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Glycosyl Trifluoroacetimidates. 2. Synthesis of Dioscin and Xiebai Saponin I

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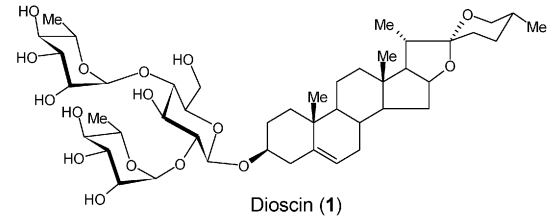
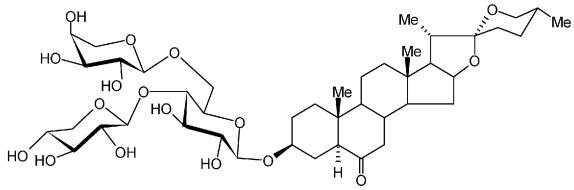
Abstract: Two trisaccharide steroidal saponins, dioscin (**1**) and Xiebai saponin I (**2**) with various bioactivities, were efficiently synthesized using the newly developed glycosyl *N*-phenyl trifluoroacetimidates (**10**−**13**) as glycosylation donors. Thus, dioscin was synthesized in five steps and a 33% overall yield from diosgenin and glycosyl trifluoroacetimidates (**10** and **11**). Xiebai saponin I was synthesized in eight steps and a 32% overall yield from laxogenin and glycosyl trifluoroacetimidates (**10**, **12**, and **13**), whereupon, the rare steroid laxogenin was prepared from diosgenin in four steps and an overall 69% yield. All the glycosylation reactions involved in the present syntheses demonstrated that glycosyl trifluoroacetimidates were successful donors comparable to the corresponding glycosyl trichloroacetimidates.

Dioscin, diosgenin-3-yl α-L-rhamnopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→4)]-β-D-glucopyranoside (**1**), which has been isolated from a number of oriental vegetables and traditionally medicinal plants, is one of the most common saponins occurring in plants. The broad spectrum of bioactivities of dioscin, which demonstrates moderate to good antitumor,[1,2] antivirus,[3] antifungal,[4] and antiinflammatory,[5−7] as well as immunostimulant activities,[8] has attracted great attention. In contrast, Xiebai saponin I, laxogenin-3-yl α-L-arabinopyranosyl-(1→6)-[β-D-xylopyranosyl-(1→4)]-β-D-glucopyranoside (**2**), has been isolated only from the bulbs of *Allium chinense*,[9] which are used as pickles and spices in China and Japan and also are the main sources of a traditional Chinese medicine "Xiebai" for treatment of chest pain, stenocardia, and heart asthma. Xiebai saponin I demonstrates inhibitory activity on cyclic AMP phosphodiesterase with an IC_{50} of 12.3×10^{-5} M and strong cytotoxic activity to Hela cells at 50 μg/mL dose.[9] Its inhibition on the hypoxia/reoxygenation (H/R)-induced protein tyrosine kinase activation and therefore protective effect on the gap junctional intercellular communication injury were also reported.[10] Because saponins exist in plants in a great heterogeneous manner, as glycoconjugates in mammals, isolation of each component is a formidable task[11] that has hampered the detailed biological and pharmacological studies of saponins. Chemical synthesis should provide a realistic way to determine the availability of homogeneous saponins, thus affording new opportunities for understanding and applying this important group of natural products. We have recently synthesized a number of saponins,[12] including dioscin,[13] by using conventional glycosylation methods, mainly with glycosyl trichloroacetimidate and thioglycoside donors. Here we focus our attention on the newly developed glycosyl trifluoroacetimidate donors [14−16] in the synthesis of dioscin (**1**) and Xiebai saponin I (**2**).



Dioscin (**1**)



Xiebai saponin I (**2**)

Saponins **1** and **2** have the aglycone of diosgenin and laxogenin, respectively. While diosgenin is a bulk material for manufacturing steroidal hormones and contra-

(1) Inhibition of growth of various tumor cells with IC_{50} at the μM level, see: (a) Hu, K.; Dong, A. J.; Yao, X. S.; Kobayashi, H.; Iwasaki, S. *Planta Med.* **1996**, *62* (6), 573. (b) Nakamura, T.; Komori, C.; Lee, Y.-y.; Hashimoto, F.; Yahara, S.; Nohara, T.; Ejima, A. *Biol. Pharm. Bull.* **1996**, *19* (4), 564.

(2) Inhibition of DNA synthesis of C6 glioma cells at 10 mg/mL, see: Chiang, H. C.; Tseng, T. H.; Wang, C. J.; Chen, C. F.; Kan, W. S. *Anticancer Res.* **1991**, *11* (5), 1911.

(3) Anti HSV-1 (herpes simlex virus type 1) activity with EC_{50} = 0.56 μg/mL, which is comparable to that of Acyclovir, see: Ikeda, T.; Ando, J.; Miyazono, A.; Zhu, X.-H.; Tsumagari, H.; Nohara, T.; Yokomizo, K.; Uyeda, M. *Biol. Pharm. Bull.* **2000**, *23* (3), 363.

(4) Antifungal activity, see: (a) Takechi, M.; Shimada, S.; Tanaka, Y. *Phytochemistry*, **1991**, *30* (12), 3943. (b) Hufford, C. D.; Liu, S.; Clark, A. M. *J. Nat. Prod.* **1988**, *51* (1), 94.

(5) Inhibition of mouse ear edema at an oral dose of 100 mg/kg, see: Kim, S. Y.; Son, K. H.; Chang, H. W.; Kang, S. S.; Kim, H. P. *Arch. Pharmacal Res.* **1999**, *22* (3), 313.

(6) Inhibitory effect on cyclic AMP phosphodiesterase, see: Mimaki, Y.; Nakamura, O.; Sashida, Y.; Nikaido, T.; Ohmoto, T. *Phytochemistry* **1995**, *38* (5), 1279.

(7) Inactivation of human pleural fluid phospholipase A2, see: Baek, S. H.; Kim, S. H.; Son, K. H.; Chung, K. C.; Chang, H. W. *Arch. Pharmacal Res.* **1994**, *17* (4), 218.

(8) Increasing of 3*H*-thymidine incorporation of Con A-stimulated lymphocytes maximally at 0.01 mg/mL, see: Chiang, H. C.; Wang, J. J.; Wu, R. T. *Anticancer Res.* **1992**, *12* (5), 1475.

(9) (a) Kuroda, M.; Mimaki, Y.; Kameyama, A.; Sashida, Y.; Nikaido, T. *Phytochemistry* **1995**, *40* (4), 1071. (b) Baba, M.; Ohmura, M.; Kishi, N.; Okada, Y.; Shibata, S.; Peng, J.; Yao, S.-S.; Nishino, H.; Okuyama, T. *Biol. Pharm. Bull.* **2000**, *23* (5), 660.

(10) (a) Zhang, Y.-W.; Morita, I.; Shao, G.; Yao, X.-S.; Murota, S.-I. *Planta Med.* **2000**, *66* (2), 114. (b) Zhang, Y.-W.; Morita, I.; Zhang, L.; Shao, G.; Yao, X.-S.; Murota, S.-I. *Planta Med.* **2000**, *66* (2), 119.

(11) Hostettmann, K.; Marston, A. *Saponins*; Cambridge University Press: Cambridge, UK, 1995.

(12) (a) Yu, B.; Hui, Y. Chemical Synthesis of Bioactive Steroidal Saponins. In *Glycochemistry: Principles, Synthesis, and Application*; Wang, P. G., Bertozzi, C. R., Eds.; Marcel Dekker: New York, 2001; p 163. (b) Yu, B.; Xie, J.; Deng, S.; Hui, Y. *J. Am. Chem. Soc.* **1999**, *121*, 12196. (c) Yu, B.; Liao, J.; Zhang, J.; Hui, Y. *Tetrahedron Lett.* **2001**, 42, 77.

(13) (a) Deng, S.; Yu, B.; Hui, Y.; Yu, H.; Han, X. *Carbohydr. Res.* **1999**, *317*, 53. (b) Deng, S.; Yu, B.; Hui, Y. *Tetrahedron Lett.* **1998**, *39*, 6511.

(14) Yu, B.; Tao, H. *Tetrahedron Lett.* **2001**, 42, 2405.

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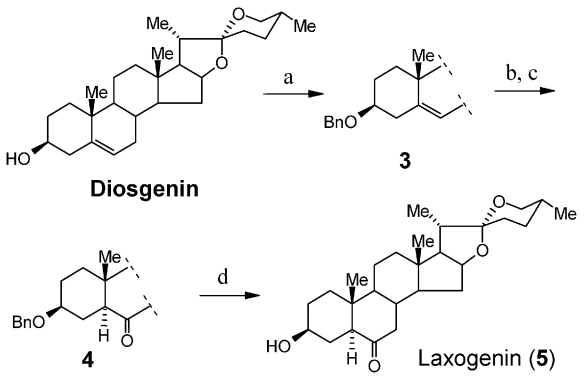
SCHEME 1. Synthesis of Laxogenin from Diosgenin[a]

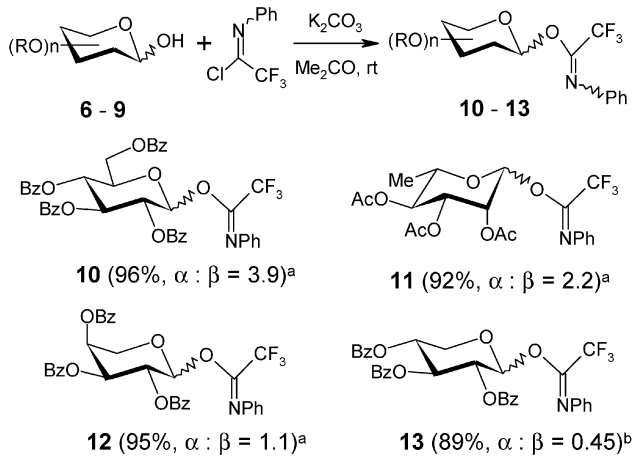


[a] Reagents and conditions: (a) BnBr, NaH, THF/DMF (1:1), rt, 97%; (b) BH_3·Me_2S, THF; then 30% NaOH, 30% H_2O_2, rt; (c) Dess−Martin periodinane, CH_2Cl_2, 30 °C, 77%; (d) H_2, Pd/C, CH_2Cl_2/EtOH (1:4), rt, 92%.

ceptives, laxogenin is rare in plants. Nevertheless, transformation of laxogenin from diosgenin is straightforward (Scheme 1). After protection of the 3-OH of diosgenin with a benzyl ether, the 5,6 double bond was subjected to hydroboration with BH_3·Me_2S, followed by oxidation with 30% H_2O_2 in the presence of aqueous NaOH to provide the corresponding 6-OH derivatives. Subsequent oxidation of the 6-OH with Dess−Martin periodinane provided the 5α-6-one product **4** in good yield (77%). The 5α stereochemistry was confirmed by X-ray crystallography.[17] Removal of the 3-OBn by hydrogenolysis afforded laxogenin. Thus laxogenin was prepared from diosgenin in four steps and an overall 69% yield, which is higher than that of a recent literature procedure without protection of the 3-OH.[18]

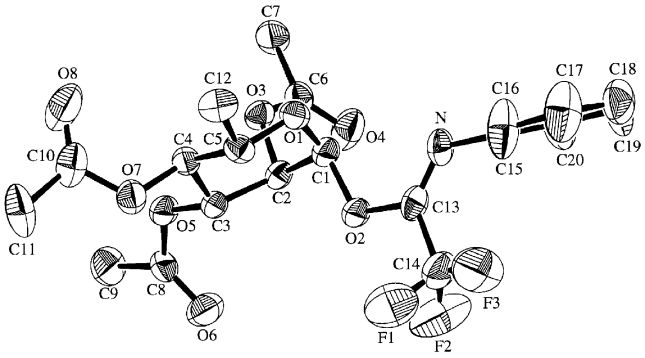
Acetyl or benzoyl protected glycopyranosyl trifluoroacetimidate donors **10**−**13**, which would be employed in the synthesis of saponins **1** and **2**, were readily prepared in excellent yields (89−96%) by treatment of the corresponding 1-hydroxyl sugars (**6**−**9**) with *N*-phenyl trifluoroacetimidoyl chloride[19] in the presence of K_2CO_3 (2.0 equiv) in acetone or CH_2Cl_2 at room temperature for 3 h (Scheme 2). It should be mentioned that moisture in the solvent is important, which might increase the solubility of K_2CO_3 and/or result in the presence of HO^- to facilitate the deprotonation of 1-OH sugars, otherwise the reaction

SCHEME 2. Preparation of Glycosyl Trifluoroacetimidate Donors



[a] The α:*β* ratio was determined after a small-scale separation. [b] Inseparable, therefore, it was determined by ^{1}H NMR.

was found sluggish.[20] While the α:*β* ratio of **10**-**12** was determined after a small-scale separation on column chromatography, the anomeric mixtures were directly used in the glycosylation reactions. It is worth noting that these glycosyl trifluoroacetimidates (**10**−**13**) were stable to storage at ambient temperature for weeks.



A single crystal of **11**α was obtained, which led to its X-ray structure as shown in the ORTEP drawing.[17,21] The *N*-phenyl group is anti to the bulky sugar moiety, and is almost perpendicular to the O2−C13−N−C14 plane with the dihedral angle being 80.36°, which shall facilitate a conjugation with the nonbonding electrons on nitrogen.[22] The conformational preference about the C1−O2 bond is such that the torsion angle C2−C1−O2−C13 is 159.9°, in agreement with the expression of an $n_O \rightarrow \delta^*_{C-O}$ orbital interaction associated with the exo-anomeric effect.[23] While the crystal structure represents a favorable conformation/configuration (for the C═N bond), the ^{1}H NMR

(15) The present protocol was developed based on the Schmidt glycosylation, which has become one of the most widely used glycosylation methods, see: (a) Schmidt, R. R. *Angew. Chem., Int. Ed. Engl.* **1986**, *25*, 212. (b) Schmidt, R. R.; Kinzy, W. *Adv. Carbohydr. Chem. Biochem.* **1994**, *50*, 21.

(16) Schmidt and co-workers also pioneered the preparation of glycosyl trifluoroacetimidates, whereupon the toxic CF_3CN with low boiling point (−63.9 °C) was used, see: Schmidt, R. R.; Michel, J.; Roos, M. *Liebigs Ann. Chem.* **1984**, 1343.

(17) Full crystallographic details of compounds **4** and **11**α have been deposited with the Cambridge Crystallographic Data Centre (CCDC 187993 and 187992, respectively). Copies of these data may be obtained free of charge from the director, CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK (fax +44-1223-336033; e-mail deposit@ccdc.cam.ac.uk or www: http://www.ccdc.cam.ac.uk).

(18) Iglesias-Arteaga, M. A.; Pérez Gil, R.; Pérez Martìnez, C. S.; Manchado, F. C. *J. Chem. Soc., Perkin Trans. 1* **2001**, (3), 261.

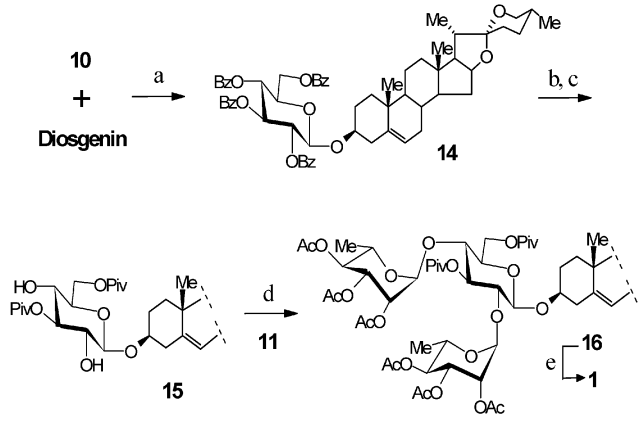
(19) Various *N*-substituted trifluoroacetimidoyl halides are readily accessible from the reaction of trifluoroacetic acid with primary amines in a $PPh_3/Et_3N/CCl_4$ system, see: Tamura, K.; Mizukami, H.; Maeda, K.; Watanabe, H.; Uneyama, K. *J. Org. Chem.* **1993**, *58*, 32.

(20) Adinolfi, M.; Barone, G.; Iadonisi, A.; Schiattarella, M. *Synlett* **2002**, 269.

(21) To the best of our knowledge, no crystal structure has so far been obtained for glycosyl trichloroacetimidate donors. For an analysis of the conformation of glycosyl trichloroacetimidates by MM2 and MNDO calculations, see: Schmidt, R. R.; Gaden, H.; Jatzke, H. *Tetrahedron Lett.* **1990**, 31, 327.

(22) (a) McCarty, C. G. In *The chemistry of the carbon−nitrogen double bond*; Patai, S., Ed.; Interscience Publishers: London, UK, 1970; p 363. (b) Perrin, C. L. In *The chemistry of amidines and imidates*; Patai, S., Rappoport, Z., Eds; John Wiley & Sons: Chichester, UK, 1991; p 147.

SCHEME 3. Synthesis of Dioscin 1[a]



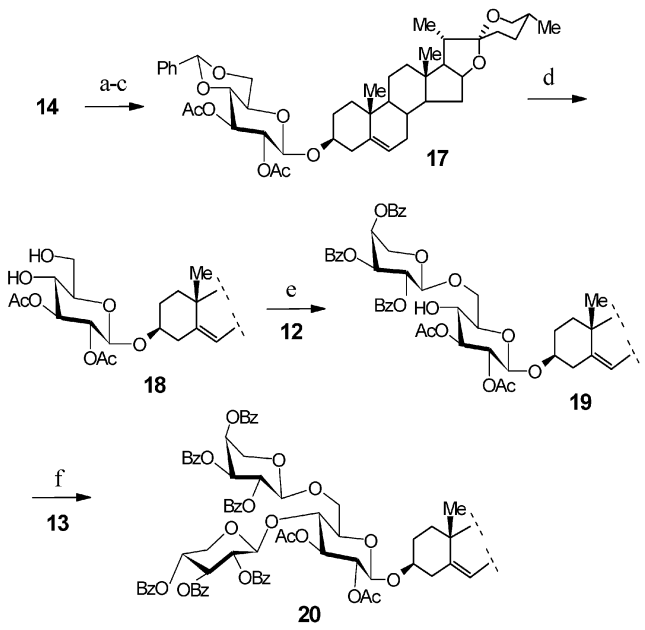
[a] Reagents and conditions: (a) TMSOTf (0.05 equiv), 4 Å MS, CH_2Cl_2, rt, 92%; (b) NaOMe, HOMe, rt; (c) PivCl, pyridine, 0 °C, 60%; (d) conditions similar to (a), 66%; (e) NaOMe, HOMe/H_2O/THF (1:1:1), rt, 90%.

spectra of these glycosyl trifluoroacetimidates (**10**−**13**), which showed broad peaks for the anomeric H-1 and one or two other signals, implicated an interconversion of different configurations via syn−anti isomerization around the carbon−nitrogen double bond,[22] or of different conformations via rotation about the single bonds, on the NMR time scale.

Glycosylation of diosgenin with benzoyl- protected glucopyranosyl trifluoroacetimidate **10** under the promotion of TMSOTf (0.05 equiv) at room temperature led to the expected coupling product **14** in excellent yield (92%) (Scheme 3). This coupling protocol has been optimized with trichloroacetimidate donors,[24] and here the corresponding trifluoroacetimidate donor gave similar results. After removal of the benzoyl groups on **14**, the resulting 3,6-OH were selectively blocked by pivaloyl groups, providing diol **15** in good yield (60%).[25] Glycosylation of **15** with acetyl- protected rhamnopyranosyl trifluoroacetimidate **11** afforded the desired trisaccharide **16** in a satisfactory 66% yield, equal to a *per* glycosylation yield of 81%. Removal of all the acyl protecting groups (acetyl, benzoyl, and pivaloyl groups) under NaOH in a solvent mixture of MeOH/THF/H_2O (1:1:1) generated dioscin **1** (90%), whose physical data were identical with those of an authentic sample.[13]

The assembly of the trisaccharide of Xiebai saponin I was first attempted with diosgenin as the aglycone (Scheme 4). Thus **14** was readily converted into 4,6-diol **18** using a routine approach for protecting group manipulation on a β-glucopyranoside (4 steps, 72% yield). Coupling of diol **18** with benzoyl-protected arabinopyranosyl trifluoroacetimidate **12** (1.1 equiv, −78 °C to rt) under the promotion of TMSOTf (0.05 equiv) gave the expected 6-OH glycosylated product **19** in 73% yield, while 12% of the 4,6-OH diglycosylated product was also

SCHEME 4. Synthesis of Diosgenyl Trisaccharide 20[a]



[a] Reagents and conditions: (a) NaOMe, HOMe/CH_2Cl_2 (1:1), rt; (b) $PhCH(OMe)_2$, *p*-TsOH, DMF, 50 °C; (c) Ac_2O, pyridine, 90% (three steps); (d) *p*-TsOH, MeOH/CH_2Cl_2, rt, 80%; (e) **12** (1.1 equiv), TMSOTf (0.05 equiv), 4 Å MS, CH_2Cl_2, −78 °C-rt, 73%; (f) **13** (1.5 equiv), conditions similar to (e), 84%.

produced. Glycosylation of **19** with a free 4-OH with the benzoyl-protected xylopyranosyl trifluoroacetimidate **13** (1.5 equiv) under similar conditions afforded the desired trisaccharide **20** in good yield (84%). Attempts to convert the 5,6-double bond of the diosgenin moiety of **20** into a 6-OH function (then into laxogenin) using $SnCl_4$−$NaBH_4$[26] failed, although a model reaction on monosaccharide **14** has been successful.

Glycosylation of laxogenin with the trifluoroacetimidate **10** (1.1 equiv) was as successful as glycosylation of diosgenin under similar conditions, affording **21** in 92% yield (Scheme 5). Only a trace amount of the presumably 3-OH, 6-enol diglycosylated product was isolated, which, after removal of the benzoyl groups, showed a molecular weight of 777.4 ($M + Na^+$) in the ESIMS spectrum and the absence of a C=O absorption on the IR spectrum.[27] Compound **21** was then transformed into 4,6-diol **22** using a similar approach for **14** → **18** conversion. At this stage, we protected the 2,3-OH of the glycosyl acceptor with the bulkier benzoyl groups instead of acetyl groups in **18**, and expected to have better regioselectivity for glycosylation. Indeed, glycosylation of diol **22** with arabinopyranosyl trifluoroacetimidate **12** under similar conditions for glycosylation of **18** afforded the desired 6-OH glycosylated product **23** in higher yield (82%, compared to the yield of 73% for **19**). However, the 3-OBz substitution also increased the hindrance of the neighboring 4-OH (compared to the influence of 3-OAc in **19**), thus glycosylation of **23** with xylopyranosyl trifluoroacetimidate **13**

(23) For the exo-anomeric effect, see: (a) Lemieux, R. U.; Koto, S. *Tetrahedron* **1974**, 30, 1933. (b) Pinto, B. M.; Leung, R. Y. N. In *The Anomeric Effect and Associated Stereoelectronic Effect*; Thatcher, G. R. J., Ed.: ACS Synp. Ser. 539; American Chemical Society: Washington, DC, 1993; p 126.

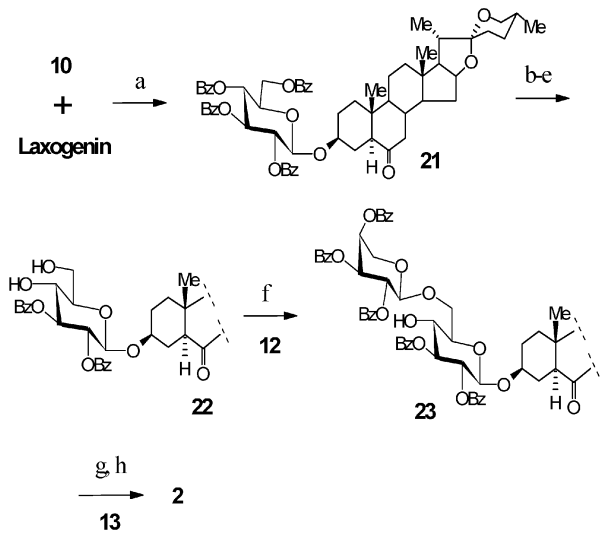
(24) Deng, S.; Yu, B.; Lou, Y.; Hui, Y. *J. Org. Chem.* **1999**, *64*, 202.

(25) Li, B.; Yu, B.; Hui, Y.; Li, M.; Han, X.; Fung, K.-P. *Carbohydr. Res.* **2001**, 331, 1.

(26) Kano, S.; Yuasa, Y.; Shibuya, S. *J. Chem. Soc., Chem. Commun.* **1979**, 796.

(27) For an enol glycosylation, see: Saito, S.; Sumita, S.; Kanda, Y.; Sasaki, Y. *Tetrahedron Lett.* **1992**, 33, 7381.

SCHEME 5. Synthesis of Xiebai Saponin I (2)[a]



[a] Reagents and conditions: (a) **10** (1.1 equiv), TMSOTf (0.05 equiv), 4 Å MS, CH_2Cl_2, rt, 92%; (b) NaOMe, HOMe/CH_2Cl_2 (1:1), reflux; (c) $PhCH(OMe)_2$, *p*-TsOH, DMF, 50 °C; (d) BzCl, pyridine, rt; (e) *p*-TsOH, CH_2Cl_2/HOMe (3:2), 60 °C, 78% (four steps); (f) **12** (1.03 equiv), conditions similar to (a), 82%; (g) **13** (4.0 equiv), conditions similar to (a), 60% (10% **23** recovered); (h) NaOMe, HOMe/CH_2Cl_2 (1:1), reflux, 90%.

(4.0 equiv) provided the desired trisacchride in lower yield (60%, compared to the yield of 84% for **20**). Removal of the benzoyl groups in the presence of NaOMe in a solvent mixture of HOMe/CH_2Cl_2 (1:1) under reflux afforded Xiebai saponin I (**2**) in 90% yield, whose data were in good accordance with those reported.[9]

In conclusion, two trisaccharide steroidal saponins, dioscin (**1**) and Xiebai saponin I (**2**) with various bioactivities, were efficiently synthesized using acetyl or benzoyl protected glycosyl trifluoroacetimidates (**10**−**13**) as glycosylation donors. Thus, dioscin was synthesized in five steps and a 33% overall yield from diosgenin and glycosyl trifluoroacetimidates (**10** and **11**). Xiebai saponin I was synthesized in eight steps and a 32% overall yield from laxogenin and glycosyl trifluoroacetimidates (**10**, **12**, and **13**), whereupon the rare steroid laxogenin was prepared from diosgenin in four steps and an overall 69% yield. All the glycosylation reactions involved in the present syntheses demonstrated that glycosyl trifluoroacetimidates were successful donors comparable to the corresponding glycosyl trichloroacetimidates used in the previous similar synthesis.[12,13,24] The dynamic conformation/configuration interconversion of the glycosyl trifluoroacetimidates, which was implicated by their ^{1}H NMR, and the substitution effect of the electron donating/withdrawing groups on the *N*-phenyl group on the glycosylation reactivities of the glycosyl trifluoroacetimidates are our current interest, and these research results will be published in due course.

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Supporting Information Available: Experimental procedures and spectral data for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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