Tetrahedron Letters 51 (2010) 1779–1781

Contents lists available at ScienceDirect

Tetrahedron Letters

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

Synthesis of 2,4,5-triaminocyclohexanecarboxylic acid as a novel 2-deoxystreptamine mimic

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ARTICLE INFO

Article history:
Received 21 December 2009
Revised 25 January 2010
Accepted 28 January 2010
Available online 2 February 2010

ABSTRACT

Since aminoglycosides have been known to exert their antibacterial activities by binding to various RNA targets, 2-deoxystreptamine served as a particularly important model in understanding RNA-small molecule interaction. Herein, 2,4,5-triaminocyclohexanecarboxylic acid was prepared as a novel 2-deoxystreptamine (2-DOS) mimic, which replaces highly flexible glycosidic bonds of aminoglycosides with amide bonds and may improve binding selectivity toward RNA targets through increased rigidity and additional hydrogen-bonding capability. This unnatural g-amino acid can also be used as a potential component for synthetic foldamers.

Aminoglycosides, a large class of clinically significant antibiotics, are known to selectively bind to various RNA targets. They are comprised of 2-deoxystreptamine (2-DOS), a highly conserved central aminocyclitol scaffold, and up to four aminosugar subunits. While established as a particularly important model in understanding the principles that decree RNA recognition processes by small molecules, aminoglycosides have shown highly promiscuous binding patterns. They bind to many different RNA secondary structural elements, such as bulges, internal loops, and stem junctions. For instance, neomycin has been shown to bind to bulge regions of unrelated RNA sequences from the 16S ribosome,[1] HIV TAR,[2] HIV RRE,[3] and Group I intron[4] with affinities in the low micromolar range.

This lack of RNA target selectivity of aminoglycosides is largely attributed to their conformational adaptability rendered by flexible glycosidic bonds, which allows aminoglycosides to adjust to fit into different shapes of RNA cavities. Tor[5] and Mobashery[6] groups reported conformationally constrained aminoglycoside derivatives to reduce the number of available conformations of these aminoglycosides and therefore increase the target selectivity.

Although aminoglycosides as a whole demonstrate poor binding selectivity, it is well established that 2-DOS, the main component of aminoglycoside, recognizes 5′-GU-3′ sequence steps in a sequence-specific manner regardless of the number of aminosugar substituents and substitution positions (Fig. 1a).[7,8] Puglisi and coworkers demonstrated that the 2-DOS itself, with no aminosugar attached, recognized 5′-GU-3′ base steps, although its binding affinity was low (~1 mM).[8] Due to the crucial role of 2-DOS in RNA recognition and biological activity and its omni existence in all clinically important aminoglycosides, mimicking the 2-DOS structure has been of great interest in preparing novel aminoglycoside analogs.[9]

Here in this Letter, 2,4,5-triaminocyclohexanecarboxylic acid **1** was prepared as a novel 2-DOS mimetic by replacing 4-OH and 6-OH of 2-DOS with amino and carboxylic acid functionalities, respectively (Fig. 1b).

This mimic will enable the introduction of rigid amide backbone in the place of the highly flexible glycosidic bonds, which may help improve sequence specificity in binding by (1) promoting additional hydrogen bonding and (2) limiting structural adaptation of the ligands to different RNA targets. The 4-OH and 6-OH are chosen for the conversion into an amine and a carboxylic acid to facilitate

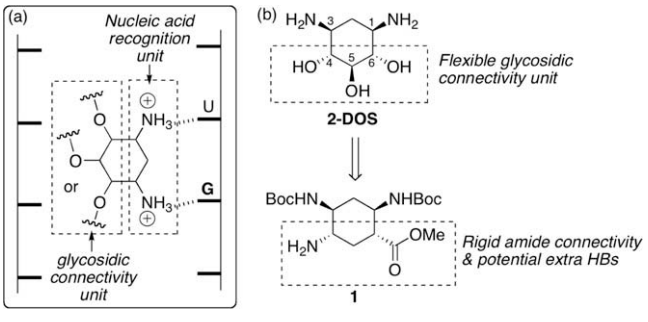

Figure 1. (a) Schematic diagram of 5'-GU-3' recognition of RNA targets by 2-DOS. (b) Rational design of 2,4,5-triaminocyclohexanes-carboxylic acid derivative (**1**) as a novel 2-DOS mimic.

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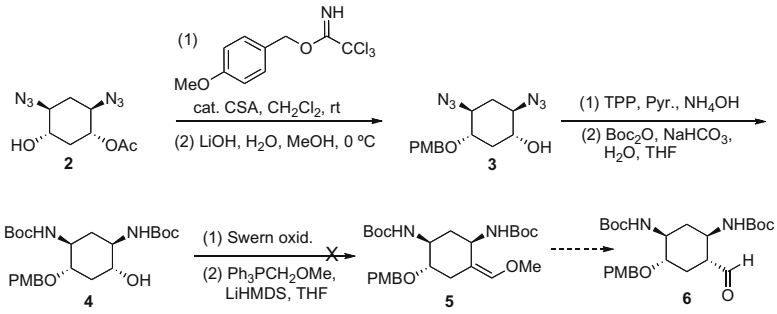
0040-4039/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2010.01.111



Scheme 1. Initial attempt to introduce a carboxylic acid group on the 6-OH position of the 2-DOS.

amide backbone as they point in the same direction as the RNA helix axis. The 5-OH is omitted in this mimic for the sake of simplicity in the synthesis because it points out of the helix axis and does not extend aminosugar substituents in the same direction as the RNA helix axis.

We have chosen the asymmetrically protected precursor **2** as the starting compound and it was prepared following the previously reported literature procedure.[10] Introduction of the carboxylic acid was initially performed after the procedures reported by Hermann and co-workers[11] (Scheme 1). The 4-OH of compound **2** was protected with PMB in acidic condition followed by hydrolysis of the acetyl group. The azides were then transformed to Boc-protected amine **4** by Staudinger reaction and subsequent treatment with Boc_2O. After oxidation of the 6-OH, the resulting ketone was treated with triphenylphosphinemethoxymethyl chloride/LiHMDS to prepare vinyl ether **5**, which was intended for the transformation to an aldehyde **6** as a precursor to a carboxylic acid. However, the Wittig reaction failed to yield the desired vinyl ether **5** even in the presence of large excess of ylide (7–14 equiv).

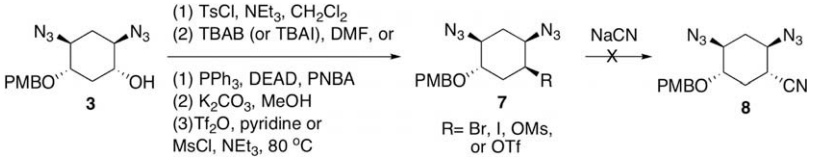
An alternative approach was attempted to introduce a nitrile as a precursor to an acid (Scheme 2). The equatorial hydroxyl group of compound **3** was converted in various reaction conditions to compound **7** that has a leaving group at the axial position. However, SN2 reaction to an equatorial nitrile resulted in the decomposition of the starting material.

Then, an attempt was made to introduce the nitrile group at the axial position from which an axially positioned aldehyde (**13**) or ester (**14b**) can be prepared followed by epimerization to the desired equatorial position (Scheme 3). The axial nitrile **11** was prepared in high yield through MOM protection of the hydroxyl group of compound **2**, saponification of the ester **9**, activation of the alcohol (**10**) to a triflate, and SN2 reaction with NaCN. Then, conversion of nitrile (**11**) to an aldehyde (**13**) was attempted using DIBAL-H after reducing two azides of compound **9** and protecting the subsequent amines with Boc. However, transformation to aldehyde **13** was not successful using various reaction conditions. Application of the same reaction condition to azide **11** led to the decomposition of the starting material. Therefore, an esterification route was pursued. Nitrile **11** was converted to methyl ester **14b** using HCl/MeOH solution (5–7 M) in about 80% yield. Slow decomposition of the starting compound was observed when the reaction was allowed to run for a period beyond 24 h at the reaction condition (70 °C). Thus, the reaction was quenched after 24 h and significant amount of the starting material was not fully converted into methyl ester **14b**. As a result, MOM-deprotected product **14a** was isolated usually in 5–15% yield, which was esterified in the same reaction condition or in cat. concd H_2SO_4 in MeOH. The latter reaction condition required prolonged reaction time (3–4 days) at 70 °C for a comparable result.

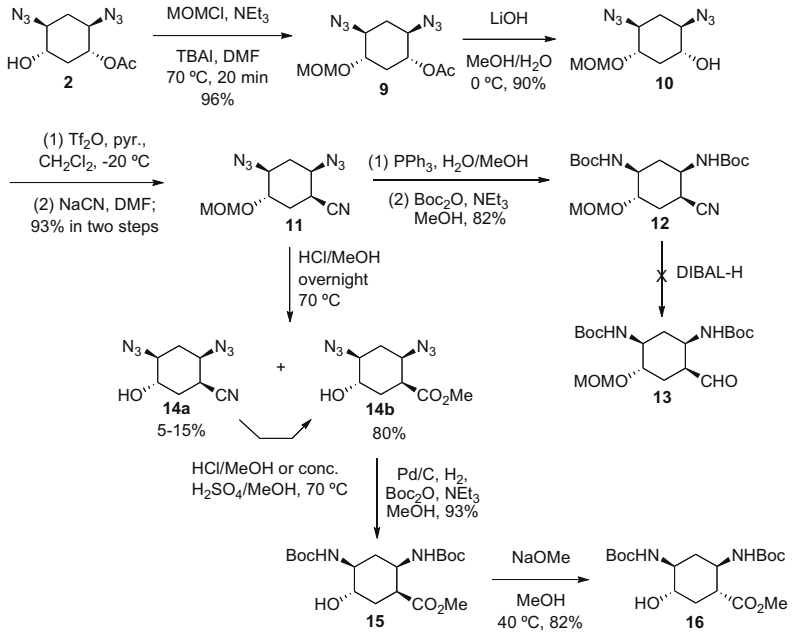
Then, the two azides of compound **14b** were transformed to Boc-protected amine **15** before epimerization with strong bases to avoid the elimination reaction of the azide located at β-position to the methyl ester. The best epimerization result to the more stable epimer **16** was obtained with NaOMe at 40 °C. No epimerization product was found at room temperature and the Boc-protecting groups became decomposed at higher temperatures (>50 °C). After 6 h at 40 °C (30 equiv of NaOMe in MeOH), equilibrium was established between epimers **15** and **16** with 1:15 respective ratio as determined by ^{1}H NMR. Epimer **16** was isolated in 75–85% yield after silica gel column chromatography. Initially, several different literature-reported conditions were employed for the epimerization using strong bases such LDA, LiHMDS, and KHMDS.[12] These strong bases either yielded no product at low temperature or led to decomposition of the starting material at an elevated temperature. NaH (5–10 equiv) has shown the formation of the product at 40 °C, but only as a minor product. Mixtures of undesired byproducts dominated while no change in TLC was found at room temperature.

The configurations of the two epimers **15** and **16** were confirmed by high-resolution NMR studies (Fig. 2). The coupling constants between the neighboring Hs provide clear evidence for the proper stereochemistry for both **15** and **16**. In the case of **15**, the coupling constants were found to be 4, 5, and 8 Hz, respectively, for *J*(Ha–Hb), *J*(Ha–Hc), and *J*(Ha–Hg), consistent with the axial conformation of the methyl ester. Similarly, the coupling constants for epimer **16** were found to be 4, 12, and 12 Hz, respectively, for *J*(Ha′–Hb′), *J*(Ha′–Hc′), and *J*(Ha′–Hg′) confirming the equatorial conformation of the methyl ester. The axial/equatorial conformations of **15** and **16** were further confirmed by NOE study among the axial protons. While **16** showed NOEs among three axial protons (Hc′, He′, and Hg), **15** showed NOEs between two protons (Hd and Hf).

After the successful introduction of an ester functional group onto the ring system, two consecutive Mitsunobu reactions were



Scheme 2. Attempts to introduce a nitrile in an equatorial position as the acid precursor.



Scheme 3. Synthesis of an ester derivative **16** as a precursor to **1**.

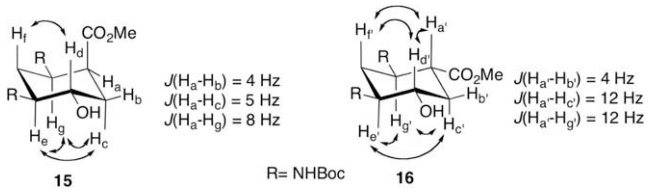
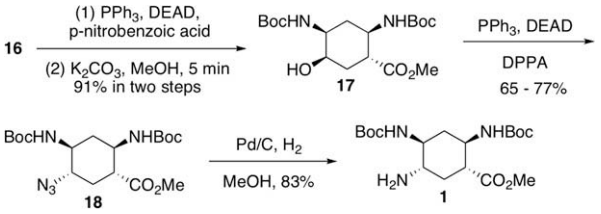


Figure 2. The coupling constants and NOE's among the neighboring H's of the two epimers **15** and **16**.



Scheme 4. Introduction of $-NH_2$ in the place of –OH of **16** using two consecutive Mitsunobu reactions.

performed to introduce $-NH_2$ in the place of the –OH of **16** (Scheme 4). Compound **17** with an axial-OH was prepared after Mitsunobu reaction and subsequent hydrolysis of the ester in overall 91% yield. Another Mitsunobu reaction was performed with PPh_3, DEAD, and DPPA to prepare azide **18**, a precursor to the desired compound **1**. In this reaction condition, the desired compound (**18**) was isolated in moderate yield and typically 5–23% of the starting compound (**17**) was recovered. When less bulky azide sources such as hydrazoic acid and nicotinyl azide were used in the place of DPPA in an effort to increase the reaction yield, the desired azide **18** was obtained in lower yield due to the formation of the undesired diastereomer. Finally, the desired 2-DOS mimic (**1**) was successfully obtained after hydrogenation of azide **18**.

In conclusion, 2,4,5-triaminocyclohexanecarboxylic acid (**1**) was designed as a novel 2-DOS mimetic based on a structure-based rational approach and successfully prepared in nine steps with overall 28% yield from the known compound **2**. It may be utilized to prepare aminoglycoside analogs that have a more rigid amide backbone than the native glycosidic linkage, which may increase the binding selectivity for any given RNA target. The amide backbone may help differentiate distinctive RNA targets through extra hydrogen-bonding capability. It may also be used to prepare novel synthetic aminoglycosides that are less prone to bacterial resistance, which is a very common resistance mechanism for aminoglycoside-based antibiotics. In addition to being a novel 2-DOS mimic, compound **1** belongs to γ-amino acid and can be used to build synthetic foldamers mimicking the structures and/or functions of natural proteins. The two amines are available for further functionalization of the foldamers.

Acknowledgments

The authors recognize BYU ORCA grant for the financial support for Brandon Leavitt. They greatly appreciate Dr. Scott Burt for his help with 2D NMR experiments.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2010.01.111.

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