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Synthesis, conformational analysis and SAR research of OSW-1 analogues

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ABSTRACT

A series of novel OSW-1 analogues were synthesized by coupling disaccharides (2-*O*-4-methoxylbenzoyl-β-D-xylopyranosyl-(1→3)-2-*O*-acetyl-α-L-arabinopyranosyl) or (2-*O*-4-(*E*)-cinnamoyl-β-D-xylopyranosyl-(1→3)-2-*O*-acetyl-α-L-arabinopyranosyl) and their 1→4 linked analogues [(2-*O*-4-methoxylbenzoyl-β-D-xylopyranosyl-(1→4)-2-*O*-acetyl-α-L-arabinopyranosyl) or (2-*O*-4-(*E*)-cinnamoyl-β-D-xylopyranosyl-(1→4)-2-*O*-acetyl-α-L-arabinopyranosyl)] with three different steroidal sapogenins at 16β-hydroxy. Their conformation was analyzed with NMR spectroscopy and molecule simulation. The arabinose moiety of 1–3 linked analogues was in chair conformation and 1–4 linked analogues was in boat conformation. 1–3 linked analogues exhibited potent *anti*-proliferation activity against a panel of human tumor cells at nanomolar concentration level, while 1–4 linked analogues did not show anti-tumor activity. This work should provide an evidence that the conformation plays an important role in the antitumor activity.

1. Introduction

OSW-1 (**1**) and its related congener **2** (Fig. 1), isolated from the bulbs of *Ornithogalum saundersiae*,[1] have attracted considerable attention because of their potent and selective antitumor activities (IC_{50}=0.25 nM for OSW-1, IC_{50}=0.20 nM for congener **2**, HL-60 cells) superior to those used in the clinical treatment of cancer such as mitomycin C, adriamycin, and taxol.[2] The biological study showed that OSW-1 can damage the membrane and cristae of cancer cell's mitochondria which played an important role in mediating the cell function, leading to the loss of transmembrane potential, a significant increase of cytosolic calcium, and activation of calcium-dependant apoptosis. The evidence disclosed that OSW-1 may cause cancer cell's death by apoptosis.[3]

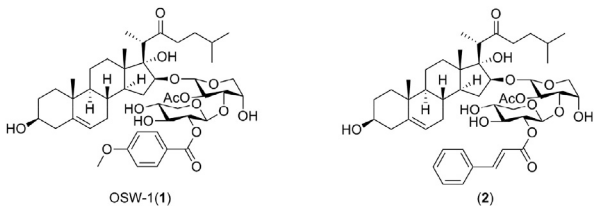

Fig. 1. Antitumor glycosylsterols OSW-1 (**1**) and its congeners (**2**).

A number of pharmacologic studies and synthetic of OSW-1 and its analogues have been reported.[4–7] Kandutsch, A.[8] and Taylor[9] discovered OSBP as a cytosolic receptor for oxysterols. Hurley[10] mentioned that sterol molecule binds within a hydrophobic tunnel in a manner consistent with a transport function for ORPs (a family of OSBP). Burgett et al.[11] identified that OSBP was a high-affinity target of OSW-1 using affinity chromatography and demonstrated that OSW-1 exerted its antitumor activity via OSBP. Yan's group also observed that ORP4L was a relevant target of OSW-1 and played a key role in maintaining the proliferation capacity of certain malignant cell types.[12] While Huang's group revealed that OSW-1 inhibited Na^+/Ca^{2+} exchanger 1, a membrane protein, which led to apoptosis through increased calcium concentration in cytosol and mitochondria.[13] The cellular targets and the molecular mechanism of their action were still controversial.

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The precise binding form of the receptor and OSW-1 remains unclear. The relationship between the disaccharide chain and its biological activity has not been well understood. For example, the necessity of carbonyl group at C-22 was still controversial. Wojtkielewice et al. commented that the presence of a carbonyl group at C-22 was pharmacophore required.[7] However, B. Yu found that the 22-deoxy-OSW-1 was slightly more potent than OSW-1.[14] The 3D structures of OSW-1 (**1**) and its closely related congener (**2**) were investigated by NMR studies and an X-ray crystallographic analysis by Sakurai et al.[15] They commented that the 3D structures of OSW-1 (**1**) and (**2**) had a flat triangular molecular shape, which was an important structural basis for its biological activity. In our previous work[16] an interesting result was found that 1–3 linked analogues showed high activity, while 1–4 linked analogues showed no activity. It was curious for us that the conformation of inactive 1–4 linked analogues. We intend to affirm the relationship between activity and conformation by comparing the conformation of 1–4 linked analogues to 1–3 linked analogues. Ten 1–3 linked (**48a**, **49a**, **50a**, **51a**, **52a**) and 1–4 linked (**48b**, **49b**, **50b**, **51b**, **52b**) OSW-1 analogues have been synthesized in our lab (Fig. 2). Their conformation was carefully analyzed with NMR spectroscopy and molecule simulation in this paper. This study should facilitate the elucidation of SAR and be useful for designing more effective analogues.

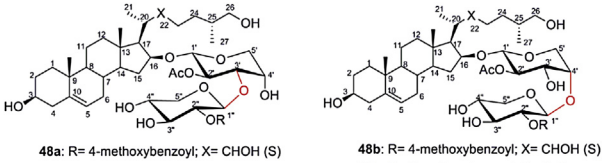
2. Results and discussion

2.1. Chemistry

Ten compounds were synthesized (**48a**, **48b**, **49a**,[16] **49b**, **50a**, **50b**, **51a**, **51b**, **52a**, **52b**) by adapting the strategy of coupling sapogenin with disaccharides (Fig. 2). Among them, cinnamoyl substituted analogues (**50a**, **50b**, **51a**, **51b**, **52a**, **52b**) and 22-OH-OSW-1 analogues (**48a**, **48b**) were reported for the first time.

Sapogenin **8** was synthesized in six steps by several groups previously.[16–18] The 3-OH of diosgenin was protected with *tert*-butyldimethylsilyl group, and the C-16 and 5(6)-double bond were oxy-functionalized with Oxone in the presence of $NaHCO_3$ to afford the mixture of epoxide diastereomers **3** and **4**.[18] In previous work,[16] the C_{26}–OH was protected with acetyl group during the F ring opened in the acidic condition, and the acetyl group was removed with CH_3OH/CH_3ONa to afford **5** and **6**. We found that treating the mixture of **3** and **4** with Zn/KI in AcOH, **5** and **6** could be directly afforded by undergoing ring-chain tautomerization in acidic condition (**5**:**6**=5:1) (Scheme 1). The mixture could be separated by flash column chromatography. **5** could be silylated to afford **7** in a yield of 92%. Dione **7** was then reduced with $NaBH_4/CeCl_3$ to provide 16β,22β-dihydroxyl aglycone **8**. The absolute configuration of C-22(S) was determined by Mosher's method.[16] Here using our method, sapogenin **8** was produced in five steps with a better yield.

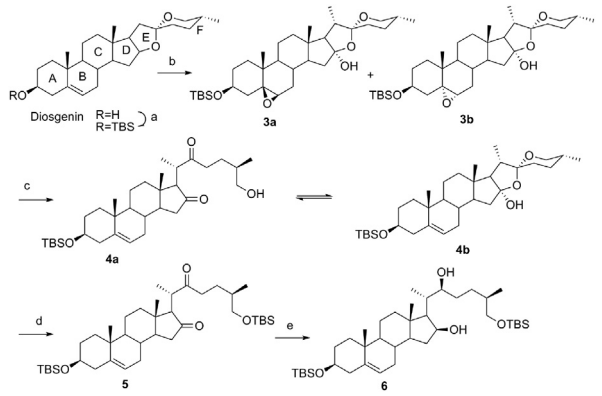
Sapogenin **10** was afforded from diosgenin via two steps.[19] Compound **9** was prepared by reaction of Zn and hydrochloric acid with diosgenin in ethanol under 50 °C in a yield of 71%. Then silylation of compound **9** with TBDMSCl in pyridine formed sapogenin **10** in a yield of 90% (Scheme 2).



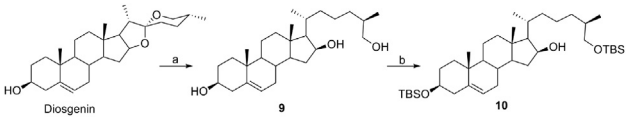
48a: R= 4-methoxybenzoyl; X= CHOH (S)
49a: R= 4-methoxybenzoyl; X= C=O
50a: R=(E)-cinnamoyl; X= CHOH (S)
51a: R=(E)-cinnamoyl; X= C=O
52a: R=(E)-cinnamoyl; X= CH_2

48b: R= 4-methoxybenzoyl; X= CHOH (S)
49b: R= 4-methoxybenzoyl; X= C=O
50b: R=(E)-cinnamoyl; X= CHOH (S)
51b: R=(E)-cinnamoyl; X= C=O
52b: R=(E)-cinnamoyl; X= CH_2

Fig. 2. Structures of synthesized OSW-1 analogues.

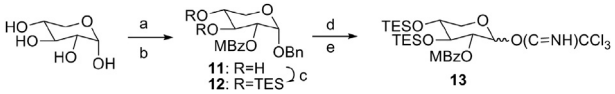


Scheme 1. Reagents and conditions: (a) TBDMSCl, DMAP, imidazole, DMF, 50 °C, 30 min, 99%; (b) Oxone, $NaHCO_3$, acetone/H_2O/CH_2Cl_2, rt, 48 h, 94%; (c) Zn powder, KI, AcOH, rt, 24 h, 64%; (d) TBDMSCl, DMAP, pyridine, rt, 6 h, 92%; (e) $NaBH_4/CeCl_3 \cdot 7H_2O$, THF, rt, 14 h, 73%.



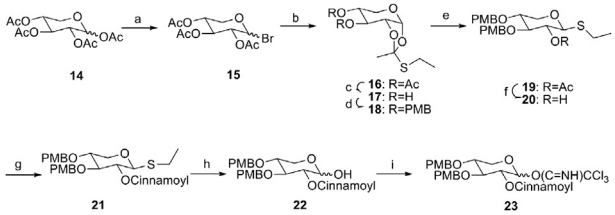
Scheme 2. Reagents and conditions: (a) Zn, HCl, ethanol, 50 °C, 20 min, 71%; (b) TBDMSCl, DMAP, pyridine, rt, 5 h, 90%.

Three monosaccharides (two donors **13**, **23** and one acceptor **29**) were designed and synthesised. The 4-methoxy benzoyl substituted xylose **13** was synthesised following Yu's route[20,21] (Scheme 3).



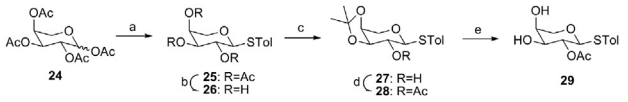
Scheme 3. Reagents and conditions: (a) BnOH, HCl, 50 °C, 24 h, 73%; (b) MBzCl, pyridine, −15 °C, 48 h, 65%; (c) TESCl, DMAP, pyridine, 93%; (d) H_2, Pd/C, MeOH, 50 atm, 50 °C, 24 h, 70%; (e) DBU, CCl_3CN, CH_2Cl_2, −5 °C, 5 h, 82%.

For synthesis of 2-cinnamoyl-D-xylose **23**, monosaccharide tetra-acetyl-D-xylose **14** was used as starting material[22] (Scheme 4). The thio ortho ester **16** was prepared via glycoside bromide **15**. After deacetylation and protection with 4-methoxybenzyl, **18** was obtained. Thio ortho ester **18** was converted to thioglycoside **19** by zinc chloride-promoted intramolecular ring opening in a yield of 87%. After deacetylation, cinnamoyl group was introduced at the C-2 position to afford **21** in a yield of 61%, sulfide at anomeric position was removed with NBS to afford **22**, which was subsequently converted to trichloroacetimidate **23** in a yield of 80%.[23] This is the first synthesis of 2-cinnamoyl-D-xylose trichloroacetimidate.



Scheme 4. Reagents and conditions: (a) 30% HBr–AcOH, CH_2Cl_2, 0 °C to rt, 4 h, 93%; (b) EtSH, 2,6-lutidine, $MeNO_2$, rt, 12 h, 82%; (c) CH_3ONa, MeOH, 25 °C, 3 h; (d) NaH, DMF, PMBCl, reflux, 4 h, 90%; (e) $ZnCl_2$, CH_2Cl_2, −60 °C to 0 °C, 87%; (f) CH_3ONa, MeOH, 25 °C, 4 h, 93%; (g) Cinnamic acid, DMAP, EDCI, CH_2Cl_2, rt, 24 h, 61%; (h) NBS, H_2O, CH_2Cl_2, rt, 1 h, 83%; (i) CCl_3CN, DBU, CH_2Cl_2, −5 °C, 5 h, 80%.

As an acceptor, the 1-thio-(4-methylphenyl)-2-*O*-acetyl-α-L-arabinopyranoside **29** was readily prepared from tetra-acetyl-L-arabinose (**24**) in five steps as illustrated[16] in Scheme 5.



Scheme 5. Reagents and conditions: (a) *p*-Thiocresol, $BF_3{\cdot}Et_2O$, CH_2Cl_2, 0 °C to rt, 8 h, 85%; (b) CH_3ONa, CH_3OH, rt, 2 h, 90%; (c) $(CH_3)_2C(OCH_3)_2$, CSA, DMF, under vacuum, 50 °C, 5 h, 95%; (d) Ac_2O, DMAP, Et_3N, CH_2Cl_2, rt, 1.5 h, 90%; (e) Amberlite IR-120(H^+), CH_3OH, 50 °C, 4 h, 81%.

Glycosylation of diol acceptor **29** with xylosyl donor **13** or **23** in presence of $BF_3{\cdot}Et_2O$ afforded corresponding disaccharides (**30** and **31**) or (**32** and **33**). Their structures were determined by 2D-NMR. Then the compounds (**30**, **31**, **32**, **33**) were exposed to silylation using TESCl in pyridine, sulfides at anomeric position were removed with NIS, disaccharides were then converted to trichloroacetimidates (**34**, **35**, **36**, **37**) under basic conditions (Scheme 6).

With the promotion of trimethylsilyl triflate (TMSOTf), coupling of 16β,22(*S*)-diol aglycone **8** with four disaccharide trichloroacetimidates (**34**, **35**, **36**, **37**) gave the corresponding 16-glycosidation saponins (**38**, **39**, **40**, **41**). Coupling of 16β-OH aglycone **10** with two disaccharide trichloroacetimidates (**35**, **37**) gave the corresponding 16-glycosidation saponins (**42**, **43**). Then removal of all protecting groups in two steps afforded target compounds **48a**, **48b**, **50a**, **50b**, **52a**, **52b** (Scheme 7). The saponins **38**, **39**, **40**, **41** were exposed to oxidation with pyridinium dichromate (PDC) to afford 22-ones (**44**, **45**, **46**, **47**). Removal of all protecting groups in two steps afforded four target compounds **49a**, **49b**, **51a**, **51b** (Scheme 8) benefit for forming such hydrophilic portion and hydrophobic cluster.

2.2. Conformation analysis

In order to study the conformational difference between 1–3 and 1–4 linked analogues, many assays had been made to cultivate crystal. Unfortunately, we did not get the single crystal because of their poor physicochemical properties. Structures of target compounds were studied with ^1H NMR, ^{13}C NMR, DEPT, NOESY, COSY, HSQC, HMBC. ^{13}C and ^1H signals for all compounds were assigned in detail. Four significant differences were observed by analyzing the NMR dates which showed the conformation was different between the 1–3 linked and 1–4 linked analogues.

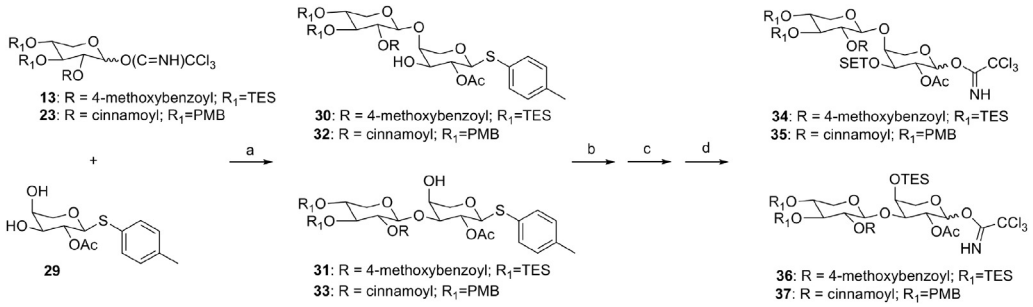
The first significant difference was that $\delta H_1'$ of C_{22}-carbonyl analogues (**49a**, **49b**, **51a**, **51b**) shifted to high-field compared with C_{22}-methylene (**52a**, **52b**) or C_{22}-hydroxymethyl (**48a**, **48b**, **50a**, **50b**) analogues. The $\delta H_1'$ of arabinose moieties was about 4.0 ppm for C_{22}-carbonyl analogues (Table 1), while the $\delta H_1'$ was about 4.3 ppm for C_{22}-methylene or C_{22}-hydroxymethyl analogues (Table 2). This shift indicated that H_1' of arabinose for C_{22}-carbonyl analogues located in shielding zone of carbonyl and implied that H_1' of arabinose was near by C_{22} of side chain.

Second, a significant difference between **48a** and **48b** was that the coupling constant of H_1' and H_5' in the portion of arabinose in ^1H NMR. **48a** arabinose moiety: δ (ppm): 4.27 (d, J=7.2 Hz, H_1'); 3.89 (dd, J=3.0 Hz, J=12.5 Hz, H_{5a}'); 3.55 (dd, J=1.3 Hz, J=12.5 Hz, H_{5b}'), while **48b**: 4.32 (d, J=5.5 Hz, H_1'); 4.00 (dd, J=5.0 Hz, J=12.0 Hz, H_{5a}'); 3.53 (dd, J=2.4 Hz, J=12.0 Hz, H_{5b}') (Table 3). The data demonstrated that the arabinose part was in chair conformation for **48a**, but approximate in boat conformation for **48b**. In addition, this result was also confirmed by NOESY. A strong H_1'–H_4' dipolar interaction could be observed from **48b** while is not expected in **48a** (Supplementary data). This phenomenon could be ascribed to a large substituent group attached at 4-position of arabinose part as pseudoequatorial in a boat conformation for **48b**. The skew boat conformation was benefit for lower molecular energy and more stable. The difference could also be observed in **50a** and **50b**, **51a** and **51b** correspondingly.

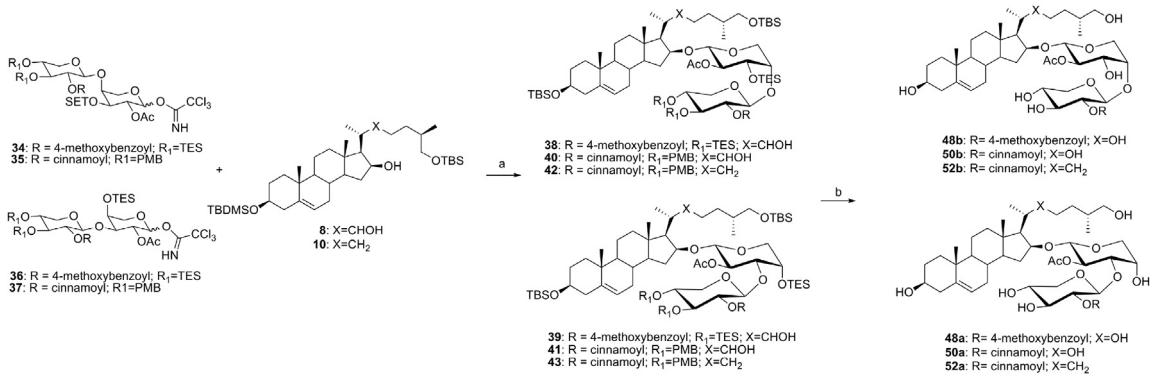
Third, compared the ^{13}C spectra of 1–3 and 1–4 linked sapogenin analogues with their relative disaccharides, a significant difference could be observed. δC_3 of arabinose of 1–3 linked sapogenins (**48a**, **50a**, **51a**) shifted to low field. The $\Delta\delta$ was approximate 3.7–7.0 ppm. However, 1–4 linked sapogenins (**48b**, **50b**, **51b**) δC_4 shifted to high field (Table 4). The $\Delta\delta$ was approximate −4.0 ppm. ^{13}C chemical shift is sensitive to stereochemistry. Space effect is an important factor to the ^{13}C chemical shift. This difference could be attributed to the conformation discrepancy of these compounds.

Fourth, in the 2D-NOESY spectra for compounds **48**–**52**, weak NOE crosspeaks were observed between H_{27} and $H_{33/35}$, $H_{32/36}$, and between H_{29} and $H_{32/36}$ in compound **48a** (Fig. 3). It indicated the close proximity between the C_2''-*p*-methoxybenzoyl (*p*-MBz) group and the C_2'-acetyl group, the C_{27} methyl groups of the C_{20}–C_{27} sterol side chain. Similarly for compound **51a**, NOEs were detected between H_{27} and H_{31}, H_{32}, $H_{35/37}$, $H_{34/38}$ and between H_{29} and H_{31}, $H_{35/37}$, $H_{34/38}$. This phenomenon was consistent with Sakurai's research report,[15] they suggested that the overall molecular shape of OSW-1 was a flat right triangle. However, such correlation was not found in **48b**, neither in **49b**, **50b**, **51b** and **52b**. This meant the conformation was different between the 1–3 linked and 1–4 linked analogues.

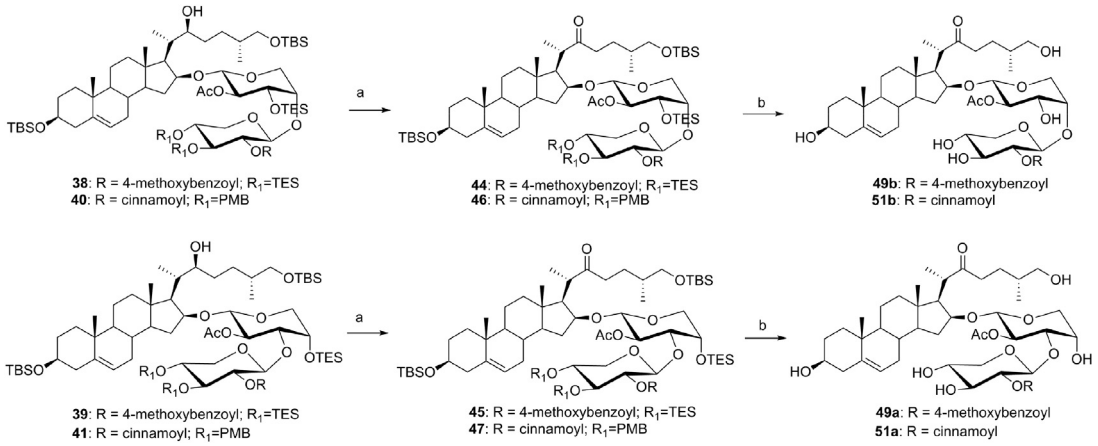
The results of molecule simulation showed that (Fig. 4): the conformation of arabinose in 1–3 linked analogues was the chair conformation, while that of 1–4 linked analogues was the skew boat conformation. These results were consistent with the analysis of ^1H NMR previously. Another phenomenon was observed in the molecule simulation, acetyl and side chain C_{27}



Scheme 6. Reagents and conditions: (a) 4 Å MS, $BF_3{\cdot}Et_2O$, CH_2Cl_2, −60 °C, 3 h, 36% for **30**, 18% for **31**, 21% for **32**, 13% for **33**; (b) TESCl, DMAP, pyridine; (c) NIS, H_2O, CH_2Cl_2, 1 h; (d) CCl_3CN, DBU, CH_2Cl_2, 3 h, three steps 56% for **34**, 61% for **35**, 64% for **35**, 52% for **37**.



Scheme 7. Reagents and conditions: (a) 4 Å MS, TMSOTf, CH_2Cl_2, −60 °C, 3 h, 64% for **38**, 58% for **39**, 51% for **40**, 47% for **41**, 55% for **42**, 43% for **43**; (b) i) DDQ, $Pd(CH_3CN)_2Cl_2$, CH_2Cl_2/actone/H_2O, rt, 5 h, 59% for **50a**, 54% for **50b**, 48% for **52a**, 61% for **52b**; ii) $Pd(CH_3CN)_2Cl_2$, actone/H_2O, rt, 3 h, 59% for **48a**, 67% for **48b**.



Scheme 8. Reagents and conditions: (a) PDC, CH_2Cl_2, rt, 6 h, 96% for **44**, 67% for **45**, 80% for **46**, 74% for **47**; (b) i) DDQ, $Pd(CH_3CN)_2Cl_2$, CH_2Cl_2/actone/H_2O, rt, 5 h, 52% for **51a**, 53% for **51b**; ii) $Pd(CH_3CN)_2Cl_2$, actone/H_2O, rt, 3 h, 62% for **49a**, 57% for **49b**.

Table 1
$\delta H_1'$ of analogues which C_{22} is carbonyl

	49a	49b	51a	51b
$\delta H_1'$	4.00	3.96	4.06	4.09

Table 2
$\delta H_1'$ of analogues which C_{22} is methylene or hydroxymethyl

	48a	48b	50a	50b	52a	52b
$\delta H_1'$	4.29	4.31	4.27	4.32	4.28	4.27

methyl and xylose substituted aromatic ring were close to each other in 1–3 linked analogues, the distance of aromatic ring to acetyl was 3.11 Å, that to H_{27} was 3.35 Å. These results were conformity with the NOESY research previously. The ^1H NMR spectra showed that C_{22} was close to H_1'. Based on the results of ^1H NMR and molecule simulation, we speculated that the 1–3 linked analogues had flat triangular structures. The flat triangular conformation of 1–3 linked analogues had a hydrophilic portion which composed by O_3'', O_4'' and O_5'' of the xylose residue and O_4' of arabinose residue. The C_2''-acylate and C_2'-acetate groups and the C_{20}–C_{27} sterol side chain pack against each other which resulted the generation of a hydrophobic cluster. This overall conformation was important for its biological functionalities. On the contrary, the results of molecule simulation showed that the distance of acetyl and the side chain and aromatic ring was farther in 1–4 linked analogues. The distance of aromatic ring to acetyl was 5.59 Å, that to H_{27} was 3.88 Å, and the arabinose was the skew boat conformation. The change of arabinose conformation led to the overall molecular conformation changed, which had a non flat triangular structure compared with 1–3 linked analogues. Those data demonstrated that the 1–4 linked analogues presented disperse structure which was not benefit for forming such hydrophilic portion and hydrophobic cluster.

Table 3
The ^1H NMR analysis of arabinose fragment for analogues (**48a**, **48b**, **50a**, **50b**, **51a**, **51b**)

	48a δ	48b δ
H_1'	4.27 (d, J=7.2)	4.32 (d, J=5.5)
H_5'	3.89 (dd, J=3.0, J=12.5)	4.00 (dd, J=5.0, J=12.0)
	3.55 (dd, J=1.3, J=12.5)	3.53 (dd, J=2.4, J=12.0)
	50a δ	**50b δ**
H_1'	4.29 (d, J=7.1)	4.31 (d, J=6.0)
H_5'	3.87 (dd, J=3.0, J=12.6)	3.99 (dd, J=4.5, J=12.1)
	3.54 (dd, J=1.3, J=12.6)	3.54 (dd, J=2.2, J=12.1)
	51a δ	**51b δ**
H_1'	4.06 (d, J=6.9)	4.09 (d, J=5.7)
H_5'	3.85 (dd, J=3.1, J=12.5)	3.94 (dd, J=4.6, J=11.9)
	3.49 (dd, J=1.6, J=12.5)	3.49 (dd, J=2.0, J=11.9)

Table 4
The ^{13}C NMR analysis of arabinose fragment

Compound	Position	Unlinked[a]	Linked[b]	$\Delta\delta$[c]
48a	C_3'	74.0	81.0	7.0
48b	C_4'	75.0	71.0	−4.0
50a	C_3'	75.9	79.6	3.7
50b	C_4'	75.3	71.1	−4.2
51a	C_3'	75.9	80.8	4.9
51b	C_4'	75.3	71.2	−4.1

[a] Disaccharide which did not attach to saponin.
[b] Target compound which disaccharide attached to saponin.
[c] $\Delta\delta=\delta_{linked}-\delta_{unlinked}$.

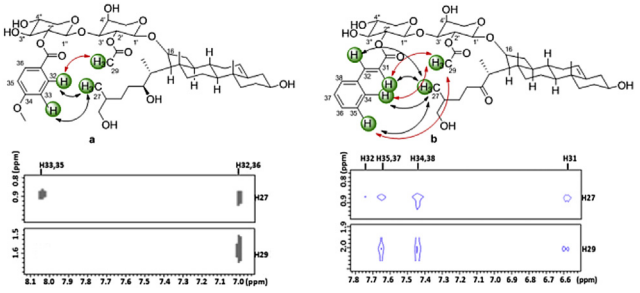


Fig. 3. Selected NOESY correlations (600 MHz, CD_3OD) for compounds **48a** (a) and **51a** (b). NOEs shown by black and red arrows (the corresponding crosspeaks in the NOESY spectra are shown in bottom panels).

2.3. Antitumor activities in vitro

The synthetic OSW-1 analogues **49a**, **49b**, **51a**, **51b**, were screened against HCT-116, MDA-MB-231, A375, BGC-823, ACHN, SW1990, A2780 and HepG2 cell lines by MTT assay. Among them **49a** and **49b** were considered as positive control. Both **49a** and **51a** were far more effective against all of the tested tumor cells than **49b** and **51b** (Table 5) in which of BGC823 and A2780 were most sensitive at nanomolar concentration level. The activity test of **49a** and **51a** were repeated three times to insure their accuracy. The cytotoxicity (IC_{50}) value of **49a** was 2.7×10^{-10} mol/L to BGC823 and 1.2×10^{-10} mol/L to A2780, the value of **51a** was 6.8×10^{-10} mol/L to BGC823 and 7.1×10^{-10} mol/L to A2780. While the value of **49b** was only 2.7×10^{-5} mol/L to BGC823 and 1.9×10^{-6} mol/L to A2780, the value of **51b** was 3.7×10^{-5} mol/L to BGC823 and 2.2×10^{-5} mol/L to A2780. In order to verify the reliability and expand the types of tumor cell lines, another test was repeated, which showed high *anti*-proliferation activities for **49a** and **51a** to SH-SY5Y, SK-N-SH, T98G, Daoy, NCI-H1975, NCI-H1703, HCT116, BGC823, Capan2, SW1990, HepG2, A375, ACHN, MDA-MB-231, A2780, PC-3 and Pfeiffer cell lines (Table 6). Pfeiffer was the most sensitive cell line to **49a** and **51a**, the IC_{50} values of **49a** and **51a** were 5.3×10^{-9} mol/L and 7.2×10^{-11} mol/L to Pfeiffer, respectively. Analogues **52a** and **52b** were tested against HCT-116, NCI-H1975, Capan2, SW1990, SK-N-SH, BGC-823, and HepG2 cell lines (Table 7). Activity of **52a** was also much higher than **52b**, the IC_{50} value of **52a** was 1.2×10^{-12} mol/L to HepG2, 2.1×10^{-12} mol/L to BGC823, while **52b** showed inactivity to all of those tumor cells.

An interesting phenomenon was observed from antitumor activity results, the higher antiproliferative activity was displayed by compounds containing 1–3 linked disaccharide which had flat triangular structures (**49a**, **51a**, **52a**) at nanomolar concentration level. 1–4 linked analogues which had non flat triangular structures (**48b**, **49b**, **51b**, **52b**) showed a lower activity. The key role of flat triangular molecular shape of 1–3 linked OSW-1 analogues for the high activity was confirmed. Notably, the activity of **52a** which aglycone C_{22} was methylene was higher compared with **49a** and **51a** which C_{22} was carbonyl. This indicated that the C_{22} of cholestane side chain which reduction form seemed could boost its activity by almost 10 times.

3. Conclusion

In conclusion, 10 OSW-1 analogues were synthesized and the conformation of analogues was studied by NMR analysis and molecule simulation. The arabinose moiety of 1–3 linked analogues was in chair conformation and 1–4 linked analogues was in boat conformation. The antitumor activity of 1–3 linked analogues which present flat triangular structure was much higher than that of 1–4 linked analogues which present non flat triangular structure. From the relationship between conformation and activity, a conclusion was presumed that the flat triangular conformation of OSW-1 analogues played a key role in their high antitumor activity. The non flat triangular conformation which showed by 1–4 linked analogues was unfavorable for its activity.

This study presents a considerable opportunity for the development of new OSW-1 analogues as antitumor candidates.

4. Experimental section

4.1. Materials and method in biological tests

4.1.1. Materials. Human cancer cell lines, SH-SY5Y, SK-N-SH, T98G, Daoy, NCI-H1975, NCI-H1703, HCT116, BGC823, Capan2, SW1990, HepG2, A375, ACHN, MDA-MB-231, A2780, PC-3, and Pfeiffer were purchased from the American Type Culture Collection (ATCC) and Cell Culture Center of Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences. All the cell lines were cultured in RPMI-1640 media supplemented with 10% fetal calf serum, penicillin (100 μg/mL) and streptomycin (100 μg/mL) (Gibco BRL, NY, USA), and incubated at 37 °C in a humidified air atmosphere

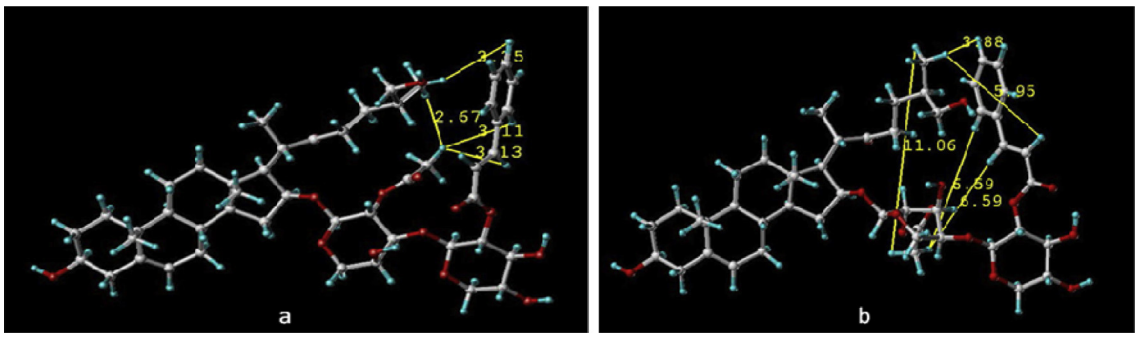


Fig. 4. Molecule simulation schematic for **49a** (a) and **49b** (b).

Table 5
Antitumor activities of compounds **49a**, **49b**, **51a**, **51b** against tumor cells[a]

OSW-1 derivative	Cell line; IC_{50} (mmol/L)							
	HCT116	MDA-MB-231	A375	BGC823	ACHN	SW1990	A2780	HepG2
49a	**0.0086**	**0.015**	**0.038**	**0.00027**	**0.03**	**0.71**	**0.00012**	**0.15**
49b	24	24	18	27	28	>50	1.9	23
51a	**0.0053**	**0.00012**	**0.045**	**0.00068**	**0.0026**	**0.026**	**0.00071**	**0.16**
51b	>50	2.60	20	37	>50	>50	22	>50

[a] The antitumor activities against HCT-116 (colon carcinoma), MDA-MB-231 (breast cancer), A375 (melanoma cell), BGC-823 (stomach carcinoma), ACHN (renal cancer), SW1990 (pancreatic cancer), A2780 (ovarian cancer) and HepG2 (liver cancer) cell lines in vitro were evaluated by the MTT assay.

Table 6
Antitumor activities of compounds **49a** and **51a** against tumor cells[a]

	IC_{50}(mmol/L)								
	SH-SY5Y	SK-N-SH	T98G	Daoy	NCI-H1975	NCI-H1703	HCT116	BGC823	Capan2
49a	0.025	0.63	0.0019	0.0035	0.075	0.021	0.024	0.00027	1.9
51a	0.0089	0.48	0.0008	0.00023	0.002	0.0092	0.073	0.00068	0.43
	SW1990	HepG2	A375	ACHN	MDA-MB-231	A2780	PC-3	Pfeiffer	—
49a	0.71	0.14	0.038	0.03	0.015	0.00018	0.13	0.0053	—
51a	0.027	0.16	0.045	0.0026	0.00012	0.00073	0.095	0.000072	—

[a] The antitumor activities against SH-SY5Y, SK-N-SH (neuroblastoma), T98G (glioblastoma), Daoy (medulloblastoma), NCI-H1975, NCI-H1703 (lung cancer), HCT116 (colon cancer), BGC823 (gastric cancer), Capan2, SW1990 (pancreatic cancer), HepG2 (HCC) A375 (melanoma), ACHN (RCC), MDA-MB-231 (breast cancer), A2780 (ovarian cancer), PC-3 (prostate cancer), and Pfeiffer (lymphoma) cell lines in vitro were evaluated by the MTT assay.

Table 7
Antitumor activities of compounds **52a** and **52b** against tumor cells[a]

OSW-1 derivative	Cell line; IC_{50} (mmol/L)						
	HCT116	NCI-H1975	Capan2	SW1990	SK-N-SH	BGC823	HepG2
52a	**0.00043**	**0.00012**	**0.024**	**0.013**	**0.0012**	**0.000021**	**0.0000012**
52b	>1	>1	>1	>1	>1	>1	>1

[a] The antitumor activities against HCT-116 (colon carcinoma), NCI-H1975 (lung cancer), Capan2 (pancreatic cancer), SW1990 (pancreatic cancer), SK-N-SH (neuroblastoma), BGC-823 (stomach carcinoma), and HepG2 (liver cancer) cell lines in vitro were evaluated by the MTT assay.

containing 5% CO_2. All cells were harvested in their exponentially growing phase.

4.1.2. MTT assay. Cells were seeded into 96-well plates at a concentration of 1000–2000 cells/well and left there to adhere the plate overnight. Then cells were incubated for another 72 h in the absence and presence of various compounds and each condition was performed with three wells. After removal of supernatant, cells were treated with 0.5 mg/mL MTT for 4 h, the purple blue sediment was dissolved in 200 μL DMSO. The relative optical density (OD)/well was determined at a test wavelength of 570 nm in a WELLSCAN MK3 ELIASA (Labsystems, Dragon, Finland) using a 450 nm reference filter. The 50% inhibitory concentration (IC_{50}) was calculated from the linear equation, which was educed by concentration versus growth inhibition regression curve.

4.2. Synthetic experiments and spectra data

General Methods: Optical rotatings were recorded in a Perkin–Elmer 341 LC polarimeter. ^{1}H NMR spectra were recorded at 300, 400, 500 or 600 MHz with chemical shifts reported in ppm (δ) in reference to the solvent peak. ^{13}C NMR spectra were recorded at 75, 100, 125 or 150 MHz with chemical shifts reported in ppm (δ) in reference to the solvent peak. The coupling constants (J) are reported in hertz (Hz). HRMS were recorded on an Agilent 1100 series LC/MSD TOF. Thin layer chromatography (TLC) was performed on silica gel GF254 plates detected by charring with 5% sulfuric acid in ethanol solution. Chromatography was performed on silica gel (HG/T2354-92) or 200–300 mesh and Sephadex LH-20 (GE Healthcare). Molecular sieves 4 Å used were dried under high vacuum at 170–180 °C for 10–15 h before use. Commercial anhydrous solvents and reagents were used without further purification.

4.3. Method of molecule simulation

The partial initial conformation of the molecule comes from the published crystal structure of **2**[15] (Fig. 1), the initial conformation of 1–3 linked analogues was constructed based on the three-dimensional crystal structures of **2**, 1–4 linked analogues was manually built from atoms using the Sketch module of Sybyl (Tripos Inc., St. Louis, MO, USA) on Dell workstation. They were minimized by the Maximin module of Sybyl using Tripos force field and partial charges of Gasteiger–Marsili method implemented in Sybyl.[24] The conformational searches were also applied before the minimization to get the possible different low energy conformation.

4.4. Chemical synthesis

4.4.1. Purity. Purity of final compounds **48a**, **48b**, **49a**, **49b**, **51a**, **51b**, **52a**, **52b** were found to be ≥95% as determined by HPLC analysis. HPLC was performed with LabAlliance apparatus comprising pumps (III Pump series), a UV–vis detector (525 dual wavelength), and injection valve (Rheodyne model 7725i). Analytical HPLC was carried out with a Phenomenex Luna C_{18} column (5 μm), 0.46 cm–25 cm. The eluting solvent was MeCN/water. The flow rate was 0.8 mL/min. The elution pattern of products was monitored continuously by UV at 272 nm. The purity and retention times of final products were as follows: **48a** (97.4%, 11.53 min, MeCN/water 60:40), **48b** (95.6%, 12.91 min, MeCN/water 60:40),

49a (95.2%, 20.78 min, MeCN/water 45:55), **49b** (97.1%, 27.39 min, MeCN/water 40:60), **51a** (98.6%, 11.00 min, MeCN/water 60:40), **51b** (95.8%, 20.17 min, MeCN/water 45:55), **52a** (99.3%, 8.78 min, MeCN/water 65:35), **52b** (98.1%, 21.95 min, MeCN/water 45:55).

*4.4.2. 3β-O-tert-Butyldimethylsilyl kryptogenin (**5**)/3β-O-tert-butyldimethylsilyl 16-hydroxyl-diosgenin (**6**).* A solution of **3** and **4** (0.50 g, 0.89 mmol) in 10 mL HOAc was added KI (0.72 g, 8.9 mmol), Zn (0.55 g, 4.45 mmol), after stirring for 20 h at room temperature and then filtered. The filtrates were diluted with 30 mL CH_2Cl_2, the solution was washed with water, saturated $NaHCO_3$, brine, dried over $MgSO_4$, the concentrate was purified by column chromatography on silica gel (petroleum ether: ethyl acetate=5:1) to afford compound **5** and **6** (0.29 g, 60% for **5**, 0.058 g, 12% for **6**).

*4.4.3. 3β,26-O-Bis(tert-butyldimethylsilyl) kryptogenin (**7**).* To a solution of **5** (1.00 g, 1.80 mmol), DMAP (0.24 g, 1.80 mmol) in dry pyridine (10 mL) was added TBDMSCl (0.55 g, 3.60 mmol). After being stirred for 6 h, then saturated Na_2SO_3 aq was added in and stirred 5 min. The solution was diluted with 50 mL CH_2Cl_2 and washed with $NaHCO_3$, brine, then dried over $MgSO_4$ and concentrated in vacuo, the residue was purified by column chromatography on silica gel (petroleum ether:ethyl acetate=8:1) to afford compound **7** (1.10 g, 92%).

*4.4.4. (22S,25R)-3β,26-Bis (tert-butyldimethylsilyloxy)-16,22-dihydroxyl-cholest-5-ene (**8**).* A solution of **7** (2.70 g, 4.00 mmol) in 15 mL dry THF was added $NaBH_4$ (7.50 g, 0.20 mol), $CeCl_3 \cdot 7H_2O$ (5.90 g, 0.016 mol), after stirring for 14 h at room temperature the solution was diluted with 100 mL CH_2Cl_2, the solution was washed with saturated $NaHCO_3$, brine, dried over $MgSO_4$, the concentrate was purified by column chromatography on silica gel (petroleum ether:ethyl acetate=3:1) to afford compound **8** (2.00 g, 73%).

*4.4.5. (25R)-Cholest-5-ene-3β,16β,26-triol (**9**).* A solution of diosgenin (2.00 g, 4.80 mmol) in 300 mL dry EtOH, Zn powder (45.00 g, 0.69 mol) was added under drastic stirring, heating to 50 °C, then dropping dilute hydrochloric acid to the solution during 40 min, after stirred for 30 min, and then filtered. The filtrates washed with water, saturated $NaHCO_3$, brine, dried over $MgSO_4$, the concentrate was purified by column chromatography on silica gel (petroleum ether:ethyl acetate=4:1) to afford compound **9** (1.40 g, 71%).

*4.4.6. (25R)-3β,26-Bis (tert-butyldimethylsilyloxy)-cholest-5-en-16β-ol (**10**).* To a solution of **9** (7.80 g, 0.019 mol) in dry Pyridine (80 mL) was added imidazole (5.40 g, 0.079 mol), DMAP (0.23 g, 0.19 mmol) and TBDMSCl (8.40 g, 0.056 mol). After being stirred for 5 h at room temperature, then saturated $NaHCO_3$ aq was added in and stirred 5 min. The solution was diluted with 500 mL CH_2Cl_2 and washed with 1 mol/L HCl, saturated $NaHCO_3$, brine, then dried over $NaSO_4$ and concentrated in vacuo, the residue was purified by column chromatography on silica gel (petroleum ether:ethyl acetate=8:1) to afford compound **10** (11 g, 90%).

*4.4.7. Ethyl 3,4-di-O-(4-methoxybenzyl)-2-O-[(E)-cinnamoyl]-1-thio-β-D-xylopyranoside (**21**).* A solution of **20** (0.20 g, 0.46 mmol) in 5.0 mL CH_2Cl_2 was added DMAP (50 mg, 0.41 mmol), EDCI(0.068 mg, 0.35 mmol) and cinnamic acid, after stirring for 24 h at room temperature, the solution was concentrated in vacuum, then the residue was purified by column chromatography on silica gel (petroleum ether:ethyl acetate=10:1) to afford compound **21** (0.16 g, 61%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.67 (d, J=16.0 Hz, 1H), 7.51 (m, 2H), 7.39 (m, 3H), 7.24 (d, J=8.6 Hz, 2H), 7.19 (d, J=8.6 Hz, 2H), 6.85 (d, J=8.6 Hz, 2H), 6.77 (d, J=8.6 Hz, 2H), 6.35 (d, J=16.0 Hz, 1H), 5.06 (t, J=8.6 Hz, 1H, H-2), 4.73 (d, AB, J=11.3 Hz, 1H), 4.65 (d, AB, J=12.0 Hz, 2H), 4.55 (d, AB, J=11.3 Hz, 1H), 4.48 (d, J=8.9 Hz, 1H, H-1), 4.06 (dd, J=4.1 Hz, J=11.6 Hz, 1H, H-5a), 3.79 (s, 3H), 3.67 (s, 3H), 3.65−3.63 (m, 2H), 3.29 (dd, J=9.3 Hz, J=11.6 Hz, 1H, H-5b), 2.67 (m, 2H), 1.23 (t, J=7.5 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 165.5, 159.3, 159.0, 145.4, 134.2, 130.3, 130.2, 130.0, 129.6, 129.4, 128.8, 128.1, 128.1, 117.5, 113.8, 113.6, 83.8, 80.9, 76.9, 74.1, 72.7, 71.1, 66.9, 55.1, 55.0, 24.0, 14.7. $C_{32}H_{36}O_7S$, HRMS (ESI): calcd for ($M+H^+$): 565.2260; found: 565.2275.

*4.4.8. 3,4-Di-O-(4-methoxybenzyl)-2-O-[(E)-cinnamoyl]-D-xylopyranose (**22**).* NBS (0.24 g, 1.10 mmol) was added in a solution of **21** (0.50 g, 0.88 mmol) in 10 mL CH_2Cl_2 with 0.50 mL H_2O, after stirring 1 h at room temperature, then saturated Na_2SO_3 aq was added in and stirred 15 min the solution was washed with $NaHCO_3$, brine, dried over $MgSO_4$, the concentrate was purified by column chromatography on silica gel (petroleum ether:ethyl acetate=5:1) to afford compound **22** (0.38 g, 83%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.75 (d, J=16.0 Hz, 1H), 7.52 (m, 2H), 7.40 (m, 3H), 7.30−7.24 (m, 4H), 6.90 (d, J=8.6 Hz, 2H), 6.87 (d, J=8.6 Hz, 2H), 6.49 (d, J=16.0 Hz, 1H), 6.20 (d, J=2.5 Hz, 1H, H-1), 4.77 (dd, AB, J=11.0, J=40.7 Hz, 2H), 4.62 (dd, AB, J=11.2, J=17.8 Hz, 2H), 4.30 (t, J=6.7 Hz, 1H, H-2), 3.86 (m, 1H), 3.80 (s, 3H), 3.80 (s, 3H), 3.73 (m, 1H), 3.62 (m, 1H), 2.60 (d, J=5.2 Hz, 1H, OH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 165.4, 159.4, 159.3, 146.3, 134.0, 130.8, 130.5, 130.2, 129.6, 129.5, 128.9, 128.8, 128.2, 117.1, 113.9, 91.9, 79.4, 76.2, 74.3, 72.5, 70.3, 65.5, 62.8, 55.2. $C_{30}H_{32}O_8$, HRMS (ESI): calcd for ($M+H^+$): 521.2175; found: 521.2181.

*4.4.9. 3,4-Di-O-(4-methoxybenzyl)-2-O-[(E)-cinnamoyl]-α/β-D-xylopyranosyl trichloroacetimidate (**23**).* The compound **22** (0.93 g, 1.80 mmol) was dissolved in dried CH_2Cl_2, added $CNCCl_3$ (1.80 mL, 0.018 mol) and DBU (0.067 mL, 0.52 mmol) under Ar at -5 °C, after stirring for 5 h, the concentrate was purified by column chromatography on silica gel (petroleum ether:ethyl acetate=10:1) to afford compound **23** (1.10 g, 80%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.53 (s, 1H, NH), 7.60 (d, J=16.0 Hz, 1H), 7.48 (m, 2H), 7.39 (m, 3H), 7.27 (d, J=8.8 Hz, 2H), 7.22 (d, J=8.8 Hz, 2H), 6.88 (d, J=8.6 Hz, 2H), 6.78 (d, J=8.6 Hz, 2H), 6.42 (d, J=3.6 Hz, 1H, H-1), 6.28 (d, J=16.0 Hz, 1H), 5.10 (dd, J=3.6, J=9.8 Hz, 1H), 4.82 (d, AB, J=11.1 Hz, 1H), 4.72 (dd, AB, J=3.9, J=11.1 Hz, 2H), 4.61 (d, AB, J=11.1 Hz, 1H), 4.30 (t, J=6.7 Hz, 1H), 4.06 (t, J=8.4 Hz, 1H), 3.83−3.74 (m, 2H), 3.80 (s, 3H), 3.68 (s, 3H). $C_{32}H_{32}Cl_3NO_8$, HRMS (ESI): calcd for ($M+H^+$): 664.1272; found: 664.1271.

*4.4.10. 4-Methylphenyl (2-O-(4-methoxybenzoyl) -3,4-di-(O-triethylsilyl)-β-D-xylopyranosyl-(1→4)-2-O-acetyl-3-hydroxy-1-α-L-arabinopyranoside(**30**)/4-methylphenyl (2-O-(4-methoxybenzoyl)-3,4-di-(O-triethylsilyl)-β-D-xylopyranosyl-(1→3)-2-O-acetyl-4-hydroxy-1-α-L-arabinopyranoside (**31**).* The mixture of compound **13** (0.58 g, 0.88 mmol) and **29** (0.40 g, 1.30 mmol) was added 4 Å MS in dried CH_2Cl_2 under Ar at −60 °C, then the $BF_3 \cdot Et_2O$ (0.011 mL, 0.044 mmol) was slowly added, after stirring for 3 h, Et_3N was added, MS was filtered and filtrate was concentrated, then the residue was purified by column chromatography on silica gel (petroleum ether:ethyl acetate=8:1) to afford compound **30** (0.25 g, 36%) and **31** (0.13 g, 18%). (**30**) ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.02 (d, J=9.0 Hz, 2H), 7.36 (d, J=8.0 Hz, 2H), 7.08 (d, J=8.0 Hz, 2H), 6.92 (d, J=9.0 Hz, 2H), 5.06 (dd, J=5.9 Hz, J=6.2 Hz, 1H, H-2), 4.99 (dd, J=6.2 Hz, J=7.2 Hz, 1H, H-2′), 4.82 (d, J=5.3 Hz, 1H, H-1), 4.66 (d, J=6.0 Hz, 1H, H-1′), 4.29 (dd, J=6.6 Hz, J=11.9 Hz, 1H, H-5a), 4.03 (dd, J=4.4 Hz, J=11.9 Hz, 1H, H-5a′), 3.91 (dd, J=3.4 Hz, J=6.6 Hz, 1H, H-4), 3.81 (s, 3H), 3.78 (t, J=7.2 Hz, 1H, H-3′), 3.70 (m, 2H, H-3, H-4′), 3.58 (dd, J=3.2 Hz, J=12.1 Hz, 1H, H-5b′), 3.28 (dd, J=7.9 Hz, J=11.6 Hz, 1H, H-5b), 2.57 (d, J=6.0 Hz, 1H, OH), 2.31 (s, 3H), 2.06 (s, 3H), 0.96 (t, J=8.0 Hz, 9H), 0.88 (t, J=8.0 Hz, 9H), 0.63 (q, J=8.1 Hz, 6H), 0.55 (q, J=8.1 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.5, 165.3, 163.5, 137.4, 132.0(2× Ar), 131.8(2× Ar), 131.4, 129.6(2× Ar),

122.0, 113.6(2× Ar), 101.5(C-1′), 86.3(C-1), 75.0(C-4), 74.1(C-3′), 73.6(C-2′), 72.1(C-2), 70.8(C-4′), 69.4(C-3), 65.0(C-5′), 63.1(C-5), 55.4(−O*C*H$_3$), 21.0(Ar−*C*H$_3$), 20.9(*C*H$_3$−CO−), 6.8(−CH$_2$−*C*H$_3$), 5.0(−*C*H$_2$−CH$_3$), 4.9(−*C*H$_2$−CH$_3$). $C_{39}H_{60}O_{11}SSi_2$, HRMS (ESI): calcd for (M+Na$^+$): 815.3293 found: 815.3288.

(**31**) ^{1}H NMR (300 MHz, CDCl$_3$): δ 8.05 (d, J=8.9 Hz, 2H), 7.11 (d, J=7.9 Hz, 2H), 7.00 (d, J=7.9 Hz, 2H), 6.89 (d, J=8.9 Hz, 2H), 5.16 (dd, J=3.4 Hz, J=4.3 Hz, 1H, H-2), 5.06 (t, J=5.9 Hz, 1H, H-2′), 4.93 (d, J=3.2 Hz, 1H, H-1), 4.84 (d, J=5.6 Hz, 1H, H-1′), 4.12 (dd, J=7.8 Hz, J=10.7 Hz, 1H, H-5a), 4.05 (dd, J=4.3 Hz, J=12.6 Hz, 1H, H-5a′), 3.99 (dd, J=3.8 Hz, J=6.6 Hz, 1H, H-3), 3.97 (m, 1H, H-4), 3.85 (s, 3H), 3.83 (t, J=7.1 Hz, 1H, H-3′), 3.69 (m, 1H, H-4′), 3.58 (dd, J=4.1 Hz, J=11.4 Hz, 1H, H-5b), 3.35 (dd, J=6.8 Hz, J=11.8 Hz, 1H, H-5b′), 2.97 (d, J=8.6 Hz, 1H, OH), 2.27 (s, 3H), 1.97 (s, 3H), 0.94 (t, J=7.8 Hz, 9H), 0.90 (t, J=7.8 Hz, 9H), 0.60 (q, J=7.8 Hz, 6H), 0.60 (q, J=7.8 Hz, 6H). ^{13}C NMR (100 MHz, CDCl$_3$): δ 169.4, 164.8, 163.3, 137.1, 132.0(2× Ar), 131.8(2× Ar), 131.6, 129.4(2× Ar), 122.8, 113.4(2× Ar), 99.0(C-1′), 85.7(C-1), 74.0(C-3), 73.1(C-3′), 72.0(C-2′), 70.4(C-4′), 70.2(C-2), 64.6(C-4), 64.3(C-5′), 62.2(C-5), 55.3(−O*C*H$_3$), 21.0(Ar−*C*H$_3$), 20.8(*C*H$_3$−CO−), 6.7(−CH$_2$−*C*H$_3$), 4.9(−*C*H$_2$−CH$_3$), 4.8(−*C*H$_2$−CH$_3$). $C_{39}H_{60}O_{11}SSi_2$, HRMS (ESI): calcd for (M+Na$^+$): 815.3293 found: 815.3299.

*4.4.11. 4-Methylphenyl {2-O-[4-(E)-cinnamoyl]-3,4-di-(O-p-methoxybenzyl)-β-D-xylopyranosyl-(1→4)-2-O-acetyl-3-hydroxy-1-α-L-arabinopyranoside}(**32**)/4-methylphenyl {2-O-[4-(E)-cinnamoyl]-3,4-di-(O-p-methoxybenzyl)-β-D-xylopyranosyl-(1→3)-2-O-acetyl-4-hydroxy-1-α-L-arabinopyranoside} (**33**).* The mixture of compound **23** (8.40 g, 0.013 mol) and **29** (3.40 g, 0.011 mol) was added 4 Å MS in dried CH_2Cl_2 under Ar at −60 °C, then the $BF_3{\cdot}Et_2O$ (0.077 mL, 0.061 mmol) was slowly added, after stirring for 3 h, Et_3N was added, MS was filtered and filtrate was concentrated, then the residue was purified by column chromatography on silica gel (petroleum ether:ethyl acetate=8:1) to afford compound **32** (2.40 g, 21%) and **33** (1.50 g, 13%). (**32**) ^{1}H NMR (400 MHz, CDCl$_3$): δ 7.65 (d, J=16.0 Hz, 1H), 7.51 (m, 2H), 7.39 (m, 3H), 7.35 (d, J=8.0 Hz, 2H), 7.25 (d, J=8.4 Hz, 2H), 7.18 (d, J=8.4 Hz, 2H), 7.07 (d, J=8.0 Hz, 2H), 6.87 (d, J=8.5 Hz, 2H), 6.78 (d, J=8.5 Hz, 2H), 6.33 (d, J=16.0 Hz, 1H), 5.06 (t, J=6.4 Hz, 1H, H-2), 5.01 (t, J=7.4 Hz, 1H, H-2′), 4.78 (d, J=5.9 Hz, 1H, H-1), 4.73 (d, J=11.3 Hz, 1H), 4.64 (dd, J=7.0 Hz, J=11.1 Hz, 2H), 4.61 (d, J=6.6 Hz, 1H, H-1′), 4.54 (d, J=11.3 Hz, 1H), 4.25 (dd, J=5.8 Hz, J=12.1 Hz, 1H, H-5a), 3.97 (dd, J=3.5 Hz, J=12.6 Hz, 1H, H-5a′), 3.93 (m, 1H, H-4), 3.80 (s, 3H), 3.74 (m, 1H, H-3), 3.67 (s, 3H), 3.64 (m, 2H, H-3′, H-4′), 3.56 (dd, J=2.5 Hz, J=11.9 Hz, 1H, H-5b), 3.29 (dd, J=8.2 Hz, J=11.4 Hz, 1H, H-5b′), 2.70 (m, 1H, OH), 2.23 (s, 3H), 2.05 (s, 3H). ^{13}C NMR (100 MHz, CDCl$_3$): δ 169.8, 165.8, 159.4, 159.2, 145.9, 137.6, 134.2, 132.3, 130.9, 130.8, 130.5, 130.2, 130.0, 129.7(2× Ar), 129.6(2× Ar), 129.5(2× Ar), 128.8(2× Ar), 128.3(2× Ar), 117.2, 113.9(2× Ar), 113.7(2× Ar), 101.7(C-1′), 86.3(C-1), 79.6(C-4′), 76.8(C-3′), 75.3(C-4), 74.0, 72.8, 72.6(C-2′), 72.0(C-2), 70.1(C-3), 64.2(C-5), 63.5(C-5′), 55.3, 55.1, 21.1, 20.9. $C_{44}H_{48}O_{12}S$, HRMS (ESI): calcd for (M+Na$^+$): 823.2764; found: 823.2773.

(**33**) ^{1}H NMR (400 MHz, CDCl$_3$): δ 7.65 (d, J=16.0 Hz, 1H), 7.46 (m, 2H), 7.37 (m, 3H), 7.21 (m, 4H), 6.92 (d, J=8.0 Hz, 2H), 6.86 (d, J=8.4 Hz, 2H), 6.77 (d, J=8.4 Hz, 2H), 6.39 (d, J=16.0 Hz, 1H), 5.17 (t, J=3.5 Hz, 1H, H-2), 5.11 (t, J=7.1 Hz, 1H, H-2′), 4.93 (d, J=3.5 Hz, 1H, H-1), 4.73 (d, J=12.0 Hz, 1H), 4.65 (d, J=10.9 Hz, 2H), 4.65 (d, J=7.1 Hz, 1H, H-1′), 4.54 (d, J=12.0 Hz, 1H), 4.09 (m, 1H, H-5a′), 3.98 (dd, J=3.8 Hz, J=11.2 Hz, 1H, H-5a), 3.95 (m, 2H, H-3, H-4), 3.79 (s, 3H), 3.67 (s, 3H), 3.64 (m, 2H, H-3′, H-4′), 3.55 (dd, J=3.8 Hz, J=11.2 Hz, 1H, H-5b), 3.33 (m, 1H, H-5b′), 2.23 (s, 3H), 2.03 (s, 3H). ^{13}C NMR (100 MHz, CDCl$_3$): δ 169.5, 165.5, 159.5, 159.2, 145.2, 137.2, 134.4, 131.7(2× Ar), 131.7, 130.3, 130.1, 129.9, 129.9 (2× Ar), 129.5 (2× Ar), 128.8 (2× Ar), 128.2 (2× Ar), 118.1, 113.9 (2× Ar), 113.7 (2× Ar), 100.4(C-1′), 85.7(C-1), 79.3(C-3′), 76.6(C-4′), 75.9(C-3), 74.0, 72.9, 71.3(C-2′), 70.8(C-2), 64.8(C-4), 63.5(C-5′), 62.5(C-5), 55.3, 55.1, 21.0, 21.0. $C_{44}H_{48}O_{12}S$, HRMS (ESI): calcd for (M+Na$^+$): 823.2764; found: 823.2757.

*4.4.12. 3β,16β,22(S),26-Tetrahydroxycholest-5-ene-3,26-di-(tert-butyldimethylsilyl) 16-O-[2-O-(4-methoxybenzoyl)-3,4-di-(O-triethylsilyl)-β-D-xylopyranosyl-(1→4)-2-O-acetyl-3-O-triethylsilyl-α-L-arabinopyranoside] (**38**)/16β,22(S),26-tetrahydroxycholest-5-ene-3,26-di-(tert-butyldimethylsilyl) 16-O-[2-O-(4-methoxybenzoyl)-3,4-di-(O-triethylsilyl)-β-D-xylopyranosyl-(1→3)-2-O-acetyl-4-O-triethylsilyl-α-L-arabinop-yranoside] (**39**)/3β,16β,22(S),26-tetrahydroxycholest-5-ene-3,26-di-(tert-butyldimethylsilyl) 16-O-{2-O-[4-(E)-cinnamoyl]-3,4-di-(O-p-methoxybenzyl)-β-D-xylopyranosyl-(1→4)-2-O-acetyl-3-O-triethylsilyl-α-L-arabinopyranoside} (**40**)/3β,16β,22(S),26-tetrahydroxycholest-5-ene-3,26-di-(tert-butyldimethylsilyl) 16-O-{2-O-[4-(E)-cinnamoyl]-3,4-di-(O-p-methoxybenzyl)-β-D-xylopyranosyl-(1→3)-2-O-acetyl-4-O-triethylsilyl-α-L-arabinopyranoside} (**41**)/3β,16β,26-tetrahydroxycholest-5-ene-3,26-di-(tert-butyldimethylsilyl) 16-O-{2-O-[4-(E)-cinnamoyl]-3,4-di-(O-p-methoxybenzyl)-β-D-xylopyranosyl-(1→4)-2-O-acetyl-3-O-triethylsilyl-α-L-arabinopyranoside} (**42**)/3β,16β,26-tetrahydroxycholest-5-ene-3,26-di-(tert-butyldimethylsilyl) 16-O-{2-O-[4-(E)-cinnamoyl]-3,4-di-(O-p-methoxybenzyl)-β-D-xylopyranosyl-(1→3)-2-O-acetyl-4-O-triethylsilyl-α-L-arabinopyranoside} (**43**).* The mixture of compound **34** (0.40 g, 0.42 mmol) and **8** (0.31 g, 0.46 mmol) was added 4 Å MS (1.00 g) in dried CH_2Cl_2 under Ar at -60 °C, then the TMSOTf (6.60 μL) was slowly added, after stirring for 3 h, Et_3N was added, MS was filtered and filtrate was concentrated, then the residue was purified by column chromatography on silica gel (petroleum ether:ethyl acetate=10:1) to afford compound **38** (0.40 g, 64%). Following the same procedure **39**(58%), **40**(51%), **41**(47%), **42**(55%), **43**(43%) were achieved. (**38**) ^{1}H NMR (400 MHz, CDCl$_3$): δ 8.03 (d, J=8.7 Hz, 2H), 6.90 (d, J=8.7 Hz, 2H), 5.30 (d, J=4.9 Hz, 1H, H-6), 4.96 (t, J=6.6, 1H, H-2′), 4.70 (dd, J=7.0 Hz, J=9.0 Hz, 1H, H-2″), 4.66 (d, J=7.0 Hz, 1H, H-1″), 4.33 (m, 1H), 4.09−3.95 (m, 3H), 3.86 (s, 3H), 3.72 (m, 4H), 3.46 (m, 4H), 3.33 (dd, J=6.9 Hz, J=9.0 Hz, 1H), 3.25 (dd, J=8.6 Hz, J=11.9 Hz, 1H), 2.28−2.20 (m, 2H), 1.97 (s, 3H), 1.81−1.70 (m, 2H), 0.64−0.44 (m, 18H), 0.06−0.02 (m, 12H). ^{13}C NMR (100 MHz, CDCl$_3$): δ 168.8, 163.7, 163.2, 141.4, 131.7(2× Ar−CH), 122.6, 120.9, 113.3(2× Ar−CH), 101.7, 101.0, 81.6, 77.1, 74.8, 74.1, 73.4, 73.1, 72.6, 71.7, 71.1, 68.4, 65.5, 65.0, 57.1, 55.3, 54.8, 51.1, 42.7, 42.0, 39.6, 37.2, 36.4, 36.2, 35.8, 34.9, 32.0, 31.8, 31.4, 30.5, 29.6, 25.9(3× *Me*$_3$CSi), 25.9(3× *Me*$_3$CSi), 22.6, 20.9, 19.3, 18.3, 18.2, 16.8, 12.7, 11.2, 6.8(6× CH$_2$*C*H$_3$), 6.7(3× CH$_2$*C*H$_3$), 4.9(3× *C*H$_2$CH$_3$), 4.9(3× *C*H$_2$CH$_3$), 4.5(3× *C*H$_2$CH$_3$), −4.6(2× *Me*$_2$Si), −5.4(2× *Me*$_2$Si). $C_{77}H_{140}O_{15}Si_5$, HRMS (ESI): calcd for (M+Na$^+$): 1467.8936; found: 1467.8945.

(**39**) ^{1}H NMR (400 MHz, CDCl$_3$): δ 8.02 (d, J=8.8 Hz, 2H), 6.91 (d, J=8.8 Hz, 2H), 5.32 (d, J=5.1 Hz, 1H, H-6), 4.96 (m, 1H), 4.90 (m, 1H), 4.81 (m, 1H), 4.32 (m, 1H), 4.21 (m, 1H), 4.03 (m, 2H), 3.91 (m, 1H), 3.87 (s, 3H), 3.78 (m, 2H), 3.60 (m, 1H), 3.45 (m, 3H), 3.27 (m, 3H), 1.92 (s, 3H), 0.64−0.55 (m, 18H), 0.07−0.04 (m, 12H). ^{13}C NMR (150 MHz, CDCl$_3$): δ 168.6, 164.5, 163.2, 141.6, 141.5, 131.8(2× Ar−CH), 122.7, 121.0, 121.0, 113.3(2× Ar−CH), 101.4, 100.8, 81.8, 78.5, 75.6, 74.5, 72.6, 72.6, 71.9, 70.2, 68.3, 68.0, 65.5, 63.0, 57.0, 55.3, 54.8, 50.1, 42.8, 42.1, 40.0, 39.4, 37.3, 36.5, 36.2, 35.9, 35.2, 32.0, 31.8, 31.5, 30.3, 25.9(3× *Me*$_3$CSi), 25.9(3× *Me*$_3$CSi), 20.8, 20.7, 19.2, 18.3, 18.2, 16.9, 13.0, 11.7, 6.9(3× CH$_2$*C*H$_3$), 6.8(3× CH$_2$*C*H$_3$), 6.8(3× CH$_2$*C*H$_3$), 4.9(6× *C*H$_2$CH$_3$), 4.8(3× *C*H$_2$CH$_3$), −4.6(2× *Me*$_2$Si), −5.3, −5.4. $C_{77}H_{140}O_{15}Si_5$, HRMS (ESI): calcd for (M+Na$^+$): 1467.8936; found: 1467.8945.

(**40**) ^{1}H NMR (400 MHz, CDCl$_3$): δ 7.64 (d, J=16.0 Hz, 1H), 7.51 (m, 2H), 7.38 (m, 3H), 7.23 (d, J=8.4 Hz, 2H), 7.23 (d, J=8.4 Hz, 2H), 6.85 (d, J=8.4 Hz, 2H), 6.74 (d, J=8.4 Hz, 2H), 6.36 (d, J=16.0 Hz, 1H), 5.29 (d, J=4.2 Hz, 1H, H-6), 5.01 (t, J=8.0 Hz, 1H), 4.72−4.51 (m, 6H), 4.36 (m, 1H), 4.30 (t, J=6.6 Hz, 1H), 4.09−3.94 (m, 3H), 3.79 (s, 3H), 3.86−3,75 (m, 2H), 3.64 (s, 3H), 3.59 (m, 2H), 3.45 (m, 2H), 3.26 (m,

2H), 1.99 (s, 3H), 0.52 (q, *J*=7.9 Hz, 6H), 0.06–0.01 (m, 12H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 168.8, 165.2, 159.3, 159.0, 145.0, 141.4, 134.4, 130.2, 130.2, 129.9, 129.8(2× Ar–CH), 129.4(2× Ar–CH), 128.8(2× Ar–CH), 128.0(2× Ar–CH), 120.9, 117.8, 113.8(2× Ar–CH), 113.6(2× Ar–CH), 102.0, 100.8, 80.1, 77.2, 75.9, 75.6, 74.9, 73.9, 73.9, 73.2, 72.7, 72.6, 72.2, 69.1, 68.4, 63.3, 57.1, 55.2, 54.9, 54.8, 50.1, 42.7, 42.0, 39.6, 37.2, 36.5, 36.2, 35.7, 34.8, 32.0, 31.8, 31.4, 30.3, 29.6, 25.9(6× Me_3CSi), 22.6, 20.9, 19.3, 18.3, 18.2, 16.8, 12.8, 11.1, 6.8(3× CH_2CH_3), 4.6(3× CH_2CH_3), −4.6(2× Me_2Si), −5.4(2× Me_2Si). $C_{82}H_{128}O_{16}Si_3$, HRMS (ESI): calcd for (M+Na^+): 1475.8408; found: 1475.8423.

(**41**) ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.65 (d, *J*=16.0 Hz, 1H), 7.50 (m, 2H), 7.37 (m, 3H), 7.23 (d, *J*=8.5 Hz, 2H), 7.23 (d, *J*=8.5 Hz, 2H), 6.81 (d, *J*=8.5 Hz, 2H), 6.75 (d, *J*=8.5 Hz, 2H), 6.37 (d, *J*=16.0 Hz, 1H), 5.29 (d, *J*=4.2 Hz, 1H, H-6), 5.00 (m, 1H), 4.71–4.54 (m, 6H), 4.28 (m, 1H), 4.10 (m, 1H), 3.97 (m, 3H), 3.75 (s, 3H), 3.70 (m, 1H), 3.68 (s, 3H), 3.59 (m, 2H), 3.45 (m, 3H), 3.33 (m, 2H), 2.02 (s, 3H), 0.60 (q, *J*=8.0 Hz, 6H), 0.06–0.01 (m, 12H). $C_{82}H_{128}O_{16}Si_3$, HRMS (ESI): calcd for (M+Na^+): 1475.8408; found: 1475.8415.

(**42**) ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.65 (d, *J*=16.0 Hz, 1H), 7.50 (m, 2H), 7.37 (m, 3H), 7.23 (d, *J*=8.2 Hz, 2H), 7.18 (d, *J*=8.2 Hz, 2H), 6.85 (d, *J*=8.2 Hz, 2H), 6.75 (d, *J*=8.2 Hz, 2H), 6.38 (d, *J*=16.0 Hz, 1H), 5.30 (m, 1H, H-6), 5.01 (t, *J*=6.8 Hz, 1H), 4.78–4.51 (m, 6H), 4.30 (m, 1H), 4.02–3.93 (m, 2H), 3.85–3.71 (m, 3H), 3.79 (s, 3H), 3.65 (s, 3H), 3.62 (m, 2H), 3.52–3.39 (m, 3H), 3.29 (m, 2H), 1.98 (s, 3H), 0.53 (q, *J*=7.92 Hz, 6H), 0.05 (s, 6H), 0.01 (s, 6H). $C_{82}H_{128}O_{15}Si_3$, HRMS (ESI): calcd for (M+Na^+): 1459.8459; found: 1459.8464.

(**43**) ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.64 (d, *J*=16.0 Hz, 1H), 7.51 (m, 2H), 7.37 (m, 3H), 7.23 (d, *J*=8.5 Hz, 2H), 7.20 (d, *J*=8.5 Hz, 2H), 6.83 (d, *J*=8.5 Hz, 2H), 6.75 (d, *J*=8.5 Hz, 2H), 6.35 (d, *J*=16.0 Hz, 1H), 5.27 (d, *J*=3.5 Hz, 1H, H-6), 5.06 (t, *J*=6.8 Hz, 1H), 4.99 (t, *J*=6.2 Hz, 1H), 4.69–4.51 (m, 6H), 4.30 (m, 1H), 4.20 (m, 1H), 3.96 (m, 1H), 3.85 (m, 2H), 3.77 (s, 3H), 3.67 (s, 3H), 3.60 (m, 2H), 3.45–3.29 (m, 5H), 1.98 (s, 3H), 0.58 (m, 6H), 0.04 (s, 6H), 0.01 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 168.7, 165.5, 159.5, 159.2, 145.1, 141.6, 134.7, 130.6, 130.4, 130.3, 129.6(2× Ar–CH), 129.6(2× Ar–CH), 129.0(2× Ar–CH), 128.3(2× Ar–CH), 121.3, 118.1, 114.0(2× Ar–CH), 113.7(2× Ar–CH), 102.9, 100.6, 81.9, 77.1, 76.2, 75.0, 73.5, 72.8, 72.7, 72.7, 68.7, 68.7, 65.7, 60.8, 60.6, 55.4, 55.4, 55.3, 55.2, 50.5, 43.0, 42.3, 39.8, 37.5, 36.7, 36.5, 36.3, 36.1, 34.4, 32.3, 31.9, 31.5, 30.8, 29.9, 26.2(3× Me_3CSi), 26.1(3× Me_3CSi), 21.3, 21.0, 19.6, 18.6, 18.5, 17.0, 14.4, 13.0, 7.0(3× CH_2CH_3), 5.0(3× CH_2CH_3), −4.3(2× Me_2Si), −5.0, −5.1. $C_{82}H_{128}O_{15}Si_3$, HRMS (ESI): calcd for (M+Na^+): 1459.8459; found: 1459.8457.

*4.4.13. 3β,16β,26-Trihydroxycholest-5-en-22-one-3,26-di-(tert-butyldimethylsilyl) 16-O-[2-O-(4-methoxybenzoyl)-3,4-di-(O-triethylsilyl)-β-D-xylopyranosyl-(1→4)-2-O-acetyl-3-O-triethylsilyl-α-L-arabinopyranoside] (**44**)/16β,26-trihydroxycholest-5-en-22-one-3,26-di-(tert-butyldimethylsilyl) 16-O-[2-O-(4-methoxybenzoyl)-3,4-di-(O-triethylsilyl)-β-D-xylopyranosyl-(1→3)-2-O-acetyl-4-O-triethylsilyl-α-L-arabinopyranoside] (**45**)/16β,26-trihydroxycholest-5-en-22-one-3,26-di-(tert-butyldimethylsilyl) 16-O-{2-O-[4-(E)-cinnamoyl]-3,4-di-(O-p-methoxybenzyl)-β-D-xylopyranosyl-(1→4)-2-O-acetyl-3-O-triethylsilyl-α-L-arabinopyranoside} **46**)/16β,26-trihydroxycholest-5-en-22-one-3,26-di-(tert-butyldimethylsilyl) 16-O-{2-O-[4-(E)-cinnamoyl]-3,4-di-(O-p-methoxybenzyl)-β-D-xylopyranosyl-(1→3)-2-O-acetyl-4-O-triethylsilyl-α-L-arabinopyranoside}(**47**).* A solution of **38** (0.087 g, 0.060 mmol) in CH_2Cl_2 (3.00 mL), was added PDC (0.069 g, 0.18 mmol), after stirring for 6 h at room temperature, the concentrate was purified by column chromatography on silica gel (petroleum ether:ethyl acetate=10:1) to afford compound **44** (0.084 g, 96%). Following the same procedure **45** (67%), **46** (80%), **47** (74%) were achieved. (**44**) ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.00 (d, *J*=8.8 Hz, 2H), 6.89 (d, *J*=8.8 Hz, 2H), 5.32 (d, *J*=4.1 Hz, 1H, H-6), 4.94 (t, *J*=6.7 Hz, 1H), 4.66 (d, *J*=6.1 Hz, 1H), 4.58 (m, 1H), 4.07 (m, 1H), 3.99 (m, 2H), 3.88 (m, 1H), 3.86 (s, 3H), 3.70 (m, 4H), 3.45 (m, 4H), 3.24 (dd, *J*=8.0 Hz, *J*=11.7 Hz, 1H), 2.87 (m, 1H), 2.61 (m, 1H), 1.95 (s, 3H), 0.54 (m, 18H), 0.09–0.01 (m, 12H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 214.3, 168.8, 164.6, 163.2, 141.4, 131.7, 122.8, 121.0, 113.4, 101.7, 100.1, 82.6, 80.5, 74.6, 73.5, 73.1, 72.6, 72.3, 71.1, 68.1, 65.0, 61.7, 56.4, 55.3, 54.1, 53.1, 50.0, 43.8, 42.8, 41.4, 39.6, 38.8, 37.3, 36.5, 35.4, 35.3, 31.9, 31.7, 31.3, 29.3, 25.9(6× Me_3CSi), 22.7, 22.6, 20.8, 19.4, 18.3, 18.2, 16.6, 13.3, 6.8(9× CH_2CH_3), 5.0(3× CH_2CH_3), 4.9(3× CH_2CH_3), 4.6(3× CH_2CH_3), −4.6(2× Me_2Si), −5.4(2× Me_2Si). $C_{77}H_{138}O_{15}Si_5$, HRMS (ESI): calcd for (M+Na^+): 1465.8780; found: 1465.8790.

(**45**) ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.04 (d, *J*=8.3 Hz, 2H), 6.90 (d, *J*=8.3 Hz, 2H), 5.30 (d, *J*=4.4 Hz, 1H, H-6), 4.91 (m, 1H), 4.80 (m, 2H), 4.2 (m, 1H), 4.07 (m, 1H), 3.93 (m, 2H), 3.86 (s, 3H), 3.85 (m, 1H), 3.76 (m, 2H), 3.60 (m, 1H), 3.46 (m, 1H), 3.37 (m, 1H), 3.25 (m, 3H), 2.84 (m, 1H), 2.42 (m, 1H), 1.93 (s, 3H), 0.56 (m, 18H), 0.05–0.00 (m, 12H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 214.1, 168.9, 164.5, 163.5, 141.4, 131.9(2× Ar–CH), 122.4, 113.5(2× Ar–CH), 99.6, 98.6, 80.7, 74.2, 72.6, 71.9, 71.4, 69.6, 69.2, 68.2, 64.7, 63.1, 61.1, 56.1, 55.3, 54.1, 50.0, 43.7, 42.8, 41.4, 39.6, 38.0, 37.2, 36.5, 35.3, 35.1, 32.0, 31.7, 31.3, 29.6, 26.9, 25.9(6× Me_3CSi), 20.7, 20.7, 19.3, 18.3, 18.2, 16.3, 16.2, 13.3, 6.8(6× CH_2CH_3), 6.7(3× CH_2CH_3), 4.8(6× CH_2CH_3), 4.7(3× CH_2CH_3), −4.6(2× Me_2Si), −5.4(2× Me_2Si). $C_{77}H_{138}O_{15}Si_5$, HRMS (ESI): calcd for (M+Na^+): 1465.8780; found: 1465.8785.

(**46**) ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.63 (d, *J*=16.0 Hz, 1H), 7.50 (m, 2H), 7.39 (m, 3H), 7.23 (d, *J*=8.0 Hz, 2H), 7.16 (d, *J*=8.0 Hz, 2H), 6.85 (d, *J*=7.8 Hz, 2H), 6.74 (d, *J*=7.8 Hz, 2H), 6.34 (d, *J*=16.0 Hz, 1H), 5.31 (d, *J*=3.4 Hz, 1H, H-6), 4.98 (t, *J*=7.64 Hz, 1H), 4.72–4.51 (m, 6H), 4.09 (m, 1H), 3.95 (m, 3H), 3.80 (m, 1H), 3.79 (s, 3H), 3.70 (m, 2H), 3.64 (s, 3H), 3.56 (m, 2H), 3.47 (m, 2H), 3.32 (m, 1H), 3.25 (m, 1H), 1.98 (s, 3H), 0.54 (q, *J*=8.0 Hz, 6H), 0.06–0.01 (m, 12H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 214.2, 168.8, 165.2, 159.3, 159.1, 145.0, 141.3, 134.3, 130.2, 130.1, 130.0, 129.8(2× Ar–CH), 129.5(2× Ar–CH), 128.8(2× Ar–CH), 128.0(2× Ar–CH), 120.9, 117.8, 113.8(2× Ar–CH), 113.6(2× Ar–CH), 102.1, 100.0, 80.1, 77.1, 75.7, 74.9, 73.9, 73.0, 72.7, 72.6, 72.2, 68.1, 66.7, 63.3, 56.4, 55.2, 55.0, 54.1, 50.0, 43.7, 42.8, 42.7, 41.3, 39.6, 38.8, 37.2, 36.5, 35.3, 32.0, 31.3, 30.5, 27.2, 25.9(3× Me_3CSi), 25.9(3× Me_3CSi), 22.6, 20.9, 19.3, 18.2, 18.2, 16.5, 16.3, 13.3, 6.8(3× CH_2CH_3), 4.6(3× CH_2CH_3), −4.6(2× Me_2Si), −5.4(2× Me_2Si). $C_{82}H_{126}O_{16}Si_3$, HRMS (ESI): calcd for (M+Na^+): 1473.8251; found: 1473.8256.

(**47**) ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.67 (d, *J*=16.0 Hz, 1H), 7.50 (m, 2H), 7.38 (m, 3H), 7.22 (d, *J*=8.0 Hz, 2H), 7.20 (d, *J*=8.0 Hz, 2H), 6.81 (d, *J*=8.6 Hz, 2H), 6.74 (d, *J*=8.6 Hz, 2H), 6.42 (d, *J*=16.0 Hz, 1H), 5.28 (d, *J*=3.4 Hz, 1H, H-6), 5.04 (t, *J*=5.5 Hz, 1H), 4.70–4.52 (m, 6H), 4.06 (m, 1H), 3.89 (m, 3H), 3.78 (m, 1H), 3.75 (s, 3H), 3.70 (m, 1H), 3.67 (s, 3H), 3.58 (m, 2H), 3.45 (m, 2H), 3.35 (m, 2H), 3.29 (m, 1H), 2.88 (m, 1H), 2.63 (m, 1H), 2.03 (s, 3H), 0.60 (m, 6H), 0.07–0.01 (m, 12H). $C_{82}H_{126}O_{16}Si_3$, HRMS (ESI): calcd for (M+Na^+): 1473.8251; found: 1473.8259.

*4.4.14. 3β,16β,22(S),26-Tetrahydroxycholest-5-en-22-hydroxy 16-O-[O-2-O-(4-methoxybenzoyl)-β-D-xylopyranosyl-(1→3)-2-O-acetyl-α-L-arabinopyranoside] (**48a**)/3β,16β,22(S),26-tetrahydroxycholest-5-en-22-hydroxy 16-O-[O-2-O-(4-methoxybenzoyl)-β-D-xylopyranosyl-(1→4)-2-O-acetyl-α-L-arabinopyranoside] (**48b**)/3β,16β,26-trihydroxycholest-5-en-22-one 16-O-[O-2-O-(4-methoxybenzoyl)-β-D-xylopyranosyl-(1→3)-2-O-acetyl-α-L-arabinopyranoside] (**49a**)/3β,16β,26-trihydroxycholest-5-en-22-one 16-O-[O-2-O-(4-methoxybenzoyl)-β-D-xylopyranosyl-(1→4)-2-O-acetyl-α-L-arabinopyranoside] (**49b**).* To a solution of **38** (0.021 g, 0.014 mmol) in acetone/H_2O (1 mL, 10: 1) was added Pd(CH_3CN)$_2Cl_2$ (0.0030 g). After the reaction was stirred at room temperature for 3 h, the solvent was removed. The residue was isolated by silica gel column chromatography (petroleum ether:ethyl acetate=12:1), then purified by Sephadex LH-20 column chromatography (chloroform: MeOH=1:1) to afford **48b** (0.0087 g, 67%). Following the same

procedure **48a** (yield: 59%), **49a** (yield: 62%), **49b** (yield: 57%) were achieved. (**48a**) ^{1}H NMR (600 MHz, CD_3OD): δ 8.04 (d, J=8.8 Hz, 2H), 7.03 (d, J=8.8 Hz, 2H), 5.34 (d, J=4.9 Hz, 1H, H-6), 5.03 (dd, J=7.2 Hz, J=9.4 Hz, 1H, H-2′), 4.94 (dd, J=7.3 Hz, J=9.0 Hz, 1H, H-2″), 4.72 (d, J=7.3 Hz, 1H, H-1″), 4.29 (d, J=7.2 Hz, 1H, H-1′), 4.14 (m, 1H, H-16), 4.05 (m, 1H, H-4′), 3.98 (m, 1H, H-5″), 3.89 (s, 3H), 3.89 (dd, J=3.0 Hz, J=12.5 Hz, 1H, H-5′), 3.80 (dd, J=2.1 Hz, J=9.4 Hz, 1H, H-3′), 3.64 (m, 2H, H-3″, H-4″), 3.55 (dd, J=1.3 Hz, J=12.5 Hz, 1H, H-5′), 3.44 (m, 1H, H-26), 3.43 (m, 1H, H-22), 3.37 (m, 1H, H-3), 3.35 (m, 1H, H-5″), 3.32 (m, 1H, H-26), 2.25 (m, 2H, H-4), 2.17 (m, 2H, H-15), 1.97 (m, 2H, H-11), 1.87 (m, 1H, H-1), 1.8 (m, 2H, H-2), 1.59 (s, 3H), 1.52 (m, 3H, H-7, H-25), 1.48 (m, 2H, H-12), 1.47 (m, 1H, H-17), 1.40 (m, 2H, H-23), 1.31 (m, 1H, H-8), 1.07 (m, 1H, H-1), 1.02 (s, 3H, H-19), 0.91 (m, 1H, H-9), 0.89 (d, J=6.6 Hz, 3H, H-27), 0.87 (m, 1H, H-14), 0.86 (d, J=7.0 Hz, 3H, H-21), 0.81 (s, 3H, H-18). ^{13}C NMR (150 MHz, CD_3OD): δ 170.9, 167.1, 165.4, 142.2(C-5), 133.2(2× Ar), 123.4, 122.3(C-6), 114.7(2× Ar), 103.6(C-1″), 103.1(C-1′), 81.4(C-16), 81.0(C-3′), 75.6(C-3″), 75.1(C-2″), 73.9(C-22), 72.4(C-2′), 72.2(C-3), 70.9(C-4″), 69.6(C-4′), 68.3(C-26), 66.5(C-5″), 66.3(C-5′), 58.7(C-17), 56.3(C-14), 56.0(−OCH_3), 51.7(C-9), 43.1(C-13), 42.9(C-4), 41.0(C-11), 38.4(C-1), 37.6(C-10), 37.2(C-25), 37.2(C-15), 36.5(C-20), 33.9(C-23), 32.8(C-7), 32.2(C-2), 31.6(C-24), 30.6(C-8), 21.8(C-12), 20.9, 19.8(C-19), 17.5(C-27), 13.1(C-18), 11.6(C-21). $C_{47}H_{70}O_{15}$, HRMS (ESI): calcd for (M+Na^+): 897.4612; found: 897.4610.

(**48b**) ^{1}H NMR (600 MHz, CD_3OD): δ 8.05 (d, J=8.9 Hz, 2H), 6.98 (d, J=8.9 Hz, 2H), 5.36 (d, J=4.7 Hz, 1H, H-6), 4.96 (dd, J=7.4 Hz, J=9.1 Hz, 1H, H-2″), 4.85 (d, J=7.4 Hz, 1H, H-1″), 4.78 (dd, J=5.5 Hz, J=7.9 Hz, 1H, H-2′),4.31 (d, J=5.5 Hz, 1H, H-1′), 4.11 (m, 1H, H-16), 4.00 (dd, J=5.0 Hz, J=12.0 Hz, 1H, H-5′), 3.93 (m, 1H, H-4″), 3.87 (s, 3H), 3.69 (dd, J=3.3 Hz, J=7.9 Hz, 1H, H-3′), 3.65 (m, 1H, H-4′), 3.64 (m, 1H, H-3″), 3.53 (dd, J=2.4 Hz, J=12.0 Hz, 1H, H-5′), 3.50 (m, 1H, H-22), 3.46 (m, 1H, H-26), 3.40 (m, 1H, H-3), 3.33 (m, 2H, H-26, H-5″), 2.25 (m, 2H, H-4), 2.15 (m, 1H, H-15), 2.00 (m, 2H, H-11), 2.00 (s, 3H), 1.87 (m, 2H, H-1, H-20), 1.80 (m, 2H, H-2), 1.56 (m, 2H, H-24, H-25), 1.52 (m, 4H, H-7, H-12), 1.50 (m, 1H, H-17), 1.42 (m, 2H, H-23), 1.31 (m, 3H, H-8, H-24), 1.07 (m, 1H, H-1), 1.05 (s, 3H, H-19), 0.93 (d, J=6.6 Hz, 3H, H-27), 0.92 (m, 1H, H-9), 0.90 (d, J=7.0 Hz, 3H, H-21), 0.86 (m, 1H, H-14), 0.82 (s, 3H, H-18). ^{13}C NMR (150 MHz, CD_3OD): δ 171.3, 167.7, 165.1, 142.2(C-5), 133.1(2× Ar), 123.5, 122.3(C-6), 114.6(2× Ar), 103.5(C-1″), 102.6(C-1′), 82.0(C-16), 77.1(C-4″), 75.8(C-3″), 75.7(C-2″), 74.1(C-22), 73.9(C-2′), 72.4(C-3), 71.1(C-3′), 71.0(C-4′), 68.4(C-26), 66.7(C-5″), 64.3(C-5′), 58.6(C-17), 56.2(C-14), 55.9(−OCH_3), 51.7(C-9), 43.1(C-4), 43.0(C-13), 41.0(C-11), 38.4(C-1), 37.6(C-10), 37.3(C-25), 37.0(C-15), 36.5(C-20), 33.9(C-23), 32.8(C-7), 32.2(C-2), 31.6(C-24), 30.6(C-8), 21.9(C-12), 20.9, 19.8(C-19), 17.4(C-27), 13.3(C-18), 11.6(C-21). $C_{47}H_{70}O_{15}$, HRMS (ESI): calcd for (M+Na^+): 897.4612; found: 897.4615.

*4.4.15. 3β,16β,22(S),26-Tetrahydroxycholest-5-en-22-hydroxy 16-O-{O-2-O-[4-(E)-cinnamoyl]-β-D-xylopyranosyl (1→3)-2-O-acetyl-α-L-arabinopyranoside} (**50a**)/3β,16β,22(S),26-tetrahydroxycholest-5-en-22-hydroxy 16-O-{O-2-O-[4-(E)-cinnamoyl]-β-D-xylopyranosyl (1→4)-2-O-acetyl-α-L-arabinopyranoside} (**50b**)/3β,16β,26-trihydroxycholest-5-en-22-one 16-O-{O-2-O-[4-(E)-cinnamoyl]-β-D-xylopyranosyl (1→3)-2-O-acetyl-α-L-arabinopyranoside} (**51a**)/3β,16β,26-trihydroxycholest-5-en-22-one 16-O-{O-2-O-[4-(E)-cinnamoyl]-β-D-xylopyranosyl (1→4)-2-O-acetyl-α-L-arabinopyranoside} (**51b**)/3β,16β,26-trihydroxycholest-5-en-16-O-{O-2-O-[4-(E)-cinnamoyl]-β-D-xylopyranosyl (1→3)-2-O-acetyl-α-L-arabinopyranoside} (**52a**)/3β,16β,26-trihydroxycholest-5-en-16-O-{O-2-O-[4-(E)-cinnamoyl]-β-D-xylopyranosyl (1→4)-2-O-acetyl-α-L-arabinopyranoside} (**52b**).* To a solution **40** (0.021 g, 0.014 mmol) in CH_2Cl_2/H_2O (1 mL, 10:1) was added DDQ (9.5 mg, 0.043 mmol). The reaction was stirred at ambient temperature for 5 h, then CH_2Cl_2 was removed, and acetone (1 mL) and $Pd(CH_3CN)_2Cl_2$ (0.0030 g) was added. After the reaction was stirred at room temperature for 3 h, the solvent was removed. The residue was isolated by silica gel column chromatography (petroleum ether:ethyl acetate=12:1), then purified by Sephadex LH-20 column chromatography (chloroform:MeOH=1:1) to afford **50b** (6.70 mg, 54%). Following the same procedure **50a** (yield: 59%), **51a** (yield: 52%), **51b** (yield: 53%), **52a** (yield: 48%), **52b** (yield: 61%) were achieved. (**50a**) ^{1}H NMR (600 MHz, CD_3OD): δ 7.75 (d, J=16.0 Hz, 1H), 7.65 (m, 2H), 7.44 (m, 3H), 6.57 (d, J=16.0 Hz, 1H), 5.34 (d, J=5.3 Hz, 1H, H-6), 5.07 (dd, J=7.1 Hz, J=9.3 Hz, 1H, H-2′), 4.84 (m, 1H, H-2″), 4.64 (d, J=7.4 Hz, 1H, H-1″), 4.27 (d, J=7.1 Hz, 1H, H-1′), 4.15 (m, 1H, H-16), 4.0 (m, 1H, H-4′), 3.96 (dd, J=5.2 Hz, J=11.7 Hz, 1H, H-5″), 3.87 (dd, J=3.0 Hz, J=12.6 Hz, 1H, H-5′), 3.79 (dd, J=3.3 Hz, J=9.3 Hz, 1H, H-3′), 3.62 (m, 1H, H-4″), 3.54 (dd, J=1.3 Hz, J=12.6 Hz, 1H, H-5′), 3.53 (t, J=8.7 Hz, 1H, H-3″), 3.48 (m, 1H, H-22), 3.41 (m, 1H, H-26), 3.38 (m, 1H, H-3), 3.30 (m, 2H, H-5″, H-26), 2.23 (m, 2H, H-4), 2.19 (m, 1H, H-15), 2.00 (s, 3H), 1.98 (m, 1H, H-11), 1.84 (m, 1H, H-15), 1.82 (m, 1H, H-20), 1.79 (m, 2H, H-2), 1.62 (m, 2H, H-24), 1.53 (m, 1H, H-25), 1.52 (m, 1H, H-23), 1.50 (m, 2H, H-7, H-17), 1.44 (m, 3H, H-12, H-25), 1.32 (m, 1H, H-8), 1.31 (m, 1H, H-23), 1.05 (m, 2H, H-1), 1.02 (s, 3H, H-19), 0.92 (m, 1H, H-9), 0.91 (d, J=6.7 Hz, 3H, H-21), 0.89 (d, J=6.6 Hz, 3H, H-27), 0.87 (m, 1H, H-14), 0.84 (s, 3H, H-18). ^{13}C NMR (150 MHz, CD_3OD): δ 169.4, 166.3, 145.3, 140.7(C-5), 134.3, 130.2, 129.4, 128.7, 127.9, 120.9(C-6), 117.5, 102.2(C-1″), 101.8(C-1′), 80.1(C-16), 79.7(C-3′), 74.3(C-3″), 73.5(C-2″), 72.5(C-22), 71.0(C-3), 71.0(C-2′), 69.3(C-4″), 68.3(C-4′), 66.8(C-26), 65.2(C-5″), 65.1(C-5′), 57.4(C-17), 55.1(C-14), 50.2(C-9), 41.7(C-13), 41.5(C-4), 39.6(C-11), 37.0(C-1), 36.4(C-10), 35.8(C-25), 35.2(C-15), 35.1(C-20), 31.6(C-23), 31.6(C-7), 30.8(C-2), 30.2(C-24), 29.4(C-8), 22.3(C-12), 20.1, 18.4(C-19), 16.1(C-27), 11.7(C-18), 10.2(C-21). $C_{48}H_{70}O_{14}$, HRMS (ESI): calcd for (M+Na^+): 893.4663; found: 893.4660.

(**50b**) ^{1}H NMR (600 MHz, CD_3OD): δ 7.76 (d, J=16.0 Hz, 1H), 7.60 (m, 2H), 7.41 (m, 3H), 6.62 (d, J=16.0 Hz, 1H), 5.34 (d, J=4.8 Hz, 1H, H-6), 4.87 (m, 2H, H-2′, H-2″), 4.81 (d, J=7.6 Hz, 1H, H-1″), 4.32 (d, J=6.0 Hz, 1H, H-1′), 4.14 (m, 1H, H-16), 3.99 (dd, J=4.5 Hz, J=12.1 Hz, 1H, H-5′), 3.97 (m, 1H, H-5″), 3.95 (m, 1H, H-4″), 3.74 (dd, J=3.3 Hz, J=8.2 Hz, 1H, H-3′), 3.63 (m, 1H, H-4′), 3.58 (m, 1H, H-3″), 3.54 (dd, J=2.2 Hz, J=12.1 Hz, 1H, H-5′), 3.52 (m, 1H, H-22), 3.47 (m, 1H, H-26), 3.41 (m, 1H, H-3), 3.35 (m, 1H, H-26), 3.30 (dd, J=1.5 Hz, J=11.7 Hz, 1H, H-5″), 2.24 (m, 2H, H-4), 2.19 (m, 1H, H-15), 1.98 (m, 1H, H-11), 1.97 (s, 3H), 1.88 (m, 1H, H-20), 1.86 (m, 1H, H-1), 1.81 (m, 1H, H-2), 1.63 (m, 1H, H-8), 1.61 (m, 1H, H-24), 1.56 (m, 1H, H-25), 1.53 (m, 2H, H-12), 1.51 (m, 1H, H-17), 1.50 (m, 2H, H-7), 1.48 (m, 2H, H-23), 1.14 (m, 1H, H-24), 1.04 (s, 3H, H-19), 1.01 (m, 1H, H-1), 0.93 (d, J=6.7 Hz, 3H, H-27), 0.92 (m, 1H, H-9), 0.87 (m, 1H, H-14), 0.86 (d, J=7.0 Hz, 3H, H-21), 0.79 (s, 3H, H-18). ^{13}C NMR (150 MHz, CD_3OD): δ 171.3, 168.2, 146.7, 142.2(C-5), 136.0, 131.3, 129.9, 129.4, 122.3(C-6), 119.1, 103.5(C-1″), 102.8(C-1′), 81.7(C-16), 77.1(C-4″), 75.8(C-3″), 75.5(C-2′), 74.1(C-2″), 73.9(C-22), 72.4(C-3), 71.4(C-3′), 71.1(C-4′), 68.4(C-26), 66.8(C-5″), 64.9(C-5′), 58.7(C-17), 56.3(C-14), 51.7(C-9), 43.1(C-4), 43.0(C-13), 41.0(C-11), 38.4(C-1), 37.6(C-10), 37.3(C-25), 37.1(C-15), 36.5(C-20), 32.9(C-23), 32.8(C-7), 32.2(C-2), 31.6(C-24), 30.6(C-8), 21.9(C-12), 21.1, 19.9(C-19), 17.4(C-27), 13.2(C-18), 11.6(C-21). $C_{48}H_{70}O_{14}$, HRMS (ESI): calcd for (M+Na^+): 893.4663; found: 893.4665.

(**51a**) ^{1}H NMR (600 MHz, CD_3OD): δ 7.77 (d, J=16.0 Hz, 1H), 7.66 (m, 2H), 7.45 (m, 3H), 6.60 (d, J=16.0 Hz, 1H), 5.34 (d, J=4.9 Hz, 1H, H-6), 4.98 (dd, J=6.9 Hz, J=9.1 Hz, 1H, H-2′), 4.84 (m, 1H, H-2″), 4.62 (d, J=7.6 Hz, 1H, H-1″), 4.06 (d, J=6.9 Hz, 1H, H-1′), 4.02 (m, 1H, H-16), 3.97 (m, 1H, H-5″), 3.96 (m, 1H, H-4′), 3.85 (dd, J=3.1 Hz, J=12.5 Hz, 1H, H-5′), 3.76 (dd, J=3.2 Hz, J=9.1 Hz, 1H, H-3′), 3.62 (m, 1H, H-4″), 3.56 (t, J=8.6 Hz, 1H, H-3″), 3.49 (dd, J=1.6 Hz, J=12.5 Hz, 1H, H-5′), 3.40 (m, 1H, H-3), 3.35 (m, 2H, H-26), 3.31 (m, 1H, H-5″), 2.85 (m, 1H, H-20), 2.65 (m, 1H, H-23), 2.45 (m, 1H, H-23), 2.24 (m, 2H, H-4), 2.14 (m, 1H, H-15), 2.07 (s, 3H), 1.93 (m, 2H, H-11), 1.85 (m, 2H, H-1), 1.8 (m, 2H, H-2), 1.69 (m, 1H, H-17), 1.59 (m, 1H, H-24), 1.57 (m, 1H, H-12), 1.52 (m, 2H, H-7), 1.50 (m, 1H, H-12), 1.48 (m, 1H, H-

25), 1.32 (m, 1H, H-8), 1.27 (m, 1H, H-24), 1.09 (d, *J*=7.2 Hz, 3H, H-21), 1.03 (s, 3H, H-19), 0.95 (m, 1H, H-9), 0.92 (m, 1H, H-14), 0.85 (d, *J*=6.7 Hz, 3H, H-27), 0.83 (s, 3H, H-18). ^{13}C NMR (150 MHz, CD_3OD): *δ* 216.4(C-22), 170.9, 167.8, 146.8, 142.2(C-5), 135.7, 131.6, 130.8, 130.1, 129.3, 122.2(C-6), 118.9, 103.7(C-1″), 102.1(C-1′), 80.9(C-16), 80.7(C-3′), 75.8(C-3″), 75.0(C-2″), 72.5(C-2′), 72.3(C-3), 70.9(C-4″), 69.4(C-5″), 68.1(C-26), 66.7(C-4′), 66.1(C-5′), 57.6(C-17), 55.5(C-14), 51.6(C-9), 44.9(C-20), 42.9(C-4), 42.8(C-13), 41.0(C-11), 39.2(C-23), 38.4(C-1), 37.6(C-10), 36.6(C-15), 36.3(C-25), 32.7(C-7), 32.2(C-2), 30.4(C-8), 28.0(C-24), 21.8(C-12), 21.5, 19.8(C-19), 17.0(C-27), 16.9(C-21), 13.5(C-18). $C_{48}H_{68}O_{14}$, HRMS (ESI): calcd for ($M+Na^+$): 891.4507; found: 891.4510.

(**51b**) ^{1}H NMR (600 MHz, CD_3OD): *δ* 7.77 (d, *J*=16.0 Hz, 1H), 7.64 (m, 2H), 7.42 (m, 3H), 6.62 (d, *J*=16.0 Hz, 1H), 5.34 (d, *J*=4.9 Hz, 1H, H-6), 4.86 (m, 1H, H-2″), 4.80 (d, *J*=7.6 Hz, 1H, H-1″), 4.77 (dd, *J*=5.7 Hz, *J*=8.0 Hz, 1H, H-2′), 4.09 (d, *J*=5.7 Hz, 1H, H-1′), 4.01 (m, 1H, H-16), 3.95 (dd, *J*=4.9 Hz, *J*=11.5 Hz, 1H, H-5″), 3.94 (dd, *J*=4.6 Hz, *J*=11.9 Hz, 1H, H-5′), 3.91 (m, 1H, H-4″), 3.72 (dd, *J*=3.1 Hz, *J*=8.0 Hz, 1H, H-3′), 3.61 (m, 1H, H-4′), 3.58 (t, *J*=8.9 Hz, 1H, H-3″), 3.49 (dd, *J*=1.9 Hz, *J*=11.9 Hz, 1H, H-5′), 3.4 (m, 1H, H-3), 3.34 (m, 2H, H-26), 3.30 (m, 1H, H-5″), 2.88 (m, 1H, H-20), 2.70 (m, 1H, H-23), 2.55 (m, 1H, H-23), 2.25 (m, 2H, H-4), 2.12 (m, 1H, H-15), 2.02 (s, 3H), 1.94 (m, 1H, H-11), 1.86 (m, 1H, H-1), 1.81 (m, 2H, H-2), 1.72 (m, 1H, H-17), 1.62 (m, 1H, H-24), 1.53 (m, 1H, H-25), 1.52 (m, 2H, H-12), 1.52 (m, 2H, H-7), 1.32 (m, 1H, H-8), 1.30 (m, 1H, H-24), 1.23 (m, 1H, H-11), 1.10 (d, *J*=7.3 Hz, 3H, H-21), 1.04 (s, 3H, H-19), 0.95 (m, 1H, H-9), 0.92 (m, 1H, H-14), 0.90 (d, *J*=6.7 Hz, 3H, H-27), 0.80 (s, 3H, H-18). ^{13}C NMR (150 MHz, CD_3OD): *δ* 216.6(C-22), 171.4, 168.3, 146.7, 142.2(C-5), 136.0, 131.4, 130.8, 129.9, 129.4, 122.2(C-6), 119.0, 103.5(C-1″), 101.8(C-1′), 81.3(C-16), 77.0(C-4″), 75.8(C-3″), 75.5(C-2″), 74.0(C-2′), 72.3(C-3), 71.1(C-3′), 71.1(C-4′), 68.2(C-26), 66.8(C-5″), 64.4(C-5′), 57.7(C-17), 55.5(C-14), 51.5(C-9), 44.8(C-20), 42.9(C-4), 42.7(C-13), 41.0(C-11), 39.3(C-23), 38.4(C-1), 37.6(C-10), 36.4(C-15), 36.4(C-25), 32.8(C-7), 32.2(C-2), 30.6(C-8), 28.0(C-24), 21.9(C-12), 21.1, 19.8(C-19), 17.0(C-21), 17.0(C-27), 13.6(C-18). $C_{48}H_{68}O_{14}$, HRMS (ESI): calcd for ($M+Na^+$): 891.4507; found: 891.4514.

(**52a**) ^{1}H NMR (600 MHz, CD_3OD): *δ* 7.74 (d, *J*=16.0 Hz, 1H), 7.65 (m, 2H), 7.45 (m, 3H), 6.57 (d, *J*=16.0 Hz, 1H), 5.34 (d, *J*=4.8 Hz, 1H, H-6), 5.10 (dd, *J*=7.5 Hz, *J*=9.7 Hz, 1H, H-2′), 4.85 (dd, *J*=7.6 Hz, *J*=9.1 Hz, 1H, H-2″), 4.65 (d, *J*=7.6 Hz, 1H, H-1″), 4.28 (d, *J*=7.5 Hz, 1H, H-1′), 4.01 (m, 2H, H-16, H-4′), 3.95 (dd, *J*=5.3 Hz, *J*=11.7 Hz, 1H, H-5″), 3.88 (dd, *J*=2.5 Hz, *J*=12.8 Hz, 1H, H-5′), 3.79 (dd, *J*=3.5 Hz, *J*=9.8 Hz, 1H, H-3′), 3.62 (m, 1H, H-3″), 3.54 (m, 2H, H-5′, H-4″), 3.36 (m, 2H, H-3, H-26), 3.33 (m, 2H, H-26, H-5″), 2.7 (m, 2H, H-11), 2.21 (m, 2H, H-4), 2.23 (m, 2H, H-15), 1.99 (m, 2H, H-2), 1.98 (s, 3H), 1.88 (m, 1H, H-1), 1.58 (m, 1H, H-25), 1.53 (m, 2H, H-12), 1.52 (m, 3H, H-7, H-20), 1.47 (m, 2H, H-22), 1.44 (m, 1H, H-24), 1.29 (m, 1H, H-8), 1.25 (m, 2H, H-23), 1.10 (m, 1H, H-17), 1.06 (m, 2H, H-1, H-24), 1.01 (s, 3H, H-19), 0.97 (m, 1H, H-9), 0.94 (d, *J*=6.9 Hz, 3H, H-27), 0.89 (d, *J*=6.6 Hz, 3H, H-21), 0.85 (m, 1H, H-14), 0.83 (s, 3H, H-18). ^{13}C NMR (150 MHz, CD_3OD): *δ* 169.5, 166.3, 145.3, 140.8(C-5), 134.3, 130.2, 129.0, 128.6, 128.2, 128.1, 127.8, 120.9(C-6), 117.5, 103.2(C-1′), 102.4(C-1″), 81.2(C-16), 79.4(C-3′), 74.4(C-4″), 73.7(C-2″), 71.1(C-2″), 71.0(C-3), 69.6(C-3″), 68.5(C-4′), 67.0(C-26), 65.3(C-5″), 65.1(C-5′), 60.6(C-17), 54.9(C-14), 50.3(C-9), 41.8(C-13), 41.5(C-4), 40.7(C-11), 37.0(C-1), 36.2(C-22), 36.1(C-15), 36.0(C-10), 35.1(C-25), 33.9(C-24), 31.5(C-20), 31.4(C-7), 30.8(C-2), 29.2(C-8), 22.1(C-23), 20.4(C-12), 20.2, 18.4(C-19), 17.3(C-27), 15.8(C-18), 11.7(C-21). $C_{48}H_{70}O_{13}$, HRMS (ESI): calcd for ($M+Na^+$): 877.4714; found: 877.4708.

(**52b**) ^{1}H NMR (600 MHz, CD_3OD): *δ* 7.72 (d, *J*=16.0 Hz, 1H), 7.59 (m, 2H), 7.38 (m, 3H), 6.50 (d, *J*=16.0 Hz, 1H), 5.30 (d, *J*=4.8 Hz, 1H, H-6), 4.92 (m, 1H, H-2′), 4.81 (m, 1H, H-2″), 4.52 (m, 1H, H-1″), 4.27 (d, *J*=6.9 Hz, 1H, H-1′), 4.01 (m, 1H, H-16), 3.95 (m, 2H, H-5′, H-5″), 3.88 (m, 1H, H-4″), 3.70 (m, 1H, H-3′), 3.54 (m, 1H, H-3″), 3.50 (m, 1H, H-5′), 3.46 (m, 2H, H-26), 3.34 (m, 1H, H-4′), 3.30 (m, 1H, H-3), 3.20 (m, 1H, H-5″), 2.19 (m, 2H, H-4), 2.05 (s, 3H), 1.97 (m, 1H, H-11), 1.80 (m, 1H, H-1), 1.76 (m, 2H, H-2), 1.54 (m, 1H, H-25), 1.47 (m, 2H, H-7), 1.46 (m, 3H, H-12, H-20), 1.40 (m, 2H, H-22), 1.31 (m, 4H, H-15, H-23), 1.28 (m, 1H, H-8), 1.11 (m, 2H, H-11, H-17), 1.02 (m, 3H, H-1, H-24), 1.00 (s, 3H, H-19), 0.93 (d, *J*=7.5 Hz, 3H, H-27), 0.88 (m, 1H, H-9), 0.81 (m, 1H, H-14), 0.81 (d, *J*=5.4 Hz, 3H, H-21). ^{13}C NMR (150 MHz, CD_3OD): *δ* 170.1, 167.6, 144.8, 140.7(C-5), 130.0, 128.5, 127.9, 127.7, 120.9(C-6), 117.1, 105.3(C-1″), 102.6(C-1′), 81.4(C-16), 78.4(C-4″), 76.3(C-3″), 73.6(C-2″), 73.5(C-3), 73.0(C-2′), 70.9(C-3′), 70.7(C-4′), 69.5(C-26), 65.2(C-5″), 64.4(C-5′), 60.6(C-17), 54.8(C-14), 50.2(C-9), 41.8(C-13), 41.5(C-4), 39.5(C-11), 37.0(C-1), 36.2(C-10), 36.0(C-15), 35.9(C-22), 35.4(C-25), 33.8(C-24), 31.4(C-20), 31.3(C-7), 30.8(C-2), 29.2(C-8), 22.2(C-23), 20.4(C-12), 19.9, 18.3(C-19), 17.2(C-27), 15.7(C-18), 11.7(C-21). $C_{48}H_{70}O_{13}$, HRMS (ESI): calcd for ($M+Na^+$): 877.4714; found: 877.4716.

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Supplementary data

Supplementary data (^{1}H NMR, ^{13}C NMR, COSY, HSQC, HMBC, NOESY, HPLC spectra of target compounds.) associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.tet.2016.05.049.

References and notes

1. Kubo, S.; Mimaki, Y.; Terao, M.; Sashida, Y.; Nikaido, T.; Ohmoto, T. *Phytochemistry* **1992**, *31*, 3969–3973.
2. Mimaki, Y.; Kuroda, M.; Kameyama, A.; Sashida, Y.; Hirano, T.; Oka, K.; Maekawa, R.; Wada, T.; Sugita, K.; Beutler, A. J. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 633–636.
3. Zhou, Y.; Garcia-Prieto, C.; Carney, D. A.; Xu, R. H.; Pelicano, H.; Kang, Y.; Yu, W. S.; Lou, C. G.; Kondo, S. J.; Liu, J. S.; Harris, D. M.; Estrov, Z.; Keating, M. J.; Jin, Z. D.; Huang, P. *J. Natl. Cancer Inst.* **2005**, *97*, 1781–1785.
4. Shi, B.; Tang, P.; Hu, X.; Liu, J. O.; Yu, B. *J. Org. Chem.* **2005**, *70*, 10354–10367.
5. (a) Morzycki, J. W.; Wojtkielewicz, A.; Wołczynski, S. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3323–3326; (b) Tang, P.; Mamdani, F.; Hu, X.; Liu, J. O.; Yu, B. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 1003–1007; (c) Ma, X.; Yu, B.; Hui, Y.; Miao, Z.; Ding, J. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2153–2156; (d) Peng, W.; Tang, P.; Hu, X.; Liu, J. O.; Yu, B. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 5506–5509; (e) Morzycki, J. W.; Wojtkielewicz, A. *Phytochem. Rev.* **2005**, *4*, 259–277.
6. Kuroda, M.; Mimaki, Y.; Yokosuka, A.; Sashida, Y.; Beutler, J. A. *J. Nat. Prod.* **2001**, *64*, 88–91.
7. Wojtkielewicz, A.; Dlugosz, M.; Maj, J.; Nowakowski, M.; Renkiewicz, J.; Strnad, M.; Swaczynova, J.; Wilczewska, A. Z.; Wojcik, J. *J. Med. Chem.* **2007**, *50*, 3667–3673.
8. Kandutsch, A. A.; Chen, H. W.; Heiniger, H. J. *Science* **1978**, *201*, 498–501.
9. Taylor, F. R.; Saucier, S. E.; Shown, E. P.; Parish, E. J.; Kandutsch, A. A. *J. Biol. Chem.* **1984**, *259*, 12382–12387.
10. Jun Im, Y.; Raychaudhuri, S.; Prinz, W. A.; Hurley, J. H. *Nat. Lett.* **2005**, *9*, 154–158.
11. Burgett, W. G. A.; Poulsen, B. T.; Wangkanont, K.; Anderson, R. D.; Kikuchi, C.; Shimada, K.; Okubo, S.; Fortner, C. K.; Mimaki, Y.; Kuroda, M.; Murphy, P. J.; Schwalb, J. D.; Petrella, C. E.; Cornella-Taracido, I.; Schirle, M.; Tallarico, A. J.; Shair, D. M. *Nat. Chem. Biol.* **2011**, *7*, 639–647.
12. Weber-Boyvat, M.; Zhong, W.; Yan, D.; Olkkonen, V. M. *Biochem. Pharmacol.* **2013**, *86*, 89–95.
13. Garcia-Prieto, C.; Ahmed, K. B. R.; Chen, Z.; Hammoudi, N.; Kang, Y.; Lou, C.; Mei, Y.; Jin, Z.; Huang, P. *J. Biol. Chem.* **2013**, *288*, 3240–3250.
14. Deng, L.; Wu, H.; Yu, B.; Jiang, M. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2781–2785.
15. Sakurai, K.; Fukumoto, T.; Noguchi, K.; Sato, N.; Asaka, H.; Moriyama, N.; Yohda, M. *Org. Lett.* **2010**, *12*, 5732–5735.
16. Zheng, D.; Zhou, L.; Guan, Y.; Chen, X.; Zhou, W.; Chen, X.; Lei, P. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 5439–5442.
17. Cheng, M. S.; Wang, Q. L.; Tian, Q.; Song, H. Y.; Liu, Y. X.; Li, Q.; Xu, X.; Miao, H. D.; Yao, X. S.; Yang, Z. *J. Org. Chem.* **2003**, *68*, 3658–3662.
18. Wang, H. X.; Su, F. Q.; Zhou, L.; Chen, X. G.; Lei, P. S. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 2796–2800.

19. Williams, J. R.; Chai, D.; Wright, D. *Steroids* **2002**, *67*, 1041–1044.
20. Deng, S. J.; Yu, B.; Lou, Y.; Hui, Y. Z. *J. Org. Chem.* **1999**, *64*, 202–208.
21. Ma, X. Q.; Yu, B.; Hui, Y. Z.; Xiao, D.; Ding. *Carbohydr. Res.* **2000**, *329*, 495–505.
22. Yu, W. S.; Jin, Z. D. *J. Am. Chem. Soc.* **2002**, *124*, 6576–6583.
23. Nicolaou, K. C.; Ohshima, T.; Hosokawa, S.; van Delft, F. L.; Vourloumis, D. *J. Am. Chem. Soc.* **1998**, *120*, 8674–8680.
24. (a) Burkert; Allinger, N. L. *Molecular Mechanics*; ACS Monograph, 1982, Vol. 177; (b) Ling, R.; Yoshida, M.; Mariano, P. S. *J. Org. Chem.* **1996**, *61*, 4439–4449.