## Synthesis of Saponins Using Partially Protected Glycosyl Donors

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A new class of glycosyl donors having unprotected 2- and 2,4-hydroxyl groups were investigated under the standard glycosylation conditions. This approach was shown to be generally effective for the synthesis of alkyl and steroidal glycosides. A natural saponin, containing 2,4-branched oligosaccharide, was prepared in 35% overall yield in four straightforward sequential reactions by taking advantage of these partially protected donors.

Recent investigations in glycobiology have revealed important roles for many glycoconjugates in the immune response, viral and bacterial infection, cell regulation, differentiation, development, inflammation, cell adhesion, and many other inter- and intracellular communication processes.<sup>1</sup> Saponins, a structurally and biologically diverse class of glycosides of steroids and triterpenes, are major components in traditional Chinese medicines and represent important examples of glycoconjugates with promising pharmaceutical and biological activities.<sup>2</sup> Dioscin (diosgenyl 2,4-di-*O*-α-L-rhamnopyranosyl- $\beta$ -D-glucopyranoside), polyphyllin D (diosgenyl  $\alpha$ -Lrhamno-pyranosyl- $(1 \rightarrow 2)$ - $[\alpha$ -L-arabinofuranosyl- $(1 \rightarrow 4)$ ]- $\beta$ -D-glucopyranoside), and balanitin 7 (diosgenyl  $\alpha$ -L-rhamnopyranosyl- $(1\rightarrow 2)$ - $[\beta$ -D-xylopyranosyl- $(1\rightarrow 3)$ - $\beta$ -D-glucopyranosyl- $(1\rightarrow 4)$ ]- $\beta$ -D-gluco-pyranoside) display cardiovascular, antifungal, and antitumor activities.<sup>3</sup> Moreover, the rhamnose moiety of solamargine (solasodinyl 2,4-di-O-aL-rhamnopyranosyl- $\beta$ -D-glucopyranoside) plays a crucial role in triggering cell death by apoptosis.<sup>4</sup> These bioactive saponins are comprised of a 2,4-branched oligosaccharide moiety, as are N-linked oligosaccharides and many plant polysaccharides.<sup>5</sup> Thus, because of their biological functions and also their unique 2,4-dibranched chain structures, the efficient synthesis of these steroidal glycosides deserves extensive exploration.

Substantial effort has been devoted to the development of novel glycosylation reactions as strategies to access natural glycoconjugate structures.<sup>6</sup> Saponins, having 2,4-branched oligosaccharides, have been traditionally assembled in three ways.<sup>7</sup> In the first approach, the reducing end sugar unit is coupled to the C-3 of a steroid or triterpene, next protection group manipulation is performed on the sugar residue, usually

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4,6-benzylidination followed by blocking the 3-hydroxyl group to give the glycosyl acceptor containing a free 2-hydroxyl group. Glycosylation at this 2-hydroxyl group, followed by selective opening of the 4,6-benzylidene, affords a free 4-hydroxyl group, which is further glycosylated to furnish a 2,4-branched saponin. The disadvantages of this method are that it involves a lengthy and low-efficiency synthesis. This is especially problematic when the aglycone is expensive or available only in limited quantities. In the second approach, a more highly convergent synthesis of saponin is carried out using a suitably modified monosaccharide donor with a participatory C-2 acyl protecting group to ensure the  $\beta$ -bond formation. However, the subsequent removal of C-2 acyl protecting groups from saponin derivatives, to expose the free 2-hydroxyl group for glycosylation, can be difficult.<sup>7d</sup> In the third approach, the 2,4-branched oligosaccharide is first prepared and then condensed with aglycone in the final step. Unfortunately, glycosylation with such an oligosaccharide results in decreased neighboring group participation and often generates  $\alpha,\beta$ -mixtures.<sup>7g</sup> With these difficulties in mind, we speculated that a partially protected glycosyl donor could be used to shorten the total synthesis of saponins.<sup>8</sup>

Model studies were first carried out on the preparation of alkyl glycosides using partially protected sugar donors and alkyl alcohol acceptors (Table 1) in  $CH_2Cl_2$  at -42 °C under

 Table 1.
 Alkyl Glycoside Synthesis Using Partially Protected

 Glycosyl Donor
 Partially

entry	donor	acceptor	product	yield
1	BZO BZO 1 S-	$+ OCH_2(CH_2)_5N_3$	BZO BZO BZO 3	80%
2		− – – – – – – – – – – – – – – – – – – –		<sub>СН₃</sub> <sup>а</sup> 83%
3		$\prec \frac{HOCH_2(CH_2)_5OTBS}{8}$		<sup>b</sup> 42% ∽отвз
	7		9	2224 6 1

<sup>*a*</sup> A 7% yield of  $\alpha$  isomer was also isolated. <sup>*b*</sup> Including 32% of the  $\beta$ -isomer and 10% of the  $\alpha$ -isomer.

promotion with *N*-iodosuccinimide (NIS) and trimethylsilyl trifluoromethanesulfate (TMSOTf). We were pleased to discover that mannopyranosyl thioglycoside **1**, containing an unprotected hydroxyl group on C-2, still acted as an excellent glycosyl donor to afford  $\alpha$ -glycoside **3** in high yield (80%).

No trace of self-condensed disaccharide was detected in our experiments. More impressively, when the galactopy-

Fable 2.	Saponin	Synthesis	Using	Partially	Protected	Glycosyl
Donors						

entry	donor	acceptor	product	yield
1		HOP TO 10	HO OBZ BZO Odios 11 OH	54%
2		10	HO OTBS TBSO 0 Odios	<sup>a</sup> 59%
3		10	complex	n.d.
4	Ph 0 FmocO s ~	10	Ph 0 FmocO Odios 15 <sup>OH</sup>	75%
5	HO OBZ BZO 16 OH	10	HO BZO 17 <sup>OH</sup>	<sup>b</sup> 54%
6	BnO OBn BnO SEt 18 OH	10	BnO OBn BnO Odios <b>19</b> OH	80%
7	Bzo 20 <sup>0H</sup>		Bzo Ochol <b>22</b> OH	68%
8		21		<sup>c</sup> 55%
9		21		<sup>d</sup> 45%

<sup>*a*</sup> An additional 6% of the  $\alpha$ -isomer was isolated. <sup>*b*</sup>  $\alpha$ :  $\beta = 1:2$ . <sup>*c*</sup>  $\alpha$ :  $\beta = 2:3$ . <sup>*d*</sup> An additional 14% yield of the disaccharide saponin derivative was also isolated.

ranosyl thioglycoside donor **4**, containing 2,4-dihydroxyl groups, was subjected to similar reaction conditions, octyl  $\beta$ -D-galactopyranoside **6** was obtained in a yield of 83% ( $\alpha$ :  $\beta = 10:1$ ). Furthermore, 2,3-dihydroxyl donor **7** afforded a modest (42%) yield of galactopyranoside **9** as a 1:3  $\alpha/\beta$  mixture.<sup>9</sup>

Encouraged by these preliminary results, we next turned our attention to saponin synthesis (Table 2). Condensation of donor **4** with diosgenin **10** in CH<sub>2</sub>Cl<sub>2</sub> at -42 °C under NIS-TMSOTf promotion afforded a 54% isolated yield of  $\beta$ -glycoside **11**. A doublet at 4.53 ppm (J = 7.7 Hz) in <sup>1</sup>H NMR spectrum clearly demonstrated the pure  $\beta$ -configuration

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<sup>(9)</sup> General Procedure. To a mixture of thioglycosyl donor (1 mmol) and ROH (1 mmol) in anhydrous dichloromethane (2 mL) at -42 °C were added 1.1 mmol of NIS and catalytic amount of TMSOTf (0.1 equiv) with N<sub>2</sub> protection. The reaction mixture was stirred under these conditions for 45 min, at which time TLC indicated the completion of the reaction. The mixture was then neutralized with Et<sub>3</sub>N and concentrated to dryness. The residue was subjected to column chromatography on silica gel with petroleum ether/EtOAc (6/1-3/1) as the eluent to give the desired product.

of 11. The  $\alpha$ -isomer might also be generated in this reaction, as the presence of inseparable contaminants did not permit us to rule out its formation. The 3,6-disilylated donor 12 gave an easily separable  $\beta$ -glycoside 13 (59%), together with a 6% yield of the  $\alpha$ -product under the same reaction conditions. The 4,6-benzylidenated donor 7 produced a complex product mixture. In contrast, the corresponding C-3 protected donors 14 and 20 provided 15 and 22, respectively, in better yields and excellent regioselectivities, indicating that appropriate protection of the 3-hydroxyl group is critical for the effective application of this type of glycosyl donor. In parallel experiments, the stereochemical outcomes of 3,4isopropylidenated donors were greatly influenced by the substitution on C-6. For example, 6-silvlated donor 23 gave cholesterol saponin 24 as an  $\alpha,\beta$ -mixture with low stereoselectivity, but 6-deoxy donor 25 afforded 45% yield of  $\beta$ -glycoside **26**. When 2,4-unprotected glucosyl donor **16** was coupled with diosgenin 10 under the same reaction conditions, only a 36% yield of  $\beta$ -glycoside 17 was obtained, much lower than that obtained with the corresponding galactosyl donor 4. Interestingly, using a partially benzylated glucosyl donor 18 significantly improved the yield (80%) and gave  $\beta$ -glycoside **19** as the sole product.

Coupling reactions between sugar residues were also investigated using thioglycoside donors with unprotected 2-hydroxyl or 2,4-hydroxyl groups (Table 3). When glucosyl

**Table 3.** Oligosaccharide Synthesis Using Partial Protected

 Glycosyl Donor

entry	donor	acceptor	product	yield
1	18	27°×	BnO BnO HO 28	77%
2	4	27	$\begin{array}{c} HO \\ BZO \\ BZO \\ 29 \\ \end{array}$	<sup>a</sup> 50%
3	23		HO HO A1 Meo	<sup>b</sup> 57%
4	18	BnO HO BnO BnO BnO BnO OMe	BnO BnO BnO HO 33 BnO BnO BnO BnO BnO BnO BnO BnO BnO BnO	20%
5	18	HO BZO 34 BZO OME	complex	n.d.

<sup>*a*</sup> An  $\alpha$ :  $\beta = 1:2$  mixture with additional trisaccharide (15%) was isolated. <sup>*b*</sup> An  $\alpha$ :  $\beta = 2:3$  mixture.

donor **18** was reacted with 1,2:3,4-di-*O*-isopropylidene- $\alpha$ -D-galactopyranose (**27**) in CH<sub>2</sub>Cl<sub>2</sub> at -42 °C in the presence of NIS-TMSOTf, a 77% yield of disaccharide **28** was isolated. However, a similar reaction between **4** and **27** gave only 50% yield of the  $\alpha$ , $\beta$ -mixture **29**. An inseparable  $\alpha$ , $\beta$ mixture of glycoside **31** ( $\beta$ :  $\alpha$  = 3:2) was also obtained in 57% yield on glycosylation of **30** with **23**. When sugar acceptors containing secondary hydroxyl groups (**32** and **34**) were examined, low yields or complex product mixtures were obtained (Table 3, entries 4 and 5). The formation of the predominantly  $\beta$ -product can be rationalized as shown in Scheme 1.<sup>10</sup> When glycosylation



with thioglycoside **A** was promoted using NIS and TMSOTf, two reactive intermediates **B** and **C** could be generated. The 1,2-anhydrosugar intermediate **C** is formed through the intermolecular ring closure of **B**. Stereoselective opening of intermediate **C** would afford  $\beta$ -product **E**, while the  $\alpha,\beta$ mixture **D** would be obtained through  $\alpha$ - and  $\beta$ -attack on oxocarbenium **B**.

To ascertain the efficiency of our new synthetic method, we next applied it to the synthesis of diosgenyl  $\alpha$ -L-rhamnopyranosyl- $(1\rightarrow 2)$ - $[\beta$ -D-glucopyranosyl- $(1\rightarrow 4)$ ]- $\beta$ -D-galactopyranoside,<sup>5f</sup> a potent drug candidate used to decrease the cholesterol level in serum.

Compound **4**, containing unprotected 2,4-hydroxyl groups, was prepared from commercially available IPTG **35** according to the method described by Chan (Scheme 2).<sup>11</sup> This



<sup>*a*</sup> Reaction conditions: (a) BzCl, Pyr,  $-10 \degree$ C, 70%. (b) **10**, NIS, TMSOTf, CH<sub>2</sub>Cl<sub>2</sub>,  $-42 \degree$ C, 54%. (c) **36**, TMSOTf, CH<sub>2</sub>Cl<sub>2</sub>,  $-42 \degree$ C; then **37**, TMSOTf, CH<sub>2</sub>Cl<sub>2</sub>,  $0 \degree$ C, 68.7% for one-pot reaction. (d) Aqueous 1 N NaOH, MeOH, 95%.

donor was condensed with diosgenin 10 in CH<sub>2</sub>Cl<sub>2</sub> at -42 °C in the presence of NIS-TMSOTF, to afford the desired

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 $\beta$ -glycoside 11 in 54% isolated yield. Compound 11 was glycosylated in one-pot with rhamnopyranosyl trichloroacetimidate 36 at -42 °C in the presence of TMSOTf, followed by glucopyranosyl imidate 37 at 0 °C, to afford the protected trisaccharide saponin derivative 38 (68.7% from 11). Natural saponin 39 was then readily obtained by deacylation with aqueous 1 N NaOH in MeOH (95%). Remarkably, this complex natural saponin was prepared in four simple steps and in 35% overall yield.

In conclusion, an efficient and practical method has been developed for the preparation of saponins having 2,4branched oligosaccharide moieties. The key to this chemistry is the use of partially unprotected thioglycosides as glycosyl donors. This results in significantly simplified protecting group manipulation and oligosaccharide assembly. The approach described is general and effective for alkyl and steroidal glycoside synthesis. More importantly, the application of this method with combinatorial chemistry might be useful as an efficient entry into libraries of more complex glycoconjugates.<sup>12</sup>

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**Supporting Information Available:** Preparation and physical data for compounds **3**, **6**, **9**, **11**, **13**, **15**, **17**, **19**, **22**, **26**, **28**, **29**, **33**, **38**, and **39**. This material is available free of charge via the Internet at http://pubs.acs.org.

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