Bioorganic & Medicinal Chemistry Letters 25 (2015) 2574–2578

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Incorporation of an acyclic alkynyl nucleoside analog into siRNA improves silencing activity and nuclease resistance

Aya Ogata [a], Yoshihito Ueno [a,b,*]

[a] United Graduate School of Agricultural Science, Gifu University, 1-1 Yanagido, Gifu 501-1193, Japan
[b] Faculty of Applied Biological Sciences, Gifu University, 1-1 Yanagido, Gifu 501-1193, Japan

ARTICLE INFO

Article history:
Received 8 December 2014
Revised 6 April 2015
Accepted 11 April 2015
Available online 17 April 2015

This Letter is dedicated to the late Professor Akio Nomoto

Keywords:
siRNA
Acyclic nucleoside
Alkyne
RNAi
Nuclease resistance

ABSTRACT

In order to improve the silencing activity and nuclease resistance of small interfering RNA (siRNA), we designed and synthesized an acyclic thymidine analog containing 4-pentyne-1,2-diol instead of D-ribofuranose. The incorporation of this analog into siRNAs at specific positions in the strands was found to enhance the silencing activity of siRNAs and to increase the resistance of the siRNA to hydrolytic degradation by a 3′ exonuclease.

Small interfering RNAs (siRNAs) are key molecules in the cellular RNA interference (RNAi) pathway of gene expression control.[1,2] In RNAi, the guide strand (antisense strand) of an siRNA forms the RNA-induced silencing complex (RISC) together with the Argonaute (Ago) protein. In RISC, the siRNA guide strand base-pairs with a complementary mRNA strand, which is then cleaved by the RNase activity of the Ago protein, thus inhibiting the mRNA function. Since siRNAs can be rationally designed and synthesized if the sequence of the target gene is known, they have considerable potential as new therapeutic drugs for intractable diseases.[3] So far, several chemically modified siRNAs have been synthesized for improved nuclease resistance and RNAi-eliciting abilities.[4]

The synthesis of oligonucleotides (ONs) comprising acyclic nucleosides has not received much attention because of the low stability of duplexes containing such nucleoside analogs.[5] Recently, however, it was reported that a site-specific incorporation of unlocked nucleic acid (UNA, **1**, Fig. 1), an acyclic analog of RNA, into siRNAs improves the specificity of gene silencing by the siRNAs by reducing unintended mRNA silencing called off-target effects.[6] Meggers and co-workers reported that an ON composed of an acyclic nucleoside analog containing glycol (GNA, **2**, Fig. 1) instead of D-ribofuranose forms a thermally stable duplex with complementary RNA,[7] and modification of ONs with alkynyl residues at the 5 positions of pyrimidine moieties is known to further stabilize the duplexes.[8]

Based on these reports, an acyclic thymidine analog (**3**, Fig. 1) containing 4-pentyne-1,2-diol instead of D-ribofuranose was designed in this study. In this Letter, we report the synthesis and thermal stability of siRNAs containing the nucleoside analog **3** as well as the silencing activity and nuclease resistance of the siRNA modified with **3**.

The synthetic route used to synthesize the thymidine analog **3** is shown in Scheme 1. A 4-pentyne-1,2-diol derivative (**4**), which was synthesized according to the reported method,[9] was coupled with 1-methyl-5-iodouracil (**5**) in the presence of CuI, $Pd(PPh_3)_4$, and Et_3N in DMF to produce the uracil analog **6** at a 58% yield. Subsequently, **6** was de-silylated by tetrabutylammonium fluoride (TBAF) in THF, and the primary hydroxy group of **3**[10] was protected with a 4,4′-dimethoxytrityl (DMTr) group to give **7** at a 92% yield. The DMTr derivative **7** was phosphitylated using a standard procedure to give rise to the corresponding phosphoramidite **8** at a 88% yield. To introduce the analog **3** at the end of an RNA strand, **7** was converted to the corresponding succinate, which was then linked to controlled pore glass (CPG) to generate the solid support **9** linked to **7** (39 μmol/g).

All RNAs containing **3** (Tables 1 and 2) were synthesized using the phosphoramidite **8** and the solid support **9**. The RNAs were

* Corresponding author. Tel./fax: +81 58 293 2919.
E-mail address: uenoy@gifu-u.ac.jp (Y. Ueno).

http://dx.doi.org/10.1016/j.bmcl.2015.04.034
0960-894X/

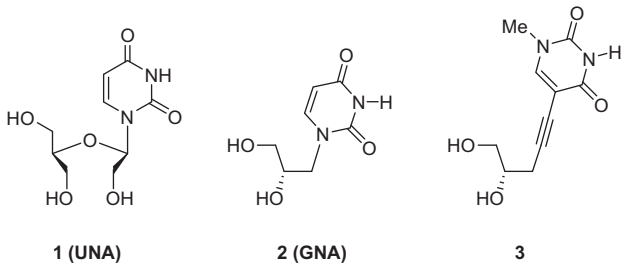
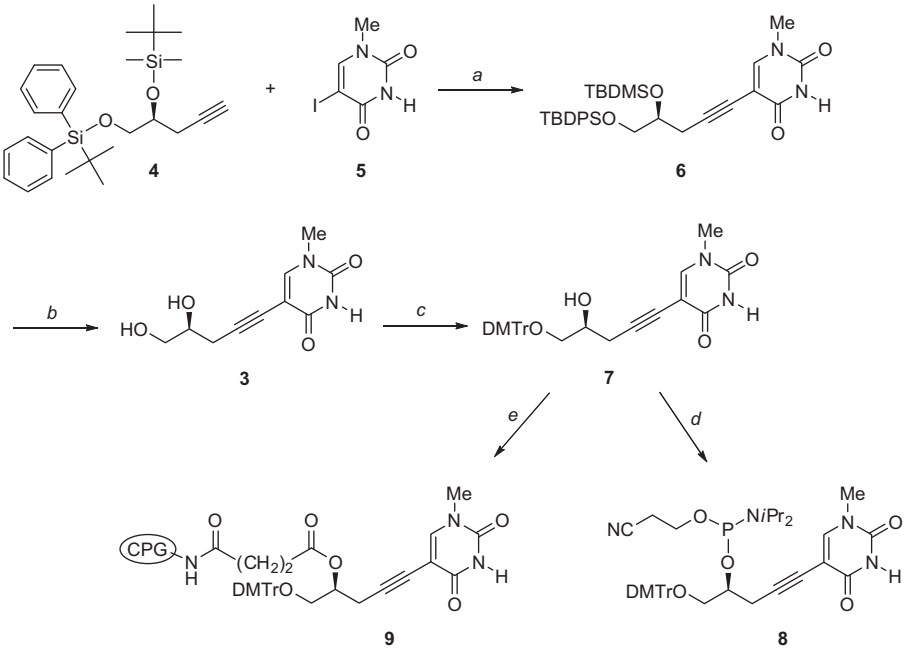


Figure 1. Structures of acyclic nucleoside analogs.

analyzed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF/MS), and the observed molecular weights from these analyses were in agreement with the structures of the RNAs.

First, the stabilities of the RNA duplexes containing **3** in the middle of the strands were evaluated by thermal denaturation in a buffer composed of 10 mM sodium phosphate (pH 7.0) and 100 mM NaCl. Melting temperatures (T_ms) and ΔT_ms (T_m of each duplex − T_m of duplex **1**) are listed in Table 1. The T_m of the natural RNA duplex was found to be 67.0 °C, whereas those of the duplexes containing **3** ranged from 60.2 °C to 64.3 °C, demonstrating that the incorporation of **3** into siRNAs thermally destabilizes RNA duplexes. The ΔT_m value of the duplex containing a **3**:A matched base pair was higher than the ΔT_ms of the duplexes containing a **3**:U, **3**:C, or **3**:G mismatched base pair. This result indicates that **3** exhibits base discrimination ability.

Next, the ability of the siRNAs containing **3** to suppress gene expression was investigated using a dual-luciferase reporter assay. The psiCHECK-2 vector encoding *Renilla* luciferase and firefly luciferase was used as the reporter[4c] and the percent *Renilla*: Firefly luciferase activities compared to transfection without siRNA are shown in Table 2. The luciferase activity ratios for an unmodified siRNA (siRNA **1**) were 28.0 and 22.3 at 1 and 10 nM, respectively. When two molecules of **3** were incorporated into each dangling end of the sense and antisense strands (siRNA **2**), the activity



Scheme 1. Reagents and conditions: (a) CuI, $Pd(PPh_3)_4$, Et_3N, DMF, 40 °C, 58%; (b) TBAF, THF, rt, 86%; (c) DMTrCl, pyridine, rt, 92%; (d) chloro(2-cyanoethoxy)(*N*,*N*-diisopropylamino)phosphine, *i*-Pr_2NEt, THF, rt, 88%; (e) (1) succinic anhydride, DMAP, pyridine, rt; (2) CPG, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide·HCl (EDCI·HCl), DMF, rt, 39 μmol/g loading amount.

Table 1
Sequences of ONs and T_m values of RNA/RNA duplexes containing **3**

Abbreviation of duplex	Abbreviation of ON	Sequence[a]	T_m (°C)	ΔT_m[b] (°C)
Duplex 1	ON 1 ON 2	5′-AGAGAUACAUUGACCUUC-3′ 3′-UCUCUAUGUAACUGGAAG-5′	67.0	—
Duplex 2	ON 3 ON 2	5′-AGAGAUACA**3**UGACCUUC-3′ 3′-UCUCUAUGUAACUGGAAG-5′	64.3	−2.7
Duplex 3	ON 3 ON 4	5′-AGAGAUACA**3**UGACCUUC-3′ 3′-UCUCUAUGUUACUGGAAG-5′	60.2	−6.8
Duplex 4	ON 3 ON 5	5′-AGAGAUACA**3**UGACCUUC-3′ 3′-UCUCUAUGUCACUGGAAG-5′	61.4	−5.6
Duplex 5	ON 3 ON 6	5′-AGAGAUACA**3**UGACCUUC-3′ 3′-UCUCUAUGUGACUGGAAG-5′	63.6	−3.4

[a] Underlined letters indicate mismatched bases.
[b] $\Delta T_m = T_m$ (each duplex) − T_m (duplex **1**).