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ARTICLE

# Total Synthesis of Pseudouridine *via* Heck-type *C*-Glycosylation†

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The reaction of 2,4-dimethoxy-5-iodopyrimidine (**8**) and 3,5-di-*O*-*tert*-butyldimethyl protected ribofuranoid glycal **4** was carried out with $Pd(OAc)_2$ as the catalyst, $PPh_3$ as the ligand and $Et_3N$ as the base in DMF at 70 °C followed by desilylation to afford exclusively the β-anomer of 5-(2,3-dideoxy-3-oxoribofuranosyl)-2,4-dimethoxypyrimidine (**11**) in a very good yield. Subsequent protecting group and functional group interconversions furnished pseudouridine (Ψ, **1**).

## Introduction

Pseudouridine (Ψ, **1**), isolated from RNA mixture in 1957[1] and characterized in 1961,[2,3] is the first *C*-nucleoside found in nature. It is the fifth nucleoside component in RNA and the most abundant natural *C*-nucleoside, which, therefore, received significant attention. Pseudouridine is the structural isomer of uridine with identical uracil base and ribosyl sugar moiety but differ only in the nucleosidic linkages (**Figure 1**). It is formed by post-translational isomerization from uridine in RNA catalyzed by pseudouridine synthase.[4] Albeit decades of efforts have been devoted, the role of pseudouridine in RNA still remains to be further clarified.[5] One of the major restrictions for the studies is due to the very limited commercial availability and expensive prices of pseudouridine. Thus, chemical synthesis of pseudouridine continues to offer an alternative supply for this biological important molecule. Nevertheless, the chemical synthesis of *C*-nucleosides (and also *C*-glycosides) is perceived to be challenging and the development of efficient syntheses for *C*-nucleosides is still an ongoing task.[6-8]

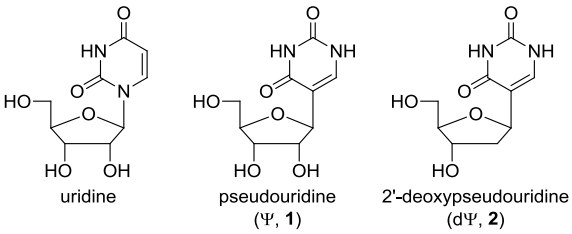
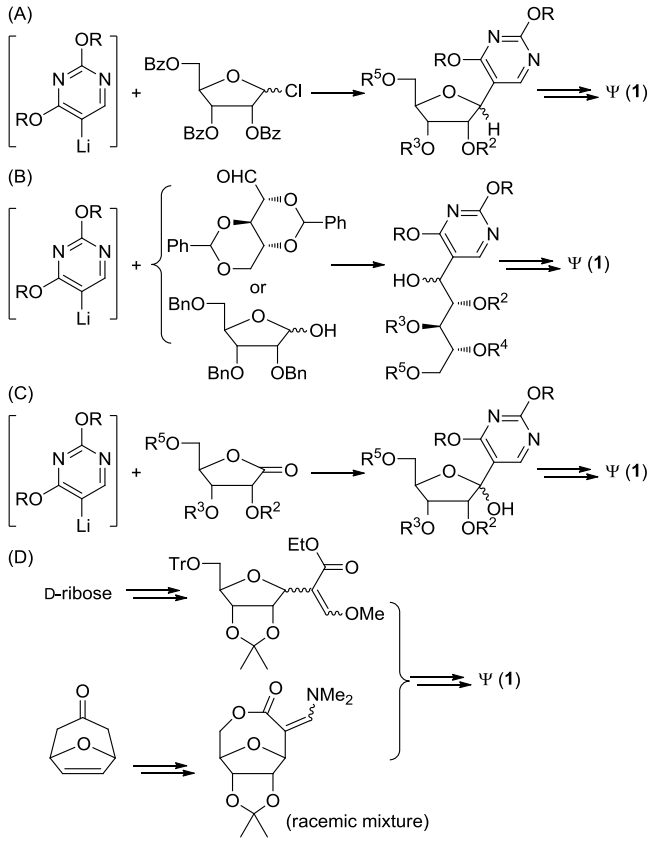



**Figure 1.** Uridine, pseudouridine (Ψ, **1**) and 2'-deoxypseudouridine (dΨ, **2**)




**Scheme 1.** Synthetic strategies toward pseudouridine (**1**)

A perusal of literature revealed that the previous synthesis of pseudouridine (**1**) was achieved mainly by four different strategies summarized in **Scheme 1**. (A) The first synthesis of pseudouridine was accomplished by Shapiro and Chambers in 1961. They exploited the substitution reaction between 5-lithiated 2,4-dimethoxypyrimidine and 2,3,5-tri-*O*-benzoylribofuranosyl chloride followed by acid-hydrolysis to afford pseudouridine.[3] (B) Later on, the addition reaction of 5-lithiated 2,4-dialkoxypyrimidine to hydroxyl-protected ribose followed by global deprotection and acid-promoted dehydrative cyclization became an early approach for the

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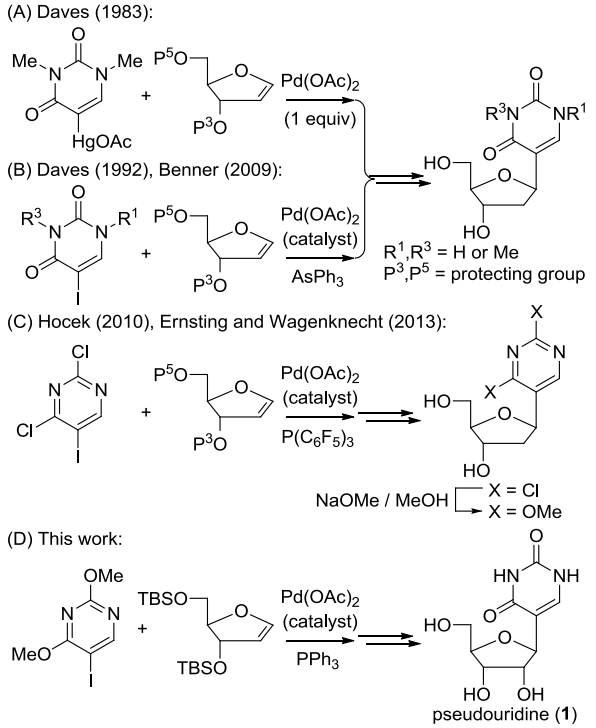
synthesis of pseudouridine.[9] However, both approaches (A) and (B) resulted in the formation of α/β-isomers in moderate yields accompanied with a minimal amount of pyranose-isomers. (C) The addition reaction of 5-lithiated 2,4-dialkoxypyrimidine to hydroxyl-protected ribonolactone was found to be a superior method. The addition reaction formed an anomeric mixture of ribonolactol and subsequent reduction followed by deprotection rendered the synthesis of pseudouridine.[10-13] It is noteworthy that the synthesis of *C*-nucleosides *via* the ribonolactone approach typically gives a mixture of α/β-stereoisomers. However, an improved protocol developed by Hanessian *et al.* allowed the synthesis of pseudouridine to be accomplished in an excellent β-stereoselectivity.[11-13] (D) Alternatively, pseudouridine can also be synthesized *via de novo* construction of the heterocyclic aglycon upon the ribofuranosyl precursors equipped with functionalized substituents at the anomeric position. This approach was demonstrated by Watanabe[14] and Noyori,[15,16] respectively.




**Scheme 2.** Heck-type glycosylation of uracil/pyrimidine derivatives

Over the past three decades, palladium-catalyzed Heck-type reaction of ribofuranoid glycals with (het)aryl iodides has been developed as a promising approach for the synthesis of 2'-deoxy-*C*-nucleosides.[8] The pioneering work was undertaken by Daves who first reported that the reaction of (1,3-dimethyluracil-5-yl)mercury(II) acetate with ribofuranoid glycals under stoichiometric amount of $Pd(OAc)_2$ led to the formation of 1,3-dimethyl-2'-deoxypseudouridine (**Scheme 2** (A)).[17] Their continuous efforts led them to develop the reaction into a practical approach which utilizes palladium-catalyzed Heck cross-coupling reaction of aryl iodides with ribofuranoid glycals for the synthesis of 2'-deoxy-*C*-nucleosides with remarkable β-stereoselectivity. In particular, Zhang and Daves demonstrated that 2'-deoxypseudouridine (d**Ψ**, **2**) can be prepared directly from 5-iodouracil and 3'-TBDPS-protected furanoid glycal under the catalysis of $Pd(OAc)_2$ and $AsPh_3$ (**Scheme 2** (B)).[18,19] Since then, the Heck-type *C*-glycosylation has been extensively applied to the synthesis of a variety of (het)arene 2'-deoxy-*C*-nucleosides.[20,21]

Nevertheless, it came to our attention that, besides 2'-deoxypseudouridine (**2**), several *C5*-(2'-deoxyribofuranosyl)pyrimidine derivatives have also been prepared by this method (**Scheme 2** (C)).[17-19,22] Despite they are structurally related to 2'-deoxypseudouridine (**2**), further elaboration of these pyrimidine 2'-deoxy-*C*-nucleosides into pseudouridine (**1**) has not been explored. Thus, we were prompted to investigate whether the palladium-catalyzed Heck-type glycosylation of 5-iodouracil derivatives followed by a sequential chemical manipulation would be a general and feasible strategy for the preparation of pseudouridine (**1**) and its derivatives (**Scheme 2** (D)).

## Results and discussion

In our attempts to study chemical elaboration of 2'-deoxypseudouridine derivatives into pseudouridine (**1**), the synthesis of 2'-deoxypseudouridine (**2**) based on the Heck reaction approach developed by Zhang and Daves was first conducted.[19] It is worth to mention that the Heck-type glycosylation usually required the use of triphenylarsine or tris(pentafluorophenyl)phosphine as the ligand in the reaction in order to achieve high stereoselectivities and yields. Since we first aimed to avoid the use of toxic arsine or expensive phosphine ligands, the reaction of 5-iodouracil (**3**) with 3,5-di-*O*-*tert*-butyldimethyl protected ribofuranoid glycal (**4**),[23] prepared from thymidine by literature procedures, was subjected to optimization with various catalysts, ligands and bases. The survey of reaction conditions showed that the Heck glycosylation adduct **5** was obtained in the maximum yield as a single stereoisomer when the reaction was carried out in DMF at 70 °C with $Pd_2(dba)_3$ as the catalyst, XantPhos as the ligand and *n*-$Bu_3N$ as the base (**Table S1** in Supporting Information). The removal of TBS groups was then accomplished by TBAF with AcOH to form the 2'-deoxy-3'-oxo-*C*-ribonucleoside **7**.[19] With the directing effect from 5'-hydroxy group, the 3'-keto group of **7** was stereoselectively reduced by $NaB(OAc)_3H$ from the β-face to give 2'-deoxypseudouridine (**2**) as a single stereoisomer in an excellent yield (**Scheme 3**).[19,24,25]

Literature survey revealed that transformations of silyl enol ethers from Heck glycosylation into ribofuranose *C*-nucleosides can be achieved mainly by three strategies, including the direct oxidation with osmium tetroxide[26] or dimethyldioxirane[27] of the silyl enol ethers to give the α-hydroxyketone, and hydroboration-oxidation of the silyl enol ethers to form the 2',3'-*trans*-diols.[28] However, our attempts to further elaborate 2'-deoxypseudouridine (**2**) or its silyl enol ether derivative **5** to pseudouridine (**1**) based on the above mentioned approaches were failed. We speculated that this failure could be attributed to the interference from the unprotected nitrogens/oxygens

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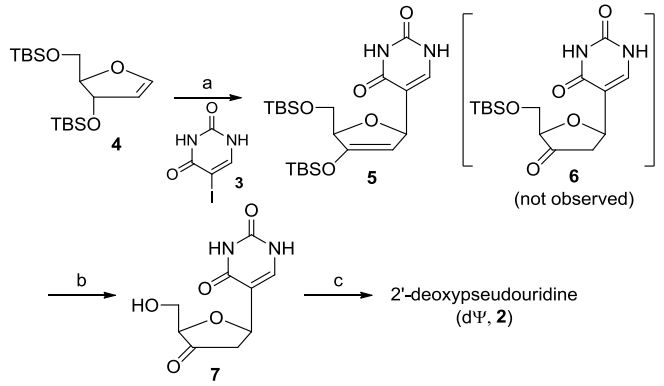
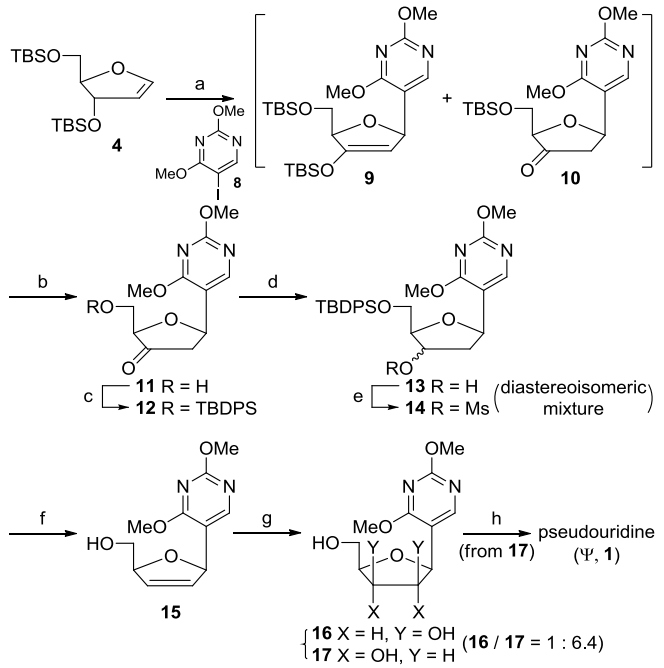
on the uracil ring. After several unsuccessful trials with *N*-protecting groups on uracil of 2′-deoxypseudouridine (**2**), we opted to protect the oxygens of uracil for subsequent transformation.




*reagents and conditions:*

(a) $Pd_2dba_3$ (0.1 equiv), XantPhos (0.2 equiv), 5-iodouracil (**3**, 2 equiv), glycal **4** (1 equiv), *n*-$Bu_3N$ (1.5 equiv), DMF (0.05 M), 70 °C, 15 h, 83%; (b) AcOH (2 equiv), TBAF (1M in THF, containing ca. 5% water, 2 equiv), THF (0.2 M), 0 °C~rt, 8 h, 85%; (c) $NaB(OAc)_3H$ (1.5 equiv), AcOH/$CH_3CN$ (12 mL, *v*/*v* = 1 : 1, 0.066 M), 0 °C~rt, 3 h, 92%

**Scheme 3.** Synthesis of 2′-deoxypseudouridine (dΨ, **2**)




*reagents and conditions:*

(a) $Pd(OAc)_2$ (0.1 equiv), $PPh_3$ (0.2 equiv), 2,4-dimethoxy-5-iodopyrimidine (**8**, 2 equiv), glycal **4** (1 equiv), $Et_3N$ (1.5 equiv), DMF (0.1 M), 70 °C, 15 h; (b) TBAF (1M in THF, containing ca. 5% water, 2.0 + 1.0 equiv), AcOH (2.0 equiv), THF (0.1 M), 0 °C~rt, overnight, 88% from **4**; (c) TBDPSCl (1.2 equiv), imidazole (2.2 equiv), DCM (0.1 M), rt, overnight, 97%; (d) $NaBH_4$ (1.5 equiv), MeOH (0.1 M), rt, 1 h, 96% (diastereoisomeric mixture); (e) MsCl (1.3 equiv), $Et_3N$ (1.5 equiv), DCM (0.1 M), rt, 1 h (quantitative); (f) DBU (18 equiv), $CH_3CN$ (0.1 M), 75 °C, 48 h, 49% from **13**; (g) $OsO_4$ (0.05 equiv), NMO (3.0 equiv), acetone (0.1 M), -20 °C~rt, overnight, 76% (**16** : **17** = 1 : 6.4); (h) NaI (4 equiv), AcOH (0.04 M), reflux, 45 min, 90%

**Scheme 4.** Synthesis of pseudouridine (Ψ, **1**)

Thus, 2,4-dimethoxy-5-iodopyrimidine (**8**),[29] an oxygen-protected equivalent of uracil, was subjected to the Heck glycosylation with ribofuranoid glycal **4**. In a continuous screening of palladium catalyst and phosphine ligand combinations, the silyl enol ether adduct **9** from Heck glycosylation was obtained as a single diastereomer in good yields (76%) when the reaction was carried out in DMF at 70 °C with $Pd_2(dba)_3$ as the catalyst and $PPh_3$ as the ligand (entries 3 and 5 in **Table S2** in Supporting Information). In contrast, when the reaction was carried out with $Pd(OAc)_2$ and $PPh_3$, only a minimum yield of the silyl enol ether **9** was obtained accompanied with the formation of the corresponding desilylated adduct **10** in 83% yield (**Table S2**). Our investigation showed that the use of $Pd(OAc)_2$ as the catalyst resulted in partial desilylation of the immediate Heck adduct **9** to form a product mixtures of **9** and **10**.[21,30] Regardless, the overall yield for the C-C bond formation is excellent and the condition is more suitable for multi-gram synthesis. Although **9** and **10** can be readily separated by column chromatography for characterization purpose, in our later work, the mixture of **9** and **10** was treated with TBAF and AcOH for global desilylation to give 5-(2′-deoxy-3′-oxoribofuranosyl)-2,4-dimethoxypyrimidine (**11**) in an excellent yield.

As a result, instead of elaborating the silyl enol ether **9**, we decided to derive the 3′-keto adduct **11** into the targeted molecule. After protecting the 5′-hydroxy group of **11** with TBDPS, the 3′-keto group was reduced with $NaBH_4$ to form an epimeric mixture of **13**. Subsequently, mesylation of the mixture **13** followed by elimination and concomitant removal of TBDPS group with DBU in $CH_3CN$ furnished the 2′,3′-dideoxydidehydro-*C*-nucleoside **15**. *cis*-Dihydroxylation of **15** with $OsO_4$ gave a mixture of lyxofuranosyl and ribofuranosyl adducts (**16** and **17**, respectively) in the ratio of 1 to 6.4.[31] Fortunately, the desired product **17** is predominant and can be readily separated.[32] Finally, *O*-demethylation of **17** was accomplished by treatment with NaI in acetic acid at reflux temperature to afford pseudouridine (**1**).[11] The spectroscopic data of our synthesized pseudouridine (**1**) is in accordance with data previously reported in the literature (**Tables S3~S6** in Supporting Information).[11,15,33-35]

## Conclusion

In summary, we herein reported a facile and practical synthesis for pseudouridine (**1**), which provides a complementary access to this important biological molecule. Further application of this approach to manipulate the Heck-type glycosylation adducts into ribonucleosides would be amenable to the synthesis of versatile *C*-nucleosides.

## Experimental section

### *General chemical procedures*

The chemical shift values are reported in δ values (parts per million, ppm) relative to the standard chemical shift for the hydrogen residue peak and carbon-13 peak in the deuterated

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solvent, $CDCl_3$, or DMSO-$d_6$.[36] The coupling constant ($J$) values are expressed in hertz (Hz). The numbers of protons directly attached to the individual carbons were determined by $^{13}C$ NMR DEPT experiments. Thin-layer chromatography (TLC) was performed on silica gel plates. Compounds on TLC were visualized by illumination under UV light (254 nm), and dipped into 10% *conc.* $H_2SO_4$ in EtOH, *p*-anisaldehyde stain (with sulfuric acid in EtOH), or 10% phosphomolybdic acid in EtOH followed by charring on a hot plate. Solvent systems are expressed with respect to the volumetric ratio of the less polar component to the more polar component (*v/v*). Silica gel (230-400 mesh) was used for flash column chromatography and this technique has been described by W. C. Still *et al.*[37] Evaporations were carried out under reduced pressure (water aspirator or vacuum pump) with the bath temperature below 50 °C unless specified otherwise. Materials obtained from commercial suppliers were used without further purification.

**3',5'-Di-*O*-(*tert*-butyldimethylsilyl)-2',3'-didehydro-2'-deoxypseudouridine (5)**

To a solution of XantPhos (0.1389 g, 0.24 mmol, 0.2 equiv) in DMF (6.0 mL) was added tris(dibenzylideneacetone)dipalladium ($Pd_2dba_3$, 0.1099 g, 0.12 mmol, 0.1 equiv) at room temperature. The $Pd_2dba_3$ solution was stirred under argon atmosphere at room temperature for 30 min. In a separate flask, to the solution of 5-iodouracil (**3**, 0.5712 g, 2.40 mmol, 2.0 equiv) and furanoid glycal **4**[23] (0.4164 g, 1.20 mmol) in DMF (18.0 mL) was added tri-*n*-butylamine (0.43 mL, 1.8.0 mmol, 1.5 equiv) under argon atmosphere at room temperature. The 5-iodouracil solution was then added to the $Pd_2dba_3$ solution and the resulting solution was stirred under argon atmosphere at 70 °C for 15 h. After cooling to room temperature, the solvent was evaporated under reduced pressure. The residue was dissolved in $CH_2Cl_2$ and the solution was washed with $H_2O$ and saturated aqueous NaCl solution. The organic layer was dried over anhydrous $MgSO_4$ and then concentrated under reduced pressure. The residue was purified by flash column chromatograghy (Hex / EtOAc = 5 : 5, $R_f$ = 0.35) to give **5** (colorless oil, 0.4539 g, 1.00 mmol, 83%). $^1H$ NMR (400 MHz, $CDCl_3$) δ 9.29 (br s, 2H), 7.68 (s, 1H), 5.68 (t, $J$ = 1.8 Hz, 1H), 4.90 (s, 1H), 4.51 (s, 1H), 3.85 and 3.73 (ABX system, $J$ = 11.3, 2.8, 1.8 Hz, 2H), 0.92 (s, 9H), 0.87 (s, 9H), 0.21 (s, 3H), 0.18 (s, 3H), 0.05 (s, 3H), 0.03 (s, 3H); $^{13}C$ NMR (400 MHz, $CDCl_3$) δ 163.2, 152.1, 150.1, 138.3 (CH), 116.6, 100.5 (CH), 83.8 (CH), 77.2 (CH), 63.2 ($CH_2$), 25.9 ($CH_3$), 25.5 ($CH_3$), 18.5, 18.0, -4.8 ($CH_3$), -5.2 ($CH_3$), -5.3 ($CH_3$), -5.4 ($CH_3$); MS (ESI-) *m/z* (%) 453 ([M - H], 100); HRMS (ESI+, TOF) calcd for $C_{21}H_{39}N_2O_5Si_2$ [M + H]: 455.2398. Found 455.2400.

**2',3'-Dideoxy-3'-oxopseudouridine[19] (7)**

To a solution of **5** (0.6223 g, 1.37 mmol) in THF (7.0 mL) at 0 °C were added AcOH (0.16 mL, 2.74 mmol, 2.0 equiv) and tetra-*n*-butylammonium fluoride (TBAF, 1 M solution in THF, containing *ca.* 5% $H_2O$, 2.74 mL, 2.74 mmol, 2.0 equiv). The reaction mixture was stirred for 8 h while the reaction temperature was allowed to rise to room temperature. The solvent was evaporated under reduced pressure and the residue was purified by flash column chromatograghy ($CH_2Cl_2$ / MeOH = 9 : 1, $R_f$ = 0.25) to give **7** (white solid, 0.2634 g, 1.16 mmol, 85%).[19] m.p. 206-208 °C; $^1H$ NMR (400 MHz, DMSO-$d_6$) δ 11.15 (br s, 2H), 7.61 (s, 1H), 5.00 (dd, $J$ = 10.0, 6.4 Hz, 1H), 4.91 (br s, 1H), 3.93 (t, $J$ = 3.0 Hz, 1H), 3.65-358 (m, 2H), 2.68 and 2.38 (ABX system, $J$ = 18.0, 10.0, 6.4 Hz, 2H); $^{13}C$ NMR (400 MHz, DMSO-$d_6$) δ 214.2, 163.5, 151.1, 139.3 (CH), 111.8, 82.1 (CH), 70.6 (CH), 60.6 ($CH_2$), 42.7 ($CH_2$); MS (ESI-) *m/z* (%) 225 ([M - H], 100); HRMS (ESI-, TOF) calcd for $C_9H_9N_2O_5$ [M - H]: 225.0511. Found 225.0510.

**2'-Deoxypseudouridine[19,24,25,38] (dΨ, 2)**

To a solution of **7** (0.1797 g, 0.79 mmol) in AcOH (6.0 mL) and acetonitrile (6.0 mL) was added sodium triacetoxyborohydride ($NaB(OAc)_3H$, 0.2522 g, 1.20 mmol, 1.5 equiv) at 0 °C. The reaction mixture was stirred for 3 h while the reaction temperature was allowed to rise to room temperature. The solvent was evaporated under reduced pressure. The residue was dissolved in EtOAc and the organic layer was washed with $H_2O$ and saturated aqueous NaCl solution. The orangic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash column chromatograghy ($CH_2Cl_2$ / MeOH = 8 : 2, $R_f$ = 0.18) to give **2** (white solid , 0.1659 g, 0.73 mmol, 92%). m.p. 220-222 °C;[24,38] $^1H$ NMR (400 MHz, DMSO-$d_6$) δ 10.95 (br s, 2H), 7.37 (s, 1H), 4.99 (d, $J$ = 3.6 Hz, 1H, OH), 4.80-4.75 (m, 2H, including 1 x OH), 4.13-4.09 (m, 1H), 3.67 (td, $J$ = 4.6, 2.4 Hz, 1H), 3.41-3.38 (m, 2H), 2.00 (ddd, $J$ = 12.6, 5.8, 1.8 Hz, 1H), 1.73 (ddd, $J$ = 12.5, 9.7, 5.7 Hz, 1H); $^{13}C$ NMR (400 MHz, DMSO-$d_6$) δ 163.5, 151.2, 137.9 (CH), 113.2, 87.1 (CH), 73.2 (CH), 72.1 (CH), 62.2 ($CH_2$), 40.9 ($CH_2$); MS (ESI-) *m/z* (%) 227 ([M - H], 100); HRMS (ESI-, TOF) calcd for $C_9H_{11}N_2O_5$ [M - H]: 227.0668. Found 227.0669.

**2,4-Dimethoxy-5-(3,5-di-*O*-*tert*-butyldimethylsilyl-2-deoxy-2,3-didehydro-β-D-ribofuranosyl)pyrimidine (9)**

To a solution of $PPh_3$ (0.0276 g, 0.11 mmol, 0.2 equiv) in DMF (1.0 mL) was added $Pd_2dba_3$ (0.0483 g, 0.053 mmol, 0.1 equiv) at room temperature. The $Pd_2dba_3$ solution was stirred under argon atmosphere at room temperature for 30 min. In a separate flask, to the solution of 2,4-dimethoxy-5-iodopyrimidine[29] (**8**, 0.2655 g, 1.00 mmol, 2.0 equiv) and furanoid glycal **4**[23] (0.1725 g, 0.50 mmol) in DMF (4.0 mL) was added triethylamine (0.11 mL, 0.79 mmol, 1.5 equiv) under argon atmosphere at room temperature. The 2,4-dimethoxy-5-iodopyrimidine solution was then added to the $Pd_2dba_3$ solution and the resulting solution was stirred under argon atmosphere at 70 °C for 15 h. After cooling to room temperature, the solvent was evaporated under reduced pressure. The residue was dissolved in EtOAc and the solution was washed with $H_2O$ and saturated aqueous NaCl solution. The organic layer was dried over anhydrous $MgSO_4$ and then concentrated under reduced pressure. The residue was purified by flash column chromatograghy (Hex / EtOAc = 20 : 1) to give **9** (oil, 0.1866 g, 0.39 mmol, 77%, $R_f$ = 0.60 (Hex / EtOAc = 2 : 1)). $^1H$ NMR (400 MHz, $CDCl_3$) δ 8.38 (s, 1H), 5.87 (dd, $J$ = 3.4, 1.4 Hz, 1H), 4.77 (dd, $J$ = 1.8 Hz, 1H), 4.54 (ddd, $J$ = 5.6, 3.8, 2.0 Hz, 1H), 3.98 (s, 3H), 3.96 (s, 3H), 3.82 and 3.70 (ABX

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system, *J* = 11.2, 4.0, 2.4 Hz, 2H), 0.93 (s, 9H), 0.83 (s, 9H), 0.22 (s, 3H), 0.20 (s, 3H), -0.0095 (s, 6H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 168.2, 164.5, 157.3 (CH), 151.0, 116.3, 99.3 (CH), 83.9 (CH), 76.8 (CH), 63.8 ($CH_2$), 54.4 ($CH_3$), 53.6 ($CH_3$), 25.8 ($CH_3$), 25.3 ($CH_3$), 18.3, 17.8, -5.1 ($CH_3$), -5.5 ($CH_3$), -5.6 ($CH_3$); MS (ESI+) *m/z* (%) 483 ([M + H], 100); HRMS (ESI+, TOF) calcd for $C_{23}H_{43}N_2O_5Si_2$ [M + H]: 483.2711. Found: 483.2707.

**2,4-Dimethoxy-5-(5-*O*-*tert*-butyldimethylsilyl-2,3-dideoxy-3-oxo-β-D-ribofuranosyl)pyrimidine (10)**

To a solution of **9** (0.9800 g, 2.03 mmol) in THF (28 mL) was added AcOH (0.13 mL, 2.16 mmol, 1.0 equiv) and TBAF (1 M solution in THF, containing *ca.* 5% $H_2O$, 4.2 mL, 4.20 mmol, 2.0 equiv) at 0 °C. The solution was stirred at 0 °C for 20 min till no starting material was detected by TLC. The solvent was evaporated under reduced pressure. The residue was purified by flash column chromatography (Hex / EtOAc = 6 : 1) to give **10** (oil, 0.6451 g, 1.75 mmol, 86%, $R_f$ = 0.35 (Hex / EtOAc = 2 : 1)). $^1$H NMR (400 MHz, $CDCl_3$) δ 8.48 (s, 1H), 5.23 (dd, *J* = 11.0, 5.8 Hz, 1H), 3.99-3.96 (m, 1H), 3.98 (s, 3H), 3.97 (s, 3H), 3.94-3.93 (m, 1H), 3.90 (dd, *J* = 11.4, 3.4 Hz, 1H), 2.86 and 2.29 (ABX system, *J* = 17.7, 10.8, 6.0 Hz, 2H), 0.82 (s, 9H), 0.040 (s, 3H), 0.021 (s, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 213.4, 168.0, 164.9, 155.7 (CH), 114.5, 82.4 (CH), 70.8 (CH), 62.5 ($CH_2$), 54.8 ($CH_3$), 53.9 ($CH_3$), 44.3 ($CH_2$), 25.7 ($CH_3$), 18.2, -5.5 ($CH_3$), -5.7 ($CH_3$); MS (ESI+) *m/z* (%) 369 ([M + H], 100); HRMS (ESI+, TOF) calcd for $C_{17}H_{29}N_2O_5Si$ [M + H]: 369.1846. Found: 369.1844.

**2,4-Dimethoxy-5-(2,3-dideoxy-3-oxo-β-D-ribofuranosyl)pyrimidine (11)**

To a solution of $PPh_3$ (0.5816 g, 2.22 mmol, 0.2 equiv) in DMF (22 mL) was added $Pd(OAc)_2$ (0.2502 g, 1.11 mmol, 0.1 equiv) at room temperature. The $Pd(OAc)_2$ solution was stirred under argon atmosphere at room temperature for 30 min. In a separate flask, to the solution of 2,4-dimethoxy-5-iodopyrimidine[29] (**8**, 5.8817 g, 22.11 mmol, 2.0 equiv) and furanoid glycal **4**[23] (3.8105 g, 11.06 mmol) in DMF (88 mL) was added triethylamine (2.4 mL, 17.21 mmol, 1.5 equiv) under argon atmosphere at room temperature. The 2,4-dimethoxy-5-iodopyrimidine solution was then added to $Pd(OAc)_2$ solution and the resulting mixture was stirred under argon atmosphere at 70 °C for 15 h. After cooling to room temperature, the solvent was evaporated under reduced pressure and the resulting residue (containing the product mixture of **9** and **10**) was used for the subsequent reaction without further purification.

To the solution of previous residue in THF (110 mL) were added AcOH (1.4 mL, 23.31 mmol, 2.0 equiv) and TBAF (1 M solution in THF, containing *ca.* 5% $H_2O$, 22 mL, 22.0 mmol, 2.0 equiv) at 0 °C. The solution was stirred overnight while the reaction temperature was allowed to rise to room temperature. Additional TBAF (1 M in THF, 11 mL, 11.00 mmol, 1.0 equiv) was added at 0 °C and the solution was stirred for additional 2 h while the reaction temperature was allowed to rise to room temperature. The solvent was evaporated under reduced pressure. The residue was dissolved in EtOAc and the solution was washed with $H_2O$ and saturated aqueous NaCl solution. The organic layer was dried over anhydrous $MgSO_4$ and then concentrated under reduced pressure. The residue was purified by flash column chromatography (Hex / EtOAc = 1 : 2, $R_f$ = 0.20) to give **11** (oil, 2.4834 g, 9.77 mmol, 88%). $^1$H NMR (400 MHz, $CDCl_3$) δ 8.34 (s, 1H), 5.20 (dd, *J* = 10.6, 6.2 Hz, 1H), 3.97 (s, 3H), 3.95 (s, 3H), 3.99-3.87 (m, 3H), 3.12 (bs, 1 H, OH), 2.81 and 2.54 (ABX system, *J* = 18.1, 10.8, 6.1 Hz, 2H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 213.5, 168.3, 165.1, 156.4 (CH), 113.4, 82.0 (CH), 71.9 (CH), 61.3 ($CH_2$), 54.9 ($CH_3$), 54.1 ($CH_3$), 43.0 ($CH_2$); MS (ESI+) *m/z* (%) 255 ([M + H], 100); HRMS (ESI+, TOF) calcd for $C_{11}H_{15}N_2O_5$ [M + H]: 255.0981. Found: 255.0981.

**2,4-Dimethoxy-5-(5-*O*-*tert*-butyldiphenylsilyl-2,3-dideoxy-3-oxo-β-D-ribofuranosyl)pyrimidine (12)**

To a mixture of **11** (2.5213 g, 9.92 mmol) and imidazole (1.4924 g, 21.92 mmol, 2.2 equiv) in $CH_2Cl_2$ (99 mL) at 0 °C was added dropwise *tert*-butylchlorodiphenylsilane (TBDPSCl, 3.4 mL, 12.37 mmol, 1.2 equiv). The reaction mixture was stirred overnight while the reaction temperature was allowed to rise to room temperature. The solvent was evaporated under reduced pressure. The residue was partitioned between $CH_2Cl_2$ and $H_2O$. The organic layer was washed with aqueous 1 N HCl solution, saturated aqueous $Na_2CO_3$ solution, saturated aqueous NaCl solution and dried over anhydrous $MgSO_4$. The solvent was evaporated under reduced pressure. The residue was purified by flash column chromatography (Hex / EtOAc = 4 : 1) to give **12** (oil, 4.7250 g, 9.59 mmol, 97%, $R_f$ = 0.45 (Hex / EtOAc = 2 : 1)). $^1$H NMR (400 MHz, $CDCl_3$) δ 8.54 (s, 1H), 7.71 (d, *J* = 6.0 Hz, 2H), 7.66 (d, *J* = 6.4 Hz, 2H), 7.44-7.36 (m, 6H), 5.27 (dd, *J* = 11.0, 5.8 Hz, 1H), 4.04-3.99 (m, 3H), 4.02 (s, 3H), 3.98 (s, 3H), 2.95 and 2.44 (ABX system, *J* = 17.4, 11.0, 5.8 Hz, 2H), 0.99 (s, 9H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 213.2, 168.0, 165.0, 155.6 (CH), 135.5 (CH), 135.4 (CH), 132.8, 132.6, 129.7 (CH), 127.7 (CH), 127.6 (CH), 114.2, 82.4 (CH), 71.0 (CH), 62.9 ($CH_2$), 54.8 ($CH_3$), 53.9 ($CH_3$), 44.3 ($CH_2$), 26.6 ($CH_3$), 19.1; MS (ESI+) *m/z* (%) 493 ([M + H], 100); HRMS (ESI+, TOF) calcd for $C_{27}H_{33}N_2O_5Si$ [M + H]: 493.2159. Found: 493.2159.

**Mixture of 2,4-dimethoxy-5-(2-deoxy-β-D-ribofuranosyl)pyrimidine and 3'-epimer (13)**

To a solution of **12** (3.7421 g, 7.60 mmol) in MeOH (76 mL) at 0 °C was added $NaBH_4$ (0.4389 g, 11.60 mmol, 1.5 equiv). The reaction mixture was stirred for 1 h till no starting material was detected by TLC while the reaction temperature was allowed to rise to room temperature. The reaction was quenched with saturated aqueous $NH_4Cl$ solution at 0 °C and the mixture was stirred for 10 min. The solvents were evaporated under reduced pressure and the residue was partitioned between EtOAc and saturated aqueous $NH_4Cl$ solution. The organic layer was washed with saturated aqueous NaCl solution and dried over anhydrous $MgSO_4$. The solvent was evaporated under reduced pressure. The residue was purified by flash column chromatography (Hex / EtOAc = 2 : 1, $R_f$ = 0.13 and 0.20) to give the product mixture of **13** (oil, 3.6205 g, 7.32 mmol, 96%). Diastereoisomeric mixture: $^1$H NMR (400 MHz, $CDCl_3$) δ 8.41 (s, 1H), 8.29 (s, 0.18H), 7.73-7.65 (m, 4H+0.72H, Ph), 7.47-7.37 (s, 6H+1.08H, Ph), 5.21 (dd, *J* = 10.0, 5.6 Hz, 0.18H), 4.97 (t, *J* = 7.4

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Hz, 1H), 4.60-4.55 (m, 1H), 4.51 (dt, *J* = 5.6, 2.7 Hz, 0.18H), 4.11 (dd, *J* = 11.0, 6.2 Hz, 1H), 4.05 (dd, *J* = 10.8, 4.0 Hz, 1H), 4.00 (dd, *J* = 3.9, 2.8 Hz, 0.18H), 3.98 (s, 3H, OMe), 3.97 (s, 3H, OMe), 3.97 (s, 0.54H, OMe), 3.95 (s, 0.54H, OMe), 3.94-3.90 (m, 1H), 3.85 and 3.69 (ABX system, *J* = 10.7, 6.4, 4.2 Hz, 0.36H), 3.08 (d, *J* = 4.4 Hz, 1H), 2.65 (ddd, *J* = 13.8, 7.8, 6.2 Hz, 1H), 2.30 (ddd, *J* = 13.2, 5.8, 2.2 Hz, 0.18H), 1.93 (ddd, *J* = 13.4, 6.6, 3.2 Hz, 2H+0.36H), 1.07 (s, 9H, *t*-butyl), 1.05 (s, 1.62H, *t*-butyl); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 167.9, 167.7, 164.5, 164.4, 155.7 (CH), 155.2 (CH), 135.4 (CH), 133.01, 132.96, 132.6, 132.5, 129.7 (CH), 129.6 (CH), 127.7 (CH), 127.6 (CH), 115.8, 115.3, 86.9 (CH), 81.7 (CH), 73.6 (CH), 73.3 (CH), 73.1 (CH), 72.6 (CH), 64.4 ($CH_2$), 62.8 ($CH_2$), 54.5 ($CH_3$), 53.7 ($CH_3$), 53.6 ($CH_3$), 41.6 ($CH_2$), 41.2 ($CH_2$), 26.6 ($CH_3$), 19.0, 18.9; MS (ESI+) *m/z* (%) 495 ([M + H], 100); HRMS (ESI+, TOF) calcd for $C_{27}H_{35}N_2O_5Si$ [M + H]: 495.2315. Found: 495.2316.

**2,4-Dimethoxy-5-(2,3-didehydro-2,3-dideoxy-β-D-ribofuranosyl)pyrimidine (15)**

To a solution of **13** (1.8260 g, 3.69 mmol) and $Et_3N$ (0.78 mL, 5.59 mmol, 1.5 equiv) in $CH_2Cl_2$ (37 mL) at 0 °C was added methanesulfonyl chloride (MsCl, 0.38 mL, 4.91 mmol, 1.3 equiv). The reaction mixture was stirred at room temperature for 1 h till no starting material was detected by TLC. The solvent was evaporated under reduced pressure and the residue was partitioned between EtOAc and $H_2O$. The organic layer was washed with saturated aqueous NaCl solution and dried over anhydrous $MgSO_4$. The solvent was evaporated under reduced pressure and the resulting residue ($R_f$ = 0.075, Hex / EtOAc = 4 : 1) was used for the subsequent reaction without further purification.

To the solution of previous residue in $CH_3CN$ (37 mL, 0.1 M) at 0 °C was added 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 10.5 mL, 68.97 mmol, 18.0 equiv). The reaction mixture was stirred at 75 °C for 48 h. After cooling to room temperature, the solvent was evaporated under reduced pressure and the residue was partitioned between EtOAc and $H_2O$. The organic layer was washed with saturated aqueous NaCl solution and dried over anhydrous $MgSO_4$. The solvent was evaporated under reduced pressure. The residue was purified by flash column chromatography (Hex / EtOAc = 1 : 2 ~ 1 : 4) to give **15** (oil, 0.4336 g, 1.82 mmol, 49%, $R_f$ = 0.35 (Hex / EtOAc = 1 : 4)). $^1$H NMR (400 MHz, $CDCl_3$) δ 8.20 (s, 1H), 5.97 (dt, *J* = 6.3, 1.9 Hz, 1H), 5.91 (ddd, *J* = 6.1, 2.3, 1.5 Hz, 1H), 5.87-5.85 (m, 1H), 4.94-4.95 (m, 1H), 3.98 (s, 3H), 3.94 (s, 3H), 3.73 and 3.63 (ABX system, *J* = 11.7, 4.6, 3.2 Hz, 2H), 2.53 (br s, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 168.5, 164.9, 156.7 (CH), 129.8 (CH), 128.1 (CH), 114.8, 87.2 (CH), 80.8 (CH), 64.7 ($CH_2$), 54.8 ($CH_3$), 54.0 ($CH_3$); MS (ESI+) *m/z* (%) 239 ([M + H], 100); HRMS (ESI+, TOF) calcd for $C_{11}H_{15}N_2O_4$ [M + H]: 239.1032. Found: 239.1031.

**2,4-Dimethoxy-5-(β-D-ribofuranosyl)pyrimidine[32] (17)**

To a mixture of **15** (0.0709 g, 0.30 mmol) and 4-methylmorpholine *N*-oxide (50 *wt*% in $H_2O$, 0.22 mL, 0.94 mmol, 3.0 equiv) in acetone (3.1 mL) at -20 °C was added dropwise $OsO_4$ (4.0 *wt*% in $H_2O$, 0.09 mL, 0.014 mmol, 0.050 equiv). The reaction mixture was stirred overnight while the reaction temperature was allowed to rise gradually to room temperature. The solvent was evaporated under reduced pressure and the residue was partitioned between $CHCl_3$ and saturated aqueous $Na_2S_2O_3$ solution. The organic layer was washed with saturated aqueous NaCl solution and dried over anhydrous $MgSO_4$. The solvent was evaporated under reduced pressure. The residue was purified by flash column chromatography ($CH_2Cl_2$ / MeOH = 20 : 1) to give two diastereoisomeric products **17** and **16** (oil, 0.0613 g, 0.23 mmol, 76%, **17** / **16** = 6.4 : 1, $R_f$ = 0.18 (more polar, the desired product, **17**) and 0.28 (less polar, the byproduct, **16**) ($CH_2Cl_2$ / MeOH = 15 : 1)).

**2,4-Dimethoxy-5-(β-D-ribofuranosyl)pyrimidine[32] (17)** (more polar product)

$^1$H NMR (500 MHz, $D_2O$) δ 8.24 (s, 1H), 4.89 (d, *J* = 5.0 Hz, 1H), 4.25 (t, *J* = 5.3 Hz, 1H), 4.14 (t, *J* = 5.8 Hz, 1H), 4.04 (dt, *J* = 5.8, 3.5 Hz, 1H), 3.99 (s, 3H), 3.95 (s, 3H), 3.91 and 3.79 (ABX system, *J* = 12.4, 5.5, 3.3 Hz, 2H); $^{13}$C NMR (125 MHz, $D_2O$) δ 168.9, 164.7, 155.7 (CH), 112.9, 83.0 (CH), 78.8 (CH), 74.5 (CH), 70.6 (CH), 61.4 ($CH_2$), 55.0 ($CH_3$), 54.3 ($CH_3$); MS (ESI+) *m/z* (%) 273 ([M + H], 100); HRMS (ESI+, TOF) calcd for $C_{11}H_{17}N_2O_6$ [M + H]: 273.1087. Found: 273.1088.

**2,4-Dimethoxy-5-(β-D-lyxofuranosyl)pyrimidine (16)** (less polar byproduct)

$^1$H NMR (400 MHz, $D_2O$) δ 8.25 (s, 1H), 5.02 (d, *J* = 4.0 Hz, 1H), 4.63 (t, *J* = 6.0 Hz, 1H), 4.44 (t, *J* = 4.6 Hz, 1H), 4.20 (dt, *J* = 6.2, 4.0 Hz, 1H), 3.96 (s, 3H), 3.95 (s, 3H), 3.89 and 3.81 (ABX system, *J* = 12.2, 5.8, 3.4 Hz, 2H); $^{13}$C NMR (100 MHz, $D_2O$) δ 168.2, 164.4, 155.5 (CH), 111.5, 79.4, 75.6 (CH), 72.0 (CH), 71.1 (CH), 60.4 ($CH_2$), 54.9 ($CH_3$), 54.3 ($CH_3$); MS (ESI+) *m/z* (%) 273 ([M + H], 100); HRMS (ESI+, TOF) calcd for $C_{11}H_{17}N_2O_6$ [M + H]: 273.1087. Found: 273.1087.

**Pseudouridine (Ψ, 1)**

To a mixture of **17** (0.0361 g, 0.13 mmol) in AcOH (3.3 mL) at room temperature was added NaI (0.0815 g, 0.54 mmol, 4.0 equiv). The reaction mixture was stirred at reflux temperature under argon atmosphere for 45 min till no starting material was detected by TLC. After cooling to room temperature, the solvent was evaporated under reduced pressure. The residue was purified by flash column chromatography ($CHCl_3$ / MeOH = 4 : 1) to give pseudouridine (Ψ, **1**, white solid, 0.0268 g, 0.12 mmol, 90%, ($R_f$ = 0.08, $CH_2Cl_2$ / MeOH = 4 : 1)). m.p. 219-222 °C;[9,14,15] $^1$H NMR (500 MHz, DMSO-$d_6$)[15,33] δ 7.52 (s, 1H), 4.46 (d, *J* = 4.5 Hz, 1H), 3.92 (t, *J* = 4.8 Hz, 1H), 3.86 (t, *J* = 5.3 Hz, 1H), 3.69 (dt, *J* = 5.5, 3.2 Hz, 1H), 3.59 and 3.44 (ABX system, *J* = 11.9, 3.7, 3.1 Hz, 2H); $^{13}$C NMR (125 MHz, DMSO-$d_6$)[15,34] δ 163.7, 151.3, 140.1 (CH), 111.0, 83.2 (CH), 78.9 (CH), 73.9 (CH), 70.4 (CH), 61.2 ($CH_2$); $^1$H NMR (500 MHz, $D_2O$)[11,33,35] δ 7.73 (d, *J* = 0.6 Hz, 1H), 4.74 (d, *J* = 5.6 Hz, 1H), 4.35 (t, *J* = 5.5 Hz, 1H), 4.20 (t, *J* = 5.4 Hz, 1H), 4.08 (dt, *J* = 5.3, 3.3 Hz, 1H), 3.90 (dd, *J* = 12.5, 3.2 Hz, 1H), 3.78 (dd, *J* = 12.5, 4.9 Hz, 1H); $^{13}$C NMR (125 MHz, $D_2O$)[11] δ 165.4, 152.9, 141.6 (CH), 110.5, 83.4 (CH), 79.1 (CH), 73.4 (CH), 70.9 (CH), 61.5 ($CH_2$); MS (ESI-) *m/z* (%) 243 ([M - H], 100); HRMS (ESI-, TOF) calcd for $C_9H_{11}N_2O_6$ [M - H]: 243.0617. Found: 243.0618.

## Conflicts of interest

There are no conflicts to declare.

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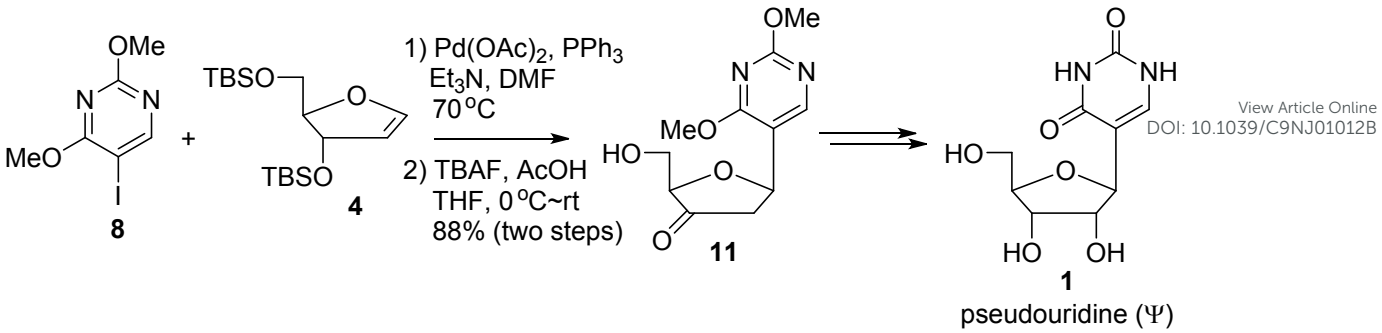


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Pseudouridine (**1**) was synthesized by functional group interconversions of the Heck adduct **11** from 2,4-dimethoxy-5-iodopyrimidine (**8**) and ribofuranoid glycal **4**.