($\underline{C}OCH_2Ph$), 170.6, 170.6, 170.0, 167.7, 143.8, 136.5, 134.4, 128.5, 128.1, 128.0, 123.7, 123.5, 122.6 (C-12), 100.7 (C-1′), 91.1, 70.8 (C-5′), 68.2 (C-3′), 66.8 (C-4′), 66.1 ($CO\underline{C}H_2Ph$), 61.7 (C-6′), 55.3, 51.7 (C-2′), 47.6, 46.9, 46.0, 41.7, 41.5, 39.4, 38.5, 38.3, 36.8, 34.0, 33.2, 32.7, 32.5, 30.8, 27.7, 27.6, 25.9, 25.7, 23.8, 23.5, 23.1, 20.9, 20.9, 20.7, 18.2, 16.9, 16.5, 15.3; HRMS (ESI TOF - MS) $[M+Na]^+$ calcd. for $C_{57}H_{73}NO_{12}$ + Na : 986.5025; found: 986.5032.

4.1.33. 3-*O*-(2′-acetylamino-2′-deoxy-*β*-D-galactopyranosyl)oleanolic acid (**66**)

Starting material **62** (112 mg, 0.118 mmol) in ethanol/ethylenediamine = 2/1 (3 mL) was stirred at 70–80 □ for 6 h. The mixture was concentrated under reduced pressure and toluene was added twice to azeotropically remove ethanol and ethylenediamine. The residue was purified by column chromatography (silica gel; CH_2Cl_2/MeOH = 10/1) to afford amine containing intermediate **63** (69 mg, 0.100 mmol, 85%). Compound **66** (28 mg, 55%), as a white solid, was prepared according to the general procedure B for amide bond coupling using **63** (53 mg, 0.076 mmol), acetic anhydride (0.1 mL), pyridine (0.5 mL) and purified by column chromatography (silica gel; CH_2Cl_2/MeOH = 20/1) to give compound **64** which was fuether deprotected using 10% Pd/C (3.0 mg) and purified by column chromatography (silica gel; CH_2Cl_2/MeOH = 10/1); m.p. 272 □; ^{1}H NMR (600 MHz, $CD_3OD/CDCl_3$ = 4/1) δ 5.26 (t, *J* = 3.5, 1H, H-12), 4.43 (d, *J* = 8.3 Hz, 1H, H-1′), 3.92 (dd, *J* = 10.7, 8.3 Hz, 1H, H-2′), 3.86

(d, *J* = 3.2 Hz, 1H, H-4′), 3.75-3.79 (m, 2H, H-6a′, H-6b′), 3.61 (dd, *J* = 10.7, 3.2 Hz, 1H, H-3′), 3.48 (t, *J* = 6.3 Hz, 1H, H-5′), 3.12 (dd, *J* = 11.7, 4.4 Hz, 1H, H-3), 2.86 (dd, *J* = 13.9, 4.2 Hz, 1H, H-18), 2.03–1.99 (m, 1H), 1.98 (s, 3H, CH_3 of Ac), 1.94–1.92 (m, 1H), 1.91–1.85 (m, 3H), 1.81–1.66 (m, 4H), 1.63–1.59 (m, 2H), 1.58–1.56 (m, 1H), 1.55–1.53 (m, 1H), 1.51–1.47 (m, 1H), 1.45–1.36 (m, 2H), 1.34–1.29 (m, 2H), 1.23–1.20 (m, 1H), 1.18–1.13 (m, 4H), 1.10–1.06 (m, 1H), 0.98 (s, 3H), 0.95 (s, 3H), 0.94 (s, 3H), 0.92 (s, 3H) , 0.81 (s, 3H) , 0.78 (s, 3H) , 0.77 ppm (s, 1H); ^{13}C NMR(150MHz, $CD_3OD/CDCl_3$ = 4/1) δ 180.7 (COOH), 172.4 (NC=O), 143.8 (C-13), 122.2 (C-12), 103.8 (C-1′), 89.6 (C-3), 74.8 (C-5′), 71.6 (C-3′), 68.1 (C-4′), 60.9 (C-6′), 55.5, 53.5 (C-2′), 46.3, 45.9, 41.5, 41.3 (C-18), 39.2, 38.6, 38.3, 36.6, 36.6, 33.6, 32.7, 32.5, 32.4, 30.3, 27.5, 27.4, 25.5, 25.3, 23.2, 22.8, 22.8, 22.0, 18.0, 16.5, 15.8, 14.7 ppm; HRMS (ESI TOF - MS) $[M+Na]^+$ calcd. for $C_{43}H_{71}NO_9$ + Na : 768.5021; found: 768.5053; HPLC purity 91.6% (t_R: 9.1 min, Mightysil, RP-18, 250 × 4.6 mm, 5μm, H_2O/ACN = 40/60, 1mL/min, 40 min)

4.1.34. 3-*O*-(2′-heptanoylamino-2′-deoxy-*β*-D-galactopyranosyl)oleanolic acid (**67**)

Compound **62** (149 mg, 0.157 mmol) was dissolved in a solution of ethanol/ethylenediamine (2 mL/1 mL), and the solution was stirred at 75 □ for 6 h. The mixture was concentrated *in vacuo* and toluene (few drops) was added twice to azeotropically remove residue solvent. The residue was purified by column chromatography (silica gel; CH_2Cl_2/MeOH = 10/1) to afford amine containing intermediate **63** (93 mg, 85%). Compound **67** (44 mg, 45%), as a white solid, was prepared according to the general procedure B for amide bond coupling using **63** (93 mg, 0.134 mmol), *n*-propinoic anhydride (0.1 mL), imidazole (46 mg, 0.670 mmol) and purified by column chromatography (silica gel; CH_2Cl_2/MeOH = 12/1) to give compound **65** which was further deprotected using 10% Pd/C (6 mg) and purified by column chromatography (silica gel; CH_2Cl_2/MeOH = 10/1); m.p. 316 □; ^{1}H NMR (400 MHz, $CD_3OD/CDCl_3$ = 4/1) δ 5.21 (t, *J* = 3.6, 1H, H-12), 4.39 (d, *J* = 8.3

Hz, 1H, H-1′), 3.88 (dd, *J* = 10.7, 8.3 Hz, 1H, H-2′), 3.80 (d, *J* = 3.2 Hz, 1H, H-4′), 3.76-3.70 (m, 2H, H-6a′, H-6b′), 3.54 (dd, *J* = 10.7, 3.2 Hz, 1H, H-3′), 3.42 (t, *J* = 6.2 Hz, 1H, H-5′), 3.07 (dd, *J* = 11.8, 4.4 Hz, 1H, H-3), 2.80 (dd, *J* = 13.6, 3.6 Hz, 1H, H-18), 2.17 (td, *J* = 8.0, 2.4 Hz, 2H, H-1″), 2.02–1.90 (m, 1H), 1.87–1.82 (m, 3H), 1.78–1.65 (m, 3H), 1.64–1.46 (m, 9H), 1.30–1.22 (m, 11H), 1.21–1.18 (m, 1H), 1.16–1.07 (m, 5H), 1.06–1.01 (m, 1H), 0.93 (s, 3H), 0.90 (s, 3H), 0.87–0.85 (m, 5H), 0.76 (s, 3H), 0.73 (s, 3H); ^{13}C NMR(100MHz, $CD_3OD/CDCl_3$ = 4/1) δ 181.0 (COOH), 175.4 (NC=O), 143.9 (C-13), 122.2 (C-12), 103.8 (C-1′), 89.4 (C-3), 74.8 (C-5′), 71.8 (C-3′), 68.2 (C-4′), 61.0 (C-6′), 55.6, 53.3 (C-2′), 46.4, 46.0, 41.6, 41.4 (C-18), 39.2, 38.7, 38.4, 36.6, 36.4, 33.7, 32.7, 32.5, 32.4, 31.5, 30.4, 29.5, 28.9, 27.7, 27.6, 25.6, 25.4, 25.3, 23.2, 22.9, 22.4, 22.3, 18.1, 16.5, 15.9, 14.7, 13.3; HRMS (ESI TOF - MS) $[M+Na]^+$ calcd. for $C_{43}H_{71}NO_9$ + Na : 768.5021; found: 768.5056; HPLC purity 86.4% (t_R: 14.3 min, Mightysil, RP-18, 250 × 4.6 mm, 5μm, H_2O/ACN = 30/70, 1mL/min, 40 min)

4.2. Cell culture and cytotoxic assay

Leukemia cell line HL-60, human prostate carcinoma cell line PC-3, and human colorectal carcinoma cell line HT29 were obtained from the American Type Culture Collection (Rockville, MD). HL-60 cells were cultured in Iscove′s modified Dulbecco′s medium (IMDM) with 10% FBS (v/v) and penicillin (100 U/mL)/streptomycin (100 μg/mL). PC-3 cells were cultured in RPMI 1640 medium with 10% FBS (v/v) and penicillin (100 U/mL)/streptomycin (100 μg/mL). HCT116 and HT29 cells were cultured in Dulbecco′s Modified Eagle Medium (DMEM) with 10% FBS (v/v) and penicillin (100 U/mL)/streptomycin (100 μg/mL). Cells were maintained in a humidified incubator at 37 □ in 5% CO_2/95% air. Cell were incubated in the absence or presence of the compounds for the indicated concentrations and times, and then the mitochondrial MTT reduction activity was assessed by enzyme-linked immunosorbent assay reader (570 nm) to obtain absorbance density values.

4.3. Cell image experiments

Copper-catalyzed click reaction: HL-60 cells were seeded in 24-well plates and treated with compound **68** (15 μM) (control treated with diluted DMSO). After 15 h, the cells were fixed with 99% alcohol, treated with 0.1% triton X-100 (in PBS) for 15 min, and then washed twice with PBS. After washing, fluorogenic probe **69** (30 μM), Na ascorbate (1 mM), TBTA (0.1 mM) in PBS were added to the cells, and then $CuSO_4$ (0.5 mM) with 0.5 mg/mL of BSA in PBS buffer were added and incubated for 6 h and cells were analyzed by fluorescence confocal microscopy; For copper-free labeling with cyclooctyne reagent (DBCO-488): HL-60 cells were seeded in 24-well plates and treated with compound **71** (15 μM) for 15 h (control treated with diluted DMSO). After treatment with compound **71**, the cells were fixed with 99% alcohol, treated with 0.1% triton X-100 (in PBS) for 15 min, and then washed twice with PBS. After washing, fluorogenic probe DBCO-488 (2 μM), with 0.5 mg/mL of BSA in PBS buffer were added and incubated for 6 h and cells were analyzed by fluorescence confocal microscopy. Nuclei were counterstained with RedDot™2 and cell membranes were counterstained with CellBrite™ Blue. Fluorescence images were detected using ZEISS, LSM 510 META Confocal Microscope.

4.4. Mitochondrial membrane potential assay

HL-60 cells were treated in the absence or presence of the compound **45**, the conditions were as mentioned in MTT assay. After 1 day, cells were treated with JC-1 (final concentration = 2 μM) under 37 □ for 30 min. Next, the cells were harvested and then were analyzed by flow cytometer (BD FACSCalibur flow cytometer).

Acknowledgment

This research was financially supported by Academia Sinica, and the Ministry of Science

and Technology [105-0210-01-13-01] and [106-0210-01-15-02].

References

[1] J. Pollier, A. Goossens, Oleanolic acid, Phytochemistry, 77 (2012) 10-15.
[2] M.B. Sporn, K.T. Liby, M.M. Yore, L. Fu, J.M. Lopchuk, G.W. Gribble, New Synthetic Triterpenoids: Potent Agents for Prevention and Treatment of Tissue Injury Caused by Inflammatory and Oxidative Stress, J. Nat. Prod., 74 (2011) 537-545.
[3] M. Masullo, C. Pizza, S. Piacente, Oleanane derivatives for pharmaceutical use: a patent review (2000-2016), Expert. Opin. Ther. Pat., 27 (2017) 237-255.
[4] J.A.R. Salvador, A.S. Leal, A.S. Valdeira, B.M.F. Gonçalves, D.P.S. Alho, S.A.C. Figueiredo, S.M. Silvestre, V.I.S. Mendes, Oleanane-, ursane-, and quinone methide friedelane-type triterpenoid derivatives: Recent advances in cancer treatment, Eur. J. Med. Chem., (2017) doi: 10.1016/j.ejmech.2017.1007.1013.
[5] J. Liu, Y. Liu, A. Parkinson, C.D. Klaassen, Effect of oleanolic acid on hepatic toxicant-activating and detoxifying systems in mice, J. Pharmacol. Exp. Ther., 275 (1995) 768-774.
[6] K.T. Liby, M.M. Yore, M.B. Sporn, Triterpenoids and rexinoids as multifunctional agents for the prevention and treatment of cancer, Nat. Rev. Cancer, 7 (2007) 357-369.
[7] P. Dzubak, M. Hajduch, D. Vydra, A. Hustova, M. Kvasnica, D. Biedermann, L. Markova, M. Urban, J. Sarek, Pharmacological activities of natural triterpenoids and their therapeutic implications, Nat. Prod. Rep., 23 (2006) 394-411.
[8] H. Nie, Y. Wang, Y. Qin, X.-G. Gong, Oleanolic acid induces autophagic death in human gastric cancer cells in vitro and in vivo, Cell. Biol. Int., (2016) 770–778.
[9] C. Lin, X. Wen, H. Sun, Oleanolic acid derivatives for pharmaceutical use: a patent review, Expert. Opin. Ther. Pat., (2016) 1-13.
[10] M.K. Shanmugam, X.Y. Dai, A.P. Kumar, B.K.H. Tan, G. Sethi, A. Bishayee, Oleanolic acid and its synthetic derivatives for the prevention and therapy of cancer: Preclinical and clinical evidence, Cancer Lett., 346 (2014) 206-216.
[11] Y.-Y. Wang, H. Zhe, R. Zhao, Preclinical evidences toward the use of triterpenoid CDDO-Me for solid cancer prevention and treatment, Mol. Cancer, 13 (2014) 30-30.
[12] B. Siewert, J. Wiemann, A. Kowitsch, R. Csuk, The chemical and biological potential of C ring modified triterpenoids, Eur. J. Med. Chem., 72 (2014) 84-101.
[13] S. Sommerwerk, L. Heller, J. Kuhfs, R. Csuk, Urea derivates of ursolic, oleanolic and maslinic acid induce apoptosis and are selective cytotoxic for several human tumor cell lines, Eur. J. Med. Chem., 119 (2016) 1-16.
[14] Q. Liu, H. Liu, L. Zhang, T. Guo, P. Wang, M. Geng, Y. Li, Synthesis and antitumor activities of naturally occurring oleanolic acid triterpenoid saponins and their derivatives, Eur.

J. Med. Chem., 64 (2013) 1-15.
[15] J. Sun, X. Han, B. Yu, Synthesis of a typical N-acetylglucosamine-containing saponin, oleanolic acid 3-yl
alpha-L-arabinopyranosyl-(1->2)-alpha-L-arabinopyranosyl-(1->6)-2-acetamido-2-d eoxy-beta-D-glucopyranoside, Carbohyd Res, 338 (2003) 827-833.
[16] M. Abdel-Kader, J. Hoch, J.M. Berger, R. Evans, J.S. Miller, J.H. Wisse, S.W. Mamber, J.M. Dalton, D.G.I. Kingston, Two Bioactive Saponins from *Albizia subdimidiata* from the Suriname Rainforest, J. Nat. Prod., 64 (2001) 536-539.
[17] Y. Seo, J. Hoch, M. Abdel-Kader, S. Malone, I. Derveld, H. Adams, M.C.M. Werkhoven, J.H. Wisse, S.W. Mamber, J.M. Dalton, D.G.I. Kingston, Bioactive Saponins from *Acacia tenuifolia* from the Suriname Rainforest, J. Nat. Prod., 65 (2002) 170-174.
[18] M.C. Yan, Y. Liu, H. Chen, Y. Ke, Q.C. Xu, M.S. Cheng, Synthesis and antitumor activity of two natural N-acetylglucosamine-bearing triterpenoid saponins: lotoidoside D and E, Bioorg Med Chem Lett, 16 (2006) 4200-4204.
[19] Y.H. Wang, H.W. Yeh, H.W. Wang, C.C. Yu, J.H. Guh, D.Z. Liu, P.H. Liang, Synthesis of a chlorogenin glycoside library using an orthogonal protecting group strategy, Carbohyd Res, 375 (2013) 118-135.
[20] Y.-B. Zeng, H.-M. Hsiao, S.-H. Chan, Y.-H. Wang, Y.-Y. Lin, Y.-H. Kuo, J.-H. Guh, P.-H. Liang, Synthesis and anti-cancer activity of a glycosyl library of N-acetylglucosamine-bearing oleanolic acid, Mol. Divers., 18 (2014) 13-23.
[21] P. Wang, J. Wang, T. Guo, Y. Li, Synthesis and cytotoxic activity of the N-acetylglucosamine-bearing triterpenoid saponins, Carbohydr. Res., 345 (2010) 607-620.
[22] R.B. Silverman, M.W. Holladay, Chapter 2 - Lead Discovery and Lead Modification, in: The Organic Chemistry of Drug Design and Drug Action (Third Edition), Academic Press, Boston, 2014, pp. 19-122.
[23] S.G. Hansen, T. Skrydstrup, Studies directed to the synthesis of oligochitosans - Preparation of building blocks and their evaluation in glycosylation studies, Eur. J. Org. Chem., (2007) 3392-3401.
[24] H.-W. Yeh, T.-S. Lin, H.-W. Wang, H.-W. Cheng, D.-Z. Liu, P.-H. Liang, S-Linked sialyloligosaccharides bearing liposomes and micelles as influenza virus inhibitors, Org. Biomol. Chem., 13 (2015) 11518-11528.
[25] A.K. Ghosh, M. Brindisi, Organic Carbamates in Drug Design and Medicinal Chemistry, J. Med. Chem., 58 (2015) 2895-2940.
[26] D. Sawada, S. Sasayama, H. Takahashi, S. Ikegami, Novel synthesis of oligosaccharides linked with carbamate and urea bonds utilizing modified Curtius rearrangement, Tetrahedron, 64 (2008) 8780-8788.
[27] M.V. Berridge, P.M. Herst, A.S. Tan, Tetrazolium dyes as tools in cell biology: New insights into their cellular reduction, in: Biotechnol. Annu. Rev., Elsevier, 2005, pp.

127-152.
[28] W.C. Huang, T.Y. Tseng, Y.T. Chen, C.C. Chang, Z.F. Wang, C.L. Wang, T.N. Hsu, P.T. Li, C.T. Chen, J.J. Lin, P.J. Lou, T.C. Chang, Direct evidence of mitochondrial G-quadruplex DNA by using fluorescent anti-cancer agents, Nucleic Acids Res., 43 (2015) 10102-10113.
[29] K. Sivakumar, F. Xie, B.M. Cash, S. Long, H.N. Barnhill, Q. Wang, A Fluorogenic 1,3-Dipolar Cycloaddition Reaction of 3-Azidocoumarins and Acetylenes, Org. Lett., 6 (2004) 4603-4606.
[30] Y. Wang, Y. Zhang, Z. Zhu, S. Zhu, Y. Li, M. Li, B. Yu, Exploration of the correlation between the structure, hemolytic activity, and cytotoxicity of steroid saponins, Bioorg. Med. Chem., 15 (2007) 2528-2532.
[31] J.H. Lorent, J. Quetin-Leclercq, M.-P. Mingeot-Leclercq, The amphiphilic nature of saponins and their effects on artificial and biological membranes and potential consequences for red blood and cancer cells, Org. Biomol. Chem., 12 (2014) 8803-8822.
[32] H.K. Kim, Y.H. Noh, B. Nilius, K.S. Ko, B.D. Rhee, N. Kim, J. Han, Current and upcoming mitochondrial targets for cancer therapy, Semin Cancer Biol, (2017) doi: 10.1016/j.semcancer.2017.1006.1006.
[33] G. Kroemer, L. Galluzzi, C. Brenner, Mitochondrial Membrane Permeabilization in Cell Death, Physiol. Rev., 87 (2007) 99-163.
[34] S.H. Chan, W.J. Leu, L.C. Hsu, H.S. Chang, T.L. Hwang, I.S. Chen, C.S. Chen, J.H. Guh, Reevesioside F induces potent and efficient anti-proliferative and apoptotic activities through Na(+)/K(+)-ATPase alpha3 subunit-involved mitochondrial stress and amplification of caspase cascades, Biochem Pharmacol, 86 (2013) 1564-1575.
[35] Y. Ye, T. Zhang, H. Yuan, D. Li, H. Lou, P. Fan, Mitochondria-Targeted Lupane Triterpenoid Derivatives and Their Selective Apoptosis-Inducing Anticancer Mechanisms, J. Med. Chem., 60 (2017) 6353-6363.

Figure Legends

Figure 1. OA and OA saponin derivatives

Figure 2. Click reaction to locate cellular uptake of (A) with compound **68** in the presence of fluorogenic probe **69**, TBTA and Cu^{+}, (B) Compound **71** in the presence of fluorogenic probe, DBCO-488. The confocal images of HL-60 cell fluorescence Green: [3 + 2] cycloaddition of the compounds **68** and **71**; Blue: CellBrite™ Blue (Cell membrane); Red: RedDot™2 (nucleus).

Figure 3. Effect of compound on $\Delta\Psi_m$ and related protein expression. HL-60 cells were incubated in the absence or dose-dependent concentration of compound **45** for the indicated times. Cells were incubated with JC-1 for the detection of $\Delta\Psi m$ using FACScan flow cytometric analysis and mitochondria membrane potential loss (%).

Highlights

Synthesis of 24 *N*-modifications of 2′-deoxy-glucosyl oleanolic saponins.

SAR studies revealed that heptyl to nonyl substitutions reached the peak activities.

56 displayed the most cytotoxic activity (IC_{50} = 0.76 μM) against HL-60 cells.

Location study suggested compounds distributed mainly in the cytosol.