Total synthesis of a furostan saponin, timosaponin BII†

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Received 12th March 2009, Accepted 15th May 2009
First published as an Advance Article on the web 15th June 2009
DOI: 10.1039/b905091d

The natural timosaponin BII, (25*S*)-26-*O*-β-D-glucopyranosyl-22-hydroxy-5β-furostane-3β,26-diol-3-*O*-β-D-glucopyranosyl-(1→2)-β-D-galactopyranoside, isolated from the rhizomes of *Anemarrhena asphodeloides* Bunge (Liliaceae), has been efficiently synthesized in ten steps and 18% overall yield. The strategy of using a partially protected glycosyl donor was applied to facilitate target synthesis. The cytotoxic activities of structurally related compounds were evaluated against HL-60 human promyelocytic leukaemia cells.

Introduction

It has been recognized that natural products are an excellent source of chemical structures with a broad range of promising pharmaceutical properties, including antitumor activity.[1] Saponins, a group of secondary metabolites present in a wide variety of plants, have opened up a new field of investigation of potential antitumor compounds, for example, OWS-1 and its analogues.[2] However, little has been known regarding the action mechanisms of saponins due to the poor accessibility to homogeneous saponins of various structures in appreciable amounts.[3] This situation has encouraged the chemical synthesis of saponins,[4] and enhances the collaboration among chemists and pharmacologists. Collaborating with others on a project involving bioactive saponin screening, we intend to find a suitable route towards the total synthesis of the natural product timosaponin BII (Fig 1).

Timosaponin BII, also called prototimosaponin AIII, was isolated from the rhizomes of *Anemarrhena asphodeloides* Bunge (Liliaceae) for the first time by Toshio Kawasaki and co-workers.[5] Its chemical structure (Fig. 1) was elucidated as (25*S*)-26-*O*-β-D-glucopyranosyl-22-hydroxy-5β-furostane-3β,26-diol-3-*O*-β-D-glucopyranosyl-(1→2)-β-D-galactopyranoside based on ^{1}H NMR and mass spectra.[6] Timosaponin BII has been reported as having useful pharmaceutical properties, including activities against dementia and stroke, and abilities to lower blood sugar levels, inhibit platelet aggregation and clear free radicals.[7] The relatively small amount of the compound from natural resources limits the potential commercial development of the compound, and also the screening potentials for other biological activities. Here we report a practical route towards the total synthesis of natural timosaponin BII starting from readily available aglycon sarsasapogenin, which was extracted and purified *via* known processes.[8]

Results and discussion

Timosaponin BII contains a β-D-glucopyranose substitute at the 26-OH and a β-D-Glc-(1→2)-β-D-Gal disaccharide unit at the 3-OH of furostan aglycon. Initially, we intended to attach a sugar unit on the 3-OH of sarsasapogenin **3** as the first step. It was reported that coupling a C-2-branched oligosaccharide to 3-OH of saponin aglycon would produce an inseparable mixture of C-1 stereo-isomers.[9] We have recently developed a new methodology to overcome this problem, *i.e.*, using partially protected thioglycoside as a glycosyl donor to facilitate the continuous C-2′ glycosylation.[10] Thus, condensation of isopropyl 3,6-di-*O*-benzoyl-1-thio-β-D-galactopyranoside[10] (**2**, Scheme 1) and **3** in dry CH_2Cl_2 in the presence of trimethylsilyl trifluoromethanesulfonate (TMSOTf) and *N*-iodosuccinimide (NIS) at –42 °C obtained the desired saponin derivative **4** in an

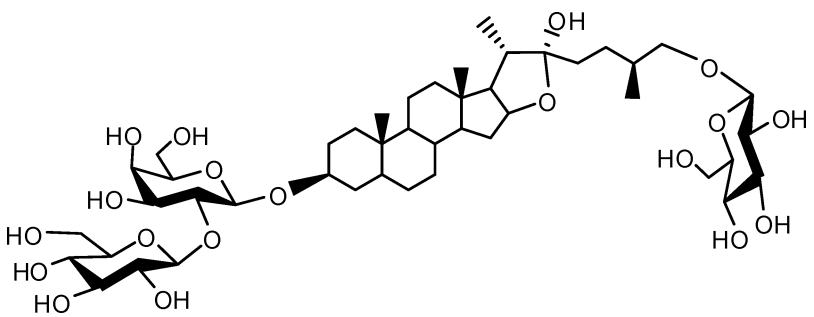

Fig. 1 Chemical structure of timosaponin BII.

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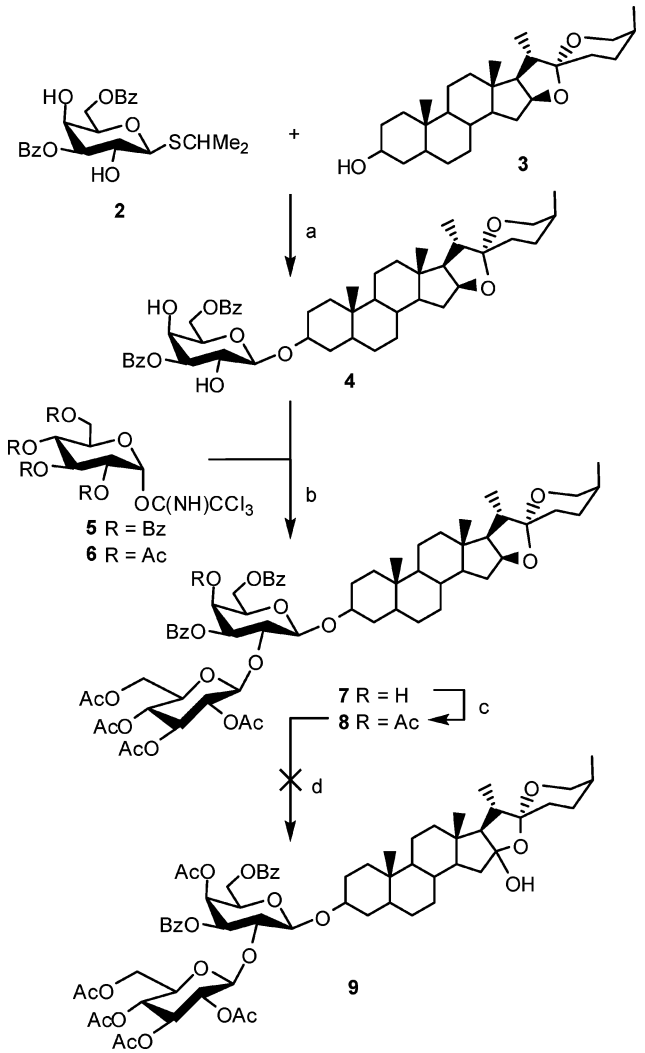
† Electronic supplementary information (ESI) available: ^{1}H and ^{13}C NMR spectra for compounds **1**, **4**, **7**, **10**, **13**, **14**, **15**, **17**, **18**, **20**. See DOI: 10.1039/b905091d



Scheme 1 Attempted synthetic strategy: (a) NIS, TMSOTf, CH_2Cl_2, –42 °C, 67%; (b) TMSOTf, CH_2Cl_2, –42 °C, no reaction using **5**, while 65% for **7** from **6**; (c) Ac_2O, Pyr, 93%; (d) oxone, $NaHCO_3$, 1.0 mM Na_2EDTA, CH_2Cl_2, acetone.

acceptable yield (67%). A small amount of α-isomer might also be generated, but the presence of inseparable contaminants did not permit us to rule out its formation. Taking advantage of the reactivity between the 2-OH and 4-OH of the galactose residue in **4**, we decided to try a regioselective coupling reaction using glycosyl trichloroacetimidate as a donor. It was found, surprisingly, that employing 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranosyl trichloroacetimidate (**5**) gave no expected product. Replacement of the donor with the sterically more favorable 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl trichloroacetimidate (**6**), the desired sarsasapogenyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(1→2)-3,6-di-*O*-benzoyl- β-D-galactopyranoside (**7**) was unambiguously obtained in 65% yield, as its acetylated derivative **8** showed H-4 of galactosyl residue at 5.58 ppm in the ^{1}H NMR spectrum. However, attempts to introduce 16-OH to compounds **7** or **8** using published Oxone chemistry[11] were frustrating, and no major component was observed under reaction conditions. We thus turned our attention to a more formal strategy (Scheme 2).

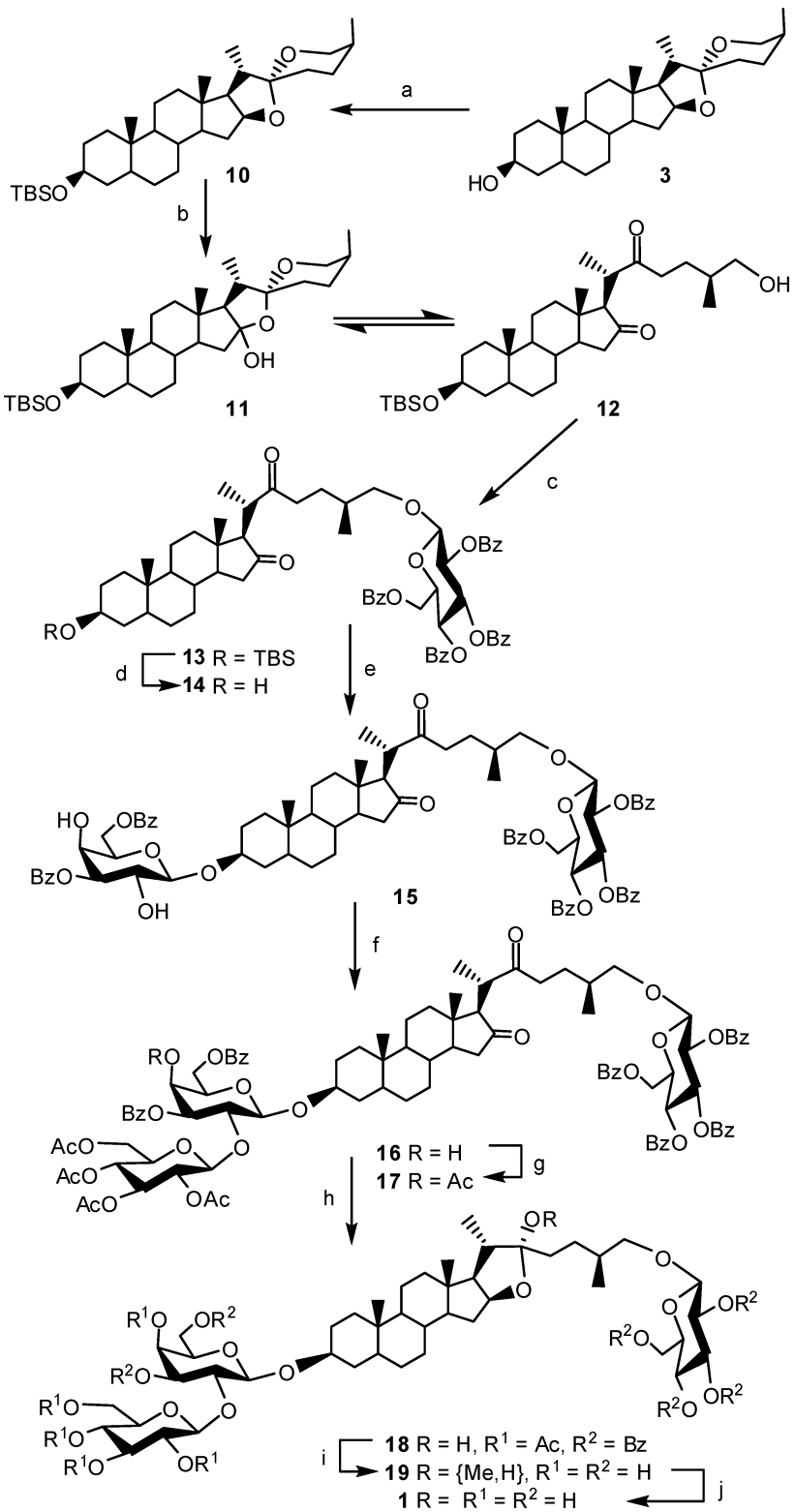
Employing a similar procedure to that developed by Bovicelli *et al.*,[12] the 3-*O*-*tert*-butyldimethylsilyl ether of sarsasapogenin (**3**) was oxidized with Oxone to afford an interconvertible mixture of compounds **11** and **12** in 81% total yield. Treatment of the mixture (**11**, **12**) with glycosyl donor **5** in the presence of a catalytic amount of TMSOTf in CH_2Cl_2 provided 26-*O*-glycosylated product **13** in a yield of 80%. TLC detection clearly indicated the transformation between **11** to **12** and **12** to **13**. Desilylation[13] of **13** with $BF_3{\cdot}Et_2O$ in CH_2Cl_2 gave **14**, which was subjected to glycosylation with **2** as described in the preparation of **4**, affording latent acceptor **15** in an acceptable yield (62%). A second glycosylation between **15** and **6** under similar conditions to those employed in the synthesis of **7**, followed by acetylation with Ac_2O in pyridine, gained the key diketone intermediate **17** (75% for two steps). It has been well documented that selective reduction of the 16-ketone of the cholestan-16,22-dione with $NaBH_4$ in *i*-PrOH provided the corresponding furostan through a concurrent intramolecular hemiketal formation.[14] Applying the same chemistry, dione **17** was successfully converted into hemiketal **18** in a moderate yield of 68%. Removal of all acyl protecting groups from **18** using NaOMe in $MeOH/CH_2Cl_2$ afforded a furostan saponin mixture **19** after column separation using MeOH/EtOAc as eluent. Refluxing of **19** in acetone/H_2O (3/7, v/v) furnished target molecule **1** as the sole product, which showed identical physical data to our authentic sample.[8]

The cytotoxic activities of (25*S*)-26-*O*-β-D-glucopyranosyl-22-hydroxy-5β-furostane-3β,26-diol-3-*O*-β-D-glucopyranosyl-(1→2)-β-D-galactopyranoside (**1**), sarsasapogenin (**3**), sarsasapogenyl β-D-glucopyranosyl-(1→2)-β-D-galactopyranoside (**20**, obtained from deacylation of compounds **7** and/or **8** (Scheme 1), and diosgenyl α-L-rhamnopyranosyl-(1→2)-β-D-galactopyranoside (**21**)[15] were evaluated against HL-60 human promyelocytic leukaemia cells (Table 1 and Fig. 2).[16] The saponins **1** and **20** from this project exhibited weak cytotoxic activity with IC_{50} values at 15.5 and 12.7 μg/mL, respectively. Aglycon **3** was not cytotoxic under our experimental conditions, while the positive control CDDP (cis-diaminodichloroplatinum) presented an IC_{50} value at 0.7 μg/mL. Compared to compound **20**, structural analogue **21** showed moderate activity under the same testing conditions, suggesting that the α-L-rhamnopyranosyl residue might play an important role in triggering cytotoxicity to the tumor cells.[17] In a parallel bioactivity screening, we also found that compound **1**, at a concentration of 10^{-5} M, could exert a direct relaxation effect on vascular smooth muscle in a significant dose-dependent way. More details will be published in due course.

Table 1 Cytotoxicity of compounds **1**, **3**, **20**, **21**, and CDDP against HL-60 cells[a]

Entry	Compound	IC_{50} (μg/mL)
1	**1**	15.5
2	**3**	> 30
5	**20**	12.7
6	**21**	7.1
7	**CDDP**	0.7

[a] The cells were continuously treated with each sample for 72 h, and the cell growth was evaluated using an MTT reduction assay. Data are mean values of three experiments performed in triplicate.



Scheme 2 Reagents and conditions: (a) TBSCl, imidazole, DMF, 97%; (b) oxone, $NaHCO_3$, 1.0 mM Na_2EDTA, CH_2Cl_2, acetone, 81%; (c) TMSOTf, CH_2Cl_2, 0 °C, 80%; (d) $Et_2O{\cdot}BF_3$, CH_2Cl_2, 95%; (e) **2**, NIS, TMSOTf, CH_2Cl_2, –20 °C, 62%; (f) **6**, TMSOTf, CH_2Cl_2, –42 °C, 30 min; (g) pyridine, Ac_2O, 75% in two steps; (h) $NaBH_4$, i-PrOH, CH_2Cl_2, 8 h, 68%; (i) NaOMe, CH_2Cl_2–MeOH (2:1, v/v); (j) acetone–H_2O (3:7, v/v), reflux, 95%.

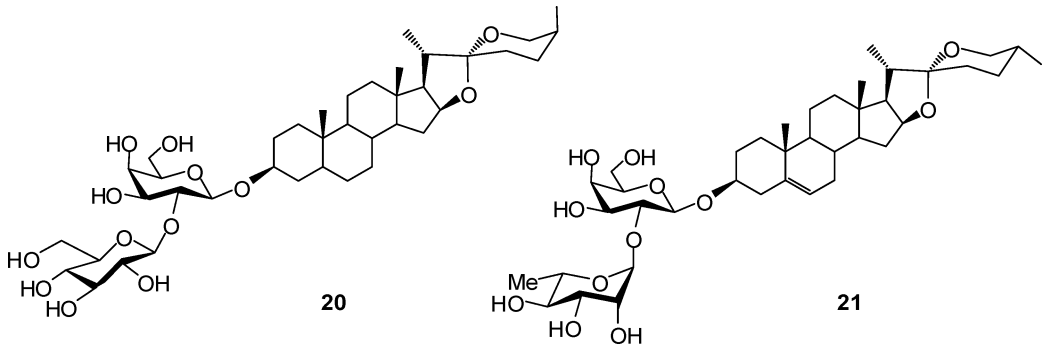


Fig. 2 Chemical structures of compounds **20** and **21**.

In conclusion, the finding of a new synthetic route to timosaponin BII opens up a possibility to develop novel physiologically active analogues and derivatives of sarsasapogenin. We have proved here that the target compound can be obtained in ten steps and in around 18% overall yield. The use of a partially protected glycosyl donor significantly simplified the tedious carbohydrate chemistry process. The current results should be valuable for related molecule design, synthesis, and bioactivity screening.[18]

General methods

Optical rotations were determined at 25 °C with a Perkin-Elmer Model 241-Mc automatic polarimeter. ^{1}H NMR and ^{13}C NMR spectra were recorded with a Bruker ARX 400 spectrometer for solutions in $CDCl_3$ or C_5D_5N. Chemical shifts are given in ppm downfield from internal Me_4Si. Mass spectra were measured using a MALDI TOF-MS with α-cyano-4-hydroxycinnamic acid (CCA) as matrix. Thin-layer chromatography (TLC) was performed on silica gel HF_{254} with detection by charring with 30% (v/v) H_2SO_4 in MeOH or in some cases by UV detector. Column chromatography was conducted by elution of a column of silica gel (100–200 mesh) with EtOAc–petroleum ether (60–90 °C) as the eluent. Solutions were concentrated at <60 °C under reduced pressure.

Synthesis of compound 4. To a mixture of compound **2** (750 mg, 1.68 mmol) and **3** (583 mg, 1.40 mmol) in anhydrous CH_2Cl_2 (20 mL) was added NIS (564 mg, 2.52 mmol) and Me_3SiOTf (30 μL, 0.17 mmol) under N_2 atmosphere at –42 °C. The mixture was stirred under these conditions for 50 min, then neutralized with TEA, and concentrated. Purification of the resulting residue on column chromatography (petroleum ether–EtOAc, 3:1) gave **4** as a white foamy solid (737 mg, 67%): $[\alpha]_D^{25}$ –21 (*c* 1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.08 (d, 2H, *J* 7.6 Hz, Bz), 8.00 (d, 2H, *J* 8.0 Hz, Bz), 7.58–7.53 (m, 2H, Bz), 7.44–7.40 (m, 4H, Bz), 5.19 (dd, 1H, *J* 3.2, 10.0 Hz, H-3), 4.62 (dd, 1H, *J* 6.6, 11.4 Hz, H-6a), 4.56 (dd, 1H, *J* 6.4, 11.3 Hz, H-6b), 4.47 (d, 1H, 7.7 Hz, H-1), 4.44–4.37 (m, 1H, H-16^{Sar}), 4.21 (d, 1H, *J* 2.3 Hz, H-4), 4.06–3.94 (m, 4H, H-2, H-5, H-3^{Sar}, H-26a^{Sar}), 3.30 (d, 1H, *J* 11.0 Hz, H-26b^{Sar}), 1.07 (d, 3H, *J* 7.1 Hz, 21-*CH*$_3$), 0.98 (d, 3H, *J* 6.7 Hz, 27-*CH*$_3$), 0.94 (s, 3H, 19-*CH*$_3$), 0.75 (s, 3H, 18-*CH*$_3$). ^{13}C NMR (100 MHz, $CDCl_3$): δ 165.8, 165.5, 132.7, 129.4, 129.2, 129.1, 127.9, 109.2, 101.4, 80.5, 74.9, 74.8, 71.8, 69.0, 66.8, 64.6, 62.3, 61.6, 59.9, 55.9, 41.6, 40.9, 40.1, 39.8, 39.6, 36.6, 34.7, 34.5, 31.2, 29.9, 29.7 26.5, 26.1, 25.4, 25.2, 23.3, 22.1, 20.5, 20.3, 15.9, 15.5, 13.8, 13.7. Anal. Calcd for $C_{47}H_{62}O_{10}$: C, 71.73; H, 7.94; Found: C, 71.50; H, 8.15%.

Synthesis of compound 7. To a mixture of compound **6** (376 mg, 0.76 mmol) and **4** (500 mg, 0.64 mmol) in anhydrous CH_2Cl_2 (15 mL) was added Me_3SiOTf (14 μL, 0.08 mmol) under N_2 atmosphere at –42 °C. The mixture was stirred under these conditions for 30 min, after which TLC (petroleum ether–EtOAc, 1:1) indicated that all starting materials were consumed. The reaction mixture was neutralized with TEA and concentrated. The syrup was purified by column chromatography (petroleum ether–EtOAc, 4:1) to give **7** as a white foamy solid (464 mg, 65%): $[\alpha]_D^{25}$ –19 (*c* 2, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.03 (d, 2H, *J* 7.6 Hz, Bz), 7.97 (d, 2H, *J* 7.6 Hz, Bz), 7.60–7.37 (m, 6H, Bz), 5.13 (dd, 1H, *J* 3.6, 9.9 Hz, H-3^{Gal}), 4.92 (t, 1H, *J* 10.4 Hz, H-3^{Glc}), 4.87 (t, 1H, *J* 9.2 Hz, H-2^{Glc}), 4.83 (d, 1H, *J* 8.4 H, H-4^{Glc}), 4.74 (d, 1H, *J*= 8.0 Hz, H-1^{Glc}), 4.55 (dd, 1H, *J* 6.4, 11.2 Hz, H-6a), 4.49 (d, 1H, *J* 7.6 Hz, H-1^{Gal}), 4.45 (dd, 1H, *J* 6.7, 11.3 Hz, H-6b), 4.39–4.36 (m, 1H, H-16^{Sar}), 4.27 (dd, 1H, *J* 5.2, 12.0 Hz, H-6a′), 4.15–3.87 (m, 6H, H-6b′, H-5, H-2^{Gal}, H-4^{Gal}, H-3^{Sar}, H-26a^{Sar}), 3.66–3.64 (m, 1H, H-5′), 3.27 (d, 1H, *J* 11.0 Hz, H-26b^{Sar}), 2.04, 1.99, 1.93, 1.86 (4 s, 4 × 3 C*H*$_3$CO), 1.04 (d, 3H, *J* 7.2 Hz, 21-*CH*$_3$), 0.96 (d, 3H, *J* 7.2 Hz, 27-*CH*$_3$), 0.94 (s, 3H, 19-*CH*$_3$), 0.72 (s, 3H, 18-*CH*$_3$). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.6, 170.2, 169.5, 168.9, 165.9, 165.2, 133.2, 132.8, 129.2, 129.1, 129.0, 128.8, 128.2, 127.9, 109.2, 100.4, 99.5, 80.5, 72.4, 71.4, 71.3, 70.7, 68.2, 66.7, 64.6, 62.1, 62.0, 61.6, 59.8, 55.9, 41.6, 40.1, 39.8, 39.6, 35.7, 34.8, 34.4, 31.2, 29.5, 26.5, 26.0, 25.7, 25.4, 25.2, 23.4, 22.1, 20.5, 20.3, 20.0, 19.6, 15.9, 15.5, 13.8, 13.6. Anal. Calcd for $C_{61}H_{80}O_{19}$: C, 65.57; H, 7.22; Found: C, 65.28; H, 7.25%.

Synthesis of compound 20. Compound **6** (100 mg, 0.089 mmol) was dissolved in anhydrous CH_2Cl_2–MeOH (18 mL, 2:1, v/v), and then 1.0 M NaOMe in MeOH (0.1 mL) was added slowly at room temperature. After stirring at room temperature for 3.5 h, TLC (*n*-BuOH : EtOH : H_2O = 4:2 : 0.5) indicated that all starting material was consumed. The reaction mixture was neutralized with Dowex 50 ion-exchange resin (H^+), then filtered and concentrated to dryness. Purification of the residue on Bio-gel P_2 column using double distilled water as eluent afforded, after freeze drying, compound **7** as an amorphous solid (64 mg, 95%): $[\alpha]_D^{25}$ –18 (*c* 1, H_2O); selected^{1}H NMR (400 MHz, C_5D_5N): δ 5.48 (d, 1H, *J* 7.6 Hz, H-1^{Glc}), 5.04 (d, 1H, *J* 8.0 Hz, H-1^{Gal}), 1.17 (d, 3H, *J* 6.8 Hz, 21-*CH*$_3$), 1.09 (d, 3H, *J* 6.8 Hz, 27-*CH*$_3$), 0.98 (s, 3H, 19-*CH*$_3$), 0.83 (s, 3H, 18-*CH*$_3$). ^{13}C NMR (100 MHz, C_5D_5N):

δ 110.1, 106.6, 103.0, 82.3, 81.8, 78.8, 77.4, 77.0, 75.9, 75.6, 72.1, 70.3, 65.6, 63.4, 63.2, 62.6, 56.9, 50.1, 42.9, 41.9, 41.3, 40.8, 40.7, 37.4, 36.0, 35.7, 32.6, 31.4, 28.0, 27.5, 27.2, 26.9, 26.7, 24.5, 21.6, 17.8, 17.4, 17.1, 16.7, 15.4. MALDITOF-MS: Calcd for $C_{39}H_{64}O_{13}$: 740.4 $[M]^+$; Found 763.4 $[M + Na]^+$.

Synthesis of compound 10. Compound **3** (13.0 g, 31.2 mmol) was dissolved in dry DMF (100 mL), and TBSCl (6.0 g, 39.8 mmol) was added to the solution. The mixture was stirred at 80 °C for 8 h, after which TLC (petroleum ether–EtOAc, 30:1) indicated that all starting materials were consumed. The reaction mixture was diluted with petroleum ether (300 mL), washed with water (450 mL × 2) and brine (300 mL). The organic phase was dried over anhydrous Na_2SO_4 and concentrated under vacuum to give a white solid **10** (16.1 g, 97%) that was directly subjected to the next reaction. Part of the crude product (50 mg) was purified with column chromatography (petroleum ether–EtOAc, 30:1) for analysis: $[\alpha]_D^{25}$ –47 (*c* 2, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.37–4.42 (m, 1H, H-16), 4.01 (br s, 1H, H-3), 3.95 (dd, 1H, *J* 2.6, 10.9 Hz, H-26a), 3.29 (d, 1H, *J* 11.8 Hz, H-26b), 1.07 (d, 3H, *J* 7.1 Hz, 21-*CH*$_3$), 0.98 (d, 3H, *J* 6.7 Hz, 27-*CH*$_3$), 0.94 (s, 3H, 19-*CH*$_3$), 0.87 (s, 9H, *t*-Bu), 0.75 (s, 3H, 18-*CH*$_3$), 0.00 (s, 6H, 2 *CH*$_3$).^{13}C NMR (100 MHz, $CDCl_3$): δ 109.6, 81.0, 67.3, 65.0, 62.1, 56.5, 42.1, 40.6, 40.0, 36.4, 35.3, 35.1, 27.0, 26.8, 26.7, 25.8, 25.7, 23.9, 20.9, 18.0, 16.4, 16.0, 14.3. Anal. Calcd for $C_{33}H_{58}O_3Si$: C, 74.66; H, 11.01; Found: C, 75.02; H, 10.91%.

Synthesis of compounds 11 and 12. To a mixture of compound **10** (16.1 g, 30 mmol) and $NaHCO_3$ (49 g, 0.58 mmol) in CH_2Cl_2–acetone–1.0 mM aqueous Na_2EDTA (450 mL, 1:1:1) was added dropwise a solution of Oxone ($2KHSO_5 \cdot KHSO_4 \cdot K_2SO_4$) (105 g, 0.169 mol) in a minimum amount of 1.0 mM aqueous Na_2EDTA. The reaction mixture was stirred at room temperature for 16 h, after which TLC (petroleum ether–EtOAc, 10:1) indicated that all starting materials were consumed. Removal of acetone and CH_2Cl_2 under diminished pressure was followed by dilution of the residue with CH_2Cl_2 and washing with water; the organic phase was dried over anhydrous Na_2SO_4 and concentrated to dryness. The crude product was purified with column chromatography (petroleum ether–EtOAc, 20:1) to afford a mixture of **11** and **12** as white solid (13.5 g, 81%). MALDITOF-MS: Calcd for $C_{33}H_{58}O_4Si$: 546.4 $[M]^+$; Found 549.2 $[M + Na]^+$.

Synthesis of compound 13. To a mixture of compounds **11**, **12** (2.25 g, 4.2 mmol), and **5** (3.56 g, 4.8 mmol) in anhydrous CH_2Cl_2 (40 mL) was added Me_3SiOTf (86 µL, 0.47 mmol) under N_2 atmosphere at 0 °C. The mixture was stirred under these conditions for 40 min, after which TLC (petroleum ether–EtOAc, 4:1) indicated that all starting materials were consumed. The reaction mixture was neutralized with triethylamine (TEA), then concentrated. Column chromatography (petroleum ether–EtOAc, 6:1) of the residue gave **13** as a foamy solid (3.78 g, 80%): $[\alpha]_D^{25}$ –26 (*c* 2, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.02–7.27 (m, 20H, 4*PhCO*), 5.90 (t, 1H, *J* 9.7 Hz, H-3^{Glc}), 5.67 (t, 1H, *J* 9.7 Hz, H-4^{Glc}), 5.53 (dd, 1H, *J* 7.8, 9.7 Hz, H-2^{Glc}), 4.86 (d, 1H, *J* 7.8 Hz, H-1^{Glc}), 4.63 (dd, 1H, *J* 3.3, 12.1 Hz, H-6a^{Glc}), 4.51 (dd, 1H, *J* 5.3, 12.1 Hz, H-6b^{Glc}), 4.19–4.14 (m, 1H, H-5^{Glc}), 4.05 (br s, 1H, H-3^{Sar}), 3.87 (dd, 1H, *J* 5.3, 9.4 Hz, H-26a^{Sar}), 3.31 (dd, 1H, *J* 7.3, 9.4 Hz, H-26b^{Sar}), 0.95 (s, 3H, 19-*CH*$_3$), 0.89 (d, 3H, *J* 6.6 Hz, 21-*CH*$_3$), 0.88 (s, 9H, *t-Bu* of TBS), 0.79 (d, 3H, *J* 6.6 Hz, 27-*CH*$_3$), 0.72 (s, 3H, 18-*CH*$_3$), 0.01 (s, 6H, 2*CH*$_3$). ^{13}C NMR (100 MHz, $CDCl_3$): δ 218.4, 213.6, 166.1, 165.8, 165.2, 165.1, 133.3, 133.1, 133.1, 133.0, 129.7, 129.7, 129.5, 129.3, 128.8, 128.7, 128.3, 128.3, 128.5, 101.4, 75.3, 72.9, 72.0, 71.9, 69.8, 67.2, 66.4, 63.2, 51.2, 43.2, 42.0, 39.8, 39.5, 39.1, 37.2, 36.3, 35.1, 34.7, 34.3, 32.6, 29.6, 28.5, 26.7, 26.6, 26.5, 25.9, 25.8, 25.8, 25.6, 23.8, 22.6, 20.5, 18.0, 16.8, 15.3, 13.1, –4.86, –4.89. Anal. Calcd for $C_{67}H_{84}O_{13}Si$: C, 71.50; H, 7.52; Found: C, 71.19; H, 7.40%.

Synthesis of compound 14. To a solution of compound **13** (3.6 g, 3.2 mmol) in dry CH_2Cl_2 (40 mL) was added $BF_3 \cdot Et_2O$ (1.0 mL, 7.3 mmol), and the mixture was stirred at room temperature for 4 h. The mixture was diluted with CH_2Cl_2 (60 mL), washed with saturated aqueous $NaHCO_3$ (100 mL) and then brine (80 mL). The organic layer was dried and concentrated. Purification by column chromatography (petroleum ether–EtOAc, 1:1) gave **14** as a white foamy solid (3.07 g, 95%): $[\alpha]_D^{25}$ –56 (*c* 2, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.02–7.27 (m, 20H, 4*PhCO*), 5.89 (t, 1H, *J* 9.7 Hz, H-3^{Glc}), 5.67 (t, 1H, *J* 9.7 Hz, H-4^{Glc}), 5.53 (dd, 1H, *J* 7.8, 9.7 Hz, H-2^{Glc}), 4.84 (d, 1H, *J* 7.8 Hz, H-1^{Glc}), 4.62 (dd, 1H, *J* 3.3, 12.0 Hz, H-6a^{Glc}), 4.50 (dd, 1H, *J* 5.1, 12.0 Hz, H-6b^{Glc}), 4.17–4.12 (m, 2H, H-5^{Glc}, H-3^{Sar}), 3.88 (dd, 1H, *J* 5.2, 9.4 Hz, H-26a^{Sar}), 3.29 (dd, 1H, *J* 7.4, 9.4 Hz, H-26b^{Sar}), 0.98 (s, 3H, 19-*CH*$_3$), 0.94 (d, 3H, *J* 6.6 Hz, 21-*CH*$_3$), 0.81 (d, 3H, *J* 6.6 Hz, 27-*CH*$_3$), 0.73 (s, 3H, 18-*CH*$_3$). ^{13}C NMR (100 MHz, $CDCl_3$): δ 218.1, 213.4, 166.0, 165.7, 165.1, 165.0, 133.3, 133.1, 133.0, 133.0, 129.7, 129.6, 129.6, 129.6, 129.5, 129.3, 128.8, 128.8, 128.3, 128.2, 128.2, 101.5, 75.28, 72.9, 72.0, 71.9, 69.9, 66.7, 66.4, 63.2, 60.3, 51.1, 43.2, 42.0, 39.6, 39.5, 39.0, 38.9, 37.1, 36.2, 35.1, 34.6, 33.4, 32.6, 29.5, 29.5, 29.4, 27.7, 26.7, 26.3, 26.3, 23.7, 22.6, 20.5, 19.1, 16.8, 15.2, 14.1, 13.1. Anal. Calcd for $C_{61}H_{70}O_{13}$: C, 72.45; H, 6.98; Found: C, 72.21; H, 7.09%.

Synthesis of compound 15. To a mixture of compound **2** (1.38 g, 3.08 mmol) and **14** (2.6 g, 2.6 mmol) in anhydrous CH_2Cl_2 (50 mL) was added NIS (1.04 g, 4.6 mmol) and Me_3SiOTf (55 µL, 0.30 mmol) under N_2 atmosphere at –20 °C. The mixture was stirred under these conditions for 60 min, then neutralized with TEA, and concentrated. Purification of the resulting residue on column chromatography (petroleum ether–EtOAc, 2:1) gave **15** as a foamy solid (2.23 g, 62%): $[\alpha]_D^{25}$ –20 (*c* 2, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.11–7.28 (m, 30H, 6*PhCO*), 5.90 (t, 1H, *J* 9.6 Hz, H-3^{Glc}), 5.67 (t, 1H, *J* 9.6 Hz, H-4^{Glc}), 5.53 (dd, 1H, *J* 7.8, 9.6 Hz, H-2^{Glc}), 5.18 (dd, 1H, *J* 3.2, 10.0 Hz, H-3^{Gal}), 4.84 (d, 1H, *J* 7.8 Hz, H-1^{Glc}), 4.64–4.48 (m, 4H, H-6a^{Glc}, H-6a^{Gal}, H-6b^{Glc}, H-6b^{Gal}), 4.48 (d, 1H, *J* 7.8 Hz, H-1^{Gal}), 4.23 (br d, 1H, *J* 3.2 Hz, H-4^{Gal}), 4.17–4.15 (m, 1H, H-5^{Gal}), 4.10 (br s, 1H, H-3^{Sar}), 4.05 (dd, 1H, *J* 7.8, 10.0 Hz, H-2^{Gal}), 3.96 (t, 1H, *J* 6.6 Hz, H-5^{Glc}), 3.88 (dd, 1H, *J* 5.2, 9.4 Hz, H-26a^{Sar}), 3.30 (dd, 1H, *J* 7.4, 9.3 Hz, H-26b^{Sar}), 0.95 (s, 3H, 19-*CH*$_3$), 0.94 (d, 3H, *J* 7.3 Hz, 21-*CH*$_3$), 0.82 (d, 3H, *J* 6.6 Hz, 27-*CH*$_3$), 0.73 (s, 3H, 18-*CH*$_3$). ^{13}C NMR (100 MHz, $CDCl_3$): δ 218.0, 213.3, 171.0, 166.2, 166.0, 165.9, 165.7, 165.1, 164.9, 133.2, 133.1, 133.1, 133.0, 132.9, 129.7, 129.6, 129.6, 129.5, 129.5, 129.2, 128.7, 128.7, 128.2, 128.1, 101.8, 101.4, 75.4, 75.2, 75.0, 72.9, 72.2, 71.9, 71.8, 69.8, 69.3, 67.1, 66.3, 63.1, 62.8, 60.2, 51.0, 43.1, 41.9, 39.7, 39.4, 38.9, 37.0, 36.7, 34.8, 34.5, 32.5, 30.1, 29.8, 26.6, 26.3, 26.2, 26.1, 23.6, 20.8, 20.4, 16.7, 15.2, 14.0, 13.0. Anal. Calcd for $C_{81}H_{88}O_{20}$: C, 70.42; H, 6.42; Found: C, 70.06; H, 6.64%.

Synthesis of compound 17. To a mixture of compound **6** (685 mg, 1.39 mmol) and **15** (1.6 g, 1.2 mmol) in anhydrous CH_2Cl_2 (15 mL) was added Me_3SiOTf (30 μL, 0.17 mmol) under N_2 atmosphere at –42 °C. The mixture was stirred under these conditions for 30 min, after which TLC (petroleum ether–EtOAc, 1:1) indicated that all starting materials were consumed. The reaction mixture was neutralized with TEA and concentrated to dryness. The resulting mixture was kept in pyridine (15 mL) and Ac_2O (5 mL) at room temperature for about 3 h, then the mixture was concentrated with the help of toluene. The syrup was purified by column chromatography (petroleum ether–EtOAc, 3:1) to give **17** as a white foamy solid (1.58 g, 75%): $[\alpha]_D^{25}$ –23 (*c* 2, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.01–7.28 (m, 30H, 6*PhCO*), 5.90 (t, 1H, *J* 9.6 Hz, H-3^{Glc-I}), 5.67 (t, 1H, *J* 9.6 Hz, H-4^{Glc-I}), 5.58 (dd, 1H, *J* 3.4, 4.1 Hz, H-4^{Gal}), 5.53 (dd, 1H, *J* 7.6, 9.6 Hz, H-2^{Glc-I}), 5.30 (dd, 1H, *J* 3.4, 9.9 Hz, H-3^{Gal}), 4.99 (t, 1H, *J* 9.2 Hz, H-3^{Glc-II}), 4.92 (t, 1H, *J* 9.2 Hz, H-4^{Glc-II}), 4.85 (dd, 1H, *J* 7.8, 9.2 Hz, H-2^{Glc-II}), 4.84 (d, 1H, *J* 7.8 Hz, H-1^{Glc-II}), 4.70 (d, 1H, *J* 7.6 Hz, H-1^{Glc-I}), 4.61 (dd, 1H, *J* 8.8, 12.0 Hz, H-6a), 4.56 (d, 1H, *J* 7.7 Hz, H-1^{Gal}), 4.53–4.48 (m, 2H, H-6a, H-6b), 4.36 (dd, 1H, *J* 7.2, 12.0 Hz, H-6a), 4.29 (dd, 1H, *J* 6.8, 11.1 Hz, H-6b), 4.19–4.12 (m, 1H, H-5), 4.10–4.04 (m, 4H, H-6b, H-3^{Sar}, H-5, H-2^{Gal}), 3.90 (dd, 1H, *J* 5.2, 9.4 Hz, H-26a^{Sar}), 3.72–3.69 (m, 1H, H-5), 3.30 (dd, 1H, *J* 7.4, 9.3 Hz, H-26b^{Sar}), 2.14, 2.08, 1.98, 1.89, 1.75 (5 s, 5 × 3 *CH*$_3$CO), 0.99 (s, 3H, 19-*CH*$_3$), 0.94 (d, 3H, *J* 7.3 Hz, 21-*CH*$_3$), 0.82 (d, 3H, *J* 6.6 Hz, 27-*CH*$_3$), 0.73 (s, 3H, 18-*CH*$_3$). ^{13}C NMR (100 MHz, $CDCl_3$): δ 218.0, 213.3, 170.5, 169.9, 169.7, 169.3, 169.2, 166.0, 165.9, 165.7, 165.3, 165.1, 165.0, 133.6, 133.3, 133.2, 133.1, 133.0, 133.0, 129.7, 129.6, 129.6, 129.5, 129.5, 129.5, 129.5, 129.4, 129.4, 129.3, 129.3, 129.1, 128.8, 128.6, 128.3, 128.3, 128.3, 128.2, 128.2, 101.4, 100.9, 99.5, 75.4, 75.2, 75.1, 73.9, 72.9, 72.8, 72.0, 71.9, 71.6, 71.1, 70.6, 69.9, 69.5, 68.7, 67.5, 66.4, 63.2, 62.5, 61.8, 60.2, 51.1, 43.2, 42.0, 41.3, 39.8, 39.5, 39.0, 37.1, 35.9, 34.9, 34.9, 34.6, 32.6, 29.7, 29.6, 26.7, 26.3, 26.2, 26.1, 25.6, 23.7, 22.5, 20.9, 20.7, 20.6, 20.5, 20.4, 20.1, 16.8, 15.2, 14.1, 13.0. Anal. Calcd for $C_{97}H_{108}O_{30}$: C, 66.43; H, 6.21; Found: C, 66.05; H, 6.27%.

Synthesis of compound 18. The mixture of compound **17** (300 mg, 0.17 mmol) and $NaBH_4$ (200 mg, 5 mmol) in *i*-propanol/CH_2Cl_2 (18 mL, 8:1, v/v) was stirred at room temperature for about 8 h. The reaction mixture was then poured into cold water, then extracted with CH_2Cl_2 (100 mL × 2). The combined organic layer was washed with water (100 mL × 3), dried over anhydrous Na_2SO_4, and concentrated to dryness. The resulting colorless oil was subjected to column chromatography using petroleum ether/EtOAc (v/v, 1:1) as eluent, to give compound **18** as a white solid (204 mg, 68%): $[\alpha]_D^{25}$ –5 (*c* 2, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.00–7.28 (m, 30H, 6*PhCO*), 5.88 (t, 1H, *J* 9.7 Hz, H-3^{Glc-I}), 5.67 (t, 1H, *J* 9.7 Hz, H-4^{Glc-I}), 5.58 (dd, 1H, *J* 3.4, 4.1 Hz, H-4^{Gal}), 5.53 (dd, 1H, *J* 7.8, 9.7 Hz, H-2^{Glc-I}), 5.30 (dd, 1H, *J* 3.4, 9.8 Hz, H-3^{Gal}), 5.00 (t, 1H, *J* 9.6 Hz, H-3^{Glc-II}), 4.93 (t, 1H, *J* 9.6 Hz, H-4^{Glc-II}), 4.86 (dd, 1H, *J* 7.8, 9.6 Hz, H-2^{Glc-II}), 4.81 (d, 1H, *J* 7.8 Hz, H-1^{Glc-II}), 4.70 (d, 1H, *J* 7.8 Hz, H-1^{Glc-I}), 4.61 (dd, 1H, *J* 3.0, 12.1 Hz, H-6a), 4.55 (d, 1H, *J* 7.5 Hz, H-1^{Gal}), 4.53–4.51 (m, 2H, H-6a, H-6b), 4.40 (dd, 1H, *J* 7.2, 12.1 Hz, H-6a), 4.30 (dd, 1H, *J* 6.8, 11.1 Hz, H-6b), 4.14–4.03 (m, 5H, H-5, H-6b, H-3^{Sar}, H-5, H-2^{Gal}), 3.87 (dd, 1H, *J* 5.2, 9.4 Hz, H-26a^{Sar}), 3.75–3.48 (m, 1H, H-5), 3.25 (dd, 1H, *J* 7.4, 9.3 Hz, H-26b^{Sar}), 2.14, 2.09, 1.98, 1.90, 1.75 (5 s, 5 × 3*CH*$_3$CO), 0.99 (s, 3H, 19-*CH*$_3$), 0.94 (d, 3H, *J* 7.3 Hz, 21-*CH*$_3$), 0.80 (d, 3H, *J* 6.6 Hz, 27-*CH*$_3$), 0.73 (s, 3H, 18-*CH*$_3$).^{13}C NMR (100 MHz, $CDCl_3$): δ 174.1, 170.9, 170.5, 169.9, 169.7, 169.2, 169.2, 165.9, 165.8, 165.6, 165.2, 165.0, 164.9, 163.9, 133.0, 133.0, 132.9, 129.6, 129.5, 129.5, 129.4, 129.3, 129.2, 129.2, 128.7, 128.5, 128.2, 128.2, 128.1, 110.1, 101.5, 100.8, 99.6, 81.1, 75.4, 75.4, 75.2, 73.8, 72.8, 72.8, 72.7, 72.0, 71.9, 71.8, 71.5, 71.0, 70.5, 69.8, 69.7, 68.6, 67.5, 63.0, 62.7, 62.3, 61.7, 60.2, 56.2, 41.45, 40.8, 39.9, 39.6, 36.0, 35.1, 34.8, 33.3, 31.5, 29.8, 29.7, 27.0, 26.3, 26.3, 26.0, 23.7, 20.8, 20.8, 20.8, 20.6, 20.5, 20.3, 20.0, 16.6, 16.5, 16.2, 15.3, 14.0, 13.5. Anal. Calcd for $C_{97}H_{110}O_{30}$: C, 66.35; H, 6.31; Found: C, 66.09; H, 6.40%.

Synthesis of compound 1. Compound **18** (202 mg, 0.114 mmol) was dissolved in anhydrous CH_2Cl_2–MeOH (36 mL, 1:2, v/v), and then 1.0 M NaOMe in MeOH (0.2 mL) was added slowly at 0 °C. After stirring at room temperature for 5 h, TLC (*n*-BuOH : EtOH : H_2O = 4:2 : 1) indicated that all starting material was consumed. The reaction mixture was neutralized with Dowex 50 ion-exchange resin (H^+), then filtered and concentrated to dryness. MS spectrum analyses suggested the resulting syrup is a mixture of **1** and its 22-methyl ether derivative. The above mixture in acetone/H_2O (40 mL, 3:7, v/v) was stirred under reflux conditions for 12 h, then concentrated to dryness under diminished pressure. Purification of the residue on Bio-gel P_2 column using double distilled water as eluent afforded, after freeze drying, target compound **1** as an amorphous solid (99 mg, 95%): $[\alpha]_D^{25}$ –6 (*c* 1, H_2O); selected ^{1}H NMR (400 MHz, C_5D_5N): δ 5.27 (d, 1H, *J* 7.5 Hz, H-1^{Glc-I}), 4.91 (d, 1H, *J* 7.5 Hz, H-1^{Gal}), 4.82 (d, 1H, *J* 7.6 Hz, H-1^{Glc-I}), 1.15 (d, 3H, *J* 6.9 Hz, 21-*CH*$_3$), 1.10 (s, 3H, 27-*CH*$_3$), 1.02 (d, 3H, *J* 6.5 Hz, 19-*CH*$_3$), 0.98 (s, 3H, 18-*CH*$_3$). ^{13}C NMR (100 MHz, C_5D_5N): δ 110.5, 105.8, 104.9, 102.3, 81.5, 81.0, 78.4, 78.2, 78.2, 77.8, 76.7, 76.4, 75.3, 75.2, 75.1, 75.0, 71.5, 71.5, 69.6, 63.8, 62.6, 62.0, 56.2, 41.0, 40.5, 40.2, 40.1, 36.9, 36.7, 35.3, 35.1, 34.2, 32.2, 30.7, 30.7, 28.1, 26.8, 26.6, 26.6, 23.8, 21.0, 17.3, 16.5, 16.3. MALDITOF-MS: Calcd for $C_{45}H_{76}O_{19}$: 920.5 $[M]^+$; Found 944.0 $[M + Na]^+$.

Cell culture assay

HL-60 cells were maintained in the RPMI 1640 medium containing 10% fetal bovin serum supplemented with L-glutamine, 100 units/mL penicillin, and 100 μg/mL streptomycin. The leukaemia cells were washed and suspended in the above medium to 1×10^5 cells/mL, and 100 μL of this cell suspension was placed in each well of a 96-well plate. The cells were incubated in 5% CO_2/air for 24 h at 37 °C. After incubation, 3 μL/mL of DMSO–H_2O (1:20, v/v) solution containing the sample was added to give the final concentration of 0.1–30 μL/mL, while 3 μL/mL of DMSO–H_2O (1:20, v/v) was added into control wells. The cells were further incubated for 72 h in the presence of each agent, and then cell growth was evaluated using a modified 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reduction assay, and the IC_{50} values were calculated accordingly.[16]

Acknowledgements

This work was partially supported by NNSF of China (Projects 20621703, 20872172, and 20732001).

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