A "Double Random" Strategy for the Preparation of Saponin Libraries

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A conspicuous feature of naturally occurring glycoconjugates is the coexistence of populations of glycosylated variants of individual aglycons (glycoforms), which result from heterogeneous in vivo enzymatic processes.[1] This phenomenon makes the complete isolation of individual components extremely difficult. In the chemical synthesis of glycoconjugates, extensive protecting group manipulation is normally required to achieve the glycosylation of a particular hydroxyl group of a polyhydroxyl saccharide residue. Consequently, the terms "lengthy" and "time-consuming" are often applied to the chemical synthesis of glycoconjugates (or the oligosaccharide residues). In contrast to this approach, the "random glycosylation" method developed by Hindsgaul in 1995[2] aims at a complete lack of regioselectivity, producing a library that contains all the possible glycosylation products, ideally in equal amounts. Preparation of glycoconjugate libraries is still not a facile process.[3] Thus, a random glycosylation strategy could provide an attractive alternative for generating glycoconjugate libraries that would significantly accelerate the initial phase of the search for new active compounds.

In Hindsgaul's seminal example,[2] random glycosylation of βGal(1 → 3)βGlcNAc → $(CH_2)_8OC_6H_5p$OCH$_3$ (with six free hydroxyl groups) with 2,3,4-tri-*O*-benzyl-L-fucopyranosyl trichloroacetimidate, after removal of the benzyl groups by hydrogenolysis and purification by reverse-phase chromatography, provided a mixture containing all six possible mono-α-fucosylated trisaccharides, with a distribution of individual saccharides in relative yields ranging from 8% to 23%. The random glycosylation consumed about 30% of the starting disaccharide and also produced difucosylated and β-fucosylated products in minor amounts. A later example described similar results.[4] We envisioned that producing all possible glycosylation products should be more feasible than producing only monoglycosylated products and should facilitate the final purification processes. We explored this idea in the preparation of saponin libraries.

Saponins,[5] glycosides of steroids and triterpenes, are widely distributed in the plant kingdom and intimately involved in our daily lives. They exist in high quantities in many significant foods, beverage plants, and forage crops. They are also active principles in many commonly used herbal medicinal plants, and saponin preparations from ginseng, notoginseng, liquorice, horse chestnut, senega, and primula have long been used as pharmaceutical agents. Like the glycoconjugates occurring in animals, saponins exist in "glycoforms" and are difficult to isolate. Using conventional chemical methods, we and others have synthesized a number of natural saponins.[6] Diosgenyl saponins **2**, **3**, **6**, and **7** have been synthesized from trillin (**1**) in five, six, seven, and eight linear steps, respectively.[6e−g] Completely random glycosylation of trillin (with four free hydroxyl groups) with a rhamnopyranosyl donor, after removal of the protecting groups on the rhamnosyl residue, should generate a library of 16 saponins,[7] including the starting monosaccharide (**1**), four disccharides (**2**−**5**), six trisaccharides (**6**−**11**), four tetrasaccharides (**12**−**15**), and one pentasaccharide (**16**) (Scheme 1).

Trillin (**1**) is soluble in DMSO, DMF, and dioxane, but only in dioxane does glycosylation proceed well, as evaluated by the consumption of starting **1** (TLC). Ethyl 2,3,4-tri-*O*-benzyl-1-thio-α-L-rhamnopyranoside (**17**)[7] was finally selected as the glycosyl donor, under the promotion of NIS/AgOTf,[6f,8] which demonstrated superior reactivity (over acetyl- or benzoyl-protected ethylthio rhamnopyranosides, trichloroacetimidates, and benzyl-protected rhamnopyranosyl trichloroacetimidate), consuming most of the trillin (**1**) in 30 min at room temperature. The benzyl groups on the rhamnosyl residue were then removed cleanly by treatment with Li/NH_3 (l), without affecting the double bond on the aglycone. The saponin mixtures were finally purified by partition in *n*-butanol and precipitation with diethyl ether, a common method for the preparation of amphiphilic saponins from natural sources.[5a] ESI-MS analysis of the resulting libraries showed five sets of peaks ($M + H^+$ and $M + Na^+$) representing the corresponding saponins containing 0−4 rhamnosyl residues. Methylation analysis was used to determine the detailed linkages of the rhamnosyl units and their relative molar proportions in the saponin mixtures.[9]

As shown in Table 1, saponin library I, prepared by direct "random glycosylation" of trillin **1** with 2.0 equiv of **17**, was obtained in 51% yield and contained all 16 possible saponins, with trisaccharides **8** (2,6-di-rha) and **10** (3,6-di-rha) being in the highest amounts (totally 40.2%) and tetrasaccharides **11** (2,4,6-tri-Rha) and **12** (2,3,4-tri-rha) in the lowest amounts (0.5% and 0.7%, respectively). "Random glycosylation" of trillin **1** with 4.0 equiv of **17** produced saponin library II in 56% yield. Compared with library I, the amount of **13** (2,3,6-tri-Rha), **14** (2,4,6-tri-Rha), and **16** (2,3,4,6-tetra-Rha) were increased greatly (20.8%, 16.2%, and 15.7%, respectively). And the overall Rha/Glc ratio in the library was increased from 2.0:1 to 2.6:1. In saponin library I, 73.7% of the components contained the 6-O−Rha linkage (**5**, **8**, **10**, **11**, and **13**−**16**), while 55.1% contained 2-O−Rha linkage, 48.8% contained 3-O−Rha linkage, and 28.3% contained 4-O linkage. These results indicate that under the random glycosylation conditions the 6-OH of the acceptor **1** is the most active and the 4-OH the least active hydroxyl group.

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Scheme 1

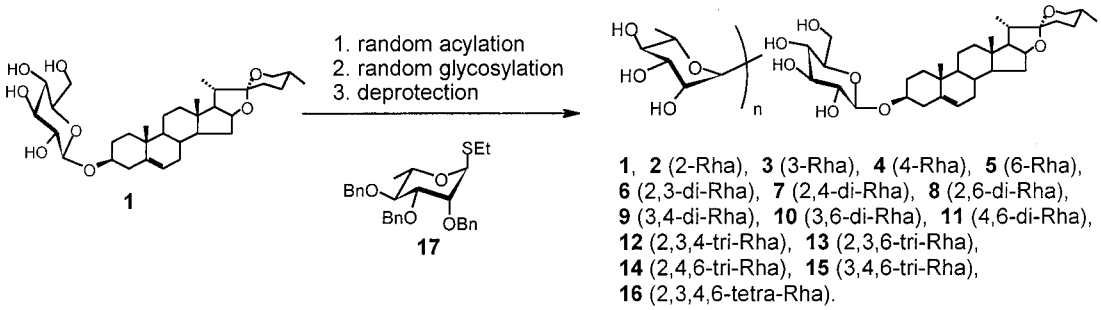

Table 1. Preparation of Saponin Libraries by "Random" and "Double Random" Strategies

				relative molar ratio of components													
library	conditions[a]	yield,[b] %	Rha/Glc	**1**	**2**	**3**	**4**	**5**	**6** + **7**[c]	**8** + **10**[c]	**9**	**11**	**12**	**13**	**14**	**15**	**16**
I	A	51	2.0:1	2.5	2.2	6.1	2.9	7.3	9.7	40.2	2.3	0.5	0.7	8.6	10.9	3.3	2.9
II	B	56	2.6:1	2.1	2.9	3.2	0	3.2	9.8	14.1	1.8	3.3	1.7	20.8	16.2	5.4	15.7
III	C	32	1.9:1	6.9	8.1	11.4	4.4	4.9	22.0	11.2	5.5	3.0	2.9	4.9	2.7	5.3	7.1
IV	D	57	1.2:1	17.7	20.7	12.3	10.8	3.2	21.6	3.2	3.8	2.0	2.0	0.6	1.0	0.6	0.7

[a] Glycosylation conditions: NIS/AgOTf, dioxane, rt. Deprotection conditions: Li/NH_3 (l), −70 °C. (A) **17** (2.0 equiv). (B) **17** (4.0 equiv). (C) (1) Ac_2O (1.6 equiv), pyridine, rt; (2) **17** (2.0 equiv). (D) (1) BzCl (1.6 equiv), pyridine, rt; (2) **17** (2.0 equiv). [b] Yields = (weight of the obtained library of compounds)/(calculated weight with consumption of all the added donor) [c] Trisaccharide saponins **6** (2,3-di-Rha) and **7** (2,4-di-Rha) could not be distinguished by methylation analysis[9] and were presumably formed in equal amounts. And so were **8** (2,6-di-Rha) and **10** (3,6-di-Rha).

These reactivity differences obviously work against the production of saponin libraries possessing ideally equalized product distribution.

To compensate for the reactivity differences of the hydroxyl groups in **1**, a "double random" strategy was applied. Specifically, we carried out a random acylation before the random glycosylation, knowing that the acyl groups could be easily removed under the final Li/NH_3 (l) debenzylation protocol. By employing 1.6 equiv of acetic anhydride or benzoyl chloride in the random acylation followed by random glycosylation with 2.0 equivalent of **17**, saponin libraries III and IV were prepared in 32% and 57% yield, respectively. To our delight, the distribution ratio of the components in library III was greatly improved, with the most abundant component (**3**, 3-Rha) in 11.4% and the least (**14**, 2,4,6-tri-Rha) in 2.7% relative yield. Most of the components possessed abundances close to the average distribution of 6.25%. Library IV, on the other hand, featured a low Rha/Glc ratio of only 1.2:1 and an unsatisfactory component distribution that was dominated by **1** and monoglycosylated products (**2**−**5**) (total of 64.7%). These results suggest that complete glycosylation of the partially benzoyl-protected trillin was considerably retarded.

In conclusion, a saponin library containing all 16 possible components in nearly equal amounts was successfully prepared by a random acetylation/random glycosylation sequence. This new "double random" strategy should be useful for the facile preparation of other glycoconjugate libraries.

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Supporting Information Available. Preparation procedures, ESI-MS spectrum, and gas chromatogram of library III. This material is available free of charge via the Internet at http://pubs.acs.org.

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