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Synthesis and properties of thymidines with six-membered amide bridge

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ABSTRACT

Artificial thymidine monomers possessing amide or N-methylamide bridges were designed, synthesized, and introduced into oligonucleotides. UV-melting experiments showed that these oligonucleotides preferred single-stranded RNA (ssRNA) to single-stranded DNA (ssDNA) in duplex formation. Both amide- and N-methylamide-modified oligonucleotides led to a significant increase in the binding affinity to ssRNA by up to +4.7 and +3.7 °C of the T_m value per modification, respectively, compared with natural oligonucleotide. In addition, their oligonucleotides showed high stability against 3′-exonuclease.

1. Introduction

Artificial nucleic acids that possess strong and sequence-selective binding affinities with single-stranded RNA (ssRNA) and/or high nuclease-resistant properties are promising tools for nucleic acid-based technologies such as the antisense method.[1,2] In fact, a large number of nucleic acid derivatives have been developed to date.[1] The breakthrough was the discovery by us[3] and Wengel's group[4] of 2′,4′-BNA/LNA with a 2′-*O*,4′-*C*-methylene-bridged structure in the sugar moiety, (Fig. 1). The 2′,4′-BNA/LNA modification of oligonucleotides leads to a great improvement in hybridizing ability with ssRNA and an increase in nuclease resistance compared with natural oligonucleotides. Since 2′,4′-BNA/LNA was reported, the development of artificial nucleic acids with additional 2′,4′-bridged structures has attracted much attention. In particular, ring-enlargement from a five-membered bridge to a six-membered one in 2′,4′-bridged structures would be a useful modification. For example, ENA, the six-membered analog of 2′,4′-BNA/LNA, shows the same level of duplex-hybridizing ability with ssRNA as does 2′,4′-BNA/LNA, and improved enzymatic stability as compared to 2′,4′-BNA/LNA (Fig. 1).[5,6] Recently, we developed AmNA with five-membered amide bridge, the duplex-forming ability of which was comparable to that of 2′,4′-BNA/LNA, and the nuclease resistance of which was superior to that of 2′,4′-BNA/LNA.[7] Against this background, we were interested in the properties of the ring-enlarged analog of AmNA shown in Figure 2. In addition, various substituents could be introduced into the nitrogen in the amide bridge to improve the functions of the oligonucleotides. In this study, two thymidines with a six-membered amide bridge, that is, NH and NMe analogs, were synthesized, and the duplex-forming abilities and the nuclease resistances of their oligonucleotides were evaluated.

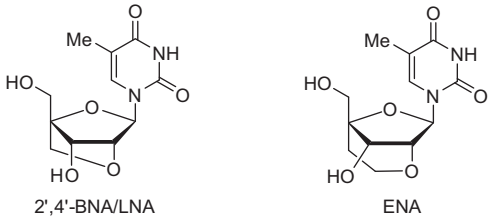

Figure 1. Structures of 2′,4′-BNA/LNA and ENA monomers.

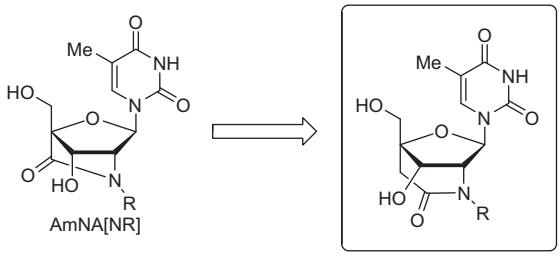

Figure 2. Design of nucleic acid monomers (R = H or Me) used in this study.

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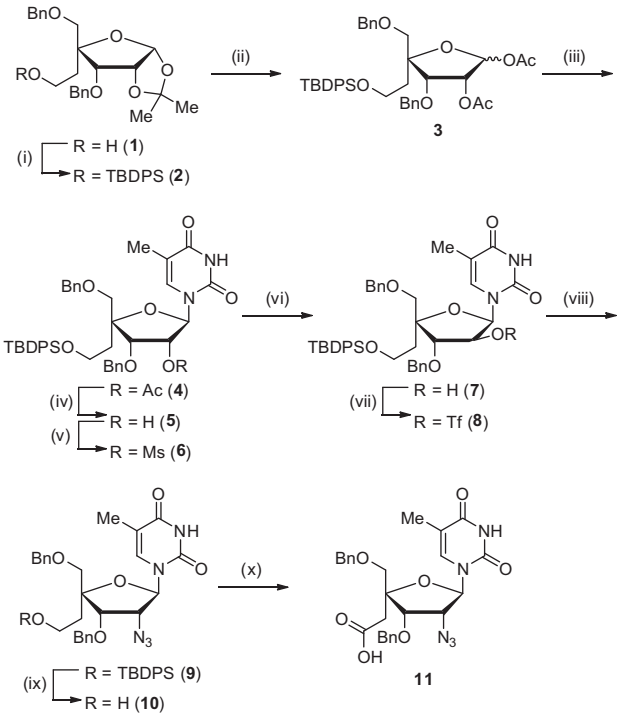


Scheme 1. Reagents and conditions: (i) TBDPSCl, DMAP, CH_2Cl_2, rt, 16 h, quant.; (ii) Ac_2O, AcOH, concd H_2SO_4, rt, 2 h, 91%; (iii) thymine, BSA, TMSOTf, MeCN, reflux, 3.5 h, 84%; (iv) K_2CO_3, MeOH, rt, 1 h, 95%; (v) MsCl, Et_3N, CH_2Cl_2, 0 °C, 1.5 h, 91%; (vi) NaOH, EtOH, rt, 12 h, 89%; (vii) Tf_2O, pyridine, CH_2Cl_2, 0 °C, 0.5 h, 83%; (viii) NaN_3, DMF, rt, 13 h, quant.; (ix) TBAF, THF, rt, 17 h, quant.; (x) PDC, MS4Å, DMF, rt, 12 h, 94%.

2. Results and discussion

2.1. Synthesis

The silylation of known compound **1**[5] using TBDPSCl gave **2** in quantitative yield (Scheme 1); **2** was converted to diacetate **3**. The reaction of **3** with silylated thymine, prepared in situ from thymine and *N,O*-bis(trimethylsilyl)acetamide (BSA), in the presence of TMSOTf produced the desired β-isomer **4**. Next, introduction of a nitrogen atom at the 2′-position was performed using a double-stereoinversion approach. After deacetylation of **4** on exposure to K_2CO_3 in MeOH, stereoinversion of the 2′-hydroxyl group was achieved by mesylation of the resulting **5**, followed by treatment with NaOH. Then, **7** underwent reaction with Tf_2O to give **8** in 83% yield, and compound **9**, with a nitrogen atom at the 2′-position, was obtained by azidation. Desilylation of **9**, followed by oxidation of **10** with PDC in DMF, efficiently produced carboxylic acid **11**.

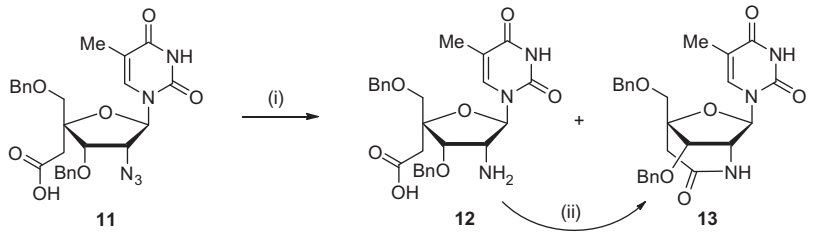
Next, reduction of the azide group in **11** was examined (Scheme 2). Reduction by $NaBH_4$ in *i*-PrOH gave the corresponding amine **12** in 55% yield. Fortunately, under Staudinger conditions using Me_3P, ring-closed **13** was produced together with **12**. Compound **12** was converted to the desired **13** using EDC or MsCl; the yields were 72% and 86%, respectively.

The synthesis of phosphoramidite **14** was carried out in three steps from **13** (Scheme 3). Diol **15** was prepared by hydrogenolysis of **13**. Then, dimethoxytritylation of **15**, followed by phosphitylation, yielded the desired phosphoramidite **14**. Concerning the *N*-methyl congener **17**, protection of the imide nitrogen of thymine in **13**, and successive methylation of **18** gave **19**, which was hydrogenolyzed to produce diol **20**. Using a similar synthetic route to **14**, **20** was converted into the desired phosphoramidite **17** with an N-methylamide bridge via dimethoxytritylated **21**. The phosphoramidites **14** and **17** obtained were used to synthesize modified oligonucleotides **22**–**29** on an automated DNA synthesizer (see the Section 4 for details). These amide bridges were stable under conventional conditions, that is, aqueous ammonia or methanolic K_2CO_3, for cleavage from the resin and removal of β-cyanoethyl groups on the phosphates.

The duplex-forming abilities of oligonucleotides **22**–**27** with ssDNA or ssRNA were evaluated and the results are summarized in Table 1. Regardless of whether it was the NH or NMe analog, the modified oligonucleotides generally showed a significantly decreased affinity for ssDNA compared with natural oligonucleotide **30**, although in the case of **24**, possessing three NH analogs, the affinity was increased by +1.7 °C per modification. Concerning ssRNA, the single-modified oligonucleotides **22** and **25** had almost the same affinities as natural oligonucleotide **30**. However, by multiple modifications, the binding affinity was greatly increased and the changes in T_m value per modification (ΔT_m/mod.) of the NH (**24**) or NMe (**27**) analogs were +4.7 and +3.7 °C, respectively. These results demonstrate that oligonucleotides containing these thymidines with six-membered amide bridges formed stable duplexes with ssRNA and recognized ssRNA more selectively than ssDNA in the duplex formation.

The binding affinities with ssDNA and ssRNA of the oligonucleotides modified by AmNA[NH] or AmNA[NMe] with a five-membered amide bridge, shown in Figure 2, and those of the oligonucleotides modified by HxNA[NMe] with a six-membered hydroxamate bridge, shown in Figure 3, were evaluated in our previous reports.[7,8] Although these analogs showed lower duplex-forming abilities with ssRNA than AmNA[NH] or AmNA[NMe], one of these analogs, the NH analog, was more stably bound to ssRNA compared with HxNA[NMe] with a six-membered bridge similar to these amide bridges.

The stabilities of oligonucleotides **28** and **29** including these analogs against 3′-exonuclease were determined and compared with those of AmNA[NH]-, AmNA[NMe]-, and HxNA[NMe]-modified oligonucleotides **31**-**33** and natural **30** (Fig. 4 and the sequences of oligonucleotides used are shown in the legend of Fig. 4). Under conditions where natural **30** decomposed completely within 5 min, over 50% and 60% of **28** and **29** remained after 40 min. The NMe analog (**29**) showed higher stability against nuclease than the NH analog (**28**) did. This is probably because ac-



Scheme 2. Reagents and conditions: (i) $NaBH_4$, *i*-PrOH, reflux, 1 h, 55% (**12**) or Me_3P, THF/H_2O (5:1), rt, 14 h, ca 35% (**12**) and 43% (**13**); (ii) EDC, DMAP, CH_2Cl_2, rt, 24 h, 72% or MsCl, Et_3N, CH_2Cl_2, 0 °C to rt, 17 h, 86%.