A Strain-Promoted Divergent Chemical Steroidation Unveils Potent Anti-Inflammatory *Pseudo*-Steroidal Glycosides

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ABSTRACT: The development of novel agents with immunoregulatory effects is keen to combat the growing threat of inflammatory storms to global health. To synthesize *pseudo*-steroidal glycosides tethered by ether bonds with promising immunomodulatory potential, we develop herein a highly effective deoxygenative functionalization of a novel steroidal donor (steroidation) facilitated by strain-release, leveraging cost-effective and readily available $Sc(OTf)_3$ catalysis. This transformation produces transient steroid-3-yl carbocation which readily reacts with *O*-, *C*-, *N*-, *S*-, and *P*-nucleophiles to generate structurally diverse steroid derivatives. DFT calculations were performed to shed light on the mechanistic details of the regioselectivity, underlying an acceptor-dependent steroidation mode. This approach can be readily extended to the etherification of sugar alcohols to enable the achievement of a diversity-oriented, pipeline-like synthesis of *pseudo*-steroidal glycosides in good-to-excellent yields with complete stereo- and regiospecific control for anti-inflammatory agent discovery. Immunological studies have demonstrated that a meticulously designed cholesteryl disaccharide can significantly suppress the interleukin-6 (IL-6) secretion in macrophages, exhibiting up to 99%-inhibition rates compared to the negative control. These findings affirm the potential of *pseudo*-steroidal glycosides as a prospective category of lead agents for the development of novel anti-inflammatory drugs.

INTRODUCTION

Bacterial infections have consistently been emphasized as a leading threat to global public health.[1] The persistent bacterial infections always induce overactive immune responses, leading to acute organ damage and potentially exacerbating to severe diseases like gastric cancer,[2] heart failure,[3] and chronic kidney disease.[4] The utility of anti-inflammation drugs is vital in modulating bacterial infection-caused inflammation dysregulation and preventing the progression of irreversible organ impairments. Currently, the most prevalent anti-inflammatory drugs are non-steroidal anti-inflammatory drugs (NSAIDs), their efficacy is, however, usually accompanied by serious adverse effects such as gastrointestinal bleeding, cardiovascular issues, and nephrotoxicity.[5] Consequently, there is a significant demand for the development of innovative and effective lead agents with anti-inflammatory properties. Widely occurring in living organisms, steroids mediate diverse physiological processes of growth, development, energy metabolism, homeostasis, and reproduction.[6] Of particular note are their abilities as immunoregulators, which are translated into another category of anti-inflammatory drugs, i.e. the steroidal anti-inflammatory drugs (SAIDs).[7] Nevertheless, their common side effects press requirements for developing new and safer steroidal anti-inflammatory medications.[8] Recent investigations demonstrated

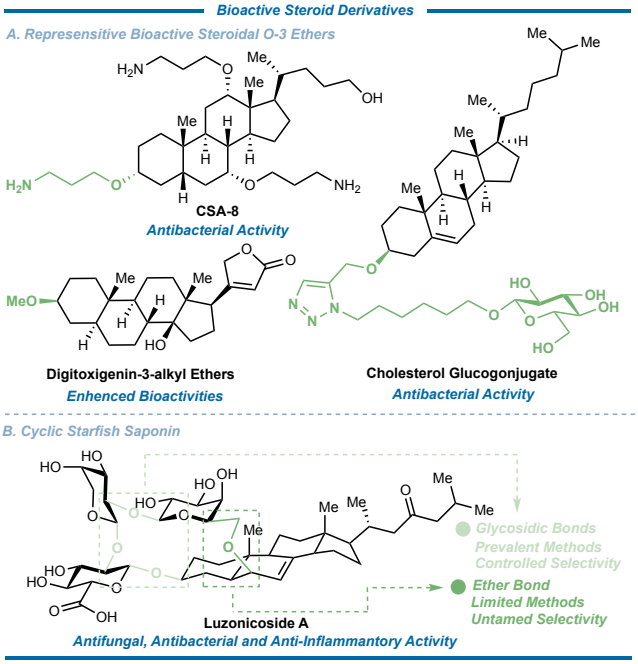

Figure 1. (A, B) Bioactive steroid derivatives.

conjugating steroids to other bioactive molecules generates essential chimeric steroid adducts with modulated biological behaviors. Among these steroidal chimeras, *C*-3 functionalized steroids are important steroidal derivatives associated with versatile antitumor, antibacterial and antifungal bioactivities (Figure 1A).[9] Moreover, naturally occurring starfish steroidal cyclic glycosides such as luzonicoside A exhibit various antifungal, antibacterial, and anti-inflammatory functions (Figure 1B).[10] The essential characteristic of these compounds is that the aglycon is linked with one sugar unit by an ether bond instead of the common acid- and enzyme-sensitive glycosidic linkage. Their unique structures therefore attracted our attention to envision directly installing sugars on *C*-3 of steroids by etherification to generate a novel category of artificial *pseudo*-steroidal glycosides that can not only mimic the immunoregulatory functions of their natural counterparts but also exhibit stability against metabolism.

Despite the advances in the biological and medicinal investigations on steroid alkyl ethers, direct translation of these encouraging results into novel anti-inflammatory lead compounds remains sluggish, mainly hampered by the difficulties in procuring these molecules with a dearth of methods for efficient etherification of steroids. Although simple alkyl groups can be incorporated into steroids employing acid- or base-mediated ether synthesis,[11] attempts to introduce densely functionalized alkyl groups like bulky or fluorinated alkyl groups, especially sugar moieties with diversified protection patterns to steroids under various basic conditions consistently gave poor outcomes (Figure 2A).[12] The regioselective modification of steroidal glycosides therefore becomes an important protocol to acquire steroidal architectures with well-defined structures for investigation of their vital biological funtions.[13] Yu's group recently conquered the odyssey towards attaining starfish cyclic steroid glycosides by successfully developing a *de novo* synthesis to generate the sugar-steroid moiety tethered by an ether bond from steroid-6-yl allylic ether,[14] attesting to the difficulty of the direct etherification of sugar alcohols with steroidal architectures. Another strategy for the etherification of sugar molecules and steroids involves the activation of steroidal donors equipped with appropriate leaving groups, i.e., a steroidation process akin to a chemical glycosylation reaction (Figure 2B). In this context, Morzycki pioneered the protocol by employing cholesteryl trichloroacetimidate, phenyl thioether, dimethyl phosphite, and diphenyl phosphate as steroidal donors to achieve the electroetherification with limited acceptors in low-to-moderate yields due to undesired hydrolysis and aglycon transfer side reactions.[15] Meanwhile, the cyclosteroid rearrangement (*i*-steroid rearrangement)[16] resulting from the mesomerically stabilized homoallylic carbocation leads to unbiased *C*-3 and *C*-6 etherification products with poor regioselectivity. Another example of steroidation of sugar alcohols was reported by Li's group using diosgenin-3-yl *ortho*-alkynylbenzoate,[17] a derivative of Yu's glycosyl *ortho*-alkynylbenzoates,[18] under Au(I)-catalysis with special tetrakis(pentafluorophenyl)borate counteranion in refluxed $PhCF_3$, exhibiting a broad acceptor scope of sugar alcohols, albeit the *i*-steroid rearrangement still dominated some examples, causing unpleasant regioselectivity.

To effectively advance toward our objective of synthesizing *pseudo*-steroidal glycosides and other *C*-3 steroid derivatives, several key issues should be keenly addressed: (1) Overcoming the conventional shared challenge of *i*-steroid rearrangement in steroid donor despite the various types of *O*-centered acceptors;

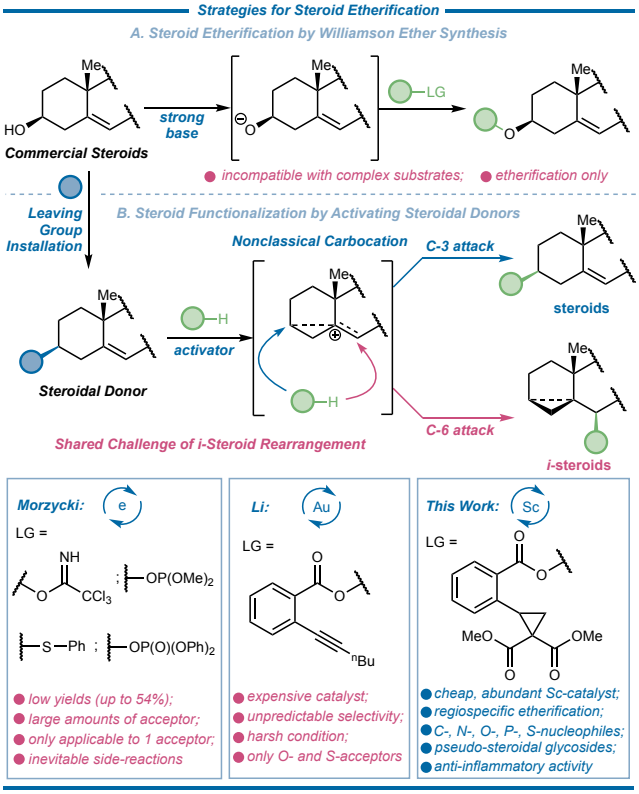

Figure 2. (A, B) Established strategies for chemical steroid etherification.

(2) Ensuring the versatility of steroidation with diverse donors and acceptors to achieve diversity-oriented steroid chemical modification; (3) Realizing the target-oriented synthesis of *pseudo*-steroidal glycosides for further biological investigations. With these in mind, we herein disclose our development of a strain-triggered steroidation with steroid-3-yl *ortho*-2,2-dimethoxycarbonylcyclopropylbenzoate (CCBz) donors employing low-cost and readily accessible Sc(III) catalyst under mild conditions.[19] Compared to all previous works, our protocol enjoys a substantially broader substrate scope of *O*-, *C*-, *N*-, *S*-, and *P*-nucleophiles and cholest-5-en-3-yl, diosgen-3-yl and pregnant-5-en-3-yl CCBz donors, producing desired steroid derivatives or *pseudo*-steroidal glycosides in good-to-excellent yields. Of note, our strain-release etherification of steroidal donors has perfect regiospecificity for all types of *O*-nucleophiles, generating *C*-3 products as the sole products. With DFT calculation, we successfully established criteria to predict regioselectivity for heteroatomic nucleophiles. A few *pseudo*-steroidal glycosides synthesized by the method were selected for further deprotection and immunological studies and results indicated that their immunoregulatory activities are on par with or even superior to naturally conformed steroidal glycosides. A rationally designed cholesteryl disaccharide stood out as the optimal candidate by inhibiting 99% of the IL-6 secretion induced by lipopolysaccharides (LPS), highlighting the promising potential applications of the strain-release steroidation for synthesizing *pseudo*-steroidal glycosides as anti-inflammatory therapeutics.

RESULTS AND DISCUSSION

Our studies commenced with the coupling reaction between the cholesterol and *ortho*-2,2-dimethoxycarbonylcyclopropylbenzoic acid (CCBzOH) in the presence of *N*,*N*'-dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP) to investigate the steroidation reactions with cholesteryl donor

because of various activities of the oxidized products of cholesterol.[20] The desired donor **1** could be prepared in good yield on a multigram scale in a single batch (For experimental details, see the SI). To test the viability of our proposal, we first conducted the coupling reaction between 1.0 equiv of donor **1** and 1.2 equiv of serine derivative **2a** in refluxed $PhCF_3$[17] in the presence of 0.1 equiv of $Sc(OTf)_3$ and 5 Å molecular sieve, and we were delighted to find that the reaction could complete in 10 minutes to deliver the desired ether **3a** in 81% yield as the sole isomer. By decreasing the temperature to room temperature, the steroidation reaction could proceed smoothly to generate **3a** in the excellent yield of 85% within 1 h, showing the exceptional reactivity of the steroidal donor **1** compared to the previous steroidal donors. Thus, this condition was selected for the study of C-O bond activation of steroids without further optimization.

Next, the acceptor scope was investigated by coupling **1** with various coupling partners ranging in *O*-, *C*-, *N*-, *S*-, and *P*-nucleophiles to give *C*-3 decorated 5-cholestenes or (3α,5α)-cyclocholestanes with synthetically satisfying yields (Figure 3). We first choose 5 typical aliphatic alcohols that are difficult to form ether bonds under basic conditions to test the mildness and regioselectivity of our strain-release steroidation. To be specific, the primary, secondary, and tertiary aliphatic alcohols **2a-e** regiospecifically formed the ether bonds at *C*-3 of the cholesterol backbone. The structure of compound **3b** is symmetric, as evidenced by this compound's single set of peaks in both ^1H and ^{13}C NMR spectra, which demonstrated that this etherification reaction is equatorial-specific (For a detailed discussion on the reaction pathway, see SI). These ethers are difficult to synthesize by base-mediated etherification due to the presence of base-sensitive functional groups like fluorenylmethyloxycarbonyl (Fmoc) group, the fixed stereocenter of cholesterol **2b,** and the bulky nature of 1-adamantanol **2c** and *tert*-butanol **2d**. Of particular note, the previous synthesis of 2,2,2-trifluoroethylcholestryl ether entailed a 2,2,2-trifluorodiazoethane with a potential hazard.[21] The synthesis of **3e** in 93% suggested that the etherification with 2,2,2-trifluoroethanol with very poor nucleophilicity took place exclusively at *C*-3 with high efficiency. The excellent yields and regiospecificities of **3c-e** demonstrated that acceptors' stereo-hindrance has little impact on both reaction efficiency and regioselectivity of the steroidation reaction. As another type of *O*-nucleophile, both electro-rich and electro-deficient phenols are competent acceptors to give the deoxygenative phenolated products **3f** and **3g** in almost quantitative yields, respectively. To our fulfillment, as a fluorescent probe with antibacterial activity,[22] the 4-methylumbelliferone could be coupled with **1** to afford cholesteryl 4-methylumbelliferone **3h** in 83% in CH_2Cl_2 due to the poor solubility of the acceptor in $PhCF_3$, realizing the efficient fluorescent labeling of cholesterol. The reaction was also applicable to the catalytic transesterification with **2i** under mild acidic conditions with high yield. Dibutyl hydrogen phosphate **2j** is a poor nucleophile due to the electro-withdrawing effect of the P=O double bond and dibutyloxy group while the phosphorylation of **1** was high-yielding without *C*-6 phosphorylation occurred. The phosphorylation of steroids is of great importance not only because many steroidal drugs have phosphate acid moiety, but the special property of this amphiphilic molecule mimicking phospholipid. Since the conventional method using steroidal phosphorochloridate to synthesize cholesteryl dihydrogen phosphate failed to give the desired product, our protocol thus offers a potential alternative.[23] With the success in the *O*-functionalization, we subsequently exposed other nucleophiles to the C-O bond activation reaction. The

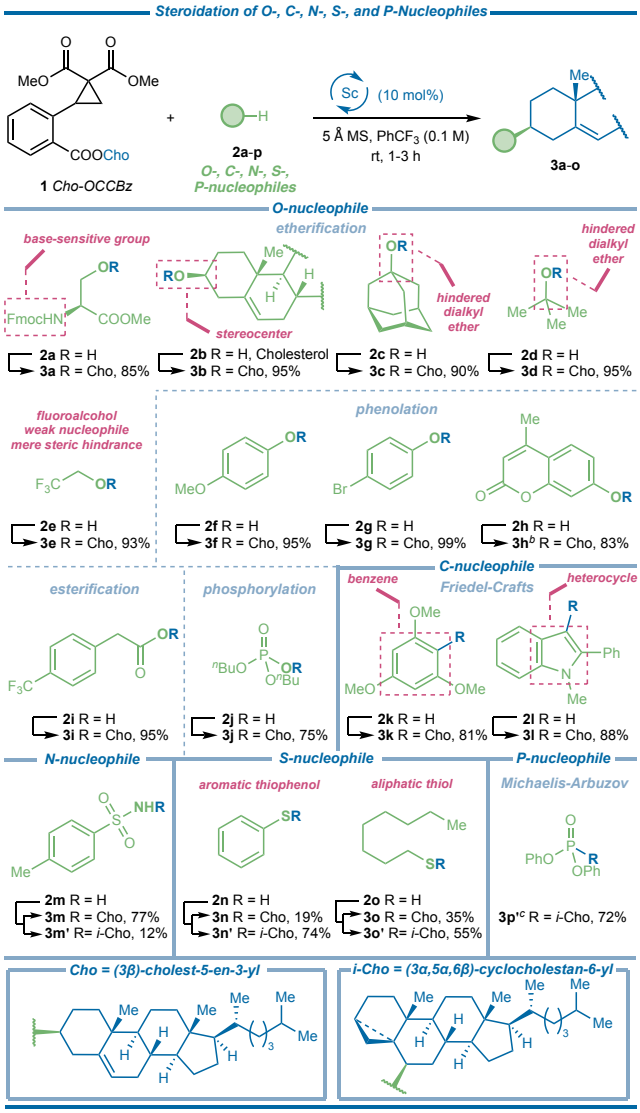

Figure 3. Acceptor scope with cholesteryl donor **1**. [a]Unless otherwise noted, all reactions were conducted on 0.1 mmol scale with respect to **1** (1.0 equiv) in the presence of **2** (1.2 equiv) and $Sc(OTf)_3$ (0.1 equiv) in $PhCF_3$ (1.0 mL) at room temperature for 1-3 h; [b]CH_2Cl_2 as the solvent; [c]1.0 equiv of $Sc(OTf)_3$ with triphenyl phosphite **2p** as the nucleophile.

Friedel-Crafts type arylation with 1,3,5-trimethoxybenzene **2k** and *N*-methyl-2-phenylindole **2l** regio- and stereospecifically generated the *C*-steroids in good yields, generating a new genre of steroidal derivatives with arene and heteroarene acceptors, respectively.

It is irrefutable that there are still some drawbacks in our current reaction. The sulfonyl amide **2m** generated the desired *C*-3 elaborated **3m** as the major product while subjecting aromatic thiophenol **2n** and aliphatic thiol **2o** reverted the regioselectivity to form *C*-6 products **3n'-o'** (*i*-Cho products) as the main isomers. The Michaelis-Arbuzov type coupling required one equiv of $Sc(OTf)_3$ to give the C-P coupling product **3p'** in a good yield of 72% albeit the reaction took place at *C*-6 exclusively. Thus, from our preliminary results included in Figure 3, we could conclude that in our hand, the strain-release steroidation with donor **1** could invariably give specific *C*-3 products with all *O*-, and *C*-nucleophiles, while *P*-nucleophile **2p** tended to generate *C*-6 *i*-steroid-conformed **3p'**. Interestingly, the *N*-nucleophile **2m**

Table 1. Calculated properties of the model nucleophiles.

Nucleophiles	E_{HOMO}[a] (eV)	f^-[b] (e)	N^-_{local}[c] (eV)	N^-_{global}[d] (eV)	NBO[e] (e)	η_{global}[f] (eV)	s^{-}[g] (e)
2f *O*-nucleophile	−8.01	0.126	0.361	2.705	−0.693	5.740	0.598
2m *N*-nucleophile	−6.90	0.174	0.519	2.687	−1.088	5.847	0.252
2n *S*-nucleophile	−6.63	0.193	0.603	2.864	−0.085	6.420	1.129
2p *P*-nucleophile	−6.49	0.266	0.631	2.266	1.612	5.575	0.427

[a]Energy of the highest occupied molecular orbital (HOMO); [b]Electrophilic Fukui function (f^-) of the nucleophilic center from CDFT; [c]Condensed local nucleophilicity index of the nucleophilic center from CDFT; [d]Overall nucleophilicity of the molecule from CDFT; [e]Natural Bonding Orbital atomic charge of the nucleophilic center; [f]Overall hardness of the molecule from CDFT; [g]Condensed local softness index of the nucleophilic center from CDFT.

and *S*-nucleophiles **2n-o** competitively led to the functionalization at *C*-3 and *C*-6 albeit *N*-nucleophile **2m** preferred the *C*-3 attack, and *S*-nucleophiles **2n-o** preferred the *C*-6 attack. The intriguing regioselectivity is presumably attributed to the complex characteristics of the nucleophiles associated with the hard-soft-acid-base (HSAB) concept, nucleophilicity, and/or match and mismatch property (*vide infra*), which could be analogously incorporated into the "acceptor-controlled glycosylation", terming acceptor-controlled steroidation.[24]

We hypothesized that the regioselectivity arises from the differentiated electrophilicity of *C*-3 and *C*-6 on the putative steroidal carbocation and the matching between the electrophilicity and/or hardness with the nucleophilicities and/or hardness of the nucleophiles. To rationalize the mechanistic details leading to the acceptor-dependent regioselectivity of the steroidation, we performed geometry optimization and frequency analysis on the steroidal carbocation, model nucleophiles phenol **2f**, benzenesulfonamide **2m**, thiophenol **2n**, and triphenyl phosphite **2p** at B3LYP/def2-TZVP/SMD (solvent = CH_2Cl_2) level of theory with Grimme's empirical dispersion D3(BJ) applied,[25] and conceptual DFT (CDFT)[26] calculations of the optimized structures at the same level of theory, using the Gaussian16 software[27] to compute the molecular and CDFT properties related to nucleophilicities. The energies of the highest occupied molecular orbitals (E_{HOMO}) and CDFT-calculated molecular nucleophilicities N_{global}, molecular hardness, as well as the natural bonding orbital (NBO) atomic charges, CDFT-calculated nucleophilic and electrophilic Fukui function (f^+ and f^-), local nucleophilicity indices N^-_{local} and local softness indices s^- of the nucleophilic center atoms were listed in Table 1. Calculated results in HOMO energies, Fukui function, and local nucleophilicity indices converged to suggest that the nucleophilicity of the model nucleophiles increases in the order of **2f** < **2m** < **2n** < **2p**, dominated by the electronegativity properties of the nucleophilic center atoms. Notably, these results indicated that the atomic charges and global nucleophilicity index may not accurately reflect the experimentally observed nucleophilicity of the nucleophiles. Moreover, the hardness and softness of the nucleophiles are not closely correlated to the calculated nucleophilicities.

Meanwhile, when the putative cholesteryl cation was subjected to geometry optimization as an isolated cation, an inevitable hydride migration from *C*-4 to *C*-3 occurred. In the resulting most stable ground state structure of the isolated cation (Figure 4, **I**), sp^2-hybridized *C*-4-*C*-5-*C*-6 forms a conjugated structure, with the partially positively charged electrophilic centers being *C*-4 and *C*-6, instead of the sp^3-hybridized *C*-3. When we performed geometry optimizations starting from a distorted 6-membered ring with *C*-3 below the approximate plane of the fused rings, we were able to identify a conformation-distorted cholesteryl cation structure with sp^2-hybridized *C*-3 and *C*-6 featuring the electrophilic centers (Figure 4, **II**). In this distorted structure, calculated f^+ Fukui function and local electrophilicity index of the atoms indicated that *C*-6 is more electrophilic compared to *C*-3. (f^+: *C*-6 = 0.174 e; *C*-3 = 0.111 e; electrophilicity index: *C*-6 = 0.949 eV, *C*-3 = 0.605 eV). On the other hand, geometry optimization of the cholesteryl cation structure together with the anionic LG (ring-opened CCBz, with the distance of *C*-3-O fixed at 3.0 Å to prevent collapse back to covalent bonding) staying close to the cation resulted in a non-distorted quasi-planar cholesteryl cation structure with sp^2-hybridized *C*-3 and *C*-6 (Figure 4, **III**). In this optimized structure *C*-3 is much more electrophilic compared to *C*-6 (f^+: *C*-6 = 0.061 e; *C*-3 = 0.236 e; electrophilicity index: *C*-6 = 0.547 eV, *C*-3 = 2.125 eV; see Figure 4, **IV** for the isosurface of f^+ function distribution on the cation), which concurs with the chemical intuition and experimental outcomes. We also calculated the relative energies of the structures **I-III** and found that in the absence of the anionic LG, **III** has the highest energy (ΔG_{III} = +24.9 kcal/mol vs ΔG_I = 0.0 kcal/mol) and **II** is also more energetic than **I** (ΔG_{II} = +10.3 kcal/mol). While with the stabilization of the anionic LG, the relative energy of **III** is significantly reduced ($\Delta G_{III}'$ = −5.4 kcal/mol vs $\Delta G_{II}'$ = +10.3 kcal/mol vs $\Delta G_I'$ = 0.0 kcal/mol), making **III** with anionic LG become the intermediate with lowest relative energy. These results demonstrated the important role of the close-contacting CCBz leaving group. Comparing these structures and results, we reasoned that the cholesteryl cation with close LG would be the most plausible reacting intermediates in the reaction system. Weak nucleophiles (alcohols/phenols) only react with the strongly electrophilic *C*-3 on the cholesteryl cation ring to give the *C*-3-ether products, while significantly stronger nucleophiles like **2m**, **2n,** and **2p** can react with the spatially more accessible but less

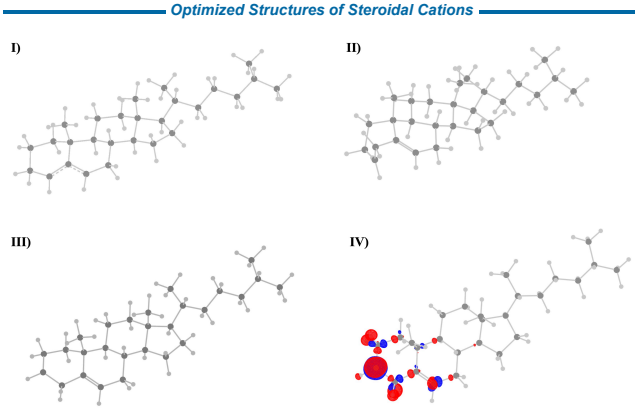

Figure 4. Three optimized structures of cholesteryl cations (**I-III**) and the nucleophilic Fukui function f^+ isosurface (**IV** = 0.018) of the most plausible structure.

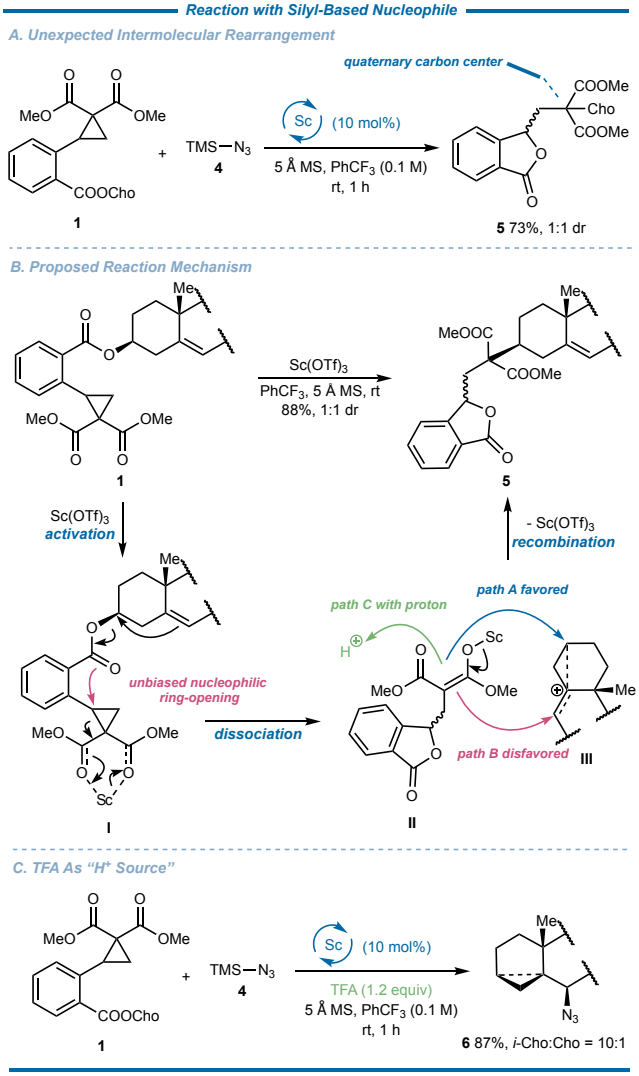

Figure 5. Reaction with silyl-based nucleophile **4**.

electrophilic *C*-6, producing *i*-Cho products. These results also suggested that the leaving group significantly contributed to the cholesteryl cation for excellent regiospecificity with *O*-nucleophiles. With this modeling, we, for the first time, achieve the quantification of the acceptor-controlled functionalization mode of steroidal cations and heteroatomic acceptors, supplying a theoretical guideline for designing novel steroidal donors and predicting the reaction results.

Silyl nucleophiles are an important class of nucleophiles with generally stronger nucleophilicity. Interestingly, when we tried to use trimethylsilyl azide **4** as the coupling partner to synthesize azido-5-cholestene, we did not obtain the desired product. To our surprise, an unexpected intermolecular rearrangement occurred to give *C*-steroid **5** as the major product in 73% yield (Figure 5A). This phenomenon might probably be rooted in the turnover dilemma of the catalyst in the absence of acidic hydrogen. To release the catalyst for the next catalytic cycle, the postulated enolate intermediate **II** had to back-attack the homoallylic carbocation **III** to form the intermolecularly rearranged **5**. Due to the poor nucleophilicity of the *C*-centered nucleophiles, the enolate preferred to attack the cholesteryl cation **III** at strongly electrophilic *C*-3. To test our hypothesis, the reaction was conducted in the absence of acceptor. As anticipated, **5** was formed in an excellent yield of 88% (Figure 5B). It is worth noting that this rearrangement generated an all-carbon quaternary carbon center which is difficult to construct.[28] The resulting 1,3-diester can readily undergo the enantioselective desymmetrization reactions to build the challenging all-carbon quaternary stereocenter.[29] Combing the highly regioselective coupling reaction of *C*-nucleophiles in our hand, the rich chemistry of 1,3-dicarbonyl compounds as well as the varied structures of steroids in nature, this serendipity should find its potential utility in the synthesis of complex steroids of biological and medicinal relevance. Of note, in the cases involving proton-releasable acceptors, no intermolecular rearrangement was observed, demonstrating that the nucleophilic attack by suitable acceptors still dominated the reaction. Upon scrutinizing the reaction process, we postulated that the intermolecular rearrangement could potentially be intercepted by an exogenous proton (reaction pathway C). The chosen proton source must be capable of releasing the proton, and the counteranion must exhibit very weak nucleophilicity, allowing the silyl nucleophiles (or *in-situ* generated nucleophiles with acidic hydrogens) to outcompete the anion. Consequently, we selected 1.2 equiv of trifluoroacetic acid (TFA) as the proton source to replicate the azidation reaction. To our delight, the azide **6** was successfully obtained with an overall yield of 87%, despite the azidation predominantly taking place at *C*-6. Such skeleton also occurs in natural products as exemplified by the dendrogenin A which can induce tumor cell-redifferentiation and death.[30] By switching the *N*-nucleophiles, we can conveniently realize the catalytic *C*-3 or *C*-6 functionalization with the same cholesteryl donor.

Cholesteryl glycosides are widely recognized as effective immunomodulators.[31] Built upon our established efficient steroidation reaction and the satisfying stereospecificity with various categories of *O*-nucleophiles, we backtracked our initial goal to capitalize on our method to craft challenging but compelling *pseudo*-steroidal glycosides by coupling the steroidal donor **1** with various sugar alcoholic acceptors **7a-l** and glycosyl thiol **7m** as well as complex *C*-glycoside drug **7n** featuring varied ring-sizes and connecting positions (Figure 6). These acceptors are equipped with different anomeric *O*-, *S*-, *C*-substituents and commonly used protecting groups in carbohydrate chemistry. Accordingly, the glucopyranose, galactopyranose, ribofuranose, mannopyranose, glucopyranuronate, glucofuranose, and glucosamine substrates **7a-l** with primary or secondary hydroxyl group were exposed to our above-mentioned optimal reaction condition and we were pleased to find that all acceptors were competent coupling partners, transmitting comprehensively novel steroidal glycosides **8a-l** in good-to-excellent yields with reaction occurring invariably at *C*-3 of 5-cholestene backbone only. These results once again underscored the remarkable stereo-, and regioselectivity of our strain-release steroidation. It is satisfying that the protecting groups including benzyl, acetyl, benzoyl, isopropylidene, benzylidene, methyl ester, and phthalimido groups, are well tolerated under this mild condition. Unlike the strongly basic conditions of conventional Williamson ether synthesis, our mild acidic conditions are crucial for preserving commonly used ester-type protecting groups in synthetic carbohydrate chemistry untouched. Of particular note, the thioglycoside **7d** was auspiciously incorporated into the etherification for the first time, delivering **8d** with synthetically acceptable yield featuring an activable anomeric phenyl thioether group for ensuing glycosylation decoration while the previous protocol only can deliver an aglycon-transferred product.[17] As a pair of armed and disarmed acceptors, **7f** and **7g** gave **8f** and **8g** in comparably high yields of 96% and 97%, respectively,

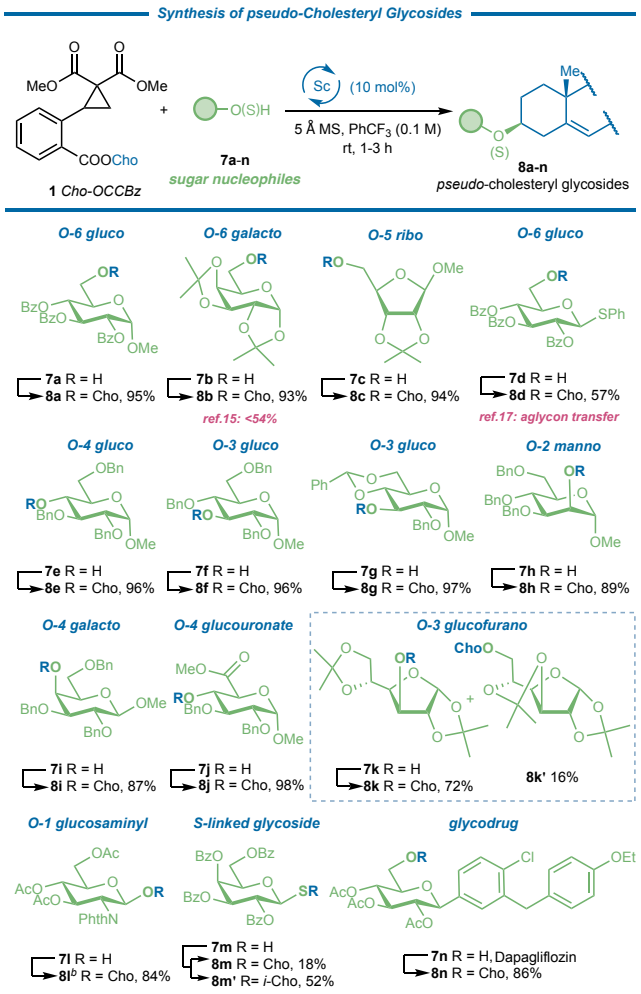

Figure 6. Synthesis of *pseudo*-cholesteryl glycosides. [a]Unless otherwise noted, all reactions were conducted on a 0.1 mmol scale with respect to **1** (1.0 equiv) in the presence of **7** (1.2 equiv) and $Sc(OTf)_3$ (0.1 equiv) in $PhCF_3$ (1.0 mL) at room temperature for 1-3 h; [b]CH_2Cl_2 as the solvent.

demonstrating the merit of the well-designed steroidal donor. Acceptors **7h-j** exhibit low reactivities mainly due to the electro-withdrawing effect of the axial-oriented C-O bond for **7h-i** and the induction effect of the carboxyl group for **7j**. Fortunately, these three reactions proceeded smoothly, generating the desired products in excellent yields, and showcasing the feasibility of our protocol with galactoside, mannoside and glucuronic acid commonly occurring in nature. Even the challenging glucose diacetonide **7k**, with significant steric hindrance and a highly acid-labile 5,6-*O*-isopropylidene group, is amenable to our protocol, and the desired product was obtained in the yield of 72%. Interestingly, during the reaction, we observed the 5-membered *O*-5,6-isopropylidene group migrated to form a generally considered less-stable 6-membered *O*-3,5-isopropylidene ring.[32] Furthermore, we attempted to use hemiacetal **7l** to showcase the "reverse glycosylation"[17,33] with our strain-release etherification. The steroidal glycoside **8l** was obtained in good yield with complete β-selectivity from the β-configurated acceptor, which underscored the configuration-retaining capability of our protocol in the "reverse glycosylation". The glycosyl thiol **7m** was also exposed to the "reverse glycosylation". Due to the stronger nucleophilicity of the thiol, the *i*-Cho product was the main product. Finally, the approved SGLT2 inhibitor

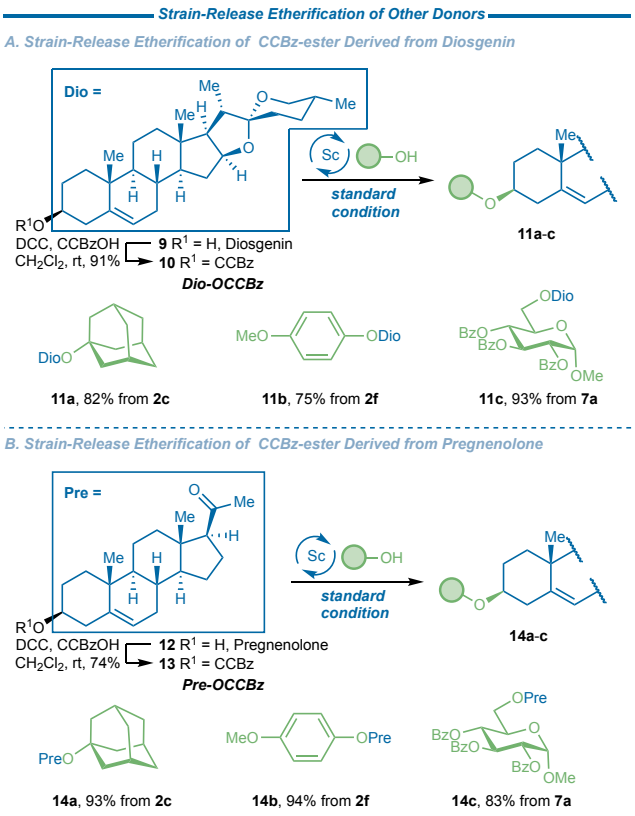

Figure 7. (A, B) Strain-release etherification of other CCBz-esters derived from other steroids. [a]All reactions were conducted on a 0.1 mmol scale to donor (1.0 equiv) in the presence of acceptor (1.2 equiv) and $Sc(OTf)_3$ (0.1 equiv) in $PhCF_3$ (1.0 mL) at room temperature for 1 h.

dapagliflozin[34] can also be used for our etherification, delivering cholesteryl glycodrug **8n** in 86% yield. Overall, conventional or natural steroidal glycosides are usually tethered by the glycosidic linkages, while our approach can offer altered bonding modes of steroids to attach them to sugars at all possible positions with excellent regio- and stereochemical controls. The generated *pseudo*-steroidal glycoside chimeras thus significantly expanded the chemical space of steroidal glycosides, and these changes might significantly affect the enzyme recognition and binding, which might have a profound impact on their activities.

Based on the satisfying results, we hypothesized that our protocol could serve as a platform to generate steroid derivatives from the corresponding donors, thus we next proceeded with the synthesis of the plant-derived diosgen-3-yl donor, resulting in the formation of **10** in an excellent yield of 91%. By subjecting hindered tertiary alcohol **2c**, phenol **2e,** and disarmed sugar alcohol **7a** to our optimal condition, we successfully obtained the desired diosgenin derivatives **11a-c** in 75 to 93% with *C*-3 exclusively etherificated, advising that the excellent selectivity is not limited to 5-cholestene (Figure 7A). Likewise, the hormone pregnenolone could be transformed into the steroidal donor, which readily underwent steroidation to give **14a-c** in excellent yields (Figure 7B). These results demonstrated that both animal- and plant-derived steroids are viable substrates for our functionalization reactions, which vividly showed the versatility of our strain-release steroidation as a powerful organic transformation to satisfy multiple needs in different scenarios.

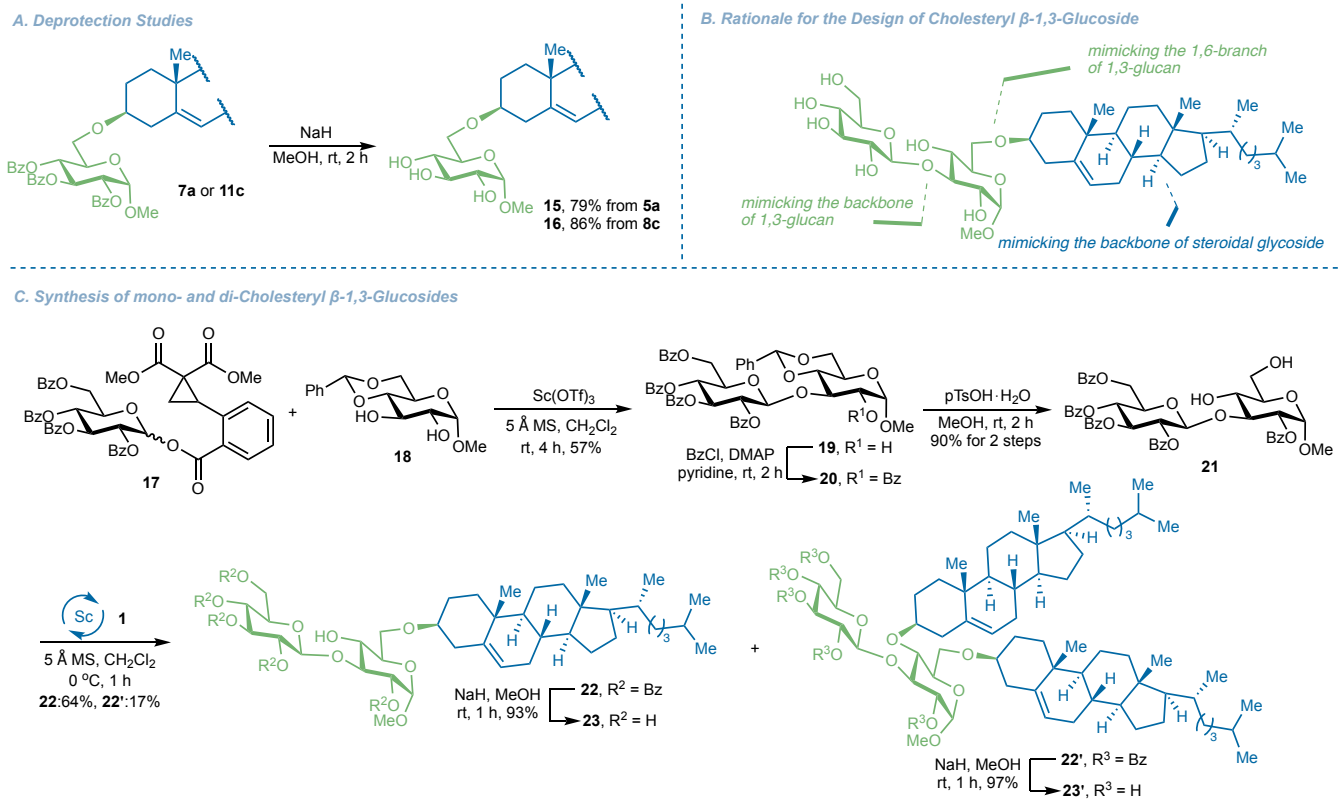

Figure 8. (A) Deprotection studies. (B) Our rationale for the design of cholesteryl β-1,3-glucoside. (C) Synthesis of mono- and di-cholesteryl β-1,3-glucosides.

With a series of *pseudo*-steroidal glycosides in hand, we proceeded with deprotection of the obtained *pseudo*-steroidal glycosides to explore further their synthetic and biological utilities (Figure 8A). Under the condition of basic transesterification, compounds **15** and **16** could be obtained in 79% and 86%, respectively with other functionalities untouched. Having successfully prepared the free *pseudo*-steroidal glycosides, we have established an efficient assembly line encompassing donor synthesis, regiospecific steroidation of sugar alcohols, and deprotection processes. This comprehensive approach allows for the highly efficient synthesis of *pseudo*-steroidal glycosides. Notably, the overall yields for compounds **15** and **16** reach 57% and 73%, respectively, utilizing commercially accessible and cost-effective sources of cholesterol and diosgenin. This route secured reliable access to sufficient amounts of *pseudo*-steroidal glycosides for ensuing biological and medicinal studies.

Subsequently, we explored the possibility of extending this protocol to complex oligosaccharide acceptors. β-Glucans behave as immunomodulators that specifically target the innate immune system.[35] Despite sharing a common β-1,3-glucan backbone, these β-glucans display notable diversity in branching patterns, insertions, and impurities, attributable to variations in sources, extraction, and purification methods. It should be noted that the immunomodulatory effects are primarily associated with β-glucans featuring a significant degree of branching, such as those derived from fungi and yeast. In this context, we devised a disaccharide incorporating a cholest-5-en-3-yl branch (Figure 8B). The designed molecule possesses a well-defined chemical skeleton of β-1,3-glucose, mimicking the backbone of β-glucans. A cholesteryl group is attached to *O*-6 on the glucosyl residue at the reducing end, not only emulating the branching nature of β-glucans but also sharing characteristics with *pseudo*-steroidal glycosides. The synthesis of this cholesteryl disaccharide aims to amalgamate the favorable attributes of β-glucans and steroidal glycosides, creating a potent lead immunomodulator through our straightforward pipeline-like program accessible to both medicinal chemists and biologists (Figure 8C). Thus, the glycosyl CCBz donor **17** was coupled with diol acceptor **18** under the catalysis of $Sc(OTf)_3$ to regioselectively give disaccharide **19** in the yield of 57% at room temperature.[19b] The unmasked *C*-2 hydroxyl group in **19** was benzoylated to afford **20**, which was treated with *p*-toluenesulfonic acid monohydrate (pTsOH·H_2O) to remove the benzylidene group to form diol acceptor **21** in an excellent yield of 90% over 2 steps. Under our optimal condition, the strain-release etherification of cholesterol donor **1** uneventfully generated our designated monocholesterated disaccharide **22** along with dicholesterated disaccharide **22'** at 0 °C in 64% and 17%, respectively. The reaction not only demonstrated that this protocol was amenable to complex oligosaccharides, but again attested to the high efficiency by the capability of installing two bulky cholesteryl residues at both *O*-4 and *O*-6 on a congested monosaccharide, offering convenient chemical diversity for structure-activity relationship investigations. Finally, both cholesteryl disaccharides were globally deprotected to deliver the *pseudo*-steroidal glycosides in excellent yields. Thus we have achieved the catalytic divergent synthesis of complex *pseudo*-steroidal glycosides of mono- and di-cholesteryl disaccharides, setting a solid foundation for the in-depth investigation of their biological functions.

To investigate the anti-inflammatory effect of our synthetic *pseudo*-steroidal glycosides, we set up an *in vitro* immunological assay by employing murine macrophage RAW 264.7 cells,

in which we treated the cells with several (*pseudo*-)steroidal glycosides (**15**, **16**, **23**, **23'** and **25**) before the addition of LPS, a strong immune activator. As depicted in Figure 9, the cytokine expression analysis by enzyme-linked immunosorbent assay (ELISA) demonstrated that the concentration of IL-6 dramatically dropped when pretreating the RAW264.7 cells with different (*pseudo*-)steroidal glycosides **15**, **16**, **23**, **23'** and **25**, while methyl α-D-glucopyranoside **24** rarely suppressed the IL-6 secretion, which is consistent with current reports that some steroidal glycosides exhibit as modulators to treat inflammatory diseases.[36] Of importance, our synthetic *pseudo*-steroidal glycosides achieved 91% to 99%-inhibition of IL-6 secretion induced by LPS, a remarkable outcome when compared to 12% to 95%-suppression reported for NSAIDs and SAIDs by Kim and coworkers,[37] highlighting the potential that these synthetic compounds can be employed as effective immunomodulators. Notably, compared to the naturally configurated steroidal glycoside **25**, our artificial steroidal glycoside **15** exhibited moderately enhanced suppression of the IL-6 expression. It is well-acknowledged that the natural steroidal glycosides composed of glycosidic linkages are sensitive to enzymatic or acidic hydrolysis *in vivo*, combining the stability of the ether bond-tethered *pseudo*-steroidal glycosides with their augmented anti-inflammatory properties, we believe that the *pseudo*-cholesteryl glycosides will be ideal lead compounds for further structural modifications. More importantly, compounds **23** and **23'** containing the disaccharide unit performed 3 to 10 times enhanced repressed effects on the IL-6 secretion than those containing monosaccharide scaffolds (**15**, **16,** and **25**), implying that merging oligosaccharide backbone and steroid aglycon may improve the anti-inflammatory activity of (*pseudo*-)steroidal glycosides. These results matched well with the previous studies abovementioned that the immunomodulatory effects can be significantly improved by branched β-1,3 glucans. Lastly, a preliminary study of the aglycon effect on the IL-6 expression was also investigated and the ELISA analysis demonstrated that the cholesteryl-substituted compound **15** showed a better-suppressed effect than the diosgenin-containing compound **16**, suggesting that the canonical cholesterol is a more promising steroid for further anti-inflammatory drugs development, also implying that the aglycon might be crucial to the immunoregulatory activities. Of note, we found the cholesteryl disaccharides **23** and **23'** have better solubilities than monosaccharide-based *pseudo*-steroidal glycosides, we speculated that the increased solubilities might also contribute to their significant immunoregulatory functions, indicating another important index to design novel *pseudo*-steroidal glycosides. With these encouraging results, we are now using our powerful strain-release etherification method to construct a library of *pseudo*-steroidal glycosides consisting of different sugar subunits, linking modes, and aglycons to study further their immunological functions, structure-effect relationships, and mechanisms, the results will be disclosed in due course.

CONCLUSIONS

In conclusion, an effective protocol to synthesize steroid derivatives using a rationally designed steroidal donor, i.e., steroidal *ortho*-2,2-dimethoxycarbonylcyclopropylbenzoates has been established for the synthesis of structurally diverse *C*-3 decorated steroid derivatives of biological and medical importance. This novel reaction proceeds under mild conditions under the catalysis of low-cost $Sc(OTf)_3$ salt and enjoys an unprecedented broad acceptor substrate scope of *O*-, *C*-, *N*-, *S*-, and *P*-

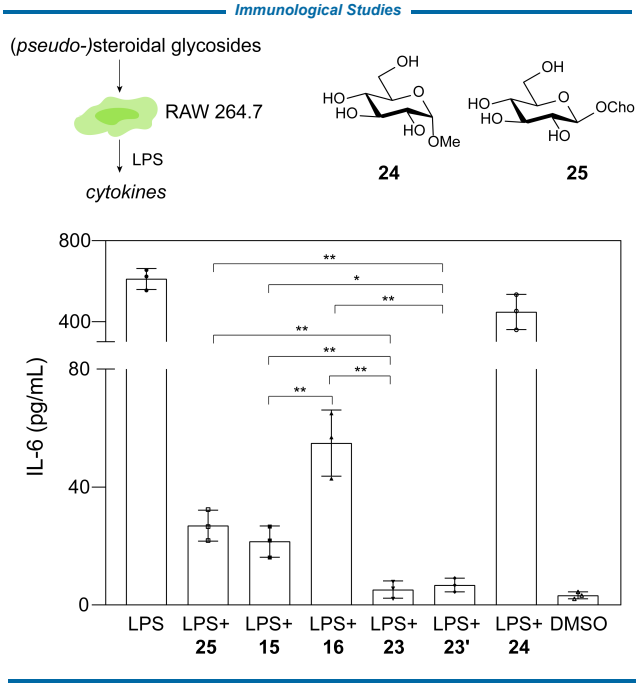

Figure 9. Different (*pseudo*-)steroidal glycosides suppress LPS-induced inflammatory responses in murine macrophage RAW 264.7 cells. The murine macrophage RAW 264.7 cells were pretreated with synthetic (*pseudo*-)steroidal glycosides for 48 h followed by the incubation with lipopolysaccharides (LPS) for 4 h. The secretion of IL-6 was analyzed by enzyme-linked immunosorbent assay (ELISA). Data are shown as mean value ± s.d. from three independent experiments. **P*<0.05, ***P*<0.01, unpaired t-test.

nucleophiles, and produced steroid *C*-3 functionalized products with exclusive regioselectivity in high yields with varied categories of *O*-nucleophiles. We investigated the mechanism of the strain-release-driven steroid etherification of acceptors with varied nucleophilicities with DFT calculations. The steroid *C*-3 regioselectivity of the reaction with *O*-nucleophiles was explained by the detailed analysis of the matching of the CDFT calculated nucleophilicity of the acceptors and the differentiated electrophilicity of the reactive sites on the steroidal cation, which combine to determine the reaction kinetics. An unexpected intermolecular rearrangement of donor in the absence of the acceptors highlighted the ability of the protocol to construct the challenging all-carbon quaternary center with *C*-3 regio- and stereospecificity. Of note, we successfully developed an easily accessible assembly line for highly efficient synthesis of mono- and oligosaccharide-based *pseudo*-steroidal glycosides for immunological studies from cheap and abundant steroids. Preliminary immunological studies of the deprotected *pseudo*-steroidal glycosides **23** and **23'** indicated that these compounds suppress the IL-6 secretion in macrophages and have potent anti-inflammatory activities. These results highlighted the potential applications of the synthetic protocol and the *pseudo*-steroidal glycosides in anti-inflammatory drug development.

ASSOCIATED CONTENT

The supporting information is available free of charge on the ACS Publications website, including experimental procedures, NMR spectroscopic, analytical data, and computational results for all compounds (PDF). The discussions on the determination of the *C*-3 configuration and plausible mechanism are also provided.

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Notes

The authors declare no competing financial interests.

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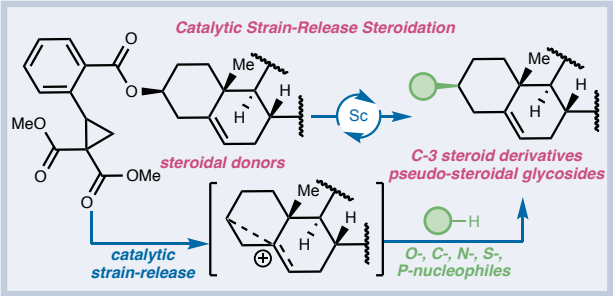
Catalytic Strain-Release Steroidation
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P-nucleophiles