ACCOUNTS
of chemical research

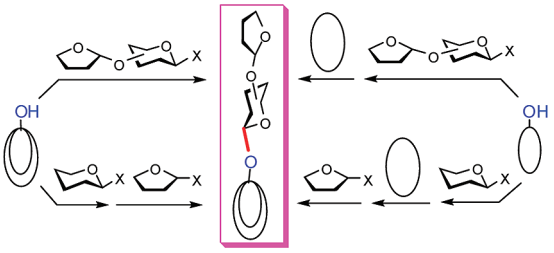
# Assembly of Naturally Occurring Glycosides, Evolved Tactics, and Glycosylation Methods

BIAO YU,* JIANSONG SUN, AND XIAOYU YANG

*State Key Laboratory of Bioorganic and Natural Products Chemistry, Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences*

RECEIVED ON NOVEMBER 18, 2011

## CONSPECTUS




Glycosylation of proteins and lipids is critical to many life processes. Secondary metabolites (or natural products), such as flavonoids, steroids, triterpenes, and antibiotics, are also frequently modified with saccharides. The resulting glycosides include diverse structures and functions, and some of them have pharmacological significance. The saccharide portions of the glycosides often have specific structural characteristics that depend on the aglycones. These molecules also form heterogeneous "glycoform" mixtures where molecules have similar glycosidic linkages but the saccharides vary in the length and type of monosaccharide unit. Thus, it is difficult to purify homogeneous glycosides in appreciable amounts from natural sources.

Chemical synthesis provides a feasible access to the homogeneous glycosides and their congeners. Synthesis of a glycoside involves the synthesis of the aglycone, the saccharide, the connection of these two parts, and the overall manipulation of protecting groups. However, most synthetic efforts to date have focused on the aglycones, treating the attachment of saccharides onto the aglycones as a dispensable topic. The synthesis of the aglycone and the synthesis of the saccharide belong to two independent categories of chemistry, and different types of the aglycones and saccharides pose as specific synthetic subjects in their own disciplines. The only reaction that integrates the broad chemistry of glycoside synthesis is the glycosidic bond formation between the saccharide and the aglycone. Focusing on this glycosylation reaction in this Account, we string together our experience with the synthesis of the naturally occurring glycosides.

We briefly describe the synthesis of 18 glycosides, including glycolipids, phenolic glycosides, steroid glycosides, and triterpene glycosides. Each molecule represents a prototypical structure of a family of the natural glycosides with interesting biological activities, and we emphasize the general tactics for the synthesis of these diverse structures. We provide a rationale for four tactics for the synthesis of glycosides, based on the stage at which the glycosidic bond is formed between the saccharide and the aglycone. This choice of tactic determines the success or failure of a synthesis, and the flexibility and the overall efficiency of the synthesis as well. Toward the synthesis of heterogeneous glycoform mixtures, we discuss successive and random glycosylation reactions. Finally, we have developed two new glycosylation protocols that address the challenges in the glycosylation of aglycones that are poorly nucleophilic, extremely acid labile, or extremely electrophilic. One of these new protocols takes advantage of glycosyl trifluoroacetimidate donors, and a second protocol uses gold(I)-catalyzed glycosylation with glycosyl *ortho*-alkynylbenzoate donors.

## Introduction

Most of the secondary metabolites in nature could occur as glycosides.[1,2] These glycosides are derived mostly via post-modification of the secondary metabolites catalyzed by glycosyltransferases.[3,4] Further modifications on the glycosides, such as acylation, oxidation, and degradation, take place frequently. In this way, Nature manages to expand the structural and functional diversity of the metabolites greatly and efficiently. Naturally, a glycoside is taken for granted to have two chemically and functionally independent parts, that is, the saccharide part and the aglycone part, of which the aglycone is the part of value while the saccharide part only increases water solubility. However, this is not always true. Although many glycosides are simply

Published on the Web 04/11/2012 www.pubs.acs.org/accounts
10.1021/ar200296m 

attached with a glucose or glucose-containing disaccharide to facilitate in vivo transportation, many others are decorated with sophisticated oligosaccharides to endow peculiar functions, in which both the aglycone and the saccharide play the role cooperatively as a whole.[1,5] The best examples are those which have already been developed into therapeutic agents, including most importantly the antibiotics, nucleosides, and cardiac glycosides.

Depending on the type of the aglycones, the saccharide parts are always characteristic and conservative in structures.[1] For those complicated glycosides, a mixture of congeners with a similar pattern of glycosidic linkage but varied length and units of monosaccharides coexist. This phenomenon is known in glycoproteins and glycolipids as "glycoform".[6] The occurrence of a microheterogeneous "glycoform" makes purification of a homogeneous glycoside component, especially in an appreciable amount, extremely difficult. Studies on the structure–activity relationships and the mechanisms of biological action of these glycosides are thus hampered. In fact, many glycosides are still remained at the folkloric usage, such as the saponin extracts from ginseng, licorice, ivy leaves, primula roots, and senega roots.[7] Access to the homogeneous glycosides and analogues in these cases could only resort to chemical[8,9] or enzymatic synthesis.[3,4]

Chemical synthesis of glycosides involves particularly the incorporation of saccharides onto aglycones. This might be extremely challenging, because the structures of the aglycones are extremely diverse, which include all of the natural product scaffolds with enormous ways of functional groups arrangement. To tackle the synthesis of the complicated glycosides would provide not only practical accesses to these biologically significant chemical entities but also opportunities to develop new glycosylation chemistry. The excellent works from the laboratories of Danishefsky,[10] Nicolaou,[11] and many other prominent chemists have well illustrated this point.[12,13] Herein we account our own experience in this topic with a focus on the formation of the *O*-glycosidic bonds between saccharides and aglycones.

## Representative Glycosides We Have Synthesized

Within the last 15 years, we have accomplished the syntheses of a number of the complicated glycosides with interesting structural features and biological activities. Listed in Figure 1 are those representative examples, including glycolipids (**1** and **2**), phenolic glycosides (**3**–**8**), steroid glycosides (**9**–**13**), and triterpene glycosides (**14**–**18**).

Tricolorin A (**1**),[14] showing allelopathic and antitumor activities, is a prototype member of the plant resin glycosides, which bears characteristically a hydroxy fatty acid aglycone forming typically a macrolactone with the saccharide backbone decorated with acyl moieties. Both our and Heathcock's synthesis employed conventional macrolactonization to construct the cyclic ester,[15−17] while Fürstner and Müller were inspired to explore ring-closing olefin metathesis to build up flexibly the resin glycosides with varied size of the macrolactones.[18,19]

Caminoside A (**2**),[20] a bacterial type III secretion inhibitor isolated from a marine sponge, possesses a hydroxy methyl ketone lipid as aglycone and a novel tetrasaccharide glycan with a fully substituted glucose residue in the middle and the rare 6-deoxy-D-talose and L-quinovose, both in the 1,2-*cis*-glycosidic linkage, at the terminals. A total of 57 steps were required to assemble this glycolipid with D-glucose, D-galactose, L-rhamnose, and 9-decenal as starting materials.[21]

The synthesis of phenol glycosides poses as a special subject.[22] Phenols with electron-withdrawing groups or *ortho*-substituents are reluctant to be *O*-glycosylated, while electron-rich phenols are prone to undergo *C*-glycosylation. CRM646-A (**3**)[23] is an unusual fungal glucuronide with a dimeric 2,4-dihydroxy-6-alkyl-benzoic acid (orcinol *para*-depside) as aglycone, which shows significant heparinase and telomerase inhibition activities. To secure the formation of the phenolic glycoside, an early stage glycosylation was performed.[24]

Flavonoid glycosides, mostly from higher plants, constitute the biggest family of the natural glycosides with several thousands of congeners that have been identified.[25] A-76202 (**4**),[26] a typical isoflavone 7-*O*-glycoside, is isolated from a *Rhodococcus* species, which shows very potent inhibitory activity against the α-glucosidases of rat liver microsome ($IC_{50}$ = 0.46 ng/mL). A later-stage glycosylation enabled us to attach a variety of the saccharides onto the isoflavone 7-OH.[27] In contrast, employing a later-stage elaboration of the isoflavone B ring, we were able to prepare isoflavone 7-*O*-α-D-arabinofuranosides with varied B rings divergently.[28]

Quercetin 3-*O*-(2″-galloyl)-α-L-arabinopyranoside (**5**)[29] is a typical acylated flavonol 3-*O*-glycoside, which shows antibacterial, antioxidant, and inhibitory activity against HIV-1 integrase. The flavonol 3-*O*-glycosidic linkage was constructed by a conventional method,[30] which employs glycosyl bromides as donors under phase-transfer-catalyzed conditions or under the promotion of a silver salt. A recent finding that the flavonol 3-OH derivatives can be glycosylated

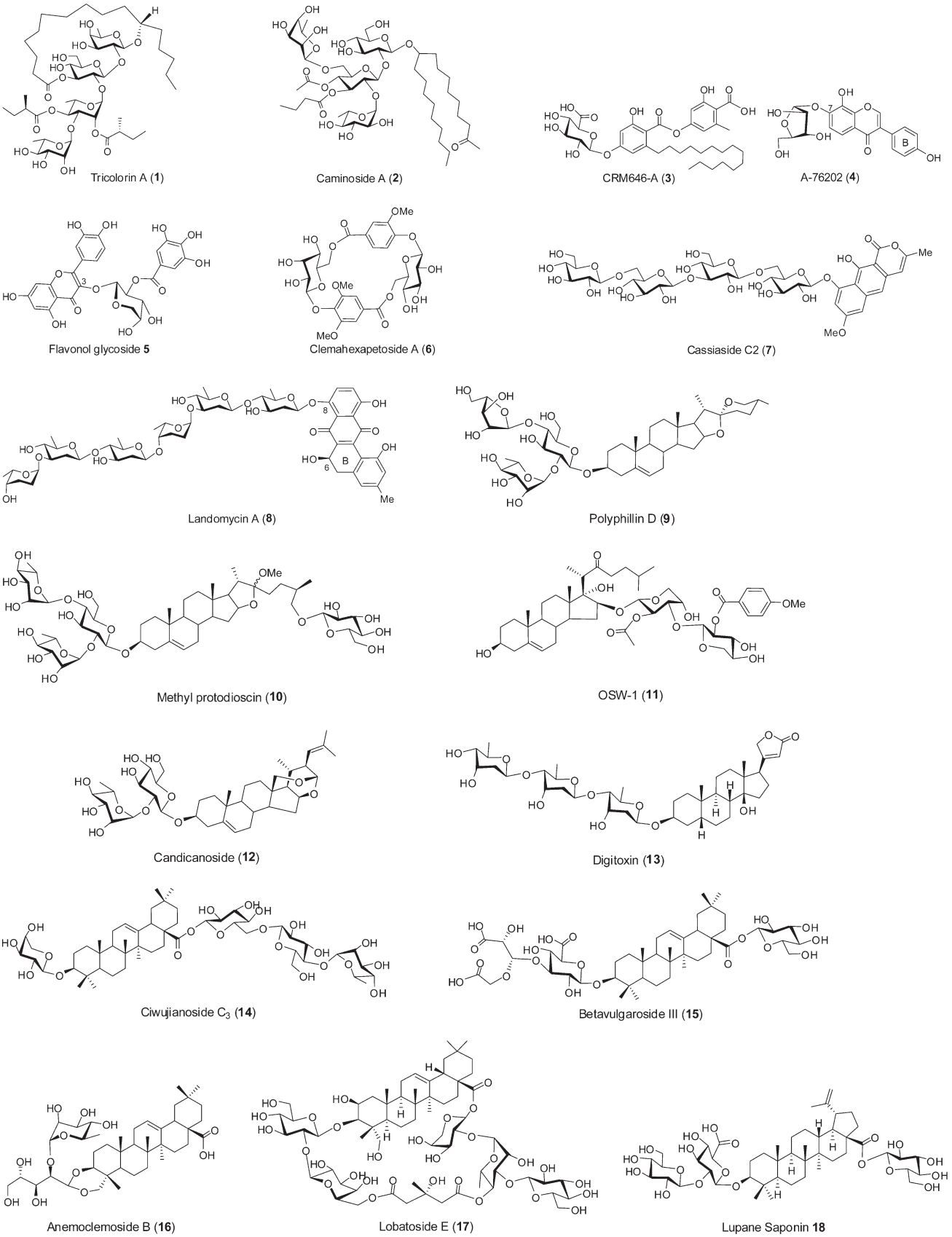



**FIGURE 1.** Representative glycosides synthesized by Yu and co-workers.

effectively with glycosyl *ortho*-alkynylbenzoates as donors under the catalysis of a gold(I) complex has greatly facilitated the synthesis of this big family of the glycosides.[31]

Clemahexapetoside A (**6**)[32] is a member of a small group of the cyclic dimers of 4-(glycosyloxy)benzoates from plants. The synthetic approach toward this natural glycoside also enabled the quick assembly of the macrocyclic trimeric, tetrameric, and pentameric congeners.[33]

Cassiaside $C_2$ (**7**)[34] is a unique naphtho-α-pyrone glycoside identified from the cassia seeds, which shows anti-inflammatory activity. The construction of the glycosidic linkage with the hydrogen-bonded poorly nucleophilic naphthol derivatives turned out to be extremely difficult. This task was finally realized at the monosaccharide level by sacrifice of 6.0 equiv of a glucosyl imidate donor.[35]

Besides the poor nucleophilicity, the vulnerability of both the aglycone and the sugar linkage makes landomycins, which feature a benz[a]anthraquinone aglycone with a dearomatized B ring and deoxyoligosaccharide chains of varied length attached at C8-OH,[36] much more formidable synthetic targets. Landomycin A (**8**), the longest and the most potent antitumor active congener, has been synthesized only recently.[37]

Steroid glycosides (e.g., **9**−**13**) can be categorized into four major subfamilies basing on the steroid aglycone, including spirostane, furostane, cholestane, and cardiac glycosides.[7] Polyphillin D (**9**)[38] is a prototypical spirostane glycoside which occurs widely in plants and shows potent antitumor activities. The synthetic route toward this representative glycoside has been adopted to the preparation of a large number of the spirostane glycosides.[8,39] Methyl protodioscin (**10**)[40] represents a furostane glycoside, which was prepared conveniently from the corresponding spirostane saponin dioscin.[41]

OSW-1 (**11**)[42] and Candicanoside A (**12**),[43] from two taxonomically related plants, are novel cholestane glycosides, of which OSW-1 and congeners show exceptionally high antitumor activities. Taking advantage of the innate 2′-*O*-acetyl substituent, a disaccharide donor was attached stereoselectively to the aglycone derivatives to achieve the synthesis of OSW-1 and analogues.[44,45] In contrast, the (1→2)-linked disaccharide in Candicanoside A discouraged a direct glycosylation with a disaccharide donor, that would lead to α/β anomers in the absence of a neighboring participation group. Thus, a stepwise glycosylation was carried out in the succeeded total synthesis.[46]

Digitoxin (**13**), a prototype of the cardenolide-type cardiac glycosides, is a widely prescribed drug for treating congestive heart failure and cardiac arrhythmia.[1] The assembly of this deoxytrisaccharide with conventional glycosylation methods was compromised with moderate stereoselectivity and yields.[47] Thus, de novo approaches via tungsten-catalyzed endoselective alkynol cyclomerization or palladium-catalyzed glycosidation of pyranone derivatives were applied to the synthesis.[48,49] Our recent synthesis employed 3,4-di-*O*-*tert*-butyldiphenylsilyl-D-digitoxosyl *ortho*-cyclopropylethynylbenzoate as donor and $Ph_3PAuOTf$ as catalyst; the formation of the β-digitoxosidic linkages was achieved in excellent yields and β/α selectivity.[50]

The most complicated and diverse glycosides in plants are triterpene glycosides.[7] Ciwujianoside $C_3$ (**14**),[51] isolated from a Chinese medicinal plant with oleanolic acid as the aglycone and two sugar moieties on the C3-OH and C28-COOH, belongs to the most common type of the triterpene saponins. This is also the first such saponin being synthesized.[52]

Betavulgaroside III (**15**),[53] from sugar beet, is a congener of a small group of the triterpene *seco*-glycosides featuring virtually an oxidative fragmentation of a terminal monosaccharide unit. The introduction of this *seco*-glycoside residue was a major concern in the synthesis.[54]

Anemoclemoside A (**16**),[55] isolated from the roots of a folk medicinal plant distributed at an altitude of 1600−3000 m in the Yangtse River valley region, is one of the two glycosides disclosed so far to have an open-chain cyclic acetal glycosidic linkage. This unique linkage was synthesized stereoselectively in an excellent yield via condensation of a hederagenin 3,23-diol derivative with a disaccharide aldehyde under the promotion of TMSOTf at low temperature.[56]

Lobatoside E (**17**)[57] is the most potent antitumor active congener of a small group of the plant triterpene saponins named cyclic bisdesmosides, which have two oligosaccharides flanked on a pentacyclic triterpene and bridged with 3-hydroxy-3-methyl glutarate. The synthesis of this complex cyclic bisdesmoside employed a highly modular approach, with a total of 73 steps starting with cheap starting materials.[58]

Betulinic acid trisaccharide **18**[59] is a usual lupane-type saponin. Acidic conditions were avoided to glycosylate the betulinic acid derivatives, which could undergo easily Wagner-Meerwein rearrangement.[60]

## Tactics for the Synthesis of Glycosides

Syntheses of saccharides and aglycones are two categories of chemistry, which are evolved and practiced separately. Synthesis of a glycoside requires integration of these two categories of chemistry, in that the attachment of the

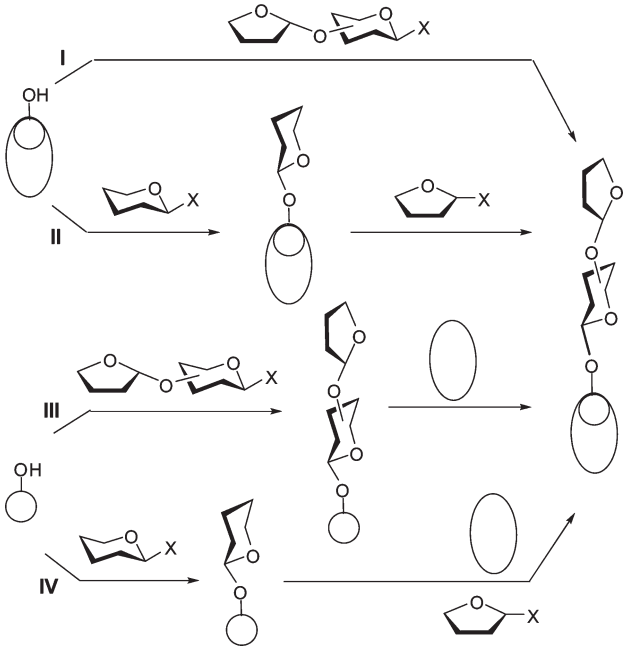
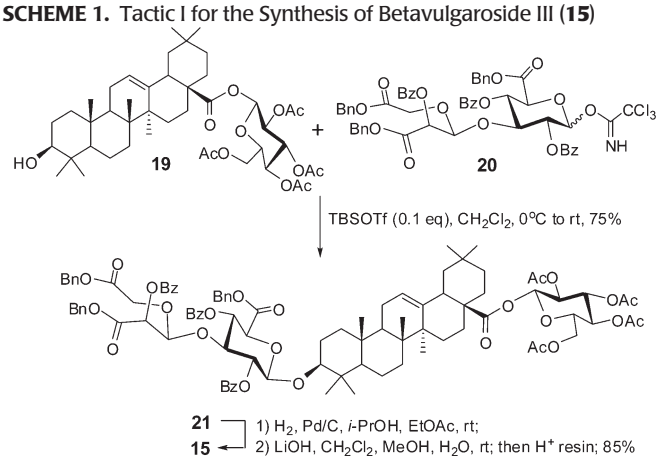



**FIGURE 2.** General tactics for the synthesis of a glycoside based on the stage at which the glycosidic bond with the aglycone is constructed.

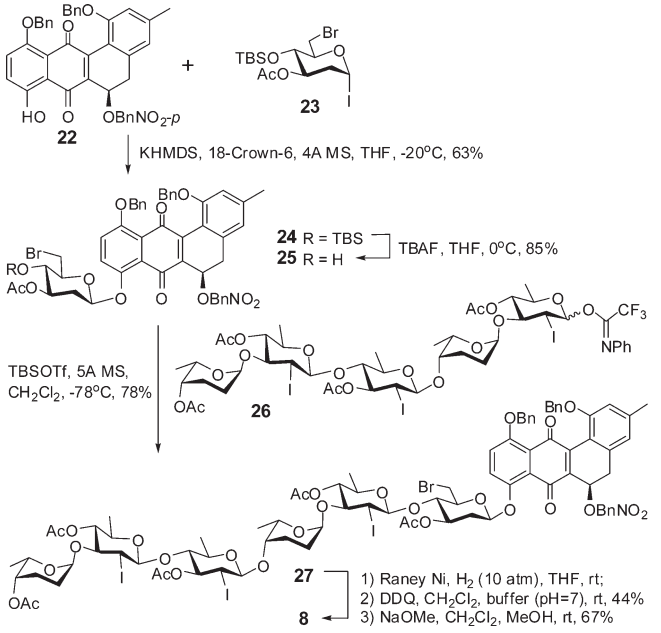
**SCHEME 1.** Tactic I for the Synthesis of Betavulgaroside III (**15**)




saccharide onto the aglycone is a major concern. The stage for performing this glycosidic bond formation reaction is decisive for a retrosynthetic consideration of the synthesis of a glycoside. Accordingly, four basic tactics are evolved (Figure 2).

The most straightforward and convergent tactic is a direct later-stage glycosylation of the aglycone with a prefabricated saccharide donor followed by global deprotection (Tactic I). The synthesis of Betavulgaroside III (**15**) illustrates the application of this tactic (Scheme 1).[54] Thus, assembly of the fully elaborated saccharide donor **20** onto oleanolic acid 28-*O*-glucoside **19** provided the advanced precursor **21**,

**SCHEME 2.** Tactic II for the Synthesis of Landomycin A (**8**)

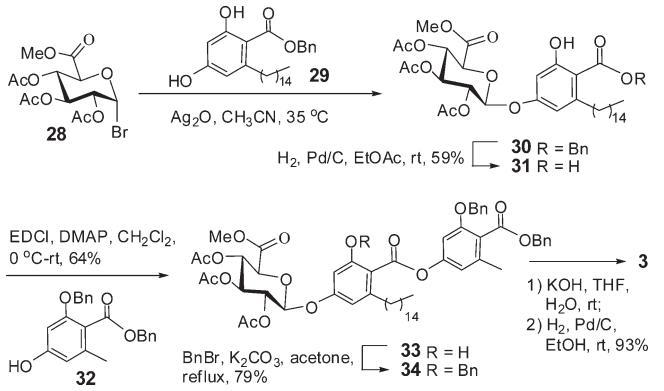



which was subjected to the final removal of benzyl and acyl protecting groups to provide the triterpene *seco*-glycoside. Otherwise, elaboration of the *seco*-sugar unit after formation of the 3-*O*-glycosidic linkage was found troublesome.

Tactic I is especially advantageous when the aglycone is precious or contains functional groups labile to the conditions for glycan elongation. However, glycosylation of the aglycone with a fully developed oligosaccharide donor is often problematic. When the required glycan contains a (1→2)-linkage at the reducing end (e.g., the glycans in glycosides **1**, **2**, **9**, **10**, **12**, **17**, and **18**), in that no neighboring group participation can be exploited, the glycosylation usually leads to a pair of the $\alpha$, $\beta$ anomers which are difficult to separate.[61]

Alternatively, the sugar moiety can be attached to the aglycone in a linear manner (Tactic II). This tactic warrants a stereospecific and high-yielding formation of the glycosidic bond to the aglycone. However, subsequent elongation of the glycan, which demands the manipulation of temporary protecting groups in between each glycosylation step, in the presence of a multifunctional aglycone becomes troublesome. In the synthesis of Landomycin A (**8**), construction of the 2-deoxy-$\beta$-glycosidic linkage with the poorly nucleophilic hydrogen-bonded C8-phenol of landomycinone is extremely difficult (Scheme 2). This task was finally realized at the monosaccharide level by an $S_N2$ type substitution of the 2,6-dideoxy-$\alpha$-D-glucopyranosyl iodide (**23**) with a naphthol anion derived from landomycinone **22**.[37]

**SCHEME 3.** Tactic III for the Synthesis of CRM646-A (**3**)

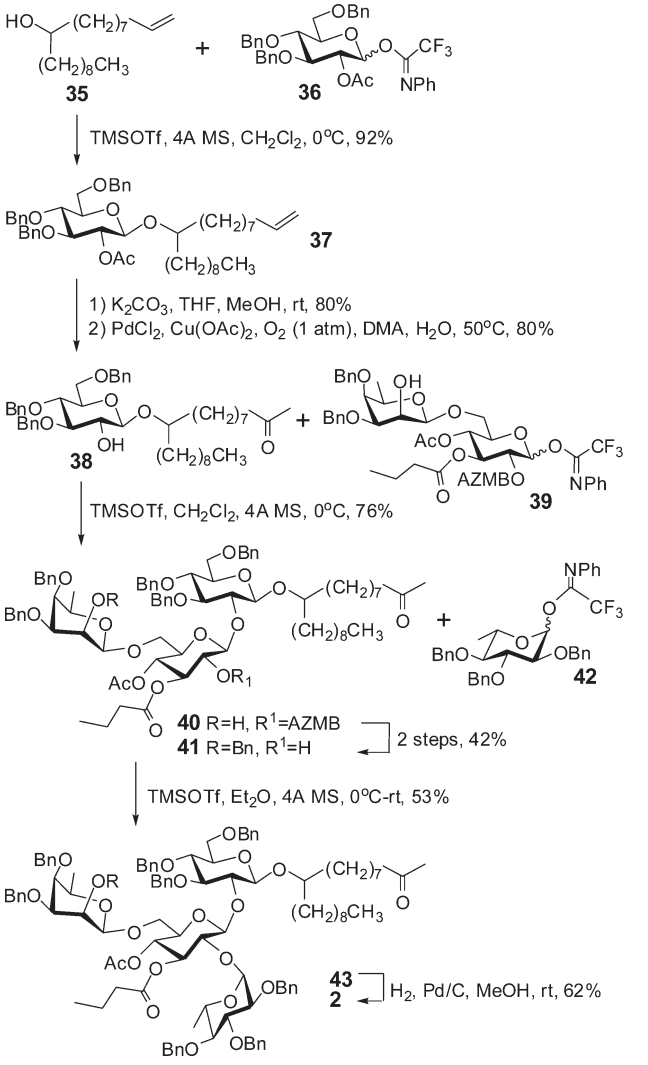



Then, elongation of the glycan can be proceeded sequentially after removal of the temporary 4′-*O*-*tert*-butyldimethylsilyl protecting group. Glycosylation of the monosaccharide **25** with pentasaccharide donor **26**, after global deprotection, afforded Landomycin A (**8**). Glycosylation of **25** with shorter saccharide donors would lead to the short congeners of the landomycin family.

When glycosylation of the aglycone, even with a compromised monosaccharide donor, is unsuccessful, then this glycosidic linkage should be built before elaboration of the full aglycone (Tactic III). In the synthesis of CRM646-A (**3**), attempts at direct glycosylation of the depside aglycone derivatives were not successful.[24] Therefore, the phenol glucuronidic linkage was synthesized via coupling of the orsellinate derivative **29** with glucuronate bromide **28** before assembly of the phenolic ester linkage in the depside aglycone (Scheme 3).

The final alternative for the assembly of a glycoside involves elaboration of both the aglycone and the glycan after construction of the glycosidic linkage (Tactic IV). Thus, the synthesis of Caminoside A (**2**) commenced with the glycosylation of 1-nonadecen-10-ol with glucopyranosyl imidate **36**, that led to $\beta$-glucoside **37** in an excellent yield.[21] The 2′-*O*-acetyl group on **37** was removed. At this stage, the methyl ketone function in the aglycone was elaborated by a Wacker oxidation. Glycosylation of the 2′-OH of **38** with disaccharide trifluoroacetimidate **39** afforded trisaccharide **40**. The difficult problem of 1,2-*cis*-glycosidic bond formation was solved at the disaccharide level (i.e., **39**). Protection of the remaining 2‴-OH with a benzyl group, followed by selective removal of the 2″-*O*-2-(azidomethyl)benzoyl (AZMB) group provided **41**. The resulting 2″-OH was then glycosylated with perbenzyl L-quinovopyranosyl trifluoroacetimidate **42** to provide the desired tetrasaccharide **43**.

**SCHEME 4.** Tactic IV for the Synthesis of Caminoside A (**2**)



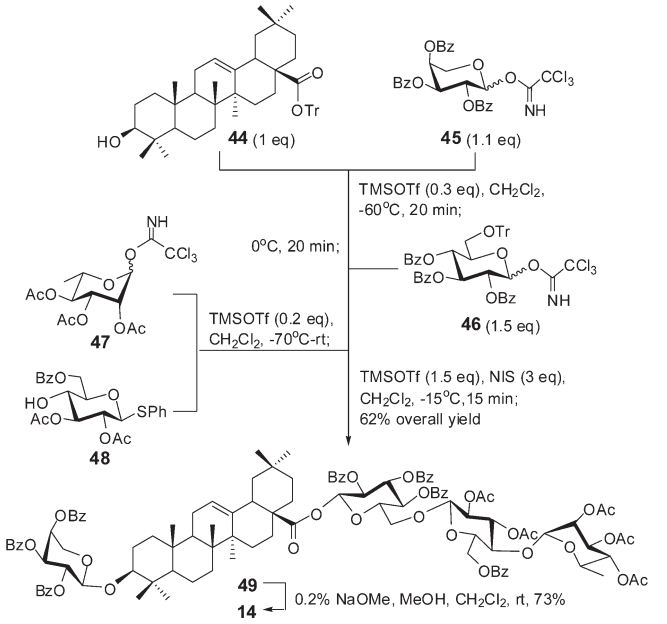

Finally, hydrogenolysis of the benzyl groups furnished the target glycolipid **2** (Scheme 4).

## Synthetic Access to the "Glycoform" of Glycosides

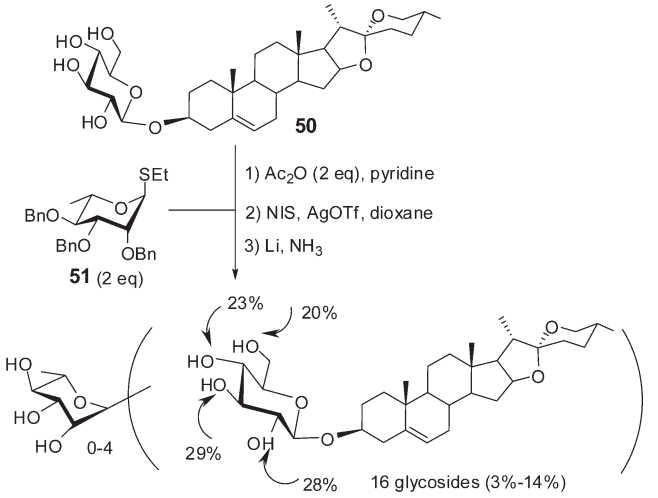
The succeeded approaches to the synthesis of the representative glycosides can be adopted easily to the synthesis of the corresponding congeners with varied saccharides. It is especially convenient to apply the divergent Tactic II for this purpose. For example, we have synthesized several dozens of the Polyphillin D (**9**) congeners via stepwise elongation of the glycan.[39,62] Thus the structure–activity relationships on the antitumor and hemolytic activities of spirostane saponins have been well recorded.[63]

Special approaches have also been developed for the glycoform-oriented synthesis. When the monosaccharide donors in the linear synthesis (Tactic II) are designed to have

**SCHEME 5.** Synthesis of Ciwujianoside $C_3$ (**14**) via Successive Glycosylation




**SCHEME 6.** Synthesis of a Library of Saponins via a Double-Random Strategy




distinguishable reactivities, sequential glycosylation can be performed to furnish the glycosides in a "one-pot" fashion.[64] The manipulation of temporary protecting groups and purification of intermediates are avoided. An extremely successful example has been provided for the synthesis of triterpene saponin **14** (Scheme 5).[52] In that, the assembly of the fully protected oleanane tetrasaccharide **49** was realized by four successive glycosylation steps (with monosaccharide donors **45**–**48** sequentially), taking advantage of the orthogonal activation of the glycosyl imidate **47** and thioglycoside **48** and the selective in situ deprotection of the trityl ester and trityl ether under the glycosylation conditions. Employing a variety of the monosaccharide donors in analogous synthesis would access quickly to a large number of the congeners with varied monosaccharide units.

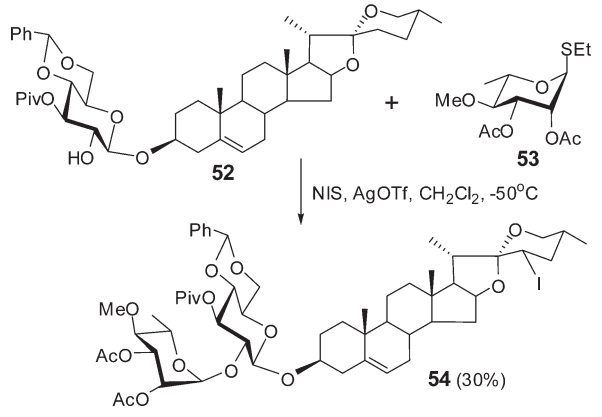
Synthesis of a targeted glycoside requires sequential manipulation of protecting groups to achieve the "complete regioselectivity". In contrast, "random glycosylation" directs toward the "completely-no-regioselectivity" to produce a library of glycosides that contains all the possible glycosylation products,[65] ideally in nearly equal amounts. Such tailor-made "glycoform" libraries might expedite the screen of active components. As shown in Scheme 6, an ideal random glycosylation of the steroid glucoside **50** with a monosaccharide donor (e.g., **51**) would provide 16 glycosides in equal amount. However, the reactivity difference of the four hydroxyl groups on **50** prohibited the ideal random glycosylation. Thus, we carried out a random acylation before the random glycosylation. This "double random" strategy, after a deprotection step, enabled the synthesis of the desired "glycoform" library with all the possible congeners in nearly statistical distribution.[66]

## New Glycosylation Protocols

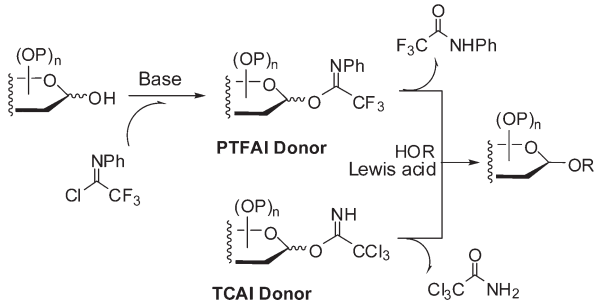
Construction of the glycosidic linkages with those multifunctional and topologically complicated aglycones is often challenging. In some cases, the challenge is expected. As in the synthesis of landomycins, the landomycinone derivative (e.g, **22**) is poorly nucleophilic and prone to aromatize in the presence of acid.[37] In others, the problems arise unexpectedly. For example, coupling of spirostane glycoside **52** with thioglycoside **53** in the presence of NIS/AgOTf led to disaccharide **54** in ~30% yield, in that the iodination took place on the $CH_2$ next to the ketal function in the aglycone (Scheme 7).[62,67] It should bear in mind that promoters in the glycosylation reaction, which are often electrophilic and used in stoichiometric amounts, could be detrimental to the aglycones.

Thus, glycosylation protocols with a catalytic amount of the promoter under mild conditions are favorable for the synthesis of naturally occurring glycosides. The Schmidt glycosylation stands out as the most favorable one, which uses glycosyl trichloroacetimidates as donors, a Lewis acid (e.g., TMSOTf and $BF_3 \cdot OEt_2$) as catalyst, and proceeds at low temperature.[68] However, when the aglycone derivatives to be glycosylated are very poorly nucleophilic or highly steric hindered, then trichloroacetamide, the leaving entity from the donors, could compete for the glycosidation. To solve

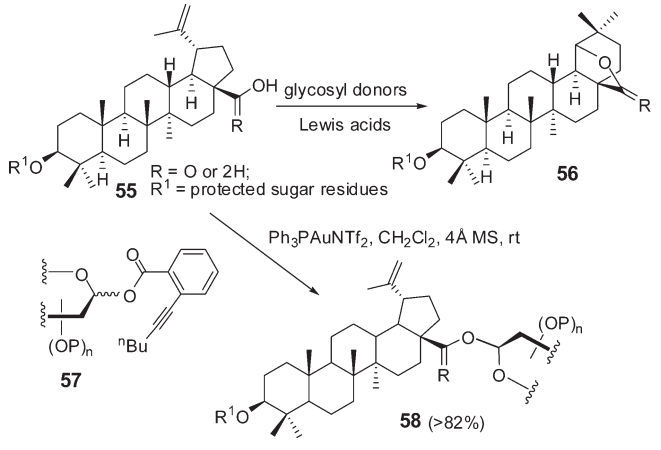
**SCHEME 7.** Unexpected Side Reaction during Glycosylation of Steroid Derivative **52** with Thioglycoside **53**




**SCHEME 8.** Glycosylation Protocol with Glycosyl *N*-Phenyltrifluoroacetimidates as Donors

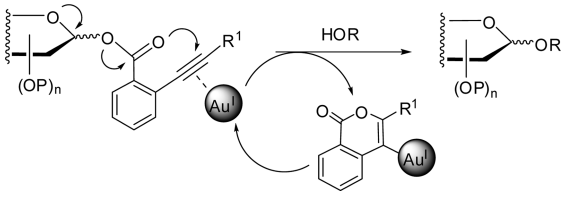



**SCHEME 9.** Glycosylation of the Acid-Labile Lupane Derivatives




this problem, we developed the glycosyl *N*-phenyltrifluoroacetimidates as donors, in that the leaving *N*-phenyltrifluoroacetamide is much less competitive as a nucleophile (Scheme 8).[69,70]

**SCHEME 10.** Glycosylation Protocol with Glycosyl *ortho*-Alkynylbenzoates as Donors and a Gold(I) Complex as Catalyst




In other cases, even a catalytic amount of the Lewis acid promoter at low temperature becomes detrimental to the aglycone derivatives. The 3-*O*-substituted betulin and betulinic acid derivatives are such substrates, which underwent Wagner-Meerwein rearrangement under the glycosylation conditions with imidate donors (Scheme 9).[60,71] This problem is solved by our newly developed glycosylation protocol with glycosyl *ortho*-alkynylbenzoates as donors and a gold(I) complex as catalyst, which proceeds under neutral conditions (Scheme 10).[72−74] Other acid-labile aglycones, such as the *N*-Boc protected purine derivatives and dammarane derivatives, have also been glycosylated effectively with this method.[75,76]

## Conclusion

Naturally occurring glycosides are extremely diverse in structures. Each type of the glycosides poses as a specific synthetic subject, in that approaches to the synthesis of the specific aglycone, the glycan, the connection of these two parts, and the overall protecting group strategy need to be implemented. Focusing on the glycosylation chemistry which connects the saccharide and the aglycone, we have pieced together our own experiences to provide herein an overview on this broad topic. Four tactical considerations for the synthesis of glycosides are suggested to facilitate the formation of the glycosidic linkages with the aglycones. Nevertheless, formation of the glycosidic linkages with those aglycones which are poorly nucleophilic or extremely labile to acid or electrophiles has demanded for innovative glycosylation devices. The challenges have provided us opportunities to develop two new glycosylation protocols. Thus, the glycosylation method with glycosyl trifluoroacetimidates as donors has shown advantageous for glycosylation of poorly nucleophilic aglycones, while the gold(I)-catalyzed glycosylation method with glycosyl *ortho*-alkynylbenzoates as donors is superior for glycosylation of aglycones extremely labile to acid or electrophiles.

Shown in the depicted examples but not yet discussed is the protecting group chemistry[77] which is also critically

important to the success of a glycoside synthesis. The protecting groups are employed not only to secure the chemo- and regioselectivity but also to secure the stereoselectivity (especially for the glycosidic bond formation) and to modulate the physical properties of the intermediates for easy handling and characterization. The overall protecting group arrangement in the synthesis of a multifunctional glycoside could be very subtle and complicated. In fact, shortage of a single protecting group often ruins the whole synthetic plan.

Although many glycosides have been successfully synthesized (our own examples discussed here represent only a very few of them), numerous others are still waiting for new chemistry to solve their synthetic challenges. The emerging demand for large-scale synthesis of glycosides which are found of industrial value calls for re-evaluation of the established glycosylation and protection–deprotection methods, which are mostly not suitable yet for practical synthesis. With these challenges ahead, the bittersweet journey toward the synthesis of the naturally occurring glycosides will continue.

*B.Y. is grateful to his co-workers whose names appear in the references for their invaluable contributions to this project, and the financial support from the National Natural Science Foundation of China, the Ministry of Science and Technology of China, the Chinese Academy of Sciences, and the E-Institute of Shanghai Municipal Education Commission (E09013).*

## BIOGRAPHICAL INFORMATION

**Biao Yu** received his B.Sc. in Radiochemistry from Peking University in 1989 and his Ph.D. (with Prof. Y. Hui) from Shanghai Institute of Organic Chemistry (SIOC), Chinese Academy of Sciences (CAS) in 1995. After a one-year postdoctoral stay in New York University, Dr. Yu returned to SIOC as an assistant professor, and became professor at the end of the last century. His laboratory is dedicated to the total synthesis, synthetic methodology, and chemical biology of glycoconjugates.

**Jiansong Sun** received his B. Sc. in Chemistry from Qufu Normal University in 2000 and his Ph.D. from Dalian Institute of Chemical Physics, CAS, in 2005 (with Prof. B. Yu and X. Han). He then joined Prof. Schmidt's group in Konstanz University as a Humboldt postdoctoral fellow. In 2007, he returned to the SIOC as an associate research professor.

**Xiaoyu Yang** received his B.Sc. in Chemistry from Nanjing University in 2007 and then joined SIOC as a graduate student (with Prof. B. Yu). He achieved the total synthesis of Landomycin A recently.

## FOOTNOTES

*To whom correspondence should be addressed. E-mail: byu@mail.sioc.ac.cn.
The authors declare no competing financial interest.

## REFERENCES

1 *Naturally Occurring Glycosides*; Ikan, R., Ed.; John Wiley & Sons Ltd.: Chichester, England, 1999.
2 Dembitsky, V. M. Chemistry and Biodiversity of the Biologically Active Natural Glycosides. *Chem. Biodiversity* **2004**, *1*, 673–781.
3 Blanchard, S.; Thorson, J. S. Enzymatic Tools for Engineering Natural Product Glycosylation. *Curr. Opin. Chem. Biol.* **2006**, *10*, 263–271.
4 Gantt, R. W.; Peltier-Pain, P.; Thorson, J. S. Enzymatic Methods for Glyco(diversification/randomization) of Drugs and Small Molecules. *Nat. Prod. Rep.* **2011**, *28*, 1811–1853.
5 Weymouth-Wilson, A. C. The Role of Carbohydrates in Biologically Active Natural Products. *Nat. Prod. Rep.* **1997**, *14*, 99–110.
6 Opdenakker, G.; Rudd, P. M.; Ponting, C. P.; Dwek, R. A. Concepts and Principles of Glycobiology. *FASEB J.* **1993**, *7*, 1330–1337.
7 *Saponins*; Hostettmann, K., Marston, A., Ed.; Cambridge University Press: Cambridge, UK, 1995.
8 Yu, B.; Zhang, Y.; Tang, P. Carbohydrate Chemistry in the Total Synthesis of Saponins. *Eur. J. Org. Chem.* **2007**, 5145–5161.
9 Yu, B.; Sun, J. Current Synthesis of Triterpene Saponins. *Chem. Asian J.* **2009**, *4*, 642–654.
10 Danishefsky, S. J.; Bilodeau, M. T. Glycals in Organic Synthesis: The Evolution of Comprehensive Strategies for the Assembly of Oligosaccharides and Glycoconjugates of Biological Consequence. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1380–1419.
11 Nicolaou, K. C.; Mitchell, H. J. Adventures in Carbohydrate Chemistry: New Synthetic Technologies, Chemical Synthesis, Molecular Design, and Chemical Biology. *Angew. Chem., Int. Ed.* **2001**, *40*, 1576–1624.
12 Toshima, K.; Tatsuta, K. Recent Progress in O-Glycosylation Methods and Its Application to Natural Products Synthesis. *Chem. Rev.* **1993**, *93*, 1503–1531.
13 Pellissier, H. Use of O-Glycosylation in Total Synthesis. *Tetrahedron* **2005**, *61*, 2947–2993.
14 Pereda-Miranda, R.; Mata, R.; Anaya, A. L.; Wickramaratne, D. B. M.; Pezzuto, J. M.; Kinghorn, A. D. Tricolorin A, Major Phytogrowth Inhibitor from *Ipomoea Tricolor*. *J. Nat. Prod.* **1993**, *56*, 571–582.
15 Lu, S.; Ouyang, Q.; Guo, Z.; Yu, B.; Hui, Y. The First Total Synthesis of Tricolorin A. *Angew. Chem.; Int. Engl. Ed.* **1997**, *36*, 2344–2346.
16 Lu, S.; Ouyang, Q.; Guo, Z.; Yu, B.; Hui, Y. Total Synthesis of Tricolorin A. *J. Org. Chem.* **1997**, *62*, 8400–8405.
17 Larson, D. P.; Heathcock, C. H. Total Synthesis of Tricolorin A. *J. Org. Chem.* **1997**, *62*, 8406–8418.
18 Fürstner, A.; Müller, T. Metathesis Route to Resin Glycosides: Formal Total Synthesis of Tricolorin A. *J. Org. Chem.* **1998**, *63*, 424–425.
19 Fürstner, A.; Müller, T. Efficient Total Syntheses of Resin Glycosides and Analogues by Ring-Closing Olefin Metathesis. *J. Am. Chem. Soc.* **1999**, *121*, 7814–7821.
20 Linington, R. G.; Robertson, M.; Gauthier, A.; Finlay, B. B.; van Soest, R.; Andersen, R. J. Caminoside A, An Antimicrobial Glycolipid Isolated from the Marine Sponge Caminus Sphaeroconia. *Org. Lett.* **2002**, *4*, 4089–4092.
21 Sun, J.; Han, X.; Yu, B. First Total Synthesis of Caminoside A, an Antimicrobial Glycolipid from Sponge. *Synlett* **2005**, 437–440.
22 Jacobsson, M.; Malmberg, J.; Ellervik, U. Aromatic O-Glycosylation. *Carbohydr. Res.* **2006**, *341*, 1266–1281.
23 Ko, H. R.; Kim, B. Y.; Oh, W. K.; Kang, D. O.; Lee, H. S.; Koshino, H.; Osada, H.; Mheen, T. I.; Ahn, J. S. CRM646-A and -B, Novel Fungal Metabolites that Inhibit Heparinase. *J. Antibiot.* **2000**, *53*, 211–214.
24 Wang, P.; Zhang, Z.; Yu, B. Total Synthesis of CRM646-A and B, Two Fungal Glucuronides with Potent Heparinase Inhibition Activities. *J. Org. Chem.* **2005**, *70*, 8884–8889.
25 *The Handbook of Natural Flavonoids*; Harborne, J. B., Baxter, H., Ed.; John Wiley & Sons: Chichester, 1999; Vol. 1 and 2.
26 Watanabe, Y.; Shiozaki, M.; Kamegai, R. Synthesis and Biological Activity of 4′,8-Dihydroxyisoflavon-7-yl D-Hexopyranosides. *Carbohydr. Res.* **2001**, *335*, 283–289.
27 Li, M.; Han, X.; Yu, B. Facile Synthesis of Flavonoid 7-O-Glycosides. *J. Org. Chem.* **2003**, *68*, 6842–6845.
28 Wei, G.; Yu, B. Isoflavone Glycosides: Synthesis and Evaluation as α-Glucosidase Inhibitors. *Eur. J. Org. Chem.* **2008**, 3156–3163.
29 Iwagawa, T.; Kawasaki, J. -I.; Hase, T.; Sako, S.; Okubo, T.; Ishida, M.; Kim, M. An Acylated Flavonol Glycoside from Lasiobema Japonica. *Phytochemistry* **1990**, *29*, 1013–1014.
30 Li, M.; Han, X.; Yu, B. Synthesis of Quercetin 3-O-(2″-Galloyl)-α-L-arabinopyranoside. *Tetrahedron Lett.* **2002**, *43*, 9467–9470.
31 Yang, W.; Sun, J.; Lu, W.; Li, Y.; Shan, L.; Han, W.; Zhang, W. -D.; Yu, B. Synthesis of Kaempferol 3-O-(3″,6″-di-O-E-p-Coumaroyl)-β-D-glucopyranoside, Efficient Glycosylation of Flavonol 3-OH with Glycosyl o-Alkynylbenzoates as Donors. *J. Org. Chem.* **2010**, *75*, 6879–6888.

32 Shi, S. -P.; Dong, C. -X.; Jiang, D.; Tu, P. -F. Macrocyclic Glycosides from Clematis Hexapetala. *Helv. Chim. Acta* **2006**, *89*, 3002–3006.

33 Li, Y.; Sun, J.; Gong, Y.; Yu, B. Synthesis of Oligomeric 4-(Glycosyloxy)benzoate Macrocyclic Glycosides. *J. Org. Chem.* **2011**, *76*, 3654–3663.

34 Kitanaka, S.; Nakayama, T.; Shibano, T.; Ohkoshi, E.; Takido, M. Antiallergic Agent from Natural Sources. Structures and Inhibitory Effect of Histamine Release of Naphthopyrone Glycosides from Seeds of Cassia Obtusifolia L. *Chem. Pharm. Bull.* **1998**, *46*, 1650–1652.

35 Zhang, Z.; Yu, B. Total Synthesis of the Antiallergic Naphtho-α-pyrone Tetraglucoside, Cassiaside C2, Isolated from Cassia Seeds. *J. Org. Chem.* **2003**, *68*, 6309–6313.

36 Weber, S.; Zolke, C.; Rohr, J.; Beale, J. M. Investigations of the Biosynthesis and Structural Revision of Landomycin A. *J. Org. Chem.* **1994**, *59*, 4211–4214.

37 Yang, X.; Fu, B.; Yu, B. Total Synthesis of Landomycin A, a Potent Antitumor Angucycline Antibiotic. *J. Am. Chem. Soc.* **2011**, *133*, 12433–12435.

38 Ma, J. C. N.; Lau, F. W. Structure Characterization of Haemostatic Diosgenin Glycosides from Paris Polyphylia. *Phytochemistry* **1985**, *24*, 1561–1565.

39 Deng, S.; Yu, B.; Hui, Y. A Facile Synthetic Approach to a Group of Structurally Typical Diosgenyl Saponins. *Tetrahedron Lett.* **1998**, *39*, 6511–6514.

40 Kawasaki, T.; Komori, T.; Miyahara, K.; Nohara, T.; Hosokawa, I.; Mihashi, K. Furostanol Bisglycosides Corresponding to Dioscin and Gracillin. *Chem. Pharm. Bull.* **1974**, *22*, 2164–2175.

41 Li, M.; Yu, B. Facile Conversion of Spirostan Saponin into Furostan Saponin: Synthesis of Methyl Protodioscin and Its 26-Thio-analogue. *Org. Lett.* **2006**, *8*, 2679–2682.

42 Mimaki, Y.; Kuroda, M.; Kameyama, A.; Sashida, Y.; Hirano, T.; Oka, K.; Maekawa, R.; Wada, T.; Sugita, K.; Beutler, J. A. Cholestane Glycosides with Potent Cytostatic Activities on Various Tumor Cells from Ornithogalum Saundersiae Bulbs. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 633–636.

43 Mimaki, Y.; Kuroda, M.; Sashida, Y.; Yamori, T.; Tsuruo, T. Candicanoside A, a Novel Cytotoxic Rearranged Cholestane Glycoside from Galtonia Candicans. *Helv. Chim. Acta* **2000**, *83*, 2698–2704.

44 Deng, S.; Yu, B.; Lou, Y.; Hui, Y. First Total Synthesis of an Exceptionally Potent Antitumor Saponin, OSW-1. *J. Org. Chem.* **1999**, *64*, 202–208.

45 Shi, B.; Tang, P.; Hu, X.; Liu, J. O.; Yu, B. OSW Saponins: Facile Synthesis Toward a New Type of Structures with Potent Antitumor Activities. *J. Org. Chem.* **2005**, *70*, 10354–10367.

46 Tang, P.; Yu, B. Total Synthesis of Candicanoside A, a Potent Antitumor Saponin with a Rearranged Steroid Side Chain. *Angew. Chem., Int. Ed.* **2007**, *46*, 2527–2530.

47 Wiesner, K.; Tsai, T. Y. R.; Jin, H. On Cardioactive Steroids. XVI. Stereoselective β-Glycosylation of Digitoxose: The Synthesis of Digitoxin. *Helv. Chim. Acta* **1985**, *68*, 300–314.

48 McDonald, F. E.; Reddy, K. S. Convergent Synthesis of Digitoxin: Stereoselective Synthesis and Glycosylation of the Digoxin Trisaccharide Glycal. *Angew. Chem., Int. Ed.* **2001**, *40*, 3653–3655.

49 Zhou, M.; O'Doherty, G. A. A Stereoselective Synthesis of Digitoxin and Digitoxigen Mono and Bisdigitoxoside from Digitoxigenin via a Palladium-Catalyzed Glycosylation. *Org. Lett.* **2006**, *8*, 4339–4342.

50 Ma, Y.; Li, Z.; Shi, H.; Zhang, J.; Yu, B. Assembly of Digitoxin by Gold(I)-catalyzed Glycosidation of Glycosyl o-Alkynylbenzoates. *J. Org. Chem.* **2011**, *76*, 9748–9756.

51 Shao, C. -J.; Kasai, R.; Xu, J. -D.; Tanaka, O. Saponins from Leaves of Acanthopanax Senticosus HARMS., Ciwujia: Structures of Ciwujianosides B, $C_1$, $C_2$, $C_3$, $C_4$, $D_1$, $D_2$ and E. *Chem. Pharm. Bull.* **1988**, *36*, 601–608.

52 Yu, B.; Xie, J.; Deng, S.; Hui, Y. First Synthesis of a Bidesmosidic Triterpene Saponin by a Highly Efficient Procedure. *J. Am. Chem. Soc.* **1999**, *121*, 12196–12197.

53 Yoshikawa, M.; Murakami, T.; Kadoya, M.; Matsuda, H.; Yamahara, J.; Muraoka, O.; Murakami, N. Betavulgarosides I, II, III, IV, and V, Hypoglycemic Glucuronide Saponins from the Roots and Leaves of Beta Vulgaris L. (Sugar Beet). *Heterocycles* **1995**, *41*, 1621–1626.

54 Zhu, S.; Li, Y.; Yu, B. Synthesis of Betavulgaroside III, a Representative Triterpene seco-Glycoside. *J. Org. Chem.* **2008**, *73*, 4978–4985.

55 Li, X.-C.; Yang, C.-R.; Liu, Y.-Q.; Kasai, R.; Ohtani, K.; Yamasaki, K.; Miyahara, K.; Shingu, K. Triterpenoid Glycosides from Anemoclema Glaucifolium. *Phytochemistry* **1995**, *39*, 1175–1179.

56 Sun, J.; Han, X.; Yu, B. Synthesis of Anemoclemoside B, the First Natural Product with an Open-chain Cyclic Acetal Glycosidic Linkage. *Org. Lett.* **2005**, *7*, 1935–1938.

57 Fujioka, T.; Iwamoto, M.; Iwase, Y.; Hachiyama, S.; Okabe, H.; Yamauchi, T.; Mihashi, K. Studies on the Constituents of Actinostemma Lobatum MAXIM. V.: Structures of Lobatosides B, E, F and G, the Dicrotalic Acid Esters of Bayogenin Bisdesmosides Isolated from the Herb. *Chem. Pharm. Bull.* **1989**, *37*, 2355–2360.

58 Zhu, C.; Tang, P.; Yu, B. Total Synthesis of Lobatoside E, a Potent Antitumor Cyclic Triterpene Saponin. *J. Am. Chem. Soc.* **2008**, *130*, 5872–5873.

59 Tapondjou, A. L.; Miyamoto, T.; Lacaille-Dubois, M. -A. Glucuronide Triterpene Saponins from *Bersama Engleriana*. *Phytochemistry* **2006**, *67*, 2126–2132.

60 Li, Y.; Sun, J.; Yu, B. Efficient Synthesis of Lupane-type Saponins via Gold(I)-Catalyzed Glycosylation with Glycosyl ortho-Alkynylbenzoates as Donors. *Org. Lett.* **2011**, *13*, 5508–5511.

61 Liu, M.; Yu, B.; Hui, Y. First Total Synthesis of 25(R)-Ruscogenin-1-yl β-D-Xylopyranosyl-(1→3)-[β-D-glucopyranosyl-(1→2)]-β-D-fucopyranoside, an Ophiopogonis Saponin from the Tuber of *Liriope Muscari* (Decne.). *Tetrahedron Lett.* **1998**, *39*, 415–418.

62 Li, M.; Han, X.; Yu, B. Synthesis of Monomethylated Dioscin Derivatives and Their Antitumor Activities. *Carbohydr. Res.* **2003**, *338*, 117–121.

63 Wang, Y.; Zhang, Y.; Zhu, Z.; Zhu, S.; Li, Y.; Li, M.; Yu, B. Exploration of the Correlation Between the Structure, Hemolytic Activity, and Cytotoxicity of Steroid Saponins. *Bioorg. Med. Chem.* **2007**, *15*, 2528–2532.

64 Yu, H.; Yu, B.; Wu, X.; Hui, Y.; Han, X. Synthesis of a Group of Diosgenyl Saponins with a Combined Use of Glycosyl Trichloroacetimidate and Thioglycoside Donors. *J. Chem. Soc., Perkin Trans. 1* **2000**, 1445–1453.

65 Kanie, O.; Barresi, F.; Ding, Y.; Labbe, J.; Otter, A; Forsberg, L. S.; Ernst, B.; Hindsgaul, O. A Strategy of `Random Glycosylation' for the Production of Oligosaccharide Libraries. *Angew. Chem., Int. Ed. Engl.* **1996**, *34*, 2720–2722.

66 Yu, B.; Li, B.; Xing, G.; Hui, Y. A Double Random Strategy for the Preparation of Saponin Libraries. *J. Comb. Chem.* **2001**, *3*, 404–406.

67 Li, M. Ph.D. Thesis, Dalian Institute of Chemical Physics, 2003.

68 Schmidt, R. R. New Methods for the Synthesis of Glycosides and Oligosaccharides: Are There Alternatives to the Koenigs-Knorr Method? *Angew. Chem., Int. Ed. Engl.* **1986**, *25*, 212–235.

69 Yu, B.; Tao, H. Glycosyl Trifluoroacetimidates. Part I. Preparation and Application as New Glycosyl Donors. *Tetrahedron Lett.* **2001**, *42*, 2405–2407.

70 Yu, B.; Sun, J. Glycosylation with Glycosyl N-Phenyltrifluoroacetimidates (PTFAI) and a Perspective of the Future Development of New Glycosylation Methods. *Chem. Commun.* **2010**, *46*, 4668–4679.

71 Gauthier, C.; Legault, J.; Lavoie, S.; Rondeau, S.; Tremblay, S.; Pichette, A. Synthesis and Cytotoxicity of Bidesmosidic Betulin and Betulinic Acid Saponins. *J. Nat. Prod.* **2009**, *72*, 72–81.

72 Li, Y.; Yang, Y.; Yu, B. An Efficient Glycosylation Protocol with Glycosyl ortho-Alkynylbenzoates as Donors under the Catalysis of $Ph_3PAuOTf$. *Tetrahedron Lett.* **2008**, *49*, 3604–3608.

73 Li, Y.; Yang, X.; Liu, Y.; Zhu, C.; Yang, Y.; Yu, B. Gold(I)-Catalyzed Glycosylation with Glycosyl ortho-Alkynylbenzoates as Donors: General Scope and Application in the Synthesis of a Cyclic Triterpene Saponin. *Chem.—Eur. J.* **2010**, *16*, 1871–1882.

74 Zhu, Y.; Yu, B. Characterization of the Isochromen-4-yl-gold(I) Intermediate in the Gold(I)-Catalyzed Glycosidation of Glycosyl ortho-Alkynylbenzoates and Enhancement of the Catalytic Efficiency Thereof. *Angew. Chem., Int. Ed.* **2011**, *50*, 8329–8332.

75 Zhang, Q.; Sun, J.; Zhu, Y.; Zhang, F.; Yu, B. An Efficient Approach to the Synthesis of Nucleosides: Gold(I)-Catalyzed N-Glycosylation of Pyrimidines and Purines with Glycosyl ortho-Alkynylbenzoates. *Angew. Chem., Int. Ed.* **2011**, *50*, 4933–4936.

76 Liao, J.; Sun, J.; Niu, Y.; Yu, B. Synthesis of Ginsenoside Rh2 and Chikusetsusaponin-LT8 via Gold(I)-Catalyzed Glycosylation with a Glycosyl ortho-Alkynylbenzoate as Donor. *Tetrahedron Lett.* **2011**, *52*, 3075–3078.

77 *Protecting Groups*; Kocieński, P. J., Ed.; Georg Thieme Verlag: Stuttgart, 2005.