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'Click' synthesis of triazole-based spirostan saponin analogs

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Dedicated to Professor Margarita Suárez Navarro on the occasion of her 65th birthday

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ABSTRACT

Novel analogs of spirostan saponins in which the glycosidic bond has been replaced by a triazole linkage are described. For this, a direct oligosaccharide–steroid conjugation approach based on the Cu^{I}-catalyzed azide–alkyne 1,3-dipolar cycloaddition was implemented, leading to diverse combinations of saponin analogs with variations in the trisaccharide moiety, the artificial linkage, and the steroid-skeleton functionalization. This 'click' process proved great efficiency for the ligation of two bulky building blocks (e.g., chacotriose derivatives and spirostanes bearing axial azides), which enabled the rapid creation of a small library of triazole-based analogs for cytotoxicity evaluation. A molecular modeling study was performed to understand the conformational and electronic differences between a natural saponin and its triazole-based analogs.

1. Introduction

Saponins[1] comprise a family of terpene and steroid glycosides with a broad variety of biological and medicinal applications.[1,2] Steroidal saponins are among the natural products of wider distribution in the plant kingdom and of greater significance in traditional medicine. Most abundant steroidal saponins contain a spirostanic aglycone and the oligosaccharide moiety directly attached to the C-3 hydroxyl group. Several members of this family have exhibited potent cytotoxic,[3] antifungal,[4] antiinflammatory,[5] and antiviral activities,[6] which makes them important targets for drug development. Indeed, the most promising property of spirostan saponins is their inhibitory activity against the growth of certain cancer cells, [3d–f,7] showing dioscin (**1**) the greatest anticancer potency.

However, a common feature of saponins is their hemolytic activity, a property that has hampered the recognition of these natural products as potential anticancer drugs. Fortunately, interesting SAR studies[8] have been accomplished and the general perception that the cytotoxicity of saponins is correlated to the hemolytic activity has been recently dissipated. In a remarkable report,[8a] Yu and co-workers evaluated dozens of spirostan saponins and showed, firstly, that the hemolytic and cytotoxic activities are highly dependent on the structural features of the glycosides, and secondly, that these activities do not correlate between them. Alternatively, the same group demonstrated that the cytotoxicity does correlate with the cellular internalization of the steroidal saponins,[9] which provides new evidences for a better understanding of the apoptosis-inducing effect of dioscin (**1**) and several of its congeners.[3d–f,7]

All these findings have been only possible as a consequence of extensive synthetic programs directed to access natural spirostan saponins and analogs for biological evaluation.[10] By introducing modifications on both the aglycone and the oligosaccharide skeletons, SAR studies[8–10] have showed many of the structural requirements needed at each moiety for these compounds to have a good cytotoxic activity. However, little attention has been paid to

Abbreviations: DIAD, diisopropylazodicarboxylate; DMAP, *N*,*N*-dimethylaminopyridine; DMF, dimethylformamide; DMPU, 1,3-dimethyl-3,4,5,6-tetrahydro-2(*1H*)-pyrimidonone; TBAB, tetra-*n*-butyl ammonium bromide; SAR, Structure–activity relationship.

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the effect of a variation of the traditional glycosidic linkage—between the oligosaccharide and the spirostan steroid—on the anticancer potency of the resulting conjugate. Will synthetic spirostan saponin with an artificial oligosaccharide–steroid linkage display good cytotoxic activity? This is an important answer to be addressed, as some alternative conjugation approaches more efficient than glycosylation could be implemented to produce analogs of steroidal saponins.

Herein we report on the use of the Cu^{I}-catalyzed 1,3-dipolar cycloaddition[11] of alkynes and azides for the direct conjugation of trisaccharides to spirostanic steroids. 'Click' chemistry refer to methods capable to join together organic molecules in high yields under mild conditions and in the presence of a diverse range of functional groups.[11c] The present 'click' approach gives rise to novel spirostan saponin analogs having a triazole ring as linkage of both building blocks. The triazole linkage comprises several features that make it a good choice in medicinal and biological chemistry, i.e., it is hydrolytically and metabolically stable, water soluble, and rigid. We also considered the easiness on the introduction of alkyne and azide groups into the steroidal and carbohydrate units, an issue that would allow for the rapid variation of the functionalization patterns on both building blocks.

The Cu^{I}-catalyzed 1,3-dipolar cycloaddition approach has been extensively used on glycoconjugation chemistry,[12] although only a few reports have dealt with such a type of ligation between steroids and sugars.[13] Very recently, a series of sulfated oligosaccharides have been conjugated to lipophilic steroids through this click process. This type of artificial ligation produced stable, amphiphilic conjugates that display potent inhibitory activity against heparin sulfate-binding proteins, and therefore, inhibition of tumor growth and metastasis.[13a] Also, cytotoxic cyclopamine-rhamnose[13c] conjugates and antibacterial cholestan-neomicin B[13b] conjugates have been prepared through standard click chemistry, proving the usefulness of this concept on diverse fields of steroid chemistry. Whereas spirostan–oligosaccharide conjugates have not been reported by means of click chemistry, we describe the first approach to various combinations of spirostan saponin analogs having the triazole ring as artificial linkage. These saponin analogs are then evaluated for their cytotoxicity and studied by molecular modeling to achieve conclusions regarding the effect of such a linkage on the biological activity.

2. Results and discussion

Fig. 1 shows the structure of dioscin (**1**) and filiasparoside A[3b] (**2**), two naturally occurring cytotoxic saponins that were chosen as models for the design of the new analogs. Dioscin is probably the saponin of greatest occurrence in the plant kingdom, and its broad spectrum of bioactivity[3f–h,4,5] makes it an interesting lead for drug development. The trisaccharide moiety of dioscin, namely chacotrioside, has been the subject of several SAR studies dealing with alkylations,[10b,d] removal or position change of some of its α-L-rhamnosyl residues,[8,10a,b] and conjugation to triterpene or lipidic aglycones.[9,10d] In contrast, filiasparoside A (**2**) possesses a less common trisaccharide moiety featuring β-D-xylopyranosyl and α-D-arabinopyranosyl units linked at positions 4 and 6 of the inner glucose, respectively. Although using α-L-rhamnosyl as branching monosaccharide, this type of connectivity will be also employed by us to construct trisaccharide moieties analogous to chacotrioside (Fig. 1).

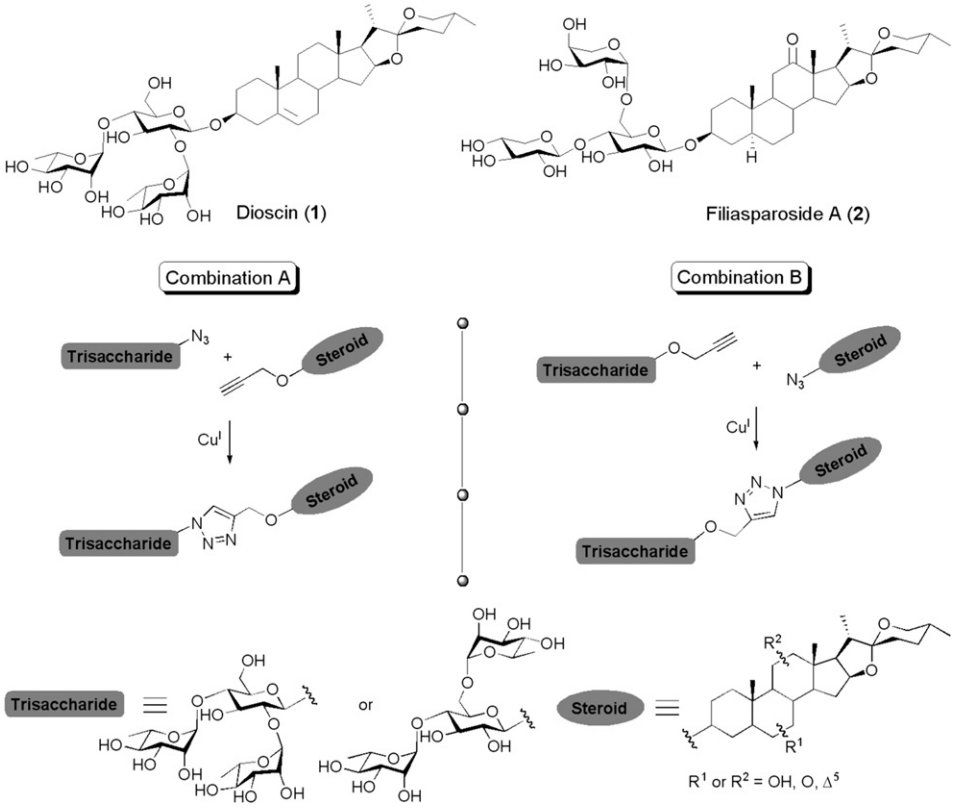


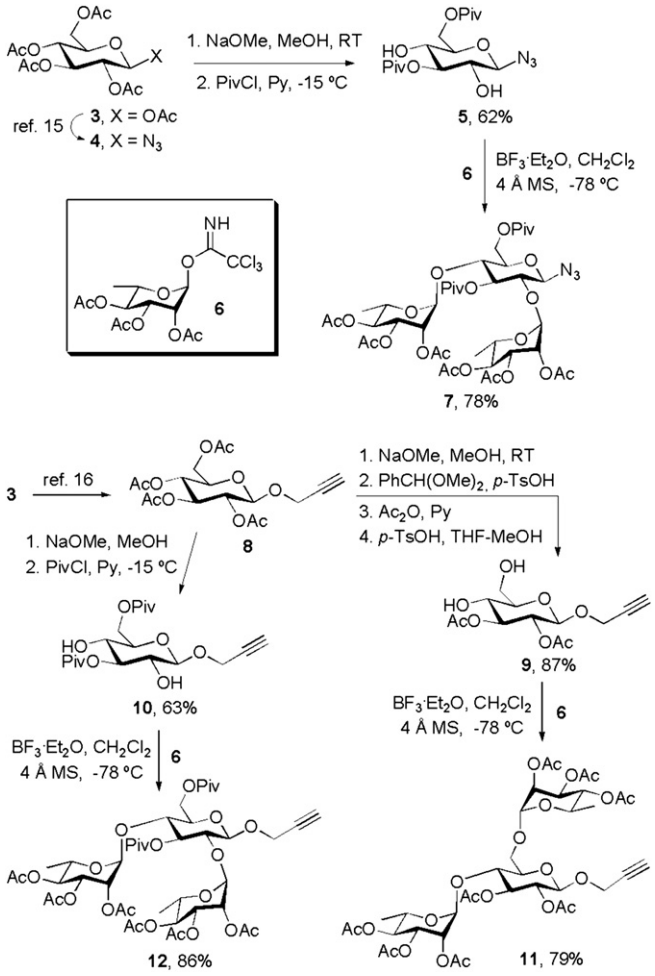
Fig. 1. Structures of naturally occurring cytotoxic saponins and strategy to produce triazole-based spirostan saponin analogs through Cu^{I}-catalyzed azide-alkyne 1,3-dipolar cycloaddition.

There are two different strategies for the incorporation of the oligosaccharide moieties of spirostan saponins to the steroidal aglycone: (i) the direct conjugation of the oligosaccharide donor to the steroidal aglycone,[10c,d] and (ii) the conjugation of the inner glucose unit to the spirostane followed by protection/deprotection protocols and further glycosylation with the branching

monosaccharide donors.[10e–i] Whereas, the first approach is more straightforward, the glycosylation step usually proceeds with low stereoselectivity when the inner glucose has position 2 capped with another sugar moiety. Nevertheless, since our conjugation process relies on a cycloaddition reaction of sugars previously functionalized at the anomeric center, the stereoselectivity issue is not a concern.

Fig. 1 illustrates the strategy to achieve analogs of spirostan saponins through the direct Cu^{I}-catalyzed 1,3-dipolar cycloaddition-based conjugation of trisaccharides to spirostanes. Combination A comprises the use of glycosyl azides and spirostanic steroids bearing a propargyl ether at C-3. Alternatively, the trisaccharide moiety can also be functionalized with propargyl alcohol and next conjugated to 3-azido-spirostanes, as depicted in combination B. The versatility of this method toward saponins-library construction relies both on the efficiency of the direct conjugation process and on the wide variety of azido-steroids and propargyl trisaccharides that can be utilized. As shown in Fig. 1, the approach includes not only the use of chacotriose (i.e., α-L-rhamnopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranose) but also an analog bearing the two rhamnoses linked at C-4 and C-6 of the glucose (i.e., α-L-rhamnopyranosyl-(1→6)-[α-L-rhamnopyranosyl-(1→4)]-β-D-glucopyranose).

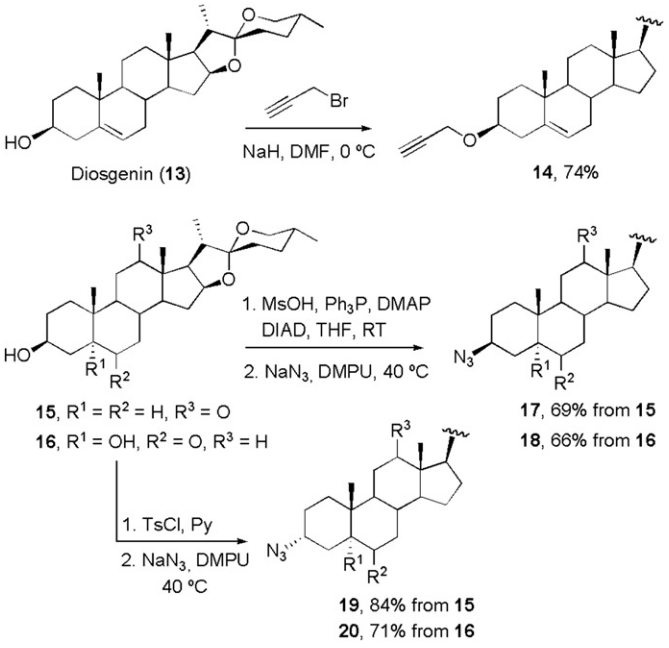
Scheme 1 depicts the preparation of the propargyl and azido trisaccharides that will be employed on the direct conjugation to spirostanes through click chemistry. Chacotriosyl azide **7** and propargyl chacotrioside **12** were prepared by an adaptation of a reported protocol designed for introducing rhamnose at positions



Scheme 1. Synthesis of trisaccharides functionalized with azido and propargyl groups.

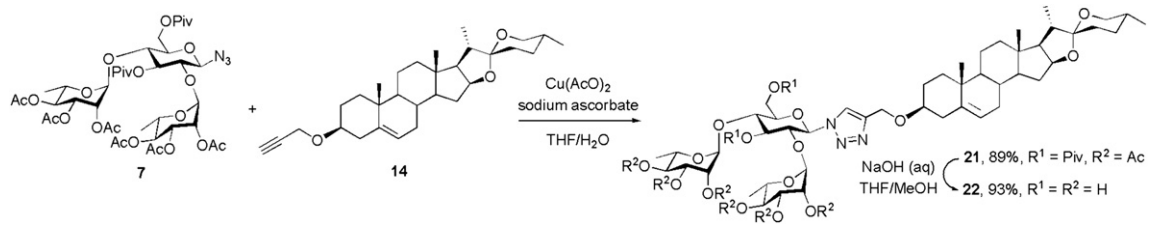
2 and 4 of the glucoside.[14] Thus, both the peracetylated glucosyl azide **4**[15] and propargyl glucoside **8**[16] were fully deprotected and next subjected to selective pivaloylation of C-3 and C-6, followed by glycosylation with rhamnopyranosyl trichloroacetimidate **6** to furnish **7** and **12**, respectively, in good overall yields. Alternatively, propargyl glucoside **8** was completely deacetylated and then capped at positions 4 and 6 as benzylidene acetal.[14] Acetylation of hydroxyls 2 and 3 followed by removal of the acetal with *p*-TsOH in the mixture THF–MeOH afforded acceptor **9**, which was subjected to glycosylation with donor **6** to give the propargyl trisaccharide **11** in 69% overall yield.

For the preparation of the alkyne and azido spirostanic building blocks, we decided to select natural spirostanic sapogenins with diverse functionalization patterns on rings B and C, but keeping their structural resemblance to the aglycones of most cytotoxic saponins. As shown in Scheme 2, the first option was diosgenin (**13**, dioscin's aglycone), which was propargylated in 74% yield upon treated with propargyl bromide in a NaH/DMF suspension. Simultaneously, the natural sapogenins hecogenin (**15**) and 5-hydroxylaxogenin (**16**) were chosen for the preparation of the corresponding azides featuring the 3α and 3β stereochemistry. Although the model saponins are endowed only with a 3β connectivity to the glycosides, we were prompted to evaluate if either the stereochemistry α or β of the artificial linkage has some influence on the bioactivity. Thus, the synthesis of the 3β-azido-spirostanes **17** and **18** required the replacement of the 3β-OH by azide with net retention of the configuration. This was accomplished according to a reported procedure by using methanesulfonic acid as the acidic/nucleophilic component of the Mitsunobu reaction, followed by displacement with NaN_3 in 1,3-dimethyl-3,4,5,6-tetrahydro-2(1*H*)-pyrimidonone (DMPU) to recover the original C-3 stereochemistry.[17] Alternatively, the 3α-azido-spirostanes **19** and **20** were easily prepared by tosylation of the 3β-OH and subsequent azide displacement with inversion of the C-3 configuration.



Scheme 2. Synthesis of 3β-propargyloxy and 3-azido-spirostanes.

As depicted in Scheme 3, the click sugar–spirostan conjugation approach (combination A, Fig. 1) was firstly implemented by utilizing chacotriosyl azide **7** and propargyloxy-diosgenin **14** on the Cu^{I}-catalyzed 1,3-dipolar cycloaddition, thus giving rise to conjugate **21** in 89% yield. Typical reaction conditions (i.e., 20% mol of



Scheme 3. Synthesis of a triazole-based dioscin analog by Cu^{I}-catalyzed 1,3-dipolar cycloaddition of chacotriosyl azide (**7**) and propargyloxy-diosgenin (**14**).

a Cu^{II} salt and 40% mol of sodium ascorbate in a THF/H_2O mixture) were employed, which proved great success on the ligation of these bulky building blocks. Complete deprotection of the trisaccharide moiety was accomplished upon treatment with standard saponification conditions, thus leading to the triazole-based dioscin analog **22** in 83% yield over the two steps. Unfortunately, dioscin analog **22** showed no significant cytotoxic activity against HL-60 cells (IC_{50}>100 μg/mL) in a preliminary biological evaluation.[18] This result made us to turn to combination B (i.e., the use of propargyl trisaccharides and 3-azido-spirostanes) as a way of creating greater structural diversity on the resulting conjugates.

It must be mentioned that the preparation of 3α-azido-spirostanes to be used in the click conjugation approach was not randomly decided. Based on conformational studies performed by molecular dynamic and molecular mechanic calculations,[8b] Mimaki et al. found that the preferred conformation of a cytotoxic diosgenyl diglycoside (i.e., α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside) possess the α-L-rhamnosyl unit in a vertical orientation against the steroidal plane. In contrast, in the conformations of non-cytotoxic diosgenyl diglycosides having the α-L-rhamnosyl unit linked at positions 3 or 4 of the glucoside, the full disaccharide moiety appear on the same plane of the steroidal aglycone. Therefore, it was suggested that the perpendicularity between the branching α-L-rhamnosyl residue and the lipophilic steroid might be important for the cytotoxicity mechanism of these saponins. Although it is be difficult to correlate this information to the case of spirostan trisaccharides,[19] we were prompted to prepare artificial conjugates derived not only from 3β-functionalized spirostanes but also from the 3α epimers. By doing this we would be populating a conformational space not accessible to the 3β-derivatives, since in the 3α epimers the trisaccharide–triazole moiety is directing completely toward the α face as a result of the axial orientation.

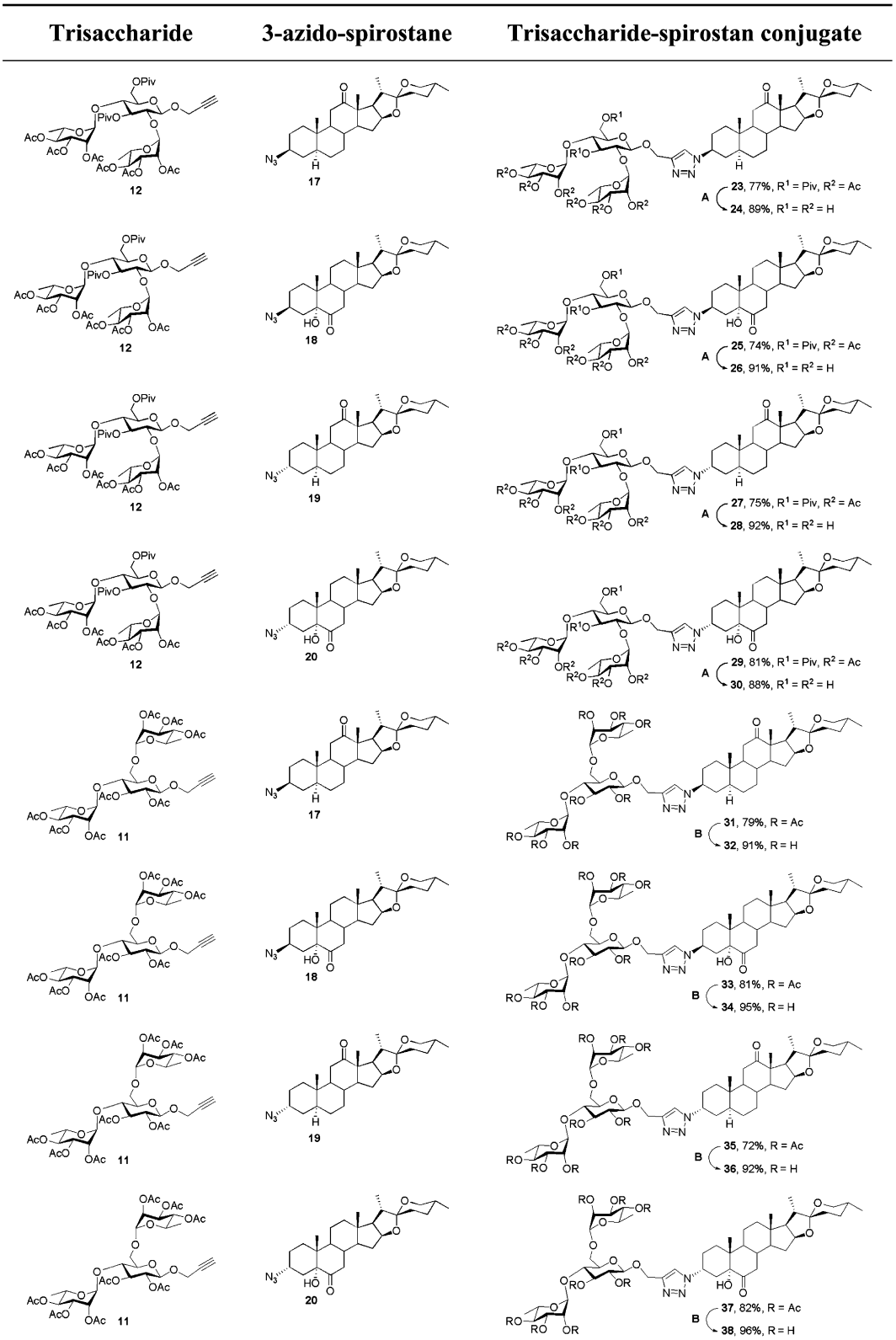
Accordingly, a small library of saponin analogs was created by the direct click conjugation of the propargyl trisaccharides **11** and **12** to the 3-azido-spirostanes **17**–**20**, which possess varied oxygenated functions on ring B and C and feature either the α or β orientation of the azide. Table 1 illustrates the results of performing the eight possible combinations between the two above oligosaccharides and the four spirostanes. As before, the approach proved great efficiency on producing the triazole-based conjugates, as most reactions were completed within a few hours with yields of isolated products higher that 70%. Remarkably, the yields of conjugates arising from the sterically demanding 3α-azido-spirostanes did not show to be lower than those of the 3β-epimers, a result otherwise impossible to achieve by using the traditional glycosylation of 3-hydroxy-spirostanes. This becomes even more intriguing for the reaction of the very hindered 3α-azido group of compound **20**, which features a 1,3-diaxial interaction with the 5α-OH group that does not seem to interfere in the reaction outcome. In general, the Cu^{I}-catalyzed azide–alkyne 1,3-dipolar cycloaddition proved to be a very reliable and efficient method for the conjugation of trisaccharides to spirostanes, and in addition allowed for a rapid access to a small library of saponin analogs for biological screening.

Sadly, none of the obtained spirostan saponin analogs showed significant cytotoxic activity against HL-60 cells (IC_{50}>100 μg/mL). This fact, although disappointing, provides new information regarding important structural elements that seem to be required for the cytotoxicity of spirostan saponins and their synthetic analogs. Firstly, while it is true that the triazole ring is rigid enough to provide conformational constrain to the resulting conjugate, it must be noticed that the methylene group of the propargyl moiety introduces considerable flexibility to the artificial linkage. As a result, the saponin analogs may have greater conformational flexibility than the natural ones. Secondly, the distance expanding the triazole-based artificial linkage is longer that the traditional glycosidic bond, which may vary the overall three-dimensional structure of the conjugate. Thirdly, the incorporation of a triazole ring introduces new electronic properties (e.g., the triazole ring is considered a peptide bond analog) into the conjugate, which might lead to mechanisms of action and/or transport in the cells completely different from those of the original spirostanyl glycosides.

To pursuit information about this subject, the conformational and electronic differences between the natural saponin and two selected analogs[20] were studied. Consequently, two conformational analyses were accomplished to obtain the most stable conformers of the natural saponin (**1**) and its analogs **22** and **24**. First, molecular mechanics with the SYBYL force field (TRIPOS, Inc.) was performed as implemented in the Spartan'02 windows program.[21] Next, the Multiple Minima Hypersurfaces Methodology (MMH)[22] featuring the semiempirical Hamiltonian AM1 was used to obtain the most stable conformers. This methodology performs a Monte Carlo simulation by randomly modifying the specified dihedral angles. In this study, 100 conformations were created for each molecule and the selected dihedrals were randomly generated for each conformation. In Table 2 appear the dihedral angles within the MMH methodology selected for the evaluation of the conformational space of dioscin (**1**) and the two saponin analogs. The angles C_2–C_3–O–C_1 (**1**), C_2–C_3–O–CH_2 (**22**), and C_2–C_3–N–N (**24**) show the relative orientation either of the carbohydrate or the carbohydrate–triazole hybrid with respect to the steroidal plane. Dihedrals Csp^2–N–C_1–O (**22**) and Csp^2–CH_2–O–C_1 (**24**) allow for the evaluation of the relative orientation of the trisaccharide with respect to the triazole ring. Single point AM1 energy calculations were performed to all conformations obtained by molecular mechanics, which enabled accessing to the whole population distribution of the three compounds.

Table 2 also shows the two calculated preferred conformations for each compound, obtained as explained before. For dioscin (**1**), conformation A contributes in 62% to the partition function, whilst conformation B does it only in 14%. Interestingly, the analysis of conformation A of dioscin (**1**) clearly agrees with the results previously reported by Mimaki et al.[8b] Therein, the conformation of a diosgenyl diglycoside having an α-L-rhamnosyl attached at position 2 of glucose possesses that rhamnose unit in a vertical orientation with respect to the steroidal plane, while the glucose unit was roughly in the same plane of the steroid. This is exactly as

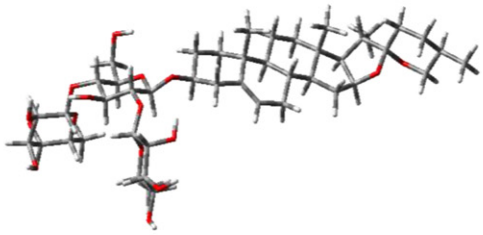
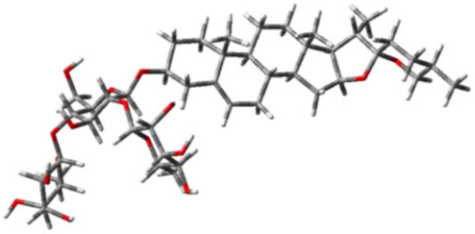
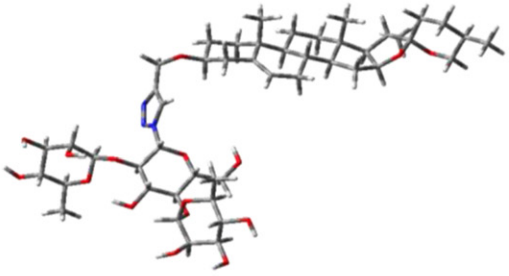
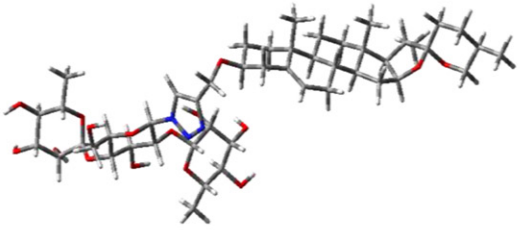
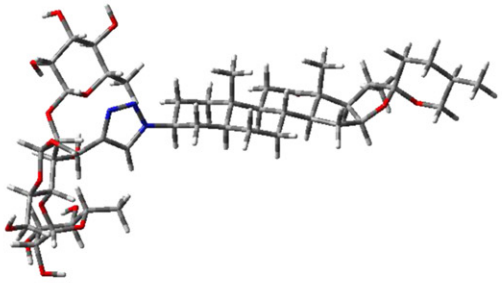
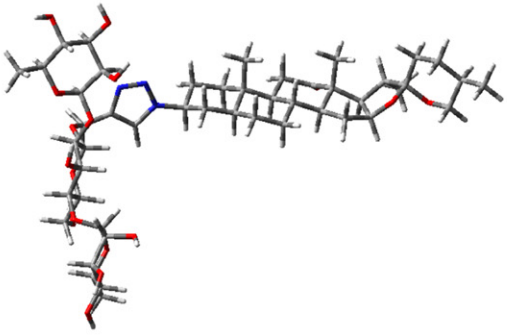
Table 1
Synthesis of spirontan saponin analogs via Cu^{I}-catalyzed 1,3-dipolar cycloaddition of propargyl trisaccharides and 3-azido-spirostanes



Conjugation conditions: 20% mol $Cu(AcO)_2 \cdot H_2O$, 40% mol sodium ascorbate, THF/H_2O.
Deprotection conditions: (A) NaOH (aq), THF/MeOH, 50 °C; (B) NaOMe, MeOH, rt.

appear in dioscin's preferred conformation A shown in Table 2, which corroborates the value of such a structural requirement for a good cytotoxicity. In contrast, most conformations of dioscin analog **22** exhibit the whole trisaccharide–triazole hybrid moiety directing toward the α face of the steroid skeleton, and with the triazole ring almost perpendicular to the steroidal plane. This system shows great conformational entropy, which is reflected by the presence of several conformers with almost the same contribution

Table 2
Calculated preferred conformations of dioscin (**1**) and saponin analogs **22** and **24**

Compound	Conformation A	Conformation B
Dioscin (**1**)		
saponin mimic (**22**)		
saponin mimic (**24**)		

to the partition function. Thus, the shown conformations A and B contribute only in 15% and 14%, respectively, and several others contribute between 8% and 12%. This result confirms our hypothesis that the methylene group expanding the linkage between the chacotriosyl triazole and the spirostanol leads to a very high conformational flexibility of the entire system. Finally, analysis of analog **24** conveys that conformation A is the most populated one with 83% of contribution, while the next preferred conformation is B with only 2%. Indeed, analog **24** has much less conformational flexibility than its analog **22**, presumably owing to the fact that the rigid triazole is directly attached to steroidal skeleton, thus lying in the same plane. In both conformations the entire trisaccharide moiety displays a vertical orientation with respect to the steroidal plane, also quite different to that of dioscin (**1**).

Fig. 2 shows the geometrical superposition of the most stable conformer of **1** (black), **22** (red) and **24** (blue), which allows for a better visualization of the conformational differences between the three molecules. As noticed from analysis of Table 2, the major differences are not obviously in the steroidal skeleton but on the orientation of the trisaccharide moiety with respect to the steroid. It is expected that such a difference in the three-dimensional structure of the trisaccharide–spirostan conjugate is responsible for the lack of significant cytotoxicity found for all analogs. In addition, it was interesting to assess further information dealing with the electronic properties of the different conjugates, with emphasis in a comparison between the triazole-based linkage and the glycosidic one. For this, the frontier orbitals were calculated for the most stable conformations of **1**, **22**, and **24** by means of the semiempirical AM1 method, and then visualized with the

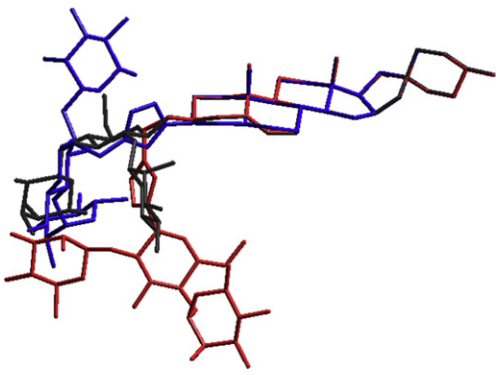
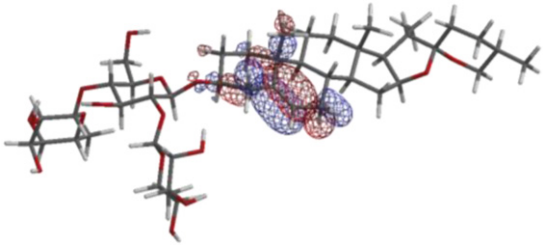
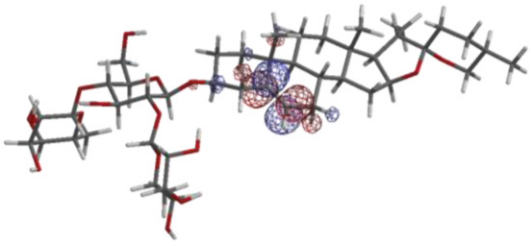
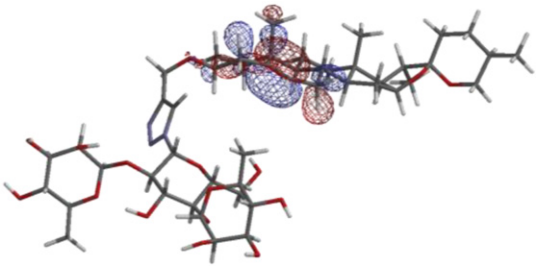
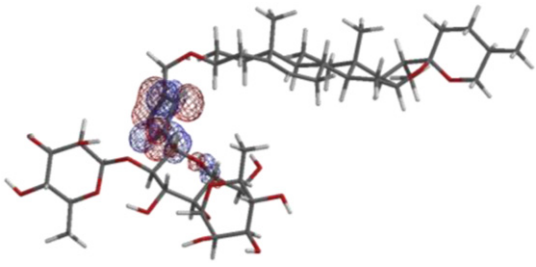
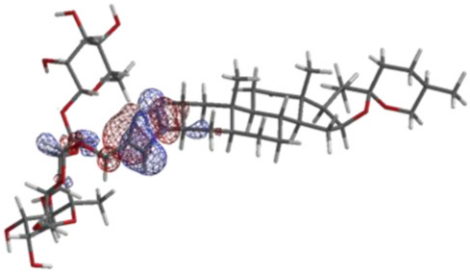
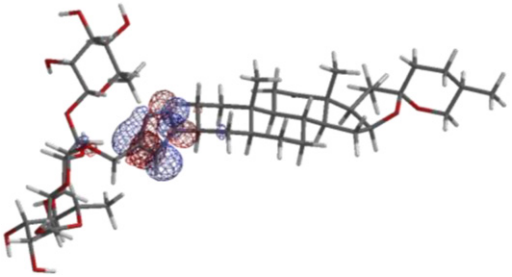


Fig. 2. Superposition of the molecular geometries of most stable conformers of dioscin (**1**, black), and analogs **22** (red) and **24** (blue).

Spartan'02 package. The location of the frontier orbitals can disclose the molecular region involved in a putative electron exchange processes during interaction with other molecules or molecular systems.

Table 3 illustrates the frontier orbitals calculated for the three molecules. Analysis of the frontier orbitals of dioscin (**1**) shows the HOMO and LUMO localized in the Δ^5 double bond of ring B, being both molecular orbitals mainly formed by the atomic p_y and p_z orbitals of the olefinic carbon atoms. Saponin analog **22** also has the HOMO localized on the Δ^5 double bond, but the LUMO is located on the triazole ring and mainly formed by the atomic p_x, p_y, and p_z orbitals of the triazole atoms. Alternatively, analog **24** has both frontier orbitals localized in the triazole ring, and as in the case of analog **22**, the atomic orbitals of the triazole atoms have the greatest contribution to the LUMO. As a summary, not only the preferred conformation of the obtained saponin analogs are unlike from that of the natural one, but also the distribution and location of frontier molecular orbitals are quite different. Since the molecular mechanism for the anticancer activity of saponins remains by far uncertain, the information provided by this theoretical study is helpful for future SAR studies.

Table 3
Calculated frontier molecular orbitals for the preferred conformations of **1**, **22**, and **24**

Compound	HOMO	LUMO
1		
22		
24		

3. Conclusions

We have implemented an oligosaccharide–steroid direct conjugation approach based on the Cu^{I}-catalyzed azide–alkyne 1,3-dipolar cycloaddition reaction included in the 'click chemistry' concept. The approach proved to be versatile and practical for the production of a series of spirostan saponin analogs bearing a triazole ring as surrogate of the traditional glycosidic linkage. Two different combinations were employed: (i) the conjugation of chacotriosyl azide to propargyl-spirostanes and (ii) the conjugation propargyl chacotrioside and a trisaccharidic analog to 3-azido-spirsotanes featuring either the 3α or 3β configuration. Both strategies resulted highly efficient—even for the direct conjugation of bulky steroids having sterically hindered axial azides—as well as adaptable for the utilization of spirostan sapogenins having diverse functionalization patterns on rings B and C. Although the obtained saponin analogs showed no significant cytotoxic activity against leukemia cells (HL-60), the wide spectrum of bioactivity exhibited by steroidal saponins shows promise toward the emergence of biological and medicinal applications for this new family of natural product analogs. A theoretical study based on molecular mechanics and semiempirical calculations was accomplished to provide more information about the conformational and electronic differences between the obtained saponins analogs and the natural one. The conformational search disclosed that there are important differences in the geometry of the preferred conformations as well as on the location of the frontier molecular orbitals between the natural product and its analogs. Indeed, this might be a cause for the lack of cytotoxic activity in the saponin analogs. Overall, this report represents the first experimental and theoretical work dealing the synthesis and structural study of spirostan saponin analogs having an artificial linkage (triazole) between the sugar and the steroid, and remarkably, the first approach on the use of steroidal azides in 'click chemistry' strategies.

4. Experimental section

4.1. General

Melting points are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded at 400 and 500 MHz for ^{1}H, 100 and 125 MHz for ^{13}C, respectively. Chemical shifts (δ) are reported in parts per million relative to the solvent signals, and coupling constants (J) are reported in hertz. NMR peak assignments were accomplished by analysis of the ^{1}H–^{1}H COSY and HSQC data. High resolution ESI mass spectra were obtained from a Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer, an RF-only hexapole ion guide and an external electrospray ion source. Flash column chromatography was carried out using silica gel 60 (230–400 mesh) and analytical thin layer chromatography (TLC) was performed using silica gel aluminum sheets. All commercially available chemicals were used without further purification.

*4.1.1. 3,6-Di-O-pivaloyl-β-D-glucopyranosyl azide (**5**).* To a suspension of compound **4** (2.12 g, 5.7 mmol) in MeOH (30 mL) was added NaOMe up to pH 9–10. The mixture was stirred for 1 h, then neutralized with acid resin Dowex-50 (H^+), filtered and the filtrate was concentrated under reduced pressure. The residue was dissolved in anhydrous pyridine (35 mL) and the solution was cooled to −15 °C. Pivaloyl chloride (3.1 mL, 28.4 mmol) was added dropwise under nitrogen atmosphere. The reaction course was monitored by TLC, and it was stirred at rt until the intermediate disappeared. The reaction mixture was then diluted with EtOAc (50 mL) and washed with dilute aq HCl, satd aq $NaHCO_3$, and brine. The organic phase was dried over anhydrous Na_2SO_4 and evaporated to dryness. The crude product was purified by flash column chromatography (hexane/EtOAc 3:1) to afford diol **5** (1.31 g, 62%) as a white solid. R_f=0.22 (hexane/EtOAc 3:1). Mp: 163–164 °C. $[\alpha]_D^{20}$ −35.3 (*c* 1.50, $CHCl_3$). IR (KBr, cm^{-1}) ν_{max}: 3447, 2961, 2925, 2853, 2373, 2346, 2118, 1724, 1288, 1160, 1052. ^{1}H NMR (400 MHz, $CDCl_3$): δ=1.21, 1.23 (2×s, 2×9H, 2× (CH_3)$_3$C); 3.39 (t, 1H, J=9.0 Hz, H-2); 3.46 (t, 1H, J=9.5 Hz, H-4); 3.65–3.70 (m, 1H, H-5); 4.33 (dd, 1H, J=5.6/12.2 Hz, H-6a); 4.41 (dd, 1H, J=2.5/12.1 Hz, H-6b); 4.65 (d, 1H, J=8.6 Hz, H-1); 4.93 (t, 1H, J=9.3 Hz, H-3). ^{13}C NMR (100 MHz, $CDCl_3$): δ=27.0, 27.1 (CH_3); 38.9, 39.0 (*C*); 62.9 (CH_2); 69.1, 72.1, 76.2, 77.6, 90.2 (CH); 179.1, 180.2 (C=O). HRMS (ESI-FT-ICR) *m/z*: 396.1749 $[M+Na]^+$ (calculated for $C_{16}H_{27}N_3O_7Na$: 396.1747).

*4.1.2. 2,4-Di-O-(2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl)-3,6-di-O-pivaloyl-β-D-glucopyranosyl azide (**7**).* A suspension of **5** (661 mg, 1.77 mmol) and 4 Å molecular sieves in CH_2Cl_2 (5 mL) was cooled to −80 °C and $BF_3{\cdot}Et_2O$ (0.99 mL, 7.79 mmol) was added under nitrogen atmosphere. Stirring was continued for 1 h at this temperature, and then a solution of rhamnopyranosyl trichloroacetimidate **6** (2.39 g, 5.49 mmol) in CH_2Cl_2 (5 mL) was added. The reaction mixture was stirred for additional 5 h at rt, then diluted with CH_2Cl_2 (30 mL) and filtered through a pad of Celite. The filtrate was washed with satd aq $NaHCO_3$ (15 mL) and brine (10 mL). The organic phase was dried over anhydrous Na_2SO_4 and evaporated to dryness. The crude product was purified by flash column chromatography (hexane/EtOAc 2:1) to afford trisaccharide **7** (1.27 g, 78%) as a white foam. R_f=0.25 (hexane/EtOAc 2:1). $[\alpha]_D^{20}$ −61.5 (*c* 1.30, $CHCl_3$). IR (KBr, cm^{-1}) ν_{max}: 2962, 2926, 2854, 2371, 2346, 2116, 1757, 1246, 1222, 1144, 1043. ^{1}H NMR (400 MHz, $CDCl_3$): δ=1.13 (d, 3H, J=6.4 Hz, CH_3 Rha); 1.15 (s, 9H, (CH_3)$_3$C); 1.20 (d, 3H, J=6.4 Hz, CH_3 Rha′); 1.21 (s, 9H, (CH_3)$_3$C); 1.93, 1.95, 2.01, 2.03, 2.08, 2.09 (6×s, 6×3H, 6× CH_3CO); 3.50 (t, 1H, J=7.9 Hz, H-2 Glc); 3.77 (t, 1H, J=7.9 Hz, H-4 Glc); 3.80–3.84 (m, 1H, H-5 Glc); 3.86–3.91 (m, 1H, H-5 Rha); 4.10–4.17 (m, 1H, H-5 Rha′); 4.27 (dd, 1H, J=4.5/12.2 Hz, H-6a Glc); 4.49 (dd, 1H, J=2.2/12.3 Hz, H-6b Glc); 4.64 (d, 1H, J=7.9 Hz, H-1 Glc); 4.81 (d, 1H, J=1.3 Hz, H-1 Rha); 4.85 (d, 1H, J=1.9 Hz, H-1 Rha′); 4.98–5.03 (m, 3H, H-2 Rha′, H-4 Rha, H-4 Rha′); 5.13–5.17 (m, 3H, H-2 Rha, H-3 Rha, H-3 Rha′), 5.25 (t, 1H, J=7.9 Hz, H-3 Glc). ^{13}C NMR (100 MHz, $CDCl_3$): δ=17.1, 17.2, 20.5, 20.6, 20.7, 26.8, 27.0 (CH_3); 38.7, 38.8 (*C*); 61.9 (CH_2); 67.4, 68.0, 68.6, 68.9, 69.4, 69.9, 70.4, 70.7, 74.2, 74.8, 75.9, 77.5, 88.0, 97.5, 98.3 (CH); 169.4, 169.6, 169.7, 169.8, 169.9, 176.3, 177.6 (C=O). HRMS (ESI-FT-ICR) *m/z*: 940.3546 $[M+Na]^+$ (calculated for $C_{40}H_{59}N_3O_{21}Na$: 940.3539).

*4.1.3. Propargyl 2,3-di-O-acetyl-β-D-glucopyranoside (**9**).* To a suspension of compound **8** (2.27 g, 5.88 mmol) in MeOH (30 mL) was added NaOMe until pH 9–10. The mixture was stirred for 1 h, then neutralized with acid resin Dowex-50 (H^+), filtered and the filtrate was concentrated under reduced pressure. The residue was dissolved in anhydrous DMF (80 mL) and benzaldehyde dimethyl acetal (6.4 mL, 42.3 mmol) was added. The pH was adjusted to 3 with *p*-TsOH and the solution was rotated in the rotavapor under reduced pressure at 50 °C for 3 h. The reaction was quenched with Et_3N and the volatiles were removed under reduced pressure. Flash column chromatography (hexane/EtOAc 1:1) afforded propargyl 4,6-*O*-benzylidene-β-D-glucopyranoside (1.66 g, 92%). The solution of this product in anhydrous pyridine (10 mL) was treated with Ac_2O (10 mL). The resulting reaction mixture was stirred at rt overnight and then poured into water and extracted with EtOAc (30 mL). The organic layer was washed with dilute aq HCl and brine, then dried over anhydrous Na_2SO_4 and evaporated to dryness. The residue was dissolved in 1:1 THF–MeOH (30 mL) and *p*-TsOH·H_2O (1.13 g, 5.96 mmol) was added. After 3 h the reaction was quenched with Et_3N and the solvents were removed under reduced pressure. The crude product was purified by flash column chromatography (hexane/EtOAc 1:4) to afford diol **9** (1.54 g, 94% from 4,6-*O*-benzylidene-β-D-glucopyranoside and 87% from **8**) as a white foam. R_f=0.38 (hexane/EtOAc 1:4). $[\alpha]_D^{20}$ −30.2 (*c* 1.20, $CHCl_3$). IR (KBr, cm^{-1}) ν_{max}: 3431, 3283, 2935, 2871, 2374, 1743, 1372, 1249, 1077, 1052. ^{1}H NMR (400 MHz, $CDCl_3$): δ=2.05, 2.08 (2×s, 2×3H, 2× CH_3CO); 2.48 (t, 1H, J=2.3 Hz, C≡CH); 2.78 (m, 2H, 2× OH); 3.42–3.46 (m, 1H, H-5); 3.76 (t, 1H, J=9.5 Hz, H-4); 3.84 (dd, 1H, J=4.3/12.1 Hz, H-6a); 3.92 (dd, 1H, J=3.2/12.1 Hz, H-6b); 4.35 (m, 2H, OCH_2); 4.76 (d, 1H, J=8.0 Hz, H-1); 4.90 (dd, 1H, J=8.0/9.6 Hz, H-2); 5.07 (t, 1H, J=9.5 Hz, H-3). ^{13}C NMR (100 MHz, $CDCl_3$): δ=20.7, 20.8 (CH_3); 56.2, 61.8 (CH_2); 69.0, 71.1 (CH); 75.4 (*C*); 75.6, 75.7, 98.5 (CH); 169.8, 171.5 (C=O). HRMS (ESI-FT-ICR) *m/z*: 325.0897 $[M+Na]^+$ (calculated for $C_{13}H_{18}O_8Na$: 325.0899).

*4.1.4. Propargyl 3,6-di-O-pivaloyl-β-D-glucopyranoside (**10**).* To a suspension of compound **8** (1.17 g, 3.03 mmol) in MeOH (15 mL) was added NaOMe until pH 9–10. The mixture was stirred for 1 h, then neutralized with acid resin Dowex-50 (H^+), filtered and the filtrate was concentrated under reduced pressure. The residue was dissolved in anhydrous pyridine (20 mL) and the solution was cooled to −15 °C. Pivaloyl chloride (1.9 mL, 15.2 mmol) was added dropwise under nitrogen atmosphere. The reaction was monitored by TLC, and it was stirring at rt until the intermediate disappeared. The reaction mixture was then diluted with EtOAc (60 mL) and washed with a dilute HCl solution, satd aq $NaHCO_3$, and brine. The organic phase was dried over anhydrous Na_2SO_4 and evaporated to dryness. The crude product was purified by flash column chromatography (hexane/EtOAc 4:1→3:1) to afford diol **10** (738 mg, 63%) as a white solid. R_f=0.32 (hexane/EtOAc 2:1). Mp: 96–97 °C. $[\alpha]_D^{20}$ −35.6 (*c* 1.35, $CHCl_3$). IR (KBr, cm^{-1}) ν_{max}: 3448, 2973, 2932, 2878, 2373, 1720, 1288, 1159, 1082, 1045. ^{1}H NMR (400 MHz, $CDCl_3$): δ=1.21, 1.24 (2×s, 2×9H, 2× (CH_3)$_3$C); 2.48 (t, 1H, J=2.4 Hz, C≡CH); 3.47 (t, 1H, J=9.6 Hz, H-4); 3.51 (dd, 1H, J=7.8/9.5 Hz, H-2); 3.57–3.61 (m, 1H, H-5); 4.30 (dd, 1H, J=6.0/12.0 Hz, H-6a);

4.33–4.44 (m, 3H, OCH_2, H-6b); 4.59 (d, 1H, J=7.7 Hz, H-1); 4.92 (t, 1H, J=9.3 Hz, H-3). ^{13}C NMR (100 MHz, $CDCl_3$): δ=27.5 (CH_3); 39.3, 39.4 (*C*); 56.3, 63.6 (CH_2); 70.1, 72.3, 74.9 (*C*H); 75.9 (*C*); 78.1, 78.7, 100.6 (*C*H); 179.1, 180.6 (*C*=O). HRMS (ESI-FT-ICR) m/z: 409.1849 $[M+Na]^+$ (calculated for $C_{19}H_{30}O_8Na$: 409.1838).

*4.1.5. Propargyl 4,6-di-O-(2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl)-2,3-di-O-acetyl-β-D-glucopyranoside (**11**).* A suspension of **9** (343 mg, 1.14 mmol) and 4 Å molecular sieves in CH_2Cl_2 (3.5 mL) was cooled to −80 °C and $BF_3 \cdot Et_2O$ (0.64 mL, 5.02 mmol) was added under nitrogen atmosphere. After stirring for 1 h at this temperature, a solution of rhamnopyranosyl trichloroacetimidate **6** (1.53 g, 3.53 mmol) in CH_2Cl_2 (3.5 mL) was added and it was stirred for 5 h at rt. The reaction mixture was diluted with CH_2Cl_2 (20 mL) and filtered through a pad of Celite. The filtrate was washed with satd aq $NaHCO_3$ (15 mL) and brine (10 mL). The organic phase was dried over anhydrous Na_2SO_4 and evaporated to dryness. The crude product was purified by flash column chromatography (hexane/EtOAc 1:1) to afford trisaccharide **11** (762 mg, 79%) as a white solid. R_f=0.34 (hexane/EtOAc 1:1). Mp: 132–134 °C. $[\alpha]_D^{20}$ −71.5 (*c* 1.30, $CHCl_3$). IR (KBr, cm^{-1}) ν_{max}: 2982, 2373, 1749, 1373, 1242, 1222, 1052, 1043. ^{1}H NMR (400 MHz, $CDCl_3$): δ=1.13 (d, 3H, J=6.2 Hz, CH_3 Rha); 1.18 (d, 3H, J=6.2 Hz, CH_3 Rha′); 1.94, 1.95, 2.01, 2.04, 2.05, 2.06, 2.08, 2.10 (8×s, 8×3H, 8× CH_3CO); 2.46 (t, 1H, J=2.4 Hz, C≡C*H*); 3.55–3.59 (m, 1H, H-5 Glc); 3.76–3.89 (m, 3H, H-5 Rha, H-4 Glc, H-6a Glc); 3.91–3.99 (m, 2H, H-5 Rha′, H-6b Glc); 4.33 (d, 2H, J=2.5 Hz, OCH_2); 4.74 (d, 1H, J=7.9 Hz, H-1 Glc); 4.80 (d, 1H, J=1.5 Hz, H-1 Rha); 4.81 (d, 1H, J=1.9 Hz, H-1 Rha′); 4.88 (dd, 1H, J=7.9/9.3 Hz, H-2 Glc); 4.99–5.07 (m, 3H, H-4 Rha, H-4 Rha′, H-2 Rha); 5.12 (dd, 1H, J=3.2/10.3 Hz, H-3 Rha′); 5.16–5.23 (m, 3H, H-2 Rha′, H-3 Rha, H-3 Glc). ^{13}C NMR (100 MHz, $CDCl_3$): δ=17.2, 17.3, 20.6, 20.7, 20.8, 20.9, 21.0 (CH_3); 55.6, 65.4 (CH_2); 66.4, 67.8, 68.8, 69.2, 69.4, 69.6, 70.4, 70.9, 71.7, 73.6, 73.7 (*C*H); 75.4 (*C*); 76.9 (*C*H); 78.1 (*C*); 97.4, 98.0, 99.8 (*C*H); 169.6, 169.7, 169.8, 169.9, 170.1, 170.2, 170.3, 170.4 (*C*=O). HRMS (ESI-FT-ICR) m/z: 869.2684 $[M+Na]^+$ (calculated for $C_{37}H_{50}O_{22}Na$: 869.2691).

*4.1.6. Propargyl 2,4-di-O-(2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl)-3,6-di-O-pivaloyl-β-D-glucopyranoside (**12**).* A suspension of **10** (620 mg, 1.60 mmol) and 4 Å molecular sieves in CH_2Cl_2 (5 mL) was cooled to −80 °C and $BF_3 \cdot Et_2O$ (0.9 mL, 7.04 mmol) was added under nitrogen atmosphere. After stirring for 1 h at this temperature, a solution of rhamnopyranosyl trichloroacetimidate **6** (2.16 g, 4.97 mmol) in CH_2Cl_2 (5 mL) was added and it was stirred for 5 h at rt. The reaction mixture was diluted with CH_2Cl_2 (30 mL) and filtered through a pad of Celite. The filtrate was washed with satd aq $NaHCO_3$ (15 mL) and brine (10 mL). The organic phase was dried over anhydrous Na_2SO_4 and evaporated to dryness. The crude product was purified by flash column chromatography (hexane/EtOAc 2:1) to afford trisaccharide **12** (1.28 g, 86%) as a white solid. R_f=0.34 (hexane/EtOAc 3:2). Mp: 127–128 °C. $[\alpha]_D^{20}$ −46.7 (*c* 1.35, $CHCl_3$). IR (KBr, cm^{-1}) ν_{max}: 2980, 2938, 2870, 2371, 1748, 1369, 1244, 1225, 1145, 1080, 1044. ^{1}H NMR (400 MHz, $CDCl_3$): δ=1.14 (d, 3H, J=6.4 Hz, CH_3 Rha); 1.15 (s, 9H, (CH_3)$_3$C); 1.17 (d, 3H, J=6.4 Hz, CH_3 Rha′); 1.21 (s, 9H, (CH_3)$_3$C); 1.93, 1.95, 2.01, 2.02, 2.08, 2.10 (6×s, 6×3H, 6× CH_3CO); 2.48 (t, 1H, J=2.4 Hz, C≡C*H*); 3.57 (t, 1H, J=7.3 Hz, H-2 Glc); 3.76 (m, 2H, H-4 Glc, H-5 Glc); 3.89 (m, 1H, H-5 Rha); 4.20 (m, 1H, H-5 Rha′); 4.24 (dd, 1H, J=4.6/11.9 Hz, H-6a Glc); 4.33 (d, 2H, J=2.5 Hz, OCH_2); 4.47 (dd, 1H, J=1.6/12.0 Hz, H-6b Glc); 4.72 (d, 1H, J=7.0 Hz, H-1 Glc); 4.79 (d, 1H, J=1.6 Hz, H-1 Rha); 4.85 (d, 1H, J=2.0 Hz, H-1 Rha′); 4.97–5.04 (m, 3H, H-2 Rha′, H-4 Rha, H-4 Rha′); 5.15–5.28 (m, 4H, H-2 Rha, H-3 Rha, H-3 Rha′, H-3 Glc). ^{13}C NMR (100 MHz, $CDCl_3$): δ=17.0, 17.1, 20.6, 20.7, 26.8, 27.1 (CH_3); 38.7, 38.8 (*C*); 55.5, 62.4 (CH_2); 66.8, 67.9, 68.7, 69.0, 69.6, 69.9, 70.5, 70.9, 72.3, 75.0, 75.6 (*C*H); 76.5 (*C*); 77.7, 78.0, 97.4, 98.1, 98.2 (*C*H); 169.5, 169.6, 169.7, 169.8, 169.9, 170.0, 176.4, 177.7 (*C*=O). HRMS (ESI-FT-ICR) m/z: 953.3623 $[M+Na]^+$ (calculated for $C_{43}H_{62}O_{22}Na$: 953.3630).

*4.1.7. (25R)-3β-Propargyloxy-spirost-5-ene (**14**).* NaH (60% in mineral oil, 362 mg, 9.05 mmol, washed with hexane) was added to a stirred solution of diosgenin **13** (2.5 g, 6.03 mmol) in dry DMF (100 mL) at 0 °C. The suspension was stirred for 10 min under nitrogen atmosphere, treated with propargyl bromide (18.1 mL, 9.06 mmol) and then stirred at rt for additional 10 h. The reaction mixture was poured into 100 mL of cold water and extracted with CH_2Cl_2 (3×50 mL). The combined organic phases were washed with aq 10% HCl (50 mL), brine (50 mL), and then dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to dryness. The crude product was purified by flash column chromatography (*n*-hexane/EtOAc 5:1) to yield compound **14** (2.02 g, 74%) as a pale yellow solid. R_f=0.34 (*n*-hexane/EtOAc 5:1). Mp: 215–217 °C. $[\alpha]_D^{20}$ −20.4 (*c* 1.40, $CHCl_3$). IR (KBr, cm^{-1}) ν_{max}: 3567, 2925, 2854, 2371, 1560, 1118. ^{1}H NMR (400 MHz, $CDCl_3$): δ=0.78 (d, 3H, J=6.9 Hz, CH_3); 0.79 (s, 3H, CH_3); 0.97 (d, 3H, J=6.9 Hz, CH_3); 1.01 (s, 3H, CH_3); 2.40 (t, 1H, J=2.3 Hz, C≡C*H*); 3.36 (t, 1H, J=10.9 Hz, H-26ax); 3.39 (m, 1H, H-3α); 3.47 (dd, 1H, J=4.1/11.0 Hz, H-26eq); 4.18 (m, 2H, OCH_2); 4.40 (m, 1H, H-16α); 5.35 (m, 1H, H-6). ^{13}C NMR (100 MHz, $CDCl_3$): δ=14.6, 16.4, 17.2, 19.4 (CH_3); 20.9, 28.1, 28.8 (CH_2); 30.3, 31.4 (*C*H); 31.5, 31.9, 32.1, 37.0 (CH_2); 37.1 (*C*); 38.7, 39.8 (CH_2); 40.3 (*C*); 41.6, 50.1 (*C*H); 55.1 (CH_2); 56.5, 62.0 (*C*H); 66.8 (CH_2); 73.9 (*C*); 78.1, 80.8 (*C*H); 109.2 (*C*); 121.5 (*C*H); 140.4 (*C*). HRMS (ESI-FT-ICR) m/z: 475.3153 $[M+Na]^+$ (calculated for $C_{30}H_{44}O_3Na$: 475.3188).

*4.1.8. (25R)-3β-Azido-5α-spirostan-12-one (**17**).* Methanesulfonic acid (0.16 mL, 2.44 mmol) was added to a stirred solution of hecogenin **15** (500 mg, 1.16 mmol), Ph_3P (913 mg, 3.48 mmol), and DMAP (298 mg, 2.44 mmol) in dry THF (3.5 mL) under nitrogen atmosphere. DIAD, diisopropylazodicarboxylate (0.69 mL, 3.48 mmol) was added dropwise over a 15 min period. The reaction mixture was stirred vigorously for 48 h at rt. The solvent was evaporated under reduced pressure and the resulting residue was partially purified by flash column chromatography (*n*-hexane/EtOAc 2:1), yielding impure 3α-methanesulfonate as a white solid (610 mg), R_f=0.68 (*n*-hexane/EtOAc 1:1). The crude methanesulfonate was dissolved in DMPU (10 mL) and NaN_3 (189 mg, 2.90 mmol) was added. The resulting mixture was stirred vigorously under nitrogen atmosphere at rt for 48 h and then diluted with Et_2O (100 mL). The organic phase was washed with aq 10% HCl (2×30 mL) and brine (30 mL), dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to dryness. The crude product was purified by flash column chromatography (*n*-hexane/EtOAc 8:1) to give the pure azide **17** (365 mg, 69% from **15**) as a white solid. R_f=0.47 (*n*-hexane/EtOAc 6:1). Mp: 231–232 °C. $[\alpha]_D^{20}$ −2.1 (*c* 1.40, $CHCl_3$). IR (KBr, cm^{-1}) ν_{max}: 3567, 2925, 2854, 2371, 2346, 1702, 1654, 1560, 1118. ^{1}H NMR (400 MHz, $CDCl_3$): δ=0.78 (d, 3H, J=6.4 Hz, CH_3); 0.89 (s, 3H, CH_3); 1.04 (s, 3H, CH_3); 1.06 (d, 3H, J=7.0 Hz, CH_3); 2.20 (dd, 1H, J=5.0/14.3 Hz, H-11β); 2.38 (t, 1H, J=14.3 Hz, H-11α); 3.22–3.29 (m, 1H, H-3α); 3.33 (t, 1H, J=10.9 Hz, H-26ax); 3.45–3.49 (m, 1H, H-26eq); 4.30–4.36 (m, 1H, H-16α). ^{13}C NMR (100 MHz, $CDCl_3$): δ=11.8, 13.2, 16.0, 17.1 (CH_3); 27.4, 28.1, 28.8 (CH_2); 30.2 (*C*H); 31.1, 31.4, 31.5, 33.8 (CH_2); 34.3 (*C*H); 36.1 (*C*); 36.6, 37.6 (CH_2); 42.2, 45.0, 53.5 (*C*H); 55.1 (*C*); 55.4, 55.7, 60.2 (*C*H); 66.9 (CH_2); 79.1 (*C*H); 109.2 (*C*); 213.2 (*C*=O). HRMS (ESI-FT-ICR) m/z: 478.3023 $[M+Na]^+$ (calculated for $C_{27}H_{41}N_3O_3Na$: 478.3046).

*4.1.9. (25R)-3β-Azido-5-hydroxy-5α-spirostan-6-one (**18**).* Ketol **16** (2.0 g, 4.48 mmol), methanesulfonic acid (0.61 mL, 9.41 mmol), Ph_3P (3.51 g, 13.4 mmol), DMAP (1.15 g, 9.41 mmol), and diisopropylazodicarboxylate (2.64 mL, 13.4 mmol) in dry THF (15 mL) were reacted in a similar way as described in the synthesis of **17**. Flash column chromatography purification (hexane/EtOAc 1:1) afforded

the corresponding 3α-mesylate as a crude product (2.24 g). This compound was subjected to nucleophilic substitution with NaN_3 (350 mg, 5.38 mmol) in DMPU (20 mL) in a similar way as described in the synthesis of **17**. The crude product was purified by flash column chromatography (hexane/EtOAc 5:1) to furnish azide **18** (1.4 g, 66% from **16**) as a white solid. R_f=0.36 (*n*-hexane/EtOAc 5:1). Mp: 255–257 °C. $[\alpha]_D^{20}$ −41.7 (*c* 0.60, $CHCl_3$). IR (KBr, cm^{-1}) ν_{max}: 2925, 2850, 2373, 2346, 2095, 1701, 1654, 1560, 1465. ^1H NMR (400 MHz, $CDCl_3$): δ=0.76 (s, 3H, C*H*$_3$); 0.79 (d, 3H, *J*=6.2 Hz, C*H*$_3$); 0.82 (s, 3H, C*H*$_3$); 0.97 (d, 3H, *J*=6.8 Hz, CH_3); 2.16 (dd, 1H, *J*=3.5/13.0 Hz, H-7β); 2.73 (t, 1H, *J*=13.0 Hz, H-7α); 3.36 (t, 1H, *J*=10.9 Hz, H-26ax); 3.46 (dd, 1H, *J*=4.3/10.9 Hz, H-26eq); 3.65 (m, 1H, H-3α); 4.41 (m, 1H, H-16α). ^{13}C NMR (100 MHz, $CDCl_3$): δ=14.0, 14.4, 16.4, 17.1 (*C*H$_3$); 21.1, 26.3, 28.7, 29.6 (*C*H$_2$); 30.7 (*C*H); 31.2, 31.4, 32.8 (*C*H$_2$); 36.6 (*C*H); 39.5 (*C*H$_2$); 41.0 (*C*); 41.5 (*C*H); 41.8 (*C*H$_2$); 42.3, 44.4, 56.0, 56.3, 61.8 (*C*H); 66.8 (*C*H$_2$); 79.8 (*C*); 80.4 (*C*H); 109.3 (*C*); 211.6 (*C*=O). HRMS (ESI-FT-ICR) *m*/*z*: 494.2986 $[M+Na]^+$ (calculated for: $C_{27}H_{41}N_3O_4Na$: 494.2995).

*4.1.10. (25R)-3α-Azido-5α-spirostan-12-one (**19**).* Hecogenin **15** (5.0 g, 11.6 mmol) was dissolved in dry pyridine (25 mL) and TsCl (6.6 g, 34.8 mmol) was added. The reaction mixture was stirred at rt until TLC (hexane/EtOAc 3:1) analysis revealed that all the hecogenin was consumed. The reaction mixture was slowly poured into 100 mL of sat aq $NaHCO_3$ and the precipitate formed was separated by filtration under reduced pressure and washed with water several times. The resulting crude product was dried over anhydrous $CaCl_2$ and recrystallized from EtOH to give the corresponding tosylate (6.3 g, 93%) as a white solid. This product was dissolved in DMPU (80 mL) and then NaN_3 (2.1 g, 32.3 mmol) was added. The reaction mixture was stirred vigorously under nitrogen atmosphere at 60 °C for 48 h and then diluted with Et_2O (500 mL). The organic phase was washed with aq 10% HCl (2×100 mL) and brine (100 mL), then dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to dryness. The crude product was purified by flash column chromatography (*n*-hexane/EtOAc 8:1) to give the azide **19** (4.4 g, 84% from **15**) as a white solid. R_f=0.58 (*n*-hexane/EtOAc 6:1). Mp: 222–223 °C. $[\alpha]_D^{20}$ −92.4 (*c* 1.10, $CHCl_3$). IR (KBr, cm^{-1}) ν_{max}: 3567, 2925, 2854, 2371, 2346, 1718, 1654, 1560, 1118. ^1H NMR (400 MHz, $CDCl_3$): δ=0.78 (d, 3H, *J*=6.5 Hz, C*H*$_3$); 0.87 (s, 3H, C*H*$_3$); 1.03 (s, 3H, C*H*$_3$); 1.06 (d, 3H, *J*=7.0 Hz, C*H*$_3$); 2.21 (dd, 1H, *J*=5.0/14.1 Hz, H-11β); 2.35 (t, 1H, *J*=14.1 Hz, H-11α); 3.34 (t, 1H, *J*=11.1 Hz, H-26ax); 3.48 (m, 1H, H-26eq); 3.89 (m, 1H, H-3β); 4.31–4.37 (m, 1H, H-16α). ^{13}C NMR (100 MHz, $CDCl_3$): δ=11.2, 13.2, 16.0, 17.1 (*C*H$_3$); 25.4, 27.8, 28.7 (*C*H$_2$); 30.2 (*C*H); 31.0, 31.4, 32.4 (*C*H$_2$); 34.2 (*C*H); 36.4 (*C*); 37.3 (*C*H$_2$); 39.8, 42.2, 53.5 (*C*H); 55.0 (*C*); 55.2, 55.7, 57.8 (*C*H); 55.1 (*C*); 55.2, 55.7, 57.8 (*C*H); 66.8 (*C*H$_2$); 79.1 (*C*H); 109.2 (*C*); 213.4 (*C*=O). HRMS (ESI-FT-ICR) *m*/*z*: 478.3013 $[M+Na]^+$ (calculated for $C_{27}H_{41}N_3O_3Na$: 478.3046).

*4.1.11. (25R)-3α-Azido-5-hydroxy-5α-spirostan-6-one (**20**).* Ketol **16** (2.0 g, 4.48 mmol) was subjected to tosylation (TsCl, 2.5 g, 13.4 mmol in dry pyridine 15 mL) and followed by nucleophilic substitution with NaN_3 (350 mg, 5.38 mmol) in DMPU (20 mL) in a similar way as described in the synthesis of **19**. The crude product was purified by flash column chromatography (*n*-hexane/EtOAc 6:1) to give the azide **20** (1.5 g, 71% from **16**) as a white solid. R_f=0.42 (hexane/EtOAc 6:1). Mp: 243–244 °C. $[\alpha]_D^{20}$ −129.2 (*c* 1.20, $CHCl_3$). IR (KBr, cm^{-1}) ν_{max}: 3567, 2925, 2854, 2371, 2346, 2115, 1718, 1561, 1458, 1240. ^1H NMR (400 MHz, $CDCl_3$): δ=0.76 (m, 9H, 3× C*H*$_3$); 0.96 (d, 3H, *J*=6.7 Hz, C*H*$_3$); 2.73 (t, 1H, *J*=12.4 Hz, H-7α); 3.35 (t, 1H, *J*=10.9 Hz, H-26ax); 3.45–3.48 (m, 1H, H-26eq); 3.69 (s, 1H, O*H*); 4.08 (m, 1H, H-3β); 4.37–4.43 (m, 1H, H-16α). ^{13}C NMR (100 MHz, $CDCl_3$): δ=13.7, 14.4, 16.4, 17.1 (*C*H$_3$); 20.8, 24.4, 25.5 (*C*H$_2$); 28.7 (*C*H); 29.5, 30.2, 31.3, 31.5, 36.9 (*C*H$_2$); 39.6 (*C*); 41.0 (*C*H); 41.5 (*C*H$_2$); 41.6, 43.3, 44.6 (*C*H); 56.1 (*C*); 57.2 (*C*H); 62.0 (*C*); 66.8 (*C*H$_2$); 79.3, 80.5 (*C*H); 109.2 (*C*); 210.6 (*C*=O). HRMS (ESI-FT-ICR) *m*/*z*: 494.2972 $[M+Na]^+$ (calculated for: $C_{27}H_{41}N_3O_4Na$: 494.2995).

*4.1.12. Click conjugation procedure: triazole-based trisaccharide–spirostan conjugate **21**.* Alkyne **14** (31 mg, 0.067 mmol) and azide **7** (51.3 mg, 0.056 mmol) were dissolved in THF (3 mL) and treated with a 8 M aqueous solution of $Cu(OAc)_2 \cdot H_2O$ (5.5 μL, 0.011 mmol) and a freshly prepared 8 M aqueous solution of sodium ascorbate (11 μL, 0.022 mmol). The reaction mixture was stirred at rt until completion as indicated by TLC, and then concentrated to dryness. The crude product was purified by flash column chromatography (hexane/EtOAc 3:2) to give conjugate **21** (69 mg, 89%) as a white solid. R_f=0.26 (hexane/EtOAc 3:2). Mp: 179–180 °C. $[\alpha]_D^{20}$ −70.0 (*c* 1.50, $CHCl_3$). IR (KBr, cm^{-1}) ν_{max}: 2927, 2854, 2371, 2346, 1750, 1246, 1222, 1143, 1052. ^1H NMR (400 MHz, $CDCl_3$): δ=0.78 (m, 6H, 2× C*H*$_3$); 0.84 (d, 3H, *J*=6.2 Hz, C*H*$_3$ Rha); 0.96 (d, 3H, *J*=6.9 Hz, C*H*$_3$); 1.01 (s, 3H, C*H*$_3$); 1.18 (m, 21H, 2× (C*H*$_3$)$_3$C, C*H*$_3$ Rha′); 1.91, 1.97, 1.98, 2.04, 2.05, 2.12 (6×s, 6×3H, 6× C*H*$_3$CO); 2.66–2.73 (m, 1H, H-5 Rha′); 3.31 (m, 1H, H-3α); 3.37 (t, 1H, *J*=10.9 Hz, H-26ax); 3.45–3.49 (m, 1H, H-26eq); 3.89–3.96 (m, 2H, H-4 Glc, H-5 Rha); 3.98–4.02 (m, 1H, H-5 Glc); 4.30 (dd, 1H, *J*=4.5/12.3 Hz, H-6a Glc); 4.35–4.45 (m, 3H, H-2 Glc, H-16α, H-6b Glc); 4.67 (s, 2H, OC*H*$_2$); 4.78 (d, 1H, *J*=1.8 Hz, H-1 Rha); 4.81 (t, 1H, *J*=10.0 Hz, H-4 Rha′); 4.92 (d, 1H, *J*=1.9 Hz, H-1 Rha′); 4.99–5.08 (m, 3H, H-2 Rha, H-3 Rha′, H-4 Rha,); 5.13 (dd, 1H, *J*=1.9/3.3 Hz, H-2 Rha); 5.18 (dd, 1H, *J*=3.3/10.1 Hz, H-3 Rha); 5.34 (m, 1H, H-6 diosgenin); 5.47 (t, 1H, *J*=7.6 Hz, H-3 Glc); 5.75 (d, 1H, *J*=8.6 Hz, H-1 Glc); 7.71 (s, 1H, H-triazole). ^{13}C NMR (100 MHz, $CDCl_3$): δ=14.5, 16.3, 17.1, 17.2, 17.5, 19.4, 20.5, 20.6, 20.7 (*C*H$_3$); 20.8 (*C*H$_2$); 26.8, 27.1 (*C*H$_3$); 28.3, 28.8 (*C*H$_2$); 30.3 (*C*H); 31.3 (*C*H$_2$); 31.4 (*C*H); 31.8, 32.1 (*C*H$_2$); 36.9 (*C*); 37.1 (*C*H$_2$); 38.8, 38.9 (*C*); 39.0, 39.8 (*C*H$_2$); 40.3 (*C*); 41.6, 50.1, 56.5 (*C*H); 61.7, 61.9 (*C*H$_2$); 62.1, 68.3, 68.6, 68.7, 69.4, 69.9, 70.4, 75.2, 75.7, 77.4, 79.1, 80.8, 86.3, 97.4, 98.1 (*C*H); 109.3 (*C*); 121.3, 121.6 (*C*H); 140.6, 146.8 (*C*); 169.1, 169.3, 169.4, 169.8, 169.9, 170.0, 176.3, 177.6 (*C*=O). HRMS (ESI-FT-ICR) *m*/*z*: 1392.6824 $[M+Na]^+$ (calculated for $C_{70}H_{103}N_3O_{24}Na$: 1392.6829).

*4.1.13. Deprotection procedure A: saponin analog **22**.* An aqueous solution of NaOH (0.5 M, 0.75 mL) was added to a solution of conjugate **21** (35 mg, 0.026 mmol) in the solvent mixture MeOH/THF/H_2O (1.6 mL, 1:1:1, v/v/v) and the reaction mixture was stirred at 50 °C overnight. The solution was neutralized with acid resin Dowex-50 (H^+) and then filtered to remove the resin. The filtrate was concentrated under reduced pressure and the resulting crude product was purified by flash column chromatography (EtOAc/MeOH 5:1) to furnish **22** (33 mg, 93%) as a white amorphous solid. R_f=0.28 (EtOAc/MeOH 5:1). $[\alpha]_D^{20}$ −82.1 (*c* 1.45, $CHCl_3$). IR (KBr, cm^{-1}) ν_{max}: 3567, 2371, 2346, 1654, 1560. ^1H NMR (500 MHz, pyridine-d_5): δ=0.71 (d, 3H, *J*=5.8 Hz, C*H*$_3$); 0.87 (s, 3H, C*H*$_3$); 0.99 (s, 3H, C*H*$_3$); 1.16 (d, 3H, *J*=7.0 Hz, C*H*$_3$); 1.55 (d, 3H, *J*=6.1 Hz, C*H*$_3$ Rha); 1.64 (d, 3H, *J*=6.2 Hz, C*H*$_3$ Rha′); 2.40 (m, 1H); 2.63 (m, 1H); 3.09–3.15 (m, 1H, H-5 Rha); 3.47–3.55 (m, 2H, H-26ax, H-3α); 3.59–3.62 (m, 1H, H-26eq); 3.89–3.93 (m, 1H, H-5 Glc); 4.03–4.21 (m, 4H, H-6a Glc, H-6b Glc, H-4 Rha, H-4 Rha′); 4.29–4.37 (m, 2H, H-3 Glc, H-3 Rha′); 4.50–4.60 (m, 3H, H-3 Rha, H-4 Glc, H-16α); 4.68 (m, 1H, H-2 Rha); 4.77 (m, 1H, H-2 Rha′); 4.84 (t, 1H, *J*=9.2 Hz, H-2 Glc); 4.88 (m, 1H, H-5 Rha′); 4.95 (d, 1H, *J*=11.9 Hz, OC*H*$_2$); 5.39 (m, 1H, H-6 diosgenin); 5.86 (d, 1H, *J*=1.3 Hz, H-1 Rha); 6.27 (d, 1H, *J*=1.3 Hz, H-1 Rha′); 6.31 (d, 1H, *J*=9.3 Hz, H-1 Glc); 8.60 (s, 1H, H-triazole). ^{13}C NMR (125 MHz, pyridine-d_5): δ=15.4, 16.8, 17.7, 18.9, 19.4, 19.8 (*C*H$_3$); 21.6, 29.2, 29.7 (*C*H$_2$); 31.0, 32.1 (*C*H); 32.2, 32.6, 32.7 (*C*H$_2$); 37.6 (*C*); 37.7, 39.8, 40.3 (*C*H$_2$); 40.9 (*C*); 42.4, 50.7, 57.1 (*C*H); 61.2, 62.5 (*C*H$_2$); 63.3 (*C*H); 67.3 (*C*H$_2$); 70.5, 70.9, 72.6, 72.7, 72.8, 73.1, 74.1, 74.2, 77.9, 78.3, 79.5, 79.6, 80.6, 81.5, 88.1, 103.3, 103.5 (*C*H); 109.7 (*C*); 122.1, 122.9 (*C*H); 141.4, 147.2 (*C*). HRMS (ESI-

FT-ICR) *m*/*z*: 972.5042 $[M+Na]^+$ (calculated for $C_{48}H_{75}N_3O_{16}Na$: 972.5045).

*4.1.14. Triazole-based trisaccharide–spirostan conjugate **23**.* Alkyne **12** (100 mg, 0.107 mmol) and azide **17** (58.3 mg, 0.128 mmol) in THF (3 mL) were reacted in the presence of $Cu(OAc)_2\cdot H_2O$ (0.021 mmol) and sodium ascorbate (0.043 mmol) according to the click conjugation procedure. Flash column chromatography purification (hexane/EtOAc 1:1) afforded conjugate **23** (114 mg, 77%) as a white solid. R_f=0.38 (hexane/EtOAc 1:2). Mp: 187–188 °C. $[\alpha]_D^{20}$ −48.7 (*c* 1.50, $CHCl_3$). IR (KBr, cm^{-1}) ν_{max}: 2955, 2925, 2857, 2375, 2346, 1748, 1460, 1369, 1244, 1222, 1042. ^1H NMR (400 MHz, $CDCl_3$): δ=0.78 (d, 3H, *J*=6.4 Hz, CH_3); 1.01 (s, 3H, CH_3); 1.06 (s, 3H, CH_3); 1.07 (d, 3H, *J*=6.9 Hz, CH_3); 1.15–1.17 (m, 15H, (CH_3)$_3$C, CH_3 Rha, CH_3 Rha′); 1.21 (s, 9H, (CH_3)$_3$C); 1.93, 1.97, 2.03, 2.05, 2.08, 2.11 (6×s, 6×3H, 6× CH_3CO); 2.25 (dd, 1H, *J*=5.0/14.3 Hz, H-11β); 2.44 (t, 1H, *J*=14.0 Hz, H-11α); 2.53 (dd, 1H, *J*=6.7/8.7 Hz); 3.34 (t, 1H, *J*=11.0 Hz, H-26ax); 3.46–3.51 (m, 1H, H-26eq); 3.58 (t, 1H, *J*=7.5 Hz, H-2 Glc); 3.77 (m, 2H, H-4 Glc, H-5 Glc); 3.87–3.94 (m, 1H, H-5 Rha); 4.01–4.08 (m, 1H, H-5 Rha′); 4.27–4.38 (m, 2H, H-16α, H-6a Glc); 4.46 (m, 1H, H-3α); 4.55 (m, 1H, H-6b Glc); 4.62 (d, 1H, *J*=7.5 Hz, H-1 Glc); 4.79 (d, 1H, *J*=1.4 Hz, H-1 Rha); 4.86 (d, 2H, *J*=1.9 Hz, H-1 Rha′); 4.92–5.07 (m, 5H, H-2 Rha, H-4 Rha, H-4 Rha′, OCH_2); 5.13–5.19 (m, 3H, H-2 Rha, H-3 Rha, H-3 Rha′); 5.26 (t, 1H, *J*=8.0 Hz, H-3 Glc), 7.68 (s, 1H, H-triazole). ^{13}C NMR (100 MHz, $CDCl_3$): δ=11.9, 13.2, 15.9, 16.9, 17.1, 17.2, 20.6, 20.7, 20.8, 20.9, 26.8, 27.2 (CH_3); 28.0, 29.7 (CH_2); 30.0 (*C*); 30.2 (CH); 31.1, 31.4 (CH_2); 34.3 (CH); 35.1 (CH_2); 36.2 (*C*); 36.9, 37.1, 37.4, 37.6 (CH_2); 38.8, 38.9 (*C*); 42.2, 45.5, 53.5 (CH); 55.1 (*C*); 55.3, 55.7 (CH); 62.3 (CH_2); 66.7 (CH); 66.9 (CH_2); 68.0, 68.8, 68.9, 69.6, 69.9, 70.5, 70.8, 72.5, 75.0, 76.6, 77.6, 79.1, 97.5, 98.0, 100.3 (CH); 109.2 (*C*); 123.2 (CH); 145.1 (*C*); 169.5, 169.6, 169.8, 169.9, 170.1, 176.4, 177.8, 213.1 (*C*=O). HRMS (ESI-FT-ICR) *m*/*z*: 1408.6794 $[M+Na]^+$ (calculated for $C_{70}H_{103}N_3O_{25}Na$: 1408.6778).

*4.1.15. Saponin analog **24**.* Compound **23** (30 mg, 0.022 mmol) was treated with NaOH (0.308 mmol) in H_2O/MeOH/THF (1.2 mL, 1:1:1, v/v/v) according to the deprotection procedure A. Flash column chromatography purification ($CHCl_3$/MeOH 4:1) afforded **24** (19 mg, 89%) as a white amorphous solid. R_f=0.20 ($CHCl_3$/MeOH 4:1). $[\alpha]_D^{20}$ −51.8 (*c* 0.85, $CHCl_3$). IR (KBr, cm^{-1}) ν_{max}: 3587, 3566, 2938, 2373, 2345, 1560. ^1H NMR (500 MHz, pyridine-d_5): δ=0.72 (d, 3H, *J*=6.0 Hz, CH_3); 0.86 (s, 3H, CH_3); 1.12 (s, 3H, CH_3); 1.39 (d, 3H, *J*=6.9 Hz, CH_3); 1.58 (d, 3H, *J*=6.0 Hz, CH_3 Rha); 1.64 (d, 3H, *J*=6.3 Hz, CH_3 Rha′); 2.24 (dd, 1H, *J*=4.9/14.3 Hz); 2.40 (t, 1H, *J*=13.9 Hz); 2.79 (dd, 1H, *J*=6.7/8.6 Hz); 3.51 (t, 1H, *J*=10.7 Hz, H-26ax); 3.61 (dd, 1H, *J*=3.7/10.7 Hz, H-26eq); 3.66 (m, 1H, H-5 Glc); 4.10 (m, 1H, H-6a Glc); 4.16–4.36 (m, 5H, H-6b Glc, H-3 Glc, H-2 Glc, H-4 Rha, H-4 Rha′); 4.41 (t, 1H, *J*=9.3 Hz, H-4 Glc); 4.49–4.60 (m, 4H, H-3 Rha, H-3 Rha′, H-3α, H-16α); 4.69 (m, 1H, H-2 Rha); 4.77 (m, 1H, H-2 Rha′); 4.80 (m, 1H, H-5 Rha); 4.98 (d, 1H, *J*=8.2 Hz, H-1 Glc); 5.08 (d, 1H, *J*=11.7 Hz, OCH_2a); 5.39 (d, 1H, *J*=11.7 Hz, OCH_2b); 5.84 (d, 1H, *J*=1.3 Hz, H-1 Rha); 6.38 (d, 1H, *J*=1.4 Hz, H-1 Rha′); 8.45 (s, 1H, H-triazole). ^{13}C NMR (125 MHz, pyridine-d_5): δ=12.1, 14.3, 16.4, 17.7, 18.9 (CH_3); 28.6, 29.4, 29.6 (CH_2); 30.9 (CH); 31.8, 31.9, 32.2 (CH_2); 34.6 (CH); 35.9 (CH_2); 36.6 (*C*); 37.2, 38.3 (CH_2); 43.0, 45.7, 54.7, 55.6 (CH); 55.8 (*C*); 56.3, 60.6 (CH); 61.5, 64.0, 67.3 (CH_2); 69.7, 70.8, 72.7, 72.9, 73.1, 73.2, 74.3, 74.6, 77.5, 77.8, 78.2, 79.0, 80.0, 102.2, 102.4, 103.3 (CH); 109.7 (*C*); 123.5 (CH); 144.9 (*C*); 213.0 (*C*=O). HRMS (ESI-FT-ICR) *m*/*z*: 988.4974 $[M+Na]^+$ (calculated for $C_{48}H_{75}N_3O_{17}Na$: 988.4994).

*4.1.16. Triazole-based trisaccharide–spirostan conjugate **25**.* Alkyne **12** (138 mg, 0.148 mmol) and azide **18** (83.6 mg, 0.177 mmol) in THF (3.2 mL) were reacted in the presence of $Cu(OAc)_2\cdot H_2O$ (0.03 mmol) and sodium ascorbate (0.059 mmol) according to the click conjugation procedure. Flash column chromatography purification (hexane/EtOAc 1:1.3) afforded conjugate **25** (154 mg, 74%) as a white solid. R_f=0.36 (hexane/EtOAc 1:2). Mp: 198–200 °C. $[\alpha]_D^{20}$ −84.4 (*c* 1.60, $CHCl_3$). IR (KBr, cm^{-1}) ν_{max}: 2955, 2873, 2372, 1747, 1374, 1245, 1224, 1146, 1075, 1050. ^1H NMR (400 MHz, $CDCl_3$): δ=0.77 (s, 3H, CH_3); 0.78 (d, 3H, *J*=6.4 Hz, CH_3); 0.91 (s, 3H, CH_3); 0.97 (d, 3H, *J*=6.7 Hz, CH_3); 1.11 (d, 3H, *J*=6.2 Hz, CH_3 Rha); 1.16 (m, 12H, (CH_3)$_3$C, CH_3 Rha′); 1.23 (s, 9H, (CH_3)$_3$C); 1.86, 1.99, 2.01, 2.08, 2.12, 2.13 (6×s, 6×3H, 6× CH_3CO); 2.33 (t, 1H, *J*=13.3 Hz); 2.78 (t, 1H, *J*=12.9 Hz); 3.36 (t, 1H, *J*=11.1 Hz, H-26ax); 3.45–3.49 (m, 1H, H-26eq); 3.66 (t, 1H, *J*=7.3 Hz, H-2 Glc); 3.77 (t, 1H, *J*=7.6 Hz, H-4 Glc); 3.81–3.85 (m, 1H, H-5 Glc); 3.88–3.92 (m, 1H, H-5 Rha); 4.00–4.04 (m, 1H, H-5 Rha′); 4.27 (dd, 1H, *J*=5.2/11.9 Hz, H-6a Glc); 4.38–4.67 (m, 2H, H-6b Glc, H-16α); 4.62 (d, 1H, *J*=7.7 Hz, H-1 Glc); 4.83 (d, 1H, *J*=1.3 Hz, H-1 Rha); 4.86 (d, 1H, *J*=1.9 Hz, H-1 Rha′); 4.90–5.06 (m, 6H, H-3 Rha, H-4 Rha, H-4 Rha′, OCH_2, H-3α); 5.08–5.10 (m, 2H, H-2 Rha, H-2 Rha′); 5.13 (dd, 1H, *J*=3.1/10.2 Hz, H-3 Rha′); 5.20 (t, 1H, *J*=7.3 Hz, H-3 Glc); 7.76 (s, 1H, H-triazole). ^{13}C NMR (100 MHz, $CDCl_3$): δ=13.9, 14.4, 16.4, 16.8, 17.1, 20.6, 20.7, 20.8 (CH_3); 21.1 (CH_2); 26.9, 27.2 (CH_3); 27.3, 28.7, 29.9 (CH_2); 30.2 (CH); 31.3, 31.5, 33.6 (CH_2); 36.5 (CH); 38.8, 38.9 (*C*); 39.5 (CH_2); 41.0 (*C*); 41.6 (CH); 41.8 (CH_2); 42.3 (*C*); 44.2, 56.0, 56.6, 62.1 (CH); 62.2, 62.7 (CH_2); 66.8 (CH); 66.9 (CH_2); 68.1, 68.8, 69.3, 69.4, 69.9, 70.3, 70.4, 73.8, 74.5, 75.7, 76.0 (CH); 79.1 (*C*); 80.5, 97.2, 98.9, 99.7 (CH); 109.3 (*C*); 121.5 (CH); 143.4 (*C*); 169.5, 169.8, 169.9, 170.1, 170.3, 171.8, 176.7, 178.1, 211.6 (*C*=O). HRMS (ESI-FT-ICR) *m*/*z*: 1424.6703 $[M+Na]^+$ (calculated for $C_{70}H_{103}N_3O_{26}Na$: 1424.6728).

*4.1.17. Saponin analog **26**.* Compound **25** (42 mg, 0.03 mmol) was treated with NaOH (0.42 mmol) in H_2O/MeOH/THF (1.8 mL, 1:1:1, v/v/v) according to the deprotection procedure A. Flash column chromatography purification ($CHCl_3$/MeOH 4:1→2:1) afforded conjugate **26** (27 mg, 91%) as a white amorphous solid. R_f=0.38 ($CHCl_3$/MeOH 4:1). $[\alpha]_D^{20}$ −96.0 (*c* 1.00, MeOH). IR (KBr, cm^{-1}) ν_{max}: 3368, 2928, 2878, 2373, 1560, 1130, 1052. ^1H NMR (500 MHz, pyridine-d_5): δ=0.70 (d, 3H, *J*=6.0 Hz, CH_3); 0.84 (s, 3H, CH_3); 0.95 (s, 3H, CH_3); 1.16 (d, 3H, *J*=6.9 Hz, CH_3); 1.52 (d, 3H, *J*=6.3 Hz, CH_3 Rha); 1.63 (d, 3H, *J*=6.0 Hz, CH_3 Rha′); 2.62 (m, 1H); 2.82 (t, 1H, *J*=13.0 Hz); 3.10 (t, 1H, *J*=12.6 Hz); 3.49 (t, 1H, *J*=10.7 Hz, H-26ax); 3.59 (dd, 1H, *J*=3.8/10.7 Hz, H-26eq); 3.64 (m, 1H, H-5 Glc); 4.07 (dd, 1H, *J*=3.2/12.0 Hz, H-6a Glc); 4.13–4.35 (m, 5H, H-3 Glc, H-6b Glc, H-2 Glc, H-4 Rha, H-4 Rha′); 4.38 (t, 1H, *J*=9.3 Hz, H-4 Glc); 4.53 (m, 2H, H-3 Rha, H-3 Rha′); 4.55–4.60 (m, 1H, H-16α); 4.67 (dd, 1H, *J*=1.6/3.2 Hz, H-2 Rha); 4.75 (dd, 1H, *J*=1.6/3.5 Hz, H-2 Rha′); 4.78 (m, 1H, H-5 Rha); 4.88 (m, 1H, H-5 Rha′); 4.94 (d, 1H, *J*=7.9 Hz, H-1 Glc); 5.04 (d, 1H, *J*=12.0 Hz, OCH_2a); 5.34 (d, 1H, *J*=12.0 Hz, OCH_2b); 5.48 (m, 1H, H-3α); 5.82 (d, 1H, *J*=1.3 Hz, H-1 Rha); 6.34 (d, 1H, *J*=1.3 Hz, H-1 Rha′); 7.69 (s, 1H, H-triazole). ^{13}C NMR (125 MHz, pyridine-d_5): δ=14.4, 15.3, 16.9, 17.7, 18.7, 18.8 (CH_3); 21.9, 29.1, 29.6 (CH_2); 30.3 (*C*); 30.8 (CH_2); 30.9 (CH); 32.1, 32.2, 35.3 (CH_2); 37.5 (CH); 40.2 (CH_2); 41.6 (*C*); 42.3 (CH); 42.6 (CH_2); 43.1 (*C*); 45.0, 56.7, 57.4 (CH); 61.5 (CH_2); 63.3 (CH); 63.8, 67.3 (CH_2); 69.7, 70.8, 72.6, 72.9, 73.0, 73.1, 74.2, 74.6, 77.4, 77.8, 78.1, 78.9 (CH); 79.7 (*C*); 81.3, 102.1, 102.2, 103.3 (CH); 109.6 (*C*); 123.5 (CH); 145.0 (*C*); 212.8 (*C*=O). HRMS (ESI-FT-ICR) *m*/*z*: 1004.4923 $[M+Na]^+$ (calculated for $C_{48}H_{75}N_3O_{18}Na$: 1004.4943).

*4.1.18. Triazole-based trisaccharide–spirostan conjugate **27**.* Alkyne **12** (100 mg, 0.107 mmol) and azide **19** (58.3 mg, 0.128 mmol) in THF (3.2 mL) were reacted in the presence of $Cu(OAc)_2\cdot H_2O$ (0.021 mmol) and sodium ascorbate (0.043 mmol) according to the click conjugation procedure. Flash column chromatography purification (hexane/EtOAc 1:1) afforded **27** (111 mg, 75%) as a white solid. R_f=0.22 (hexane/EtOAc 1:1). Mp: 180–182 °C. $[\alpha]_D^{20}$ −23.8 (*c* 1.30, $CHCl_3$). IR (KBr, cm^{-1}) ν_{max}: 2932, 2852, 2373, 2346, 1749, 1242, 1225, 1148, 1052. ^1H NMR (400 MHz, $CDCl_3$): δ=0.94 (d, 3H, *J*=6.1 Hz, CH_3); 0.97 (s, 3H, CH_3); 1.03 (s, 3H, CH_3); 1.04 (d, 3H, *J*=7.0 Hz, CH_3); 1.15 (m, 15H, (CH_3)$_3$C, CH_3 Rha, CH_3 Rha′); 1.21 (s, 9H,

(CH_3)$_3$C); 1.93, 1.97, 2.03, 2.05, 2.08, 2.11 (6×s, 6×3H, 6× CH_3CO); 2.18 (dd, 1H, J=5.5/14.4 Hz, H-11β); 2.36 (t, 1H, J=14.2 Hz, H-11α); 2.48 (dd, 1H, J=6.7/8.8 Hz); 3.33 (t, 1H, J=11.0 Hz, H-26ax); 3.45–3.49 (m, 1H, H-26eq); 3.58 (t, 1H, J=7.6 Hz, H-2 Glc); 3.72–3.78 (m, 2H, H-4 Glc, H-5 Glc); 3.87–3.94 (m, 1H, H-5 Rha); 4.03–4.11 (m, 1H, H-5 Rha′); 4.26–4.36 (m, 2H, H-16α, H-6a Glc); 4.54 (dd, 1H, J=1.3/11.8 Hz, H-6b Glc); 4.63 (d, 1H, J=7.4 Hz, H-1 Glc); 4.66 (m, 1H, H-3β); 4.79 (d, 1H, J=1.6 Hz, H-1 Rha); 4.86 (d, 1H, J=1.9 Hz, H-1 Rha′); 4.89–5.07 (m, 5H, H-2 Rha, H-4 Rha, H-4 Rha′, OCH_2); 5.14–5.18 (m, 3H, H-2 Rha′, H-3 Rha, H-3 Rha′); 5.26 (t, 1H, J=8.0 Hz, H-3 Glc), 7.70 (s, 1H, H-triazole). ^{13}C NMR (100 MHz, $CDCl_3$): δ=11.5, 13.2, 15.9, 16.9, 17.1, 17.2, 20.6, 20.7, 20.8, 20.9 (CH_3); 25.5 (CH_2); 26.8, 27.2 (CH_3); 27.8, 28.8 (CH_2); 30.2 (*C*H); 31.0, 31.1, 31.4, 32.6, 32.8 (CH_2); 34.2 (*C*H); 36.3 (*C*); 37.2 (CH_2); 39.6, 42.2, 53.5, 55.0, 55.6, 56.2 (*C*H); 62.3, 62.6 (CH_2); 66.8 (*C*H); 66.9 (CH_2); 68.0, 68.8, 68.9, 69.6, 69.9, 70.5, 70.9, 72.6, 74.9, 79.1, 97.5, 98.0, 100.3 (*C*H); 109.2 (*C*); 122.8 (*C*H); 144.7 (*C*); 169.5, 169.6, 169.8, 169.9, 170.0, 176.4, 177.8, 212.9 (*C*=O). HRMS (ESI-FT-ICR) m/z: 1408.6747 [M+Na]$^+$ (calculated for $C_{70}H_{103}N_3O_{25}Na$: 1408.6778).

*4.1.19. Saponin analog **28**.* Compound **27** (80 mg, 0.058 mmol) was treated with NaOH (0.81 mmol) in H_2O/MeOH/THF (3.6 mL, 1:1:1, v/v/v) according to the deprotection procedure A. Flash column chromatography purification ($CHCl_3$/MeOH 4:1) afforded **28** (52 mg, 92%) as a white amorphous solid. R_f=0.35 ($CHCl_3$/MeOH 3:1). $[\alpha]_D^{20}$ −38.8 (*c* 0.85, $CHCl_3$). IR (KBr, cm^{-1}) ν_{max}: 3487, 2928, 2878, 2373, 1560, 1130, 1052. ^{1}H NMR (500 MHz, pyridine-d_5): δ=0.70 (d, 3H, J=6.0 Hz, CH_3); 0.81 (s, 3H, CH_3); 1.09 (s, 3H, CH_3); 1.36 (d, 3H, J=6.9 Hz, CH_3); 1.56 (d, 3H, J=6.3 Hz, CH_3 Rha); 1.63 (d, 3H, J=6.3 Hz, CH_3 Rha′); 2.37 (t, 1H, J=13.6 Hz); 2.48 (m, 1H); 2.78 (dd, 1H, J=6.7/8.6 Hz); 3.49 (t, 1H, J=10.7 Hz, H-26ax); 3.60 (dd, 1H, J=3.8/10.7 Hz, H-26eq); 3.64 (m, 1H, H-5 Glc); 4.07 (m, 1H, H-6a Glc); 4.14–4.35 (m, 5H, H-2 Glc, H-3 Glc, H-6b Glc, H-4 Rha, H-4 Rha′); 4.38 (t, 1H, J=9.2 Hz, H-4 Glc); 4.46–4.54 (m, 3H, H-3 Rha, H-3 Rha′, H-16α); 4.67 (m, 1H, H-2 Rha); 4.70 (m, 1H, H-3β); 4.75–4.81 (m, 2H, H-2 Rha′, H-5 Rha); 4.85–4.91 (m, 1H, H-5 Rha′); 4.97 (d, 1H, J=7.9 Hz, H-1 Glc); 5.09 (d, 1H, J=12.0 Hz, OCH_2a); 5.40 (d, 1H, J=12.0 Hz, OCH_2b); 5.82 (d, 1H, J=1.6 Hz, H-1 Rha); 6.35 (d, 1H, J=1.6 Hz, H-1 Rha′); 8.50 (s, 1H, H-triazole). ^{13}C NMR (125 MHz, pyridine-d_5): δ=11.7, 14.3, 16.5, 17.7, 18.9 (CH_3); 25.9, 28.3, 29.6 (CH_2); 30.9 (*C*H); 31.7, 32.2, 33.1, 33.3 (CH_2); 34.5 (*C*H); 36.8 (*C*); 37.9 (CH_2); 40.3, 43.0, 54.6, 55.4 (*C*H); 55.7 (*C*); 56.1, 56.6 (*C*H); 61.5, 64.0, 67.3 (CH_2); 69.7, 70.8, 72.7, 72.9, 73.1, 73.2, 74.3, 74.6, 77.5, 77.9, 78.2, 79.0, 80.0, 102.2, 102.4, 103.3 (*C*H); 109.7 (*C*); 124.4 (*C*H); 145.0 (*C*); 213.0 (*C*=O). HRMS (ESI-FT-ICR) m/z: 988.4992 [M+Na]$^+$ (calculated for $C_{48}H_{75}N_3O_{17}Na$: 988.4994).

*4.1.20. Triazole-based trisaccharide–spirostan conjugate **29**.* Alkyne **12** (100 mg, 0.107 mmol) and azide **20** (60.3 mg, 0.128 mmol) in THF (3.0 mL) were reacted in the presence of $Cu(OAc)_2 \cdot H_2O$ (0.021 mmol) and sodium ascorbate (0.043 mmol) according to the click conjugation procedure. Flash column chromatography purification (hexane/EtOAc 2:3) afforded conjugate **29** (121.4 mg, 81%) as a white solid. R_f=0.20 (hexane/EtOAc 1:1). Mp: 186–187 °C. $[\alpha]_D^{20}$ −50.0 (*c* 1.40, $CHCl_3$). IR (KBr, cm^{-1}) ν_{max}: 2954, 2935, 2873, 2371, 1750, 1374, 1369, 1244, 1224, 1146, 1074, 1050. ^{1}H NMR (400 MHz, $CDCl_3$): δ=0.75 (s, 3H, CH_3); 0.77 (d, 3H, J=6.4 Hz, CH_3); 0.85 (s, 3H, CH_3); 0.95 (d, 3H, J=6.8 Hz, CH_3); 1.01 (d, 3H, J=6.2 Hz, CH_3 Rha); 1.14 (m, 12H, (CH_3)$_3$C, CH_3 Rha′); 1.20 (s, 9H, (CH_3)$_3$C); 1.92, 1.96, 2.02, 2.04, 2.07, 2.10 (6×s, 6×3H, 6× CH_3CO); 2.60 (dd, 1H, J=6.9/16.6 Hz); 2.87 (t, 1H, J=12.5 Hz); 3.34 (t, 1H, J=10.9 Hz, H-26ax); 3.44–3.47 (m, 1H, H-26eq); 3.56 (t, 1H, J=7.7 Hz, H-2 Glc); 3.73 (m, 2H, H-4 Glc, H-5 Glc); 3.86–3.92 (m, 1H, H-5 Rha); 4.02–4.09 (m, 1H, H-5 Rha′); 4.25 (dd, 1H, J=3.9/11.9 Hz, H-6a Glc); 4.37–4.42 (m, 1H, H-16α); 4.56–4.60 (m, 2H, H-1 Glc, H-6b Glc); 4.78 (d, 1H, J=1.2 Hz, H-1 Rha); 4.84–4.90 (m, 4H, H-1 Rha′, H-3β, OCH_2); 4.95 (t, 1H, J=9.8 Hz, H-4 Rha′); 5.01 (t, 1H, J=9.9 Hz, H-4 Rha); 5.05 (dd, 1H, J=2.1/3.1 Hz, H-2 Rha′); 5.10–5.13 (m, 2H, H-2 Rha, H-3 Rha); 5.15 (dd, 1H, J=3.1/10.0 Hz, H-3 Rha′); 5.25 (t, 1H, J=8.2 Hz, H-3 Glc); 7.76 (s, 1H, H-triazole). ^{13}C NMR (100 MHz, $CDCl_3$): δ=13.8, 14.4, 16.4, 16.9, 17.0, 17.1, 20.5, 20.6, 20.7, 20.8 (CH_3); 20.9, 25.7, 26.4 (CH_2); 26.8, 27.1 (CH_3); 28.7, 29.4 (CH_2); 30.2 (*C*H); 31.3, 31.5 (CH_2); 37.1 (*C*H); 38.8, 38.9 (*C*); 39.6 (CH_2); 41.1 (*C*); 41.6 (*C*H); 43.2 (*C*); 44.2, 54.6, 56.1, 61.9 (*C*H); 62.0, 62.8, 66.8 (CH_2); 66.9, 68.0, 68.7, 68.9, 69.5, 69.9, 70.4, 70.8, 72.6, 75.0, 76.5 (*C*H); 76.9 (*C*); 77.3, 80.5, 97.5, 97.8, 100.4 (*C*H); 109.2 (*C*); 123.6 (*C*H); 144.2 (*C*); 169.5, 169.7, 169.8, 169.9, 170.0, 176.4, 177.9, 211.6 (*C*=O). HRMS (ESI-FT-ICR) m/z: 1424.6715 [M+Na]$^+$ (calculated for $C_{70}H_{103}N_3O_{26}Na$: 1424.6728).

*4.1.21. Saponin analog **30**.* Compound **29** (48 mg, 0.035 mmol) was treated with NaOH (0.49 mmol) in H_2O/MeOH/THF (2.1 mL, 1:1:1, v/v/v) according to the deprotection procedure A. Flash column chromatography purification ($CHCl_3$/MeOH 4:1) afforded **29** (30 mg, 88%) as a white amorphous solid. R_f=0.28 ($CHCl_3$/MeOH 4:1). $[\alpha]_D^{20}$ −86.9 (*c* 1.60, $CHCl_3$). IR (KBr, cm^{-1}) ν_{max}: 3567, 3033, 2374, 2346, 1560. ^{1}H NMR (500 MHz, pyridine-d_5): δ=0.68 (d, 3H, J=6.0 Hz, CH_3); 0.84 (s, 3H, CH_3); 0.86 (s, 3H, CH_3); 1.14 (d, 3H, J=6.9 Hz, CH_3); 1.58 (d, 3H, J=6.3 Hz, CH_3 Rha); 1.62 (d, 3H, J=6.3 Hz, CH_3 Rha′); 2.67 (d, 2H, J=3.5 Hz); 3.19 (t, 1H, J=12.6 Hz); 3.47 (t, 1H, J=10.5 Hz, H-26ax); 3.57 (dd, 1H, J=3.3/10.7 Hz, H-26eq); 3.63 (m, 1H, H-5 Glc); 4.06–4.15 (m, 2H, H-3 Glc, H-6a Glc); 4.20–4.34 (m, 4H, H-2 Glc, H-6b Glc, H-4 Rha, H-4 Rha′); 4.38 (t, 1H, J=9.3 Hz, H-4 Glc); 4.45–4.53 (m, 3H, H-3 Rha, H-3 Rha′, H-16α); 4.66 (m, 1H, H-2 Rha); 4.69–4.75 (m, 2H, H-2 Rha′, H-5 Rha); 4.84–4.90 (m, 1H, H-5 Rha′); 4.95 (d, 1H, J=7.9 Hz, H-1 Glc); 5.01 (d, 1H, J=12.0 Hz, OCH_2); 5.06 (m, 1H, H-3β); 5.30 (d, 1H, J=11.7 Hz, OCH_2); 5.81 (d, 1H, J=1.1 Hz, H-1 Rha); 6.31 (d, 1H, J=1.2 Hz, H-1 Rha′); 8.60 (s, 1H, H-triazole). ^{13}C NMR (125 MHz, pyridine-d_5): δ=14.2, 15.4, 17.0, 17.7, 18.9 (CH_3); 21.8, 27.5, 29.6 (CH_2); 31.0 (*C*H); 32.1, 32.2, 32.3 (CH_2); 37.8 (*C*H); 40.4 (CH_2); 41.7 (*C*); 42.4 (*C*H); 42.5 (CH_2); 43.6 (*C*); 45.1, 54.9, 56.9 (*C*H); 61.5 (CH_2); 63.2 (*C*H); 63.7, 67.3 (CH_2); 69.8, 70.8, 72.8, 72.9, 73.0, 73.1, 74.3, 74.6, 77.5, 77.9 (*C*H); 78.1 (*C*); 78.2, 79.0, 81.3, 102.3, 103.3 (*C*H); 109.7 (*C*); 124.7 (*C*H); 144.5 (*C*); 213.1 (*C*=O). HRMS (ESI-FT-ICR) m/z: 1004.4935 [M+Na]$^+$ (calculated for $C_{48}H_{75}N_3O_{18}Na$: 1004.4943).

*4.1.22. Triazole-based trisaccharide–spirostan conjugate **31**.* Alkyne **11** (80 mg, 0.095 mmol) and azide **17** (51.9 mg, 0.114 mmol) in THF (3.0 mL) were reacted in the presence of $Cu(OAc)_2 \cdot H_2O$ (0.019 mmol) and sodium ascorbate (0.038 mmol) according to the click conjugation procedure. Flash column chromatography purification (hexane/EtOAc 2:3) afforded conjugate **31** (98 mg, 79%) as a white solid. R_f=0.19 (hexane/EtOAc 2:3). Mp: 193–195 °C. $[\alpha]_D^{20}$ −91.1 (*c* 1.35, $CHCl_3$). IR (KBr, cm^{-1}) ν_{max}: 2929, 2864, 2375, 1750, 1372, 1238, 1222, 1044. ^{1}H NMR (500 MHz, $CDCl_3$): δ=0.79 (d, 3H, J=6.3 Hz, CH_3); 1.01 (s, 3H, CH_3); 1.06 (s, 3H, CH_3); 1.07 (d, 3H, J=6.0 Hz, CH_3); 1.15 (d, 3H, J=6.3 Hz, CH_3 Rha); 1.22 (d, 3H, J=6.3 Hz, CH_3 Rha′); 1.96, 1.97, 1.99, 2.03, 2.05, 2.06, 2.10, 2.12 (8×s, 8×3H, 8× CH_3CO); 2.25 (dd, 1H, J=5.0/14.5 Hz); 2.44 (t, 1H, J=13.7 Hz); 2.52 (dd, 1H, J=6.8/8.7 Hz); 3.35 (t, 1H, J=10.9 Hz, H-26ax); 3.49 (ddd, 1H, J=2.2/8.8/11.0 Hz, H-26eq); 3.57–3.60 (m, 1H, H-5 Glc); 3.79–3.84 (m, 1H, H-5 Rha′); 3.85–3.89 (m, 2H, H-6a Glc, H-4 Glc); 3.96–4.03 (m, 2H, H-6b Glc, H-5 Rha); 4.33–4.37 (m, 1H, H-16α); 4.40–4.46 (m, 1H, H-3α); 4.62 (d, 1H, J=7.9 Hz, H-1 Glc); 4.76 (d, 1H, J=12.3 Hz, OCH_2a); 4.83 (d, 1H, J=1.9 Hz, H-1 Rha); 4.84 (d, 1H, J=1.6 Hz, H-1 Rha′); 4.89 (dd, 1H, J=7.9/9.1 Hz, H-2 Glc); 4.93 (d, 1H, J=12.4 Hz, OCH_2b); 5.02–5.08 (m, 3H, H-4 Rha, H-4 Rha′, H-2 Rha); 5.14 (dd, 1H, J=3.2/10.1 Hz, H-3 Rha′); 5.17–5.21 (m, 2H, H-3 Glc, H-2 Rha′); 5.24 (dd, 1H, J=3.6/10.2 Hz, H-3 Rha); 7.52 (s, 1H, H-triazole). ^{13}C NMR (125 MHz, $CDCl_3$): δ=12.0, 13.2, 16.0, 17.1, 17.2, 17.3, 20.6, 20.7, 20.8, 20.9, 21.0, 21.1 (CH_3); 28.0, 28.7, 28.8 (CH_2); 30.2 (*C*H); 31.1, 31.3, 31.4 (CH_2); 34.3 (*C*H); 35.2 (CH_2); 36.2 (*C*); 36.8, 37.6

(CH_2); 42.2, 45.4, 53.6 (*C*H); 55.1 (*C*); 55.3, 55.7, 60.1 (*C*H); 62.4, 65.8 (CH_2); 66.5 (*C*H); 66.9 (CH_2); 67.8, 68.8, 69.2, 69.5, 69.6, 70.4, 70.9, 72.0, 73.6, 74.0, 77.2, 79.1, 97.7, 99.4, 99.8 (*C*H); 109.2 (*C*); 120.6 (*C*H); 143.5 (*C*); 169.6, 169.7, 169.8, 169.9, 170.1, 170.2, 170.3, 213.0 (*C*=O). HRMS (ESI-FT-ICR) *m*/*z*: 1324.5825 $[M+Na]^+$ (calculated for $C_{64}H_{91}N_3O_{25}Na$: 1324.5839).

*4.1.23. Deprotection procedure B: saponin analog **32**.* To a solution of **31** (74 mg, 0.057 mmol) in THF/MeOH (4 mL, 1:1, v/v) was added NaOMe up to pH 9–10. The reaction mixture was stirred at rt overnight, then neutralized with acid resin Dowex-50 (H^+) and filtered under reduced pressure. The filtrate was concentrated to dryness and the crude product purified by flash column chromatography (EtOAc/MeOH 6:1) to afford **32** (50 mg, 91%) as a white amorphous solid. R_f=0.70 (EtOAc/MeOH 3:1). $[\alpha]_D^{20}$ −40.0 (*c* 1.50, MeOH). IR (KBr, cm^{-1}) ν_{max}: 3384, 2928, 2873, 2371, 1703, 1450, 1376, 1097, 1060. ^{1}H NMR (400 MHz, CD_3OD): δ=0.83 (d, 3H, *J*=6.4 Hz, CH_3); 1.07 (d, 3H, *J*=7.0 Hz, CH_3); 1.10 (s, 3H, CH_3); 1.13 (s, 3H, CH_3); 1.29 (d, 6H, *J*=6.1 Hz, CH_3 Rha, CH_3 Rha′); 2.25 (dd, 1H, *J*=4.7/14.3 Hz, H-11β); 2.51 (dd, 1H, *J*=6.7/8.8 Hz); 2.58 (t, 1H, *J*=13.7 Hz, H-11α); 3.28 (dd, 1H, *J*=7.9/8.8 Hz, H-2 Glc); 3.33–3.61 (m, 7H, H-26ax, H-26eq, H-4 Rha, H-4 Rha′, H-3 Glc, H-4 Glc, H-5 Glc); 3.66–3.79 (m, 4H, H-3 Rha, H-3 Rha′, H-5 Rha′, H-6a Glc); 3.86 (dd, 1H, *J*=1.7/3.2 Hz, H-2 Rha′); 3.89 (dd, 1H, *J*=1.6/3.2 Hz, H-2 Rha); 3.96–4.05 (m, 2H, H-5 Rha, H-6b Glc); 4.34–4.40 (m, 1H, H-16α); 4.43 (d, 1H, *J*=7.8 Hz, H-1 Glc); 4.57 (m, 1H, H-3α); 4.77 (d, 1H, *J*=1.3 Hz, H-1 Rha); 4.78 (d, 1H, *J*=12.3 Hz, OCH_2a); 4.96 (d, 1H, *J*=12.4 Hz, OCH_2b); 8.10 (s, 1H, H-triazole). ^{13}C NMR (100 MHz, CD_3OD): δ=12.2, 13.7, 16.6, 17.5, 17.9, 18.1 (CH_3); 29.3, 29.8, 29.9 (CH_2); 31.4 (*C*H); 32.0, 32.5, 32.6 (CH_2); 35.6 (*C*H); 36.3 (CH_2); 37.3 (*C*); 37.9, 38.7 (CH_2); 43.5, 46.6, 55.1 (*C*H); 56.4 (*C*); 56.8, 57.2, 61.7 (*C*H); 63.2, 67.3, 67.9 (CH_2); 69.9, 70.7, 72.2 (*C*H); 72.3 (*C*); 72.4, 72.5, 73.8, 74.0, 75.2, 75.7, 76.6, 79.6, 80.6, 101.9, 102.8, 103.5 (*C*H); 110.5 (*C*); 123.4 (*C*H); 145.3 (*C*); 215.7 (*C*=O). HRMS (ESI-FT-ICR) *m*/*z*: 988.4986 $[M+Na]^+$ (calculated for $C_{48}H_{75}N_3O_{17}Na$: 988.4994).

*4.1.24. Triazole-based trisaccharide–spirostan conjugate **33**.* Alkyne **11** (80 mg, 0.095 mmol) and azide **18** (53.5 mg, 0.113 mmol) in THF (3.0 mL) were reacted in the presence of $Cu(OAc)_2 \cdot H_2O$ (0.019 mmol) and sodium ascorbate (0.038 mmol) according to the click conjugation procedure. Flash column chromatography purification (hexane/EtOAc 2:3) afforded conjugate **33** (100 mg, 81%) as a white solid. R_f=0.33 (hexane/EtOAc 1:2). Mp: 201–203 °C. $[\alpha]_D^{20}$ −83.1 (*c* 1.30, $CHCl_3$). IR (KBr, cm^{-1}) ν_{max}: 2951, 2874, 2370, 1751, 1374, 1367, 1242, 1224, 1075, 1051. ^{1}H NMR (400 MHz, $CDCl_3$): δ=0.77 (s, 3H, CH_3); 0.78 (d, 3H, *J*=6.4 Hz, CH_3); 0.91 (s, 3H, CH_3); 0.97 (d, 3H, *J*=6.7 Hz, CH_3); 1.14 (d, 3H, *J*=6.2 Hz, CH_3 Rha); 1.22 (d, 3H, *J*=6.1 Hz, CH_3 Rha′); 1.95, 1.96, 2.02, 2.03, 2.04, 2.05, 2.10, 2.12 (8×s, 8×3H, 8× CH_3CO); 2.82 (t, 1H, *J*=12.1 Hz); 3.36 (t, 1H, *J*=11.0 Hz, H-26ax); 3.45–3.49 (m, 1H, H-26eq); 3.65 (m, 1H, H-5 Glc); 3.77–3.88 (m, 3H, H-4 Glc, H-6a Glc, H-5 Rha′); 3.93–4.04 (m, 2H, H-5 Rha, H-6b Glc); 4.42 (m, 1H, H-16α); 4.62 (d, 1H, *J*=7.6 Hz, H-1 Glc); 4.81–4.96 (m, 6H, H-1 Rha, H-1 Rha′, H-2 Glc, H-3α, OCH_2); 5.01–5.06 (m, 3H, H-2 Rha, H-4 Rha, H-4 Rha′); 5.14 (dd, 1H, *J*=3.2/10.3 Hz, H-3 Rha′); 5.16–5.21 (m, 3H, H-3 Rha, H-2 Rha′, H-3 Glc); 7.67 (s, 1H, H-triazole). ^{13}C NMR (100 MHz, $CDCl_3$): δ=14.1, 14.4, 16.4, 17.1, 17.2, 17.4, 20.6, 20.7, 20.8, 20.9, 21.0, 21.1 (CH_3); 27.9, 28.8, 29.9 (CH_2); 30.2 (*C*H); 31.3, 31.5, 34.1 (CH_2); 36.7 (*C*H); 39.5 (CH_2); 41.1 (*C*); 41.6 (*C*H); 41.9 (CH_2); 42.5 (*C*); 44.4, 56.0, 62.0, 66.6 (*C*H); 66.9 (CH_2); 67.9, 68.8, 69.3, 69.5, 69.7, 70.4, 70.8, 71.9, 73.6, 73.9, 77.4 (*C*H); 79.4 (*C*); 80.4, 97.9, 99.7, 99.8 (*C*H); 109.3 (*C*); 169.7, 169.9, 170.1, 170.2, 170.3, 170.4, 211.3 (*C*=O). HRMS (ESI-FT-ICR) *m*/*z*: 1340.5802 $[M+Na]^+$ (calculated for $C_{64}H_{91}N_3O_{26}Na$: 1340.5778).

*4.1.25. Saponin analog **34**.* Compound **33** (93 mg, 0.071 mmol) was treated with NaOMe in THF/MeOH (6 mL, 1:1, v/v) according to the deprotection procedure B. Flash column chromatography purification (EtOAc/MeOH 5:1) afforded **34** (66 mg, 95%) as a white amorphous solid. R_f=0.65 (EtOAc/MeOH 2:1). $[\alpha]_D^{20}$ −80.0 (*c* 1.20, $CHCl_3$). IR (KBr, cm^{-1}) ν_{max}: 3567, 2372, 2346, 1654, 1560. ^{1}H NMR (400 MHz, CD_3OD): δ=0.81 (d, 3H, *J*=6.4 Hz, CH_3); 0.83 (s, 3H, CH_3); 0.95 (s, 3H, CH_3); 0.98 (d, 3H, *J*=6.9 Hz, CH_3); 1.27 (d, 3H, *J*=6.1 Hz, CH_3 Rha); 1.29 (d, 3H, *J*=6.3 Hz, CH_3 Rha′); 2.35 (t, 1H, *J*=13.2 Hz); 2.84 (t, 1H, *J*=12.6 Hz); 3.26 (dd, 1H, *J*=7.8/8.9 Hz, H-2 Glc); 3.35–3.59 (m, 6H, H-26ax, H-26eq, H-4 Rha, H-4 Rha′, H-3 Glc, H-4 Glc); 3.63–3.75 (m, 5H, H-3 Rha, H-3 Rha′, H-5 Rha′, H-5 Glc, H-6a Glc); 3.84 (dd, 1H, *J*=1.8/3.3 Hz, H-2 Rha′); 3.86 (dd, 1H, *J*=1.7/3.3 Hz, H-2 Rha); 3.94–4.02 (m, 2H, H-5 Rha, H-6b Glc); 4.38–4.47 (m, 2H, H-1 Glc, H-16α); 4.75 (d, 1H, *J*=1.3 Hz, H-1 Rha); 4.77 (d, 1H, *J*=12.4 Hz, OCH_2); 4.82 (d, 1H, *J*=1.6 Hz, H-1 Rha′); 4.84–4.94 (m, 2H, H-3α, OCH_2); 8.11 (s, 1H, H-triazole). ^{13}C NMR (100 MHz, CD_3OD): δ=14.4, 14.9, 16.9, 17.5, 17.9, 18.1 (CH_3); 22.3, 29.3, 29.9, 31.2 (CH_2); 31.5 (*C*H); 32.5, 34.8 (CH_2); 38.4 (*C*H); 40.9 (CH_2); 42.3 (*C*); 42.9 (*C*H); 43.0 (CH_2); 43.7 (*C*); 45.9, 57.5, 58.2 (*C*H); 63.2 (CH_2); 63.8 (*C*H); 67.4, 67.9 (CH_2); 69.9, 70.7, 72.3, 72.4, 72.6, 73.8, 74.1, 75.3, 75.7, 76.6, 79.6 (*C*H); 80.3 (*C*); 82.0, 102.0, 102.8, 103.5 (*C*H); 110.6 (*C*); 123.6 (*C*H); 145.5 (*C*); 213.9 (*C*=O). HRMS (ESI-FT-ICR) *m*/*z*: 1004.4949 $[M+Na]^+$ (calculated for $C_{48}H_{75}N_3O_{18}Na$: 1004.4943).

*4.1.26. Triazole-based trisaccharide–spirostan conjugate **35**.* Alkyne **11** (80 mg, 0.095 mmol) and azide **19** (51.9 mg, 0.114 mmol) in THF (3.0 mL) were reacted in the presence of $Cu(OAc)_2 \cdot H_2O$ (0.019 mmol) and sodium ascorbate (0.038 mmol) according to the click conjugation procedure. Flash column chromatography purification (hexane/EtOAc 2:3) afforded conjugate **35** (88.9 mg, 72%) as a white solid. R_f=0.25 (hexane/EtOAc 2:3). Mp: 188 °C. $[\alpha]_D^{20}$ −65.0 (*c* 1.40, $CHCl_3$). IR (KBr, cm^{-1}) ν_{max}: 2933, 2874, 2369, 1751, 1374, 1240, 1224, 1076, 1043. ^{1}H NMR (400 MHz, $CDCl_3$): δ=0.77 (d, 3H, *J*=6.2 Hz, CH_3); 0.97 (s, 3H, CH_3); 1.03 (s, 3H, CH_3); 1.06 (d, 3H, *J*=7.0 Hz, CH_3); 1.14 (d, 3H, *J*=6.2 Hz, CH_3 Rha); 1.21 (d, 3H, *J*=6.2 Hz, CH_3 Rha′); 1.95, 1.96, 1.97, 2.03, 2.04, 2.06, 2.09, 2.11 (8×s, 8×3H, 8× CH_3CO); 2.46 (dd, 1H, *J*=6.8/8.7 Hz); 3.32 (t, 1H, *J*=11.0 Hz, H-26ax); 3.45–3.48 (m, 1H, H-26eq); 3.57–3.61 (m, 1H, H-5 Glc); 3.77–3.82 (m, 1H, H-5 Rha′); 3.84–3.90 (m, 2H, H-6 Glc, H-4 Glc); 3.94–4.03 (m, 2H, H-5 Rha, H-6b Glc); 4.29–4.34 (m, 1H, H-16α); 4.64 (m, 2H, H-1 Glc, H-3β); 4.76 (d, 1H, *J*=12.5 Hz, OCH_2); 4.83 (m, 2H, H-1 Rha, H-1 Rha′); 4.88 (dd, 1H, *J*=7.9/9.4 Hz, H-2 Glc); 4.94 (d, 1H, *J*=12.5 Hz, OCH_2); 5.00–5.07 (m, 3H, H-4 Rha, H-4 Rha′, H-2 Rha); 5.13 (dd, 1H, *J*=3.2/10.3 Hz, H-3 Rha′); 5.16–5.25 (m, 3H, H-2 Rha′, H-3 Glc, H-3 Rha); 7.58 (s, 1H, H-triazole). ^{13}C NMR (100 MHz, $CDCl_3$): δ=11.4, 13.2, 16.0, 17.1, 17.2, 17.3, 20.6, 20.7, 20.8, 20.9, 21.0 (CH_3); 25.4, 27.7, 28.8 (CH_2); 30.2 (*C*H); 31.0, 31.1, 31.4, 32.5, 32.7 (CH_2); 34.2 (*C*H); 36.3 (*C*); 37.2 (CH_2); 39.7, 42.2, 53.5, 55.0, 55.5, 56.0 (*C*H); 62.4, 65.7 (CH_2); 66.5 (*C*H); 66.8 (CH_2); 67.8, 68.8, 69.2, 69.5, 69.6, 70.4, 70.9, 72.0, 73.6, 73.9, 77.1, 79.1, 97.6, 99.6, 99.8 (*C*H); 109.2 (*C*); 121.9 (*C*H); 143.5 (*C*); 169.5, 169.7, 169.8, 169.9, 170.1, 170.2, 170.3, 170.4, 212.9 (*C*=O). HRMS (ESI-FT-ICR) *m*/*z*: 1324.5819 $[M+Na]^+$ (calculated for $C_{64}H_{91}N_3O_{25}Na$: 1324.5839).

*4.1.27. Saponin analog **36**.* Compound **35** (55 mg, 0.042 mmol) was treated with NaOMe in THF/MeOH (4 mL, 1:1, v/v) according to the deprotection procedure B. Flash column chromatography purification (EtOAc/MeOH 5:1) afforded **36** (37 mg, 92%) as a white amorphous solid. R_f=0.67 (EtOAc/MeOH 3:1). $[\alpha]_D^{20}$ −40.0 (*c* 1.15, $CHCl_3$). IR (KBr, cm^{-1}) ν_{max}: 3567, 2371, 2346, 1654, 1560. ^{1}H NMR (500 MHz, pyridine): δ=0.69 (d, 3H, *J*=5.8 Hz, CH_3); 0.80 (s, 3H, CH_3); 1.09 (s, 3H, CH_3); 1.36 (d, 3H, *J*=6.9 Hz, CH_3); 1.65 (d, 3H, *J*=6.1 Hz, CH_3 Rha); 1.70 (d, 3H, *J*=6.2 Hz, CH_3 Rha′); 2.35 (t, 1H, *J*=13.8 Hz); 2.79 (dd, 1H, *J*=6.7/8.6 Hz); 3.49 (t, 1H, *J*=10.8 Hz, H-26ax); 3.58–3.61 (m, 1H, H-26eq); 3.86–3.90 (m, 1H, H-4 Glc); 3.94–4.01 (m, 2H, H-2 Glc, OCH_2); 4.08–4.18 (m, 2H, H-3 Glc, H-4 Rha′); 4.24 (t, 1H, *J*=9.3 Hz, H-4 Rha); 4.31–4.39 (m, 3H, H-5 Glc,

OCH_2, H-5 Rha); 4.47–4.56 (m, 4H, H-3 Rha, H-3 Rha′, H-16α, H-3β); 4.59 (dd, 1H, J=1.6/3.2 Hz, H-2 Rha); 4.65 (dd, 1H, J=1.4/3.1 Hz, H-2 Rha′); 4.85–4.93 (m, 1H, H-5 Rha′); 4.97 (d, 1H, J=7.8 Hz, H-1 Glc); 5.17 (d, 1H, J=12.2 Hz, H-6a Glc); 5.37 (d, 1H, J=12.3 Hz, H-6b Glc); 5.40 (d, 1H, J=1.2 Hz, H-1 Rha); 5.61 (d, 1H, J=1.2 Hz, H-1 Rha′); 8.18 (s, 1H, H-triazole). ^{13}C NMR (125 MHz, pyridine): δ=11.7, 14.3, 16.5, 17.7, 18.9, 19.1 (CH_3); 25.9, 28.3, 29.6 (CH_2); 30.9 (CH); 31.7, 32.2, 33.1, 33.2 (CH_2); 34.6 (CH); 36.8 (C); 37.9 (CH_2); 40.3, 43.0, 54.7, 55.4 (CH); 55.7 (C); 56.1, 56.5 (CH); 63.5, 67.3, 67.5 (CH_2); 70.3, 71.0, 72.6, 72.9, 73.1, 74.2, 74.4, 75.4, 76.1, 76.9, 79.9, 80.1, 102.5, 103.3, 104.2 (CH); 109.7 (C); 123.7 (CH); 145.2 (C); 213.1 (C=O). HRMS (ESI-FT-ICR) m/z: 988.4976 $[M+Na]^+$ (calculated for $C_{48}H_{75}N_3O_{17}Na$: 988.4994).

*4.1.28. Triazole-based trisaccharide–spirostan conjugate **37**.* Alkyne **11** (80 mg, 0.095 mmol) and azide **20** (53.5 mg, 0.113 mmol) in THF (3.0 mL) were reacted in the presence of $Cu(OAc)_2 \cdot H_2O$ (0.019 mmol) and sodium ascorbate (0.038 mmol) according to the click conjugation procedure. Flash column chromatography purification (hexane/EtOAc 1:2) afforded conjugate **37** (102 mg, 82%) as a white solid. R_f=0.24 (hexane/EtOAc 1:2). Mp: 196–197 °C. $[\alpha]_D^{20}$ −98.5 (c 1.30, $CHCl_3$). IR (KBr, cm^{-1}) ν_{max}: 2951, 2873, 2372, 1751, 1374, 1369, 1242, 1223, 1075, 1050. ^{1}H NMR (400 MHz, $CDCl_3$): δ=0.75 (s, 3H, CH_3); 0.77 (d, 3H, J=6.4 Hz, CH_3); 0.85 (s, 3H, CH_3); 0.95 (d, 3H, J=6.7 Hz, CH_3); 1.14 (d, 3H, J=6.2 Hz, CH_3 Rha); 1.21 (d, 3H, J=6.1 Hz, CH_3 Rha′); 1.96 (s, 6H, 2× CH_3CO); 1.99, 2.03, 2.05, 2.06, 2.09, 2.11 (6×s, 6×3H, 6× CH_3CO); 2.60 (dd, 1H, J=6.5/16.3 Hz); 2.86 (t, 1H, J=12.4 Hz); 3.35 (t, 1H, J=11.0 Hz, H-26ax); 3.44–3.48 (m, 1H, H-26eq); 3.58 (m, 1H, H-5 Glc); 3.77–3.88 (m, 3H, H-4 Glc, H-6a Glc, H-5 Rha); 3.96–4.02 (m, 2H, H-5 Rha′, H-6b Glc); 4.40 (m, 1H, H-16α); 4.63 (d, 1H, J=7.6 Hz, H-1 Glc); 4.74–4.96 (m, 6H, H-1 Rha, H-1 Rha′, H-2 Glc, OCH_2, H-3β); 4.87 (m, 1H, H-2 Glc); 5.01–5.08 (m, 3H, H-2 Rha, H-4 Rha, H-4 Rha′); 5.13 (dd, 1H, J=3.2/10.3 Hz, H-3 Rha′); 5.17–5.21 (m, 2H, H-3 Glc, H-2 Rha′); 5.24 (dd, 1H, J=3.5/10.2 Hz, H-3 Rha); 7.64 (s, 1H, H-triazole). ^{13}C NMR (100 MHz, $CDCl_3$): δ=13.8, 14.4, 16.4, 17.1, 17.2, 17.3, 20.6, 20.7, 20.8, 20.9, 21.0 (CH_3); 25.7, 28.8, 29.5 (CH_2); 30.2 (CH); 31.3, 31.5 (CH_2); 37.0 (CH); 39.6 (CH_2); 41.1 (C); 41.5 (CH_2); 41.6 (CH); 43.2 (C); 44.2, 54.8, 56.1, 62.0 (CH); 62.2, 65.8 (CH_2); 66.6 (CH); 66.8 (CH_2); 67.8, 68.8, 69.2, 69.5, 69.6, 70.4, 70.9, 72.1, 73.6, 73.9 (CH); 76.9 (C); 77.1, 80.5, 97.7, 99.4, 99.8 (CH); 109.2 (C); 122.1 (CH); 144.2 (C); 169.7, 169.9, 170.0, 170.1, 170.2, 170.3, 211.5 (C=O). HRMS (ESI-FT-ICR) m/z: 1340.5773 $[M+Na]^+$ (calculated for $C_{64}H_{91}N_3O_{26}Na$: 1340.5778).

*4.1.29. Saponin analog **38**.* Compound **37** (80 mg, 0.061 mmol) was treated with NaOMe in THF/MeOH (5 mL, 1:1, v/v) according to the deprotection procedure B. Flash column chromatography purification (EtOAc/MeOH 4:1) afforded **38** (57 mg, 96%) as a white amorphous solid. R_f=0.68 (hexane/EtOAc 2:1). $[\alpha]_D^{20}$ −96.3 (c 1.35, MeOH). IR (KBr, cm^{-1}) ν_{max}: 3366, 2928, 2874, 2374, 1708, 1653, 1449, 1380, 1239, 1174, 1060. ^{1}H NMR (400 MHz, CD_3OD): δ=0.80 (d, 3H, J=6.6 Hz, CH_3); 0.81 (s, 3H, CH_3); 0.90 (s, 3H, CH_3); 0.98 (d, 3H, J=6.9 Hz, CH_3); 1.27 (d, 3H, J=6.1 Hz, CH_3 Rha); 1.29 (d, 3H, J=6.2 Hz, CH_3 Rha′); 2.51 (dd, 1H, J=5.7/12.1 Hz, H-7β); 2.77 (t, 1H, J=11.8 Hz, H-7α); 3.26 (dd, 1H, J=7.9/9.1 Hz, H-2 Glc); 3.36–3.59 (m, 7H, H-26ax, H-26eq, H-4 Rha, H-4 Rha′, H-3 Glc, H-4 Glc, H-5 Glc); 3.63–3.78 (m, 4H, H-3 Rha, H-3 Rha′, H-5 Rha, H-6a Glc); 3.84 (dd, 1H, J=1.6/3.2 Hz, H-2 Rha′); 3.88 (dd, 1H, J=1.7/3.4 Hz, H-2 Rha); 3.97–4.02 (m, 2H, H-6b Glc, H-5 Rha′); 4.33 (d, 1H, J=7.7 Hz, H-1 Glc); 4.43 (m, 1H, H-16α); 4.76 (d, 1H, J=1.5 Hz, H-1 Rha); 4.81 (d, 1H, J=12.6 Hz, OCH_2a); 4.82 (d, 1H, J=1.3 Hz, H-1 Rha′); 4.91–4.95 (m, 2H, H-3β, OCH_2b); 8.13 (s, 1H, H-triazole). ^{13}C NMR (100 MHz, CD_3OD): δ=14.1, 14.8, 16.9, 17.5, 17.9, 18.1 (CH_3); 22.1, 24.7, 27.9, 29.9 (CH_2); 31.5 (CH); 32.3, 32.4, 32.5 (CH_2); 38.6 (CH); 40.9 (CH_2); 42.3 (C); 42.7 (CH_2); 42.9 (CH); 44.1 (C); 45.9, 55.7, 57.6 (CH); 62.7 (CH_2); 63.6 (CH); 67.3, 67.9 (CH_2); 69.9, 70.7, 72.2 (CH); 72.3 (C); 72.4, 72.5, 73.8, 74.0, 75.3, 75.7, 76.6 (CH); 78.7 (C); 79.6, 81.9, 102.0, 102.6, 102.9 (CH); 110.6 (C); 125.0 (CH); 144.6 (C); 214.5 (C=O). HRMS (ESI-FT-ICR) m/z: 1004.4950 $[M+Na]^+$ (calculated for $C_{48}H_{75}N_3O_{18}Na$: 1004.4943).

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