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Synthesis and cytotoxicities of icogenin analogues with disaccharide residues

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ABSTRACT

For further structure–activity relationships (SAR) research of furostan saponin, two icogenin analogues: (25*R*)-22-*O*-methyl-furost-5-en-3β,26-diol-3-*O*-α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranoside **1** and (25*R*)-22-*O*-methyl-furost-5-en-3β,26-diol-3-*O*-α-L-rhamnopyranosyl-(1 → 2)-α-D-glucopyranoside **2**, with valuable disaccharide moieties, were synthesized from diosgenin through eight steps. Both of the analogues behaved the similar cytotoxic activities with icogenin, towards nine types of human tumor cells herein.

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As a common and significative feature, the cytotoxicity of furostan saponin has been given particular attention by researchers in recent years.[1,2] Icogenin,[3] namely (25*R*)-22-*O*-methyl-furost-5-en-3β,26-diol-3-*O*-α-L-rhamnopyranosyl-(1 → 2)-[β-D-gluco-pyanosyl-(1 → 3)]-β-D-glucopyranoside, a typical furostan saponin isolated from *Dracaena draco*, showed potent cytotoxic activity on the growth of HL-60, the IC_{50} was 2.6 ± 0.9 μM at 72 h, owing to the induction of apoptosis in HL-60 cells. In 2006, icogenin and its α-epimer were first synthesized by Hou in our lab.[4,5] Icogenin consists of a biggish trisaccharide moiety and a furostan sapogenin (Fig. 1). In order to decipher the structure–activity relationships and mechanism of action of furostan saponin, we employed the simplified disaccharide [α-L-Rha*p*-(1-2)-β-D-Glc*p*], which commonly existed in the oligosaccharides' skeletons of icogenin and many natural saponins,[6–8] and contributed to the cytotoxic activity of saponin molecule,[9] replacing the trisaccharide moiety of icogenin to design two icogenin analogues (**1** and **2**, Fig. 1). Herein, a facile and efficient way for the synthesis of these two analogues and their cytotoxicities against human tumor cells were reported.

Inspired by the report about the synthesis of methyl protodioscin[2] by Chen, we designed a concise and efficient approach in Scheme 1 for the synthesis of icogenin analogues. The analogues were attempted to construct with **3** and **4**. To get both 1,2-trans and 1,2-cis products in the reaction of glycosylation, the thioglycoside **3** having a non-neighbouring group-active substituent at C-2 position was synthesized following the modified method of Hou[4,5] as shown in Scheme 2. In the presence of trimethylsilyl trifluoromethanesulfonate (TMSOTf), glucosyl pentaacetate **5** was treated with ethanethiol to give two epimers: **6** and **7** (**6:7** = 1:1). The latter was deacetylated, then treated with benzaldehyde dimethylacetal and *p*-toluenesulfonic acid monohydrate (*p*-TsOH·H_2O) in DMF to afford **8**. Under the condition of *tert*-butyldimethylsiyl chloride (TBDMSiCl), imidazole and catalytic amount of 4-dimethylamino pyridine (DMAP), the TBDMS group masked product **9** was formed. Using Triethylsilyl trifluoromethanesulfonate (TESOTf) as a promoter,[10] the remaining 2-OH of **9** coupled with rhamnopyranosyl trichloroacetimidate **10**,[11] the expected product **11** was obtained. Treated with acetic acid-water (4:1, v/v) at 80 °C, the benzylidene group and TBDMS group were removed in one pot, followed with acetylation, **11** was transformed into thioglycoside **3** in 70% yield.

The sapogenin **4** was prepared from disogenin (Scheme 3) by the approach of Chen.[2] Diosgenin was protected with TBDMSiCl at 3-OH provided **12** in a satisfactory 98% yield. Oxyfunctionalization of C-16 and 5,6 double bond with oxone in the presence of $NaHCO_3$ afford two isomer: **13** and **14** (**13**:**14** = 1:1), which were readily distinguished by their ^{1}H NMR spectra. In isomer **13**, the signal of 3-proton appeared at 3.63 ppm, while it was shifted downfield to 3.85 ppm in **14**, because of the affection of 5,6-epoxy ring. As both isomers could be used in the next step, treatment of **13** and **14** with Zn/KI in Ac_2O/HOAc gave 16,22-dione **15** successfully. Removal of the 3-TBDMS group of **15** provided the sapogenin **4** at last.

In Scheme 4, glycosylation of the 3-OH in sapogenin **4** with thioglycoside **3** under the promotion of *N*-iodosuccinimide (NIS) and

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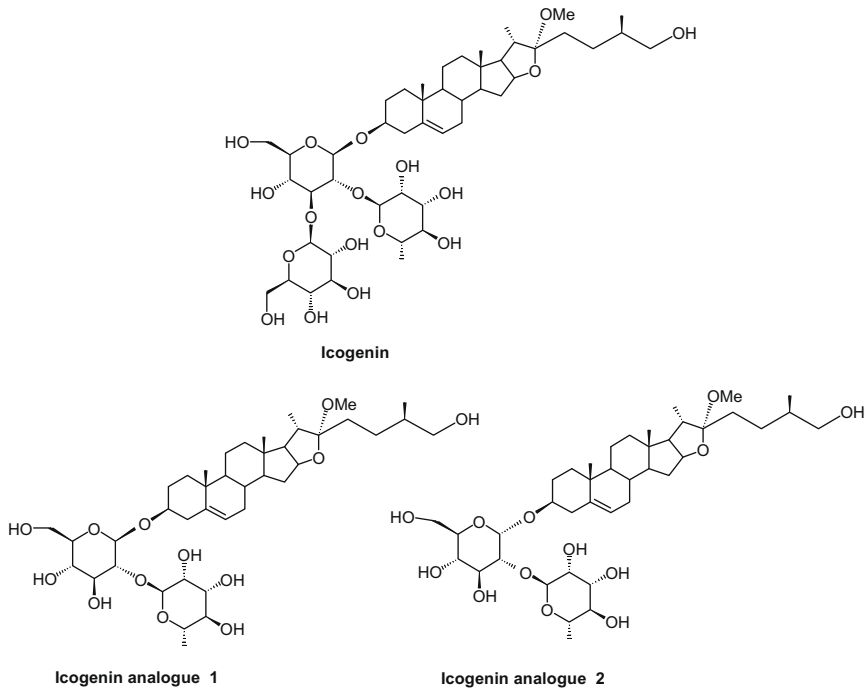
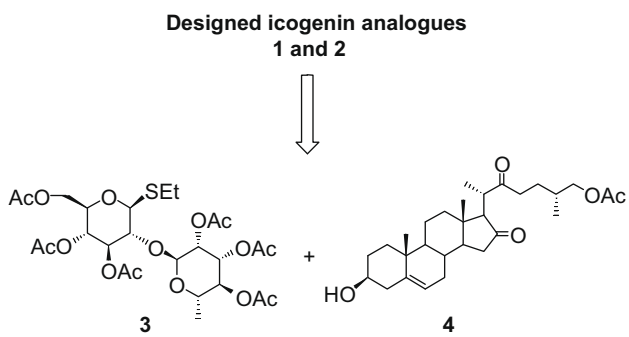
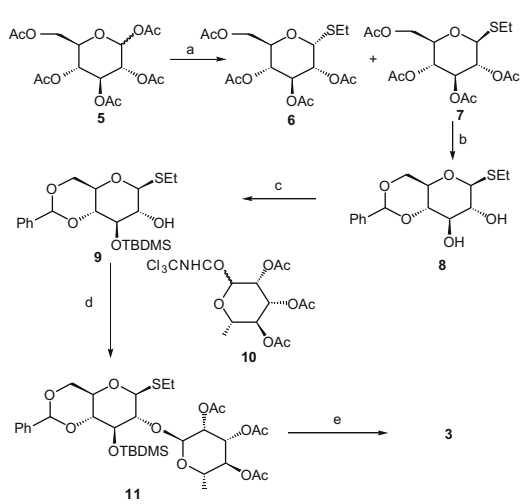


Figure 1. Icogenin and its analogues.



Scheme 1. The concise approach for the synthesis of analogues.



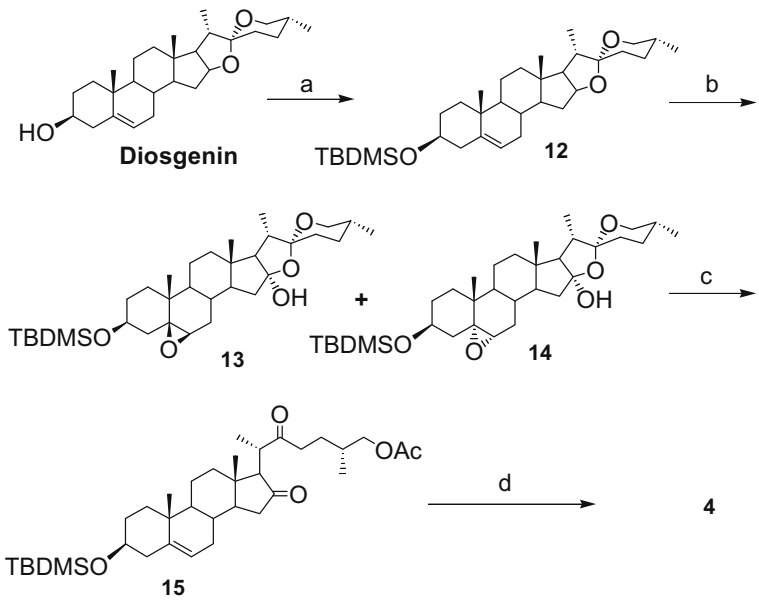
Scheme 2. Reagents and conditions: (a) EtSH, TMSOTf, CH_2Cl_2, rt, 12 h, 66%; (b) MeONa(cat.), MeOH, 3 h, rt; then $Ph(MeO)_2CH$, p-TsOH·H_2O, 50 °C, 5 h, 70%; (c) TBDMSiCl, DMAP, imidazole, rt, 10 h, 85%; (d) TESOTf, CH_2Cl_2, −20 °C, 52%; (e) 80%HOAc, 80 °C, 6 h; $(Ac)_2O$, pyridine, rt, 12 h, 70%.

TMSOTf led to the desired mixture of two epimers: **16** and **17**. Without further separation, the subsequent reduction[2,12] of the epimeric mixture with $NaBH_4$ in *i*-PrOH gave two hemiketals: **18** and **19** (**18**:**19** = 2:3). The ^{1}H NMR spectra showed that the signal of 1′-β-anomeric proton of epimer **18** appeared at δ ~5.04 ppm (d, 1 H, $J_{1,2}$ = 7.8 Hz), and the 1′-α-anomeric proton of epimer **19** appeared at δ ~5.48 ppm (d, 1 H, $J_{1,2}$ = 3.6 Hz). After the methoxylation of C_{22}–OH and the deprotection of acetyl groups, the designed icogenin analogues **1** and **2** were finally obtained efficiently.

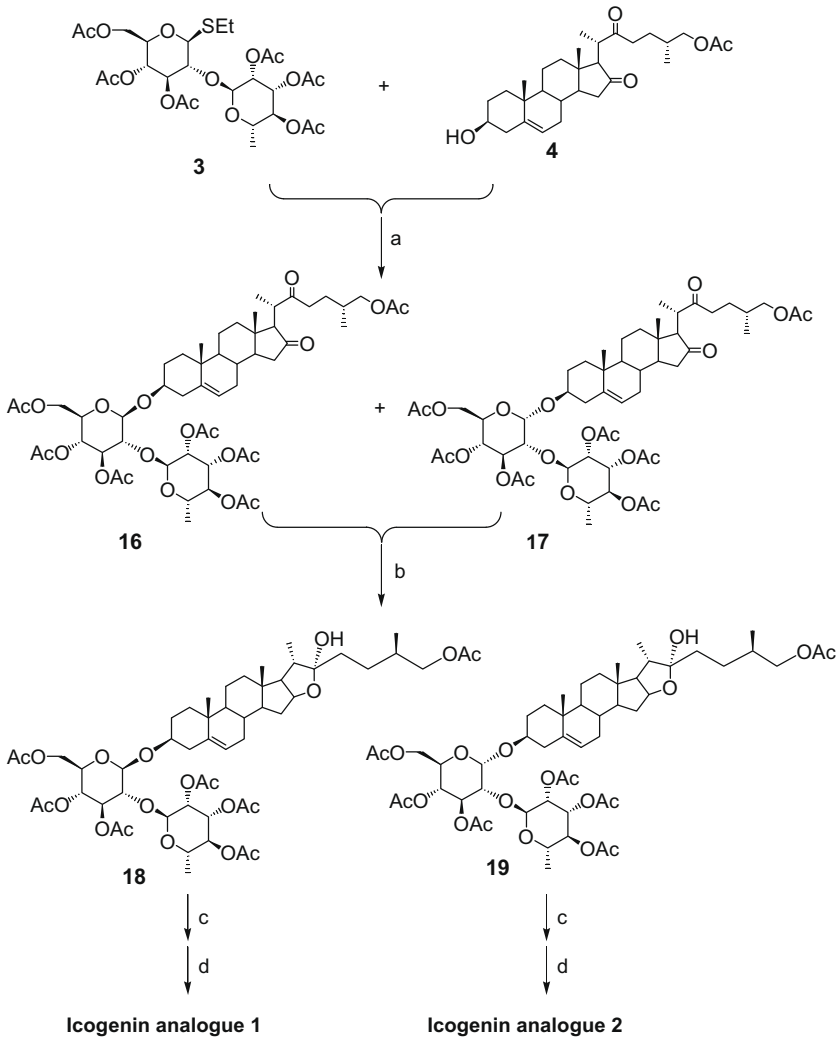
It was also found that the C_{16}–ketone was more active than the C_{22}–ketone in dione **15**,[13] the preferential reduction[2,14] of the C_{16}–ketone in **15** with $NaBH_4$ generated a secondary alcohol in C-16 which cyclized with C_{22}–ketone to give hemiketal **20** concurrently (Scheme 5). The methyloxylation of C_{22}–OH and removal of TBDMS group were achieved in the presence of p-TsOH·H_2O in MeOH/CH_2Cl_2, furnishing the intact icogenin sapogenin **21** in one pot. When the coupling of **21** and **3** under the promotion of NIS and TMSOTf was tried, no expected product was found while the byproduct **22** was formed (Fig. 2). Removal of the 26-acetyl with NaOMe in MeOH provided a novel sapogenin **23**.

When amberlite IR-120 (H^+) (ion-exchange) was used to change for the remnant Na^+ in the deprotection of **19**, another byproduct **24** was gotten (Fig. 2). This phenomenon combining with the formation of **22** demonstrated the furostan sapogenin of icogenin was instable in weak acid condition. The similar process happened in the acetylation of icogenin also.[3]

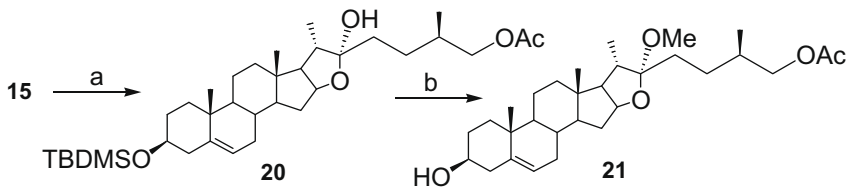
The cytotoxic activities of icogenin analogues (**1** and **2**) and diosgenyl saponin **24** against the growth of nine human tumor cell lines were evaluated comparing with icogenin and three typical diosgenyl saponins (dioscin,[7,15] gracillin[8] and polyphillin C,[16] Fig. 3), which broadly exist in the nature and exhibit valuable cytotoxicities. The results were listed in Table 1. It was showed that the diosgenyl saponin **24** did not exhibit considerable inhibition at a concentration of 10 μM towards all nine tumor cell lines, while the designed icogenin analogues (**1** and **2**) behaved as potent as icogenin (α and β) and three typical diosgenyl saponins. Similarly, isomers **1** and **2** were almost same cytotoxic towards all tested tumor cell lines.



Scheme 3. Reagents and conditions: (a) TBDMSiCl, DMAP, imidazole, DMF, 50 °C, 30 min, 98%; (b) Oxone, $NaHCO_3$, H_2O/CH_2Cl_2, rt, 48 h, 85%; (c) Zn powder, KI, HOAc/$(Ac)_2O$, rt, 24 h, 86%; (d) *p*-TsOH·H_2O, CH_2Cl_2/MeOH, rt, 1 h, 92%.



Scheme 4. Reagents and conditions: (a) NIS, TMSOTf, 4 Å molecular sieves, CH_2Cl_2, −15 °C, 30 min, 41%; (b) $NaBH_4$, *i*-PrOH/CH_2Cl_2, −15 °C, overnight, 92%; (c) MeOH/CH_2Cl_2, 80 °C, 24 h; (d) MeONa, MeOH, 80 °C, 24 h.



Scheme 5. Reagents and conditions: (a) $NaBH_4$, $CeCl_3 \cdot 7H_2O$, CH_2Cl_2, −15 °C, 1 h, 36%; (b) *p*-TsOH·H_2O, MeOH/CH_2Cl_2, rt, 1 h, 90%.

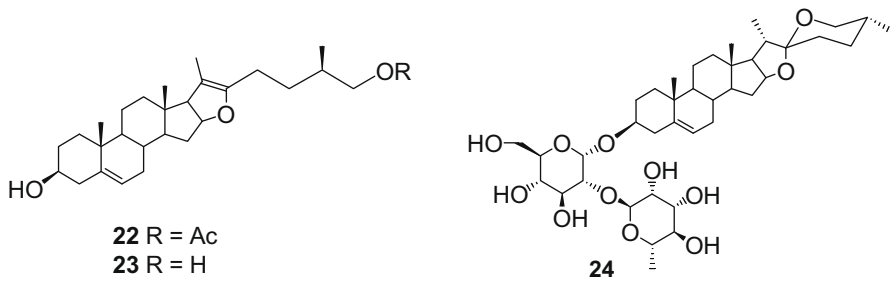
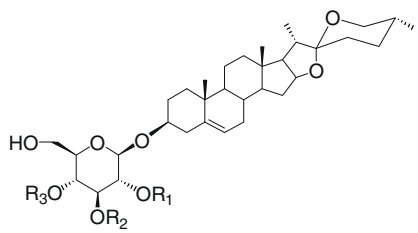


Figure 2. The structures of the byproducts.



Compounds	R_1	R_2	R_3
Dioscin	α-L-rha	H	α-L-rha
Gracillin	α-L-rha	β-D-glu	H
Polyphillin C	H	α-L-rha	H

Figure 3. Three typical diosgenyl saponins.

The present results indicated that the icogenin analogues with simplified disaccharide residues (**1** and **2**) reserved the cytotoxic activities of icogenin, and the epimerization of the anomeric carbon in the analogues made no noticeable difference in the inhibition towards all nine tumor cell lines herein.

In summary, ground on the previous structure–activity relationships research on the cytotoxicity of furostan saponin, especially icogenin, a pair of icogenin analogues (**1** and **2**) with disaccharide residues was rationally designed and synthesized from diosgenin in 8 steps. This pair of epimeric analogues was achieved in one route. In the succedent experiments, two byproducts (**23** and **24**) were obtained also. Both of the icogenin analogues (**1** and **2**) showed similar potent cytotoxicities as icogenin at the concentration of 10 μM against nine tumor cell lines. These results suggested that the disaccharide residues in the analogues (**1** and **2**) could preserve the cytotoxic activities of icogenin and the configuration of the anomeric carbon was not essential to the cytotoxicities. While comparing with the typical trisaccharide diosgenyl saponins (dioscin, gracillin and polyphillin C) herein, the loss of the cytotoxicities of disaccharide diosgenyl saponin **24** in the concentration of 10 μM, indicated the disaccharide residue might not keep the cytotoxicities of diosgenyl saponin. The precise molecular mechanism underlying these disparities in potency among saponin analogues remains to be further studied.

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Supplementary data

Supplementary data (the experimental procedures for synthesis, analytical data for key compounds and cell proliferation assay)

Table 1
The in vitro cytotoxicities (IC_{50}, μM) of synthetic icogenin analogues and saponin analogues[a]

Cell lines	Icogenin (β)	α-Icogenin	Dioscin	Gracillin	Polyphillin C	Gemcitabine	Icogenin analogue **1**	Icogenin analogue **2**	Diosgenyl saponin **24**
SW1990	1.0	0.9	0.50	1.1	5.4	1.2	0.66	0.73	>10
BxPC3	1.1	1.0	0.70	1.3	>10	2.9	1.01	1.04	>10
Capan2	1.1	1.1	0.05	1.3	2.7	1.7	1.07	1.36	>10
PANC1	0.84	0.82	0.81	0.70	11.8	5.6	0.83	0.87	>10
A549	1.86	0.89	0.70	0.96	2.3	1.4	1.08	1.48	>10
Bel7402	0.86	0.92	0.23	1.1	>10	0.84	0.90	0.94	>10
A2780	0.92	7.1	4.46	4.3	>10	0.31	7.2	7.8	>10
HCT-8	0.93	1.3	0.56	0.92	4.16	1.74	1.02	1.26	>10
MCF-7	1.07	1.2	1.03	17.5	3.45	3.28	2.89	2.65	>10

[a] The in vitro cytotoxic activities against SW1990 (pancreatic cancer), BxPC3 (pancreatic cancer), Capan2 (pancreatic cancer), PANC1 (pancreatic cancer), A549 (lung carcinoma), Bel7402 (liver cancer), A2780 (ovarian cancer), HCT-8 (colon carcinoma) and MCF-7 (breast cancer) cell lines were evaluated by the standard MTT assay using Gemcitabine, a clinical antitumor drug, as a positive control.

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