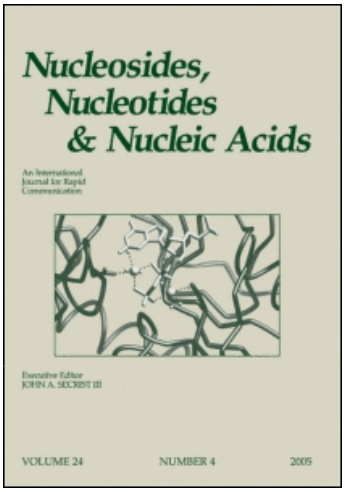

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Synthesis of Uridylyl (3'-5') Uridine Derivatives Containing 5-(Methylamino-Methyl) Uridine as A Modified Nucleoside Found from *E. COLI* Minor tRNA^{Arg}

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SYNTHESIS OF URIDYLYL (3'-5') URIDINE DERIVATIVES CONTAINING 5-(METHYLAMINO-METHYL)URIDINE AS A MODIFIED NUCLEOSIDE FOUND FROM *E. COLI* MINOR $tRNA^{Arg}$

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Abstract: 5-(Methylaminomethyl)uridine-containing uridylyl (3'-5') uridine derivatives (**14**, **26**, and **29**) , which were the original and modified sequences corresponding to the first letter (position 34) and the 5'-upper ribonucleoside (position 33) in the anticodon loop of minor $tRNA^{Arg}$, have been synthesized *via* 5-(methylaminomethyl)uridine derivatives (**4** and **24**).

INTRODUCTION

A number of modified ribonucleosides have been discovered in tRNAs.[1-3] Particularly, uridines located at the first position of anticodons are modified at their base residues with few exceptions[4] and such modifications have been elucidated to play an important role in codon-anticodon recognition.[5-9] As a typical modified uridine derivative, 5-(methylaminomethyl)-2-thiouridine (mnm^5s^2U)[10] was discovered as the first letter of the anticodons of *E. coli* $tRNA^{Gln}$,[11] $tRNA^{Lys}$,[12] and $tRNA^{Glu}$.[13] This modified nucleoside has been synthesized by several research groups.[14-16] Malkiewicz reported the synthesis of anticodon sequences having mnm^5s^2U.[17,18] ^1H NMR studies[6] of mnm^5s^2U revealed that it takes the "rigid" *C3'-endo* conformation capable of base paring with A and G, especially, more favorably with the former.[19] Quite recently, Malkiewicz has described the synthesis of a 2-thio uridylate derivative ($Upmnm^5s^2U$) and the oxidative conversion of $Upmnm^5s^2U$ to $Upmnm^5U$.[20]

A previous study on molecular modeling of tRNAs reported by Hillen[21] suggested that tRNAs containing mnm^5s^2U as the first letter could be conformationally stabilized due to an interresidue hydrogen bonding between the 5-methylaminomethyl group of mnm^5s^2U (position 34) and the 2'-hydroxyl group of uridine (position 33) at the 5'-upper side.

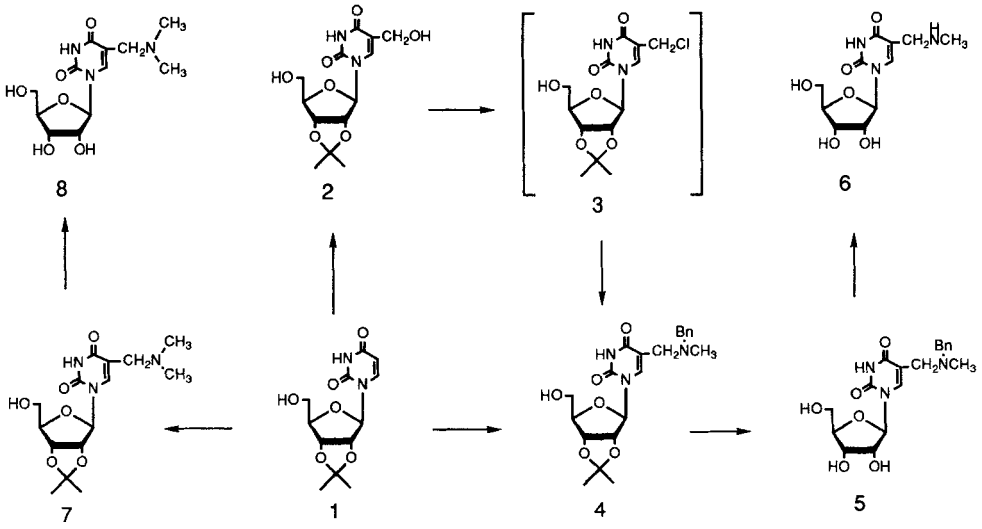
In connection with these precedent studies, Yokoyama[22] has recently disclosed that the first letter of the anticodon of minor $tRNA^{Arg}$ from *E. coli* was a "non-thiolated" uridine derivative, *i.e.*, 5-(methylaminomethyl)uridine (mnm^5U). Therefore, it becomes more important to understand how the 5-substituent and/or the 2-thiocarbonyl group work precisely for molecular recognition of nucleic acids.

In this paper, we wish to report the chemical synthesis of mnm^5U and three kinds of mnm^5U containing uridylates: Uridylyl (3'-5') 5-(methylaminomethyl)uridine ($Upmnm^5U$), its 5'-phosphorylated dimer ($pUpmnm^5U$), and 2'-*O*-methyluridylyl (3'-5') 5-(methylaminomethyl)uridine ($Umpmnm^5U$). The former two uridylates are the partial structures of *E. coli* minor $tRNA^{Arg}$ corresponding to the dimer sequence involving the first letter (position 34) and its 5'-neighboring nucleoside (position 33) in the anticodon region.[22] The last was synthesized in order to study the conformational effect of the 2'-*O*-methyl group, the presence of which has recently been found to stabilize the *C3'-endo* conformation in Um of UmpU.[23] These synthetic uridylyl (3'-5') uridine derivatives would be useful as substrates for conformational analysis by means of NMR spectroscopy to clarify the structure and function of the original and modified sequences in the anticodon loop as well as the artificial synthesis of recombinant tRNA molecules.

RESULTS AND DISCUSSION

Synthesis of 5-(methylaminomethyl)uridine (6: mnm^5U) and 5-(dimethylaminomethyl)uridine (8)

For introduction of the (methylamino)methyl group to position 5 of uridine we employed 5-hydroxymethyl-2',3'-*O*-isopropylideneuridine (**2**) which was easily prepared by hydroxymethylation of 2',3'-*O*-isopropylideneuridine (**1**).[24] We have recently found that **2** could be converted to a 5-(chloromethyl)uridine derivative (**3**)[26] by treatment with 5 equiv of chlorotrimethylsilane in dioxane at 60 °C for 3 h.[26] The compound **2** thus obtained was allowed to react *in situ* with 2 equiv each of *N*-benzylmethylamine and ethyldiisopropylamine in DMF at room temperature for 25 h. Consequently, this reaction gave 5-[(*N*-benzyl)methylaminomethyl]-2',3'-*O*-isopropylideneuridine (**4**) in 67% yield (Method 1). The pure material was treated with 80% acetic acid to remove the isopropylidine group. Reverse-phase column chromatography of the mixture gave two fractions. The faster eluted product was the acetate salt (20%) of 5-[(*N*-



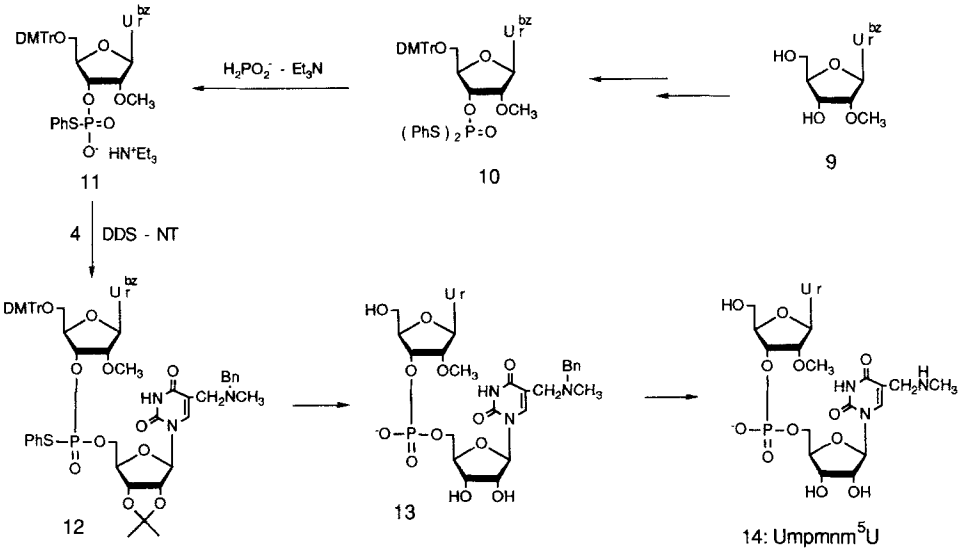
Scheme 1

benzyl)methylaminomethyl)uridine (**5**). The product obtained from the second fraction was the free 5-[(*N*-benzyl)methylaminomethyl]uridine (**5**) (40%). The total yield of **5** was 60%. Compound **4** could be also synthesized on a 3 mmol scale by the Mannich reaction (27) of **1** with *N*-benzylmethylamine and 37% aqueous formalin (Method 2). However, crude product **4** contaminated with some impurities was obtained in this case. The crude material **4** was similarly converted to **5** as the free form in 50% yield from **1** by pretreatment with sodium carbonate before reverse-phase column chromatography. Compound **5** thus obtained was treated with H_2 on Pd/C to afford the desired product (**6**: mnm^{5}U) in 52% yield. When the hydrogenolysis on Pd/C was performed prior to deprotection of the isopropylidene group, considerable side reactions such as hydrogenation of the 5,6-double bond and the N-C(5) bond cleavage were observed.

In connection with the present study, 5-(dimethylaminomethyl)uridine (**8**)[28] was also prepared. Although this compound has not yet been found from natural products involving tRNAs, it would be useful as reference material for comprehensive and physicochemical studies using a series of related compounds. The Mannich reaction of **1** with 5 equiv of dimethylamine and 37% aqueous formalin followed by removal of the isopropylidene group gave **8** as the acetate salt.

Synthesis of 2'-*O*-methyluridylyl (3'-5') 5-(methylaminomethyl)uridine (14)

The uridine building block (**10**) was prepared by the 5'-*O*-dimethoxytritylation of N^3-benzoyl-2'-*O*-methyluridine (**9**) followed by the successive 3'-phosphorylation using



Scheme 2

cyclohexylammonium *S,S*-diphenyl phosphorodithioate (PSS, 1.5 equiv) / isodurenedisulfonyl dichloride (DDS, 3 equiv) / tetrazole (Tet, 4 equiv).[29] The selective removal of one of the two phenylthio groups from **10** (0.08 mmol) by the action of pyridinium phosphinate in pyridine-triethylamine (1.1 : 0.5, v/v, 2.6 ml) gave quantitatively the *O,S*-diester (**11**) as the triethylammonium salt.[30] The *O,S*-diester was further condensed with **4** in the presence of DDS and 1,2,4-1*H*-nitrotriazole (NT) to afford the fully protected dimer (**12**) in 78% yield. The *N*-benzyl dimer derivative (**13**) was obtained by the following successive treatments of **12**: 1) 0.75 M $Bu_3SnOSnBu_3$[31] (15 equiv) in pyridine at room temperature for 1.5 h; 2) Me_3SiCl (30 equiv) at room temperature for 5 min; 3) concentrated ammonia-pyridine (1 : 1, v/v) at 60 °C for 3 h; 4) 80% acetic acid at 100 °C for 3 h; 5) paper chromatogaphy using iPrOH-concentrated ammonia-H_2O (7 : 1 : 2, v/v/v). The purified material **13** was hydrogenated on 10% Pd/C to give 2'-*O*-methyluridylyl (3'-5') 5-(methylaminomethyl)uridine (**14**: Umpmnm^{5}U) which was obtained in 16% yield by paper chromatography. The dimer was further purified by reverse-phase HPLC. The low yield of **14** was due to mainly to the competitive C-N bond fission and the hydrogenation of the 5,6-double bonds. The structure of **14** was confirmed by 500 MHz ^{1}H NMR spectroscopy.

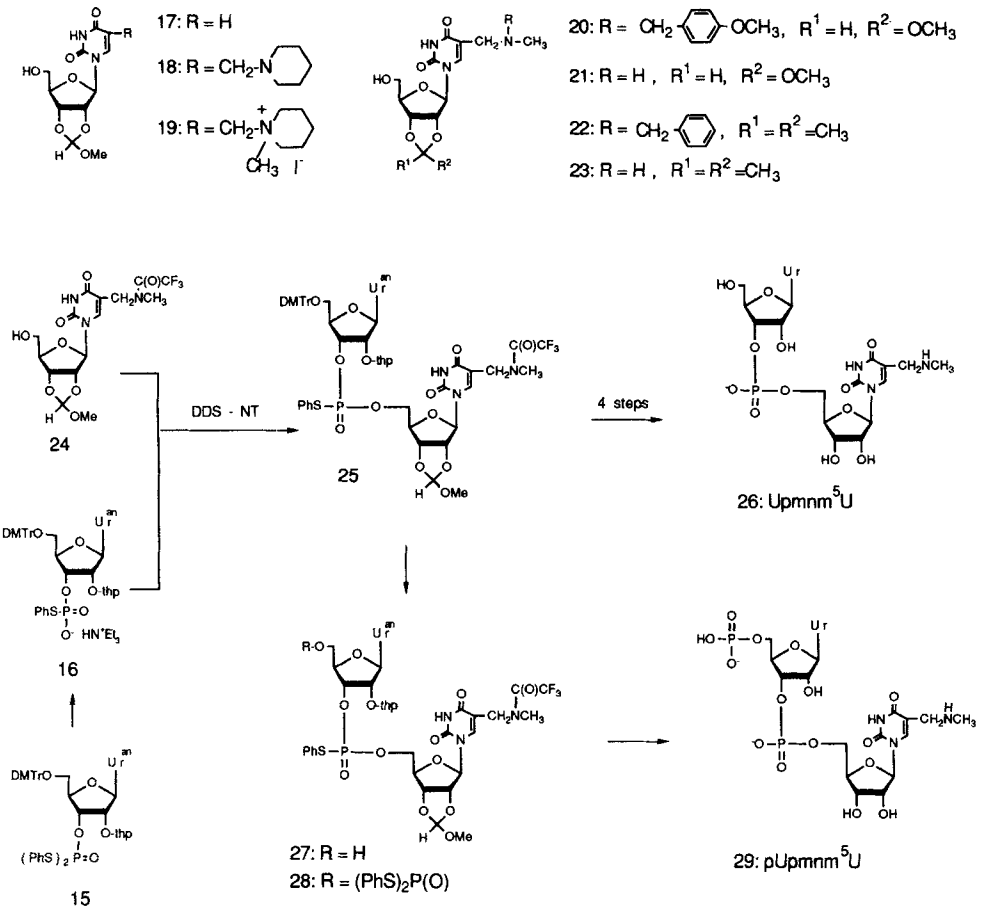
Synthesis of uridylyl (3'-5') 5-(methylaminomethyl)uridine (26: Upmnm^{5}U) and its 5'-phosphorylated derivative (29: pUpmnm^{5}U)

The 2'-unsubstituted derivative, Upmnm^{5}U (**26**) was synthesized by the use of *S,S*-diphenyl 5'-*O*-(4,4'-dimethoxytrityl)-2'-*O*-(tetrahydro-2*H*-pyran-2-yl)-*N*^{3}-anisoyluridine 3'-phosphorodithioate (**15**)[32] and 2',3'-*O*-methoxymethylene-5-[(*N*-trifluoroacetyl)-methylaminomethyl]uridine (**24**) as the 5'- and 3' terminal uridine blocks, respectively. The latter had a base-labile*N*-trifluoroacetyl group at the 5-substituent, since the palladium-catalyzed hydrogenolysis of the *N*-benzyl group at the last step of the synthesis of **14** caused simultaneous saturation of the 5,6-double bond. The trifluoroacetyl group has been originally used by Malkiewicz for the synthesis of oligoribonucleotides containing 2-thio-5-(methylaminomethyl)uridine.[17,18] In the present study, we have developed a new effective method for debenzylation which was required for the preparation of **24** from 2',3'-*O*-methoxymethyleneuridine (**17**).

The Mannich reaction of **17** with piperidine in the presence of 37% formalin gave the 5-(piperidinomethyl)uridine derivative (**18**), which was *in situ* converted to the quaternary amine derivative (**19**) by treatment with methyl iodide.[33,34] This salt was in turn allowed to react with *N*-(4-methoxybenzyl)methylamine to give the 5-substituted product (**20**) in an overall yield of 47% from **17**.

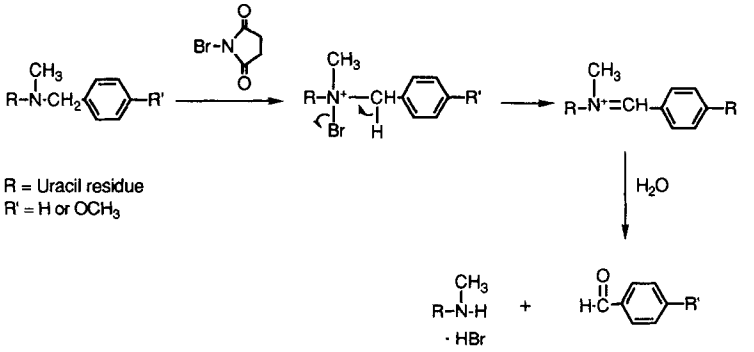
When **20** was subjected to hydrogenolysis on Pd/C to remove selectively the 4-methoxybenzyl group, a nonselective reaction was also observed giving a complex mixture. After several screenings of reagents for the selective debenzylation, it was ultimately found that *N*-bromosuccinimide (NBS) was effective as a reagent for conversion of **20** to the 5-methylaminomethyluridine derivative (**21**). This idea arose from consideration based on the fact that tertiary amines such as trimethylamine was converted to secondary amines by treatment with bromine.[35] The use of 2 equiv of NBS in dioxane-water (4 : 3, v/v) gave **21** as the main product in 56% yield. Under these conditions, the methoxymethylene group was found to be stable. This procedure proved to be also effective for the debenzylation from a similar but *N*-benzyl group-containing compound, 2',3'-*O*-isopropylidene-5-(*N*-benzylaminomethyl)uridine (**22**) to give the debenzylated product **23** in 46% yield.

A number of procedures for dealkylation of tertiary amines have been reported up to date.[35,36] Most of them, however, involved the use of peracids,[36] acyl halides,[36] and alkoxycarbonyl chlorides,[36-39] which could not be applied to the debenzylation of **20** and **22** because of their drastic conditions or undesirable side reactions such as 5'-acylations and oxidation. Generally, much difficulty in removing the benzyl group from aliphatic tertiary benzylamines (BnNR^{1}R^2: R^1, R^2 = aliphatic groups) like our case has been



Scheme 3

encountered when debenzylation methods other than hydrogenolysis were employed.[40] This is rationalized in terms of the extremely poor leaving ability of the dialkylamino group. To our knowledge, alternative methods superior to the present NBS procedure have not been described except for the usual hydrogenolysis which was ineffective in our study. Therefore, it should be emphasized that the new procedure described here would be generally useful for debenzylation of secondary amines masked with the benzyl or 4-methoxybenzyl group. We propose that the present debenzylation proceeds *via N*-bromination followed by formation of an iminium salt intermediate which is easily hydrolized to give a secondary amine hydrobromide and an aldehyde, as shown in Scheme 4.



Scheme 4

Full acylation of **21** with trifluoroacetic anhydride (6 equiv) in pyridine followed by partial hydrolysis by treatment with aqueous 10% sodium carbonate solution[17] afforded the 5'-free 3'-terminal uridine component (**24**) in 53% yield. Condensation of **24** with the *O,S*-phosphodiester uridine unit (**16**) in the presence of DDS and NT gave the fully protected dimer (**25**) in 83% yield. Upmnm^{5}U (**26**) was finally obtained in 54% yield by full deprotection of this material: 1) $Bu_3SnOSnBu_3$ (15 equiv / one PhS group) in pyridine at room temperature for 2 h; 2) Me_3SiCl (30 equiv) in pyridine at room temperature for 5 min; 3) concentrated ammonia-pyridine (3:1, v/v) at 60 °C for 24 h; 4) 80% acetic acid at room temperature for 5 h .

For incorporation of synthetic Upmnm^{5}U (**26**) into tRNAs at the first letter and 5'-neighboring site to study the structure and function of modified tRNAs, we needed 5'-phosphorylated pUpmnm^{5}U (**29**), which could be utilized as a donor in ligation catalyzed by T4 RNA ligase. Treatment of the fully protected uridylate dimer (**25**) with 0.5% trifluoroacetic acid in CH_2Cl_2 for 60 min gave the 5'-free product (**27**) which was obtained in 97% yield and further phosphorylated in the usual way using PSS/DDS/Tet as mentioned previously to give the product (**28**) in 47% yield. This compound was deblocked and purified by HPLC to afford pUpmnm^{5}U (**29**) in the usual manner: 1) Silver acetate (50 equiv/one PhS) in pyridine at room temperature for 1.5 h; 2) concentrated ammonia-pyridine (1:1, v/v) at r. t. for 18 h; 3) 0.01 M HCl at room temperature for 30 h. These synthetic dinucleotides were characterized by 270 MHz ^{1}H NMR as well as 500 ^{1}H NMR.

Preliminary studies exhibited that the ribose moiety of the 3'-terminal mnm^{5}U residue dominantly takes the ordinary *C3'-endo* form. It has been reported that nucleoside mnm^{5}U itself does not exhibit the preference to the *C3'-endo* form.[41] Therefore, this

preliminary result suggests that the 5'-terminal uridylyl group contributes to the stabilization of the *C3'-endo* form of the mnm^{5}U residue. Full details of NMR studies will be shortly reported elsewhere.

EXPERIMENTAL

Melting points were determined on a Mitamura MELT-POINTER apparatus and are uncorrected. ^{1}H NMR spectra were recorded at 60 MHz on a Hitachi 24B spectrometer, at 100 MHz on a JEOL JNM PS-100 spectrometer, at 270 MHz on a JEOL EX270 spectrometer, at 400 MHz on a Bruker AM-400 spectrometer, and at 500 M Hz on a JEOL GX500 spectrometer with Me_4Si (for water-insoluble materials) or DSS (for water-soluble materials) as the external reference. UV spectra were obtained on a Hitachi 220A spectrophotometer. Paper chromatography was performed by use of a descending technique with Whatman 3MM papers and Toyo Roshi 51 papers using the following solvent system: 2-propanol-conc. aqueous ammonia-water, 7 : 1 : 2, v/v/v. Column chromatography was performed with silica gel C-200 purchased from Wako Co., Ltd., and a minipump for a goldfish bowl was conveniently used to attain sufficient pressure for rapid chromatographic separation. Reverse-phase column chromatography was performed by the use of mBondapak C-18 silica gel (Prep S-500, Waters). TLC was performed on precoated TLC plates of silica gel 60 F-254 (Merck). Reverse-phase HPLC was performed on a Waters Model A25 using a mBondasphere C-18 column with a linear gradient starting from 0.1 M NH_4OAc, pH 7.0 and applying CH_3CN at a flow rate of 1.0 ml/min for 30 min. Uridine was purchased from Yamasa Co., Ltd. Pyridine was distilled two times from *p*-toluenesulfonyl chloride and from calcium hydride and then stored over molecular sieves 3A. Elemental analyses were performed by the Microanalytical Laboratory, Tokyo Institute of Technology, at Nagatsuta.

2',3'-*O*-Isopropylidene-5-[(*N*-benzyl)methylamino]methyluridine (4). Method 1. Compound **2** (598 mg, 1.9 mmol) was dissolved in dioxane (10 ml) under argon atmosphere and chlorotrimethylsilane (1.2 ml, 9.5 mmol) was added. After being stirred at 60 °C for 3 h, the mixture was evaporated under reduced pressure. The residue was dissolved in DMF (5 ml), and benzylamine (0.49 ml, 3.8 mmol) and ethyldiisopropylamine (0.66 ml, 3.8 mmol) were successively added to the solution. After being stirred at room temperature for 2.5 h, the solution was evaporated under reduced pressure. The residue was chromatographed on a column of silica gel eluted with CH_2Cl_2-MeOH-pyridine (99.5 : 0 : 0.5 ~ 98 : 1.5 : 0.5, v/v/v) to give **4** (521 mg, 66%): ^{1}H NMR (60 MHz, $CDCl_3$) δ 1.35 and 1.57 (6H, s, isp), 2.21 (3 H, s, *N*-CH_3), 3.28 (2H, s, CH_2Ph), 3.53 (2H, s, *N*-CH_2), 4.45 (3H, m, 4',5'-H), 4.49 (2H, m, 2',3'-H), 5.54

(1H, d, J = 1.8 Hz, 1'-H), 7.18 (5H, s, Ph), 7.39 (1H, s, 6-H). Anal. Calcd for $C_{21}H_{27}N_3O_6$: C, 60.42; H, 6.52; N, 10.07. Found: C, 60.53; H, 6.73; N, 9.96.

Method 2. Compound **1** (0.85 g, 3 mmol) was dissolved in a mixture of ethanol (11 ml), water (10 ml), 35% formalin (2 ml, 28 mmol), and *N*-methylbenzylamine (3.9 ml, 30 mmol). The resulting mixture was refluxed for 44 h. Then the solution was cooled to room temperature and evaporated under reduced pressure. The excess formalin was removed by repeated coevaporations with water (X 5), pyridine (X 2), and toluene. The resulting foam was chromatographed by silica gel column chromatography to give crude **4** (1.39 g) which contained some impurities.

5-[(*N*-Benzyl)methylamino]methyluridine (5). Method 1. The compound **4** (334 mg, 0.8 mmol) obtained *via* Method 2 in the above experiment was heated with 80% acetic acid (60 ml) at 100 °C for 4 h. The mixture was evaporated under reduced pressure and repeated coevaporations with water were continued until the odor of acetic acid could no longer be detected. The residue was chromatographed on a column of reverse-phase C_{18} silica gel (Waters) eluted with water-MeOH (100 : 0 ~ 70 : 30, v/v) to give two fractions containing the acetate salt and the free amine of **5**. The early fractions were collected, combined, and evaporated under reduced pressure. The residue was freeze-dried to give the acetate salt of **5** (74 mg, 20%): ^{1}H NMR (60 MHz, $CDCl_3$) δ 1.73 (3H, s, AcO^-), 2.60 (3 H, s, *N*-CH_3), 3.81 (2H, s, CH_2Ph), 3.83 (2H, s, *N*-CH_2), 5.60 (1H, br, 1'-H), 7.21 (5H, s, Ph), 7.80 (1H, s, 6-H). Anal. Calcd for $C_{18}H_{23}N_3O_6 \cdot C_2H_4O_2 \cdot H_2O$: C, 52.74; H, 6.42; N, 9.23. Found: C, 52.56; H, 6.37; N, 9.61. The second fractions which was eluted with water-MeOH (70:30, v/v) were collected, combined, and evaporated under reduced pressure. The residue was freeze-dried to give the free amine (130 mg, 40%) of **5**: ^{1}H NMR (60 MHz, $CDCl_3$) δ 2.13 (3 H, s, *N*-CH_3), 3.24 (2H, s, CH_2Ph), 3.53 (2H, s, *N*-CH_2), 4.00 (3H, m, 4',5'-H), 5.65 (1H, d, J = 2.4 Hz, 1'-H), 7.17 (5H, s, Ph), 7.80 (1H, s, 6-H). Anal. Calcd for $C_{18}H_{23}N_3O_6 \cdot 1.5H_2O$: C, 53.46; H, 6.47; N, 10.39. Found: C, 53.65; H, 6.32; N, 10.40.

Method 2. The crude compound **4** (808 mg) obtained *via* Method 2 in the above experiment was heated with 80% acetic acid (145 ml) at 100 °C for 4 h. The mixture was evaporated under reduced pressure and repeated coevaporation with water was continued until the odor of acetic acid could no longer be detected. The residue was dissolved with water and sodium carbonate (400 mg) was added. The mixture was chromatographed on a column of reverse-phase C_{18} silica gel (Waters) eluted with water-MeOH (100 : 0 ~ 70 : 30, v/v) to give the free amine of **5** (351 mg, 50% from **1**).

5-(Methylaminomethyl)uridine (6). Compound **5** (1.5 hydrate, 300 mg, 0.74 mmol) was dissolved in ethanol-water (9:1, v/v, 15 ml) and 10% Pd/C (150 mg) was added. After being vigorously stirred under hydrogen atmosphere at room temperature for 3 h, the mixture was filtered by using hyflosupercel. The filtrate was evaporated under reduced pressure and the residue was coevaporated several times with water to remove ethanol. The ethanol free material was purified by reverse-phase C_{18} silica gel (Waters) eluted with water to give fractions containing **6**. The fractions were collected, combined, and freeze-dried to give **6** (116 mg, 52%): UV (H_2O) λmax 263.5 nm, λmin 228 nm; ^1H NMR (400 MHz, D_2O) δ 2.70 (3 H, s, *N*-CH_3), 3.81 (1H, m, 5'-Ha), 3.94 (1H, m, 5'-Hb), 3.95 (2H, s, *N*-CH_2), 4.13 (1H, m, 4'-H), 4.21 (1H, t, J = 5.6 Hz, 3'-H), 4.32 (1H, t, J = 4.6 Hz, 2'-H), 5.87 (1H, d, J = 4.0 Hz, 1'-H), 8.18 (1H, s, 6-H). Anal. Calcd for $C_{11}H_{17}N_3O_6$: C, 43.28; H, 6.27; N, 13.76. Found: C, 43.50; H, 5.83; N, 13.10.

5-[(*N*,*N*-Dimethylamino)methyl]uridine (8). Compound **1** (1.99 g, 7 mmol) was dissolved in a mixture of 37% formalin (2.84 g, 35 mmol) and 50% dimethylamine aqueous solution (3.16 g, 35 mmol). The resulting mixture was stirred at room temperature for 10 days and then at 80 °C for 24 h. The mixture was evaporated under reduced pressure and the residue was coevaporated successively with water (X 10), ethanol (X 2), and acetone (X 2). The formalin-free material was chromatographed on a column of silica gel eluted with CH_2Cl_2-MeOH-pyridine (99.5 : 0 : 0.5 ~ 90.5 : 9 : 0.5, v/v/v) to give crude **7** (0.99 g, 42%). The crude material was dissolved in 80% acetic acid and the solution was refluxed for 4 h. The mixture was evaporated and the residue was coevaporated several times with water until the odor of acetic acid could no longer be detected. The residue was chromatographed on a column of reverse-phase C_{18} silica gel (Waters) eluted with water to give the acetate salt of **8** (114 mg, 22% from **1**): ^1H NMR (400 MHz, D_2O) δ 2.84 (3 H, s, *N*-CH_3), 3.80 (1H, m, 5'-Ha), 3.94 (1H, m, 5'-Hb), 4.04 (2H, s, *N*-CH_2), 4.13 (1H, m, 4'-H), 4.20 (1H, t, J = 5.8 Hz, 3'-H), 4.32 (1H, t, J = 4.4 Hz, 2'-H), 5.85 (1H, d, J = 3.7 Hz, 1'-H), 8.23 (1H, s, 6-H).

***S*,*S*-Diphenyl N^3-benzoyl-5'-*O*-(4,4'-dimethoxytrityl)-2'-*O*-methyluridine 3'-Phosphorodithioate (10).** Compound **9** (421 mg, 1.16mmol) was rendered anhydrous by repeated coevaporations with dry pyridine and finally dissolved in dry pyridine (10 ml). Dimethoxytrityl chloride (471 mg, 1.39 mmol) was added, and the solution was stirred for 14 h. In a different flask, a mixture of cyclohexylammonium S,S-diphenyl phosphorodithioate (PSS, 664 mg, 1.74 mmol) and tetrazole (330 mg, 4.64 mmol) was rendered anhydrous by repeated coevaporations with dry pyridine and

dissolved in the same solvent. The solution was poured into the former reaction mixture and isodurenedisulfonyl dichloride (DDS, 1.15 g, 3.48 mmol) was added. After being stirred for 1h, the mixture was partitioned between CH_2Cl_2-H_2O. The CH_2Cl_2 extract was washed successively with H_2O, saturated $NaHCO_3$ (X 2), and H_2O, dried over Na_2SO_4, filtered, and evaporated to dryness under reduced pressure. After being coevaporated with toluene, the residue was chromatographed on a silica gel column with hexane-CH_2Cl_2 (1 : 1 v/v) to give **10** (627 mg, 59 %): ^1H NMR (270 MHz, $CDCl_3$) δ 3.44 (3H, s, OCH_3), 3.57 (1H, dd, $J_{4',5'}$ = 4.5 Hz, J_{gem} = 14 Hz, 5'-H), 3.62 (1H, d, 5''-H), 3.78 (3H, s, $ArOCH_3$), 3.79 (3H, s, $ArOCH_3$), 4.02 (1H, m, 4-H), 4.21 (1H, d, $J_{2',3'}$ = 6.3 Hz, 3'-H), 6.00 (1H, d, $J_{1',2'}$ = 2.9 Hz, 1'-H), 6.84~7.98 (28H, m, ArH), 8.05 (1H, d, $J_{5,6}$ = 8.2 Hz, 6-H). Anal. Calcd for $C_{50}H_{45}N_2O_{10}PS_2 \cdot 1/4\ H_2O$: C, 64.6; H, 4.86; N, 3.01; S, 6.90. Found: C, 64.4; H, 5.03; N, 3.38; S, 7.16.

Synthesis of Fully Protected Dimer Block (12). Compound **10** (100 mg, 0.108 mmol) was dissolved in 5 M pyridinium phosphinate in pyridine-triethylamine (1.1 : 0.5, v/v, 1.6 ml) and stirred at room temperature for 45 min. The mixture was partitioned between CH_2Cl_2-H_2O. The CH_2Cl_2 extract was washed with H_2O (X 3), triethylammonium bicarbonate (TEAB) (X 2), and H_2O (X 2) , dried over Na_2SO_4, filtered, and evaporated to dryness under reduced pressure. The residue **11** was dissolved in dry pyridine, 3-nitro-1*H*-1,2,4-triazole (NT, 37.0 mg, 0.324 mmol) and **4** (37.6 mg, 0.09 mmol) was added. The mixture was coevaporated with dry pyridine. DDS (107 mg, 0.324 mmol) was added and the mixture was stirred at room temperature. After 45 min, the reaction was quenched by addition of H_2O (1 ml) and the solution was partitioned between CH_2Cl_2-H_2O. The CH_2Cl_2 extract was washed with H_2O, saturated $NaHCO_3$ (X 2), and H_2O, dried over Na_2SO_4, filtered, and evaporated to dryness under reduced pressure. After being coevaporated with toluene, the residue was chromatographed on a silica gel column to give the fully protected dimer **12** (87.4 mg, 78%).

Synthesis of *N*-Benzyl Derivative of Umpmnm^{5}U (13). Compound **12** (31.4 mg, 25.2 mmol) was dissolved in a 0.75 M solution of $(Bu_3Sn)_2O$ in pyridine (760 ml) and stirred at room temperature. After 1.5 h, chlorotrimethylsilane (81.9 mg, 755 mmol) was added and then kept at room temperature for 5 min. The mixture was diluted with pyridine-H_2O (1 : 1, 10 ml) and washed 3 times with hexane. The aqueous layer was evaporated under reduced pressure, dissolved in pyridine-concentrated ammonia (5 ml-5 ml) and stirred at 60 °C for 3h. After the solvent was removed by evaporation, the residue was dissolved in 80 % acetic acid (5 ml) and the solution was stirred at 100 °C for 3h. The mixture was coevaporated 5 times with H_2O and concentrated under reduced pressure. The

residue was chromatographed on Whatman 3MM papers with iPrOH-concentrated ammonia-H_2O (7:1:2, v/v/v) to give the *N*-benzyl dimer **13** (273 A_{262}): UV (H_2O) λmax 262 nm, λmin 234.5 nm.

Synthesis of Fully deblocked Umpmnm^{5}U (14). The *N*-benzyl dimer **13** (273 A_{262}) was dissolved in H_2O-EtOH (1 ml-1 ml) and hydrogenated on 10 % Pd/C (8 mg) at room temperature for 15 h. The powder of Pd/C was filtered and the filtrate was concentrated under reduced pressure. The residue was chromatographed on papers (Whatman 3MM) with iPrOH-concentrated ammonia-H_2O (7 : 1 : 2, v/v/v) to give a band containing **14**. The band was eluted with water and the eluate was concentrated under reduced pressure. Further purification was performed by reverse-phase HPLC to give pure **14** (64$A_{264.5}$, 15 % yield): UV(H_2O) λmax, 264.5 nm, λmin 239.5 nm; ^{1}H NMR (500 MHz, D_2O) δ 2.75 (3H, s, *N*-CH_3), 3.48 (3H, s, *O*-CH_3), 3.82 (1H, dd, J_{gem} = 13.1 Hz, $J_{4',5'a}$ = 3.82 Hz, 5'-H of Um), 3.92 (1H, dd, $J_{4',5'b}$ = 2.44, 5"-H of Um), 4.00 (2H, s, CH_2-*N*), 5.88 (1H, d, J = 7.93 Hz, 5-H of Um), 5.94 (1H, d, J = 2.75 Hz, 1'-H of mnm^{5}U), 5.98 (1H, d, J = 4.56 Hz, 1'-H of Um), 7.91 (1H, d, 6-H of Um), 8.22 (1H, s, 6-H of mnm^{5}U); HPLC retention time 16.1 min.

2',3'-*O*-Methoxymethylene-5-[*N*-(4-methoxybenzyl)methylamino-methyl]uridine (20). Compound **17** (572 mg, 2.0 mmol) was dissolved in H_2O-EtOH (5 ml-5 ml), 37% formaldehyde (1.4 ml, 18 mmol) and piperidine (1.70 g, 20 mmol) was added. The mixture was stirred under reflux for 7 h. After the reaction was completed, the mixture was coevaporated with H_2O (10 ml x 5), pyridine (10 ml x 3), DMF (10 ml x 3), and evaporated under reduced pressure to dryness. The residue containing **18** was dissolved in DMF (20 ml). MeI (2.83 g, 20 mmol) was added and stirred at 40 °C. After 1 h, the excess MeI was removed under reduced pressure for 5 min. To the DMF solution containing the quaternary salt **19**, 4-methoxybenzylmethylamine (372 mg, 2.4 mmol) and ethyldiisopropylamine (310 mg, 2.4 mmol) were added, and the mixture was stirred at 70 °C for 160 min. The solution was diluted with CH_2Cl_2 and partitioned between CH_2Cl_2-H_2O. The CH_2Cl_2 extract was washed successively with H_2O, saturated $NaHCO_3$ (X 2) and H_2O, dried over Na_2SO_4, filtered, and evaporated to dryness under reduced pressure. After being coevaporated with toluene, the residue was chromatographed on a silica gel column to give **20** (899 mg, 47 %): ^{1}H NMR (270 MHz, $CDCl_3$) δ 2.27 (3H, s, *N*-CH_3), 3.33 (3H, s, *O*-CH_3), 3.38 (2H, s, 5-methylene), 3.59 (2H,s, *N*-CH_2Ar), 3.80 (3H, s, ArOCH_3), 3.94 (1H, J_{gem} = 12 Hz, $J_{4',5'}$ = 2.3 Hz, 5'-H), 4.28 (1H, m, 4'-H), 5.12~5.13 (2H, m, 2'-H, 3'-H), 5.60 (1H, d, $J_{1',2'}$ = 2.0 Hz, 1'-H), 6.01 (1H, s, methyne), 7.25 (2H, m, ArH), 6.87 (2H, m, ArH), 7.59 (1H, s, 6-

H). Anal. Calcd for $C_{21}H_{27}N_3O_8 \cdot H_2O$: C, 53.97; H, 6.25; N, 8.98. Found: C, 54.33; H, 5.97; N, 8.57.

2',3'-*O*-Methoxymethylene-5-(methylaminomethyl)uridine (21). Compound **20** (224 mg, 0.5 mmol) was dissolved in dioxane-H_2O (4 ml-3 ml) and *N*-bromosuccimide (178 mg, 1.0 mmol) was added. The mixture was stirred at room temperature for 40 min. $NaHCO_3$ (84 mg, 1.0 mmol) was added and then the mixture was partitioned between CH_2Cl_2-H_2O. The CH_2Cl_2 extract was back-extracted 3 times with H_2O and the aqueous layer was concentrated under reduced pressure. The residue was chromatographed on a reverse-phase C_{18} column with H_2O-methanol (100 : 5, v/v) to give the debenzylated derivative **21** (92 mg, 56%): ^1H NMR (270 MHz, $CDCl_3$) δ 2.68 (3H, s, *N*-CH_3), 3.35 (3H, s, *O*-CH_3), 3.72~3.92 (3H, m, 5', 5"-H, methylene), 4.30 (1H, m, 4'-H), 4.95~5.20 (2H, m, 2'-H, 3'-H), 5.82 (1H, d, $J_{1',2'}$ = 2.65 Hz, 1'-H), 6.23 (1H, s, methyne), 8.02 (1H, s, H-6) .

2',3'-*O*-Isopropylidene-5-(methylaminomethyl)uridine (23). Compound **22** (169 mg, 0.4 mmol) was debenzylated in the same procedure as described in the case of the synthesis of **21** to give **23** (61 mg, 46 %): ^1H NMR (270 MHz, D_2O), δ 2.40 (3H, s, *N*-CH_3), 3.72 (1H, dd, 5'-Ha, $J_{4',5'a}$ = 4.00 Hz, J_{gem} = 10.8 Hz), 3.80 (1H, dd, 5'-Hb, $J_{4',5'b}$ = 3.62 Hz), 4.21 (1H, dd, 4'-H), 5.89 (1H, d, 1'-H, $J_{1',2'}$ = 2.3 Hz), 7.85 (1H, s, 6-H).

2',3'-*O*-Methoxymethylene-5-[*N*-(trifluoroacetyl)methylaminomethyl] uridine (24). Compound **21** (84.2 mg, 250 mmol) was rendered anhydrous by repeated coevaporations with dry pyridine and finally dissolved in dry pyridine (5 ml). Trifluoroacetic anhydride (216.3 mg, 1.03 mmol) was added. After being stirred for 40 min, the mixture was treated with 10 % Na_2CO_3 (50 ml). Stirring was continued for an additional 10 min and the mixture was partitioned between CH_2Cl_2-H_2O. The CH_2Cl_2 extract was washed 3 times with H_2O, dried over Na_2SO_4, filtered, and evaporated to dryness under reduced pressure. The residue was coevaporated with toluene and chromatographed on a silica gel column with CH_2Cl_2-MeOH to give the trifluoroacetylated derivative **24** (54.5 mg, 50 %): ^1H NMR (270 MHz, $CDCl_3$) δ 3.34 (3H, s, *O*-CH_3 or *N*-CH_3), 3.36 (3H, s, *O*-CH_3 or *N*-CH_3), 3.82 (1H, d, 5"-H), 4.00 (1H, d, J_{gem} = 12 Hz, 5'-H), 4.17 (1H, d, J_{gem} = 14.2 Hz, methylene a), 4.26 (1H, d, J_{gem} = 14.2 Hz, methylene b), 4.45 (1H, m, 4'-H), 4.98 (1H, dd, $J_{1',2'}$ = 3.0 Hz, $J_{2',3'}$ = 6.3 Hz, 2'-H), 5.03 (1H, dd, $J_{2',3'}$ = 6.3 Hz, $J_{3',4'}$ = 1.65 Hz,3'-H), 5.81 (1H, d, $J_{1',2'}$ = 3.0 Hz, 1'-H), 5.99 (1H, s, methylene), 8.00 (1H, s, 6-H). Anal. Calcd for $C_{15}H_{18}N_3O_8F_3$: C, 42.37; H, 4.27; N, 9.88. Found: C, 42.60; H, 4.22; N, 9.62.

Synthesis of Fully Protected Dimer Block (25). Compound **15** (153.9 mg, 0.15 mmol) was dissolved in 5M pyridinium phosphinate in pyridine-triethylamine (1.1: 0.5, v/v, 2.3 ml) and the resulting mixture was stirred at room temperature for 30 min. The mixture was partitioned between CH_2Cl_2-H_2O. The CH_2Cl_2 extract was washed with H_2O (X 3), TEAB (X 2) and H_2O (X 2), dried over Na_2SO_4, filtered, and evaporated to dryness under reduced pressure. A mixture of the residue **16**, NT (42.8 mg, 0.38 mmol), and **24** (53.1 mg, 0.13 mmol) was rendered anhydrous by repeated coevaporations with dry pyridine and finally dissolved in dry pyridine (2 ml). Then DDS (124.2 mg, 0.38 mmol) was added and the mixture was stirred at room temperature for 40 min. The mixture was partitioned between CH_2Cl_2-H_2O. The CH_2Cl_2 extract was washed successively with H_2O, saturated $NaHCO_3$ (X 2) and H_2O, dried over Na_2SO_4, dried over Na_2SO_4, filtered, and evaporated to dryness under reduced pressure. After being coevaporated with toluene, the residue was chromatographed on a silica gel column with CH_2Cl_2-MeOH (100 : 0.5) to give the fully protected dimer **25** (167.4 mg, 83 %).

Synthesis of Upmnm^{5}U (26). Compound **25** (50.0 mg, 37.3 mmol) was dissolved in a 0.75 M solution of $(Bu_3Sn)_2O$ in pyridine (1.12 ml, 0.56 mmol) and stirred at room temperature. After 2 h, TMSCl (121.6 mg, 1.12 mmol) was added and then kept at room temperature for 5 min. The mixture was diluted by pyridine-H_2O (1 : 1, 10 ml) and washed 3 times with hexane. The aqueous layer was evaporated under reduced pressure, dissolved in pyridine-concentrated ammonia (10 ml-30 ml) and stirred at 60 °C for 24 h. The mixture was evaporated, dissolved in 80 % acetic acid (10 ml) and stirred at room temperature for 3h. The mixture was coevaporated 5 times with H_2O, and concentrated under reduced pressure. The residue was chromatographed on papers (Whatman 3MM) with iPrOH-concentrated ammonia-H_2O (7 : 1 : 2, v/v/v) to give fully deblocked Upmnm^{5}U. The product was further purified by reverse-phase HPLC to give **26** ($350A_{261}$, 54 %): UV(H_2O) λmax, 261 nm, λmin 232 nm; ^{1}H NMR (500 MHz, D_2O) δ 2.72 (3H, s, *N*-CH_3), 3.81 (1H, dd, J_{gem} = 10.2 Hz, $J_{4'5'a}$ = 3.4 Hz, 5'-H of U), 3.91 (1H, dd, $J_{4'5'b}$ = 2.2 Hz, 5'-H of U), 4.00 (2H, s, CH_2-*N*), 4.47 (1H, t, $J_{1'2'}$ = 4.2 Hz, $J_{2'3'}$ = 4.2 Hz, 2'-H of mnm^{5}U), 5.92 (1H, d, 1'-H of mnm^{5}U), 5.94 (1H, d, $J_{1'2'}$ = 2.7 Hz, 1'-H of U), 7.86 (1H, d, J = 6.4 Hz, 6-H of U), 8.17 (1H, s, 6-H of mnm^{5}U); HPLC retention time 21 min.

Synthesis of pUpmnm^{5}U (29). A solution of the fully protected Upmnm^{5}U **25** (28 mg, 20.9 mmol) in dry CH_2Cl_2 (20 ml) was cooled to 0 °C and trifluoroacetic acid (100 μl) was added. After 1 h, pyridine (1ml) was added and the mixture was partitioned between CH_2Cl_2-H_2O. The CH_2Cl_2 extract was washed subsequently with H_2O, saturated

$NaHCO_3$ (X 2) and H_2O, dried over Na_2SO_4, filtered, and evaporated to dryness under reduced pressure. The residue was chromatographed on preparative TLC plates (Merck, Art. No. 5717) with CH_2Cl_2-MeOH (9:1) to give 5'-hydroxyl derivative of Upmnm^{5}U **27** (22 mg, 96%). A mixture of **27** (19.2 mg, 18.5 mmol), PSS (10.6 mg, 27.8 mmol) and 1-*H*-tetrazole (5.2 mg, 74.3 mmol) was rendered anhydrous by repeated coevaporations with dry pyridine. The mixture was dissolved in dry pyridine (2 ml), and DDS (18.4 mg, 55.6 mmol) was added. After being stirred at room temperature for 30 min, the mixture was partitioned between CH_2Cl_2-H_2O. The CH_2Cl_2 extract was washed successively with H_2O, saturated $NaHCO_3$ (X 2) and H_2O, dried over Na_2SO_4, filtered, and evaporated to dryness under reduced pressure. The residue was chromatographed on preparative TLC plates with CH_2Cl_2-MeOH (9:1) to give the 5'-phosphorylated derivative of Upmnm^{5}U **28** (11.3mg, 47%). Compound **28** (11.3 mg, 8.73 mmol) was dissolved in pyridine (1 ml). Silver acetate (218.7 mg, 1.31 mmol) was added and the mixture was stirred at room temperature for 1.5 h. After the reaction was completed, hydrogen sulfide gas was bubbled into the mixture. The resulting silver sulfide was filtered off, and the filtrate was evaporated to dryness. The residue was dissolved in pyridine-aqueous ammonia (2 ml-2 ml) and stirred at room temperature for 18 h. The mixture was coevaporated 5 times with H_2O. The residue was dissolved in 0.01 N HCl (5 ml) and the solution was adjusted to pH 2.0. The mixture was stirred at room temperature for 30 h. Then the solution was neutralized by addition of pyridine (2 ml) and evaporated to dryness under reduced pressure. The residue was chromatographed on papers (Whatman 3MM) with iPrOH-concentrated ammonia-H_2O (7 : 1 : 2, v/v/v) to give pUpmnm^{5}U **29**. The dimer was further purified by reverse-phase HPLC to give the pure material of **29** ($19A_{256}$, 15%): UV(H_2O) λmax, 262 nm, λmin 230 nm; ^{1}H NMR (270 MHz, D_2O) δ 2.70 (3H, s, *N*-CH_3), 4.00 (2H, s, CH_2-*N*), 6.0 (3H, m, 1'-H, 5-H), 8.04 (1H, d, J = 7.56 Hz, 6-H of pU), 8.24 (1H, s, 6-H of mnm^{5}U), ^{31}P NMR (109.25 MHz, D_2O) δ 0.03 (3'-5' phosphodiester), 3.20 (5' phosphate) ; HPLC retention time 4.2 min.

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