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Synthesis of bisdesmosidic kryptogenyl saponins using the 'random glycosylation' strategy and evaluation of their antitumor activity

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Abstract—A bisdesmosidic steroidal saponins library, composed of 16 novel kryptogenin glycosides, was set up via six random glycosylation procedures, wherein two compounds showed their antitumor activity against HeLa cell in the preliminary pharmacological research.

Steroidal saponins comprise a diverse class of plant glycosides, which possess a broad range of promising biological property, such as antitumor activity.[1,2] As an important access to find novel steroidal glycoside structures or to scale up some interesting natural products which are scarce in nature, chemical synthesis plays a dominant role and receives increasing attention. However, particular protecting group manipulation is often required to accomplish the regioselective glycosylation of a certain multi-hydroxyl aglycone to produce bisdesmosidic or tridesmosidic saponins.[3] Therefore, it is usually time-consuming for the lengthy course of stepwise glycosylation work.

As an alternative approach, 'random glycosylation' strategy developed by Hindsgaul and co-workers [4] was proved to be effective for the rapid construction of oligosaccharide libraries. In the successful application of this method, completely unprotected saccharide receptor was glycosylated to generate a mixture of glycosides containing all the possible products for further bioactivity screening research.[5] In addition, a process centered upon the inherent promiscuity of secondary metabolite-associated glycosyltransferases is one of the latest promising developments toward the same goal.[6] Although the lack of regioselectivity in the two approaches makes the target individual compound difficult to be isolated from the library,[7] the 'randomization' strategy can accelerate the rate of finding new lead in the initial phase of drug discovery.

To the best of our knowledge, the random glycosylation strategy was mainly used in the reactions where sugar donor glycosylated randomly with other unprotected sugar moiety.[5] We, herein report the work of building a bisdesmosidic steroidal saponins library, containing 16 diverse kryptogenin glycosides, by using different monosaccharide donors to glycosylate with kryptogenin randomly. The preliminary antitumor activity of these synthetic saponins was then investigated.

Kryptogenin **1**, namely 3β,26-dihydroxy-25(*R*)-cholest-5-en-16,22-dione, was used to be an intermediate in the research of metabolism of bile acid in last century.[8] As a natural sapogenin, **1** owns a basic chemical structure of cholesterol, which ensures its reliability of being the mimetics in the biosynthesis research. Furthermore, by far researches on cholestanol glycosides are not as much as those on spirostanol or furostanol saponins, either in synthetic method or in bioactivity evaluation. Hence, we chose **1** as the aglycone to investigate the bioactivity of the target bisdesmosidic cholestanol saponins bearing different sugar moiety on both side chains. Since **1** is no longer commercially available and the reported

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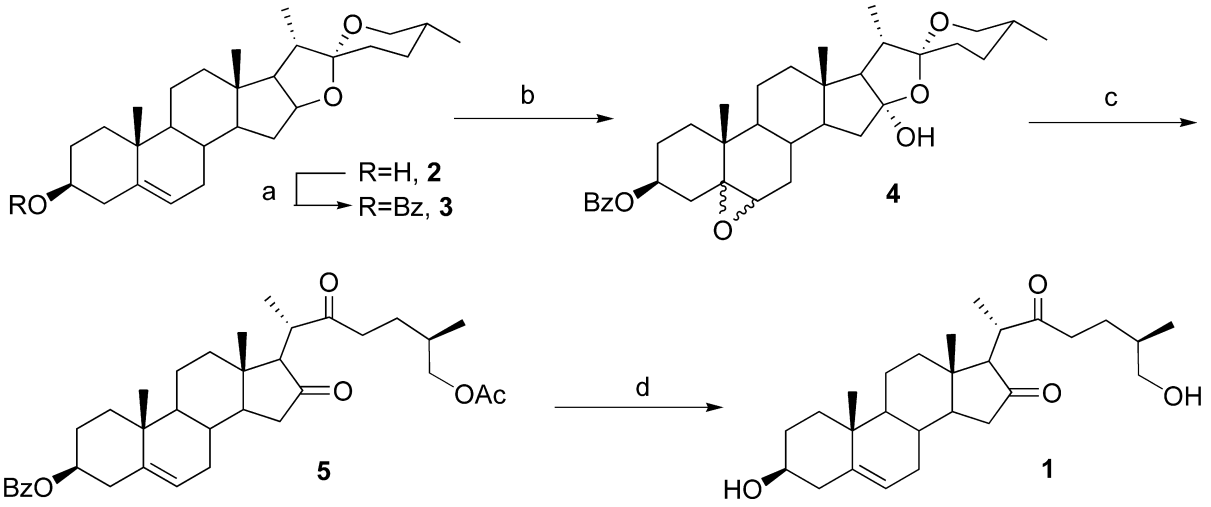
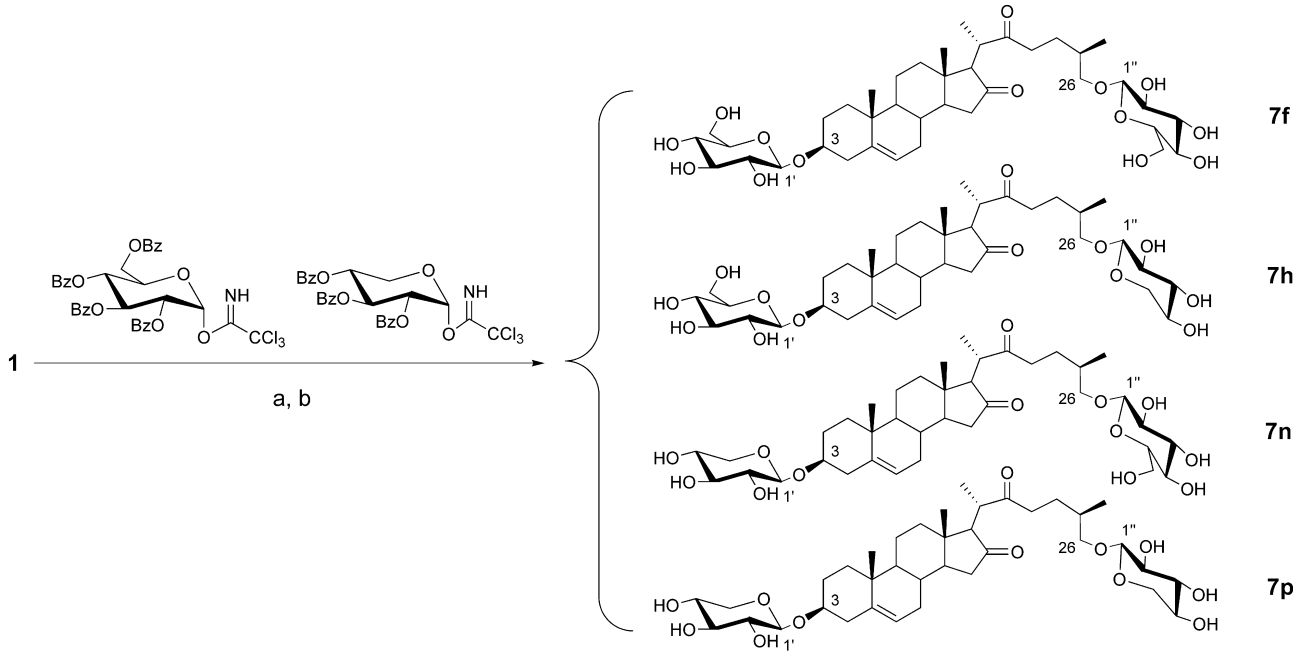


Scheme 1. Preparation of kryptogenin **1**. Reagents and conditions: (a) BzCl, pyridine, CH_2Cl_2, rt, 12 h; (b) oxone, $NaHCO_3$, acetone, H_2O, CH_2Cl_2, rt, 12 h, 90%; (c) Zn, KI, Ac_2O, AcOH, 50 °C, 12 h, 66%; and (d) MeONa, MeOH, CH_2Cl_2, rt, 2 h, 98%.

preparation methods require harsh conditions,[9] we developed a new route to convert diosgenin **2** into **1** facilely based on our former work (Scheme 1).[10] The hydroxyl group at C-3 of **2** was first protected by BzCl in dry pyridine—CH_2Cl_2 to give **3** quantificationally. Under the stepwise oxidation with oxone–acetone system in CH_2Cl_2–H_2O using $NaHCO_3$ as a buffer for controlling pH ~ 7.5, **3** was converted to 5,6-epoxy-16α-ol hemiketal **4** in 12 h with high yield of 90%. The reduction and the acetylation with Zn, KI and Ac_2O–AcOH in one pot transformed **4** into 16,22-dione **5** in a yield of 66% after purification through silica gel column chromatography. Finally, the *O*-acetyl and the *O*-benzoyl groups of **5** were removed by MeONa–MeOH in CH_2Cl_2 to afford **1**. The overall yield for the four steps from **2** to **1** was 58%.

Using **1** as the aglycone, we attempted to attach different sugar moieties to its hydroxyl groups at C-3 and C-26. According to the traditionally sequential chemical methods, at least two steps (protection and de-protection of either hydroxyl group) must be introduced additionally to furnish the target bisdesmosidic saponins bearing various sugars on both sides. Furthermore, the 'one by one' synthetic manner makes the compound library difficult to build. Alternatively, random glycosylation of **1** with two sugar donors, after removal of the protecting groups on the sugar moieties, should generate a mixture of four bisdesmosidic saponins, which can be separated completely via chromatography technique. Consequentially, the latter strategy should show more superiority in saving time and labor.



Scheme 2. An example of random glycosylation. Reagents and conditions: (a) TMSOTf, CH_2Cl_2, 4 Å MS, 0 °C to rt, 2 h; and (b) MeONa, MeOH, rt, 2 h.

A random glycosylation procedure was performed as shown in Scheme 2. To a magnetically stirred solution of **1** and two glycosyl trichloroacetimidates **6b** and **6d** (0.8 equiv each) in redistilled CH_2Cl_2 under Nitrogen at 0 °C, was added the powder of freshly activated 4 Å molecular sieves. After stirring for 15 min, a solution of TMSOTf (0.1 equiv) in CH_2Cl_2 was added dropwise to promote the reaction. The cooling bath was removed 30 min later and the stirring continued for 1.5 h until all the glycosyl donors were consumed, when Et_3N was added to quench the reaction. The molecular sieves were filtered off and the solvent was evaporated to give an oily residue, which was treated on a flash silica gel column chromatography with a step gradient to remove the unreacted aglycone and minor monodesmosidic chemicals.[11] The collected bisdesmosidic saponins fractions then were treated with a solution of MeONa in MeOH and the mixture was stirred for 2 h before being neutralized with Dowex 50 (H^+) and filtered. The filtrate was concentrated in vacuo and then anhydrous Et_2O was added to result in a white precipitate of the target molecules (**7f**, **7h**, **7n**, and **7p**) as a sublibrary.

As far as bioactivity evaluation is concerned, an individual sample can present a result more exactly and directly than the mixture does. Therefore, we try to separate the four compounds in the sublibrary for further studies. Primary attempt using the column chromatography ended in failure because of incomplete separation of the two isomeric compounds **7h** and **7n**. Afterwards the recycling preparative HPLC was applied to achieve collecting every component clean and without loss.[12] The chemical structures of the products were characterized via ESI-MS, ^1H and ^{13}C NMR, and the isomeric compounds were identified by HMBC confirming the attached position of the sugar residue.[13]

By this means, we chose four benzoyl-protected glycosyl trichloroacetimidates (**6a**–**d**, Fig. 1), which were all readily prepared according to the reported methods,[14] to apply to the glycosylation with **1** in pairs.[15] Thus, in every entry only two chemical steps were required to produce four bisdesmosidic saponins. Consequently 16 novel kryptogenyl saponins (**7a**–**p**, Fig. 1) in high purity were

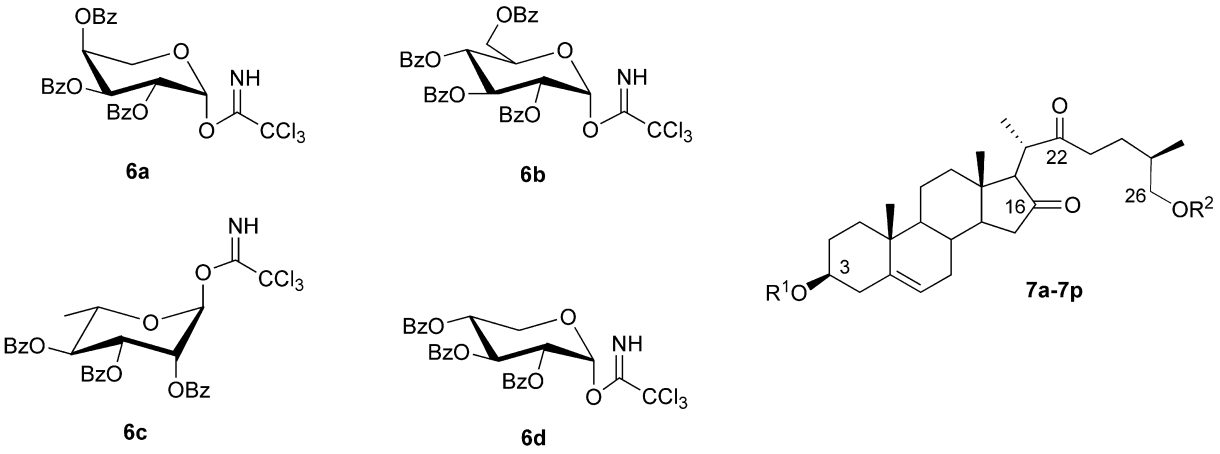


Figure 1. Four sugar donors (**6a**–**d**) chosen for random glycosylation and the library of the synthetic bisdesmosidic saponins (**7a**–**p**).

Table 1. Structures and physical data of the synthetic kryptogenyl saponins[a,b]

Compound	R^1	R^2	Formula	ESI-MS $(M+Na)^+$	Mp (°C)	$[\alpha]_D$ (MeOH)
7a	α-L-Ara*p*-	α-L-Ara*p*-	$C_{37}H_{58}O_{12}$	717.3	237.7–239.0	−93.8 (*c* 0.16)
7b	α-L-Ara*p*-	β-D-Glc*p*-	$C_{38}H_{60}O_{13}$	747.6	244.3–245.5	−110.7 (*c* 0.10)
7c	α-L-Ara*p*-	α-L-Rha*p*-	$C_{38}H_{60}O_{12}$	731.6	236.5–238.3	−118.8 (*c* 0.15)
7d	α-L-Ara*p*-	β-D-Xyl*p*-	$C_{37}H_{58}O_{12}$	717.6	231.4–233.6	−114.7 (*c* 0.10)
7e	β-D-Glc*p*-	α-L-Ara*p*-	$C_{38}H_{60}O_{13}$	747.6	249.0–250.7	−115.1 (*c* 0.13)
7f	β-D-Glc*p*-	β-D-Glc*p*-	$C_{39}H_{62}O_{14}$	777.7	260.5–262.2	−119.6 (*c* 0.05)
7g	β-D-Glc*p*-	α-L-Rha*p*-	$C_{39}H_{62}O_{13}$	761.6	255.8–257.6	−124.7 (*c* 0.07)
7h	β-D-Glc*p*-	β-D-Xyl*p*-	$C_{38}H_{60}O_{13}$	747.6	254.9–256.1	−120.0 (*c* 0.07)
7i	α-L-Rha*p*-	α-L-Ara*p*-	$C_{38}H_{60}O_{12}$	731.6	244.8–246.7	−153.3 (*c* 0.08)
7j	α-L-Rha*p*-	β-D-Glc*p*-	$C_{39}H_{62}O_{13}$	761.7	257.5–259.0	−150.0 (*c* 0.05)
7k	α-L-Rha*p*-	α-L-Rha*p*-	$C_{39}H_{62}O_{12}$	745.7	234.3–235.8	−153.5 (*c* 0.05)
7l	α-L-Rha*p*-	β-D-Xyl*p*-	$C_{38}H_{60}O_{12}$	731.7	232.0–233.8	−152.7 (*c* 0.05)
7m	β-D-Xyl*p*-	α-L-Ara*p*-	$C_{37}H_{58}O_{12}$	717.6	232.8–234.0	−117.7 (*c* 0.05)
7n	β-D-Xyl*p*-	β-D-Glc*p*-	$C_{38}H_{60}O_{13}$	747.6	245.9–247.0	−119.5 (*c* 0.08)
7o	β-D-Xyl*p*-	α-L-Rha*p*-	$C_{38}H_{60}O_{12}$	708.6	239.3–240.6	−121.9 (*c* 0.07)
7p	β-D-Xyl*p*-	β-D-Xyl*p*-	$C_{37}H_{58}O_{12}$	717.6	227.2–229.1	−119.2 (*c* 0.05)

[a] ESI-MS spectra were recorded with an Agilent 1100 mass spectrometer. Melting points were determined on a BÜCHI Melting Point B-540. Optical rotations were measured at room temperature with a Perkin-Elmer 241 MC polarimeter at the sodium D line.

[b] α-L-Ara*p*-, α-L-arabinopyranosyl; β-D-Glc*p*-, β-D-glucopyranosyl; α-L-Rha*p*-, α-L-rhamnopyranosyl; β-D-Xyl*p*-, β-D-xylopyranosyl.

Table 2. Antitumor activity of the synthetic compounds against HeLa tumor cell line

Compound	Inhibition rate at different concentration (%)				IC_{50}(μM)
	10^{-5} M	10^{-6} M	10^{-7} M	10^{-8} M	
1	22.34	17.29	13.80	9.49	>10
5	18.47	11.03	12.05	8.33	>10
7a	62.02	4.34	1.64	0.26	4.25
7b	9.71	4.79	3.84	3.25	>10
7c	10.33	9.25	7.51	6.36	>10
7d	11.77	2.84	1.79	0.98	>10
7e	4.36	3.67	1.05	0.87	>10
7f	3.46	2.31	1.78	0.46	>10
7g	10.48	9.94	7.16	3.42	>10
7h	24.78	14.48	12.76	7.95	>10
7i	60.13	6.34	6.93	1.35	8.36
7j	15.13	1.67	1.66	0.16	>10
7k	26.18	21.19	14.28	7.19	>10
7l	28.47	20.70	20.06	13.64	>10
7m	30.95	10.64	5.45	0.68	>10
7n	30.86	22.97	19.26	17.47	>10
7o	28.14	26.30	22.29	15.84	>10
7p	17.59	16.27	14.23	6.00	>10

generated through total six manipulations, whose structures are shown in Table 1.

The antitumor activity in vitro of these synthetic saponins against HeLa tumor cells was investigated by the standard MTT assay. As shown in Table 2, only saponins **7a** and **7i** presented definite inhibitions against HeLa cells below the concentration of 10 μM. However, other 14 saponins, the ester form of kryptogenin **5**, and the aglycone **1** itself showed relatively lower effect at the same condition. This result indicates that some sugar moieties are important for inducing the antitumor activity of cholestanol saponins. Nevertheless, there is still a large amount of work to do for probing such effective sugar residues.

In summary, a library of 16 novel bisdesmosidic steroidal saponins was built for the evaluation of antitumor activity. Applied to random glycosylation strategy, two sugar donors and kryptogenin successfully generated all four possible bisdesmosidic glycosides in a simpler and faster manner. This idea should be useful for the facile preparation of other multi-*O*-glycosyl saponins library. Since biological ligands are generally two to four sugars in size, oligosaccharide donors need to be introduced to steroidal aglycone randomly to prepare a potentially useful library for bioactivity test and structure–activity relationship (SAR) research. Further studies in this area are in progress and will be reported in due courses.

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References and notes

1. Hostettmann, K.; Marston, A. *Saponins*; Cambridge University Press: Cambridge, 1995; pp. 288–304.
2. Lasztity, R.; Hidvegi, M.; Bata, A. *Food Rev. Int.* **1998**, *14*, 371.
3. For some examples reported, see (a) Hou, S.; Xu, P.; Zhou, L.; Yu, D.; Lei, P. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 2454; Wang, P.; Li, C.; Zang, J.; Song, N.; Zhang, X.; Li, Y. *Carbohydr. Res.* **2005**, *340*, 2086; Zhang, Y.; Li, Y.; Zhu, S.; Guan, H.; Lin, F.; Yu, B. *Carbohydr. Res.* **2004**, *339*, 1753; Suhr, R.; Lahmann, M.; Oscarson, S.; Thiem, J. *Eur. J. Org. Chem.* **2003**, *20*, 4003.
4. Kanie, O.; Barresi, F.; Ding, Y.; Labbe, J.; Otter, A.; Forsberg, L. S.; Ernst, B.; Hindsgaul, O. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 2720.
5. Ding, Y.; Kanie, O.; Labbe, J.; Palcic, M. M.; Ernst, B.; Hindsgaul, O. *Adv. Exp. Med. Biol.* **1995**, *376*, 261; Ding, Y.; Labbe, J.; Kanie, O.; Hindsgdul, O. *Bioorg. Med. Chem.* **1996**, *4*, 683; Boons, G. J.; Heskamp, B.; Hour, F. *Angew. Chem., Int. Ed.* **1996**, *35*, 2845; Izumi, M.; Ichikawa, Y. *Tetrahedron Lett.* **1998**, *39*, 2079; Yu, B.; Li, B.; Xing, G.; Hui, Y. *J. Comb. Chem.* **2001**, *3*, 404.
6. Fu, X.; Albermann, C.; Zhang, C.; Thorson, J. S. *Org. Lett.* **2004**, *7*, 1513; Yang, J.; Fu, X.; Jia, Q.; Shen, J.; Biggins, J. B.; Jiang, J.; Zhao, J.; Schmidt, J. J.; Wang, P. G.; Thorson, J. S. *Org. Lett.* **2003**, *5*, 2223.
7. The mixture generated via random glycosylation usually composed of a series of isomeric compounds, which made the isolation of individual chemicals extremely difficult. Moreover, the deconvolution of the library was also a complicated course if the active compounds needed to be identified.
8. Shoda, J.; Axelson, M.; Sjovall, J. *Steroids* **1993**, *58*, 119; Javitt, N. B.; Kok, E.; Lloyd, J.; Benscath, A.; Field, F. H. *Biomed. Mass Spectrom.* **1982**, *9*, 61; Mui, M. M.; Kamat, S. Y.; Elliott, W. H. *Steroids* **1974**, *24*, 239; Noll, B. W.; Doisy, E. A.; Elliott, W. H. *J. Lipid Res.* **1973**, *14*, 391.
9. Alessandrini, L.; Ciuffreda, P.; Santaniello, E.; Terraneo, G. *Steroids* **2004**, *69*, 789; Kessar, S. V.; Gupta, Y. P.; Mahajan, R. K.; Joshi, G. S.; Rampal, A. L. *Tetrahedron* **1968**, *24*, 899.
10. 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.
11. Petroleum ether–EtOAc (6:1, v/v) was used to collect the fractions including all the possible bisdesmosidic glycosides. Afterwards minor monodesmosidic saponins were eluted by petroleum ether–EtOAc (3:1, v/v), followed by the fraction of starting kryptogenin with a recovery of 10%.
12. A JAI-LC 9103 recycling preparative HPLC equipped with an ODS reverse-phase column was applied to isolate the components with MeOH–H_2O (80:20, v/v) at a flow rate of 9.0 ml/min. The relative abundance ratio of each component was 27:23:18:31 (**7f**:**7h**:**7n**:**7p**).
13. NMR spectral data were listed as following: for **7h**: ^{1}H NMR (600 MHz, pyridine-d_5, ppm): δ 5.28 (br s, 1H, H-6), 5.06 (d, J = 7.7 Hz, 1H, H-1′), 4.72 (d, J = 7.8 Hz, 1H, H-1″), 4.59–4.57 (d, J = 11.5, 1H, CH_2-6′-1), 4.44–4.42 (m, 1H, CH_2-6′-2), 4.38–4.35 (dd, J = 11.2, 5.2, 1H, CH_2-5″-1), 4.31–4.29 (m, 2H, H-3′, H-4″), 4.24–4.23 (m, 1H, H-4′), 4.18–4.15 (t, J = 8.6, 1H, H-3″), 4.07 (m, 1H, H-2′), 4.02–4.00 (m, 2H, H-3, H-5′), 3.96–3.93 (m, 2H, H-2″, CH_2-26-1), 3.74–3.70 (t, J = 10.5, CH_2-5″-2), 3.65–3.63 (dd, J = 14.9, 4.0, CH_2-26-2), 2.92–2.91 (m, 1H, H-20), 2.79–2.77 (m, 3H, H-17, CH_2-4), 2.68–2.66 (m, 1H, CH_2-15-1), 2.45 (m, 1H, CH_2-23-1), 2.14–2.12 (m, 1H, CH_2-15-

2), 2.05–2.01 (m, 3H, CH_2-2-1, CH_2-24-1, H-25), 1.86 (br s, 1H, CH_2-2-2), 1.76–1.62 (m, 5H, CH_2-7-1, CH_2-1-1, CH_2-12-1, CH_2-23-2, CH_2-24-2), 1.45–1.43 (m, 2H, CH_2-11), 1.41–1.32 (m, 4H, H-8, CH_2-7-2, H-14, CH_2-12-2), 1.05–1.04 (d, J = 6.6 Hz, 3H, CH_3-27), 1.02–1.01 (d, J = 6.8 Hz, 3H, CH_3-21), 0.96–0.93 (m, 2H, CH_2-1-2, H-9), 0.91 (s, 3H, CH_3-19), 0.67 (s, 3H, CH_3-18); ^{13}C NMR (150 MHz, pyridine-d_5, ppm): δ 217.7 (C-16), 212.9 (C-22), 141.1 (C-5), 121.4 (C-6), 105.4 (C-1′), 102.6 (C-1″), 78.7 (C-3′), 78.6 (C-5′), 78.5 (C-3″), 78.0 (C-3), 75.4 (C-2′), 75.0 (C-26), 74.8 (C-2″), 71.7 (C-4′), 71.2 (C-4″), 67.3 (C-5″), 66.4 (C-17), 62.9 (C-6′), 51.1 (C-14), 49.9 (C-9), 43.7 (C-20), 41.7 (C-13), 40.1 (C-15), 39.3 (C-4), 38.6 (C-12), 37.3 (C-23), 37.1 (C-1), 37.0 (C-10), 33.5 (C-25), 31.9 (C-2), 30.9 (C-7), 30.2 (C-8), 27.7 (C-24), 20.7 (C-11), 19.4 (C-19), 17.5 (C-27), 15.6 (C-18), 12.9 (C-21); for **7n**: ^{1}H NMR (600 MHz, pyridine-d_5, ppm): δ 5.31 (br s, 1H, H-6), 4.93 (d, J = 7.5 Hz, 1H, H-1″), 4.86 (d, J = 7.8 Hz, 1H, H-1′), 4.58–4.56 (d, J = 11.5, 1H, CH_2-6″-1), 4.42–4.38 (m, 2H, CH_2-6″-2, CH_2-5′-1), 4.29–4.25 (m, 3H, H-3″, H-4′, H-4″), 4.22-4.19 (t, J = 8.8, 1H, H-3′), 4.07–4.02 (m, 2H, H-2″, H-3), 3.99–3.96 (m, 2H, H-5″, H-2′), 3.90–3.87 (m, 1H, CH_2-26-1), 3.77–3.74 (t, J = 10.6, CH_2-5′-2), 3.62-3.60 (dd, J = 15.0, 3.8, CH_2-26-2), 2.92–2.91 (m, 1H, H-20), 2.76–2.66 (m, 4H, H-17, CH_2-4, CH_2-15-1), 2.45 (m, 1H, CH_2-23-1), 2.14–2.12 (m, 1H, CH_2-15-2), 2.06–1.96 (m, 3H, CH_2-2-1, CH_2-24-1, H-25), 1.85 (br s, 1H, CH_2-2-2), 1.76–1.63 (m, 5H, CH_2-7-1, CH_2-1-1, CH_2-12-1, CH_2-23-2, CH_2-24-2), 1.47–1.34 (m, 6H, CH_2-11, H-8, CH_2-7-2, H-14, CH_2-12-2), 1.05–1.04 (d, J = 6.0 Hz, 3H, CH_3-27), 1.02-1.01 (d, J = 6.5 Hz, 3H, CH_3-21), 0.95–0.93 (m, 2H, CH_2-1-2, H-9), 0.92 (s, 3H, CH_3-19), 0.67 (s, 3H, CH_3-18); ^{13}C NMR (150 MHz, pyridine-d_5, ppm): δ 217.7 (C-16), 213.0 (C-22), 141.1 (C-5), 121.4 (C-6), 104.9 (C-1″), 103.5 (C-1′), 78.7 (C-3″), 78.6 (C-5″), 78.5 (C-3′), 78.2 (C-3), 75.3 (C-2″), 75.2 (C-26), 75.1 (C-2′), 71.7 (C-4″), 71.3 (C-4′), 67.3 (C-5′), 66.4 (C-17), 62.9 (C-6″), 51.1 (C-14), 50.0 (C-9), 43.7 (C-20), 41.7 (C-13), 40.2 (C-15), 39.3 (C-4), 38.6 (C-12), 37.4 (C-23), 37.3 (C-1), 37.0 (C-10), 33.5 (C-25), 31.9 (C-2), 30.9 (C-7), 30.4 (C-8), 27.8 (C-24), 20.7 (C-11), 19.4 (C-19), 17.5 (C-27), 15.6 (C-18), 12.9 (C-21). In the HMBC spectrum of **7h**, long range correlations were observed between H-1 (δ 5.06) of glucose and C-3 (δ 78.0) of the aglycone, and H-1 (δ 4.72) of xylose and C-26 (δ 75.0) of the aglycone. While the HMBC spectrum of **7n** displayed long rang correlations between H-1 (δ 4.86) of xylose and C-3 (δ 78.2) of the aglycone, and H-1 (δ 4.93) of glucose and C-26 (δ 75.2) of the aglycone. The similar coupling constants (J = 7.5–7.8 Hz) of the anomeric protons signals in the ^{1}H NMR spectra indicated the β-configuration of anomeric carbons of sugar units.

14. Schmidt, R. R. *Modern Methods in Carbohydrate Synthesis*; Harwood Academic: Netherlands, **1996**; pp. 20-s-54.

15. We also attempted to use three glycosyl donors in one random reaction. Unfortunately, the corresponding sub-library generated was too complex to be analyzed and separated. So far, at best three components are suitable in our research.