Application of the Glycal Assembly Strategy to the Synthesis of a Branched Oligosaccharide: The First Synthesis of a Complex Saponin

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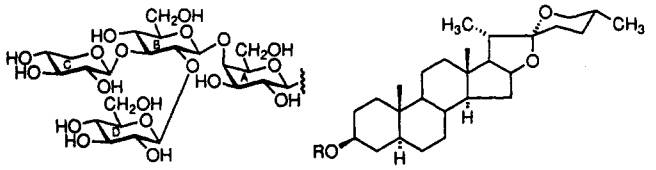
Two general classes of steroidal glycosides are isolated from the purple foxglove (*Digitalis purpurea* L.).[1,2] Differentiating features of these two structural types are found in both the aglycone (steroidal) and the oligosaccharide sectors. The more clinically advanced family is the cardenolides, in which the aglycone bears a β-oriented butenolide attached at C-17 and a β-hydroxyl group at C-14. Glycosides of this type have been used for many years in the treatment of cardiac insufficiencies (cf. digitalis).[1a,3]

The other group, called saponins, is one in which the steroid aglycone contains a spiroketal linkage at C-21.[4] Separation of the individual components of a crude extract of saponins is a painstaking task. Investigation of the therapeutic potential of individual saponins has thus been impeded. Nonetheless, members of this class have been shown to have antiviral[5] as well as antitumor properties.[6]

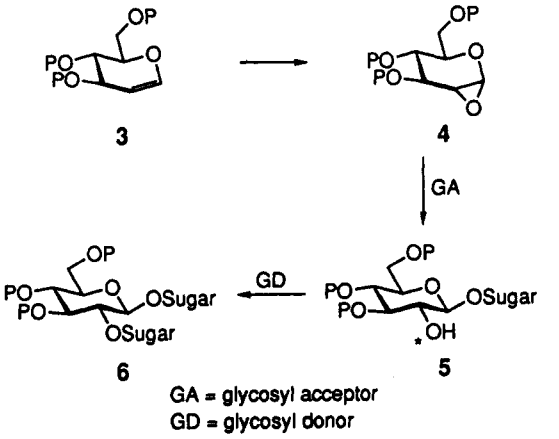
Our interest in the synthesis of steroidal glycosides was spurred by the prospect that such an effort could provide new insights into the construction of branched oligosaccharides. It was also hoped that, through this chemistry, novel counterparts to current cardiotonic agents could be assembled and evaluated. For this purpose, we directed our first foray in this area toward the synthesis[7] of the naturally occurring desgalactotigonin (**1**).[2b,8]



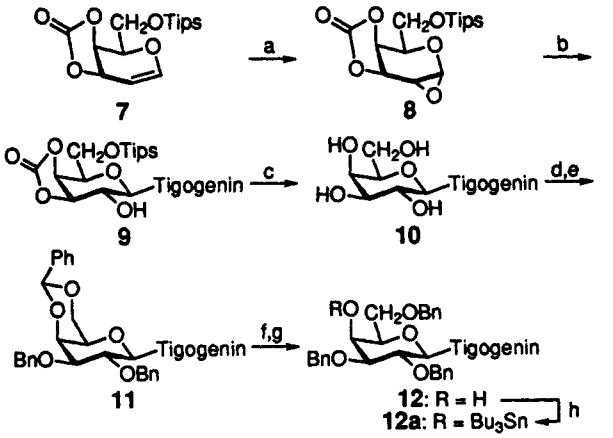
1: desgalactotigonin (R = tetrasaccharide)
2: tigogenin (R = H)

This compound represented a suitable goal for synthesis from several standpoints. First, the steroidal aglycone, tigogenin (**2**), is commercially available.[9] Moreover, its glycosidic ensemble is sufficiently complicated to properly test our strategy for reaching complex branched oligosaccharides. The guiding logic of the proposal is summarized in Scheme I. Glycal epoxide **4**, derived from **3**, is subjected to the action of a glycosyl acceptor (GA). It is seen that the product **5** contains a unique hydroxyl at C-2 of the erstwhile pyranose donor (see asterisk in Scheme I). This hydroxyl group serves as the acceptor in a reaction with a glycosyl donor (GD). Branched **6** is thus produced. While Scheme I illustrates the assemblage of an oligosaccharide, successful outcome could surely find ready application to other branched glycoconjugates. Herein we report the first synthesis of the branched saponin **1**. The work is illustrative of the power of the glycal assembly method for constructing such complex targets. We note that Thiem and co-workers were the first to demonstrate the usefulness of glycals in the synthesis of steroidal glycosides.[10] However, the 1,2-anhydrosugar methodology, described herein, constitutes a major expansion of the role of glycals in that it leads to fully-oxygenated branched glycosides.

Scheme I



Scheme II[a]



[a] Reagents: (a) 3,3-dimethyldioxirane, quantitative; (b) **2**, $ZnCl_2$, THF, 89%; (c) (1) TBAF, THF, (2) LiOH, 94%; (d) PhCHO, HCO_2H, 88%; (e) NaH, BnBr, DMF, 77%; (f) 80% aqueous AcOH, 60 °C, 92%; (g) (1) $(Bu_3Sn)_2O$, $PhCH_3$, reflux, (2) BnBr, *N*-methylimidazole, 91%; (h) $(Bu_3Sn)_2O$, *o*-xylene, reflux.

Reaction of D-galactal derivative **7**[11] with 3,3-dimethyldioxirane[12] generated **8** (Scheme II). Without benefit of purification,

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(2) For isolation and characterization of *Digitalis* saponins, see: (a) Tschesche, R.; Wulff, G. *Tetrahedron* **1963**, *19*, 621. (b) Kawasaki, T.; Nishioka, I. *Chem. Pharm. Bull.* **1964**, *12*, 1311. (c) Yang, Y. M.; Lloyd, H. A.; Pannell, L. K.; Fales, H. M.; Macfarlane, R. D.; McNeal, C. J.; Ito, Y. *Biomed. Mass Spectrom.* **1986**, *13*, 439.

(3) Smith, T. W. *N. Engl. J. Med.* **1988**, *318*, 358.

(4) For examples of the use of *Digitalis* saponins as biological detergents and complexing agents, see: (a) Miller, R. G. *Biochim. Biophys. Acta* **1984**, *774*, 151. (b) Repke, H. *Biochim. Biophys. Acta* **1987**, *929*, 47. (c) Hayashi, T.; Koyama, T.; Matsuda, K. *Plant Physiol.* **1988**, *87*, 341.

(5) Balashova, I. T.; Verderevsky, T. D.; Kintya, P. K.; Kosakovskaya, O. I. *Izv. Akad. Nauk Mold. SSR, Ser. Biol. Khim. Nauk* **1982**, 63.

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(7) For alternative approaches to the synthesis of saponin oligosaccharides, see: (a) Yamada, H.; Nishizawa, M. *Synlett* **1993**, 54. (b) Bing, H. X.; Schmidt, R. R. *Liebigs Ann. Chem.* **1992**, 817. (c) Jiang, Z.-H.; Schmidt, R. R. *Liebigs Ann. Chem.* **1992**, 975. (d) Takeo, K.; Nakaji, T.; Shinmitsu, K. *Carbohydr. Res.* **1984**, *133*, 275.

(8) (a) Nagumo, S.; Kishi, S.; Inoue, T.; Nagai, M. *Yakugaku Zasshi* **1991**, *111*, 306. (b) Sharma, S. C.; Chand, R.; Sati, O. P.; Sharma, A. K. *Phytochemistry* **1983**, *22*, 1241.

(9) Research Plus, Inc., Bayonne, NJ, catalog no. 5009-19.

(10) (a) Thiem, J.; Kopper, S. *Angew. Chem., Int. Ed. Engl.* **1982**, *21*, 779. (b) Thiem, J.; Ossowski, P. *Liebigs Ann. Chem.* **1983**, 2215.

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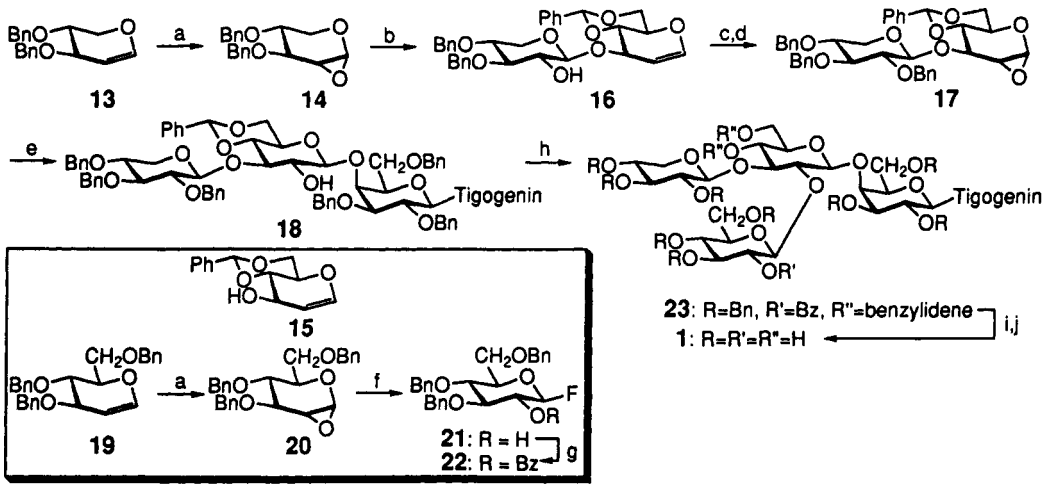
Scheme III[a]



[a] Reagents: (a) 3,3-dimethyldioxirane, 4:1 mixture of **14**:β-epoxide; (b) **15**, $ZnCl_2$, THF, 59% (mixture of glycosides, see ref 17); (c) NaH, BnBr, DMF, 94%; (d) 3,3-dimethyldioxirane; (e) **12a**, $Zn(OTf)_2$, THF, 46%; (f) TBAF, THF; (g) BzCl, pyridine; (h) **22**, $Sn(OTf)_2$, 4-Å molecular sieves, Et_2O, 56%; (g) NaOH, MeOH, 97%; (h) Pd black/H_2, 1:1 AcOH:MeOH, 95%.

8 was used to stereoselectively glycosylate[13] aglycone **2** (1.2 equiv) to afford β-glycoside **9** in 89% yield. We have found[11] the *cis*-3,4-cyclic carbonate linkage to be particularly effective in promoting highly stereoselective epoxidation (see **7** → **8**) and β-galactosylation. It was now necessary to reconstitute the protecting pattern on the galactosyl moiety to identify the C-4 axial hydroxyl as the glycosyl acceptor. This was accomplished as shown via intermediates **10**, **11**, **12**. The latter was converted to its uncharacterized tri-*n*-butylstannyl ether **12a**,[14] which was to serve as the coupling partner with disaccharide donor **17**.

Indeed, glycal assembly was used in fashioning **17** (Scheme III). The D-xylal derivative **13**[15] was converted to its α-epoxide **14** by the action of 3,3-dimethyldioxirane. The epoxidation was stereoselective in the desired sense (α), though only to the extent of 4:1. Without separation, **14** was coupled to **15**[16] (1.5 equiv) under the usual conditions to afford disaccharide glycal **16**,[17] which, after benzylation followed by epoxidation, gave **17**. Coupling of the in situ generated **12a** with **17** (0.7 equiv) was mediated by zinc triflate, giving rise to **18** in 46% yield. This was an important step in that it gave access to the β-glycoside of the rather hindered axial hydroxyl center at C-4 of D-galactose. Again, glycal epoxide chemistry was used to generate **22**, the coupling partner of **18**. Toward this end, epoxidation of glycal **19** by the dioxirane method afforded **20**,[18] which was subjected to our fluoridolysis protocol[19] to yield **21**.[20] The unique C-2 hydroxyl was protected as its benzoate **22**. The strategically placed benzoate served as a neighboring group director to promote β-attack by the glycosyl acceptor. The stage was at hand to implement the strategy implied in Scheme I. In the event, coupling of **18** and **22**, mediated by stannous triflate,[21] gave a 56% yield of **23**. Finally, sequential deacylation and debenzylation afforded the target saponin **1** in 92% yield.[22]

The synthesis described herein required a solution to several otherwise formidable problems in complex oligosaccharide synthesis. Most notable were the glycosidic linkage to the axial C-4 hydroxyl of the A ring and the "ortho" branched glycoside pattern of the B ring.[23] The successful application of glycal epoxides to solve all of the glycosidations involved in this construction speaks well for their potentiality in simplifying the assembly of complex oligosaccharides and glycoconjugates. Such applications have recently been enhanced by applying the glycal methodoloy in a synthesis of oligosaccharides on a solid support.[24]

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Supplementary Material Available: ^1H NMR (300 MHz) spectrum, ^{13}C NMR spectrum, FTIR, optical rotation, melting point, and FABMS of the goal compound desgalactotigonin (**1**) (3 pages). Ordering information is given on any current masthead page.

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(14) Attempts to couple **12** with **17** using the standard procedure (ref 13) failed to produce the desired trisaccharide **18**.
(15) Weygand, F. *Methods Carbohydr. Chem.* **1962**, *1*, 182.
(16) Sharma, M.; Brown, R. K. *Can. J. Chem.* **1966**, *44*, 2825.
(17) This glycosidation reaction afforded a 4:1 mixture (59% overall yield) of **16** along with the α-*lyxo*-hexopyranoside, formed from reaction of **15** with the β-epoxide. Following benzylation, these two stereoisomers were separated by column chromatography on silica gel.
(18) Several attempts were made to couple **20** with **18** using a number of different conditions. However, these attempts failed to provide the desired tetrasaccharide.
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(20) For the use of glycosyl fluoride donors, see: (a) Mukaiyama, T.; Murai, Y.; Shoda, S. *Chem. Lett.* **1981**, 431. (b) Mukaiyama, T.; Hashimoto, Y.; Shoda, S. *Chem. Lett.* **1983**, 935. (c) Nicolaou, K. C.; Dolle, R. E.; Chucholowski, A.; Randall, J. L. *J. Chem. Soc., Chem. Commun.* **1984**, 1153. (d) Nicolaou, K. C.; Chucholowski, A.; Dolle, R. E.; Randall, J. L. *J. Chem. Soc., Chem. Commun.* **1984**, 1155. (e) Nicolaou, K. C.; Randall, J. L.; Furst, G. T. *J. Am. Chem. Soc.* **1985**, *107*, 5556.
(21) Ito, Y.; Ogawa, T. *Tetrahedron Lett.* **1987**, *28*, 6221.
(22) The structure of **1** was confirmed by ^1H and ^{13}C NMR (see supplementary material), and by high-resolution FAB mass spectroscopy (calcd. for $C_{50}H_{82}O_{22}Na$, 1057.520, found, 1057.524). While comparison with an authentic sample has not yet been carried out, the structure of our final synthetic product is secure from its spectral properties and from comparison with the literature data. Also, the rotation and melting point data are in reasonable agreement with literature data, although this is complicated as a result of divergencies in reports of these physical properties from the various isolation papers.
(23) For recent examples illustrating the difficulties associated with assembling the "ortho" branching pattern, see refs 7b–d.
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