Tetrahedron Letters 54 (2013) 1602–1606

Contents lists available at SciVerse ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

Synthesis and conformational study of triazole-linked bis-spirostanic conjugates

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ARTICLE INFO

Article history:
Received 8 December 2012
Revised 11 January 2013
Accepted 14 January 2013
Available online 23 January 2013

Keywords:
Steroids
Spirostanes
Click chemistry
Cycloaddition reactions
Conformational study

ABSTRACT

A general approach based on the Cu[I]-catalyzed azide–alkyne 1,3-dipolar cycloaddition reaction was implemented for the conjugation of two spirostanic steroids. This process provides rapid access to a small library of triazole-based bis-spirostanic conjugates with varied functionalization patterns as well as different stereochemistry of the linkage. The approach proved high efficiency even with the use of sterically hindered 3α-azido-spirostanes. A molecular modeling study was performed to determine the conformational characteristics of the bis-spirostanic conjugates as well as to address the structural resemblance with the potent anticancer bis-spirostanic conjugates cephalostatins and ritterazines. Considering its efficiency and versatility, this approach shows promise for the discovery of novel bis-steroidal conjugates with applications in medicinal chemistry.

In the last decade, the Cu[I]-catalyzed 1,3-dipolar cycloaddition approach[1] has been extensively exploited in conjugation chemistry and in the construction of hybrid scaffolds integrating two different structural fragments.[2] The triazole moiety provided by this process has proven success in medicinal and biological chemistry, as the rigid, metabolically stable and hydrogen bonding features of this heterocycle usually modify the activity and properties of biologically relevant molecules while increasing the solubility of the resulting triazole hybrid. In the field of steroid chemistry, such a click approach has been used for the conjugation of sugars[3] and *pseudo*-peptides[4] to a variety of steroids, and for the preparation of oligosteroidal conjugates derived from bile acids.[5] Several applications have been reported for the 1,2,3-triazole-linked bile acid dimers and higher oligomers, including the preparation of antimicrobial β-lactam/triazole/bile acid dimers,[5d,e] the design of macrocyclic scaffolds for ion-pair recognition,[5c] and the use of amphiphilic oligomers for transporting hydrophilic molecules across lipid membranes[5b] and for the solubilization of hydrophilic dyes in non-polar solvents.[5f]

Our interest on utilizing click chemistry for the conjugation of two steroidal units differs from those previously reported, as we aim at the preparation and conformational study of a series of bis-spirostanic conjugates with certain resemblance to the antitumor bis-steroids cephalostatins[6] and ritterazines (Fig. 1).[7]

These marine natural products contain either two spirostanic or spirofuranic units linked by a pyrazine ring, forming an unsymmetrical polycyclic system. Owing to the difficult total synthesis of these bis-steroids[8] and their remarkable anticancer activity, the preparation of analogs and related bis-spirostanic conjugates stands as a promising strategy. Accordingly, we were prompted to evaluate the efficacy of such a triazole-forming reaction in the generation of structurally varied bis-spirostanic conjugates, thus providing an alternative strategy to the classic pyrazine-formation mediated synthesis.

Herein we report on the use of the Cu[I]-catalyzed 1,3-dipolar cycloaddition of stereochemically dissimilar spirostanic azides and alkynes as the way to conjugate two different spirostanic skeletons. The selection of this type of chemical process also relies on its capability to rapidly provide a variety of compounds for both structural analysis and future biological studies. Hence, a conformational

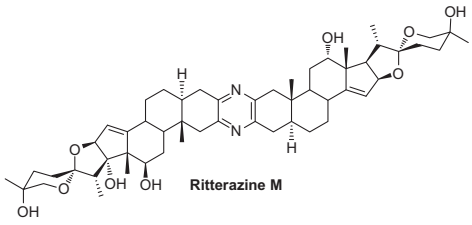

Figure 1. The natural bis-spirostane ritterazine M.

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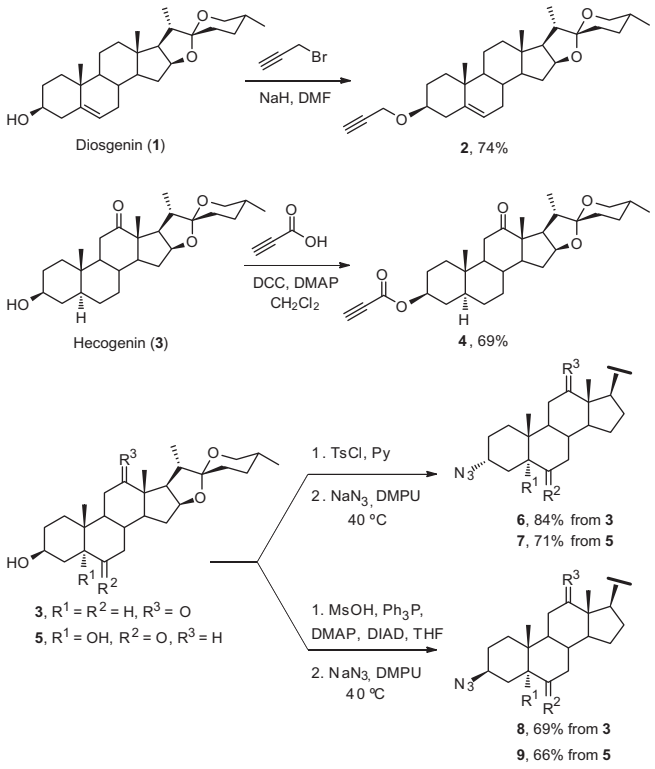
http://dx.doi.org/10.1016/j.tetlet.2013.01.058



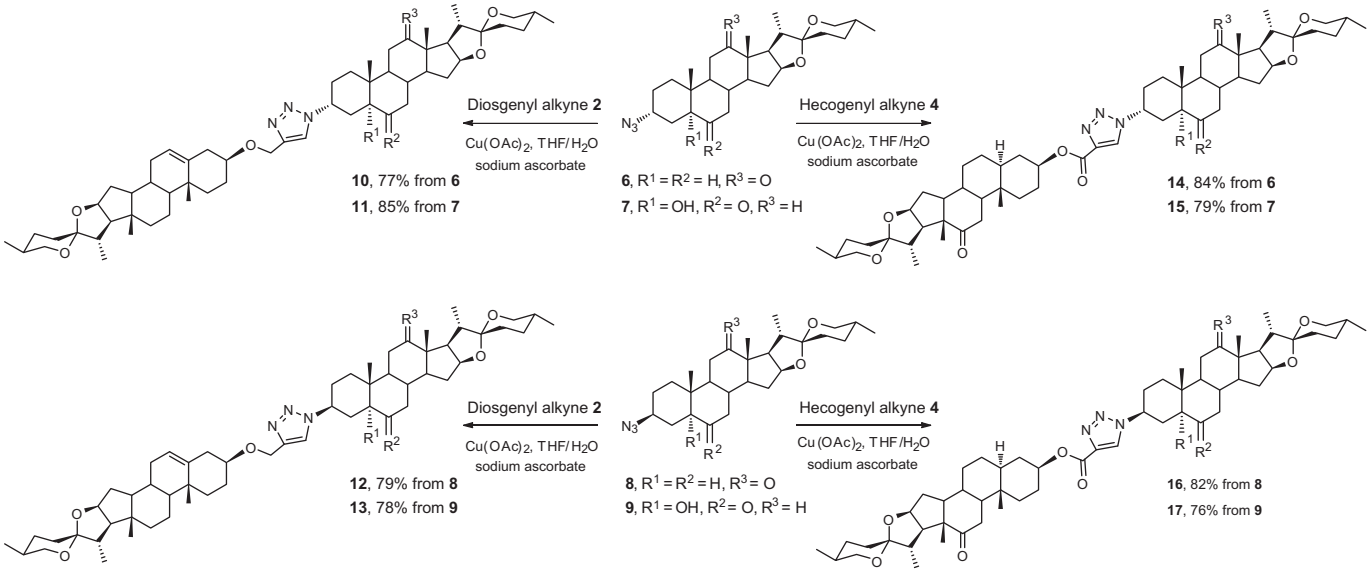
Scheme 1. Synthesis of spirostanic alkynes and azides as substrates of the click process.

study of geometrically different compounds derived from the use of 3α and 3β-azido-spirostanes is also accomplished, thus providing a clue of their three-dimensional structural resemblance with the natural pyrazine-linked bis-steroids. As several approaches are already known for accessing the original side-chain functionalities of these natural products from spirostanic sapogenins,[9] our focus relied on evaluating the efficiency of the click process using these very bulky steroidal substrates as well as analyzing the conformational space accessible by including a triazole moiety instead of a pyrazine one as the linkage unit.

The strategy to prepare triazole-linked bis-spirostanic conjugates comprises the preparation of 3-azido-spirostanes as well as spirostanes bearing either propargyl ether or propiolate ester at C-3. For this, natural sapogenins with diverse functionalization patterns on rings B and C were selected as starting materials. Hence, diosgenin (**1**) was propargylated to alkyne **2** in 74% as reported before,[3a] while hecogenin (**3**) was esterified with propiolic acid in the presence of DCC/DMAP to furnish the 3β-hecogenyl propiolate (**4**) in 69% yield.

We also accomplished the synthesis of 3-azido-spirostanes featuring either the 3α or 3β configuration, so that the influence of the different C-3 stereochemistry on the conjugation efficiency and the conformation of the resulting conjugates could be evaluated. As shown in Scheme 1, hecogenin (**3**) and 5α-hydroxy-laxogenin (**5**) [i.e., (25*R*)-3β,5-dihydroxy-5α-spirostan-6-one] were chosen for the preparation of the corresponding azides.[3a] Thus, the 3α-azido-spirostanes **6** and **7** were readily prepared by tosylation of the 3β-OH and subsequent azide displacement with inversion of the C-3 configuration. Alternatively, the synthesis of the 3β-azido-spirostanes **8** and **9** required the replacement of the 3β-OH by azide with net retention of the configuration.[3a] This was accomplished according to a reported Mitsunobu protocol using methanesulfonic acid as the acidic/nucleophilic component,[10] followed by displacement with NaN_3 to recover the 3β configuration at C-3.

The click steroid–steroid conjugation approach was firstly implemented by using 3α-hecogenyl azide **6** and propargyloxy-diosgenin **2**, thus leading to conjugate **10** in 77% yield.[11] Typical reaction conditions (i.e., 20% mol of a Cu^{II} salt and 40% mol of sodium ascorbate in a THF/H_2O mixture) were employed, which proved great success on the ligation of these bulky building blocks. As shown in Scheme 2, these conditions were also used for the reaction of alkyne **2** with 3-azido-spirostanes **7**–**9** bearing different oxygenated functions on rings B and C and featuring either the α or β stereochemistry at C-3. Importantly, we were pleased to find that the efficiency of the conjugation process—in terms of reaction yield and rate—was not decreased when using the sterically hindered 3α-azido-spirostanes **6** and **7** (axial azido group) as compared with the 3β-isomers **8** and **9**. This becomes even more interesting for the case of 3α-azido-spirostane **7**, which features a 1,3-diaxial interaction with the 5α-OH group that usually decreases reactivity of functional groups at C-3.



Scheme 2. Synthesis of triazole-linked bis-spirostanic conjugates by the Cu^{I}-catalyzed 1,3-dipolar cycloaddition.