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Synthesis and Solution Conformation Studies of 3-Substituted Uridine and Pseudouridine Derivatives

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Abstract

A series of 3-substituted uridine and pseudouridine derivatives, based on the naturally occurring 3-(3-amino-3-carboxypropyl) modification, were synthesized. Their aqueous solution conformations were determined by using circular dichroism and NMR spectroscopy. Functional group composition and chain length were shown to have only a subtle influence on the distribution of *syn/anti* conformations of the modified nucleosides. The dominating factor appears to be the glycosidic linkage (C– vs. N–glycoside) in determining the nucleoside conformation.

Introduction

Modified nucleosides have the ability to regulate the function, stability, or structures of RNA.[1,2] Over 100 different post-transcriptional modifications occur in natural RNAs.[3] Synthesis of the individual modified nucleosides is useful in order to examine the unique influences of the modifying groups on base and/or sugar conformation.[4] Modified nucleosides also have the ability to function as antiviral reagents by interfering with polymerases or reverse transcriptase;[5] however, a large number of synthetic analogues have low activity or excessive toxicity. Therefore, it is important to determine the level of conformational flexibility of the modified nucleosides and to correlate this information with active site binding to a target protein or enzyme in order to design better antiviral agents.[6]

Methylation is the most common and simple nucleoside modification.[7] More complex modifications, such as L-homoserine addition, are found in both uridine (3-(3-amino-3-carboxypropyl)uridine, or acp^{3}U) and pseudouridine (1-methyl-3-(3-amino-3-carboxypropyl) pseudouridine, or m^{1}acp$^3\Psi$) at the N3 position in natural RNA sequences.[8,9] Early studies revealed the presence of acp^{3}U in *Escherichia coli* tRNA^{Phe},[10] as well as other RNAs. The role of acp^{3}U is still unclear, since the translation process is not affected by the presence or absence of this modified nucleoside in tRNA.[11] The biological function of the related derivative m^{1}acp$^3\Psi$ is also not known. This hypermodified nucleoside is found in 18S rRNAs of eukaryotes,[8,9,12,13] including humans and yeast. It is also the only known modified nucleoside in helix 31 of 18S rRNA in eukaryotes.[14] A methylated pseudouridine derivative (m$^1\Psi$) was found to be a metabolite in *Streptomyces platensis*, then later discovered in tRNA of archaebacteria.[15,16] A 2′-*O*-methylpseudouridine derivative (Ψm) is found in 18S and 28S wheat embryo tRNAs and rRNAs.[17]

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In this study, a series of 3-subsitiuted uridine and pseudouridine analogues (acp^{3}U, m^{1}acp$^3\Psi$, 3-(4-amino-4-carboxybutyl)uridine or acb^{3}U, 3-(3-carboxypropyl)uridine or cp^{3}U, 3-(4-hydroxybutyl)uridine or hb^{3}U, and 1-methyl-3-(4-hydroxybutyl)pseudouridine or m^{1}hb$^3\Psi$,Figure 1) were prepared by using a Mitsunobu reaction in the key step, and their solution conformations were examined by using a variety of biophysical techniques, namely circular dichroism (CD) and NMR nuclear Overhauser effect (NOE) spectroscopy. The uridine analogue cp^{3}U was synthesized and compared with acp^{3}U to better understand the role of the homoserine amino group in influencing sugar/base conformation. For the hb^{3}U and m^{1}hb$^3\Psi$ derivatives, the homoserine carboxylate and amino groups were replaced with a hydroxyl group. The acb^{3}U derivative was prepared to study the role of the amino-acid side-chain length (propyl vs. butyl). In addition, two methylated pseudouridine derivatives, 1-methylpseudouridine (m$^1\Psi$) and 2′-*O*-methylpseudouridine (Ψm), were prepared for comparison with Ψ and m^{1}acp$^3\Psi$.

Results and Discussion

Synthesis of Pseudouridine Derivatives

Pseudouridine was synthesized from compound **1**,[18] which was stereoselectively reduced with $ZnCl_2$ and L-Selectride followed by cycloetherification using the Mitsunobu reaction as adapted from Hanessian and coworkers[19] to give the protected nucleoside **3** in >80% yield (Scheme 1). The pyrimidine protective groups were removed by sodium iodide treatment under acidic and refluxing conditions to give compounds **4** and **5**, followed by sugar deprotection with trifluoroacetic acid (9/1 TFA/H_2O) solution[20] to give β-pseudouridine **6** (Scheme 1).

1-Methylpseudouridine was prepared from compound **5** (Scheme 1), which was treated with the bulky base *N*,*O*-bis(trimethylsilyl)acetamide (BSA) and iodomethane under refluxing conditions or at room temperature to selectively methylate the N1 position.[21,22] The protective groups of compound **7** were removed under acidic conditions to give the desired 1-methylpseudouridine (m$^1\Psi$) **8** in 90% yield. Next, the Mitsunobu reaction[23] was utilized to couple a suitable amino acid or alcohol moiety to compound **7** and generate the 3-substituted derivatives. The main advantage of this approach is that the reaction is carried out under mild conditions with high efficiency and without any side reactions on the homoserine or alcohol functionalities. A boc-protected homoserine methyl ester **9** was prepared from commercially available homoserine using a literature procedure,[24] and coupled with protected 1-methylpseudouridine **7** using the Mitsunobu reaction to give compound **10** in >90% yield. The deprotection of compound **10** was accomplished by saponification of the ester with 0.67 N $NaOH_{(aq)}$ and acid hydrolysis with TFA/H_2O (9/1) to give compound **11**, m^{1}acp$^3\Psi$(Scheme 1).

The m^{1}hb$^3\Psi$ derivative (deamino and decarbonyl analogue of m^{1}acp$^3\Psi$) was synthesized in four steps using the same procedure as for compound **11**. Prior to coupling, commercially available 1,4-butanediol was monosilylated with *tert*-butyldimethylsilyl chloride to give compound **12** in 68% yield (Supplementary, Scheme S1). The alcohol derivative **12** was then reacted with protected 1-methylpseudouridine **7** to give compound **13** in 77% yield (Scheme 1). The silyl protecting groups were removed with tetrabutylammonium fluoride (TBAF) followed by hydrolysis under acidic conditions to afford the desired product **15**. The 2′-*O*-methylpseudouridine (Ψm) derivative was prepared from pseudouridine following a literature procedure.[25]

Synthesis of Uridine Derivatives

Synthesis of the modified uridine analogues began with commercially available uridine. The 2′ and 3′ hydroxyl groups of uridine were protected with isopropylidene, to generate

intermediate **16**, followed by 5′ silylation with *tert*-butyldiphenylsilyl chloride and imidazole to give uridine derivative **17** (Supplementary, Scheme S2).[26] Synthesis of 3-(3-amino-3-carboxypropyl)uridine (acp^3U) followed the protocol for synthesis of $m^1acp^3\Psi$.[20,24] The protected uridine **17** and homoserine derivative **9** were coupled under Mitsunobu conditions to give **18** in >90% yield. The two-step deprotection employed 0.67 N $NaOH_{(aq)}$ followed by treatment with TFA/H_2O to give the desired acp^3U **19** (Supplementary, Scheme S3).

To investigate the relationship between the functional group modification and nucleoside conformation, a series of uridine derivatives was synthesized. The homologue 3-(4-amino-4-carboxybutyl)uridine (acb^3U) was synthesized to study the role of amino-acid chain length. A protected amino alcohol was used in the coupling reaction. Compound **20**[27] was treated with benzoyl chloride to protect the 1 position followed by desilylation to give the alcohol derivative **22** (Scheme 2). Compound **22** was coupled with uridine derivative **17** followed by removal of the benzoyl group under basic conditions (>90% yield) (Scheme 3). The resulting alcohol **24** was oxidized to the corresponding carboxylate **25** in high yield using pyridinium dichromate (PDC).[28] The protective groups were removed under acidic conditions (TFA/H_2O) to give the desired product **26** in good yield (71% from compounds **17** and **22**).

The 3-(4-hydroxybutyl)uridine (hb^3U) was synthesized to determine the effects of removing the amino and carbonyl groups of acp^3U on nucleoside conformation. Synthesis of hb^3U employed the same protocol as $m^1hb^3\Psi$ synthesis. Protected uridine **17** was used in place of $m^1\Psi$ (**7**, Scheme 1), and coupled with **12** to give protected hb^3U (**27**) in 92% yield (Supplementary, Scheme S4). The silyl protecting groups were removed by treatment with TBAF followed by hydrolysis under acidic conditions to afford the desired product **29**.

To further utilize the Mitsunobu reaction, the acid derivative cp^3U was synthesized from a dimethoxytrityl protected alcohol and protected uridine. 1,4-Butanediol was treated with dimethoxyltrityl chloride to give monoprotected compound **30** (Supplementary, Scheme S5). This compound was coupled with uridine derivative **17** under Mitsunobu conditions followed by removal of the dimethoxytrityl group to give alcohol **31** in one pot (Scheme 3). The dimethoxytrityl protecting group was used on 1,4-butanediol, because it is acid labile and can be removed without affecting the fluoride-labile 5′-*tert*-butyldiphenylsilyl group on uridine. The alcohol derivative **31** was oxidized to the corresponding acid with PDC to give carboxylic acid **32**. The protective groups were removed in one step under acidic conditions to give the desired product **33** (Scheme 3). Although the synthesis of 3-(3-carboxypropyl)uridine (cp^3U) was previously reported by Seela and coworkers[29], we chose to use the Mitsunobu reaction to extend the substrate scope and further demonstrate the generality of the method.

Circular Dichroism Studies of Modified Nucleosides

The solution conformations of the modified pseudouridine and uridine nucleosides were studied by using circular dichroism (CD) spectroscopy. The CD spectrum of pseudouridine (Ψ) has a peak minimum at 276 nm (Figure 2), consistent with previous studies.[30] This key feature is suggestive of a *syn* conformation for pseudouridine.[31] In the case of $m^1\Psi$, a peak minimum occurs at 281 nm, also consistent with the *syn* orientation. The 5 nm shift in the peak minimum compared to Ψ is likely due to the presence of the methyl group, and is similar to that observed with $m^3\Psi$ (282 nm).[32] Overall, the presence of the methyl group at the N1 position does not appear to influence the preference of Ψ for the *syn* conformation. In contrast, the modified nucleoside m^3U prefers the *anti* conformation,[32] consistent with the fact that molecular orbital interactions play a role in the nucleoside conformation.[4] It has been implicated that the orbital of the H5–H6 double bond in the pyrimidine ring of uridine has interactions with the lone pair of the sugar O4′ which results in an *anti* conformational preference.[33,34] The orbital overlap would clearly be different for Ψ and corresponding derivatives, due to the C-glycosidic linkage and altered spacial arrangement of the pyrimidine

ring. The CD spectra of the modified nucleosides $m^1acp^3\Psi$, $m^1hb^3\Psi$ and Ψm (Figure 2) show peak minima at 280, 279, and 276 nm, respectively. Our results reveal that the conformations of the four modified Ψ nucleosides are predominately *syn*. This study shows that the conformation of pseudouridine is not significantly influenced by the presence of functional groups on the pyrimidine ring (N1 or N3) or sugar moiety, and that the dominate contribution comes from the glycosidic linkage between the base and ribose (*i.e.*, C– vs. N–glycoside).

Similarly, N3 modifications have minimal effects on the CD spectra of the uridine derivatives (Figure 3). The spectrum for the modified nucleoside acp^3U has a peak maximum at 269 nm, and the other uridine modified nucleosides, acb^3U, cp^3U, and hb^3U, show CD peak maxima at 268 nm. For comparison, the uridine spectrum also has a peak maximum at 268. These data suggest that the uridine nucleosides are all predominately in the *anti* conformation in solution. The shapes of the CD spectra of the uridine analogues are similar, suggesting that the functional groups do not make a significant contribution to the conformational preference. The derivatives of pseudouridine and uridine consistently show opposite absorption bands due to the C-C and C-N linkages between the sugar and base, which lead to different molecular electronic dipole and molecular magnetic dipole transition moments. The opposing CD spectra for the pseudouridine and uridine derivatives are in agreement with previous results.[31,35] In addition, the CD spectra of the naturally modified nucleosides, $m^1\Psi$, $m^1acp^3\Psi$, and acp^3U, do not show significant changes with pH (data are not shown), providing further support that the amino and carboxyl functionalities have little influence on the nucleoside conformations.

NMR Studies of Uridine and Pseudouridine Derivatives

NMR NOE experiments were done to study the effects of modification on the base orientation and sugar pucker. The *syn* and *anti* conformations of the bases can be determined by four protons (H6, H1′, H2′, and H3′).[36,37] NOE experiments on the pseudouridine derivatives were done in D_2O at room temperature. The uridine derivatives were studied in D_2O at 40 °C because of overlap between the H1′ and H5 peaks at the lower temperature. The *syn* conformation is expected to give a strong NOE at H1′ when H6 is irradiated. In contrast, the same irradiation typically gives a strong NOE to H2′ and H3′ if the nucleoside is in the *anti* conformation (Figure 4).

The derivatives $m^1\Psi$, $m^1acp^3\Psi$, and $m^1hb^3\Psi$ show stronger H1′ NOE effects when H6 is irradiated compared to Ψ (Table 1), revealing a preference for the *syn* conformation. The strongest H1′ NOE effect is observed for $m^1\Psi$, suggesting that the *syn* conformation is more highly favorable for this nucleoside. These data are consistent with the results from CD spectroscopy. The observation of weaker NOEs between H1′ and H6 of Ψm reveals that the *syn* conformation is not as strongly preferred for the 2′-*O*-methylated derivative. In contrast, strong NOE effects at H2′ and H3′ are observed for acp^3U, acb^3U, and hb^3U upon irradiation at H6 (Table 1). This suggests that the *anti* conformation is preferred for those nucleosides, which again is in agreement with the CD data. The cp^3U nucleoside shows stronger H1′ and weaker H2′ and H3′ NOE effects than the other modified uridines. Thus, the *anti* conformation is less preferred for cp^3U compared to the other modified uridines. Also, the H1′ to H6 NOEs are greater for $m^1\Psi$, Ψ, and $m^1hb^3\Psi$ compared to $m^1acp^3\Psi$. This trend suggests that the *syn* conformation is slightly more favored when N3 substituents are less bulky or absent. A similar trend is observed with the uridine derivatives in which the more bulky substituents more strongly prefer the *anti* conformation.

The ribose moieties of RNA normally adopt C_2'-*endo* (S) or C_3'-*endo* (N) conformations (Figure 4). The conformations or sugar puckers can be defined by the $J_{H1'-H2'}$ and $J_{H3'-H3'}$ coupling constants.[37] The percentage of these two conformers can be derived from the equations: $[C_2'\text{-}endo] = J_{1',2'}/(J_{1',2'} + J_{3',4'})$ and $[C_3'\text{-}endo] = 1 - [C_2'\text{-}endo]$. The coupling constants for the modified nucleosides are shown in Table 2. The uridine derivatives show a

slight preference for C_3'-*endo* (N) conformers (55 to 60%) compared to pseudouridine derivatives (43 to 52%) (Table 3). The C-glycoside nucleosides normally favor C_2'-*endo* (S) compared to N-glycoside nucleosides due to the decreased anomeric effect.[4,38] The conformation of sugars in the Ψ and U analogues is in agreement with previous conformational studies of other C-C and C-N nucleosides.[33,36,39] The exception is $m^1hb^3\Psi$, which has a K_{eq} (N/S) value of 1.1.

Conclusions

A simple and efficient experimental procedure for synthesizing 3-subsitituted uridine and pseudouridine derivatives has been presented. The Mitsunobu coupling reaction between a simple alcohol and nucleoside gives excellent yields (over 90% yield) and high efficiency compared to the somewhat harsher conditions using brominated or tosylated substrates (typically ~70% yields).[10b,35] A number of recent studies have shown that modification of nucleobases can achieved in an efficient and practical way using the Mitsunobu reaction.[39] The mild reaction conditions of this procedure are highly tolerated by a broad range of nucleoside functional or modified groups. Furthermore, the highly water-soluble phosphine reagent can be used to facilitate the purification process.[40] The CD and NMR NOE studies allow the *syn* and *anti* conformational assignments for the modified nucleosides to be made and compared. In $m^1\Psi$, the N1 methyl group provides steric constraints to suppress the *anti* conformation and the *syn* conformation is highly favored. This was observed in circular dichroism spectra and NMR NOE studies. The functional group and length of the side chain at N3 have only a slight influence on the *syn/anti* conformations of the modified uridine and pseudouridine nucleosides examined in this study. The conformation of the ribose sugars was also investigated. The C-C nucleosides show a slightly higher population of the C_2'-*endo* (S) conformation; whereas, the C_3'-*endo* (N) conformation is slightly more favored for C-N nucleosides. This observation is in agreement with the conformation studies of other C-C and C-N nucleosides. The development of efficient syntheses of modified nucleosides will allow for generation of phosphoramidites or nucleotide triphosphates, followed by incorporation onto oligoribonucleotides for further biophysical studies of modified RNA sequences.

Experimental Section

Synthesis of Modified Nucleosides

5-[(1*R*,2*S*,3*R*,4*S*)-5′-*O*-(*tert*-Butyldiphenylsilyl)-2′,3′-*O*-isopropylidene-1,4-pentandiol]-2,4-dimethoxypyrimidine (2)—A 1.0 M solution of $ZnCl_2$ (7.15 mL, 7.15 mmol) in diethyl ether was added dropwise to a solution of **1α** and **1β** (2.70 g, 4.77 mmol) in anhydrous dichloromethane (110 mL) at −72 °C under argon. After stirring at −72 °C for 30 min, a 1.0 M solution of L-Selectride (18.12 mL, 18.12 mmol) in THF was added slowly over 30 min. The reaction was warmed to rt and stirred for 19 h. The reaction mixture was quenched by addition of EtOH (5 mL), water (5 mL), 30% H_2O_2 (5 mL), and 5 N NaOH (5 mL). After workup, the crude product was purified by column chromatography using EtOAc/hexane (30–50%) to give **2** (2.50 g, 92%) as a colorless oil: R_f 0.25 (EtOAc/hexane 1:1); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.38 (s, 1 H), 7.68 (m, 4 H), 7.41 (m, 6 H), 5.29 (d, *J* = 4.0 Hz, 1 H), 4.36 (m, 1 H), 4.26 (m, 2 H), 3.96 (m, 6 H), 3.91 (dd, *J* = 10.0, 3.0 Hz, 1 H), 3.83 (dd, *J* = 10.0, 5.0 Hz, 1 H), 3.24 (d, *J* = 6.0 Hz, 1 H), 3.12 (d, *J* = 3.5 Hz, 1 H), 1.48 (s, 3 H), 1.31 (s, 3 H), 1.08 (s, 9 H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 168.0, 164.8, 157.2, 135.8, 135.7, 133.14, 133.07, 130.2, 130.1, 128.1, 128.0, 115.6, 108.8, 78.2, 77.6, 76.5, 69.9, 65.6, 64.9, 54.9, 54.3, 27.1, 27.0, 25.1, 19.5; ESI-MS (ES^+) m/z calcd for $C_{30}H_{40}N_2O_7Si$ 568.26, found 575.19 ($M+Li^+$), 607.20 ($M+K^+$); HRMS calcd for $C_{26}H_{31}N_2O_7Si$ ($M^+–C_4H_9$) 511.1901, found 511.1896.

5-[5′-*O*-(*tert*-Butyldiphenylsilyl)-2′,3′-*O*-isopropylidene-β-D-ribofuranosyl]-2,4-dimethoxypyrimidine (3)—Diisopropyl azodicarboxylate (1.38 mL, 7.14 mmol) was added

to a stirred solution of **2** (2.03 g, 3.57 mmol) and triphenylphosphine (1.87 g, 7.14 mmol) in anhydrous THF (200 mL) at 0 °C under argon. The reaction was slowly warmed to rt and stirred for 19 h. The solvent was removed under reduced pressure. The residue was purified by column chromatography using EtOAc/hexane (15–30%) to give **3** (1.79 g, 91%) as a colorless oil: R_f 0.58 (EtOAc/hexane 1:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.30 (s, 1 H), 7.66 (m, 4 H), 7.39 (m, 6 H), 4.98 (d, *J* = 4.0 Hz, 1 H), 4.71 (dd, *J* = 6.4, 4.8 Hz, 1 H), 4.65 (dd, *J* = 6.4, 4.8 Hz, 1 H), 4.13 (m, 1 H), 3.98 (s, 3 H), 3.94 (s, 3 H), 3.89 (m, 1 H), 3.82 (dd, *J* = 11.2, 4.8 Hz, 1 H), 1.59 (s, 3 H), 1.35 (s, 3 H), 1.06 (s, 9 H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 168.8, 165.4, 157.2, 135.9, 135.8, 133.41, 133.37, 130.00, 129.97, 128.0, 114.5, 113.4, 85.4, 84.9, 81.8, 80.6, 77.5, 64.2, 55.1, 54.3, 27.9, 27.1, 25.9, 22.0, 19.5; ESI-MS (ES^+) m/z calcd for $C_{30}H_{38}N_2O_6Si$ 550.25, found 551.19 ($M+H^+$), 573.15 ($M+Na^+$); HRMS calcd for $C_{30}H_{38}N_2O_6Si$ ($M^+-C_4H_9$) 493.1795, found 493.1790.

5′-*O*-(*tert*-Butyldiphenylsilyl)pseudouridine (4) 5′-*O*-(*tert*-Butyldiphenylsilyl)-2′, 3′-*O*-(isopropylidene)pseudouridine (5), 5-(β-D-Ribofuranosyl)uracil (6, Ψ), 1-Methyl-5′-*O*-(*tert*-butyldiphenylsilyl)-2′,3′-*O*-(isopropylidene)pseudouridine (7), and 1-Methylpseudouridine (8, m^{1}Ψ)—Compounds **4**–**8** were prepared according to literature procedures.[18,21,22]

1-Methyl-3-[(*S*)-3-*N*-(*tert*-butoxycarbonyl)-amino-3-methyl-carboxypropyl]-5′-*O*-(*tert*-butyldiphenylsilyl)-2′,3′-*O*-(isopropylidene)pseudouridine (10)—Diisopropyl azodicarboxylate (0.12 mL, 0.80 mmol, 1.3 eq) was added to a stirred solution of **7** (0.33 g, 0.61 mmol, 1.0 eq), boc-protected homoserine methyl ester **9** (0.14 g, 0.61 mmol, 1.0 eq), and triphenylphosphine (0.19 g, 0.74 mmol, 1.2 eq) in anhydrous THF (20 mL) at rt under argon. The reaction mixture was stirred for 1 h. The solvent was removed under reduced pressure. The residue was purified by column chromatography using EtOAc/hexane (40–60%) to give **10** (0.43g, 94%) as a white foam: R_f 0.23 (EtOAc/hexane 1:1); mp 53–56 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.66 (m, 4 H), 7.44 (m, 2 H), 7.37 (m, 4 H), 7.28 (d, *J* = 1.0 Hz, 1 H), 5.44 (d, *J* = 9.0 Hz, 1 H), 4.91 (d, *J* = 3.5 Hz, 1 H), 4.74 (dd, *J* = 6.5, 4.5 Hz, 1 H), 4.65 (dd, *J* = 6.5, 3.5 Hz, 1 H), 4.37 (m, 1 H), 4.13 (dd, *J* = 7.5, 4.0 Hz, 1 H), 4.04 (m, 2 H), 3.98 (dd, *J* = 11.5, 3.5 Hz, 1 H), 3.84 (dd, *J* = 12.0, 4.5 Hz, 1 H), 3.65 (s, 3 H), 3.07 (s, 3 H), 2.12 (m, 2 H), 1.59 (s, 3 H), 1.45 (s, 9 H), 1.37 (s, 3 H), 1.06 (s, 9 H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 172.9, 162.0, 155.8, 151.6, 140.3, 135.7, 135.6, 133.7, 133.2, 130.2, 130.2, 128.1, 128.0, 114.4, 112.4, 85.6, 85.0, 81.2, 80.9, 80.1, 64.1, 52.5, 51.7, 37.9, 37.0, 29.7, 28.6, 27.8, 27.1, 25.8, 19.6; ESI-MS (ES^+) m/z calcd for $C_{39}H_{53}N_3O_{10}Si$ 751.4, found 774.4 ($M+Na^+$); HRMS calcd for $C_{39}H_{53}N_3O_{10}SiNa^+$ $(M+Na)^+$ 774.3392, found 774.3424.

1-Methyl-3-(3-amino-3-carboxypropyl)pseudouridine (11, m^{1}acp^{3}Ψ)—To a solution of compound **10** (0.48 g, 0.64 mmol) in dioxane (3 mL) was added 0.67 M (aq) NaOH (2.37 mL, 1.59 mL). The reaction was stirred for 0.5 h at rt. The mixture was diluted with water (15 mL) and acidified with 10% HCl (aq). The aqueous solution was extracted with ethyl acetate (3 × 50 mL). The combined organic extracts were dried over Na_2SO_4 and concentrated under reduced pressure to give a colorless oil. The remaining protecting groups were removed using TFA/H_2O solution to give compound **11** (0.21 g, 92%) as a white solid: mp 205–208 °C; IR (KBr) ν 3184, 1700, 1661, 1624, 1460, 1105 cm^{-1}; ^{1}H NMR (500 MHz, D_2O) δ 7.61 (s, 1H), 4.57 (d, *J* = 5.5 Hz, 1 H), 4.13 (t, *J* = 5.0 Hz, 1 H), 4.00 (t, *J* = 6.5 Hz, 1 H), 3.95 (m, 2 H), 3.87 (m, 1 H), 3.73 (dd, *J* = 13.0, 3.5 Hz, 1 H), 3.61 (m, 2 H), 3.27 (s, 3 H), 2.10 (m, 2 H); ^{13}C NMR (500 MHz, D_2O) δ 173.0, 164.1, 152.7, 144.4, 110.2, 83.2, 79.5, 73.7, 70.7, 61.4, 52.0, 37.6, 37.3, 27.8; ESI-MS (ES^+) m/z calcd for $C_{14}H_{21}N_3O_8$ 359.13, found 360.13 ($M+H^+$), 382.09 ($M+Na^+$); Anal. Calcd for $C_{14}H_{21}N_3O_8$: C, 46.80; H, 5.89; N, 11.69; O, 35.62. Found: C, 44.66; H, 5.68; N, 10.08; O, 35.08.

1-*O*-(*tert*-Butyldimethylsilyl)-butane-4-ol (12) and 1-*O*-(dimethoxytrityl)-butane-4-ol (30)—Compounds **12** and **30** were prepared using excess amount of 1,4-butanediol relative to the protective groups (Supplement).

Compounds 13, 18, 23, and 27—The procedure for generating compounds **13**, **18**, **23**, and **27** was the same as for **10** using the appropriate alcohol derivative and nucleoside **7** or **17** (Supplement).

1-Methyl-3-[4-hydroxybutyl]-2′,3′-*O*-(isopropylidene)pseudouridine (14)—To a solution of compound **13** (0.20 g, 0.28 mmol) in dry THF (10 mL) was added tetrabutylammonium fluoride (1.0 M solution in THF, 0.61 mL, 0.61 mmol) at rt under argon. The reaction mixture was stirred for 2.5 h. The solvent was removed under reduced pressure and the crude product was purified by column chromatography using methanol/methylene chloride (0–20%) to give **14** (91 mg, 89%) as a colorless oil: R_f 0.38 (MeOH/CH_2Cl_2 1:9); ^{1}H NMR (400 MHz, CD_3OD) δ 7.70 (s, 1 H), 4.72 (m, 3 H), 4.04 (m, 1 H), 3.94 (m, 2 H), 3.75 (m, 1 H), 3.65 (dd, *J* = 12.0, 4.8 Hz, 1 H), 3.56 (t, *J* = 6.4 Hz, 2 H), 3.38 (s, 3 H), 1.66 (m, 2 H), 1.55 (m, 5 H), 1.32 (s, 3 H); ^{13}C NMR (400 MHz, CD_3OD) δ 162.8, 151.8, 143.1, 114.0, 110.7, 85.2, 84.4, 82.0, 81.8, 62.2, 61.4, 40.9, 36.1, 29.8, 26.7, 24.6, 24.0; ESI-MS (ES^+) m/z calcd for $C_{17}H_{26}N_2O_7$ 370.2, found 393.4 ($M+Na^+$); HRMS calcd for $C_{17}H_{26}N_2O_7$ (M^+) 370.1740, found 370.1754.

1-Methyl-3-(4-hydroxybutyl)pseudouridine, (15, m^{1}hb^{3}Ψ)—To a solution of compound **14** (86 mg, 0.23 mmol) in H_2O/acetone (9:1, 7 mL) was added TFA (3 mL) and stirred for 2 h. The solvent was evaporated under reduced pressure in a hot water bath and coevaporated with toluene. The residue was purified by column chromatography using MeOH/methylene chloride (10–30%) to give **15** (76 mg, 99%) as a light yellow oil: R_f 0.44 (MeOH/CH_2Cl_2 1:4); ^{1}H NMR (500 MHz, CD_3OD) δ 7.72 (s, 1 H), 5.62 (d, *J* = 5.0 Hz, 1 H), 4.14 (m, 1 H), 4.07 (t, *J* = 5.0 Hz, 1 H), 3.93 (m, 3 H), 3.81 (dd, *J* = 12.5, 3.0 Hz, 1 H), 3.67 (dd, *J* = 12.0, 3.5 Hz, 1 H), 3.56 (t, *J* = 6.0 Hz, 2 H), 3.38 (s, 3 H), 1.66 (m, 2 H), 1.55 (m, 2 H); ^{13}C NMR (500 MHz, CD_3OD) δ 163.2, 151.8, 142.9, 111.0, 84.2, 80.5, 74.4, 71.2, 61.8, 61.4, 40.9, 36.1, 29.8, 24.0; ESI-MS (ES^+) m/z calcd for $C_{14}H_{22}N_2O_7$ 330.1, found 353.3 ($M+Na^+$); HRMS calcd for $C_{14}H_{22}N_2O_7$ (M^+) 330.1427, found 330.1436.

2′,3′-*O*-(Isopropylidene)uridine (16) and 5′-*O*-(*tert*-Butyldiphenylsilyl)-2′,3′-*O*-(isopropylidene)uridine (17)—Compounds **16** and **17** were generated according to literature procedures.[26]

3-(3-Amino-3-carboxypropyl)uridine (19, acp^{3}U)—The same procedure for compound **11** was employed for compound **18**.

5-*O*-(*tert*-Butyldiphenylsilyl)-1-*O*-benzoyl-(*S*)-2-*N*-(*tert*-butoxycarbonyl)-pentane (21)—To a solution of compound **20** (0.51 g, 1.12 mmol) in pyridine (6 mL) was added benzoyl chloride (0.16 mL, 1.34 mmol) and stirred for 16 h at rt. The solvent was evaporated under reduced pressure in a hot water bath. The residue was taken up by ethyl acetate (50 mL) and water (10 mL). The organic layer was washed with brine and dried over Na_2SO_4. The solvent was evaporated to yield crude product that was purified by column chromatography using ethyl acetate/hexane (10–25%) to give **21** (0.61 g, 98%) as a colorless oil: R_f 0.34 (EtOAc/hexane 1:4); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.05 (m, 2 H), 7.66 (m, 4 H), 7.56 (m, 1 H), 7.40 (m, 8 H), 4.64 (d, *J* = 9.0 Hz, 1 H), 4.32 (m, 2 H), 4.00 (m, 1 H), 3.70 (t, *J* = 6.0 Hz, 1 H), 1.68 (m, 4 H), 1.42 (s, 9 H), 1.04 (s, 9 H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 166.7, 155.7, 135.8, 134.0, 133.3, 130.2, 129.9, 129.9, 128.6, 127.9, 79.7, 67.1, 63.6, 49.9, 29.11, 28.6, 27.1, 19.43; ESI-MS (ES^+) m/z calcd for $C_{33}H_{43}NO_5Si$ 561.3, found 584.2 (M

+Na^+), 600.2 (M+K^+); HRMS calcd for $C_{29}H_{34}NO_4Si$ (M^+–C_4H_9O) 488.2257, found 488.2267.

3-[5-Hydroxy-(*S*)-4-*N*-(*tert*-butoxycarbonyl)-amino-pentyl]-5′-*O*-(*tert*-butyldiphenylsilyl)-2′,3′-*O*-(isopropylidene)uridine (24)—To a solution of compound **23** (570 mg, 0.688 mmol) in anhydrous methanol (5 mL) was added sodium methoxide (1.0 M solution in MeOH, 1.38 mL, 1.38 mmol) and stirred for 16 h at rt. The solvent was removed under reduced pressure. The residue was purified by column chromatography using MeOH/ CH_2Cl_2 (1–10%) to give **24** (420 mg, 84%) as a white foam: R_f 0.58 (MeOH/ CH_2Cl_2 1:9); mp 66–69 °C; ^{1}H NMR (500 MHz, CD_3OD) δ 7.67 (m, 5 H), 7.39 (m, 6 H), 5.82 (d, *J* = 2.0 Hz, 1 H), 5.53 (d, *J* = 8.0 Hz, 1 H), 4.91 (dd, *J* = 6.5, 2.0 Hz, 1 H), 4.78 (dd, *J* = 6.0, 3.5 Hz, 1 H), 4.27 (m, 1 H), 3.95 (dd, *J* = 11.5, 3.5 Hz, 1 H), 3.81 (m, 3 H), 3.45 (m, 3 H), 3.30 (m, 1 H), 1.57 (m, 6 H), 1.42 (s, 9 H), 1.32 (s, 3 H), 1.04 (s, 9 H); ^{13}C NMR (500 MHz, CD_3OD) δ 163.7, 157.1, 150.8, 140.8, 135.6, 135.5, 133.2, 132.8, 130.0, 130.0, 127.8, 127.8, 113.8, 100.9, 94.4, 88.0, 85.1, 81.0, 78.8, 64.4, 64.2, 52.4, 52.3, 40.8, 28.5, 27.7, 26.4, 26.3, 24.5, 24.1, 21.2, 18.9; ESI-MS (ES^+) m/z calcd for $C_{38}H_{53}N_3O_9Si$ 723.4, found 762.2 (M+K^+); HRMS calcd for $C_{37}H_{50}N_3O_9Si$ 708.3316, found 708.3294.

3-[(*S*)-4-*N*-(*tert*-Butoxycarbonyl)-amino-4-carboxybutyl]-5′-*O*-(*tert*-butyldiphenylsilyl)-2′,3′-*O*-(isopropylidene)uridine (25)—To a solution of compound **24** (392 mg, 0.54 mmol) in anhydrous *N*,*N*-dimethylformamide (10 mL) was added pyridinium dichromate (1.22 g, 3.25 mmol) and stirred for 20 h at rt. Water (50 mL) was added to the reaction mixture and then extracted with ethyl acetate (3 × 50 mL). The combined organic layers were dried over Na_2SO_4. The solvent was evaporated under reduced pressure. The residue was purified by column chromatography using MeOH/CH_2Cl_2 (5–15%) to give **25** (370 mg, 94%) as a colorless oil: R_f 0.22 (MeOH/ CH_2Cl_2 1:9); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.61 (m, 5 H), 7.42 (m, 6 H), 5.92 (m, 1 H), 5.53 (d, *J* = 8.4 Hz, 1 H), 5.12 (br s, 1 H), 4.75 (s, 2 H), 4.36 (br s, 1 H), 4.30 (m, 1 H), 3.96 (m, 3 H), 3.82 (dd, *J* = 12.0, 4.0 Hz, 1 H), 1.70 (m, 4 H), 1.58 (s, 3 H), 1.44 (s, 9 H), 1.35 (s, 3 H), 1.06 (s, 9 H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 163.2, 150.9, 138.7, 135.8, 135.6, 132.9, 132.5, 130.4. 130.4, 128.2, 128.2, 114.4, 102.0, 93.2, 86.9, 85.7, 80.5, 64.2, 40.7, 29.9, 28.6, 27.5, 27.2, 25.6, 23.8, 19.5; ESI-MS (ES^+) m/z calcd for $C_{38}H_{51}N_3O_{10}Si$ 737.3, found 776.2 (M+K^+).

3-(4-Amino-4-carboxybutyl)uridine, TFA Salt (26, acb^{3}U)—The protective groups of compound **25** were removed using TFA/H_2O solution (Supplement).

3-(4-Hydroxybutyl)-2′,3′-*O*-(isopropylidene)uridine (28) and 3-(4-Hydroxybutyl) uridine (29, hb^{3}U)—The procedures were the same as for compounds **14** and **15**.

3-(Butan-4-ol)-5′-*O*-(*tert*-butyldiphenylsilyl)-2′,3′-*O*-(isopropylidene)uridine (31)—Diisopropyl azodicarboxylate (0.26 mL, 1.33 mmol) was added to a stirred solution of **17** (0.53 g, 1.02 mmol), **30** (0.40 g, 1.02 mmol), and triphenylphosphine (0.240 g, 1.22 mmol) in anhydrous THF (15 mL) at rt under argon. The reaction was stirred for 1 h. The solvent was removed under reduced pressure. The residue was dissolved in EtOAc (100 mL) and washed with saturated solution of $NaHCO_3$ and brine. The organic layer was dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure and the residue was dissolved in chloroform (10 mL). TFA (3 mL) was added to the solution dropwise and stirred for 30 min. The orange-red solution was evaporated to dryness and the residue was purified by column chromatography using ethyl acetate/hexane (50–70%) to give **31** (0.31 g, 51%) as a colorless oil: R_f 0.24 (EtOAc/hexane 3:7); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60 (m, 4 H), 7.55 (d, *J* = 8.0 Hz, 1 H), 7.40 (m, 6 H), 5.92 (d, *J* = 2.4 Hz, 1 H), 5.50 (d, *J* = 8.0 Hz, 1 H), 4.74 (m, 2 H), 4.29 (dd, *J* = 6.4, 2.4 Hz, 1 H), 3.93 (m, 3 H), 3.80 (dd, *J* = 11.2, 4.0 Hz, 1 H), 3.66 (t, *J* = 6.4 Hz,

2 H), 1.62 (m, 7 H), 1.33 (s, 3 H), 1.05 (s, 9 H), ^{13}C NMR (400 MHz, $CDCl_3$) δ162.9, 151.0, 138.5, 135.8, 135.6, 132.9, 132.5, 130.4, 130.3, 128.2, 128.2, 114.4, 102.1, 93.2, 86.9, 85.7, 80.6, 64.2, 62.7, 40.9, 29.9, 27.5, 27.2, 25.6, 24.2, 19.5; ESI-MS (ES^+) m/z calcd for $C_{32}H_{42}N_2O_7Si$ 594.3, found 617.2 ($M+Na^+$), 633.1 ($M+K^+$); HRMS calcd for $C_{31}H_{39}N_2O_7Si$ (M^+–CH3) 579.2527, found 579.2537.

3-(3-Carboxypropyl)-5′-*O*-(*tert*-butyldiphenylsilyl)-2′,3′-*O*-(isopropylidene) uridine (32) and 3-(3-Carboxypropyl)uridine (33, cp^{3}U)—The procedures are the same as for compounds **25** and **26**.

CD and NMR Studies of Modified Nucleosides

Sample Preparation—Each modified nucleoside was dissolved in ddH_2O for circular dichroism studies. Their concentrations were determined using Beer-Lambert's law: $A = \varepsilon \cdot C \cdot \lambda$, in which λ is the pathlength of the cuvette. The extinction coefficient used for pseudouridine nucleosides Ψ, $m^1\Psi$, Ψm, $m^1acp^3\Psi$, and $m^1hb^3\Psi$ is 1.09×10^4 $cm^{-1}M^{-1}$ at 260 nm. The extinction coefficient used for uridine nucleosides U, acp^3U, acb^3U, cp^3U, and hb^3U is 1.33×10^4 $cm^{-1}M^{-1}$ at 260 nm. The molar ellipticity was normalized using equation $\Delta\varepsilon = \theta/(32.98 \times C)$, in which θ is the CD absorbance of each nucleoside. The modified nucleosides were dissolved in deuterium oxide (0.25 M concentrations) for NMR experiments.

Circular Dichroism and NMR Spectroscopy—CD spectra were recorded on a Chirascan circular dichroism spectrometer equipped with a water bath to control the temperature at 25 °C. The molar ellipticities were normalized from a concentration of 10 mM for each nucleoside. 1D NMR NOE spectra were recorded on a 500 MHz NMR spectrometer (Varian-500S). The NMR experiments of pseudouridine derivatives were performed at room temperature. The uridine derivatives were done at 40 °C in order to avoid overlap of the H1′ and H5 peaks. 1D NOE data at 40 °C and room temperature show no significant changes; for example, the derivative acp^3U gave NOEs to H1′ (4.0%), H2′ (7.1%), and H3′ (2.9%) when H6 was irradiated at room temperature compared to H1′ (4.0%), H2′ (7.0%), and H3′ (2.7%) at 40 °C.

Supporting Information

Schemes for compounds **15, 17, 19, 29,** and **30**, experimentals for compounds **12, 13, 18, 19, 22, 23, 26–30, 32, 33** and NMR spectra for compounds **15, 26, 29**, and **33** are available.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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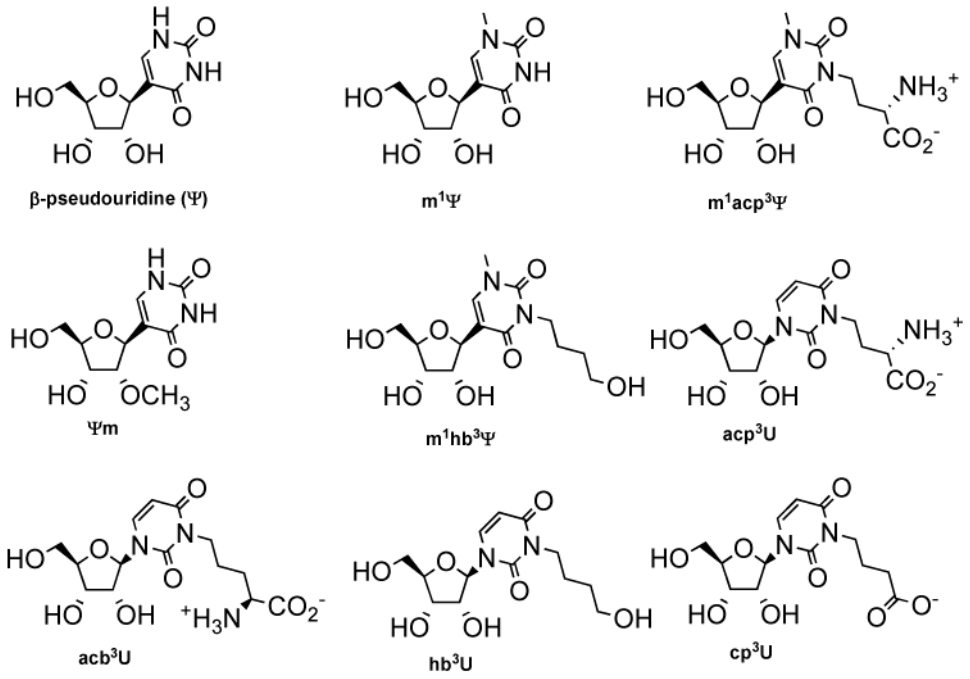


Figure 1.

The structures of pseudouridine (Ψ), 1-methylpseudouridine ($m^1\Psi$), 1-methyl-3-(3-amino-3-carboxypropyl)pseudouridine ($m^1acp^3\Psi$), 2′-*O*-methylpseudouridine (Ψm), 1-methyl-3-(4-hydroxybutyl)pseudouridine ($m^1hb^3\Psi$), 3-(3-amino-3-carboxypropyl)uridine (acp^3U), 3-(4-amino-4-carboxybutyl)uridine (acb^3U), 3-(4-hydroxybutyl)uridine (hb^3U), and 3-(3-carboxybutyl)uridine (cp^3U) are shown.

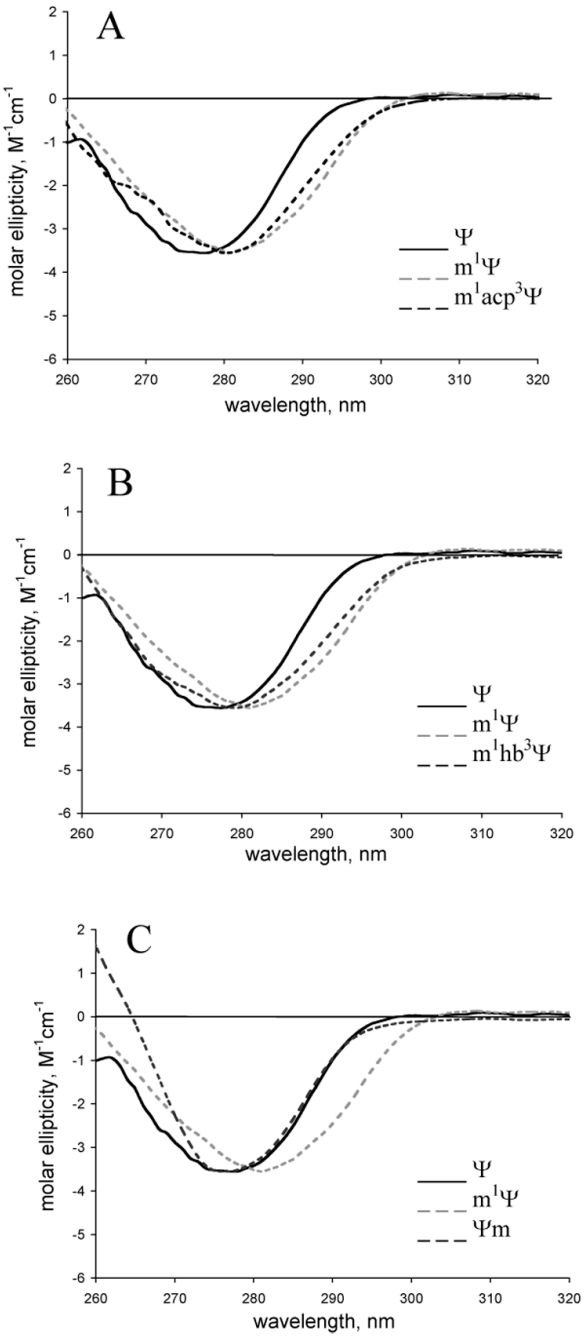


Figure 2.

CD spectra of $m^1acp^3\Psi$ (A), $m^1hb^3\Psi$ (B), and Ψm (C) (dashed black lines) in H_2O are compared with Ψ (solid lines) and $m^1\Psi$ (dashed grey lines) at room temperature. The molar ellipticities were normalized from concentrations of 10, 60, and 20 mM, respectively, based on an extinction coefficient of 1.09×10^4 cm^{-1} M^{-1} at 260 nm. Each curve represents the average of five scans.

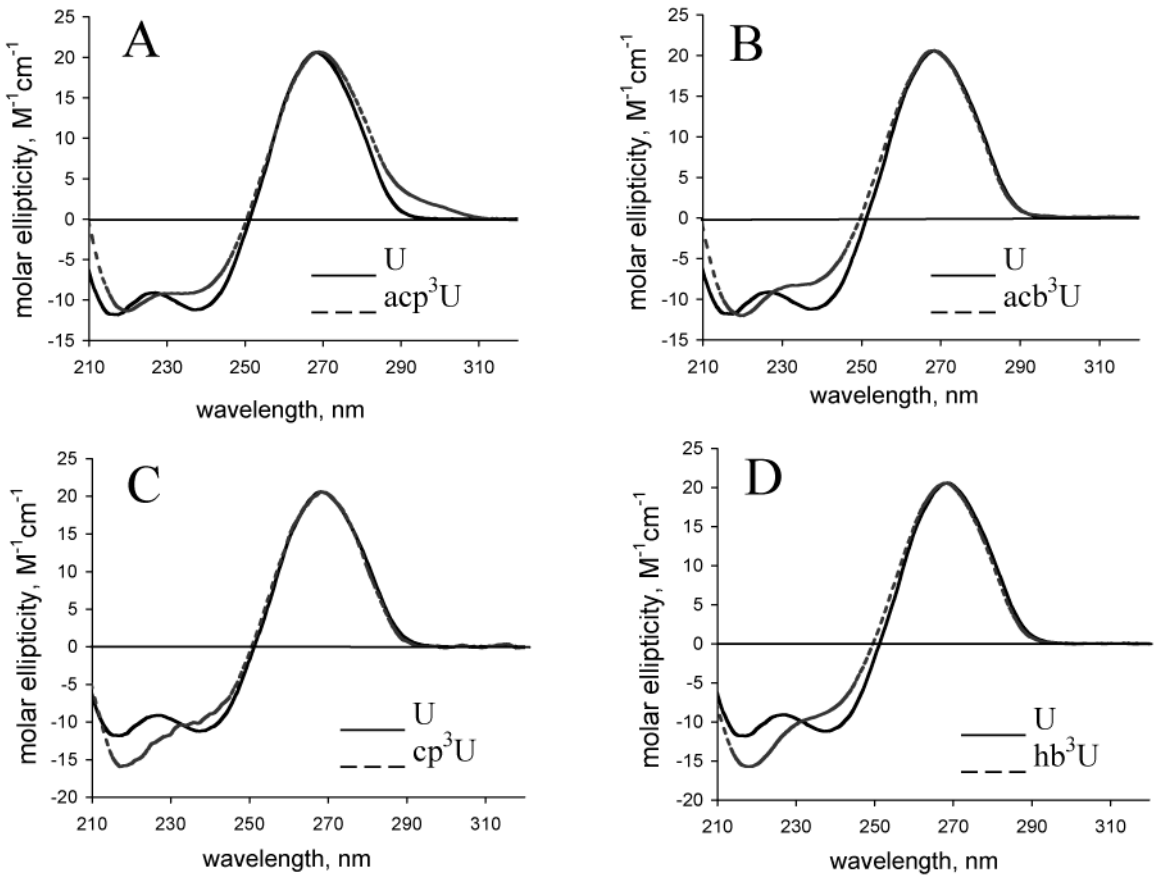


Figure 3.

CD spectra of acp^3U (A), acb^3U (B), cp^3U (C), and hb^3U (D) (dashed lines) in H_2O are compared with U (solid lines) at room temperature. The molar ellipticities were normalized from concentrations of 10, 14, 37, and 17 mM, respectively, based on an extinction coefficient of 1.33×10^4 cm^{-1} M^{-1} at 260 nm. Each curve represents the average of five scans.

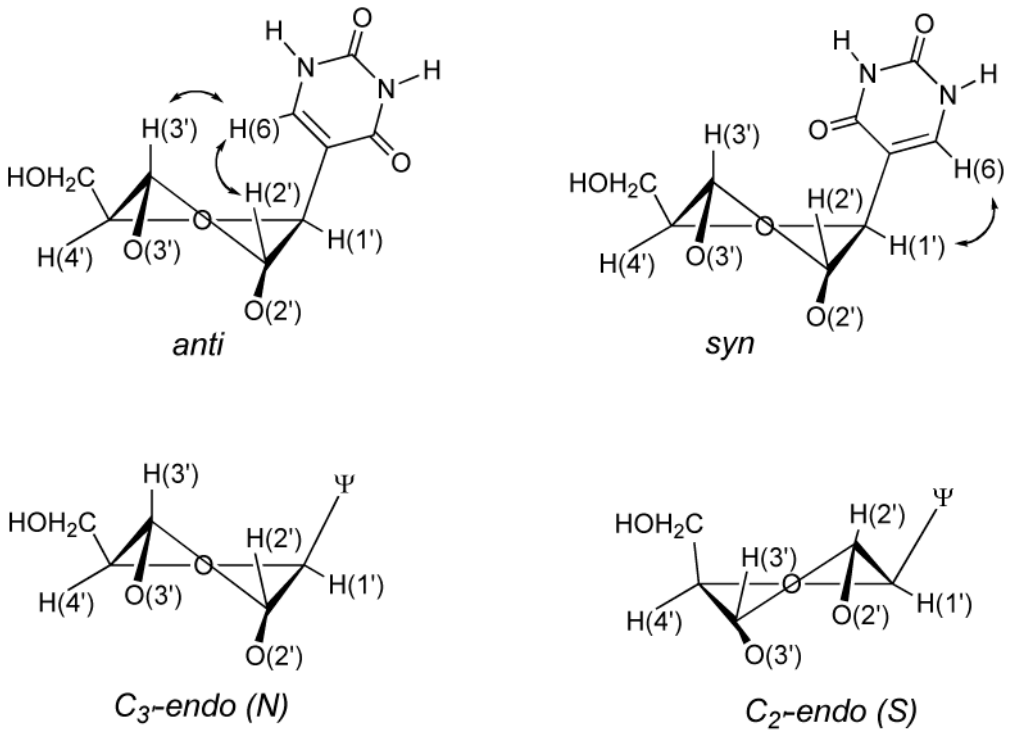
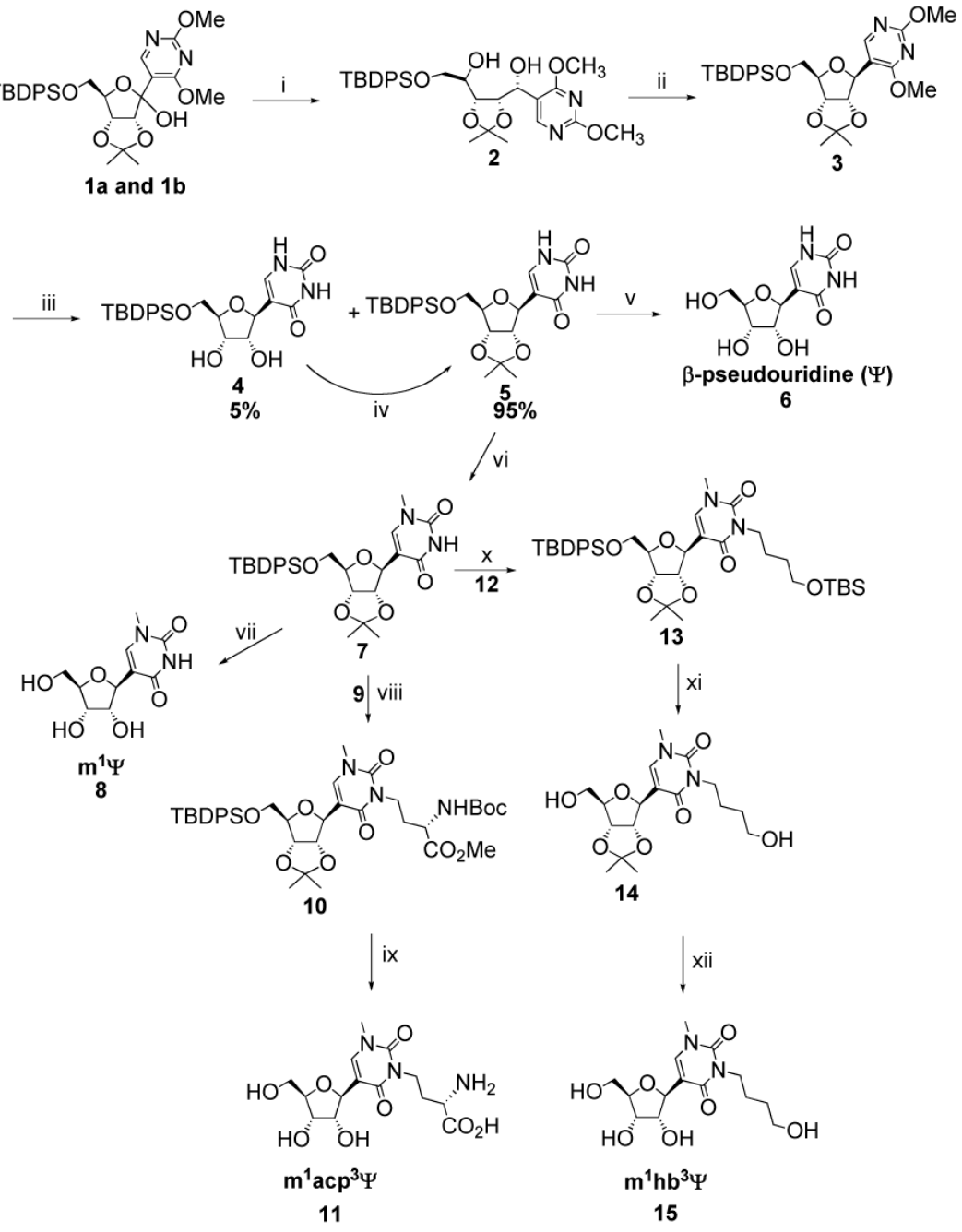
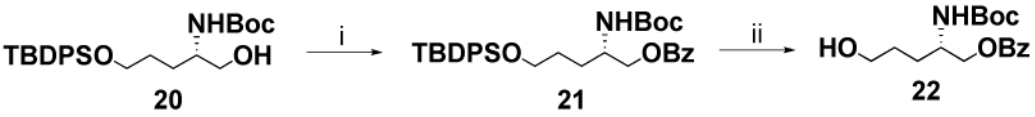


Figure 4.
The possible conformations (*anti* and *syn*; $C_{3'}$-*endo* (*N*) and $C_{2'}$-*endo* (*S*)) of pseudouridine, which can be determined by 1D NOE experiments, are represented.



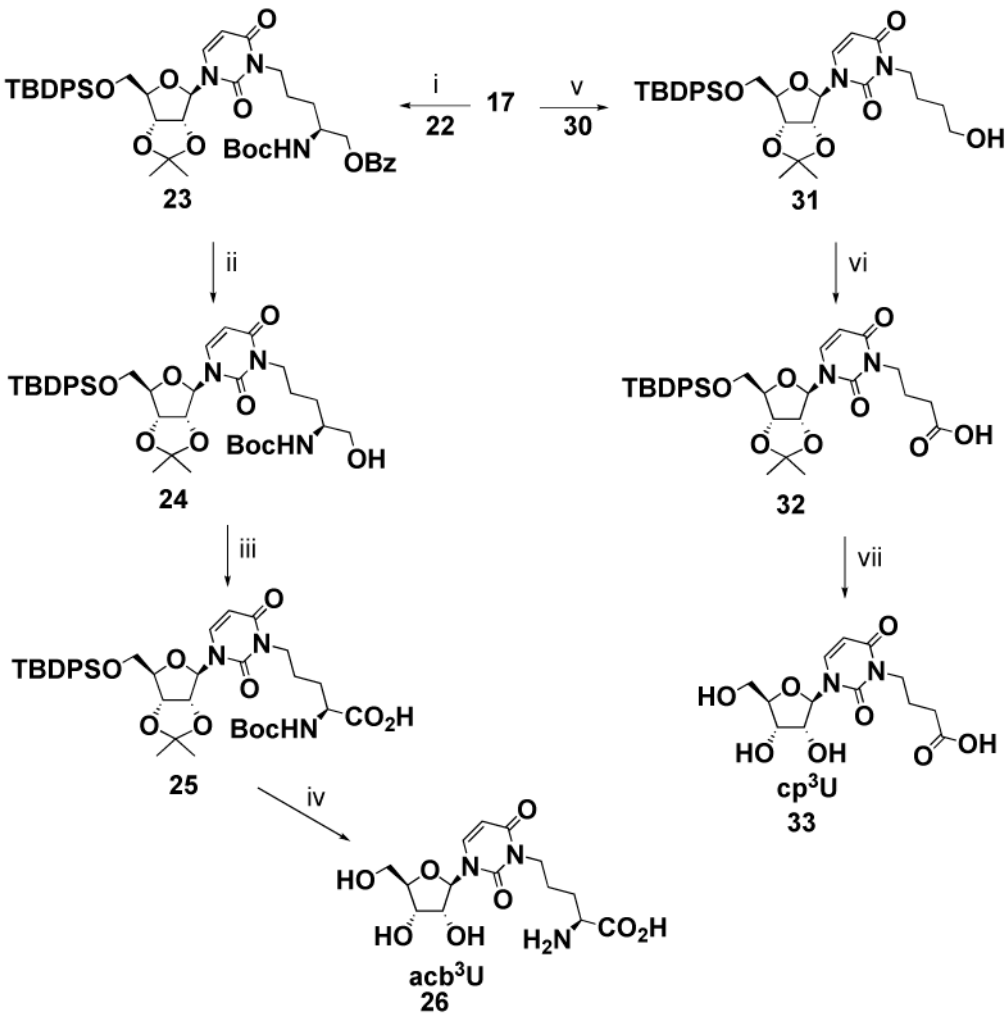
Scheme 1. Reagents and conditions

(i) $ZnCl_2$, L-Selectride, CH_2Cl_2, −72 °C, 19 h, 92% yield; (ii) DIAD, PPh_3, THF, 0 °C to rt, 19 h, 91% yield; (iii) CH_3COOH, NaI, reflux, 35 min; (iv) *conc.* H_2SO_4, acetone, rt, 1 h, 94%; (v) TFA/H_2O (9/1), 1 h, rt, 100%; (vi) a) BSA (2.5 eq), CH_2Cl_2, rt, 1 h; b) CH_3I (1.5 eq), reflux, 120 h, 80%; (vii) TFA/H_2O (9/1), 1 h, rt, 90%; (viii) **9**, DIAD, PPh_3, THF, rt, 1 h, 94%; (ix) a) 0.67 N $NaOH_{(aq)}$, dioxane, rt, 25 min; b) TFA/H_2O (9/1), 1 h, rt, 92% in two steps; (x) **12**, DIAD, PPh_3, THF, rt, 1 h, 77%; (xi) tetrabutylammonium fluoride, THF, rt, 2.5 h, 89%; (xii) TFA, H_2O/acetone (9/1, v/v), rt, 2 h, 99%.



Scheme 2. Reagents and conditions

(i) Benzoyl chloride, pyridine, rt, 16 h, 98%; (ii) tetrabutylammonium fluoride, THF, rt, 1.5 h, 83%.



Scheme 3. Reagents and conditions

(i) **22**, DIAD, PPh_3, THF, rt, 1 h, 96%; (ii) NaOMe, MeOH, rt, 16 h, 84%; (iii) PDC, DMF, rt, 20 h, 94%; (iv) TFA/H_2O (9/1), 1 h, rt, 94%; (v) **30**, DIAD, PPh_3, THF, rt, 1 h; b) TFA, CH_3Cl, rt, 30 min, 51% yield in two steps; (vi) PDC, DMF, rt, 20 h, 80%; (vii) TFA/H_2O (9/1), 2 h, rt, 90%.

Table 1

Irradiation and NOE data for pseudouridine at 25 °C and uridine derivatives at 40 °C

Compound	Proton irradiated	NOE (%)	Compound Proton	irradiated	NOE (%)
Ψ	H6	H1′ (5.8)	U	H6	H1′ (4.3)
syn		H2′ + H3′ (1.9)	*anti*		H2′ + H3′ (10.2)
	H1′	H6 (5.9)		H1′	H6 (4.5)
	H2′	H6 (2.2)		H2′	H6 (6.9)
	H3′	H6 (0.8)		H3′	H6 (2.5)
$m^1\Psi$	H6	H1′ (9.1)	acp^3U	H6	H1′ (4.0)
syn		H2′ + H3′ (2.4)	*anti*		H2′ + H3′ (9.7)
	H1′	H6 (7.6)		H1′	H6 (4.0)
	H2′	H6 (2.6)		H2′	H6 (6.7)
	H3′	H6 (2.1)		H3′	H6 (2.1)
$m^1acp^3\Psi$	H6	H1′ (6.5)	acb^3U	H6	H1′ (5.0)
syn		H2′ + H3′ (3.6)	*anti*		H2′ + H3′ (9.2)
	H1′	H6 (5.1)		H1′	H6 (4.9)
	H2′	H6 (2.2)		H2′	H6 (7.3)
	H3′	H6 (0.7)		H3′	H6 (2.1)
$m^1hb^3\Psi$	H6	H1′ (7.9)	hb^3U	H6	H1′ (4.4)
syn		H2′ + H3′ (4.3)	*anti*		H2′ + H3′ (8.1)
	H1′	H6 (5.5)		H1′	H6 (4.0)
	H2′	H6 (2.5)		H2′	H6 (5.5)
	H3′	H6 (0.5)		H3′	H6 (1.9)
Ψm	H6	H1′ (3.5)	cp^3U	H6	H1′ (5.2)
syn		H2′ + H3′ (1.2)	*anti*		H2′ + H3′ (7.5)
	H1′	H6 (1.4)		H1′	H6 (4.8)
	H2′	ND		H2′	H6 (6.5)
	H3′	ND		H3′	H6 (2.1)

ND: not detectable

Table 2

^{1}H-^{1}H coupling constants for pseudouridine and uridine derivatives at 25 °C

Compound'	$J_{1',2'}$	$J_{2',3'}$	$J_{3',4'}$	$J_{4',5'}$	$J_{4',5''}$
$m^{1}\Psi$	5.6	5.2	4.8	3.2	4.4
$m^{1}acp^{3}\Psi$	5.5	5.0	5.0	3.0	3.5
$m^{1}hb^{3}\Psi$	5.0	5.5	5.5	3.5	4.5
Ψm	6.4	5.2	4.8	3.2	4.4
$acp^{3}U$	4.0	5.5	6.0	3.0	4.5
$acb^{3}U$	4.5	5.5	5.5	3.0	4.5
$hb^{3}U$	4.0	6.0	5.5	3.0	4.5
$cp^{3}U$	4.0	5.5	5.5	3.0	4.5

Table 3

Percentages of $C_{3'}$-*endo* (N) versus $C_{2'}$-*endo* (S) conformers and equilibrium constants (N/S) for pseudouridine and uridine derivatives at 25 °C

Compound	% $C_{3'}$-*endo* (*N*)	% $C_{2'}$-*endo* (S)	K_{eq} (*N/S*)
Ψ[a]	48	52	0.9
$m^1\Psi$	46	54	0.9
$m^1acp^3\Psi$[a]	48	52	0.9
$m^1hb^3\Psi$	52	48	1.1
Ψm	43	57	0.8
U[b]	53	47	1.1
acp^3U[a]	60	40	1.5
acb^3U	55	45	1.2
hb^3U	58	42	1.4
cp^3U	58	42	1.4

*a)*refs. 35, 36