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Carbohydrate–steroid conjugation by Ugi reaction: one-pot synthesis of triple sugar/*pseudo*-peptide/spirostane hybrids

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ABSTRACT

The one-pot synthesis of novel molecular chimeras incorporating sugar, *pseudo*-peptide, and steroidal moieties is described. For this, a new carbohydrate–steroid conjugation approach based on the Ugi four-component reaction was implemented for the ligation of glucose and chacotriose to spirostanic steroids. The approach proved wide substrate scope, as both mono and oligosaccharides functionalized with amino, carboxy, and isocyano groups were conjugated to steroidal substrates in an efficient, multicomponent manner. Two alternative strategies based on the hydrazoic acid variant of the Ugi reaction were employed for the synthesis of tetrazole-based chacotriose–diosgenin conjugates resembling naturally occurring spirostan saponins. This is the first time that triple sugar/*pseudo*-peptide/steroid hybrids are produced, thus opening up an avenue of opportunities for applications in drug discovery and biological chemistry.

1. Introduction

The conjugation of steroids to other biomolecules is a common strategy employed both by nature and chemists to modulate the biological and chemical behavior of these molecules.[1] An important family of naturally occurring steroidal conjugates are the saponins, which possess an oligosaccharide moiety attached to the steroidal skeleton.[2] These steroidal glycosides have found significant applications in traditional and modern medicine[2,3] owing to their cytotoxic,[4–6] antifungal,[7] antiinflammatory,[8] and antiviral activities.[9]

The interest on conjugating lipophilic scaffolds like steroids to oligosaccharides also derives from the recognized capability of the resulting amphipathic hybrids to interact with phospholipid membranes and liposomes.[10] Very recently, facially amphiphilic steroid-disaccharide hybrids have proven success on the solubilization and stabilization of membrane proteins.[11] This has opened up new perspectives for the extensive manipulation and characterization of membrane proteins, as many of the hybrid amphiphiles are synthetically available in multigram scale. Alternatively, the growing development of 'click chemistry' has had also an impact on the development of novel sugar/steroids hybrid architectures.[12] Different types of lipophilic steroids have been ligated to saccharides by the Cu^{I}-catalyzed azide–alkyne 1,3-dipolar cycloaddition, thus producing amphipathic hybrids with anticancer[12c] and antimicrobial activities.[12f] Considering the growing importance of sugar/steroid hybrids in drug discovery and biological chemistry, we were prompted to pursuit a novel carbohydrate–steroid conjugation approach alternative to the traditional glycosylation and 'click' processes and capable to produce unique types of molecular chimeras.

Here we report on the use of the Ugi four-component reaction (Ugi-4CR),[13] and its hydrazoic acid variant,[14] for the conjugation of sugars to spirostanic steroids. The Ugi-4CR is one of the most versatile and explored isocyanide-based multicomponent reactions (MCRs), owing to the great level of molecular diversity and complexity that generates with low synthetic cost.[13,15] This MCR has been utilized for the assembly of steroidal macrocycles,[16] for the conjugation of carbohydrates to amino acids[17] and proteins[18] and for the construction of glycoconjugate libraries.[19] However, its utilization in the synthesis of any type of carbohydrate/steroid hybrid has remained completely unexplored. Accordingly, we describe the first multicomponent approach for the direct conjugation of sugars to steroids, thus producing new types of triple hybrids including a *pseudo*-peptidic (i.e., peptoid or tetrazole) moiety in-between the sugar and steroidal skeletons.

The simplest version of the Ugi-4CR comprises the condensation of a primary amine, and aldehyde or ketone, a carboxylic acid and an isocyanide to afford an N-substituted dipeptide.[13] An

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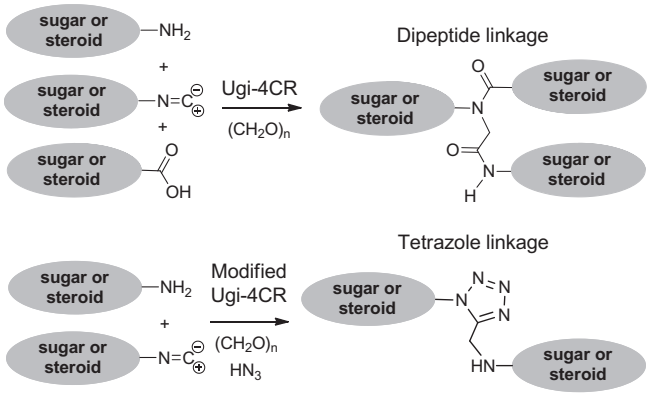


Figure 1. Strategy for the conjugation of sugars to steroids by the Ugi-4CR and its hydrazoic acid variant.

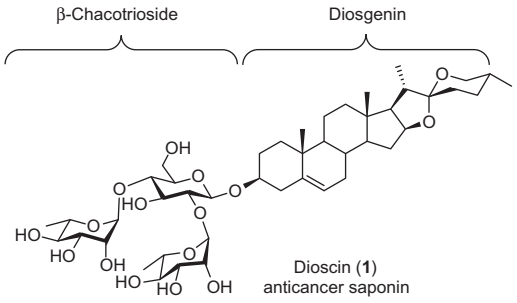


Figure 2. Structure of the spirostan saponin dioscin, a diosgenyl trisaccharide.

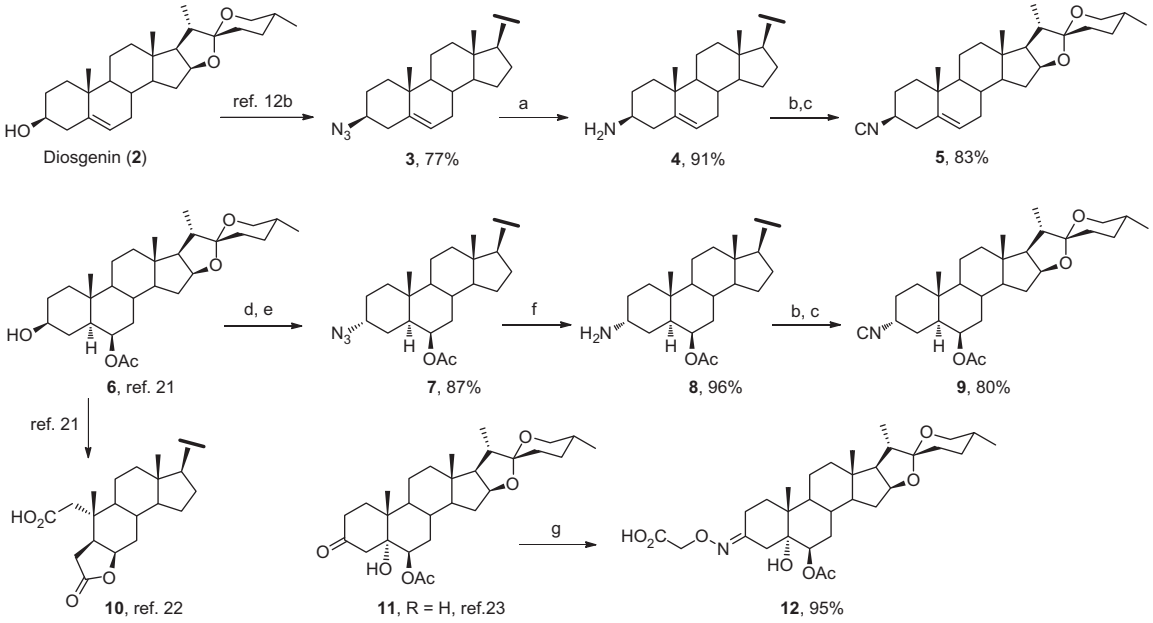
important variation of this approach encompasses the utilization hydrazoic acid as the acid component, which upon reaction with an amine, an oxo-compound, and an isonitrile furnishes a 1,5-disubtituted tetrazole,[14] which is commonly considered as an special type of peptide bond mimetic. Both processes are chemically related and include the formation of an α-adduct, which in the classic Ugi-4CR undergoes the Mumm rearrangement while in the hydrazoic acid variant performs an electrocyclic ring closure.

Figure 1 depicts the strategy toward carbohydrate/steroid hybrids using Ugi-type MCRs, which give rise either to N-substituted peptide or tetrazole frameworks. Both motifs are considered as *pseudo*-peptidic backbones, as they feature structurally modified peptide bonds or mimics of them. Also, they may allow for accessing a high level of diversity by varying the combinations of carbohydrate and steroidal functional groups reacting on the multicomponent conjugation, that is, 16 combinations for the Ugi-4CR and 9 combinations for the Ugi-tetrazole reaction (hydrazoic acid is a fixed component). As shown in Figure 1, it is possible to use both the sugar and the steroid as each one of the Ugi-components, though in this work the oxo-component will be fixed to formaldehyde in order to avoid the formation of diastereomers of difficult separation.

2. Results and discussion

To assess the scope of the Ugi-4CR-based conjugation approach, we chose spirostanes as the steroidal Ugi-components owing to their high incidence in bioactive saponins. Most spirostan saponins[4] incorporate an inner D-glucose unit conjugated to ring A of the spirostanic skeleton and additionally glycosylated with other glycosyl units. For example, dioscin (**1**, Fig. 2) is a spirostanyl glycoside combining high occurrence in the plant kingdom and a broad spectrum of bioactivity,[4,7–9] including potent cytotoxicity against cancer cells. This compound possesses a 4,6-di-*O*-(α-L-rhamnopyranosyl)-β-D-glucopyranoside unit, namely chacotriose, linked to C-3 of diosgenin (**2**). Taking dioscin (**1**) as model compound, the aim was posed on addressing the multicomponent conjugation approach utilizing glucose, chacotriose, and spirostanic derivatives bearing Ugi-reactive functional groups.

Glucose derivatives having amino, carboxylic, and isocyano groups at C-1 have been previously prepared and utilized in Ugi-4CRs.[17a,b,19b] On the other hand, spirostanes functionalized with amino and carboxylic groups are less common,[16c,d] while



Scheme 1. Synthesis of spirostanes functionalized with amino, carboxylic, and isocyano groups. Reagents and conditions: (a) PPh_3, THF/H_2O; (b) HCO_2H/Ac_2O, Et_3N, THF; (c) $POCl_3$, Et_3N, THF, −60→0 °C; (d) MsCl, Et_3N, CH_2Cl_2; (e) NaN_3, DMPU; (f) H_2 (g), 10% Pd/C, EtOAc/AcOH; (g) $HCl{\cdot}H_2NOCH_2CO_2H$, pyridine.

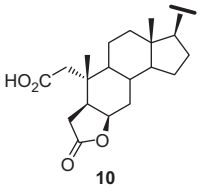
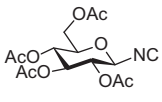
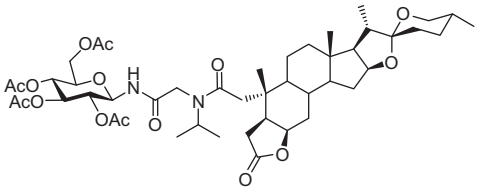
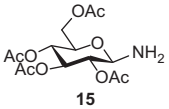
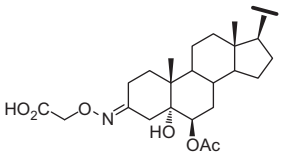
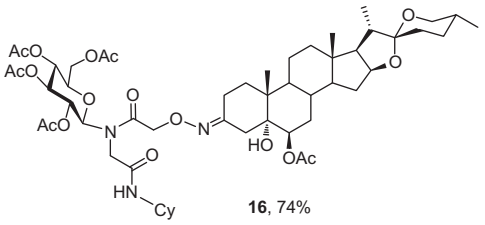
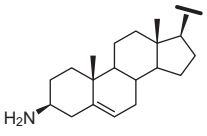
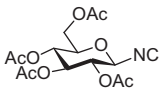
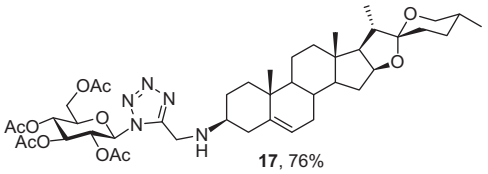
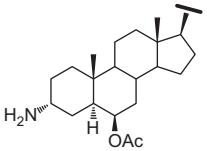
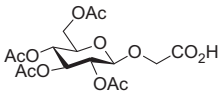
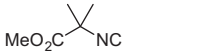
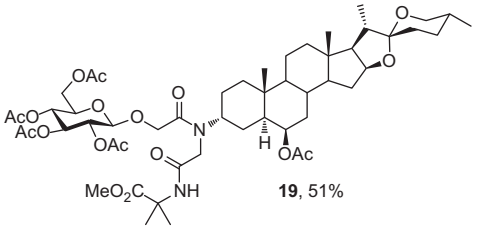
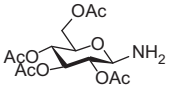
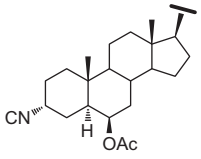
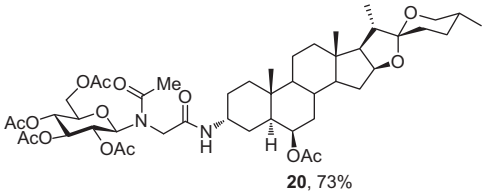
spirostanic isocyanides have not been described. Scheme 1 shows the synthesis of spirostanes functionalized with the Ugi-reactive groups. Initially, 3β-diosgenyl azide (**3**) was prepared from diosgenin (**2**) as previously described,[12b] following a protocol developed for the synthesis of its analogous 3β-cholestanyl azide.[20] Subsequent Staudinger azide reduction with PPh_3 produced the 3β-diosgenyl amine (**4**) in 91% yield after column chromatography. This spirostanic amine was then subjected to formylation by treatment with acetic–formic mixed anhydride followed by phosphorylchloride mediated dehydration to afford the 3β-diosgenyl isocyanide (**5**) in 83% yield.

We next turned to the synthesis of 3-amino and 3-isocyanospirostanes having the α disposition at C-3, so that the influence of the different C-3 stereochemistry on the conjugation efficiency could be evaluated. For this, the B-ring functionalized 3β-spirostanol **6**[21] was subjected to mesylation and nucleophilic replacement with azide to furnish the 3α-azidospirostane **7** in 87% yield. This azide was next subjected to Pd-catalyzed hydrogenation over 24 h to afford amine **8** in 96% yield. Conversion of amine **8** into the 3α-isocyanospirostane **9** was accomplished as before in 80% yield over two steps. Importantly, configuration of C-3 was not affected during the synthesis of the two spirostanic isocyanides.

Two different types of spirostanic acids were prepared for the conjugation to isocyano and aminosugars. The seco-spirostanic acid **10**, which is a naturally occurring sapogenin,[22] was obtained from spirostanol **6** according to a reported procedure.[21] Alternatively, condensation of ketone **11**[23] with *O*-(carboxymethyl)-hydroxylamine gave rise to acid **12**, which thus bears the carboxy functionality attached to C-3 through an oxime linker. Accordingly, each pair of functionalized spirostanes could be utilized to evaluate the substrate scope on the Ugi-conjugation approach to glucose derivatives.

Table 1 shows the results of the conjugation of glucose derivatives to a variety of spirostanic steroids by means of the Ugi-4CR and its hydrazoic acid variant. Based on our experience on Ugi-4CRs with bulky substrates,[16] reaction times were fixed to 24 h for the classical four-component process and 72 h for the hydrazoic acid variant.[13b] Examples were chosen to assess the conjugation efficiency and to prove the structural diversity of triple sugar/*pseudo*-peptide/steroid hybrids that can be accessed in one-pot

Table 1
Conjugation of glucose derivatives to spirostanic steroids through the Ugi-4CR and its hydrazoic acid variant[a]

Amine	Acid	Isocyanide	Sugar-spirostan hybrid
	 **10**	 **13**	 **14**, 87%
 **15**	 **12**		 **16**, 74%
 **4**	HN_3	 **13**	 **17**, 76%
 **8**	 **18**		 **19**, 51%
 **15**	AcOH	 **9**	 **20**, 73%

[a] All reactions were conducted in MeOH using 1 equiv of each component. Paraformaldehyde was employed as the oxo component in all cases. HN_3 was formed in situ from $TMSN_3$/MeOH.

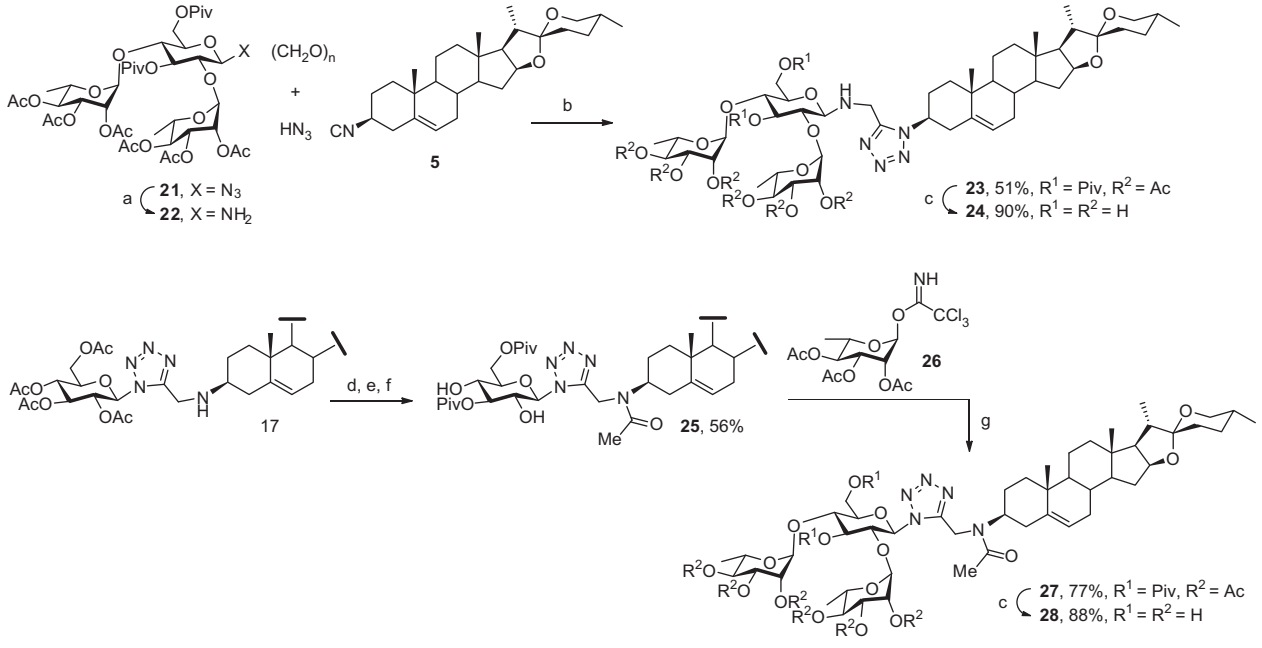
processes. Thus, the spirostanic acids **10** and **12** were conjugated to glucosyl isocyanide[17] **13** and glucosyl amine[17] **15** to furnish hybrids **14** and **16**, respectively, in good yields. As noticed, the structural differences between both types of conjugates rely not only in the spirostanic skeleton—that is, **10** is a *seco*-spirostane while acid **12** bears the carboxy functionality attached to the steroid through an oxime linker—but on the linear or branched nature of the *pseudo*-peptidic backbone connecting the two structures. Thus, hybrid **14** incorporates the N-glucoside as a secondary amide, while **16** incorporates the same moiety as a tertiary amide resulting in a branched peptidic connectivity.

We next turned to the conjugation of glucose derivatives to steroidal amines featuring either α or β stereochemistry. Thus, the equatorially-disposed 3β-diosgenyl amine (**4**) was conjugated to glucosyl isocyanide **13** by the hydrazoic acid variant of the Ugi-4CR, giving rise to the triple glucose/tetrazole/spirostane hybrid **17** in 76% yield after column chromatography. In this process, hydrazoic acid is formed in situ by methanolysis of azidotrimethylsilane, and behaves as acid component upon reaction with the imine and the isocyanide. Alternatively, the conjugation of the axially-disposed spirostanyl amine **8** to the carboxymethyl glucoside **18** led to hybrid **19** in only 51% yield after column chromatography. This is an expected result considering that axially-disposed steroidal amines are less reactive than equatorial ones in all types of transformations.

In contrast, we found that the different stereochemistry of 3-isocyanosteroids does not have a profound effect of the reaction yield. Hence, in several initial experiments (not shown) with simple amino, aldehyde, and carboxylic components, it turned out that either the equatorial steroidal isocyanide **5** or the axial isocyanide **9** led to Ugi-products in good yields (i.e., 85–90%). Of course, yields drops significantly when utilizing bulkier substrates as any of the other components. For example, the axial spirostanic isocyanide **9** was conjugated to glucosyl amine **15** to afford the hybrid **20** in 73% yield after column chromatography (see Table 1). Alternatively, Scheme 2 depicts the conjugation of β-chacotriosyl amine **22** to the 3β-diosgenyl isocyanide **5** by means of the hydrazoic acid variant of the Ugi-4CR, thus leading to hybrid **23** in 51% yield after 72 h. Indeed, the bulky structure of the trisaccharidic amine **22** is the main cause of the moderate yield obtained in this procedure, since other initial experiments with the same isocyanide and smaller amines proved to proceed in up to 90%.

As mentioned above, an important aim of this report is to illustrate applications of the multicomponent conjugation approach in the synthesis of analogs of bioactive sugar/steroids hybrids. To this end, two different strategies were implemented for the synthesis of chacotriose/spirostane hybrids resembling the natural spirostan saponin dioscin (**1**). As shown in Scheme 2, the β-chacotriosyl azide **21**[12a] was reduced by catalytic hydrogenation to amine **22**, which was then conjugated to diosgenyl isocyanide **5** to afford the triple chacotriose/tetrazole/diosgenin hybrid **23**. This hybrid was subjected to global deprotection to furnish **24** in overall 46% yield. As noticed from Scheme 2, compound **24** may be considered as a dioscin analog wherein the traditional glycosidic bond is replaced by an aminomethyltetrazole linkage, thus encompassing a unique type of hybrid architecture.

Considering the moderate yield of the direct conjugation of β-chacotrioside moiety to spirostanic steroids, we turned to evaluate an alternative route to access analogs of the target saponin. This second approach comprises the construction of the 2,4-branched trisaccharide moiety starting from the previously obtained glucose/diosgenin hybrid **17**, a route that requires the regioselective incorporation of two L-rhamnose units. The sequence commenced with acetylation of the aminomethyltetrazole moiety of **17**—to avoid its participation in next steps—followed by removal of the glucose acetyl groups under typical Zemplen conditions (NaOMe/MeOH). Further selective pivaloylation at OH-3 and OH-6 according to a reported procedure[24] furnished compound **25** in 56% yield over three steps. 2,3,4-Tri-*O*-acetyl-α-L-rhamnopyranosyl trichloroacetimidate (**26**)[25] was next used as glycosyl donor for the double glycosylation step following the Schmidt's inverse procedure,[26] thus leading to the triple chacotriose/tetrazole/diosgenin hybrid **27** in 77% yield. Final deprotection furnished the spirostan saponin analog **28**, which—apart from the acetamide



Scheme 2. Synthesis of tetrazole-based spirostan saponin analogs. Reagents and conditions: (a) H_2 (g), Pd/C, MeOH; (b) MeOH (HN_3 is formed in situ from $TMSN_3$); (c) aq NaOH, THF/MeOH, 50 °C; (d) Ac_2O, Et_3N, CH_2Cl_2; (e) NaOMe, MeOH; (f) PivCl, Py, −15 °C→rt; (g) $BF_3{\cdot}Et_2O$, CH_2Cl_2, 4 Å MS, −78 °C→rt.

functionality—comprises the same structure of hybrid **24** but with the aminomethyltetrazole linkage in a reverse manner.

3. Conclusions

We have shown that the Ugi-4CR and its hydrazoic acid variant are suitable procedures for the conjugation of mono and oligosaccharides to steroids. The approach proved wide substrate scope, since both the sugar and the steroidal substrates were utilized with success as amino, carboxylic, and isocyano components of the Ugi reactions. These procedures give rise to unique types of conjugates featuring triple sugar/*pseudo*-peptide/steroid hybrid architectures. To our knowledge, this is the first time that multicomponent reactions are employed for the conjugation of carbohydrates to steroidal derivatives. The approach was utilized for the synthesis of tetrazole-based spirostan saponin analogs, which proved that applications in the synthesis of natural product analogs are possible. Considering the remarkable diversity-oriented character of isocyanide-based MCRs, the present approach shows promise for the combinatorial production and biological screening of libraries of saponin analogs.

4. Experimental

Melting points were determined on a Leica DM LS2 apparatus and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on Varian Mercury spectrometers. Chemical shifts (δ) are reported in ppm relative to the TMS (^{1}H NMR) and to the solvent signal (^{13}C NMR). High resolution ESI mass spectra were obtained from a Bruker Apex III Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer equipped with an Infinity™ cell, a 7.0 Tesla superconducting magnet, an RF-only hexapole ion guide, and an external electrospray ion source (Agilent, off axis spray). ESI-MS spectra were recorded on a Finnigan TSQ 7000, capillary temperature: 220 °C, ESI positive ion mode: spray voltage 4.5 kV, collision energy −40 eV, CID pressure 1.8 mT, collision gas: Ar, ESI negative ion mode: spray voltage 4.0 kV. Unless otherwise stated, flash column chromatography was carried out using Merck Silica Gel 60 (0.015–0.040 nm) and analytical thin layer chromatography (TLC) was performed using Merck Silica Gel 60 F_{254} aluminium sheets. Solid compounds were recrystallized from selected solvents for the melting points measurements. All commercially available chemicals were used without further purification. Spirostanes **3**, **6**, and **11** were obtained as described in Refs.[12b,21,23], respectively.

4.1. (25*R*)-3β-Amino-5-spirostene (4)

Azide **3** (1.2 g, 2.9 mmol) was dissolved in 20 mL of THF and PPh_3 (1.13 g, 4.3 mmol) was added. The reaction mixture was stirred at room temperature for 8 h, then treated with H_2O (180 μL, 10 mmol), and stirred for additional 48 h. The reaction mixture was concentrated under reduced pressure and the crude product purified by flash column chromatography (CH_2Cl_2/Et_3N 10:0.1) to furnish amine **4** (1.09 g, 91%) as a white solid. Mp (MeOH): 166–167 °C. $[\alpha]_D^{20}$ −91.4 (*c* 0.5, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.78 (3H, d, *J* = 6.4 Hz, H-27); 0.79 (3H, s, H-18); 0.97 (3H, d, *J* = 6.7 Hz, H-21); 1.09 (3H, s, H-19); 2.70 (1H, m, H-3α); 3.36 (1H, t, *J* = 10.8 Hz, H-26ax); 3.49 (1H, dd, *J* = 10.8/4.0 Hz, H-26eq); 4.41 (1H, br m, H-16α); 5.29 (1H, m, H-6). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 13.3, 16.0, 17.1, 18.5 (*C*H_3); 26.8, 28.8 (*C*H_2); 30.1, 30.6 (*C*H); 31.1, 31.3, 31.5, 32.7 (*C*H_2); 36.8 (*C*); 37.3, 37.9, 39.9 (*C*H_2); 40.4 (*C*); 42.0 (*C*H); 51.6, 53.1, 55.6, 55.9 (*C*H); 66.9 (*C*H_2); 79.8 (*C*H); 109.3 (*C*); 124.2 (*C*H); 136.0 (*C*). HRMS (ESI-FT-ICR) *m/z*: 414.3369 $[M+H]^+$; calcd for $C_{27}H_{44}O_2N$: 414.3373.

4.2. (25*R*)-3β-Isocyano-5-spirostene (5)

A mixture of acetic anhydride (2.4 mL, 25 mmol) and formic acid (1.4 mL, 37 mmol) was stirred at 60 °C for 3 h. The mixture was cooled to room temperature and added dropwise to a solution of diosgenyl amine **4** (1.0 g, 2.5 mmol) and Et_3N (1.5 mL) in 30 mL of THF. The reaction mixture was stirred at room temperature overnight and then diluted with 60 mL of EtOAc. The organic phase was washed with satd aq $NaHCO_3$ (3 × 50 mL), aq 10% HCl and brine (50 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to dryness. The resulting formamide was dissolved in dry THF (30 mL) and the solution was treated with 4 mL of Et_3N and cooled to −60 °C. A solution of $POCl_3$ (0.35 mL, 3.75 mmol) in 3 mL of THF was added dropwise under nitrogen atmosphere and the reaction mixture was stirred at −60 °C for 3 h and then allowed to reach room temperature. The mixture was poured into 80 mL of cold water and extracted with Et_2O (2 × 50 mL). The organic layer was washed with satd aq $NaHCO_3$ (2 × 50 mL), brine (50 mL), dried over anhyd 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 afford the diosgenyl isocyanide **5** (878 mg, 83%) as a white solid. Mp (MeOH): 135–137 °C. $[\alpha]_D^{20}$ −10.2 (*c* 0.95, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.79 (3H, d, *J* = 6.2 Hz, H-27); 0.80 (3H, s, H-18); 0.96 (3H, d, *J* = 6.8 Hz, H-21); 1.06 (3H, s, H-19); 2.57 (1H, dd, *J* = 6.8/8.8 Hz); 3.35 (1H, t, *J* = 10.9 Hz, H-26ax); 3.49 (1H, dd, *J* = 4.1/10.7 Hz, H-26eq); 3.68 (1H, br m, H-3α); 4.35–4.40 (1H, m, H-16α); 5.33 (1H, m, H-6). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 13.2, 15.9, 17.1, 18.6 (*C*H_3); 26.6, 28.8 (*C*H_2); 30.2, 30.6 (*C*H); 31.2, 31.3, 31.4, 32.8 (*C*H_2); 36.9 (*C*); 37.3, 37.6, 39.8 (*C*H_2); 42.2 (*C*H); 51.5 (t, *J* = 5.2 Hz, *C*H); 51.7, 53.2 (*C*H); 54.6 (*C*); 55.6 (*C*H); 66.9 (*C*H_2); 79.2 (*C*H); 109.2 (*C*); 124.2 (*C*H); 136.0 (*C*); 155.1 (t, *J* = 5.2 Hz, *C*N). HRMS (ESI-FT-ICR) *m/z*: 446.3042 $[M+Na]^+$; calcd for $C_{28}H_{41}NO_2Na$: 446.3035.

4.3. (25*R*)-3α-Azido-5α-spirostan-6β-yl acetate (7)

Spirostanol **6** (4.36 g, 7.4 mmol) was dissolved in dry CH_2Cl_2 (80 mL) and treated with Et_3N (4.6 mL, 33.3 mmol) and mesyl chloride (1.32 mL, 11.1 mmol) at 0 °C. The reaction mixture was stirred at 0 °C for 1 h, then diluted with 200 mL of CH_2Cl_2, and washed with brine (2 × 100 mL). The organic phase was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to dryness. The resulting crude product was dissolved in DMPU (50 mL) and the solution was treated with NaN_3 (828 mg, 14.8 mmol). The reaction mixture was stirred vigorously under nitrogen atmosphere at 50 °C for 48 h and then diluted with 300 mL of Et_2O. The organic phase was washed with aq 10% HCl (2 × 60 mL) and brine (100 mL), dried over anhyd Na_2SO_4 and evaporated under reduced pressure to dryness. The crude product was purified by flash column chromatography (*n*-hexane/EtOAc 3:1) to give the pure spirostanyl azide **7** (3.21 g, 87%) as a white solid. Mp (MeOH): 152–154 °C. $[\alpha]_D^{20}$ −124.8 (*c* 1.30, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.78 (3H, d, *J* = 6.4 Hz, H-27); 0.79 (3H, s, H-18); 0.96 (3H, d, *J* = 6.8 Hz, H-21); 1.00 (3H, s, H-19); 2.04 (3H, s, CH_3CO); 3.37 (1H, t, *J* = 10.9 Hz, H-26ax); 3.47 (1H, dd, *J* = 4.0/10.7 Hz, H-26eq); 3.94 (1H, m, H-3β); 4.39 (1H, br m, H-16α); 4.92 (1H, d, *J* = 2.7 Hz, H-6α). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 14.5, 14.7, 16.5, 17.1 (*C*H_3); 20.3 (*C*H_2); 21.4 (*C*H_3); 25.4, 28.7, 29.7 (*C*H_2); 30.3, 30.5 (*C*H); 31.3, 31.6, 34.3 (*C*H_2); 36.0 (*C*); 36.4, 39.8 (*C*H_2); 40.5 (*C*); 41.6, 41.8, 53.7, 55.8, 58.0, 62.0 (*C*H); 66.8 (*C*H_2); 73.3, 80.6 (*C*H); 109.2 (*C*); 170.6 (*C*=O). HRMS (ESI-FT-ICR) *m/z*: 522.3293 $[M+Na]^+$; calcd for $C_{29}H_{45}O_4N_3Na$: 522.3302.

4.4. (25*R*)-3α-Amino-5α-spirostan-6β-yl acetate (8)

Azide **7** (1.5 g, 3.0 mmol) was dissolved in 40 mL of the solvent mixture EtOAc/AcOH 9:1 and treated with 10% Pd/C (150 mg). The reaction mixture was treated successively with hydrogen and vacuum and finally stirred under hydrogen atmosphere for 48 h, when TLC analysis revealed that all of the azide had been consumed. The catalyst was removed by filtration over a pad of Celite and the resulting solution was evaporated under reduced pressure to give the crude amine **8** (1.36 g, 96%). Re-crystallization from MeOH provided an analytical sample for characterization. Mp (MeOH): 186–187 °C. $[\alpha]_D^{20}$ −86.7 (*c* 0.45, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.78 (3H, d, *J* = 6.2 Hz, H-27); 0.79 (3H, s, H-18); 0.96 (3H, d, *J* = 6.6 Hz, H-21); 0.99 (3H, s, H-19); 2.05 (3H, s, C*H*$_3$CO); 3.28 (1H, m, H-3β); 3.37 (1H, t, *J* = 10.8 Hz, H-26ax); 3.47 (1H, dd, *J* = 3.4/10.8 Hz, H-26eq); 4.38 (1H, br m, H-16α); 4.90 (1H, d, *J* = 2.3 Hz, H-6α). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 14.6, 15.6, 16.5, 17.1 (*C*H$_3$); 20.3 (*C*H$_2$); 21.4 (*C*H$_3$); 26.4, 28.7, 30.0 (*C*H$_2$); 30.2, 30.6 (*C*H); 31.3, 31.6, 34.1 (*C*H$_2$); 36.3 (*C*); 36.5, 39.9 (*C*H$_2$); 40.6 (*C*); 41.2, 41.6, 52.1, 53.8, 56.0, 62.0 (*C*H); 66.8 (*C*H$_2$); 74.0, 80.7 (*C*H); 109.2 (*C*); 170.8 (*C*=O). HRMS (ESI-FT-ICR) *m/z*: 474.8535 $[M+H]^+$; calcd for $C_{29}H_{48}O_4N$: 474.8535.

4.5. (25*R*)-3α-Isocyano-5α-spirostan-6β-yl acetate (9)

A mixture of acetic anhydride (2.4 mL, 25 mol) and formic acid (1.43 mL, 37 mmol) was stirred at 60 °C for 3 h. The mixture was cooled to room temperature and added dropwise to a solution of amine **8** (1.2 g, 2.5 mmol) and Et_3N (1.5 mL) in 30 mL of THF. The reaction mixture was stirred at room temperature overnight and then diluted with 80 mL of EtOAc. The organic phase was washed with satd aq $NaHCO_3$ (3 × 50 mL), aq 10% HCl and brine (50 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to dryness. The resulting formamide was dissolved in dry THF (30 mL) and the solution was treated with 4 mL of Et_3N and cooled to −60 °C. A solution of $POCl_3$ (0.35 mL, 3.75 mmol) in 3 mL of THF was added dropwise under nitrogen atmosphere and the reaction mixture was stirred at −60 °C for 3 h and then allowed to reach room temperature. The mixture was poured into 80 mL of cold water and extracted with Et_2O (2 × 50 mL). The organic layer was washed with satd aq $NaHCO_3$ (2 × 50 mL), brine (50 mL), dried over anhyd Na_2SO_4, and concentrated under reduced pressure to dryness. The crude product was purified by flash column chromatography (*n*-hexane/EtOAc 3:1) to afford the spirostanyl isocyanide **9** (960 mg, 80%) as a white solid. Mp (MeOH): 158–160 °C. $[\alpha]_D^{20}$ −73.2 (*c* 0.30, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.77 (3H, d, *J* = 6.4 Hz, H-27); 0.80 (3H, s, H-18); 0.96 (3H, d, *J* = 6.8 Hz, H-21); 0.99 (3H, s, H-19); 2.02 (3H, s, C*H*$_3$CO); 3.36 (1H, t, *J* = 10.8 Hz, H-26ax); 3.47 (1H, dd, *J* = 3.7/10.8 Hz, H-26eq); 3.95 (1H, m, H-3β); 4.40 (1H, br m, H-16α); 4.90 (1H, d, *J* = 2.4 Hz, H-6α). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 14.5, 15.1, 16.4, 17.1 (*C*H$_3$); 20.4 (*C*H$_2$); 21.4 (*C*H$_3$); 25.7, 28.8, 29.8 (*C*H$_2$); 30.2, 30.6 (*C*H); 31.3, 31.7, 34.4 (*C*H$_2$); 36.1 (*C*); 36.4, 39.7 (*C*H$_2$); 40.6 (*C*); 41.7, 41.9 (*C*H); 51.9 (t, *J* = 5.1 Hz, *C*H); 55.8, 56.7, 62.0 (*C*H); 66.8 (*C*H$_2$); 73.4, 80.5 (*C*H); 109.3 (*C*); 154.3 (t, *J* = 5.1 Hz, *C*N), 170.5 (*C*=O). HRMS (ESI-FT-ICR) *m/z*: 484.3430 $[M+H]^+$; calcd for $C_{30}H_{46}O_4N$: 484.3427.

4.6. (25*R*)-6β-Acetoxy-3*E*-[*O*-(carboxymethyl)oximino]-5α-spirostan-5-ol (12)

O-(Carboxymethyl)-hydroxylamine hydrochloride (114 mg, 1.39 mmol) was added to a solution of ketone **11** (488 mg, 1.0 mmol) in 20 mL of dry pyridine. The reaction mixture was stirred at room temperature for 10 h and then diluted with 100 mL of EtOAc. The organic phase was washed with aq 10% HCl (3 × 50 mL), dried over anhyd Na_2SO_4, and concentrated under reduced pressure to afford the spirostanic acid **12** (532 mg, 95%) as a white solid. Re-crystallization from EtOAc provided an analytical sample for characterization. Mp (EtOAc): 235–236 °C.

$[\alpha]_D^{20}$ −25.4 (*c* 0.50, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.78 (6H, m, H-18 + H-27); 0.95 (3H, d, *J* = 6.6 Hz, H-21); 1.21 (3H, s, H-19); 2.07 (3H, s, C*H*$_3$CO); 3.36 (1H, t, *J* = 10.9 Hz, H-26ax); 3.46 (1H, m, H-26eq); 4.37 (1H, m, H-16α); 4.52 (1H, m, H-6α); 4.64–4.78 (2H, m, OC*H*$_2$). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 14.5, 15.6, 16.5, 17.1, 20.7 (*C*H$_3$); 21.4, 21.5, 27.2, 28.7, 30.1, 30.2, 31.3, 31.6, 32.6 (*C*H$_2$); 33.9 (*C*H); 39.7 (*C*); 39.8, 40.6 (*C*H); 41.6 (*C*); 45.0, 55.4, 62.0 (*C*H); 66.8 (*C*H$_2$); 74.6 (*C*H); 76.4 (*C*H$_2$); 77.2 (*C*); 80.7 (*C*H); 109.2 (*C*); 162.3 (*C*=N); 170.1, 174.3 (*C*=O). HRMS (ESI-FT-ICR) *m/z*: 584.3196 $[M+Na]^+$; calcd for $C_{31}H_{47}NO_8Na$: 584.3199.

4.7. General procedure for the Ugi-4CR-based conjugation approach

A solution of paraformaldehyde (0.5 mmol) and the amine (0.5 mmol) in MeOH (30 mL) is stirred for 2 h at room temperature. The carboxylic or hydrazoic acid (0.5 mmol) and the isonitrile (0.5 mmol) are then added and the reaction mixture is stirred at room temperature. The mixture is concentrated under reduced pressure and the crude product is purified by flash column chromatography on silica gel to afford the corresponding sugar-steroid hybrid.

4.8. Glucose/*pseudo*-peptide/spirostane hybrid 14

Isopropylamine (21 μL, 0.25 mmol), paraformaldehyde (7.5 mg, 0.25 mmol), the spirostanic acid **10** (115 mg, 0.25 mmol), and glucosyl isocyanide **13** (90 mg, 0.25 mmol) were reacted for 24 h according to the general Ugi-4CR procedure described in Section 4.7. Flash column chromatography purification (*n*-hexane/EtOAc 2:1) afforded the pure conjugate **14** (193 mg, 87%) as a white solid. Mp (EtOAc): 207–209 °C. $[\alpha]_D^{20}$ −54.5 (*c* 0.75, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.75 (3H, s, H-18); 0.77 (3H, d, *J* = 5.7 Hz, H-27); 0.95 (3H, d, *J* = 6.7 Hz, H-21); 1.20 (3H, s, H-19); 1.24 (6H, d, *J* = 6.6 Hz, (C*H*$_3$)$_2$CH); 2.01, 2.02, 2.03, 2.04 (4 × 3H, 4 × s, 4 × C*H*$_3$CO); 3.19 (1H, m); 3.35 (1H, t, *J* = 10.9 Hz, H-26ax); 3.46 (1H, dd, *J* = 4.4/11.0 Hz, H-26eq); 3.65 (2H, m); 3.97 (1H, m); 4.10–4.18 (2H, m); 4.13 (1H, dd, *J* = 2.2/12.5 Hz); 4.23 (1H, dd, *J* = 4.9/12.6 Hz); 4.40 (1H, m, H-16α); 4.63 (1H, m); 4.86 (1H, t, *J* = 9.3 Hz); 4.92 (1H, t, *J* = 9.3 Hz); 5.06 (1H, t, *J* = 9.4 Hz); 5.26 (1H, m); 7.44 (1H, d, *J* = 9.2 Hz, N*H*). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 14.5, 16.3, 16.9, 17.1, 20.2, 20.3, 20.4, 20.7, 20.8, 20.9 (*C*H$_3$); 28.8 (*C*H$_2$); 29.0 (*C*H); 30.2, 30.3, 31.4, 31.6, 35.0 (*C*H$_2$); 36.8 (*C*H); 39.6, 40.2 (*C*H$_2$); 40.3, 41.6 (*C*); 43.2 (*C*H); 45.6, 56.5 (*C*H$_2$); 58.4, 61.5 (*C*H); 62.0, 66.9 (*C*H$_2$); 67.4, 68.5, 70.2, 72.9, 73.5, 75.2, 78.1, 80.3 (*C*H); 109.3 (*C*); 169.7, 170.2, 170.7, 171.0, 171.7, 172.1, 178.1 (C=O). HRMS (ESI-FT-ICR) *m/z*: 911.4521 $[M+Na]^+$; calcd for $C_{46}H_{68}N_2O_{15}Na$: 911.4517.

4.9. Glucose/*pseudo*-peptide/spirostane hybrid 16

Glucosyl amine **15** (88 mg, 0.25 mmol), paraformaldehyde (7.5 g, 0.25 mmol), spirostanic acid **12** (140 mg, 0.25 mmol), and cyclohexylisocyanide (22 μL, 0.25 mmol) were reacted for 24 h according to the general Ugi-4CR procedure described in Section 4.7. Flash column chromatography purification (*n*-hexane/EtOAc 3:1) furnished the pure sugar–spirostan conjugate **16** (190 mg, 74%) as a white foam. $[\alpha]_D^{20}$ −82.1 (*c* 1.20, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.74 (3H, d, *J* = 6.2 Hz, H-27); 0.77 (3H, s, H-18); 0.91 (3H, d, *J* = 6.8 Hz, H-21); 1.20 (3H, s, H-19); 1.98, 1.99, 2.00, 2.02, 2.03 (5 × 3H, 5 × s, 5 × C*H*$_3$CO); 3.26 (1H, t, *J* = 10.9 Hz, H-26ax); 3.40–3.44 (2H, m); 3.55 (1H, m); 3.90–4.08

(3H, m); 4.15–4.22 (1H, m); 4.36 (1H, m); 4.63 (1H, d, J = 4.5 Hz); 4.70 (1H, m); 4.85 (1H, m); 4.90–5.02 (2H, m); 5.04–5.12 (2H, m); 5.33–5.45 (2H, m); 6.76 (1H, m, N*H*). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 15.0, 16.3, 17.1, 17.6, 20.7, 20.8, 21.2, 21.5, 21.8 (*C*H$_3$); 22.9, 23.8, 25.9, 26.0, 26.1, 26.6, 26.7 (*C*H$_2$); 29.9, 31.4 (*C*H); 31.5, 32.4, 32.6, 32.7, 33.7, 40.5, 40.7, 41.1 (*C*H$_2$); 41.8 (*C*); 42.9, 46.4, 56.9 (*C*H); 63.7, 67.8 (*C*H$_2$); 70.2, 71.4, 72.7, 74.1, 74.2, 75.0, 75.1, 77.3 (*C*H); 79.5 (*C*); 82.0 (*C*H$_2$); 90.9 (*C*H); 110.4 (*C*); 166.1, 169.6, 171.3, 171.5, 171.8, 172.1, 172.8, 174.0 (*C*=O). HRMS (ESI-FT-ICR) *m/z*: 1052.5258 [M+Na]$^+$; calcd for $C_{53}H_{79}NaO_{17}N_3$: 1052.5307.

4.10. Glucose/tetrazole/spirostane hybrid 17

Spirostanyl amine **4** (206 mg, 0.5 mmol), paraformaldehyde (15 mg, 0.5 mmol), azidotrimethylsilane (66 μL, 0.5 mmol), and glucosyl isocyanide **13** (180 mg, 0.5 mmol) were reacted for 72 h according to the general Ugi-4CR procedure described in Section 4.7. Flash column chromatography purification (*n*-hexane/EtOAc 2:1) afforded the pure conjugate **17** (314 mg, 76%) as a white solid. Mp (EtOAc): 211–212 °C. $[\alpha]_D^{20}$ −60.9 (*c* 1.10, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.77 (3H, d, J = 6.4 Hz, H-27); 0.78 (3H, s, H-18); 0.97 (3H, d, J = 6.5 Hz, H-21); 0.97 (3H, s, H-19); 2.02, 2.03, 2.05, 2.06 (3 × 4H, 4 × s, 4 × C*H*$_3$CO); 2.27 (1H, m); 2.40 (1H, ddd, J = 2.1/2.3/15.4 Hz); 3.29 (1H, tt, J = 4.4/11.2 Hz); 3.37 (1H, t, J = 10.9 Hz, H-26ax); 3.44–3.50 (1H, m); 3.98 (1H, ddd, J = 2.1/5.0/10.1 Hz); 4.13 (1H, dd, J = 2.0/12.5 Hz); 4.28 (1H, dd, J = 5.1/12.7 Hz); 4.40 (1H, m, H-16α); 4.67 (1H, s); 5.23 (1H, m); 5.32–5.38 (1H, m); 5.41 (1H, t, J = 9.3 Hz); 5.46 (1H, t, J = 9.0 Hz); 5.87 (1H, d, J = 9.2 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 14.5, 16.2, 17.1, 19.31, 20.2, 20.5, 20.5, 20.7, 20.8 (*C*H$_3$); 28.2, 28.7 (*C*H$_2$); 30.2 (*C*H); 31.3, 31.4 (*C*H$_2$); 31.8 (*C*H); 32.0 (*C*H$_2$); 36.9 (*C*); 37.1, 38.9, 39.7 (*C*H$_2$); 40.2 (*C*); 41.5, 45.0, 50.0, 56.4 (*C*H); 61.5 (*C*H$_2$); 62.0 (*C*H); 66.8 (*C*H$_2$); 70.1, 72.7, 75.0, 78.9, 80.8, 85.6 (*C*H); 109.2 (*C*); 121.5 (*C*H); 140.6, 159.6 (*C*); 168.9, 169.3, 169.9, 170.5 (*C*=O). HRMS (ESI-FT-ICR) *m/z*: 848.4428 [M+Na]$^+$; calcd for $C_{43}H_{63}N_5O_{11}Na$: 848.4422.

4.11. Glucose/*pseudo*-peptide/spirostane hybrid 19

The spirostanyl amine **8** (119 mg, 0.25 mmol), paraformaldehyde (7.5 mg, 0.25 mmol), acid **18** (102 mg, 0.25 mmol), and methyl 2-isocyano-2-methylpropanoate (32 mg, 0.25 mmol) were reacted for 24 h according to the general Ugi-4CR procedure described in Section 4.7. Flash column chromatography purification (*n*-hexane/EtOAc 3:1) afforded the pure conjugate **19** (130 mg, 51%) as a white solid. Mp (from MeOH): 237–238 °C. $[\alpha]_D^{20}$ −49.1 (*c* 0.80, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.78 (3H, d, J = 6.3 Hz, H-27); 0.79 (3H, s, H-18); 0.97 (3H, d, J = 6.6 Hz, H-21); 1.00 (3H, s, H-19); 1.50 (2 × 3H, 2 × s, 2 × C*H*$_3$); 2.05, 2.03, 2.02, 2.01 (3 × 5H, 5 × s, 5 × C*H*$_3$CO); 3.37 (1H, t, J = 11.0 Hz, H-26ax); 3.47 (1H, m, H-26eq); 3.52 (1H, m, H-3β); 3.70 (3H, s, C*H*$_3$O); 4.04–3.98 (1H, m); 4.14 (1H, m); 4.26–4.22 (2H, m, C*H*$_2$); 4.29 (1H, dd, J = 4.5/12.3 Hz); 4.40–4.36 (2H, m); 4.43 (1H, br m, H-16α); 4.69 (1H, d, J = 9.8 Hz); 4.91 (1H, m, H-6α); 5.26 (1H, t, J = 9.6 Hz); 5.36–5.41 (2H, m); 6.95 (1H, br s, N*H*). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 14.6, 15.6, 16.5, 17.0, 20.1, 20.2, 20.5, 20.6, 20.8, 21.2, 21.4 (*C*H$_3$); 26.5, 28.8 (*C*H$_2$); 30.0 (*C*H); 30.2, 30.7, 31.3 (*C*H$_2$); 34.9 (*C*H); 36.5 (*C*H$_2$); 39.8 (*C*); 40.4 (*C*H$_2$); 41.3 (*C*); 41.6, 45.4 (*C*H); 52.5 (*C*H$_3$); 53.6, 56.2 (*C*H); 58.6 (*C*); 61.6 (*C*H); 62.0, 62.4, 66.8 (*C*H$_2$); 70.1, 72.6, 74.2, 75.0, 80.7, 100.4 (*C*H); 109.2 (*C*); 168.6, 169.9, 169.7, 169.5, 170.5, 170.8, 171.4, 174.7 (*C*=O). HRMS (ESI-FT-ICR) *m/z*: 1041.5150 [M+Na]$^+$; calcd for $C_{52}H_{78}N_2O_{18}Na$: 1041.5146.

4.12. Glucose/*pseudo*-peptide/spirostane hybrid 20

Glucosyl amine **15** (88 mg, 0.25 mmol), paraformaldehyde (7.5 mg, 0.25 mmol), acetic acid (15 mg, 0.25 mmol), and the spirostanyl isocyanide **9** (120 mg, 0.25 mmol) were reacted according for 24 h to the general Ugi-4CR procedure described in Section 4.7. Flash column chromatography purification (*n*-hexane/EtOAc 3:1) furnished the pure conjugate **20** (185 mg, 73%) as a white solid. Mp (from MeOH): 224–226 °C. $[\alpha]_D^{20}$ −63.7 (*c* 0.50, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.77 (3H, d, J = 6.5 Hz, H-27); 0.81 (3H, s, H-18); 0.96 (3H, d, J = 6.8 Hz, H-21); 1.12 (3H, s, H-19); 2.02, 2.04, 2.05, 2.06, 2.08, 2.27 (6 × 3H, 6 × s, 6 × C*H*$_3$CO); 3.36 (1H, t, J = 10.9 Hz, H-26ax); 3.44 (1H, m, H-26eq); 4.04–4.08 (2H, m); 4.13 (1H, dd, J = 2.5/12.3 Hz); 4.21 (1H, d, J = 9.0 Hz); 4.23 (1H, dd, J = 4.9/12.3 Hz); 4.27–4.36 (3H, m); 4.38 (1H, m); 4.84 (1H, m); 4.91 (1H, m); 6.72 (1H, m, N*H*). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 14.7, 15.3, 16.5, 17.2, 20.2, 20.3, 20.5, 20.7, 21.0, 21.4 (*C*H$_3$); 25.5 (*C*H$_2$); 28.3 (*C*H); 28.4, 30.4, 31.4, 31.6 (*C*H$_2$); 34.7 (*C*H); 36.3 (*C*H$_2$); 36.8 (*C*); 39.9 (*C*H$_2$); 40.4 (*C*); 41.5 (*C*H$_2$); 41.8 (*C*H); 46.7 (*C*H$_2$); 53.6, 55.8, 58.1, 61.7 (*C*H); 62.0, 66.8 (*C*H$_2$); 69.8, 71.1, 71.3, 72.4, 73.3, 80.6, 80.8, 81.5, 84.7 (*C*H); 109.2 (*C*); 169.9, 170.2, 170.5, 170.6, 171.0, 171.5 (*C*=O). HRMS (ESI-FT-ICR) *m/z*: 1040.5316 [M+Na]$^+$; calcd for $C_{52}H_{79}N_3NaO_{17}$: 1040.5309.

4.13. Chacotriose/tetrazole/spirostane hybrid 23

β-Chacotriosyl azide **21** (230 mg, 0.25 mmol) was dissolved in 25 mL of MeOH and treated with 25 mg of 10% Pd/C. The reaction mixture was treated successively with hydrogen and vacuum and finally stirred under hydrogen atmosphere for 48 h, when TLC analysis revealed that all of the azide had been consumed. The catalyst was removed by filtration over a pad of Celite and the resulting solution was evaporated under reduced pressure to furnish β-chacotriosyl amine (**22**), which was identified by ESI-MS and used in the Ugi-4CR conjugation procedure without further purification. The crude amine **22** (223 mg, 0.25 mmol), paraformaldehyde (7.5 mg, 0.25 mmol), azidotrimethylsilane (33 μL, 0.25 mmol), and the spirostanyl isocyanide **5** (106 mg, 0.25 mmol) were reacted in MeOH for 72 h according to the general Ugi-4CR procedure described in Section 4.7. Flash column chromatography purification (*n*-hexane/EtOAc 2:1) furnished the pure conjugate **23** (175 mg, 51%) as a white foam. $[\alpha]_D^{20}$ −70.0 (*c* 1.25, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.78 (6H, m, H-18 + H-27); 0.84 (3H, d, J = 6.2 Hz, C*H*$_3$ Rha); 0.97 (3H, d, J = 6.9 Hz, H-21); 1.03 (3H, s, H-19); 1.15–1.18 (21H, m, 2 × (C*H*$_3$)$_3$C, C*H*$_3$ Rhá); 1.91, 1.95, 1.97, 2.03, 2.04, 2.11 (6 × 3H, 6 × s, 6 × C*H*$_3$CO); 2.65–2.72 (1H, m, H-5 Rhá); 3.38 (1H, t, J = 10.9 Hz, H-26ax); 3.43–3.48 (1H, m, H-26eq); 3.88–3.97 (2H, m, H-4 Glc, H-5 Rha); 3.99–4.04 (1H, m, H-5 Glc); 4.06–4.12 (2H, m, NC*H*$_2$); 4.29 (1H, dd, J = 4.4/12.2 Hz, H-6a Glc); 4.33–4.43 (3H, m, H-2 Glc, H-16α, H-6b Glc); 4.48–4.52 (1H, m, H-1 Glc); 4.66 (1H, m, H-3α); 4.77 (1H, d, J = 1.7 Hz, H-1 Rha); 4.81 (1H, t, J = 10.0 Hz, H-4 Rhá); 4.94 (1H, d, J = 1.8 Hz, H-1 Rhá); 4.99–5.08 (3H, m, H-2 Rha, H-3 Rhá, H-4 Rha,); 5.15 (1H, dd, J = 1.8/3.2 Hz, H-2 Rha); 5.21 (1H, dd, J = 3.2/10.1 Hz, H-3 Rha); 5.34 (1H, m, H-6 diosgenin); 5.47 (1H, t, J = 7.6 Hz, H-3 Glc). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 14.9, 16.4, 17.4, 17.5, 17.8, 19.4, 20.4, 20.5, 20.6 (*C*H$_3$); 20.8 (*C*H$_2$); 26.9, 27.1 (*C*H$_3$); 28.3, 28.7 (*C*H$_2$); 30.6 (*C*H); 31.5 (*C*H$_2$); 31.6 (*C*H); 31.8, 32.1 (*C*H$_2$); 36.9 (*C*); 37.5 (*C*H$_2$); 38.8, 38.9 (*C*); 39.0, 39.9 (*C*H$_2$); 40.4 (*C*); 41.6, 50.1, 56.5 (*C*H); 61.7, 61.9 (*C*H$_2$); 62.3, 68.5, 68.6, 68.7, 69.4, 69.9, 70.7, 75.5, 75.7, 77.4, 79.4, 80.8, 86.7, 97.5, 98.3 (*C*H); 109.3 (*C*); 124.3 (*C*H); 139.6 (*C*); 169.1, 169.2, 169.4, 169.8, 169.9, 170.1, 177.3, 177.6 (*C*=O). HRMS (ESI-FT-ICR) *m/z*: 1392.6938 [M+Na]$^+$; calcd for $C_{69}H_{103}N_5O_{23}Na$: 1392.6942.

4.14. Tetrazole-based dioscin analogue 24

An aqueous solution of NaOH (1 M, 1 mL) was added to a solution of hybrid **23** (140 mg, 0.1 mmol) in THF/MeOH (4 mL, 1:1, 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 filtrates were concentrated under reduced pressure and the resulting crude product was purified by flash column chromatography ($CHCl_3$/MeOH 5:1) to afford the saponin analogue **24** (86 mg, 90%) as a white amorphous solid. $[\alpha]_D^{20}$ −82.1 (*c* 0.90, MeOH). ^{1}H NMR (400 MHz, pyridine): δ = 0.73 (3H, d, *J* = 5.9 Hz, H-27); 0.89 (3H, s, H-18); 1.01 (3H, s, H-19); 1.18 (3H, d, *J* = 6.9 Hz, H-21); 1.57 (3H, d, *J* = 6.1 Hz, C*H*$_3$ Rha); 1.65 (3H, d, *J* = 6.4 Hz, C*H*$_3$ Rhá); 3.08–3.14 (1H, m, H-5 Rha); 3.48–3.56 (1H, m, H-26ax); 3.59–3.63 (1H, m, H-26eq); 3.90–3.94 (1H, m, H-5 Glc); 4.04–4.18 (6H, m, H-6a Glc, H-6b Glc, H-4 Rha, H-4 Rhá, NC*H*$_2$); 4.28–4.35 (2H, m, H-3 Glc, H-3 Rhá); 4.40–4.44 (1H, m, H-1 Glc); 4.49–4.58 (4H, m, H-3 Rha, H-4 Glc, H-16α, H-3α); 4.69 (1H, m, H-2 Rha); 4.77 (1H, m, H-2 Rhá); 4.83 (1H, t, *J* = 9.2 Hz, H-2 Glc); 4.88 (1H, m, H-5 Rhá); 5.49 (1H, m, H-6 diosgenin); 5.88 (1H, d, *J* = 1.3 Hz, H-1 Rha); 6.12 (1H, d, *J* = 1.5 Hz, H-1 Rhá). ^{13}C NMR (100 MHz, pyridine): δ = 15.6, 16.8, 17.9, 18.8, 19.5, 19.9 (*C*H$_3$); 21.6, 29.4, 29.9 (*C*H$_2$); 31.0, 32.1 (*C*H); 32.3, 32.5, 32.8 (*C*H$_2$); 37.6 (*C*); 37.9, 39.8, 40.3 (*C*H$_2$); 40.9 (*C*); 42.4, 50.9, 56.9 (*C*H); 61.2, 62.6 (*C*H$_2$); 63.3 (*C*H); 67.3 (*C*H$_2$); 70.5, 70.8, 72.5, 72.6, 72.7, 73.1, 74.3, 74.4, 77.9, 78.6, 79.6, 79.7, 80.8, 81.9, 90.4, 101.8, 102.2 (*C*H); 109.5 (*C*); 124.1 (*C*H); 139.4 (*C*). HRMS (ESI-FT-ICR) *m/z*: 972.5152 $[M+Na]^+$; calcd for $C_{47}H_{75}N_5O_{15}$Na: 972.5157.

4.15. Glucose/tetrazole/spirostane hybrid 25

To a solution of hybrid **17** (300 mg, 0.36 mmol) in 20 mL of CH_2Cl_2 was added Et_3N (100 μL, 0.72 mmol) and acetic anhydride (85 μL, 0.9 mmol). The reaction mixture was stirred at room temperature for 4 h and then diluted with 50 mL of CH_2Cl_2. The solution was washed with aq 10% HCl (2 × 50 mL), brine (50 mL), then dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The crude product was dried in vacuo for 2 h, then dissolved in MeOH (30 mL, 1:1, v/v) and treated with NaOMe up to pH 9–10. The mixture was stirred overnight, then neutralized with acid resin Dowex-50 (H^+) and filtered to remove the resin. The filtrate was concentrated under reduced pressure to dryness and. The residue was dried in vacuo and then dissolved in anhydrous pyridine (20 mL). The solution was cooled to −15 °C under nitrogen atmosphere and pivaloyl chloride (0.22 mL, 1.8 mmol) was added dropwise. The reaction mixture was stirred at room temperature until the intermediate disappeared as indicated by TLC. The mixture was then diluted with EtOAc (40 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 (*n*-hexane/EtOAc 2:1) to afford the dipivaloylated conjugate **25** (172 mg, 56%) as a white solid. Mp (EtOAc): 208–210 °C. $[\alpha]_D^{20}$ −5.3 (*c* 1.45, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.78 (3H, d, *J* = 6.9 Hz, H-27); 0.79 (3H, s, H-18); 0.97 (3H, d, *J* = 6.9 Hz, H-21); 1.01 (3H, s, H-19); 1.19, 1.22 (2 × 9H, 2 × s, 2 × (*CH*$_3$)$_3$C); 2.09 (3H, s, *CH*$_3$CO); 3.36 (1H, t, *J* = 10.9 Hz, H-26ax); 3.41–3.48 (3H, m, H-2 Glc, H-4 Glc, H-26eq); 3.54–3.59 (1H, m, H-5 Glc); 4.22 (1H, dd, *J* = 6.9/11.7 Hz, H-6a Glc); 4.30–4.34 (1H, m, H-16α); 4.40–4.43 (1H, m, H-6b Glc); 4.46 (1H, m, H-3α); 4.86 (1H, t, *J* = 9.1 Hz, H-3 Glc); 4.89–4.95 (2H, m, NC*H*$_2$); 5.32 (1H, m, H-6 diosgenin); 5.78 (1H, d, *J* = 9.1 Hz, H-1 Glc). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 11.9, 13.2, 15.9, 17.1, 20.6, 27.0, 27.1 (*C*H$_3$); 28.3, 28.7, 29.2 (*C*H$_2$); 30.1 (*C*H); 31.1, 31.4, 31.5 (*C*H$_2$); 34.3 (*C*H); 36.1 (*C*); 36.4, 37.7 (*C*H$_2$); 42.2, 44.6, 53.5 (*C*H); 55.1 (*C*); 55.4, 55.7 (*C*H); 63.7, 66.8 (*C*H$_2$); 70.0, 72.1, 74.2, 77.8, 78.7, 79.1, 101.1 (*C*H); 109.2 (*C*); 178.5, 180.1 (*C*═O). HRMS (ESI-FT-ICR) *m/z*: 890.5250 $[M+Na]^+$; calcd for $C_{47}H_{73}N_5O_{10}$Na: 890.5255.

4.16. Chacotriose/tetrazole/spirostane hybrid 27

A suspension of hybrid **25** (140 mg, 0.16 mmol) and 4 Å molecular sieves in dry CH_2Cl_2 (30 mL) was cooled to −80 °C and treated with $BF_3{\cdot}Et_2O$ (80 μL, 0.64 mmol) under nitrogen atmosphere. After stirring for 1 h at this temperature, a solution of rhamnopyranosyl trichloroacetimidate **26** (208 mg, 0.48 mmol) in CH_2Cl_2 (10 mL) was added and the stirring was continued for 5 h at room temperature. 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 $NaHCO_3$ (2 × 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 (*n*-hexane/EtOAc 2:1) to afford **27** (173 mg, 77%) as a white foam. $[\alpha]_D^{20}$ −48.7 (*c* 0.25, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.78 (6H, m, H-18 + H-27); 0.84 (3H, d, *J* = 6.2 Hz, C*H*$_3$ Rha); 0.96 (3H, d, *J* = 6.9 Hz, H-21); 1.03 (3H, s, H-19); 1.19–1.23 (21H, m, 2 × (*CH*$_3$)$_3$C, C*H*$_3$ Rhá); 1.98, 1.99, 2.02, 2.04, 2.06, 2.08, 2.12 (7 × 3H, 7 × s, 7 × *CH*$_3$CO); 2.66–2.73 (1H, m, H-5 Rhá); 3.37 (1H, t, *J* = 10.9 Hz, H-26ax); 3.45–3.49 (1H, m, H-26eq); 3.89–3.96 (2H, m, H-4 Glc, H-5 Rha); 3.98–4.02 (1H, m, H-5 Glc); 4.30 (1H, dd, *J* = 4.5/12.3 Hz, H-6a Glc); 4.35–4.45 (3H, m, H-2 Glc, H-16α, H-6b Glc); 4.48 (1H, m, H-3α); 4.78 (1H, d, *J* = 1.8 Hz, H-1 Rha); 4.81 (1H, t, *J* = 10.0 Hz, H-4 Rhá); 4.92 (1H, d, *J* = 1.9 Hz, H-1 Rhá); 4.99–5.08 (5H, m, H-2 Rha, H-3 Rhá, H-4 Rha, NC*H*$_2$); 5.13 (1H, dd, *J* = 1.9/3.3 Hz, H-2 Rha); 5.18 (1H, dd, *J* = 3.3/10.1 Hz, H-3 Rha); 5.34 (1H, m, H-6 diosgenin); 5.47 (1H, t, *J* = 7.6 Hz, H-3 Glc); 5.89 (1H, d, *J* = 8.6 Hz, H-1 Glc). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 14.5, 16.3, 17.1, 17.2, 17.5, 19.4, 20.5, 20.6, 21.8 (*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 (*C*H); 143.2 (*C*); 169.1, 169.3, 169.4, 169.8, 169.9, 170.0, 171.4, 176.3, 177.6 (*C*═O). HRMS (ESI-FT-ICR) *m/z*: 1434.7050 $[M+Na]^+$; calcd for $C_{71}H_{105}N_5O_{24}$Na: 1434.7047.

4.17. Tetrazole-based dioscin analogue 28

An aqueous solution of NaOH (1 M, 1 mL) was added to a solution of hybrid **23** (130 mg, 0.09 mmol) in THF/MeOH (4 mL, 1:1, 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 filtrates were concentrated under reduced pressure and the resulting crude product was purified by flash column chromatography ($CHCl_3$/MeOH 5:1) to afford the saponin analogue **28** (80 mg, 88%) as a white amorphous solid. $[\alpha]_D^{20}$ −61.9 (*c* 0.85, MeOH). ^{1}H NMR (400 MHz, pyridine-d_5): δ = 0.71 (3H, d, *J* = 5.8 Hz, H-27); 0.87 (3H, s, H-18); 0.99 (3H, s, H-19); 1.16 (3H, d, *J* = 7.0 Hz, H-21); 1.55 (3H, d, *J* = 6.1 Hz, C*H*$_3$ Rha); 1.64 (3H, d, *J* = 6.2 Hz, C*H*$_3$ Rhá); 2.11 (3H, s, C*H*$_3$CO); 3.09–3.15 (1H, m, H-5 Rha); 3.47–3.55 (1H, m, H-26ax); 3.59–3.62 (1H, m, H-26eq); 3.89–3.93 (1H, m, H-5 Glc); 4.03–4.21 (4H, m, H-6a Glc, H-6b Glc, H-4 Rha, H-4 Rhá); 4.29–4.37 (2H, m, H-3 Glc, H-3 Rhá); 4.46 (1H, m, H-3α); 4.50–4.60 (3H, m, H-3 Rha, H-4 Glc, H-16α); 4.68 (1H, m, H-2 Rha); 4.77 (1H, m, H-2 Rhá); 4.84 (1H, t, *J* = 9.2 Hz, H-2 Glc); 4.88 (1H, m, H-5 Rhá); 5.39 (1H, m, H-6 diosgenin); 5.86 (1H, d, *J* = 1.3 Hz, H-1 Rha); 6.27 (1H, d, *J* = 1.3 Hz, H-1 Rhá); 6.31 (1H, d, *J* = 9.3 Hz, H-1 Glc). ^{13}C NMR (100 MHz, pyridine): 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 (*C*H); 141.4 (*C*). HRMS (ESI-FT-ICR) *m*/*z*: 1014.5267 $[M+Na]^+$; calcd for $C_{49}H_{77}N_5O_{16}Na$: 1014.5263.

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