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Synthesis and antitumor activity of icogenin and its analogue

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Abstract—Natural saponin icogenin, namely 25(*S*)-22-*O*-methyl-furost-5-en-3β,26-dio-3-*O*-α-L-rhamnopyranosyl-(1 → 2)-[β-D-glucopyranosyl-(1 → 3)]-β-D-glucopyranoside, and one of its analogues, 25(*S*)-22-*O*-methyl-furost-5-en-3β,26-dio-3-*O*-α-L-rhamnopyranosyl-(1 → 2)-[β-D-glucopyranosyl-(1 → 3)]-α-D-glucopyranoside, were first synthesized via line strategy and convergent approach, respectively. It was observed that icogenin and its analogue show potent antitumor activity in vitro.

It was well known 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 presented in a wide variety of plants, have been opened as a new field of investigation of potential anticancer compounds, for example, OSW-1.[2]

Icogenin, isolated from *Dracaena draco*,[3] shows significant cytotoxic effect on the growth of HL-60 with an IC_{50} of 2.6 ± 0.9 μM.[3] In order to further study the anticancer activity of icogenin, icogenin and one designed analogue were first synthesized.

Icogenin was synthesized from diosgenin via line strategy as shown in Scheme 1. According to the reported method,[4] **4** was synthesized in a facile way. Deprotection of the benzylidene group from **4**, followed by the sequence of oxidation with oxone and reduction, acetylation with Zn, KI, and HOAc–Ac_2O, the diosgenyl glycoside was transformed into 16,22-dione **5** within one-pot. Treatment of **5** with hydrazine acetate in CH_2Cl_2, then glycosylation with sugar donor **3**[5] under the promotion of NIS and a catalytic amount of TMSOTf, trisaccharide **6** was provided in a yield of 74% over two steps. The reduction of the C_{16}-ketone in compound **6** with $NaBH_4$ in *i*-PrOH was executed, and the newly generated secondary hydroxyl group currently cyclized with C_{17}-ketone to produce the hemiketal **7** in a yield of 42%. Methylation for the C_{22}–OH of **7** under the condition of reflux in methanol for 36 h, then deprotection of acetyl groups in the presence of MeO-Na–MeOH, gave the target compound **1** in a yield of 41%. The spectral data of the synthesized saponin **1** were identical to those of natural icogenin.[3]

During synthesis of the analogue of **11**, TBDPS has been used to protect diosgenin at rt by Cheng et al.[6] to give the corresponding protected diosgenin. Herein, taking cheaper TBDMSiCl instead of TBDPSiCl, the silyl ether **8** was synthesized at 50 °C in a yield of 95% within 15 min (Scheme 2). During the process of transformation of **8** into **10** in the presence of oxone and $NaHCO_3$, intermediate **9** was first provided in a yield of 98% with a 3:2 mixture of inseparable diastereomers which was confirmed by the data of ^1H NMR. Because of the interconversion between the opening and closure of the E and F rings,[7] the epoxide hemiketal **10** was also as a mixture of four compounds. Using Cheng's method, only a trace of **11** was produced from **10**. However, at the condition of 70 °C, **11** was provided in a yield of 65%. In the presence of CAN, **12** was furnished in a yield of 86%.

In order to get the α isomer of icogenin, one trisaccharide donor **19** was designed and synthesized as described in Scheme 3. According to the method of Nicolaou,[8] **13** was selectively masked by TBDMS to give **14**, which was coupled with **15**,[9] and disaccharide **16** was furnished in a yield of 92%. In the presence of HF–pyridine, **16**

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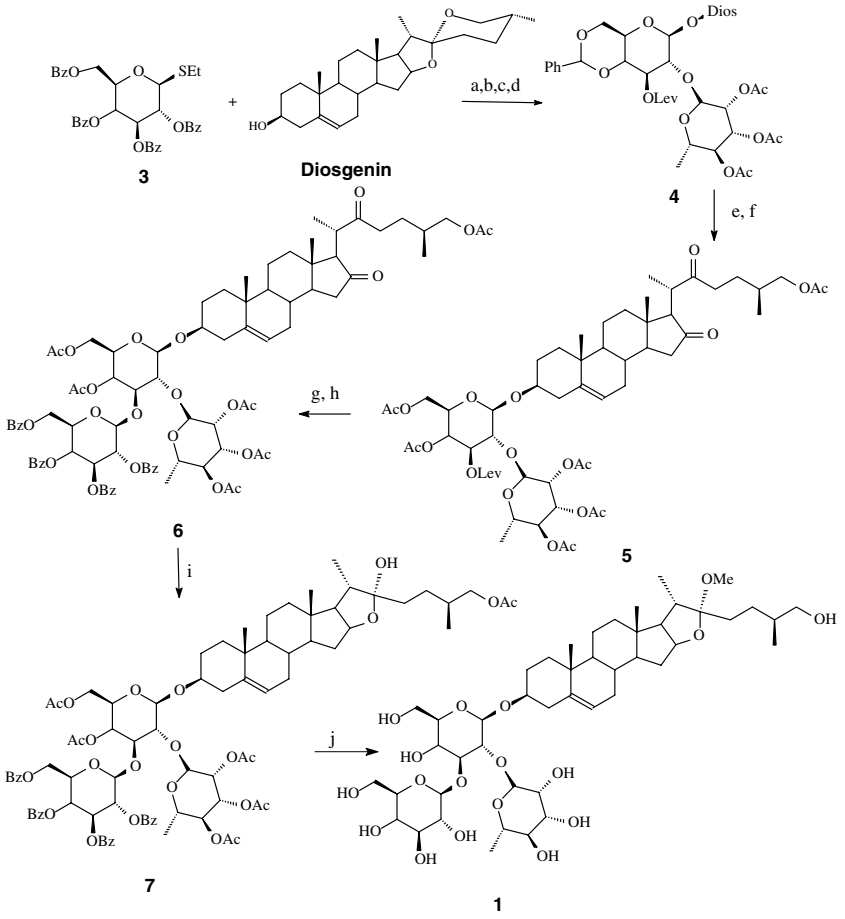


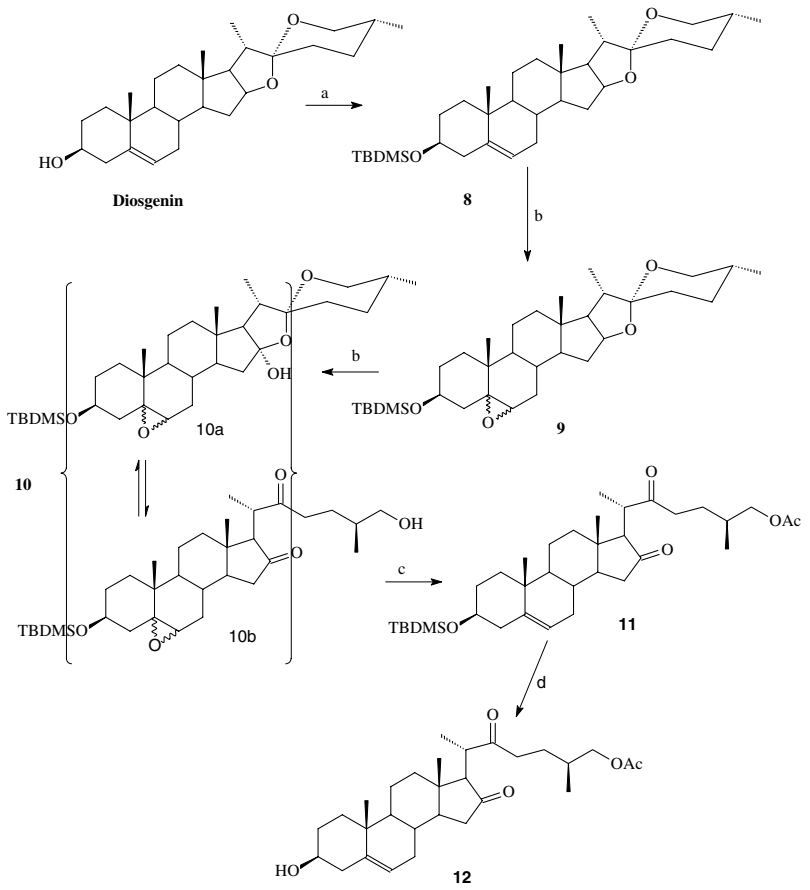
Scheme 1. Synthesis of icogenin (**1**). Reagents and conditions: (a) NIS-TMSOTf, 4 Å MS, CH_2Cl_2, −15 °C to rt, 94%; (b) 1 mol/L MeONa–MeOH, reflux, 92%; (c) $PhCH(OMe)_2$, DMF, *p*-TsOH, 75%; (d) i—levulinic acid, DCC, DMAP, CH_2Cl_2, rt, 73%; ii—NIS-TMSOTf, 4 Å MS, CH_2Cl_2, −30 °C to rt, 87%; (e) 80% HOAc, 70 °C, 82%; (f) oxone, $NaHCO_3$, CH_2Cl_2–acetone–H_2O, 24 h, rt and then Zn, KI, HOAc–Ac_2O, 50 °C, 12 h, 63%; (g) $H_2N{\cdot}NH_2$–HOAc, CH_2Cl_2–MeOH, rt, 89%; (h) NIS-TMSOTf, 4 Å MS, CH_2Cl_2, −10 °C to rt, 83%; (i) $NaBH_4$, *i*-PrOH, rt, 36 h, 42%; (j) MeOH, reflux, 36 h then 0.1 mol/L MeONa–MeOH, reflux, 12 h, 41%.

was converted into **17** in a yield of 93%. Under the promotion of $BF_3{\cdot}Et_2O$, sugar receptor **17** was glycosylated by donor **18**[10] to give trisaccharide **19**. Because of the steric hindrance of hydroxyl in **17**, the yield of **19** was 51%.

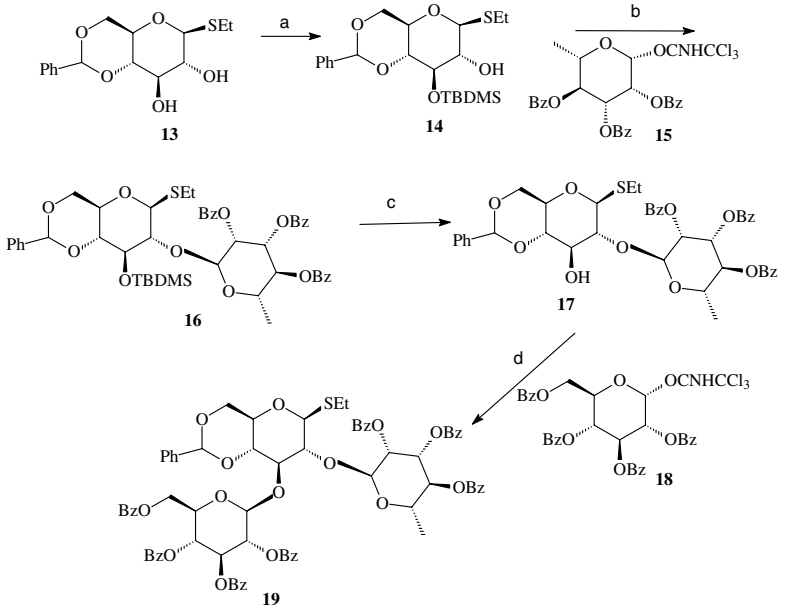
With trisaccharide donor **19** and modified sapogenin **12** efficiently synthesized in hand, their union via an idonium ion promoted glycosylation[11] was executed. Treatment of **12** and **19** with NIS-TMSOTf in mild condition gave the desired trisaccharide saponin **20** in a yield of 51%. The ^1H NMR analysis of **20** showed the signals for the three anomeric protons at δ 5.05 (d, $J = 3.6$ Hz, H-1′), 5.20 (br s H-1″), and 5.52 (d, $J = 7.6$ Hz, H-1‴), respectively. Contrasting these data to those of diosgenyl 2,3,4-tri-*O*-benzoyl-α-L-rhamanopyranosyl-(1 → 2)-[2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(1 → 3)-4,6-benzylidene-β-D-glucopyranoside[12](δ 4.68, d, $J = 8.0$ Hz, H-1′), it was concluded that the bond between the sugar and sapogenin in **20** is a form of 1,2-*cis* but 1,2-*trans*. Treatment with 80% HOAc at 70 °C, then benzoylation, followed by reduction with $NaBH_4$ in *i*-PrOH, **20** was transformed into hemiketal **21** in an overall yield of 38%. The designed target saponin **2** was eventually afforded by methoxylation at C_{22} position and then deprotection of all of acyl groups in a yield of 21% (Scheme 4).

The in vitro antitumor activities of the synthesized icogenin (**1**) and the designed analogue (**2**) against KETR3, PANC-1, PC-3M, H460, HCT8, BEL7402, BGC-823, and A431 were evaluated by the standard MTT assay.[13] As shown in Table 1, both icogenin (**1**) and the synthesized analogue (**2**) showed a broad spectrum of antitumor activities to the chosen cancer cells. Though these two furostan saponins show potent antitumor activity to PANC-1 with the IC_{50} values of 0.27 and 0.38 μM, respectively, analogue **2** having an α sugar moiety was more potent than **1** against H460, BGC-823 and A431 cell lines (Fig. 1).

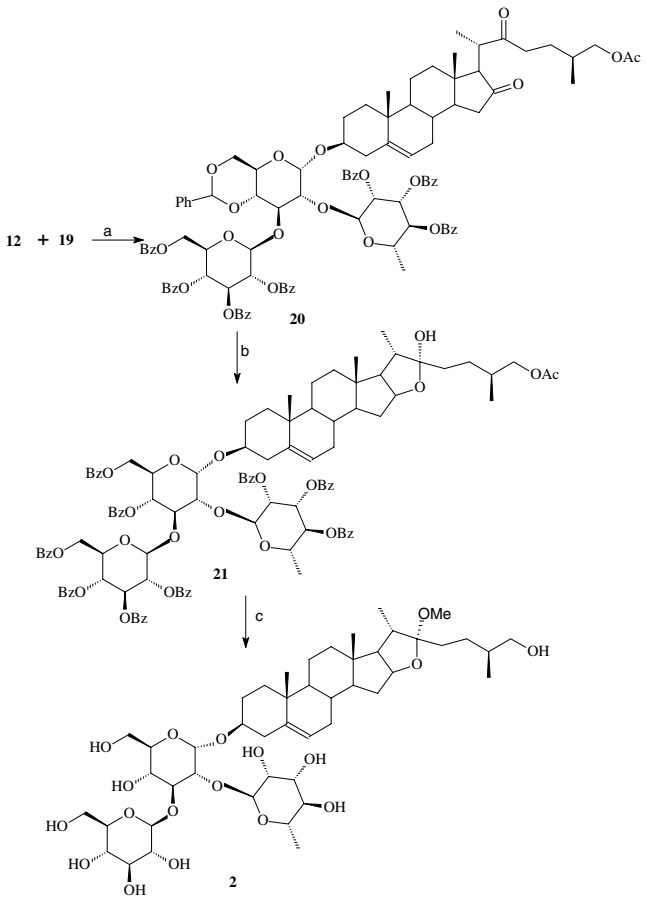
In summary, the total synthesis of icogenin and one of its analogue was carried out using line strategy and highly convergent approach, respectively. The antitumor activities of these two saponins were evaluated in vitro. Antitumor activity tests of these compounds demonstrated that the sugar moiety plays an important role in the antitumor action. Further studies on the



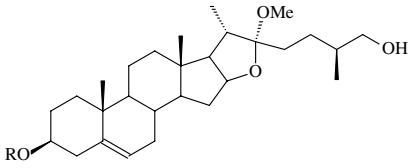
Scheme 2. Synthesis of modified saopgenin **12**. Reagents and conditions: (a) TBDMSiCl, DMAP, imidazole, DMF, 95%; (b) $NaHCO_3$, oxone, CH_2Cl_2–acetone–H_2O, 24 h, rt, 65%; (c) Zn, KI, HOAc–Ac_2O, 54%; (d) CAN, CH_2Cl_2–MeOH, 86%.



Scheme 3. Synthesis of trisaccharide donor (**19**). Reagents and conditions: (a) TBDMSiCl, DMAP, imidazole, DMF, 81%; (b) $BF_3{\cdot}Et_2O$, CH_2Cl_2, 4 Å MS, 92%; (c) HF–pyridine, THF, 93%; (d) $BF_3{\cdot}Et_2O$, CH_2Cl_2, 4 Å MS, 51%.



Scheme 4. Synthesis of icogenin analogue **2**. Reagents and conditions: (a) TMSOTf-NIS, CH_2Cl_2, 4Å MS, rt, 51%; (b) i—80% HOAc, 70 °C; ii—pyridine, BzCl; iii—$NaBH_4$, *i*-PrOH, 38% overall three steps; (c) MeOH, reflux, 36 h then 0.1 mol/L MeONa–MeOH, reflux, 12 h, 21%.



Icogenin: R=α-L-rhamnopyranosyl-(1→2)-[β-D-glucopyranosyl(1→3)]-β-D-glucopyranosyl (**1**);

The designed analogue of icogenin: R=α-L-rhamnopyranosyl-(1→2)-[β-D-glucopyranosyl(1→3)]-α-D-glucopyranosyl (**2**);

Figure 1. Icogenin and its designed analogue.

structure–activity relationship of icogenin and its analogue are in progess.

Acknowledgment

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References and notes

1. Cragg, G. M.; Newman, D. J. *Expert Opin. Invest. Drugs* **2000**, *9*, 2738.
2. Mimaki, Y.; Kuroda, M.; Kameama, A.; Sashida, Y.; Hirano, T.; Oka, K.; Maekawa, R.; Wada, T.; Sugita, K.; Beutler, J. A. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 633.
3. Hernandea, J. C.; Leon, F.; Quintana, J.; Estevez, F.; Bermejo, J. *Bioorg. Med. Chem.* **2004**, *12*, 4423.
4. Hou, S. J.; Zou, C. C.; Zhou, L.; Lei, P. S.; Yu, D. Q. *Chem. Lett.* **2005**, *34*, 1220.
5. Akulski, Z.; Pierozynski, D.; Zamojski, A. *Tetrahedron* **1994**, *50*, 2975.
6. 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.
7. Yu, B.; Liao, J. C.; Zhang, J. B.; Hui, Y. Z. *Tetrahedron Lett.* **2001**, *42*, 77.
8. Nicolaou, K. C.; Winssinger, N.; Pastor, J.; DeRoose, F. *J. Am. Chem. Soc.* **1997**, *119*, 449.
9. Ziegle, T.; Bien, F.; Jurish, C. *Tetrahedron: Asymmetry* **1998**, *9*, 795.
10. Fukase, K.; Winarno, H.; Kusumoto, S. *Chem. Express* **1993**, *8*, 409.
11. Hou, S. J.; Zou, C. C.; Lei, P. S.; Yu, D. Q. *Chin. Chem. Lett.* **2005**, *16*, 593.
12. Zou, C. C.; Hou, S. J.; Shi, Y.; Lei, P. S.; Liang, X. T. *Carbohydr. Res.* **2003**, *338*, 721.
13. Kuroda, M.; Mimaki, Y.; Sashida, Y.; Hirano, T.; Oka, K.; Dobashi, A.; Li, H.; Harada, N. *Tetrahedron* **1997**, *53*, 11549.

Further reading

14. Selective data for the key intermediate: compound **5**: ^{1}H NMR (400 MHz, $CDCl_3$), δ 5.38 (d, J = 4.8 Hz, 1 H, H-6), 5.29–5.23 (m, 3H, H-1″, H-2″, H-3″), 5.06 (t, J = 10.0 Hz, 1H, H-4′), 4.82 (t, J = 9.6 Hz, 1H, H-4″), 4.50 (d, J = 7.6 Hz, 1H, H-1′), 4.40–4.36 (m, 1H, H-5″), 4.29–4.24 (dd, J = 4.8 Hz, 12.0 Hz, 1H, H-6′$_a$), 4.10–4.07 (dd, J = 1.8 Hz, 12.0 Hz, 1H, H-6′$_b$), 3.94–3.90 (m, 1H, H-5′), 3.83–3.77 (m, 1H, H-3′), 3.63–3.56 (m, 3H), 2.12 (s, 3H, OAc), 2.09 (s, 3H, OAc), 2.04 (s, 3H, OAc), 2.02 (s, 3H, OAc), 2.01 (s, 3H, OAc), 1.97 (s, 3H, OAc), 1.20 (d,

Table 1. Cytotoxicity activity of icogenin and its designed analogue against tumor cells[a,b]

Compound	IC_{50} (μM)							
	KETR3	PANC-1	PC-3M	H460	HCT8	BEL7402	BGC-823	A431
1	5.15	0.270	4.35	>10	1.45	0.75	>10	3.44
2	3.78	0.380	2.76	8.16	1.42	0.95	2.52	0.67

[a] The standard MTT assay was followed.
[b] All the cell lines come from ATCC.

J = 6.4 Hz, 3H, H-6″), 1.06 (d, J = 6.4 Hz, 3H), 1.02 (s, 3H), 0.96 (d, J = 6.8 Hz, 3H), 0.92–0.84 (m, 6H), 0.79 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): 217.90, 213.29, 171.27, 170.98, 170.69, 170.15 (2× C), 169.84, 140.38, 121.35, 99.64, 97.37, 79.21, 76.30, 71.51, 71.34, 71.14, 69.54, 69.16, 69.00, 66.32, 66.13, 62.43, 51.17, 49.67, 43.32, 41.63, 39.67, 38.54, 38.44, 37.15, 36.78, 2.04, 31.55, 30.89, 29.52, 26.67, 22.77, 20.94, 20.89, 20.79, 20.50, 20.12, 19.14, 17.21, 16.78, 15.35, 14.37, 14.08, 12.96, 11.36. ESI-MS: 1014.6 $(M+Na)^+$.

Compound **7** ^{1}H NMR (400 MHz, $CDCl_3$), δ 8.01–7.30 (m, 20H, OBz), 6.04 (t, J = 9.6 Hz, 1H, 1H, H-3‴), 5.71 (dd, J = 9.9, 9.6 Hz, 1H, H-4‴), 5.44–5.36 (m, 3H, H-6, H-1″, H-2″), 5.33–5.29 (dd, J = 3.3, 10.5 Hz, 1H, H-3″), 5.16–5.04 (m, 2H, H-1‴, H-2‴), 4.86 (t, J = 9.9, 9.3 Hz, 1H, H-4″), 4.66–4.61 (dd, J = 3.0, 12.3 Hz, 1H, H-6a‴), 4.56–4.50 (dd, J = 4.8, 12.3 Hz, 1H, H-6b‴), 4.43–4.38 (m, 2H, H-1′, H-5″), 4.25–4.19 (m, 1H), 4.17–4.00 (m, 2H), 3.69 (t, J = 8.0 Hz, 1H), 3.48–3.33 (m, 3H), 2.18 (s, 3H, OAc), 2.08 (s, 3H, OAc), 2.05 (s, 3H, OAc), 2.02 (s, 3H, OAc), 2.03 (s, 3H, OAc), 1.90 (s, 3H, OAc), 1.23 (s, 3H), 1.08 (d, J = 6.0 Hz, 3H, H-6″), 1.01 (s, 3H), 0.97 (d, J = 6.6 Hz, 3H), 0.79 (d, J = 2.4 Hz, 3H), 0.78 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): 170.84, 170.68, 170.17, 169.95, 169.34, 166.08, 165.44, 165.15, 165.09, 140.19, 133.32, 133.19, 132.99, 129.89, 129.79, 129.70, 129.60, 129.24, 129.08, 128.84, 128.44, 128.33, 128.19, 128.12, 121.96, 109.28, 99.22 (2× C), 96.58, 80.76, 80.23, 78.73, 76.10, 72.75, 72.58, 72.17, 71.32, 71.07, 69.87, 66.76, 68.81, 68.15, 66.83, 66.54, 62.94, 62.43, 62.06, 56.44, 50.02, 41.59, 40.24, 39.70, 38.27, 37.11, 36.87, 32.07, 31.82, 31.39, 30.27, 29.67, 29.46, 28.78, 20.81, 20.75, 20.67, 19.26, 17.12, 16.27, 14.50. ESI-MS: 1595.8$(M+Na)^+$.

Compound **16**: ^{1}H NMR (400 MHz, $CDCl_3$), δ 8.07–7.23 (m, 20H, OBz), 5.81 (br s, 1H, H-2′), 5.78 (dd, J = 3.2, 9.6 Hz, 1H, H-3′), 5.68 (s, 1H, H-1′), 5.60 (t, J = 10.0 Hz, 1H, H-4′), 5.46 (s, 1H, PhCH), 4.93 (m, 1H, H-5′), 4.65 (d, J = 9.6 Hz, 1H, H-1), 4.36 (dd, J = 4.4, 10.8 Hz, 1H, H-6_a), 4.10 (t, J = 8.0 Hz, 1H), 3.83 (t, J = 8.0 Hz, 1 H), 3.73 (t, J = 10.0 Hz, 1H), 3.55–3.44 (m, 2H), 2.87–2.80 (m, 2H), 1.40–1.30 (m, 6H, SCH_2CH_3, H-6′), 0.65 (br s, 9H, CMe_3), −0.01 (2s, 6H, $SiMe_2$). ESI-MS: 910.2 $(M+Na)^+$.

Compound **21**: ^{1}H NMR (400 MHz, $CDCl_3$), δ 8.70–6.88 (m, 40H, OBz), 6.70 (dd, J = 2.4, 10.4 Hz, 1H, H-3″), 6.51 (br s, 1H, H-2″), 6.37 (d, J = 8.4 Hz, 1H, H-1‴), 6.30 (t, J = 10.0 Hz, 1H, H-4″), 6.13 (t, J = 9.2, 8.8 Hz, 1H), 5.99 (t, J = 9.6 Hz, 1H), 5.78 (t, J = 9.6 Hz, 1H), 5.72 (br s, 1H, H-1″), 5.64 (d, J = 3.6 Hz, 1H, H-1′), 5.35 (dd, J = 9.2, 9.6 Hz, 1H), 5.29 (br s, 1H, H-6), 4.96–4.87 (m, 10H), 4.82–4.75 (m, 2H), 4.70–4.59 (m, 3H), 4.37–4.34 (dd, J = 3.6, 9.6 Hz, 1H), 4.12–4.08 (dd, J = 6.4, 10.8 Hz, 1H), 4.09–3.96 (dd, J = 6.4, 10.8 Hz, 1H), 3.87 (m, 1H), 2.92 (m, 1H), 2.77 (m, 1H), 2.29–2.24 (m, 2H), 2.00 (s, 3H, $COCH_3$), 1.59 (d, J = 7.0 Hz, 3H), 1.34 (d,J = 6.8 Hz, 3H, H-6″), 1.09 (s, 3H), 0.95 (s, 3H), 0.92 (d, J = 6.4 Hz, 3H). ^{13}C NMR (400 MHz, $CDCl_3$): 169.60, 165.48, 165.15, 164.99, 164.91, 164.68, 164.57, 164.33, 139.13, 133.65, 133.36, 109.31, 99.41, 98.58, 96.05, 80.84, 79.98, 79.01, 74.43, 72.20, 71.01, 70.68, 70.42, 70.14, 68.19, 67.35, 62.60, 55.45, 49.06, 39.68, 39.50, 38.77, 35.83, 32.13, 31.31, 30.50, 29.30, 27.75, 27.05, 19.98, 19.56, 18.29, 17.04, 15.86, 15.32, 15.21. ESI-MS:1905 $(M+Na+1)^+$(base).

Compound **2**: ^{13}C NMR (100 MHz, pyridine-d_5): 140.71, 121.77, 109.22, 105.20, 101.79, 99.91, 81.94, 81.06, 78.46, 78.24, 78.04, 77.70, 77.25, 76.18, 74.93, 74.07, 72.72, 72.43, 71.14, 69.46, 66.82, 62.84, 62.01, 61.83, 56.58, 50.22, 41.92, 40.41, 39.80, 38.87, 37.44, 37.08, 32.26, 32.19, 31.79, 31.64, 30.57, 30.10, 29.96, 29.23, 21.06, 19.36, 18.64, 17.31, 16.31, 15.03. ESI-MS: 907 $(M+Na-MeOH)^+$.