ACS Combinatorial Science

Research Article

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Multicomponent Ligation of Steroids: Creating Diversity at the Linkage Moiety of Bis-spirostanic Conjugates by Ugi Reactions

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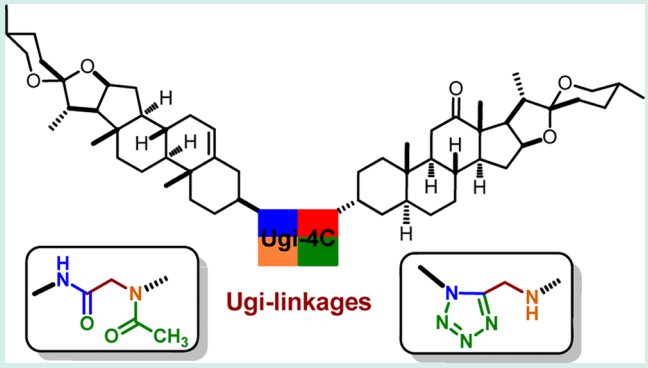
Supporting Information

ABSTRACT: The diversity-oriented synthesis of novel bis-spirostanic conjugates utilizing two different Ugi four-component reactions (Ugi-4CR) is described. Spirostanic steroids were functionalized with Ugi-reactive groups, that is, amines, isocyanides, and carboxylic acids, and next were subjected to multicomponent ligation approaches leading to bis-steroidal conjugates featuring pseudo-peptidic and heterocyclic linkage moieties. Both the classic Ugi-4CR and its hydrazoic acid variant were implemented, proving good efficiency for the ligation of isocyanosteroids to spirostanic acids and equatorial amines. Axially oriented amines showed poorer results, although model studies proved that chemical efficiency could be significantly improved while increasing reaction times. Overall, the method comprises the rapid generation of molecular diversity at the bis-steroid linkage moiety and, consequently, shows promise toward the production of combinatorial libraries of bis-spirostanes for biological screening.



KEYWORDS: *steroids, chemical ligation, conjugation, multicomponent reactions, Ugi reaction*

INTRODUCTION

Bis-steroidal conjugates have attracted considerable attention in the last two decades in view of their remarkable applications in biological, medicinal, and supramolecular chemistry. Examples of naturally occurring bis-steroids are the cephalostatins and ritterazines,[1,2] that is, marine natural products containing either two spirostanic or spirofuranic units linked by a pyrazine ring (Figure 1). Other impressively exploited classes of bis-steroidal conjugates are those composed by two cholanic skeletons, which, depending on the nature of both the axially disposed functionalities and the linker, have found important applications in molecular and ion pair recognition,[3] in the discovery of antibacterial agents,[4] in the encapsulation and transportation of hydrophilic agents in lipophilic media,[5] and in the solubilization of membrane proteins,[6] among others.[7]

Despite the immense progress achieved in this field, the ligation of the two steroidal units usually relies just on a few types of reactions. Thus, the classical amide and ester bonds formation reactions using coupling agents have been the synthetic choice for most bis-steroidal conjugates found in the literature. More recently, the Cu^{I}-catalyzed 1,3-dipolar cycloaddition (click) approach has been also used for this purpose, with the resulting 1,2,3-triazole-linked bis-steroids showing interesting medicinal and chemical applications.[8−10] Nevertheless, despite the great efficiency of both ligation methods, a relevant drawback is the low level of molecular diversity that can be installed in the linkage moiety, which certainly limits applicability in, for example, the combinatorial generation of compounds libraries for screening of bioactivity and properties.

In this sense, synthetic approaches that rapidly generate molecular diversity and complexity in the linkage moiety of bis-steroidal conjugates may be of great interest for medicinal chemistry, whereas examples of both antibacterial bis-cholanes[4] and anticancer bis-spirostanes are well documented.[1,2] Considering the unraveling way by which isocyanide-based multicomponent reactions (I-MCRs) generate structural diversity with minimized synthetic cost, we envisioned the implementation of these reactions in the synthesis of structurally diverse bis-steroidal conjugates. Spirostanic steroids were chosen to address the efficiency and diversity-oriented character of such a multicomponent ligation process, as synthetic approaches toward bis-spirostanes have attracted significant attention in the last years.[11]

Herein we report on the utilization of Ugi four-component reactions (Ugi-4CRs)[12] as a general multicomponent ligation approach toward bis-spirostanic conjugates. The Ugi-4CR is currently considered one of the most versatile synthetic tools for the generation of molecular diversity and complexity.[13−15]

Received: March 5, 2013
Revised: April 2, 2013
Published: April 10, 2013

   dx.doi.org/10.1021/co400028e | ACS Comb. Sci. 2013, 15, 320−330

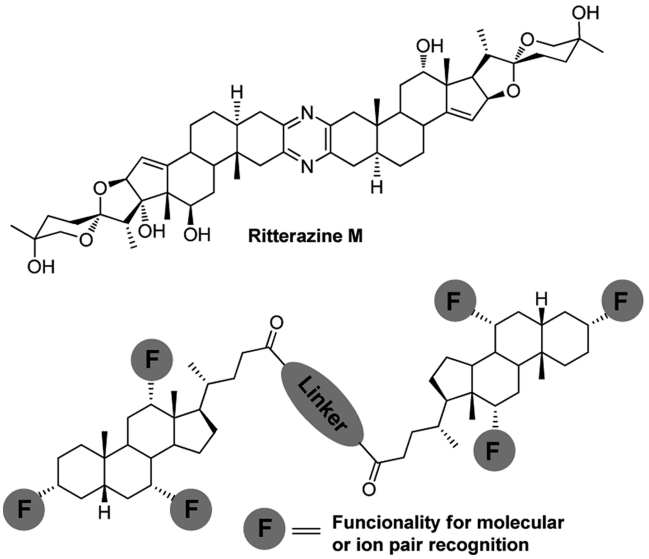


Figure 1. Structures of natural bis-spirostanic and synthetic bis-cholanic conjugates.

In the field of steroid chemistry, such a MCR has been used for the synthesis of steroidal hybrids incorporating sugars,[16] peptidomimetics,[17] and β-lactams,[18] as well as for the assembly of steroidal macrocycles.[19−22] However, an assessment of its versatility and efficiency in the construction of a small library of bis-spirostanic conjugates has not been described so far.

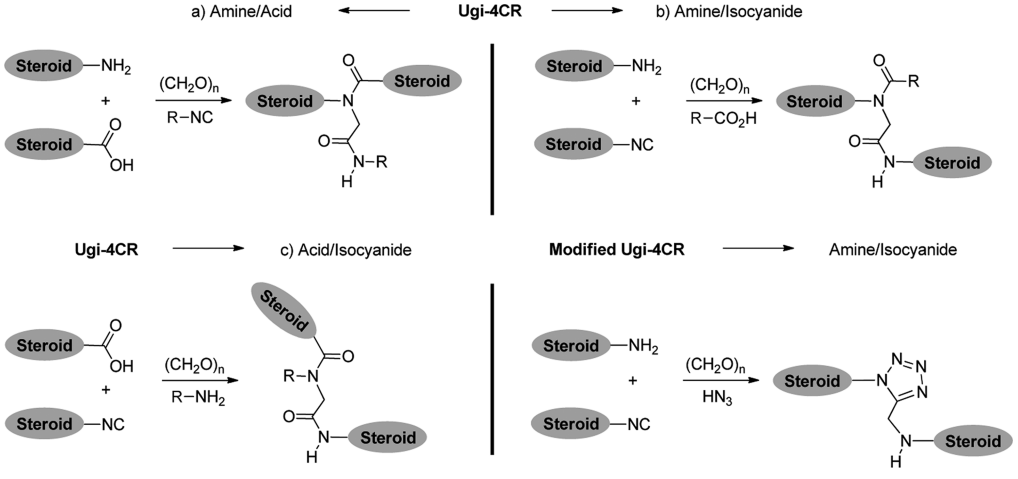
RESULTS AND DISCUSSION

The simplest version of the Ugi-4CR comprises the one-pot condensation of an oxo compound (i.e., ketone or aldehyde), a primary amine, a carboxylic acid, and an isocyanide to give an *N*-substituted dipeptide backbone.[12] An important variation of this approach encompasses the utilization of hydrazoic acid as the acid component, which upon reaction with the amine, the oxo-compound, and the isocyanide furnishes a 1,5-disubtituted tetrazole *via* an electrocyclic ring closure.[23] The selection of these processes relies on their capability to form stable chemical linkages between two spirostanic units, which is indeed an alternative to the pyrazine-linkage of the natural bis-spirostanes. Moreover, a key feature is the high diversity of substitution patterns at the Ugi-type linkage resulting from the variation of the functionalities (i.e., Ugi-components) at the reacting steroidal skeletons. This combinatorial principle derived from the multicomponent nature of the Ugi-4CR has been implemented for other families of compounds,[12−14] but it has not been exploited in the MCR-based modifications of steroids. Accordingly, this work attempts to illustrate the many possibilities derived from the use of two dissimilar Ugi-4CRs for the ligation of steroids.

Scheme 1 depicts the strategy toward bis-steroidal conjugates using Ugi-4CRs, which give rise either to peptidic or tetrazole linking platforms. In this work, the oxo-component will be fixed to formaldehyde in order to avoid formation of diastereomers, while the steroids will be functionalized with each the three remaining Ugi-functionalities. Consequently, three different combinations may be implemented for the classical Ugi-4CR, while only one combination is possible for the Ugi-tetrazole reaction, as hydrazoic acid is also a fixed component.[24] As it will be shown, further skeletal diversity can be generated by variation of the position and stereochemistry of the Ugi-reactive functionalities, which shall lead to dissimilar conformations and topologies of the resulting conjugates.

Scheme 2 shows the synthesis of spirostanes functionalized with the Ugi-reactive groups. Initially, the focus was posed on the synthesis of 3-amino-spirostanes having varied stereochemistry and diverse oxygen-functionalities in rings B and C. Hence, hecogenin (**1**) was converted to 3α-hecogenyl azide (**2**) as previously described,[25] which was further subjected to Staudinger azide reduction with PPh_3 to produce the 3α-hecogenyl amine (**3**) in 81% yield after column chromatography. In a similar way, 5α-hydroxy-laxogenin (**5**)[26] was transformed into 3α-amino-spirostane **7** also by the traditional tosylation/azide displacement and subsequent reduction to amine. Alternatively, we turned to the synthesis of spirostanic amines with the 3β configuration, aiming to address the influence of the stereochemistry in the efficiency of the Ugi-conjugation. For this, spirostanetriol **9**, readily obtained[27] from the highly available diosgenin (**13**), was subjected to Mitsunobu reaction using methanesulfonic acid as the acidic/nucleophilic component, followed by replacement with azide to recover the original C-3 stereochemistry in the 3β-azido-spirostane **10**.

Scheme 1. Combinations of the Multicomponent Ligation Approach Derived from Both the Variation of the Ugi-Reactive Functional Groups and the Type of Ugi-4CR



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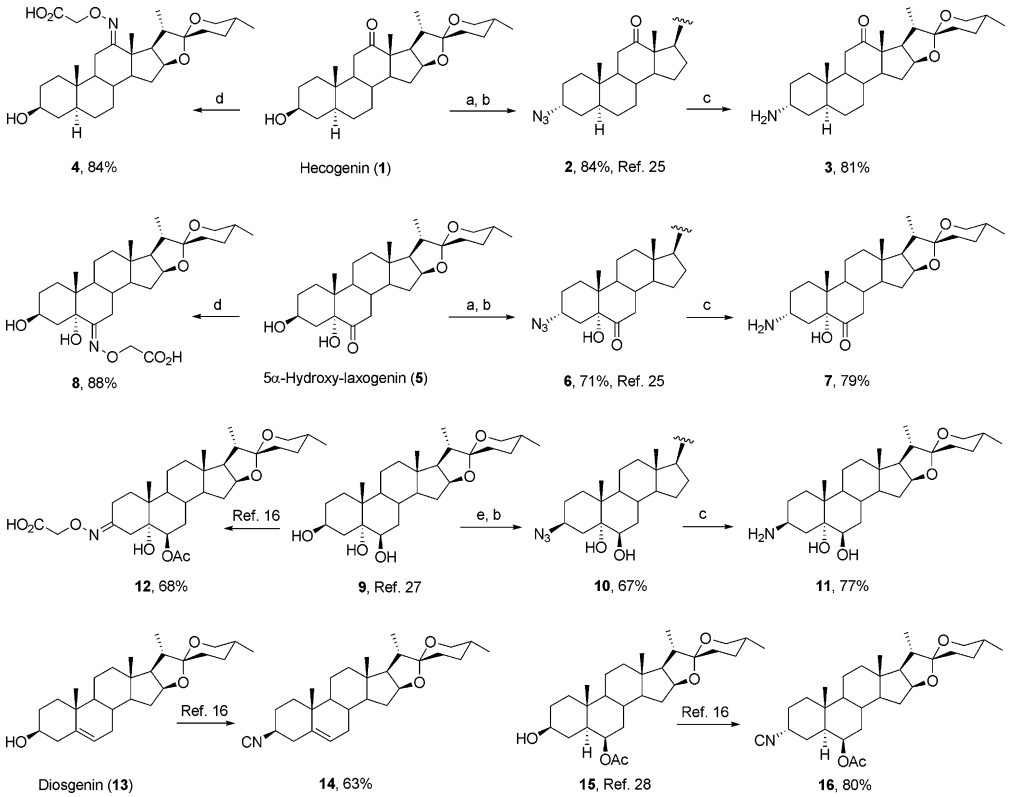
Scheme 2. Synthesis of Spirostanes Functionalized with Amino, Carboxylic, and Isocyano Groups[a]



[a]Reagents and conditions: (a) TsCl, pyridine; (b) NaN_3, DMPU, 60 °C; (c) PPh_3, THF/H_2O; (d) $HCl{\cdot}H_2NOCH_2CO_2H$, pyridine; (e) MsOH, PPh_3, DMAP, DIAD, THF.

Subsequent Staudinger reduction with PPh_3 produced the 3β-amine **11** in 48% yield over two steps.

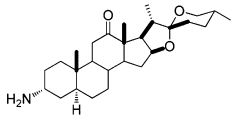
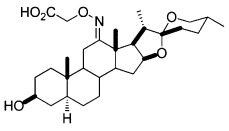
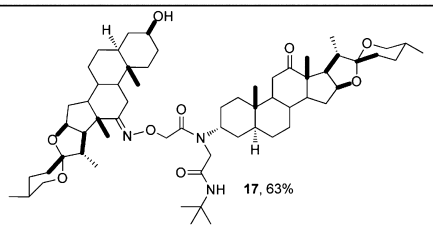
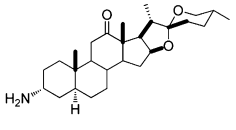
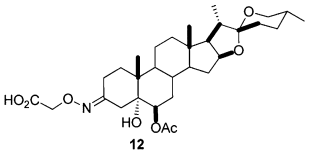
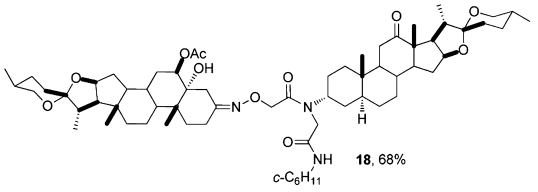
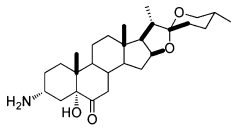
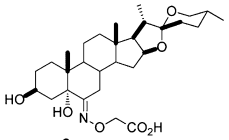
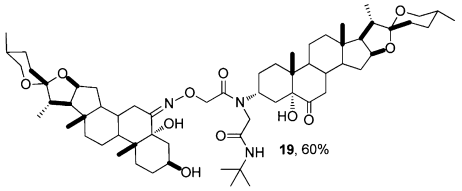
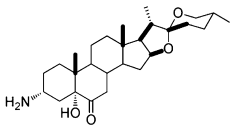
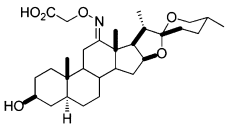
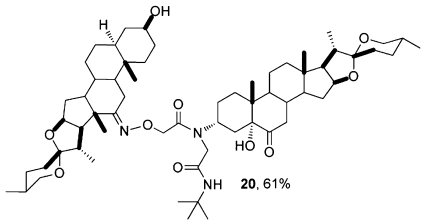
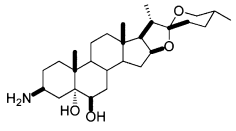
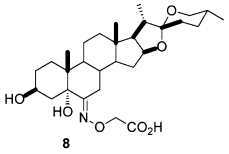
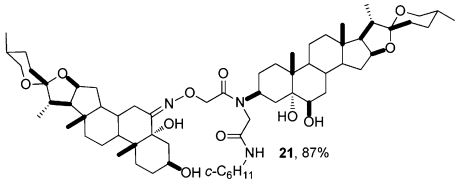
We were next engaged in the synthesis of spirostanic acids having the carboxylic functionality at different positions of the steroidal skeleton. This was accomplished by condensation of ketones **1** and **5** with *O*-(carboxymethyl)-hydroxylamine to give rise to **4** and **8** having the oxime-linked carboxylic acids attached at positions 12 and 6, respectively. Spirostanic acid **12** was similarly prepared from triol **9** in a sequence including oxidation to the 3-keto intermediate followed by condensation with *O*-(carboxymethyl)-hydroxylamine.[16]

To complete the preparation of Ugi-functionalized spirostanes, we proceeded to the synthesis of 3-isocyanospirostanes having either α or β stereochemistry at C-3. Hence, the 3β-isocyanide **14** and the 3α-isocyanide **16** were prepared from diosgenin (**13**) and the 3β-spirostanol **15**,[28] respectively, according to a reported procedure[16] including amination at C-3, formylation using mixed formic-acetic anhydride and subsequent phosphorylchloride mediated dehydration of the corresponding formamides. With the set of both equatorially and axially oriented amino and isocyano groups attached at the A/B-trans steroidal nucleus, we should be able not only to evaluate the efficiency of the different Ugi-combinations in the conjugation of such sterically crowed building blocks but also to access to a more diverse conformational space derived either from the flat (i.e., using equatorial functionalities) or the perpendicular (i.e., using axial functionalities) topologies of the resulting conjugates.[29]

Table 1 illustrates the implementation of the amine/acid combination for the Ugi-4CR-based conjugation of two spirostanic steroids, always using commercially available isocyanides. Initially, hecogenyl amine **3**, endowed with an axially oriented amino group, was combined with the spirostanic acids **4** and **12** to furnish the bis-spirostanes **17** and **18** in 63% and 68% yield, respectively. Similarly, the axially oriented amine **7** was reacted with acids **8** and **4** to produce the conjugates **19** and **20** in 60% and 61% yield, respectively. It must be noticed that despite amine **7** features and 1,3-diaxial interaction with the 5α-hydroxyl group, its reactivity was not significantly hampered with regard to hecogenyl amine **3**. Alternatively, the reaction of the equatorially oriented amine **11**, also having a 5α-hydroxyl group, with spirostanic acid **8** afforded conjugate **21** in 87% yield, almost a 30% more than the combination of the same acid with the axial amine **7**. Two different conclusions may arise from analysis of Table 1: (i) the position of the oxime-linked carboxylic functionality does not have significant influence in the efficiency of the Ugi-4CR-based conjugation and (ii) the stereochemistry of the amine functionality plays a crucial role in the reaction outcome. That equatorial amines show higher reactivity than axial ones is in agreement with the classic reactivity of steroid functionalities, albeit in the case of the Ugi-4CR there might be several factors influencing such a behavior. As known from the mechanism of the Ugi-4CR,[12,24] crucial steps are the imine formation and the intramolecular acylation step that requires the migration of the amine nitrogen atom once the α-adduct has been formed (i.e., Mumm rearrangement). Indeed, both processes are more favorable for equatorially oriented amines than for axial ones. Another factor likely affecting the yield of the Ugi-conjugation with axial amines may be the fact that the resulting sterically crowded tertiary amide is less stable with an axial disposition than with an equatorial one.

dx.doi.org/10.1021/co400028e | ACS Comb. Sci. 2013, 15, 320−330

Table 1. Synthesis of Amide-Linked Bis-spirostanes by the Amine/Acid Combination of the Ugi-4CR[a]

Amine	Acid	Isocyanide	Bis-spirostanic Conjugates
 3	 4		 **17**, 63%
 3	 12		 **18**, 68%
 7	 8		 **19**, 60%
 7	 4		 **20**, 61%
 11	 8		 **21**, 87%

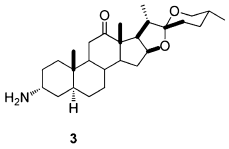
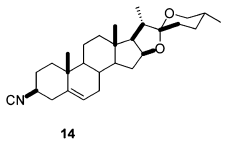
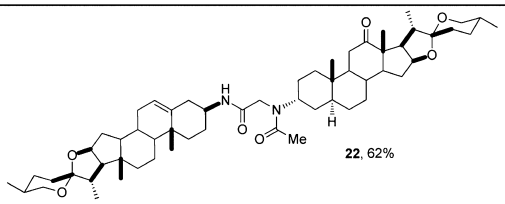
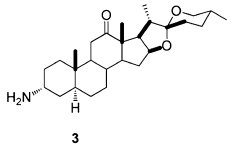
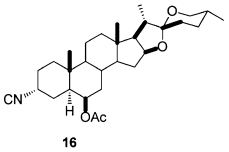
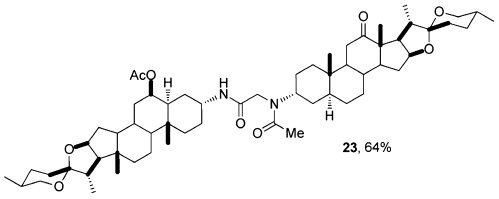
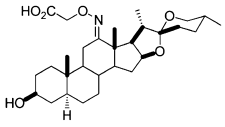
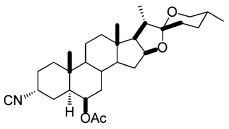
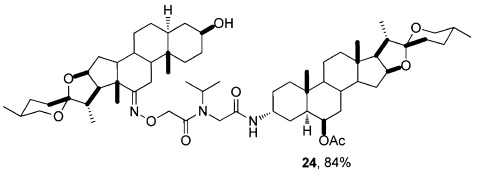
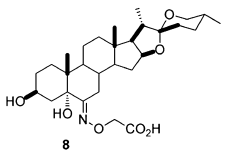
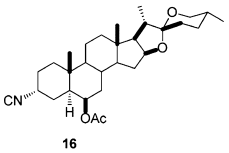
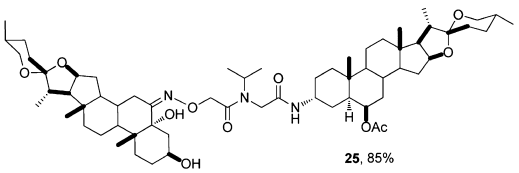
[a]All reactions were conducted in MeOH at room temperature for 24 h using paraformaldehyde as the oxo component.

With the aim to install molecular diversity not only derived from the variation of the stereochemistry and positions of the functionalities, we turned to ligation approaches based on the two remaining combinations of Ugi-functionalities. Thus, Table 2 illustrates the implementation of the amine/isocyanide and the acid/isocyanide combinations of the Ugi-conjugation process,[30] in all cases using highly reactive aliphatic amines and carboxylic acids as the other two components. Accordingly, the goal was also to assess the efficiency of the ligation process while using axially- and equatorially oriented spirostanic isocyanides. To this end, amine **3** was reacted with the equatorial isocyanosteroid **14** and the axial one **16** in the presence of acetic acid to furnish bis-spirostanes **22** and **23** in 62% and 64% yield, respectively. In a parallel manner, isocyanosteroid **16** was conjugated to spirostanic acids **4** and **8** in the presence of *iso*-propylamine to produce the bis-spirostanes **24** and **25** in 84% and 85% yield, respectively, ~20% more than the reaction of the same sterically hindered isocyanide with the steroidal amine **3**.

Results of Table 2 confirm our hypothesis that the efficiency of this ligation process mostly depends on the reactivity of the steroidal amine (i.e., herein addressed by its equatorial or axial disposition) and not much on that of the isocyanosteroid. These results also suggest that both equatorial and axial steroidal isocyanides are as reactive as other aliphatic isocyanides (i.e., *tert*-butyl and cyclohexyl) typically employed in MCR chemistry. To gain more information about the reactivity of axial aminosteroids, we decided to address the factor time in the reaction of amine **3** with different substrates. Thus, parallel experiments showed that conjugate **18**, resulting of reaction of amine **3** with spirostanic acid **12**, could be obtained in 79% and 91% yield after 3 and 5 days of reaction, respectively. Similarly, reaction of amine **3** with isocyanide **14** afforded conjugate **22** in 83% and 94% yield after 3 and 5 days of reaction, respectively. This confirms that upon reaction of

dx.doi.org/10.1021/co400028e | ACS Comb. Sci. 2013, 15, 320−330

Table 2. Synthesis of Amide-Linked Bis-spirostanes by the Amine/Isocyanide and Acid/Isocyanide Combinations of the Ugi-4CR[a]

Amine	Acid	Isocyanide	Bis-spirostanic Conjugates
 3	CH_3CO_2H	 14	 **22**, 62%
 3	CH_3CO_2H	 16	 **23**, 64%
	 4	 16	 **24**, 84%
	 8	 16	 **25**, 85%

[a]All reactions were conducted in MeOH at room temperature for 24 h using paraformaldehyde as the oxo component.

axial amines with bulky steroidal substrates, long times are needed to achieve yields suitable, for example, combinatorial chemistry or automated synthesis.

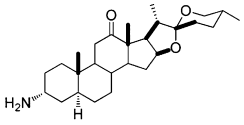
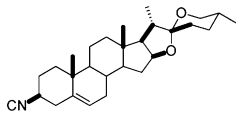
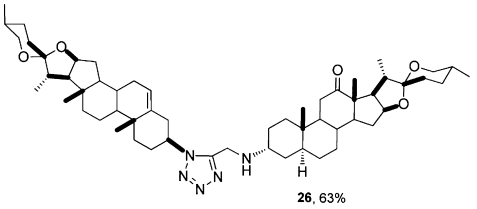
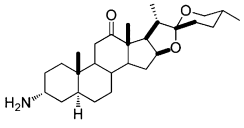
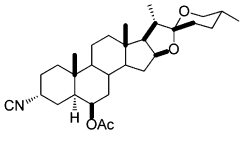
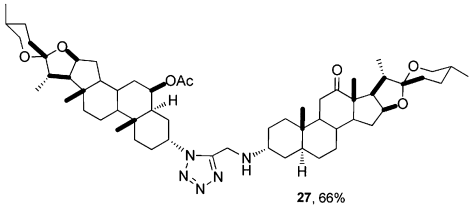
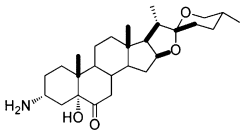
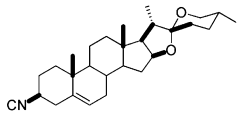
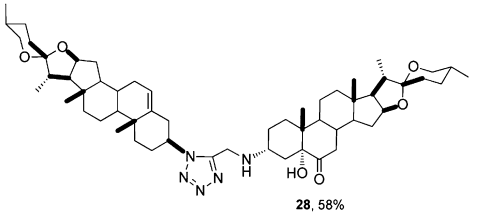
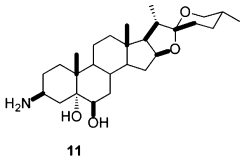
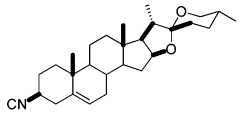
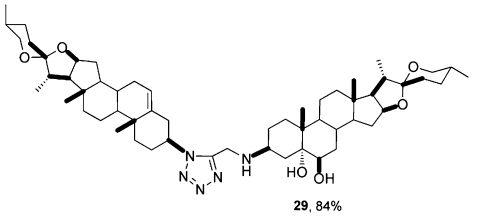
As shown in Table 3, the multicomponent ligation process based on the hydrazoic acid variant of the Ugi-4CR enables accessing a structurally different type of bis-spirostanes bearing a tetrazole linkage. In this case, reaction times were fixed to 72 h as a result of the known slower kinetic of this process compared to the classic Ugi-4CR.[24] As before, spirostanic amine **3** was initially reacted with isocyanosteroids **14** and **16** in the presence of paraformaldehyde and hydrazoic acid to furnish the tetrazole-linked bis-spirostanes **26** and **27** in 63% and 66% yield, respectively. Alternatively, both the axial amine **7** and the equatorial one **11** were subjected to conjugation to isocyanosteroid **14** to produce bis-spirostanes **28** and **29** in 58% and 84% yield, respectively. As noticed, the same tendency of the higher reactivity of equatorial amines was found in the implementation of the hydrazoic acid variant of the Ugi-4CR. This shows that steroidal amines likely present a general behavior in their reactivity in isocyanide-based MCRs, although other archetypal MCRs should be studied to achieve more concluding elements. To gain deeper insights into this field, steroids bearing the amino functionality at other positions of the polycyclic skeleton and even being subjected to dissimilar steric constrains (e.g., amines in the β face having steric interaction with the axial methyl groups) should be also studied for their behavior in diverse Ugi-4CRs. Nevertheless, this issue lies away from the goal of the present report, which is more focused on the scope of Ugi-4CRs as general approaches for the synthesis of pseudo-peptide and tetrazole-linked bis-spirostanes.

CONCLUSIONS

We have demonstrated that structurally diverse bis-spirostanes can be easily synthesized by the utilization of the Ugi-4CR and its hydrazoic acid variant as multicomponent ligation processes. The process proved good efficiency considering the bulky and complex nature of the steroidal building blocks, as well as the fact that four covalent bonds are formed in one pot. Additionally, it was proven that yields can be significantly improved with the increment of the reaction time, especially for less reactive axially oriented amines. We believe that a feature of great potential of this method is its diversity-oriented character upon the construction of the linkage moiety between the two steroidal scaffolds. In this report, we have addressed the creation of structural diversity varying the stereochemistry, nature, and position of the Ugi-functionalities, as well as the type of isocyanide-based MCR employed. Whereas this rapid generation of molecular diversity is an intrinsic characteristic of Ugi-4CRs, other types of MCRs can be equally utilized because of the easy functionalization and manipulation of steroidal substrates. As the method shows great promise toward the combinatorial production of compounds for biological screening, we envisioned further applications in the synthesis of

dx.doi.org/10.1021/co400028e | ACS Comb. Sci. 2013, 15, 320−330

Table 3. Synthesis of Tetrazole-Linked Bis-spirostanes by the Hydrazoic Acid Variant of the Ugi-4CR[a]

Amine	Isocyanide	Bis-spirostanic conjugates
 3	 14	 **26**, 63%
 3	 16	 **27**, 66%
 7	 14	 **28**, 58%
 11	 14	 **29**, 84%

[a]All reactions were conducted in MeOH for 72 h using paraformaldehyde and HN_3 as the oxo and acid components, respectively. HN_3 was formed in situ from $TMSN_3$/MeOH.

interesting conjugates and hybrids derived not only from steroids, but also from other families of natural products like terpenes, alkaloids, lipids, etc.

EXPERIMENTAL PROCEDURES

General. Melting points are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded at 400 and 500 MHz for ^{1}H and 100 and 125 MHz for ^{13}C, respectively. Chemical shifts (δ) are reported in parts per million relative to the residual solvent signals, and coupling constants (J) are reported in hertz. 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. Hecogenin (**1**) and diosgenin (**13**) are commercially available sapogenins, while spirostanols **9** and **15** were obtained as described in refs 27 and 28, respectively.

*(25R)-3α-Amino-5α-spirostan-12-one (**3**).* Azide **2** (500 mg, 1.10 mmol) was dissolved in THF (10 mL) and treated with PPh_3 (430 mg, 1.65 mmol) and H_2O (0.3 mL). The reaction mixture was stirred at room temperature until completion as indicated by TLC. The reaction mixture was concentrated under reduced pressure and the residue was dissolved in $CHCl_3$ (20 mL) and washed with 1 M aqueous solution of NaOH (3 × 10 mL). The organic phase was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to dryness. The crude product was purified by flash column chromatography (EtOAc/MeOH 3:1) to furnish the amine **3** (383 mg, 81%) as a white solid. mp: 182−184 °C. $[\alpha]_D^{20}$: −94.7 (c 0.95, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.78 (d, 3H, J = 6.5 Hz, CH_3); 0.87 (s, 3H, CH_3); 1.03 (s, 3H, CH_3); 1.06 (d, 3H, J = 7.0 Hz, CH_3); 2.21 (dd, 1H, J = 5.0/14.1 Hz); 2.35 (t, 1H, J = 14.1 Hz); 3.34 (t, 1H, J = 11.1 Hz, H-26ax); 3.36 (m, 1H, H-3β); 3.48 (m, 1H, H-26eq); 4.39 (m, 1H, H-16α). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 10.8, 13.2, 15.6, 17.1 (CH_3); 26.4, 27.8, 28.7 (CH_2); 30.2 (CH); 31.1, 31.4, 31.6, 31.8 (CH_2); 34.3 (CH); 35.6 (CH_2); 36.7 (C); 37.4 (CH_2); 38.9, 42.2, 44.2, 53.6 (CH); 55.1 (C); 55.5, 55.8 (CH); 66.9 (CH_2); 79.2 (CH); 109.2 (C); 213.6 (C═O). HRMS (ESI-FT-ICR) m/z: 430.3319 [M + H]$^+$. Calcd for $C_{27}H_{44}O_3N$: 430.3321.

*(25R)-12E-[O-(carboxymethyl)oximino]-5α-spirostan-3β-ol (**4**).* O-(carboxymethyl)hydroxylamine hydrochloride (157 mg, 1.44 mmol) was added at room temperature to a stirred solution of hecogenin (**1**) (500 mg, 1.21 mmol) in dry pyridine

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(3 mL). The reaction mixture was stirred at room temperature overnight and then poured into a cold solution of aqueous 5% HCl (15 mL). The resulting precipitate was filtered, washed twice with cold water and dried to give the acid **4** (510 mg, 84%) as a white solid. mp: 257−258 °C. $[\alpha]_D^{20}$: −32.1 (*c* 0.50, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$/CD_3OD/DMSO): δ = 0.65 (d, 3H, *J* = 6.0 Hz, CH_3); 0.74 (s, 3H, CH_3); 0.82 (s, 3H, CH_3); 0.88 (d, 3H, *J* = 6.8 Hz, CH_3); 3.11 (dd, 1H, *J* = 5.0/15.2 Hz); 3.22 (t, 1H, *J* = 11.1 Hz, H-26ax); 3.35 (m, 1H, H-26eq); 3.42 (m, 1H, H-3α); 4.40 (m, 1H, H-16α); 4.48 (m, 2H, OCH_2CO_2H). ^{13}C NMR (100 MHz, $CDCl_3$/CD_3OD/DMSO): δ = 11.9, 13.0, 15.8, 17.1 (CH_3); 28.2, 28.4, 31.2, 31.5, 31.6, 31.8 (CH_2); 34.3, 35.4 (CH); 36.1 (*C*); 36.5, 37.7, 37.8 (CH_2); 41.6, 44.6 (CH); 55.5 (*C*); 55.6, 55.9, 62.2 (CH); 65.7 (CH_2); 70.8 (CH); 71.9 (CH_2); 81.7 (CH); 109.8 (*C*); 164.9 (*C*═N); 174.1 (*C*═O). HRMS (ESI-FT-ICR) *m*/*z*: 502.3169 $[M - H]^-$. Calcd for $C_{29}H_{44}O_6N$: 502.3169.

*(25R)-3α-Amino-5-hydroxy-5α-spirostan-6-one (**7**).* Azide **6** (500 mg, 1.12 mmol), PPh_3 (440 mg, 1.69 mmol), and H_2O (0.3 mL) in THF (10 mL) were reacted in a manner similar to that described in the synthesis of **3**. Flash column chromatography purification (EtOAc/MeOH 2:1) afforded the amine **7** (387 mg, 79%) as a white solid. mp: 178−179 °C. $[\alpha]_D^{20}$: −116.2 (*c* 1.05, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.78 (m, 9H, 3 × CH_3); 0.96 (d, 3H, *J* = 6.7 Hz, CH_3); 2.74 (t, 1H, *J* = 12.4 Hz); 3.36 (t, 1H, *J* = 10.9 Hz, H-26ax); 3.45−3.48 (m, 1H, H-26eq); 3.40 (m, 1H, H-3β); 4.41 (m, 1H, H-16α). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 13.7, 14.4, 16.4, 17.1 (CH_3); 20.8, 24.4, 25.5 (CH_2); 28.7 (CH); 29.5, 30.2, 31.3, 31.5, 36.9 (CH_2); 39.6 (*C*); 41.0 (CH); 41.5 (CH_2); 43.3, 44.2, 44.6, 54.7 (CH); 56.1, 62.0 (CH); 66.8 (CH_2); 79.3 (*C*); 80.5 (CH); 109.2 (*C*); 210.6 (*C*═O). HRMS (ESI-FT-ICR) *m*/*z*: 446.3276 $[M + H]^+$. Calcd for $C_{27}H_{44}O_4N$: 446.3270.

*(25R)-6E-[O-(Carboxymethyl)oximino]-5-hydroxy-5α-spirostan-3β-ol (**8**).* 5α-Hydroxy-laxogenin (**5**) (500 mg, 1.12 mmol) and *O*-(carboxymethyl)hydroxylamine hydrochloride (147 mg, 1.35 mmol) were reacted in dry pyridine (3 mL) in a similar way as described in the synthesis of **4** to afford the acid **8** (510 mg, 88%). mp: 224−225 °C. $[\alpha]_D^{20}$: −17.9 (*c* 0.65, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$, MeOD): δ = 0.77 (s, 3H, CH_3); 0.80 (d, 3H, *J* = 6.3 Hz, CH_3); 0.86 (s, 3H, CH_3); 0.97 (d, 3H, *J* = 6.8 Hz, CH_3); 3.34 (t, 1H, *J* = 11.0 Hz, H-26ax); 3.48 (m, 1H, H-26eq); 3.72 (m, 1H, H-3α); 4.41 (m, 1H, H-16α); 4.50 (m, 2H, OCH_2CO_2H). ^{13}C NMR (100 MHz, $CDCl_3$, MeOD): δ = 13.8, 16.0, 16.5, 20.8 (CH_3); 25.5, 28.2, 29.2, 29.3 (CH_2); 29.5 (CH); 29.6, 29.8, 30.9 (CH_2); 31.1 (CH); 33.9 (CH_2); 40.2 (*C*); 40.5 (CH); 40.6 (CH_2); 41.2 (*C*); 44.3, 55.7, 61.6, 66.5 (CH); 69.4, 76.0 (CH_2); 77.2 (*C*); 80.6 (CH); 109.2 (*C*); 162.3 (*C*═N); 173.0 (*C*═O). HRMS (ESI-FT-ICR) *m*/*z*: 542.3072 $[M + Na]^+$. Calcd. for $C_{29}H_{45}O_7NNa$: 542.3094.

*(25R)-3β-Azido-5α-spirostan-5,6β-diol (**10**).* Methanesulfonic acid (0.15 mL, 2.33 mmol) was added to a stirred solution of triol **9** (500 mg, 1.11 mmol), Ph_3P (873 mg, 3.33 mmol), and DMAP (285 mg, 2.33 mmol) in dry THF (4 mL) under nitrogen atmosphere. DIAD (0.65 mL, 3.33 mmol) was then added dropwise over a 15 min period at 0 °C, and the reaction mixture was stirred vigorously for 48 h at room temperature. The solvent was evaporated under reduced pressure and the resulting residue was chromatographed (*n*-hexane/EtOAc 3:1) to yield the partially impure 3α-methanesulfonate as a white solid (590 mg, R_f = 0.68, *n*-hexane/EtOAc 1:1). This intermediate was dissolved in DMPU (10 mL) and treated with NaN_3 (110 mg, 1.70 mmol). The resulting mixture was stirred vigorously under nitrogen atmosphere at room temperature for 48 h and then diluted with Et_2O (50 mL). The organic phase was washed with aqueous 10% HCl (2 × 20 mL) and brine (20 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 corresponding 3β-azide **10** (352 mg, 67%) as a white solid. mp: 224−225 °C. $[\alpha]_D^{20}$: − 62.7 (*c* 0.60, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.79 (s, 3H, CH_3); 0.79 (d, 3H, *J* = 6.2 Hz, CH_3); 0.96 (d, 3H, *J* = 6.9 Hz, CH_3); 1.18 (s, 3H, CH_3); 3.36 (t, 1H, *J* = 10.9 Hz, H-26ax); 3.47 (dd, 1H, *J* = 4.3/10.7 Hz, H-26eq); 3.54 (m, 1H, H-6α); 3.78 (m, 1H, H-3α); 4.40 (m, 1H, H-16α). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 14.4, 16.5, 16.7, 17.1 (CH_3); 20.8, 26.7, 28.7 (CH_2); 29.7, 30.2 (CH); 31.2, 31.6, 32.2, 35.0, 37.1 (CH_2); 38.4 (*C*); 39.9 (CH_2); 40.6 (*C*); 41.6 (CH); 55.7, 55.8, 60.4, 61.9 (CH); 66.8 (CH_2); 75.3 (CH); 75.5 (*C*); 80.7 (CH); 109.4 (*C*). HRMS (ESI-FT-ICR) *m*/*z*: 496.3157 $[M + Na]^+$. Calcd. for $C_{27}H_{43}O_4N_3Na$: 496.3151.

*(25R)-3β-Amino-5α-spirostan-5,6β-diol (**11**).* Azide **10** (325 mg, 0.69 mmol) was dissolved in THF (6 mL) and treated with PPh_3 (267 mg, 1.04 mmol) and H_2O (0.2 mL) in a similar way as described in the synthesis of **3**. Flash column chromatography purification ($CHCl_3$/MeOH 5:1) afforded the 3β-amine **11** (237 mg, 77%). mp: 201−202 °C. $[\alpha]_D^{20}$: −155 (*c* 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.76 (s, 3H, CH_3); 0.79 (d, 3H, *J* = 6.2 Hz, CH_3); 0.96 (d, 3H, *J* = 6.6 Hz, CH_3); 1.17 (s, 3H, CH_3); 3.38 (t, 1H, *J* = 10.8 Hz, H-26ax); 3.48 (dd, 1H, *J* = 3.5/10.7 Hz, H-26eq); 3.88 (m, 1H, H-6α); 3.97 (m, 1H, H-3α); 4.40 (m, 1H, H-16α). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 14.2, 16.3, 16.4, 16.9 (CH_3); 20.8, 28.8 (CH_2); 29.8, 30.3 (CH); 30.6, 31.1, 31.4, 32.1, 34.1, 39.9 (CH_2); 40.5 (*C*); 41.4 (CH); 42.6 (*C*); 44.6, 45.3 (CH); 45.5 (CH_2); 55.7, 61.9 (CH); 67.0 (CH_2); 75.2 (CH); 75.5 (*C*); 80.8 (CH); 109.4 (*C*). HRMS (ESI-FT-ICR) *m*/*z*: 448.3423 $[M + H]^+$. Calcd for $C_{27}H_{46}O_4N$: 448.3427.

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 to afford the corresponding bis-steroid conjugate.

*Bis-spirostanic Conjugate **17**.* The spirostanic amine **3** (100 mg, 0.23 mmol), paraformaldehyde (6.9 mg, 0.23 mmol), the spirostanic acid **4** (116 mg, 0.23 mmol), and *tert*-butylisocyanide (26 μL, 0.23 mmol) were reacted for 24 h according to the general procedure for the Ugi-4CR-based conjugation approach. Flash column chromatography purification (*n*-hexane/EtOAc 1:4) afforded the pure bis-spirostanic conjugate **17** (159 mg, 67%) as a white solid. mp: 205−206 °C. $[\alpha]_D^{20}$ −15.1 (*c* 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.76 (d, 6H, *J* = 6.3 Hz, 2 × CH_3); 0.84 (s, 3H, CH_3); 0.85 (s, 3H, CH_3); 0.92 (s, 3H, CH_3); 0.98 (d, 3H, *J* = 6.9 Hz, CH_3); 1.02 (s, 3H, CH_3); 1.04 (d, 3H, *J* = 6.9 Hz, CH_3); 1.30 (s, 9H, (CH_3)$_3$C); 3.28−3.32 (m, 2H, 2 × H-26ax); 3.44−3.48 (m, 2H, 2 × H-26eq); 3.54 (m, 1H, H-3α); 3.94 (s, 2H, NCH_2CO); 4.28−4.36 (m, 2H, 2 × H-16α); 4.58−4.65 (m, 2H, OCH_2CO); 6.80 (m, 1H, N*H*). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 11.0, 11.9, 13.0, 13.2, 15.6, 15.8, 17.1 (CH_3); 26.4,

dx.doi.org/10.1021/co400028e | *ACS Comb. Sci.* 2013, 15, 320−330

27.8, 28.2, 28.4, 28.7 (CH_2); 28.9 (CH_3); 30.2 (*C*H); 31.1, 31.2, 31.4, 31.5, 31.6, 31.8 (CH_2); 32.9 (*C*); 34.3, 35.4 (*C*H); 35.6 (CH_2); 36.1 (*C*); 36.5 (CH_2); 36.7 (*C*); 37.4, 37.7, 37.8 (CH_2); 38.9, 41.6, 42.2, 44.2, 44.6 (*C*H); 52.7 (CH_2); 53.6 (*C*H); 55.1, 55.5 (*C*); 55.6, 55.9, 62.2 (*C*H); 65.7, 66.9 (CH_2); 70.8, 71.6 (*C*H); 71.9 (CH_2); 79.2, 81.7 (*C*H); 109.2, 109.8 (*C*); 164.9 (*C*═N); 168.5, 170.6, 213.1 (*C*═O). HRMS (ESI-FT-ICR) *m/z*: 1050.7119 $[M + Na]^+$. Calcd. for $C_{62}H_{97}O_9N_3Na$: 1050.7123.

Bis-spirostanic Conjugate ***18***. The spirostanic amine **11** (98 mg, 0.22 mmol), paraformaldehyde (6.7 mg, 0.22 mmol), the spirostanic acid **12** (123 mg, 0.22 mmol), and cyclohexylisocyanide (27 μL, 0.22 mmol) were reacted for 24 h according to the general procedure for the Ugi-4CR-based conjugation approach. Flash column chromatography purification (*n*-hexane/EtOAc 1:5) afforded the pure bis-spirostanic conjugate **18** (156 mg, 68%) as a white solid. mp: 227−229 °C. $[\alpha]_D^{20}$: −98.7 (*c* 1.1, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.79 (d, 6H, *J* = 6.6 Hz, 2 × CH_3); 0.88 (s, 3H, CH_3); 0.96 (d, 3H, *J* = 7.0 Hz, CH_3); 1.04 (s, 3H, CH_3); 1.06 (d, 3H, *J* = 7.0 Hz, CH_3); 1.24 (m, 3H, CH_3); 1.25 (m, 3H, CH_3); 2.08 (s, 3H, CH_3); 3.32−3.40 (m, 2H, 2 × H-26ax); 3.45−3.51 (m, 2H, 2 × H-26ec); 3.70 (m, 1H); 3.82 (m, 1H); 3.97−4.12 (m, 2H, CH_2); 4.36 (m, 2H, 2 × H-16α); 4.59 (m, 1H, H-6α); 4.64−4.90 (m, 2H, CH_2). ^{13}C NMR ($CDCl_3$) δ = 12.7; 13.2; 14.1; 14.5; 15.6; 16.0; 16.5; 17.1; 20.7 (CH_3); 21.5; 22.9; 23.7; 23.8; 24.6; 24.7; 25.5; 25.9; 27.4; 28.2; 28.7; 29.0; 29.6; 30.1; 30.3; 31.0; 31.3; 31.6 (CH_2); 31.7; 31.8; 31.9 (*C*H); 32.7; 33.0; 33.6; 34.0; 34.3 (CH_2); 35.2 (*C*H); 37.3 (CH_2); 38.7; 39.8 (*C*); 40.5; 41.6; 42.1 (*C*H); 44.9 (*C*); 47.9; 48.3; 49.6; 53.4; 54.9; 55.0; 55.1 (*C*H); 55.2 (*C*); 55.3 (*C*H); 55.4; 62.0; 66.8 (CH_2); 66.9 (*C*H); 74.9 (*C*); 79.1; 80.7 (*C*H); 109.1; 109.2 (*C*); 163.5 (*C*═N); 168.6; 170.0; 171.3; 213.0 (*C*═O). HRMS (ESI-FT-ICR) *m/z*: 1134.7329 $[M + Na]^+$. Calcd for $C_{66}H_{101}O_{11}N_3Na$: 1134.7334.

Bis-spirostanic Conjugate ***19***. The spirostanic amine **7** (100 mg, 0.22 mmol), paraformaldehyde (6.7 mg, 0.22 mmol), the spirostanic acid **8** (116 mg, 0.22 mmol), and *tert*-butylisocyanide (25 μL, 0.22 mmol) were reacted for 24 h according to the general procedure for the Ugi-4CR-based conjugation approach. Flash column chromatography purification (*n*-hexane/EtOAc 1:6) afforded the pure bis-spirostanic conjugate **19** (145 mg, 60%) as a white solid. mp: 235−237 °C. $[\alpha]_D^{20}$: −134 (*c* 0.95, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.76 (s, 3H, CH_3); 0.79 (s, 3H, CH_3); 0.80 (d, 6H, *J* = 6.3 Hz, 2 × CH_3); 0.86 (s, 3H, 2 × CH_3); 0.96 (d, 3H, *J* = 6.8 Hz, CH_3); 0.98 (d, 3H, *J* = 6.8 Hz, CH_3); 1.29 (s, 9H, $(CH_3)_3C$); 3.16 (m, 1H, H-3α); 3.30−3.40 (m, 2H, 2 × H-26ax); 3.44−3.47 (m, 2H, 2 × H-26eq); 3.84 (m, 1H, H-3β); 4.00−4.08 (m, 2H, NCH_2CO); 4.32−4.40 (m, 2H, 2 × H-16α); 4.60−4.72 (m, 2H, OCH_2CO); 6.78 (m, 1H, N*H*). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 13.7, 14.1, 14.4, 16.1, 16.4, 17.1 (CH_3); 20.8, 21.4, 24.4, 25.5 (CH_2); 28.3 (*C*H); 28.5 (CH_3); 29.2, 29.5, 30.0, 30.2 (CH_2); 30.3 (*C*H); 31.3, 31.4, 31.5, 31.6 (CH_2); 32.3 (*C*); 36.9 (CH_2); 37.1 (*C*H); 39.6 (*C*); 39.7 (CH_2); 41.0 (*C*H); 41.2 (*C*); 41.5 (CH_2); 41.6, 41.7 (*C*H); 42.0 (CH_2); 42.6 (*C*); 43.3, 44.2, 44.5, 44.6 (*C*H); 52.1 (CH_2); 56.1 (*C*); 56.2 (*C*H); 62.0 (*C*); 62.1 (*C*H); 66.8 (CH_2); 66.9 (*C*H); 67.0, 72.4 (CH_2); 73.1 (*C*H); 80.1 (*C*); 80.5, 80.8 (*C*H); 109.2, 109.5 (*C*); 165.1 (*C*═N); 169.2, 172.3, 212.2 (*C*═O). HRMS (ESI-FT-ICR) *m/z*: 1082.7026 $[M + Na]^+$. Calcd for $C_{62}H_{97}O_{11}N_3Na$: 1082.7021.

Bis-spirostanic Conjugate ***20***. The spirostanic amine **7** (100 mg, 0.22 mmol), paraformaldehyde (6.7 mg, 0.22 mmol), the spirostanic acid **4** (113 mg, 0.22 mmol), and *tert*-butylisocyanide (25 μL, 0.22 mmol) were reacted for 24 h according to the general procedure for the Ugi-4CR-based conjugation approach. Flash column chromatography purification (*n*-hexane/EtOAc 1:4) afforded the pure bis-spirostanic conjugate **20** (150 mg, 61%) as a white solid. mp: 221−222 °C. $[\alpha]_D^{20}$: −97.2 (*c* 1.0, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.71 (s, 3H, CH_3); 0.74 (s, 3H, CH_3); 0.77 (d, 3H, *J* = 6.3 Hz, CH_3); 0.78 (d, 3H, *J* = 6.3 Hz, CH_3); 0.87 (s, 3H, CH_3); 0.94 (s, 3H, CH_3); 0.95 (d, 3H, *J* = 6.9 Hz, CH_3); 0.99 (d, 3H, *J* = 6.9 Hz, CH_3); 1.28 (s, 9H, $(CH_3)_3C$); 3.31−3.37 (m, 2H, 2 × H-26ax); 3.44−3.46 (m, 2H, 2 × H-26eq); 3.56 (m, 1H, H-3α); 3.93−4.02 (m, 2H, NCH_2CO); 4.31−4.41 (m, 3H, 2 × H-16α, H-3β); 4.57−4.64 (m, 2H, OCH_2CO); 6.78 (m, 1H, N*H*). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 11.9, 13.4, 14.4, 16.4, 17.1, 17.2 (CH_3); 21.0, 21.2, 28.3 (CH_2); 28.5 (CH_3); 28.7, 28.8 (CH_2); 30.1, 30.2 (*C*H); 30.8, 31.2, 31.3, 31.5, 31.6 (CH_2); 34.7 (*C*H); 36.0 (*C*); 36.7 (CH_2); 36.9 (*C*H); 37.0 (*C*); 37.9, 39.6 (CH_2); 40.9 (*C*); 41.6 (*C*H); 41.7 (CH_2); 42.1, 44.7 (*C*H); 47.2, 51.4 (*C*); 53.6, 56.1, 56.3, 62.0 (*C*H); 66.8 (CH_2); 70.9 (*C*H); 72.4 (CH_2); 79.8, 80.5 (*C*H); 109.2 (*C*); 165.8 (*C*═N); 169.6, 170.8, 212.2 (*C*═O). HRMS (ESI-FT-ICR) *m/z*: 1066.7068 $[M + Na]^+$. Calcd for $C_{62}H_{97}O_{10}N_3Na$: 1066.7072.

Bis-spirostanic Conjugate ***21***. The spirostanic amine **12** (100 mg, 0.22 mmol), paraformaldehyde (6.7 mg, 0.22 mmol), the spirostanic acid **8** (116 mg, 0.22 mmol), and cyclohexylisocyanide (27 μL, 0.22 mmol) were reacted for 24 h according to the general procedure for the Ugi-4CR-based conjugation approach. Flash column chromatography purification (*n*-hexane/EtOAc 1:8) afforded the pure bis-spirostanic conjugate **21** (212 mg, 87%) as a white solid. mp: 243−245 °C. $[\alpha]_D^{20}$: −156.1 (*c* 0.80, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.77 (d, 6H, *J* = 6.0 Hz, 2 × CH_3); 0.78 (s, 6H, 2 × CH_3); 0.85 (s, 3H, CH_3); 0.93 (s, 3H, CH_3); 0.95 (d, 3H, *J* = 6.9 Hz, CH_3); 0.99 (d, 3H, *J* = 7.2 Hz, CH_3); 3.33−3.38 (m, 2H, 2 × H-26ax); 3.41−3.46 (m, 2H, 2 × H-26eq); 3.54−3.59 (m, 1H, H-3α); 3.95−4.01 (m, 2H, NCH_2CO); 4.22−4.27 (m, 1H, H-3α); 4.36−4.42 (m, 3H, 2 × H-16α); 4.63−4.67 (m, 2H, OCH_2CO); 6.61 (m, 1H, N*H*). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 12.1, 13.2, 13.6, 14.5, 16.0, 16.4, 17.0 (CH_3); 21.2, 21.5, 24.8, 25.9, 28.7, 28.9 (CH_2); 29.7 (*C*H); 30.3, 30.6 (CH_2); 30.8 (*C*H); 31.4, 31.6, 31.7, 32.3, 34.8, 35.5 (CH_2); 37.1 (*C*H); 37.8 (CH_2); 39.6 (*C*); 39.7, 39.8 (CH_2); 40.6, 41.2 (*C*); 41.7 (*C*H); 42.0 (CH_2); 42.6 (*C*); 44.5, 45.4 (*C*H); 45.6 (CH_2); 45.9, 49.7, 52.9, 55.0, 56.2, 62.1, 64.8 (*C*H); 66.7, 66.9, 72.8 (CH_2); 75.4 (*C*H); 75.8, 80.4 (*C*); 80.5, 80.9 (*C*H); 109.3, 109.7 (*C*); 164.8 (*C*═N); 169.7, 173.1 (*C*═O). HRMS (ESI-FT-ICR) *m/z*: 1110.7337 $[M + Na]^+$. Calcd for $C_{64}H_{101}O_{11}N_3Na$: 1110.7334.

Bis-spirostanic Conjugate ***22***. The spirostanic amine **3** (100 mg, 0.23 mmol), paraformaldehyde (7.0 mg, 0.23 mmol), acetic acid (13 μL, 0.23 mmol), and the spirostanic isocyanide **14** (98.7 mg, 0.23 mmol) were reacted for 24 h according to the general procedure for the Ugi-4CR-based conjugation approach. Flash column chromatography purification (*n*-hexane/EtOAc 1:4) afforded the pure bis-spirostanic conjugate **22** (134 mg, 62%) as a white solid. mp: 196−198 °C. $[\alpha]_D^{20}$: −26.4 (*c* 0.45, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.79 (d, 3H, *J* = 6.4 Hz, CH_3); 0.80 (s, 3H, CH_3); 0.87 (s, 3H, CH_3); 0.96 (d, 3H, *J* = 6.8 Hz, CH_3); 1.03 (s, 6H, 2 × CH_3); 1.06 (d, 6H, *J* = 6.6 Hz, 2 × CH_3); 1.98 (s, 3H, CH_3CO); 3.33−3.37 (m, 2H, 2 × H-26ax); 3.45−3.49 (m, 2H, 2 × H-26eq); 3.70−3.74 (m, 1H, H-3α); 4.01−4.05 (m, 2H,

dx.doi.org/10.1021/co400028e | *ACS Comb. Sci.* 2013, 15, 320−330

NCH_2CO); 4.22−4.27 (m, 1H, H-3β); 4.32−4.40 (m, 2H, 2 × H-16α); 5.33 (m, 1H, H-6); 6.89 (m, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 13.2, 14.2, 14.8, 14.9, 16.7, 16.8, 17.5, 20.8 (CH_3); 21.3, 21.4, 26.0, 26.8, 28.7, 29.2, 29.7 (CH_2); 30.7 (CH); 31.7, 31.8 (CH_2); 31.9 (CH); 32.0, 32.2, 32.5 (CH_2); 37.4 (C); 37.5 (CH); 37.6, 39.4, 40.0, 40.2 (CH_2); 40.7, 41.5 (C); 41.9 (CH_2); 42.0 (CH); 43.7 (C); 44.6, 50.5, 55.1, 56.5, 56.9 (CH); 62.0 (CH_2); 62.4, 62.5 (CH); 67.3 (CH_2); 77.2 (C); 77.6, 79.5, 80.9, 81.2 (CH); 109.6, 109.7 (C); 122.0 (CH); 141.0 (C); 169.1, 173.4, 212.9 (C═O). HRMS (ESI-FT-ICR) m/z: 947.6484 [M + Na]$^+$. Calcd for $C_{58}H_{88}O_7N_2Na$: 947.6489.

Bis-spirostanic Conjugate ***23***. The spirostanic amine **3** (100 mg, 0.23 mmol), paraformaldehyde (7.0 mg, 0.23 mmol), acetic acid (13 μL, 0.23 mmol), and the spirostanic isocyanide **16** (113 mg, 0.23 mmol) were reacted for 24 h according to the general procedure for the Ugi-4CR-based conjugation approach. Flash column chromatography purification (*n*-hexane/EtOAc 1:4) afforded the pure bis-spirostanic conjugate **23** (174 mg, 64%) as a white solid. mp: 202−204 °C. $[\alpha]_D^{20}$: −37.1 (*c* 0.90, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.76 (d, 6H, *J* = 6.4 Hz, 2 × CH_3); 0.80 (s, 3H, CH_3); 0.87 (s, 3H, CH_3); 0.96 (d, 3H, *J* = 6.8 Hz, CH_3); 0.99 (s, 3H, CH_3); 1.04 (m, 6H, 2 × CH_3); 2.00, 2.06 (s, 6H, 2 × CH_3CO); 3.35−3.38 (m, 2H, 2 × H-26ax); 3.43−3.48 (m, 2H, 2 × H-26eq); 3.84−3.87 (m, 1H, H-3β); 4.02−4.07 (m, 2H, NCH_2CO); 4.20−4.25 (m, 1H, H-3β); 4.34−4.42 (m, 2H, 2 × H-16α); 4.90 (m, 1H, H-6α); 6.91 (m, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 11.8, 13.2, 14.5, 16.1, 16.4, 17.0, 20.6 (CH_3); 20.8 (CH_2); 21.5 (CH_3); 28.0, 28.3, 28.8 (CH_2); 28.9 (C); 29.0 (CH_2); 30.2, 30.3 (CH); 31.1, 31.3, 31.4, 31.5 (CH_2); 31.6 (CH); 31.8, 32.1 (CH_2); 34.3 (CH); 35.2 (CH_2); 36.2 (C); 36.8 (CH_2); 37.0 (C); 37.1, 37.6, 39.0; 39.8 (CH_2); 40.2 (C); 42.2, 45.0, 45.4, 50.1, 53.5 (CH); 55.1 (C); 55.2, 55.8, 56.9, 60.2 (CH); 61.8 (CH_2), 62.1 (CH); 66.8, 66.9 (CH_2); 76.7, 77.0, 77.3 (C); 79.3, 80.8 (CH); 109.2, 109.3 (C); 169.9, 171.1, 172.6, 213.4 (C═O). HRMS (ESI-FT-ICR) m/z: 1007.6708 [M + Na]$^+$. Calcd for $C_{60}H_{92}O_9N_2Na$: 1007.6701.

Bis-spirostanic Conjugate ***24***. *iso*-Propylamine (26 μL, 0.30 mmol), paraformaldehyde (9.0 mg, 0.30 mmol), the spirostanic acid **4** (151 mg, 0.30 mmol), and the spirostanic isocyanide **16** (145 mg, 0.30 mmol) were reacted for 72 h according to the general procedure for the Ugi-4CR-based conjugation approach. Flash column chromatography purification (*n*-hexane/EtOAc 1:5) afforded the pure conjugate **24** (317 mg, 84%) as a white solid. mp: 207−208 °C. $[\alpha]_D^{20}$: −33.4 (*c* 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.78 (s, 3H, CH_3); 0.83 (d, 6H, *J* = 6.4 Hz, 2 × CH_3); 0.87 (m, 9H, 3 × CH_3); 0.96 (s, 3H, CH_3); 1.01 (m, 3H, CH_3); 1.19 (m, 6H, (CH_3)$_2$CH); 2.06 (s, 3H, CH_3CO); 3.29−3.38 (m, 2H, 2 × H-26ax); 3.43−3.47 (m, 2H, 2 × H-26eq); 3.58−3.62 (m, 1H, H-3α); 3.72 (m, 1H, H-3α); 3.95 (s, 2H, NCH_2CO); 4.01 (m, 1H, (CH_3)$_2$CH); 4.36−4.41 (m, 2H, 2 × H-16α); 4.61−4.65 (m, 2H, OCH_2CO); 4.88 (m, 1H, H-6α); 6.93 (m, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 11.8, 13.0, 14.5, 15.1, 15.8, 16.4, 17.1, 19.8 (CH_3); 20.4 (CH_2); 21.4 (CH_3); 25.7, 28.2, 28.4, 28.8, 29.8 (CH_2); 30.2, 30.6 (CH); 31.2, 31.3, 31.5, 31.6, 31.7, 31.8, 34.4 (CH_2); 34.6, 35.4 (CH); 36.1 (C); 36.4, 36.5, 37.7, 37.8, 39.7 (CH_2); 40.6 (C); 41.6, 41.7, 41.9, 44.6 (CH); 52.7 (CH_2); 55.5 (C); 55.6, 55.8, 55.9, 56.7, 62.0, 62.2 (CH); 65.7, 66.8 (CH_2); 70.8, 71.8 (CH); 71.9 (CH_2); 73.4, 80.5, 81.7 (CH); 109.3, 109.8 (C); 164.9 (C═N); 169.1, 170.5, 173.9 (C═O). HRMS (ESI-FT-ICR) m/z: 1094.7389 [M + Na]$^+$. Calcd for $C_{64}H_{101}O_{10}N_3Na$: 1094.7385.

Bis-spirostanic Conjugate ***25***. *iso*-Propylamine (26 μL, 0.30 mmol), paraformaldehyde (9.0 mg, 0.30 mmol), the spirostanic acid **8** (156 mg, 0.30 mmol), and the spirostanic isocyanide **16** (145 mg, 0.30 mmol) were reacted for 72 h according to the general procedure for the Ugi-4CR-based conjugation approach. Flash column chromatography purification (*n*-hexane/EtOAc 1:6) afforded the pure conjugate **25** (324 mg, 85%) as a white solid. mp: 215−217 °C. $[\alpha]_D^{20}$: −42.1 (*c* 0.85, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.76 (s, 6H, 2 × CH_3); 0.85 (m, 6H, 2 × CH_3); 0.87 (m, 3H, CH_3); 0.96 (s, 3H, CH_3); 1.01 (d, 6H, *J* = 6.6 Hz, 2 × CH_3); 1.22 (m, 6H, (CH_3)$_2$CH); 2.04 (s, 3H, CH_3CO); 3.30−3.39 (m, 2H, 2 × H-26ax); 3.41−3.47 (m, 2H, 2 × H-26eq); 3.57−3.63 (m, 1H, H-3α); 3.76 (m, 1H, H-3$\acute{\alpha}$); 3.98 (s, 2H, NCH_2CO); 4.03 (m, 1H, (CH_3)$_2$CH); 4.36−4.41 (m, 2H, 2 × H-16α); 4.63−4.66 (m, 2H, OCH_2CO); 4.91 (m, 1H, H-6α); 6.95 (m, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 14.1, 14.4, 14.5, 15.1, 16.1, 16.4, 17.1, 19.9 (CH_3); 20.4 (CH_2); 21.4 (CH_3); 25.7, 28.8, 29.8, 30.0, 30.2 (CH_2); 30.4, 30.6, 30.7 (CH); 31.3, 31.4, 31.6, 31.7, 34.4, 35.5 (CH_2); 36.1 (C); 36.4 (CH_2); 37.1 (CH); 39.7 (CH_2); 40.6, 41.2 (C); 41.7, 41.9 (CH); 42.2 (CH_2); 42.6 (C); 44.5 (CH); 52.7 (CH_2); 55.8, 56.2, 56.7, 62.0, 62.1, 64.8 (CH); 66.7, 66.9 (CH_2); 71.2 (CH); 72.3 (CH_2); 73.4 (CH); 80.1 (C); 80.5, 80.8 (CH); 109.2, 109.7 (C); 165.1 (C═N); 168.9, 170.5, 172.4 (C═O). HRMS (ESI-FT-ICR) m/z: 1110.7329 [M + Na]$^+$. Calcd for $C_{64}H_{101}O_{11}N_3Na$: 1110.7334.

Bis-spirostanic Conjugate ***26***. The spirostanic amine **3** (100 mg, 0.23 mmol), paraformaldehyde (7.0 mg, 0.23 mmol), azidotrimethylsilane (30 μL, 0.23 mmol), and the spirostanic isocyanide **14** (98.7 mg, 0.23 mmol) were reacted for 72 h according to the general procedure for the Ugi-4CR-based conjugation approach. Flash column chromatography purification (*n*-hexane/EtOAc 2:3) afforded the pure conjugate **26** (135 mg, 63%) as a white solid. mp: 193−195 °C. $[\alpha]_D^{20}$: −56.2 (*c* 1.2, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.75 (s, 3H, CH_3); 0.78 (m, 9H, 3 × CH_3); 0.85 (s, 3H, CH_3); 0.96 (m, 6H, 2 × CH_3); 1.01 (s, 3H, CH_3); 3.25−3.40 (m, 3H, 2 × H-26ax, H-3α); 3.42−3.49 (m, 2H, 2 × H-26eq); 3.97 (s, 2H, NCH_2); 4.32 (m, 1H, H-16α); 4.35 (m, 1H, H-16$\acute{\alpha}$); 4.65 (m, 1H, H-3β); 5.35 (m, 1H, H-6). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 14.2, 14.8, 14.9, 16.7, 16.8, 17.5 (CH_3); 21.3, 21.4, 26.0, 26.8, 28.7, 29.2, 29.7 (CH_2); 30.7 (CH); 31.7, 31.8 (CH_2); 31.9 (CH); 32.0, 32.2, 32.5 (CH_2); 37.4 (C); 37.5 (CH); 37.6, 39.4, 40.0, 40.2 (CH_2); 40.7, 41.5 (C); 41.9 (CH_2); 42.0 (CH); 43.7 (C); 44.6, 50.5, 55.1, 56.5, 56.9 (CH); 62.0 (CH_2); 62.4, 62.5 (CH); 67.3 (CH_2); 77.2 (C); 77.6, 79.5, 80.9, 81.2 (CH); 109.6, 109.7 (C); 121.2 (CH); 141.0, 155.1 (C); 212.1 (C═O). HRMS (ESI-FT-ICR) m/z: 945.6687 [M + Na]$^+$. Calcd for $C_{57}H_{88}N_5O_5Na$: 945.6683.

Bis-spirostanic Conjugate ***27***. The spirostanic amine **3** (100 mg, 0.23 mmol), paraformaldehyde (7.0 mg, 0.23 mmol), azidotrimethylsilane (30 μL, 0.23 mmol), and the spirostanic isocyanide **16** (113 mg, 0.23 mmol) were reacted for 72 h according to the general procedure for the Ugi-4CR-based conjugation approach. Flash column chromatography purification (*n*-hexane/EtOAc 2:3) afforded the pure conjugate **27** (151 mg, 66%) as a white solid. mp: 195−197 °C. $[\alpha]_D^{20}$: −88.4 (*c* 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.77−0.79 (m, 12H, 4 × CH_3); 0.93 (d, 3H, *J* = 6.6 Hz, CH_3); 1.24 (d, 3H, *J* = 5.4 Hz, CH_3); 1.27 (s, 3H, CH_3); 1.29 (s, 3H, CH_3); 2.05 (s, 3H, CH_3CO); 3.36 (t, 2H, *J* = 10.9 Hz, 2 × H-26ax);

3.39−3.43 (m, 1H, H-3β); 3.44−3.48 (m, 2H, 2 × H-26eq); 3.97 (s, 2H, NCH_2); 4.38−4.42 (m, 2H, 2 × H-16α); 4.60−4.66 (m, 1H, H-3β); 4.90−4.93 (m, 1H, H-6α). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 14.3, 14.6, 15.0, 16.4, 16.6, 17.2 (CH_3); 20.4, 20.9 (CH_2); 21.5 (CH_3); 22.8, 25.5, 28.4, 28.8, 29.4 (CH_2); 29.7, 29.8, 30.0, 30.3 (CH); 30.5, 31.4, 31.5, 31.6, 31.8, 31.9, 32.1, 34.7 (CH_2); 35.9, 36.3 (C); 37.0, 37.2, 39.1, 39.8 (CH_2); 40.3, 40.6 (C); 41.6, 41.7, 41.9, 50.1, 53.6, 55.8, 56.0, 56.5, 61.9, 62.0 (CH); 66.8 (CH_2); 72.9, 77.2, 78.9, 80.6, 80.8 (CH); 109.1, 109.2 (C); 155.4 (C); 170.4, 213.0 (C═O). HRMS (ESI-FT-ICR) m/z: 1005.6889 $[M + Na]^+$. Calcd for $C_{59}H_{92}O_7N_5Na$: 1005.6894.

Bis-spirostanic Conjugate ***28***. The spirostanic amine **7** (100 mg, 0.22 mmol), paraformaldehyde (6.7 mg, 0.22 mmol), azidotrimethylsilane (30 μL, 0.22 mmol), and the spirostanic isocyanide **14** (94 mg, 0.22 mmol) were reacted for 72 h according to the general procedure for the Ugi-4CR-based conjugation approach. Flash column chromatography purification (*n*-hexane/EtOAc 2:3) afforded the pure conjugate **28** (122 mg, 58%) as a white solid. mp: 198−200 °C. $[\alpha]_D^{20}$: −163.1 (*c* 0.85, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.78 (m, 9H, 3 × CH_3); 0.96 (d, 3H, *J* = 6.9 Hz, CH_3); 0.97 (d, 3H, *J* = 6.9 Hz, CH_3); 1.01 (s, 3H, CH_3); 1.09 (s, 3H, CH_3); 1.12 (s, 3H, CH_3); 3.26−3.40 (m, 3H, 2 × H-26ax, H-3α); 3.44−3.50 (m, 2H, 2 × H-26eq); 3.98 (s, 2H, NCH_2); 4.40 (m, 2H, 2 × H-16α); 4.78 (m, 1H, H-3β); 5.34 (m, 1H, H-6). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 14.1, 14.4, 14.5, 16.3, 16.4, 17.1, 17.2 (CH_3); 21.0, 28.3, 28.5, 28.9 (CH_2); 29.8, 30.4 (CH); 31.3, 31.4 (CH_2); 31.5 (CH); 31.8, 31.9, 32.2, 32.5, 35.0 (CH_2); 37.0 (C); 37.1, 38.5, 38.6, 39.0, 39.8, 40.2 (CH_2); 40.4, 40.8 (C); 41.6, 41.7, 45.7, 50.1, 55.8, 56.5, 56.9 (CH); 61.6 (CH_2); 62.2 (CH); 66.8 (CH_2); 75.5, 77.2, 79.1 (CH); 80.1 (C); 80.7, 80.8 (CH); 109.4, 109.5 (C); 121.8 (CH); 140.8, 154.9 (C); 212.2 (C═O). HRMS (ESI-FT-ICR) m/z: 961.6639 $[M + Na]^+$. Calcd for $C_{57}H_{88}O_6N_5Na$: 961.6632.

Bis-spirostanic Conjugate ***29***. The spirostanic amine **11** (100 mg, 0.22 mmol), paraformaldehyde (6.7 mg, 0.22 mmol), azidotrimethylsilane (30 μL, 0.22 mmol), and the spirostanic isocyanide **14** (94 mg, 0.22 mmol) were reacted for 72 h according to the general procedure for the Ugi-4CR-based conjugation approach. Flash column chromatography purification (*n*-hexane/EtOAc 1:2) afforded the pure conjugate **29** (172 mg, 84%) as a white solid. mp: 203−205 °C. $[\alpha]_D^{20}$: −168.7 (*c* 0.90, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.78 (m, 9H, 3 × CH_3); 0.96 (s, 3H, CH_3); 0.97 (d, 3H, *J* = 6.7 Hz, CH_3); 1.01 (s, 3H, CH_3); 1.27 (s, 6H, 2 × CH_3); 3.24−3.36 (m, 2H, 2 × H-26ax); 3.40−3.48 (m, 3H, 2 × H-26eq, H-3α); 3.60 (m, 1H, H-6α); 3.98 (s, 2H, NCH_2); 4.39 (m, 2H, 2 × H-16α); 5.00 (m, 1H, H-3α); 5.35 (m, 1H, H-6). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 14.5, 14.6, 16.3, 16.6, 16.7, 17.1, 19.4 (CH_3); 20.9, 28.3, 28.5, 28.8 (CH_2); 29.9, 30.3 (CH); 31.3, 31.4 (CH_2); 31.5 (CH); 31.7, 31.8, 32.1, 32.4, 35.0 (CH_2); 37.0 (C); 37.1, 38.4, 38.5, 39.0, 39.8, 40.0 (CH_2); 40.2, 40.7 (C); 41.6, 41.7, 45.7, 50.1, 55.8, 56.5, 56.9 (CH); 61.6 (CH_2); 62.1 (CH); 66.8 (CH_2); 75.0 (C); 75.5, 77.2, 79.1, 80.7, 80.8 (CH); 109.2, 109.3 (C); 121.9 (CH); 140.8, 155.3 (C). HRMS (ESI-FT-ICR) m/z: 963.6785 $[M + Na]^+$. Calcd. for $C_{57}H_{90}O_6N_5Na$: 963.6789.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

^{1}H and ^{13}C NMR spectra of selected building blocks and final products. This material is available free of charge via the Internet at http://pubs.acs.org.

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Funding

K.P.-L. gratefully acknowledges MAEC-AECID for a doctoral scholarship.

Notes

The authors declare no competing financial interest.

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