Regioselective Mitsunobu Reaction of Partially Protected Uridine

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ABSTRACT
Mitsunobu reaction of partially acylated uridine proceeds with high regioselectivity for intramolecular $S_N2$ anhydro linkage closing. Under the reaction conditions, an isomeric mixture of diacyl uridine derivatives with either free 2'- or 3'-hydroxyl group was transformed into a single cyclonucleosidic product, 2,2'-anhydro-3',5'-di-O-acyluridine. This paper presents a possible mechanism of the reactions, the explanation of observed phenomenon based on semiempirical and density functional theory (DFT) calculations and possible utility of this synthetic pathway.

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Introduction

Functionalization of the 2',3'-cis-diole system in ribonucleosides is still a challenging and attractive topic, because nucleosides modified in positions C-2' and C-3' of the sugar moiety are extremely important in the search of new therapeutic agents, acting as DNA/RNA elongation terminators or antisense oligonucleotides. One of the possible ways to differentiate between the 2'- and 3'-hydroxyl groups is the application of anhydro nucleosides. Although they have been known for many years, their potential does not seem to be fully explored. The synthesis of 2,2'-anhydouridine was reported, for the first time, by Brown et al. in 1950's,\cite{1} and since then the preparation methods and synthetic usefulness of the anhydro nucleosides have been extensively studied. Because of their susceptibility to $S_N2$ substitution with a variety of nucleophiles, the anhydro synthons can be applied in the synthesis of nucleoside analogs with a modified sugar portion, e.g., 3'-azido-2',3'-dideoxythymidine (AZT)\cite{2} or 2'-O-methyluridine.\cite{3} Furthermore, the anhydro nucleosides can readily be transformed into xylofuranosyl and arabinofuranosyl derivatives through simple hydrolysis of an appropriate (2,3' or 2,2', respectively) anhydro linkage under acidic\cite{1,4} or basic conditions.\cite{5–7}

A number of synthetic methods for the preparation of anhydro nucleosides have been reported. In the ribonucleosides series, the method involving the application of diphenyl carbonate, originally developed by Hampton and Nichol as a result
of an attempted synthesis of uridine 2′,3′-cyclic carbonate, offers the best results in the formation of 2,2′-anhydro linkage. The method is simple, efficient (90% yield on kilogram scale), and does not require any protection of the sugar portion. In turn, the intramolecular cyclization reaction of 2′(3′)-tosyl, -mesyl, or -trifluoromethanesulfonyl substrates has commonly been applied for synthesis of the anhydro nucleosides in the both ribo and 2′-deoxyribo series. However, the latter method requires the use of protecting groups for the preparation of substrates. Its usefulness can be exemplified here by the synthesis of 2,3′-anhydro-2′-C-methyluridine or 2′,3′-bis-modified derivatives of uridine.

The third common approach to anhydro nucleosides involves a tandem Mitsunobu reaction and it is mainly applied for the 2′-deoxy series. The Mitsunobu reaction is widely used as a versatile regioselective method for the dehydrative condensation of pronucleophile with primary or secondary alcohols, and requires the combination of a reducing phosphine reagent together with an oxidizing azo reagent. The general mechanism of Mitsunobu reaction is well examined.

In this article, we would like to present an interesting regioselectivity, discovered during anhydrouridine synthesis under Mitsunobu conditions. We would like to propose a probable mechanism of this reaction, based on current state of knowledge, and the explanation of the observed regioselectivity, supported by molecular modeling, which has not been presented yet and gives rise to a better understanding of widely utilized synthesis of 2,2′-anhydrouridine with diphenyl carbonate.

Results and discussion

Mitsunobu reaction

Recently, 4-N,2′,5′-O,O-tripivaloylcytidine has successfully been obtained in crystalline form in good yields. In the case of uridine, a similar, partial acylation results in a mixture of diacylated 3′-hydroxyl and 2′-hydroxyl isomers. In presented experiment, a mixture of two dipivaloylated isomers was obtained (2a, 3a; Scheme 1) in a ratio of 4:1, respectively. The structure of the two isomers in a mixture was elucidated, and characteristic 1H NMR signals were assigned (Figure 1) with the COSY method supported with HSQC and HMBC techniques. Such a mixture was presented to Mitsunobu reaction. Both isomers could give anhydrocyclic derivatives and, because of substantial structure differentiation between 2,2′-anhydro-3′,5′-di-O-pivaloyluridine and 2,3′-anhydro-2′,5′-di-O-pivaloyluridine, their separation could be easy. However, Mitsunobu reaction performed on the isomeric mixture gave 2,2′-anhydro-3′,5′-di-O-pivaloyluridine (4a; Scheme 1; overall yield from uridine 56%), as a single product, which could readily be isolated as a pure precipitate from ether. Such a regioselectivity of Mitsunobu reaction has never been reported in the literature, what prompted us to further examine the nature of observed phenomenon. To prove its structure, the product (4a) was treated with NH3/MeOH, causing cleavage of pivaloyl groups, and then subjected to 2D NMR spectral analysis, which indicated that the observed compound was 2,2′-anhydrouridine (5).
Scheme 1. Reagents and conditions: (i) 2 eq. PivCl(a)/BzCl(b), Py, t < 5°C; (ii) DEAD, Ph3P, DMF, rt (4a 56% from uridine, 4b 68% from uridine); (iii) NH3/MeOH, rt (92%); (iv) DEAD, Ph3P, 60°C (23%).

The procedure was repeated with benzoyl protective group giving analogous result—the mixture of two dibenzoyl precursors 2b and 3b in a ratio 2:1 and only one product 2,2′-anhydro-3′,5′-di-O-benzoyluridine (Scheme 1), although with a better yield (68% from uridine). It suggests that Mitsunobu reaction leading to

Figure 1. Characteristic 1H NMR signals in the isomeric mixture of 2a and 3a.
Scheme 2. The proposed mechanism of regioselective formation of 4a–b.

3′,5′-bis-protected 2,2′-anhydrouridine may be of general character for acyl protective groups suitable for partial protection of nucleosides. Furthermore, in order to establish the regioselectivity of anhydro linkage formation, in the case of 2′,3′-O,O-unprotected substrate, we conducted the Mitsunobu reaction with 5′-O-(4,4′-dimethoxytrityl)uridine (6). As a result, we obtained a single product, 2,2′-anhydro-5′-O-(4,4′-dimethoxytrityl)uridine (7; 23%, Scheme 1). One can observe the influence of bulkiness of the DMT group in position O-5′ on the yield of 2,2′-anhydrouridine formation and the demand for energy. This result may indicate that the presented Mitsunobu reaction regioselectivity is not driven by the labile acyl protective group, but rather occurs despite its presence within a cis-diole system. The labile character of acyl protective groups, under the conditions of Mitsunobu reaction, is implied by the ratio of diacylated precursors, in favor of O2′,O5′ ones, however this does not interrupt the formation of only 2,2′-anhydro product in both situations.

The probable mechanism of the regioselective formation of 4a–b can be depicted (Scheme 2) analogously to the silyl migration in diol system reported recently. At the first stage, the proton from heterocyclic base residue is detached by 8 generating ion pairs (9 and 10a–b; 9 and 11a–b; Scheme 2), because \( pK_a \) values of hydroxyl groups in positions 2′ and 3′ are higher (\( pK_{aOH2′} = 12.7 \), \( pK_{aOH3′} = 13 \).
than amide group in uracil ($pK_a = 9.7$). The compound 8 can act as basic catalyst. In the presence of 8, compounds 2a–b and 3a–b are isomerizing, and anions 10a–b and 11a–b can isomerize as well, while the amount of 11a–b is depleting because of the formation of oxyphosphonium ion 13a–b. An alternative oxyphosphonium ion 14a–b can form as well. The observed product 4a–b is formed from 13a–b, in intramolecular $S_{N2}$ substitution. This is a crucial step because it is practically irreversible. The regioselectivity of the reaction could be caused by different factors—steric hindrance, electron density on the centers of nucleophilic attack, and geometrical feasibility of closing the anhydro bridge. To estimate the factor magnitudes, we have performed semiempirical and density functional theory (DFT) calculations.

Further reactions

We tested 2,2′-anhydro-3′,5′-di-O-pivaloyluridine (4a) for acidic hydrolysis and for substitutions with three different nucleophiles (Scheme 3). As it was summarized by Piccirilli et al., some nucleophiles substitute to a position C-2′ of a sugar moiety giving a ribose like product and some attack a position C-2 of nucleobase what results in C-2-substituted 1-(β-D-arabinofuranosyl)uracil derivatives. First substitution to 4a was performed with magnesium methoxide in absolute methanol and resulted with a good yield (84%), but it was followed by full deprotection of hydroxyl groups giving 2′-methoxyuridine (15). The synthesis of 2′-methoxyuridine from 2,2′-anhydouridine was presented inter alia by Roy and Tang with 92% yield. Second reaction of 4a was with lithium azide and gave a C-2′-substituted product with yield 72% (16), while reaction of 5 with the same nucleophile gave an expected product with yield 68% (17). Third nucleophile was generated from 4-methylthiophenol with catalytic amount of potassium phthalimide in DMF. In the reaction with 4a it is substituted to the C-2′ position giving product 18 with quantitative yield, however the analogous reaction with 5 occurred equally good (TLC), although standard purification gave some losses (19). The conducted substitutions indicate that 4a does not have any significant advantage over unprotected 5 in nucleophilic substitutions. The acidic hydrolysis of 4a occurred correctly with retention of protective groups giving a desired product 1-(3′,5′-di-O-pivaloyl-β-D-arabinofuranosyl)uracil (20) with yield 74%. Isolation and purification of 20 did not encounter any difficulties, because the migration of pivalic group within 2′,3′-trans-diole system could not take place. The configuration of sugar moiety was confirmed by NOESY. Finally, we wanted to answer an obvious question if straight acylation of 2,2′-anhydouridine (5) is a better way to obtain compounds like 4a or 4b. Shen et al. synthesized 4b by simple benzylation of 5 with 3 eq. of benzoyl chloride in room temperature with yield >88%. In the same manner, we have conducted pivaloylation of 5 obtaining 4a with yield 87%. Assuming 90% efficacy in obtaining 5 with diphenyl carbonate, yield from uridine would be 78% for 4a and >79% for 4b.
Calculations

Because of the size of the molecules, only carbon backbones of two anhydouridines were calculated with the DFT (B3LYP/6-31++G(d,p)) method. Elimination of neighboring side groups enabled the estimation of energetic cost of anhydro ring closure only. The calculated total energy figures are \(-644.863\) hartree for 2'-anhydro backbone (2'AB) and \(-644.852\) hartree for 3'-anhydro backbone (3'AB), which gives 6.9 kcal/mol difference in favor of 2'AB (Figure 2).
To find a rationale for the calculated energies, the analysis of intrinsic structural strains was made. Generally, sugar residue of $3'\text{AB}$ was more compressed and the lengths of diagonals of a furanose backbone total $d_{C3'-C1'} = 2.272 \text{ Å}$ and $d_{C4'-C2'} = 2.377 \text{ Å}$ for $3'\text{AB}$, whereas, for $2'\text{AB}$, they total $d_{C3'-C1'} = 2.428 \text{ Å}$ and $d_{C4'-C2'} = 2.392 \text{ Å}$. In the models of THF, constructed on the basis of crystallographic bond longitudes and the maximum and minimum size of the bond angles[16] the diagonals ranged from 2.370 Å to 2.426 Å. In the case of the $3'\text{AB}$ model, one diagonal was smaller and out of the range, while both diagonals were smaller than in the $2'\text{AB}$ model. The calculations might suggest that the geometric strains were the cause of the energy difference. However, comparison of the bond lengths of $2'\text{AB}$ and $3'\text{AB}$, computed with the DFT method, with literature data[16] and the bond angles with ideal values, did not reveal which deformation had a crucial role.

To estimate the electron effect on the described reaction, two simplified systems were taken into consideration. To compare which hydroxyl group is more susceptible to interaction with phosphonium intermediate (9), two structures of $2'$-fluorouridine and $3'$-fluorouridine were calculated with the DFT (B3LYP/6-31G(d,p)) method. The substitution with fluoride was done to eliminate the contribution of undesired $2',3'$ OH···O hydrogen bonds. The analysis of partial charges gave $\delta_{O2'} = -0.31 \text{ e}$ and $\delta_{O3'} = -0.33 \text{ e}$, which had a small but counteracting effect. Therefore, this factor was not responsible for the discussed regioselectivity. To estimate which center of nucleophilic attack was more preferable for the anhydro bridge closing, uridine $2',3'$-cyclic carbonate was optimized with the DFT (B3LYP/6-31G(d,p)) method. Uridine carbonate is an intermediate product of the reaction of uridine with diphenylcarbonate and sodium hydrogen carbonate, which only leads to $2,2'$-anhydouridine formation. The global minimum on potential energy surface for $2',3'$-cyclic carbonate corresponded to a structure with hydrogen bond between $5'$-OH and O-2 (Figure 3). The partial charges located in C-2' and C-3' within the calculated structure were equal ($+0.05 \text{ e}$). For the $2'$-fluorouridine and $3'$-fluorouridine, the comparison of partial charges on carbons bonded to hydroxyl
Figure 3. Optimized structure of uridine 2',3'-cyclic carbonate.

groups gave a similar result: for C-3' in 2'-fluorouridine $\delta_{C3'} = +0.03$ e, for C-2' in 3'-fluorouridine $\delta_{C2'} = +0.02$ e, and $\Delta\delta_{C3'/C2'} = 0.01$ e. Consequently, the electron density effect did not play any significant role in the observed regioselectivity.

Uridine carbonate is a relatively simple structure, which allowed to calculate the energy barrier of anhydro bridge closing, using the PM7 semiempirical method. The structure corresponding to the lowest local minimum without hydrogen bond was used as a starting point. The energy of a substrate was set at 0 kcal/mol, as the reference value. The calculated energy of the transition state for 2'-anhydro bridge closing ($\text{TS}_{2'}$) was lower by 8.5 kcal/mol than that of 3'-anhydro bridge ($\text{TS}_{3'}$), which is consistent with previous conclusions. Schematic illustration of potential energy surface for both, theoretically possible, reaction directions is presented in Figure 4. Furthermore, the example of regioselective reaction of uridine with diphenyl carbonate indicated that steric hindrance of side groups and large phosphonium intermediate was not a contributing factor in the presented Mitsunobu reaction either. However, the optimized structure of uridine carbonate displayed another factor that turned out to be relevant. The distances between the C2'-O2 and C3'-O2 atoms were 2.970 Å and 3.680 Å, respectively. For the intramolecular reaction, it is a significant determinant that controls the reaction kinetically, which explains the difference in the calculated transition states energy values. It is clear that the sugar puckering of uridine carbonate is unique, and the example has only an illustrative value. However, this effect should hold true meaning for the presented Mitsunobu reaction.

**Summary**

Our study presents the regioselective 2,2'-anhydro linkage formation in partially acylated uridine by the Mitsunobu reaction. The molecular modeling helped us to
comprehend the observed regioselectivity and suggested that geometrical feasibility of locking the anhydro bridge preferably to the C-2′ position, than to the C-3′ is a leading factor, responsible for different energy values of transition states and products. The effect seems to apply to all methods of generating 2,2′-anhydronucleosides, where both C-3′ and C-2′ are available. The observed regioselectivity is interesting, however, it has not escaped our notice, that presented synthetic solution probably will not find an application since an 2,2′-anhydouridine can be easily obtained with the Hampton and Nichol’s method with high yield, unprotected 2,2′-anhydouridine (5) undergoes substitutions equally good to 2,2′-anhydro-3′,5′-di-O-pivaloyluridine (4a) and straight acylation of 5 leads to 4a and 4b with better yields. Acidic hydrolysis of 4a allowed to obtain 1-(3′,5′-di-O-pivaloyl-β-D-arabinofuranosyl)uracil.

**Experimental section**

**Calculations**

Conformational analyses were made using the HyperChem 8.0 package\textsuperscript{[17]} by molecular mechanics in the AMBER force field. Geometry optimization calculations were performed, using the NWChem 6.1 package\textsuperscript{[18]} employing DFT (B3LYP/6-31++G(d,p)) and DFT (B3LYP/6-31G(d,p)) levels of theory. The IR spectra for all of the resulting structures were calculated to check if the obtained structures meet their corresponding energy minima. The Mulliken and Lowdin analyses were carried out in the GAMESS software\textsuperscript{[19]} at the DFT (B3LYP/6-31G(d,p)) level of theory, in order to calculate partial charges and assess bond

**Figure 4.** Schematic representation (energy vs. reaction coordinate) of two alternative anhydro linkage formation.
order in the optimized structures. Semiempirical calculations were performed using the HyperChem 8.0 package and MOPAC2012 package,\cite{20} employing the PM7 method.

**Chemistry**

All reagents were purchased from commercial suppliers and were used without further purification. 5’-O-(4,4’-dimethoxytrityl)uridine (6) was obtained according to Ref.\cite{21} Melting points were determined on a Laboratory Devices Mel-Temp II micromelting points apparatus (Laboratory Devices, Holliston, MA, USA) and remain uncorrected. The NMR spectra were recorded on a Bruker Avance 400 MHz spectrometer in DMSO-$d_6$ with tetramethylsilane as an internal standard: chemical shifts are reported in $\delta$-values (ppm) and coupling constants are given in Hz. For assigning the NMR signals to specific atom positions COSY, \textsuperscript{1}H–\textsuperscript{13}CH S Q C and \textsuperscript{1}H–\textsuperscript{13}C HMBC techniques were used. To establish sugar moiety structure of 1-(3’,5’-di-O-pivaloyl-$\beta$-D-arabinofuranosyl)uracil (20) NOESY technique was employed. Mass spectra (MS) were measured on a Bruker microTOF-Q spectrometer. The RP-HPLC analysis was performed on Waters 1525 Binary HPLC with Waters 2487 UV Detector on LiChrospher RP18–5 Endcapped C18 (5.0 $\mu$m) 4.6 mm $\times$ 250 mm column from Supelco Analytical using solvent system A—0.1 M triethylammonium acetate and B—A/acetonitrile 1:4 (v/v) and gradient B in A 60%–100% within 5 min, held 100% B for 8 min and then reversed gradient B in A 100%–60% within 5 min with a flow rate of 1 mL/min at 25°C. For compound 19, the RP-HPLC analysis was made using gradient B in A 5%–90% within 25 min. Thin-layer chromatography (TLC) was conducted on the Merck silica gel 60 F\textsubscript{254} plates, using the following solvent systems (measured by volume): C—dichloromethane-methanol (95:5), D—dichloromethane-methanol (9:1), E—dichloromethane-acetone (2:1), F—dichloromethane-methanol (4:1), G—dichloromethane-methanol (98:2), H—dichloromethane-acetone (4:1), I—dichloromethane-methanol (99:1). For preparative column chromatography, the Merck silica gel 60 (0.040–0.063 mm) was used along with the solvent systems mentioned above.

\textbf{2’,5’-Di-O-pivaloyluridine and 3’,5’-di-O-pivaloyluridine (2a,3a)}

Uridine (4.4913 g, 18.39 mmol) was added to dry pyridine (80 mL). The solution was concentrated to ca. 70 mL and cooled down to 0°C. Then, pivaloyl chloride (4.52 mL, 36.78 mmol) was added in nine portions at a temperature below 5°C, and the solution was maintained at 4°C for 18 hours. The solvent was coevaporated with toluene under diminished pressure. The resulting oil was dissolved in methylene chloride (80 mL) and washed with cold, saturated solution of NaHCO$_3$ (200 mL). The organic layer was separated, dried over Na$_2$SO$_4$, concentrated to thick oil and dried in vacuum. The product (6.9778 g) was analyzed with the NMR spectroscopy ($^1$H NMR and COSY), showing the presence of two isomeric compounds (2a,3a) in ratio 4:1, respectively.
2′,5′-Di-O-benzoyluridine and 3′,5′-di-O-benzoyluridine (2b,3b)

Uridine (1 g, 4.09 mmol) was added to dry pyridine (30 mL). The solution was concentrated to ca. 25 mL and cooled down to 0°C. Then, benzoyl chloride (0.950 mL, 8.18 mmol) was added in five portions at a temperature below 5°C, and the solution was maintained at 4°C for 18 hours. The solvent was coevaporated with toluene under diminished pressure. The resulting oil was dissolved in methylene chloride (80 mL) and washed with cold, saturated solution of NaHCO₃ (200 mL). The organic layer was separated, dried over Na₂SO₄, concentrated to thick oil, and dried in vacuum. The product (1.5728 g) was analyzed with the NMR spectroscopy (¹H NMR and COSY), showing the presence of two isomeric compounds (2b,3b) in ratio 2:1, respectively.

2,2′-Anhydro-3′,5′-di-O-pivaloyluridine (4a)

The obtained mixture of 2a and 3a and Ph₃P (5.3314 g, 20.33 mmol) was dissolved in dry DMF (100 mL) and concentrated to ca. 90 mL. Then, the DEAD (3.5363 g, 3.2 mL, 20.3 mmol) was added and the solution was stirred at room temperature for 21 hours. The solvent was evaporated under diminished pressure, and the residue was suspended in diethyl ether (80 mL). The white precipitate was collected and dried in vacuum yielding 4.074 g of the product (10.33 mmol, 56% from uridine). The product was 99% pure by RP-HPLC; m.p. 223–224°C; Rᵣ 0.42 (D); ¹H NMR: δ 7.89 (d, J = 7.6 Hz, 1H, H₆), 6.40 (d, J = 5.2 Hz, 1H, H₁′), 5.91 (d, J = 7.6 Hz, 1H, H₅), 5.49 (d, J = 5.6 Hz, 1H, H₂′), 5.32 (d, J = 2.4 Hz, 1H, H₃′), 4.46 (m, 1H, H₄′), 4.05 (dd, J = 4.8, 12.4 Hz, 1H, H₅′a), 3.94 (dd, J = 6.8, 12.4 Hz, 1H, H₅′b), 1.19 (s, 9H, (CH₃)₃), 1.08 (s, 9H, (CH₃)₃); ¹³C NMR: δ 177.3, 177.0 (2 × C=O), 171.2 (C₄), 159.8 (C₂), 137.1 (C₆), 109.4 (C₅), 90.1 (C₁′), 86.5 (C₂′), 82.5 (C₄′), 76.4 (C₃′), 62.4 (C₅′), 38.6 (2 × C(Me)₃), 27.1 ((CH₃)₃), 27.0 ((CH₃)₃). Mass (ESI-MS) m/z calcd for [C₁₉H₂₆N₂O₇ + H]⁺ 395.1813, found 395.1801, err 3.0 ppm.

2,2′-Anhydro-3′,5′-di-O-pivaloyluridine (4a from 5)

Dried 5 (2.150 g, 9.51 mmol) was dissolved in dry pyridine (35 mL), concentrated to ca. 32 mL and cooled down in ice bath. Then, the pivaloyl chloride was added in portions (3.4401 g, 28.53 mmol, 3.5104 mL). The mixture was allowed to warm up to rt and stirred overnight. The solvent was coevaporated with toluene, the residual solid dissolved in methylene chloride and extracted with saturated aqueous solution of NaHCO₃. Organic layer was dried over Na₂SO₄, concentrated, and the product was precipitated with diethyl ether. The crude product was contaminated with a compound of smaller Rᵣ—probably product of monoacylation. Therefore, it was subsequently purified by silica gel column chromatography in solvent system G giving 3.2698 g (8.29 mmol, 87%). The product was 96% pure by RP-HPLC, m.p. 223–224°C, NMR and MS data were consisted with those of 4a.

2,2′-Anhydro-3′,5′-di-O-benzoyluridine (4b)

The obtained mixture of 2b and 3b and Ph₃P (1.5029 g, 5.73 mmol) was dissolved in dry DMF (25 mL) and concentrated to ca. 20 mL. Then, the DEAD (0.9982 g,
0.9 mL, 5.73 mmol) was added and the solution was stirred at room temperature for 21 hours. The solvent was evaporated under diminished pressure, and the residue was suspended in diethyl ether (30 mL). The white precipitate was collected and dried in vacuum yielding 1.2137 g of the product (2.79 mmol, 68% from uridine). The product was 97% pure by RP-HPLC; m.p. 263 – 264°C; Rf 0.42 (D); 1H NMR: δ 8.05 (d, J = 7.2 Hz, 2H, Ph), 7.92 (d, J = 7.2 Hz, 1H, H6), 7.90 (d, J = 7.2 Hz, 2H, Ph), 7.72 (t, J = 7.2 Hz, 1H, Ph), 7.66 (t, J = 7.2 Hz, 1H, Ph), 7.58 (t, J = 7.6 Hz, 2H, Ph), 7.50 (t, J = 7.6 Hz, 2H, Ph), 6.49 (d, J = 5.6 Hz, 1H, H1′), 5.89 (d, J = 7.6 Hz, 1H, H5), 5.77 (d, J = 5.6 Hz, 1H, H2′), 5.71 (d, J = 2.8 Hz, 1H, H3′), 4.88 (m, 1H, H4′), 4.36 (m, 2H, H5′a,b); 13C NMR: δ 170.7 (C4), 165.2 (C=O), 164.9 (C=O), 159.4 (C2), 136.7 (C6), 134.0 (Ph), 129.6 (Ph), 129.2 (Ph), 129.0 (Ph), 128.8 (Ph), 128.7 (Ph), 128.6 (Ph), 109.0 (C5), 89.9 (C1′), 86.1 (C2′), 82.2 (C4′), 77.2 (C3′), 63.1 (C5′). Mass (ESI-MS) m/z calcd for [C23H18N2O7 + H]+ 435.1187, found 435.1184, err. −0.6 ppm.

2,2′-Anhydouridine (5) 4a (100 mg, 0.25 mmol) was dissolved in 2 mL of MeOH saturated with NH3. The solution was stirred for 3 hours at 0°C, the solvent was evaporated, and the product was purified on previously washed silica gel column in solvent system D. The column was washed with CH2Cl2:MeOH 1:1 v/v. The fractions containing the product were collected and evaporated under diminished pressure. The product was crystallized from DMF and flushed with MeOH resulting in 52.5 mg (0.23 mmol, yield 92%) of 5. The product was 99% pure by RP-HPLC; m.p. 242 – 243°C; Rf 0.27 (F); 1H NMR: δ 7.83 (d, J = 7.2 Hz, 1H, H6), 6.30 (d, J = 5.6 Hz, 1H, H1′), 5.87 (s, 1H, 3′OH), 5.84 (d, J = 7.6 Hz, 1H, H5), 5.20 (d, J = 5.6 Hz, 1H, H2′), 4.97 (s, 1H, 5′OH), 4.38 (s, 1H, H3′), 4.07 (t, J = 4.8 Hz, 1H, H4′), 3.28 (dd, J = 4.8, 11.6 Hz, 1H, H5′a), 3.19 (dd, J = 5.6, 11.2 Hz, 1H, H5′b); 13C NMR: δ 171.2 (C4), 159.8 (C2), 136.8 (C6), 108.6 (C5), 90.0 (C1′), 89.2 (C2′), 88.7 (C4′), 74.7 (C3′), 60.8 (C5′); Mass (ESI-MS) m/z calcd for [C9H10N2O5 + H]+ 227.0662, found 227.0666, err. −1.7 ppm.

2,2′-Anhydro-5′-O-(4,4′-dimethoxytrityl)uridine (7) Dried O5′-(4,4′-dimethoxytrityl)uridine (305.0 mg, 0.56 mmol) and Ph3P (204.6 mg, 0.78 mmol) were dissolved in dry DMF (12 mL) and concentrated to ca. 10 mL. Then, the DEAD (135.9 mg, 123 μL, 0.78 mmol) was added and stirred for 21 hours. The solvent was evaporated under diminished pressure and the mixture was purified on a silica gel column in solvent system C. The fractions containing the product were collected, evaporated, precipitated from toluene, flushed with hexane, and dried in vacuum resulting in 70.9 mg of 7 (0.13 mmol, yield 23%). The product was 98% pure by RP-HPLC; m.p. 129.5 – 130°C; Rf 0.34 (D); 1H NMR: δ 7.93 (d, J = 7.6 Hz, 1H, H6), 7.27 (m, 4H, Ph), 7.21 (m, 1H, Ph), 7.14 (dd, J = 2.8, 8.8 Hz, 4H, Ph), 6.83 (dd, J = 6.0, 8.8 Hz, 4H, Ph), 6.33 (d, J = 5.6 Hz, 1H, H1′), 5.98 (d, J = 4.8 Hz, 1H, 3′OH), 5.88 (d, J = 7.2 Hz, 1H, H5), 5.21 (d, J = 5.6 Hz,
1H, H2′), 4.30 (m, 1H, H3′), 4.23 (m, 1H, H4′), 2.94 (dd, J = 4.4, 10.4 Hz, 1H, H5′a), 2.81 (dd, J = 7.2, 10.4 Hz, 1H, H5′b); 13C NMR: δ 170.9 (C4), 159.3 (C2), 158.1 (Ph), 158.0 (Ph), 158.1 (Ph), 158.0 (Ph), 144.6 (Ph), 136.7 (C6), 135.2 (Ph), 135.1 (Ph), 129.5 (Ph), 129.4 (Ph), 127.8 (Ph), 127.4 (Ph), 126.7 (Ph), 113.2 (Ph), 108.87 (C5), 89.7 (C1′), 88.4 (C2′), 86.8 (C4′), 85.4 (C(MeOPh)2Ph), 74.7 (C3′), 62.8 (C5′), 55.0 (CH3O), 54.9 (CH3O); Mass (ESI-MS) m/z calcd. for [C30H28N2O7]+= 529.1969, found 529.1989, err. −3.7 ppm.

2′-Deoxy-2′-methoxyuridine (15)
Dried 4a (200 mg, 0.51 mmol) was added to freshly prepared solutions of magnesium methoxide (88.1 mg, 1.02 mmol) in absolute methanol (6 mL) with catalytic amount of iodine (6.5 mg, 0.051 mmol). The solution was refluxed for 5 hours. Then the mixture was cooled to room temperature and pH was neutralized by slow addition of glacial acetic acid. The solvent was evaporated under diminished pressure and refluxed with ethanol (5 mL) for 2 hours. The solid magnesium salts were filtered off and residual solvent was evaporated giving 120.1 mg of a crude product. Final purification was made by silica gel column chromatography with solvent system C. Product was obtained with yield 84% giving 111.2 mg (0.43 mmol). The product was 95% pure by RP-HPLC; m.p. 158–158.5 °C; Rf 0.75 (F); 1H NMR: δ 11.38 (s, 1H, NH), 7.94 (d, J = 7.6 Hz, 1H, H6), 5.85 (d, J = 4.8 Hz, 1H, H1′), 5.63 (d, J = 8.0 Hz, 1H, H5), 5.31 (bs, 2H, 3′OH, 5′OH), 4.12 (t, J = 4.8 Hz, 1H, H3′), 3.84 (dd, J = 2.8, 12.4 Hz, 1H, H5′a), 3.56 (dd, J = 3, 12.2 Hz, 1H, H5′b), 3.36 (s, 3H, CH3); 13C NMR: δ 163.25 (C2), 150.6 (C4), 140.34 (C6), 101.78 (C5), 85.96 (C1′), 85.05 (C4′), 74.7 (C3′), 68.12 (C5′), 60.34 (C2′), 57.48 (CH3); Mass (ESI-MS) m/z calcd. for [C10H13N2O6−]− 257.0776, found 257.0779, err. 1.1 ppm.

2′-Azido-2′-deoxy-3′,5′-di-O-pivaloyluridine (16)
Dried 4a (200 mg, 0.51 mmol) and LiN3 (49.9 mg, 1.02 mmol) were added to distilled and dried DMF (6 mL), which was then concentrated to ca. 5 mL. The solution was stirred in 135°C for 18 hours. Then the solvent was evaporated and the residual oil was separated by silica gel chromatography with solvent system E. The fractions with product were collected and the product was recrystallized from MeOH/H2O giving 160.5 mg (0.37 mmol) of white solid with yield 72%. The product was 95% pure by RP-HPLC; m.p. 115–116 °C, Rf 0.74 (D); 1H NMR: δ 11.50 (s, 1H, NH), 7.43 (d, J = 8.4 Hz, 1H, H6), 5.79 (d, J = 6.0 Hz, 1H, H1′), 5.72 (d, J = 8.0 Hz, 1H, H5), 5.32 (t, J = 6.0 Hz, 1H, H3′), 4.78 (t, J = 6.0 Hz, 1H, H2′), 4.23 (m, 3 H, H4′, H5′a, H5′b), 1.22 (s, 9H, 3′(CH3)3), 1.15 (s, 9H, 5′(CH3)3); 13C NMR: δ 177.11 (5′(C=O)), 176.61 (3′(C=O)), 162.90 (C4), 150.25 (C2), 140.81 (C6), 102.44 (C5), 87.60 (C1′), 79.14 (C4′), 71.22 (C3′), 62.71 (C5′), 62.23 (C2′), 38.37 (3′(CH3)3), 38.24 (5′(CH3)3), 26.76 (5′CH3Piv), 26.62 (3′CH3Piv); Mass (ESI-MS) m/z calcd. for [C19H27N5O7]− 436.1838, found 436.1859, err. −4.8 ppm.
2'-Azido-2'-deoxyuridine (17)

Dried 5 (200 mg, 0.88 mmol) and LiN$_3$ (86.2 mg, 1.76 mmol) were added to distilled and dried DMF (6 mL), which was then concentrated to ca. 5 mL. The solution was stirred in 135°C for 18 hours. Then the solvent was evaporated and the residual oil was separated by silica gel chromatography with solvent system C. The fractions with product were collected and the solvent was evaporated. The product was suspended twice in dichloromethane and dried on an evaporator. Any attempts to recrystallize failed. The product was a sticky, yellowish solid. After drying in vacuum it became more powdery yielding 161.0 mg (0.60 mmol, 68%), however melting point measurement was uncertain. The product was 95% pure by RP-HPLC; $R_f$ 0.40 (D); $^1$H NMR: $\delta$ 11.41 (s, 1H, NH), 7.86 (d, $J = 7.6$ Hz, 1H, H6), 5.96 (d, $J = 5.2$ Hz, 1H, H1'), 5.88 (d, $J = 5.6$ Hz, 1H, 3'OH), 5.67 (d, $J = 8.0$ Hz, 1H, H5), 5.17 (t, $J = 4.8$ Hz, 1H, 5'OH), 4.30 (dd, $J = 5.2$, 10.4 Hz, 1H, H2'), 4.05 (t, $J = 5.2$ Hz, 1H, H3'), 3.89 (dd, $J = 3.2$, 7.6 Hz, 1H, H4'); $^{13}$C NMR: $\delta$ 163.0 (C4), 150.4 (C2), 140.0 (C6), 102.0 (C5), 85.6 (C1'), 85.2 (C4'), 70.4 (C2'), 64.6 (C2'), 60.2 (C5); Mass (ESI-MS) $m/z$ calcd. for [C$_9$H$_{11}$N$_5$O$_5$+Na]$^+$ 292.0652, found 292.0662, err. $-$3.3 ppm.

2'-Deoxy-3',5'-di-O-pivaloyl-2'-[(4-tolylthio)uridine (18)

Dried 4a (200 mg, 0.51 mmol) and 4-methylthiophenol (126.7 mg, 1.02 mmol) were added to distilled and dried DMF (6 mL) and it was concentrated to ca. 5 mL. Then a potassium phthalimide was added (9.2 mg, 0.05 mmol) and the solution was stirred in 120°C for 4 hours. The reaction went quantitative (TLC). The solvent was evaporated and residual mixture was purified on silica gel column chromatography with solvent system I and crystallized from MeOH with water. After the purification product was obtained with yield 97% giving 256.7 mg (0.495 mmol). The product was 96% pure by RP-HPLC; m.p. 134.5–135°C, $R_f$ 0.69 (H); $^1$H NMR: $\delta$ 11.29 (s, 1H, NH), 7.39 (d, $J = 8.0$ Hz, 1H, H6), 7.26 (d, $J = 8.0$ Hz, 2H, Ph$^o$), 7.08 (d, $J = 8.0$ Hz, 2H, Ph$^m$), 6.11 (d, $J = 9.2$ Hz, 1H, H1'), 5.50 (d, $J = 8.0$ Hz, 1H, H5), 5.40 (d, $J = 6.0$ Hz, H1, H3'), 4.22 (m, 4H, H5'a, H5'b, H2', H4'), 2.23 (s, 3 H, CH3), 1.22 (s, 9H, 3'(CH$_3$)$_3$), 1.13 (s, 9H, 5'(CH$_3$)$_3$); $^{13}$C NMR: $\delta$ 177.10 (5'C=O), 176.20 (3'C=O), 162.39 (C4), 150.31 (C2), 139.51 (C6), 137.81 (Ph$^o$), 132.45 (Ph$^o$), 129.79 (Ph$^m$), 128.11 (Ph$^{C-S*}$), 102.64 (C5), 88.29 (C1'), 80.47 (C4'), 73.48 (C3'), 63.20 (C5'), 51.44 (C2'), 38.53 (3'C(CH$_3$)$_3$), 38.22 (5'C(CH$_3$)$_3$), 26.76 (CH$_3$)$_{3Piv}$), 20.55 (CH$_3$); Mass (ESI-MS) $m/z$ calcd. for [C$_{26}$H$_{34}$N$_2$O$_7$S + H]$^+$ 519.2159, found 519.2149, err. 1.9 ppm. *Abbreviations: “m”—meta, “or”—ortho, “p”—para, “C-S”—carbon bonded to sulfur in 4-tolylthio group.

2'-Deoxy-2'-(4-tolylthio)uridine (19)

Dried 5 (200 mg, 0.88 mmol) and 4-methylthiophenol (218.6 mg, 1.76 mmol) was added to distilled and dried DMF (6 mL) and it was concentrated to ca. 5 mL. Then a potassium phthalimide was added (16.7 mg, 0.09 mmol) and the solution was stirred in 120°C for 4 hours. The reaction was quantitative (TLC). The solvent was evaporated and residual mixture was purified on silica gel column chromatography with...
solvent system C and crystallized from MeOH with water. After the purification product was obtained with yield 82% giving 252.3 mg (0.72 mmol). The product was 99% pure by RP-HPLC; m.p. 209–210°C, Rf 0.55 (D); 1H NMR: δ 11.12 (s, 1H, NH), 7.55 (d, J = 7.6 Hz, 1H, H6), 7.22 (d, J = 8.0 Hz, 2H, Ph^or*), 7.04 (d, J = 8.0 Hz, 2H, Ph^m*), 6.16 (d, J = 9.2 Hz, 1H, H1'), 5.86 (d, J = 5.6 Hz, 1H, 3'OH), 5.44 (d, J = 8.0 Hz, 1H, H5), 5.10 (t, J = 5.2 Hz, 1H, 5'OH), 4.31 (t, J = 4.4 Hz, 2H, H5'a,b), 2.21 (s, 3 H, CH3); 13C NMR: δ 162.6 (C4), 150.5 (C2), 139.8 (C6), 137.0 (Ph^p*), 132.1 (Ph^or*), 129.6 (Ph^m*), 129.5 (Ph^C-S*), 102.3 (C5), 87.8 (C1'), 86.7 (C4'), 72.4 (C3'), 61.5 (C5',a,b), 20.5 (CH3); Mass (ESI-MS) m/z calcd. for [C16H18N2O5S+Na]^+ 373.0829, found 373.0840, err. -2.9 ppm.

Abbreviations: “m”—meta, “or”—ortho, “p”—para, “C-S”—carbon bonded to sulfur in 4-tolylthio group.

1-(3',5'-Di-O-pivaloyl-β-D-arabinofuranosyl)uracil (20)

4a (75.0 mg, 0.19 mmol) was dissolved in DMF (3 mL) and 2 mL of 2N HCl was added. The solution was heated to 80°C and after next 2.5 hours the reaction was neutralized with saturated NaHCO3aq, extracted with methylene chloride and purified by silica gel column chromatography with solvent system G. Product was crystallized from toluene giving 57.2 mg (0.14 mmol) with yield 74%. The product was 94% pure by RP-HPLC; m.p. 139–140°C, Rf 0.71 (D); 1H NMR: δ 11.37 (s, 1H, NH), 7.51 (d, J = 8.0 Hz, 1H, H6), 6.11 (d, J = 4.8 Hz, 1H, 2'OH), 6.00 (d, J = 4.0 Hz, 1H, H1'), 5.59 (d, J = 8.0 Hz, 1H, H5), 4.96 (s, 1H, H3'), 4.37 (m, 1H, H5'a), 4.22 (m, 1H, H5'b), 4.15 (s, 1H, H2'), 4.11 (s, 1H, H5'), 1.18 (s, 9H, (CH3)3), 1.16 (s, 9H, (CH3)3); 13C NMR: δ 177.2 (C=O), 176.5 (C=O), 163.1 (C4), 150.3 (C2), 141.7 (C6), 100.2 (C5), 85.6 (C1'), 79.5 (C4'), 78.0 (C3'), 72.4 (C2'), 62.9 (C5'), 26.8 (CH3^Piv'), 26.6 (CH3^Piv); Mass (ESI-MS) m/z calcd. for [C19H28N2O8+Na]^+ 435.1738, found 435.1725, err. 3.1 ppm.

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