Chemical Synthesis and Properties of an Interresidually Cyclized Uridylyl(3′–5′)uridine as a Model of tRNA U-Turn Structure Having a Sharp Bend

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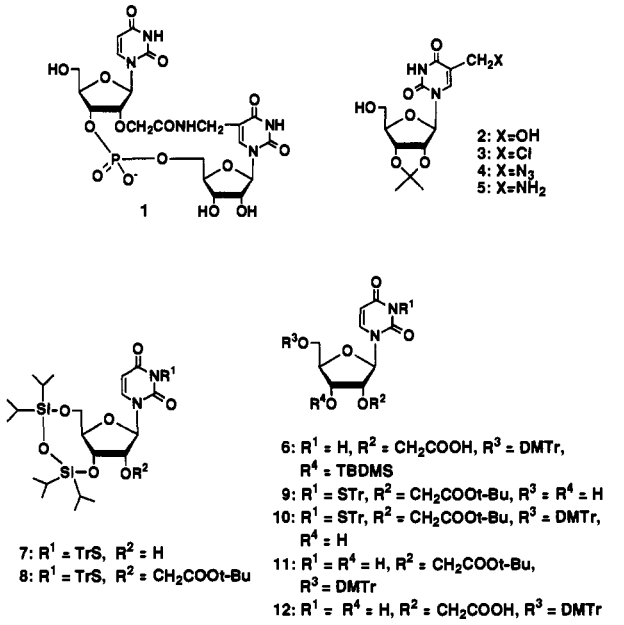
Many biologically important RNAs have loop structures. The importance of such structures has been demonstrated in connection with studies on the structure–function relationships of RNAs.[1] In common with other RNA loops found to date, the anticodon loop in tRNAs has a sharply bent structure, called a "U turn", seen between the thirty-third uridine residue (U_{33}) and the thirty-fourth nucleoside (N_{34}).[2] The latter corresponds to the first anticodon nucleoside; this is greatly modified in the base or sugar moieties.[3] Since this local structure is known to play an important role in codon–anticodon recognition on the ribosome, extensive conformational studies of this region have been carried out at the monomer and dimer levels using NMR techniques.[4,5] Several interresidual interactions have also been discussed with regard to stabilization of the U-turn structure.[6] The crystallographic study[6a] of 5-[(methylamino)methyl]-2-thiouridine (mnm^5s^2U), *i.e.*, the thirty-fourth minor nucleoside of several tRNAs from *Escherichia coli*,[7] suggested that the 5-aminomethyl group could approach closely to the 2′-hydroxyl of the thirty-third uridine residue to form a hydrogen bond between the NH of mnm^5s^2U and the 2′-oxygen. In this paper, we report the first example of a conformationally restrained U-turn structure between thirty-third and thirty-fourth uridines, which may be useful in understanding the nature of structure in RNAs.

Chart 1



We have synthesized a mimic (**1**, Chart 1) which had an interresidually cyclized structure through an amide bond and was expected to exist in a bent conformation like the U turn in tRNAs.[8] First, we prepared 5-(aminomethyl)-2′,3′-*O*-isopropylideneuridine **5** as the 3′ terminal nucleoside unit for the synthesis of **1**. Reaction of readily available **2**[9] with 5 equiv of chlorotrimethylsilane in dioxane at 60 °C for 2.5 h gave the 5-chloromethylated uridine derivative (**3**).[10] The 5-(azidomethyl)uridine derivative **4** was prepared in 50% yield from **2** by treatment of **3** with 6 equiv of NaN_3 in DMF at 60 °C for 3 h and then converted to the 5-(aminomethyl)uridine derivative **5**[11] in 71% yield by Letsinger's method using PPh_3.[12]

On the other hand, a synthetic unit **6** at the 5′-upstream side was prepared by a six-step reaction from 3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-*N*-[(triphenylmethyl)thio]uridine (**7**)[13,14] as follows: Reaction of **7** with 4 equiv of NaH in THF for 30 min followed by treatment with 4 equiv of *tert*-butyl bromoacetate gave the 2′-*O*-alkylated product (**8**) in 78% yield. Desilylation of **8** with 2.2 equiv each of $Bu_4NF{\cdot}H_2O$ and acetic acid[15] in THF gave the diol derivative **9** in 98% yield. The reaction of **9** with 4,4′-dimethoxytrityl chloride afforded **10** in 88% yield. Reaction of **10** with 2.5 equiv of Bu_3SnH in the presence of 0.1 equiv of AIBN in refluxing toluene for 5 min[16] gave the deprotected product **11** in 70% yield. The *tert*-butyl ester of **11** was hydrolyzed by treatment with 0.2 M KOH–pyridine (1:1, v/v) at room temperature for 1.5 h to give compound **12**. *In situ* reaction of **12** with 3 equiv each of *tert*-butyldimethylsilyl chloride and

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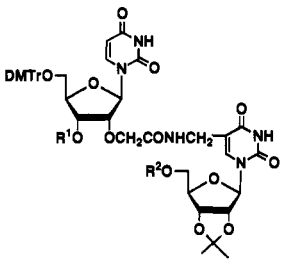
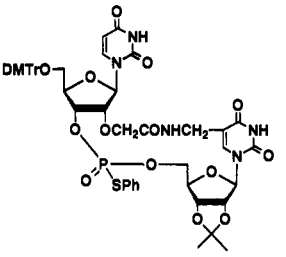
Chart 2



13: R^1 = TBDMS, R^2 = H
14: R^1 = TBDMS, R^2 = $(PhS)_2P(O)$
15: R^1 = H, R^2 = $PhSP(O)O^-$



16

imidazole in DMF at room temperature for 60 h followed by partial hydrolysis of the simultaneously formed silyl ester linkage gave **6** in 93% yield.

The 5′ terminal synthetic unit **6** thus prepared was coupled with the 5-aminomethylated 3′-terminal uridine unit **5** by the use of *N*-ethyl-*N*′-[3-(dimethylamino)propyl]carbodiimide·HCl[17] as a condensing agent to give the amide **13** in 69% yield. The product **13** was phosphorylated with cyclohexylammonium *S*,*S*-diphenyl phosphorodithioate in the presence of 2 equiv of isodurenedisulfonyl dichloride (DDS)[18] and 4 equiv of 1*H*-tetrazole in pyridine at room temperature for 30 min to give compound **14** in 82% yield. The selective dephenylthiolation of **14** with Et_3N–H_2O–pyridine (2:1:2, v/v/v)[19] at room temperature for 1 h followed by treatment with 2.5 equiv each of TBAF·H_2O and acetic acid in THF[15] at room temperature for 3 h afforded the suitably deprotected dimer **15** for the next cyclization. Compound **15** was allowed to react with 3 equiv each of DDS and 1*H*-tetrazole in pyridine at room temperature for 1.5 h to give the fully protected cyclic uridylate dimer **16** in an overall yield of 50% from **14**. This yield seems rather high for such a large-membered (17-membered) ring formation, with additional devices such as high dilution technique usually employed in the field of macrolide synthesis[20] not having been tried. Therefore, this result suggested that such a cyclic structure was not so strained and that the 5-substituent of the 3′-downstream uridine could approach the 2′-hydroxyl of the 5′-upstream uridine as discussed in the interresidual hydrogen bonding in the U-turn structure.[6a] Finally **16** was deprotected by treatment with an excess amount (20 equiv) of bis(tributyltin) oxide[21] in pyridine at room temperature for 3 h, followed by hydrolysis with 60% formic acid at room temperature for 20 h. The fully deprotected dimer **1** was obtained in 61% yield. The structure of **1** was characterized by UV, ^1H (^1H–^1H COSY, NOESY), ^{13}C, and ^{31}P NMR[22] and the following enzymatic assay.

We examined the enzymatic property of cyclic dimer **1** by the use of snake venom phosphodiesterase (SVP) and calf spleen phosphodiesterase (CSP). Consequently, it was found that the dimer was not digested in both cases. It is well-known that such enzymes recognize nucleoside bases of the substrates.[23] This resistance to enzymatic cleavage of the internucleotide linkage probably resulted from a highly restricted base conformation of **1**, especially owing to the 3′-terminal uridine unit. In contrast to this result, we found that nuclease P1 could digest this dimer completely to produce only a new single peak in reverse phase HPLC.[24] This conversion of a single peak to another single peak clearly suggested the presence of a cyclic structure in **1**.[25]

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Supplementary Material Available: ^1H, ^{13}C, and ^{31}P NMR spectra of the synthetic intermediates and the final product **1** (26 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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(22) ^1H NMR (270 MHz, D_2O, TMS): δ 3.81 (1H, dd, J_{gem} = 13.2 Hz, $J_{4',5'}$ = 3.8 Hz, 5′-H_{a1}), 3.93 (1H, dd, J_{gem} = 13.2 Hz, $J_{4',5'}$ = 2.8 Hz, 5′-H_{a2}), 4.2–4.3 (8H, m, 2′-H_b, 3′-H_b, 4′-H_b, 5′-H_b, CH_2N), 4.35 (3H, m, 4′-H_a, CH_2-CO), 4.40 (2H, m, 2′-H_a), 4.83 (1H, m, 3′-H_a), 5.88 (1H, d, $J_{5,6}$ = 7.9 Hz, 5-H_a), 6.00 (1H, d, $J_{1',2'}$ = 3.9 Hz, 1′-H_b), 6.07 (1H, d, $J_{1',2'}$ = 4.6 Hz, 1′-H_a), 7.52 (1H, s, 6-H_b), 7.87 (1H, d, $J_{5,6}$ = 7.9 Hz, 6-H_b). ^{31}P NMR (109 Hz, D_2O, H_3PO_4): δ 0.61. UV (H_2O): λ_{max} 261.5 nm, λ_{min} 231 nm.

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