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Synthesis of Triazole-linked Analogues of RNA (^{TL}RNA)

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A triazole-linked analogue of RNA (^{TL}RNA) has been developed. Monomeric ribonucleoside analogues bearing natural nucleobases were prepared from D-xylose to afford the elongating units in gram quantities. The puckering of the monomeric analogues was similar to that of natural ribonucleosides and preferred the North-type conformation. An efficient elongation reaction via the copper-catalyzed Huisgen cycloaddition on a solid support was used for the synthesis of trinucleotides.

Albeit subtle at a glance, the presence of the hydroxy group at the 2′-position in a furanose ring is essential for the discrimination of the structures and functions of RNA from those of DNA (Figure 1).[1] For the design of artificial variants with modified internucleotide linkages, it is therefore important to decide whether to incorporate the 2′-hydroxy group. Presently, ribonucleic analogues have been much less explored than deoxyribonucleic analogues.[2] In nature, both types of monomeric nucleosides are readily available, as the removal of the 2′-hydroxy groups of ribonucleosides is readily accessible through enzymatic dehydroxylation.[3] In contrast, the reverse transformation, i.e., the 2′-hydroxylation of deoxyribonucleosides, is not synthetically accessible, which forced us to return to the starting point of the synthesis of ribonucleic analogues after the development of a triazole-linked analogue of DNA (^{TL}DNA).[4] We herein report the synthesis of triazole-linked analogues of RNA (^{TL}RNA) that possess 2′-hydroxy groups in the nucleosidic structures in addition to the six-bond periodicity in the oligonucleotide form.[4,5] The monomeric ribonucleoside analogues, each of which can bear one of the four natural nucleobases, were prepared from a natural monosaccharide, D-xylose, through a common glycosylation reaction of the corresponding nucleobases. The presence of a pseudo-2′-hydroxy group was advantageous for the β-selective introduction of nucleobases and also for maintaining the North-type puckering of the monomeric analogues. An efficient elongation reaction via solid-phase synthesis was demonstrated for the preparation of trinucleotides.

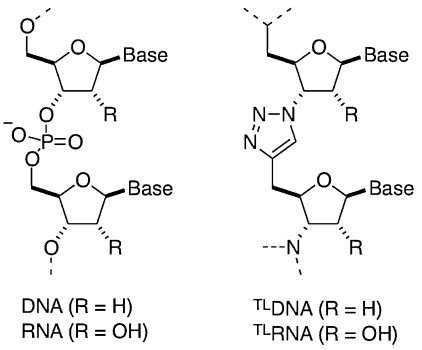
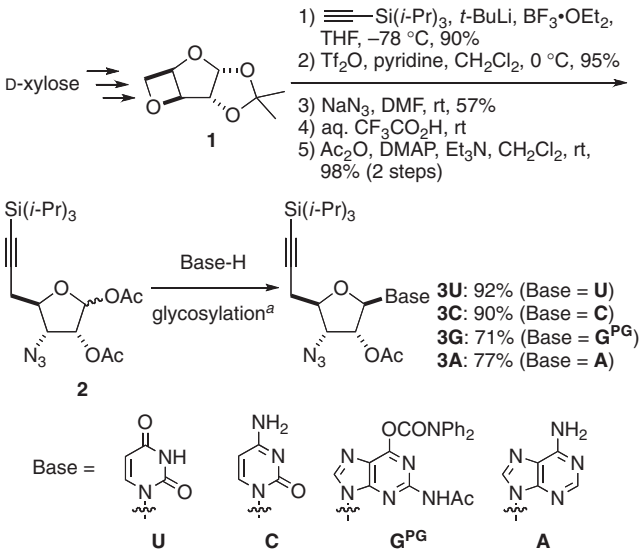


Figure 1. Structures of DNA, RNA, ^{TL}DNA, and ^{TL}RNA.

The synthesis of ribonucleoside analogues turned out to be more robust than that of deoxyribonucleoside analogues. Unlike the deoxyribonucleoside analogues, which required both relatively expensive deoxythymidine for the starting material and nonselective transglycosylation for the nucleobase replacement,[4,6,7] the ribonucleoside analogues were readily accessible from inexpensive D-xylose[8] and required one common glycosidic donor for the introduction of various nucleobases. Oxetane **1** was obtained through a 4-step synthetic procedure from D-xylose[9] and was converted into glycosidic donor **2** through a synthetic route similar to that for deoxyribonucleoside analogues (Scheme 1).[4,10] The subsequent glycosylation of the nucleobases proceeded in a stereoselective manner as the result of the anchimeric assistance of the acetoxy group at the pseudo-2′-position. The pyrimidine analogues **3U** and **3C** were obtained in good yield (92% and 90%) using a TMSOTf-promoted glycosylation reaction of silylated nucleobases.[6,11] Although the silylation of the guanine derivative required an alternate reagent,[12,13] the glycosylation proceeded after silylation with *N*,*O*-bis(trimethylsilyl)acetamide (BSA) to afford the corresponding analogue **3G** in 71% yield. The glycosylation reaction of N^6-octanoyladenine proceeded in a similar manner but gave the adenosine analogue in poorer yield (42% with TMSCl/TMSOTf). We therefore switched to the $SnCl_4$-promoted



Scheme 1. Synthesis of ribonucleoside analogues. [a]For Base = **U** and **C**: 1) TMSCl, $NH(SiMe_3)_2$, reflux, 2) TMSOTf, $ClCH_2CH_2Cl$, reflux. For Base = $\mathbf{G^{PG}}$: 1) BSA, $ClCH_2CH_2Cl$, reflux, 2) TMSOTf, $ClCH_2CH_2Cl$, reflux. For Base = **A**: $SnCl_4$, CH_3CN, rt.

Table 1. Vicinal coupling constants and puckering population in DMSO

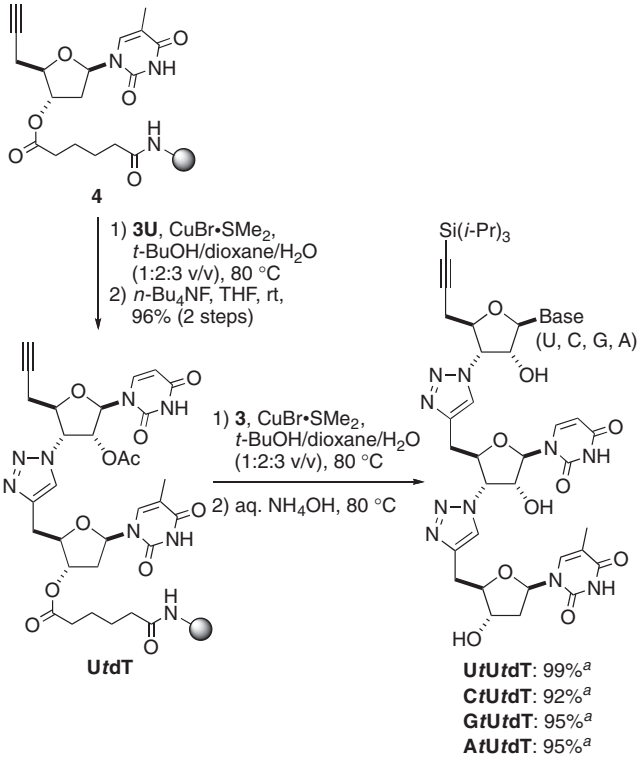
Analogue	$^{3}J_{H1',H2'}$/Hz	$^{3}J_{H3',H4'}$/Hz	%North
3U	4.9	6.1	55
3C	4.4	6.3	59
3G	2.6	7.0	73
3A	4.7	6.0	60

glycosylation of adenine[14] and obtained **3A** in 77% yield. All glycosylation reactions afforded the desired β-anomers exclusively.

Analysis of the ribonucleoside analogues by ^{1}H NMR showed that the puckering preference was similar to that of the natural congeners. One of the important structural differences between ribo- and deoxyribonucleosides is found in their puckering conformations: ribonucleosides prefer a North-type (3′-*endo*) conformation, whereas deoxyribonucleosides prefer a South-type (2′-*endo*) conformation.[1,15] In our previous experience with ^{TL}DNA, deoxyribonucleoside analogues, despite bearing nonnatural acetylene/azide linking moieties, also prefer the South-type conformation in solution.[6] When we recorded the ^{1}H NMR spectra of the ribonucleoside analogues **3** in DMSO at 25 °C, all of them showed smaller vicinal coupling constants (^{3}J) for H1′ and H2′ than for H3′ and H4′ (Table 1). This observation indicated the preference for the North-type conformation, and the Altona–Sundaralingam analysis of the ^{3}J-values indeed showed a larger population of furanose rings with the North-type conformation, with the values of 55% for **3U**, 59% for **3C**, 73% for **3G**, and 60% for **3A**.[15,16] The results may indicate the dominant contribution of the 2′-hydroxy group to the puckering preference of the furanose ring.[1,17]

Finally, we examined the elongation reaction with the ribonucleoside analogues using a deoxythymidine analogue on polystyrene resins as a 3′-terminus substrate **4**.[7] The elongation reaction, i.e., the copper-catalyzed Huisgen cycloaddition between acetylene and azide, proceeded with **3U** and **4**, and the dinucleotide **U*t*dT** was obtained in 96% yield (Scheme 2).[18,19] The elongation reaction between ribonucleoside analogues was then conducted with **U*t*dT** and each of the four ribonucleoside analogues, and the corresponding trinucleotides were obtained in excellent yields: The fully deprotected trinucleotides after the hydrolytic cleavage from the resins were obtained in 99% yield for **U*t*U*t*dT**, 92% yield for **C*t*U*t*dT**, 95% yield for **G*t*U*t*dT**, and 95% yield for **A*t*U*t*dT**. The elongation efficiency was comparable to that of deoxyribonucleic analogues,[4,7] a result that shows that neither the nucleobases nor the 2′-acetoxy group severely hampers the click coupling reactions for the extension of triazole-linked oligonucleotides.

In conclusion, we have developed a new ribonucleic analogue, ^{TL}RNA. Starting from an abundantly available monosaccharide, D-xylose, the synthesis of ribonucleoside analogues is sufficiently concise to be conducted on a gram scale. The puckering preference, which is similar to that of natural ribonucleosides, may help ^{TL}RNA mimic the functions of RNA. Moreover, as ^{TL}DNA has been demonstrated to be a competent enzymatic substrates,[20–22] the biologically non-degradable linkage of ^{TL}RNA may provide an intriguing opportunity to explore the sophisticated function of RNA, for instance, RNA interference, that require the enzymatic recognition of the oligonucleotides. The design and synthesis of such oligonucleotides will be investigated in the near future.



Scheme 2. Solid-phase synthesis of trinucleotide analogues. [a]Yield after the two-step synthetic operation from **U*t*dT**.

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