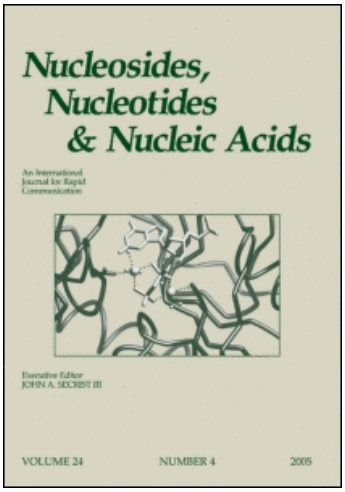

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Natalia Dyatkina[ab]; Alexander Khorlin[ab]; Kyoichi A. Watanabe[ac]
[a] Codon Pharmaceuticals, Inc., Gaithersburg, MD [b] Genelabs Technologies, Inc., Redwood City, CA [c] Pharmasset, Inc., Tucker, GA

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FORMATION AND REACTIVITY OF 2,4-DITRIAZOLYL PYRIMIDINE *C*-NUCLEOSIDE DERIVED FROM PSEUDOURIDINE

Natalia Dyatkina,[a] Alexander Khorlin*[a] and Kyoichi A. Watanabe[b]

Codon Pharmaceuticals, Inc., 200 Perry Parkway, Gaithersburg, MD 20877

ABSTRACT: Thymine and 2',3',5'-tri-*O*-acetyl-ψ-uridine (**1**) was converted into the corresponding 2,4-ditriazolyl derivatives **5** and **2**, respectively. Of these two substituents, the C4-triazolyl group was found to be quite susceptible to nucleophilic substitution while the other triazolyl is resistant.

In recent years, considerable attention has been directed towards the development of methods for substitution at the C4 position of the pyrimidine ring in nucleosides. A 1,2,4-triazol-1-yl substituent has been successfully introduced for modification by Reese.[1] The 4-(1,2,4-triazol-1-yl)nucleoside derived from thymidine, after incorporation into oligonucleotide, can be used as a convertible base for post-synthetic modification of oligomers.[2] We are interested in preparation of oligonucleotides containing C2-modified nucleotides. To our best knowledge, no convenient methods have been developed to prepare C2 substituted pyrimidine *N*-nucleosides. However, geometrical shapes of C2 substituted pyrimidine *N*-nucleosides are quite similar to that of C4 substituted *C*-nucleosides (FIG. **1**). During the course of our study to develop convertible bases from a *C*-nucleoside, we discovered that ψ-uridine has an interesting property, which we wish to report herein.

We investigated the behavior of tri-*O*-acetyl-ψ-uridine[3] (**1**) in the reaction with phosphoro-tris(1,2,4-trazolide) prepared *in situ* from $POCl_3$ and triazole in the presence of Et_3N.

Dedicated to the memory of Professor Alexander A. Krayevsky, 1932-1999.

Present address: a) Genelabs Technologies, Inc., 505 Penobscot Drive, Redwood City, CA 94063; b) Pharmasset, Inc., 1860 Montreal Road, Tucker, GA 30084

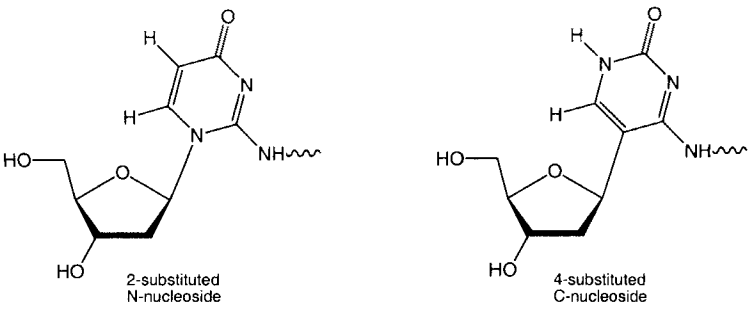
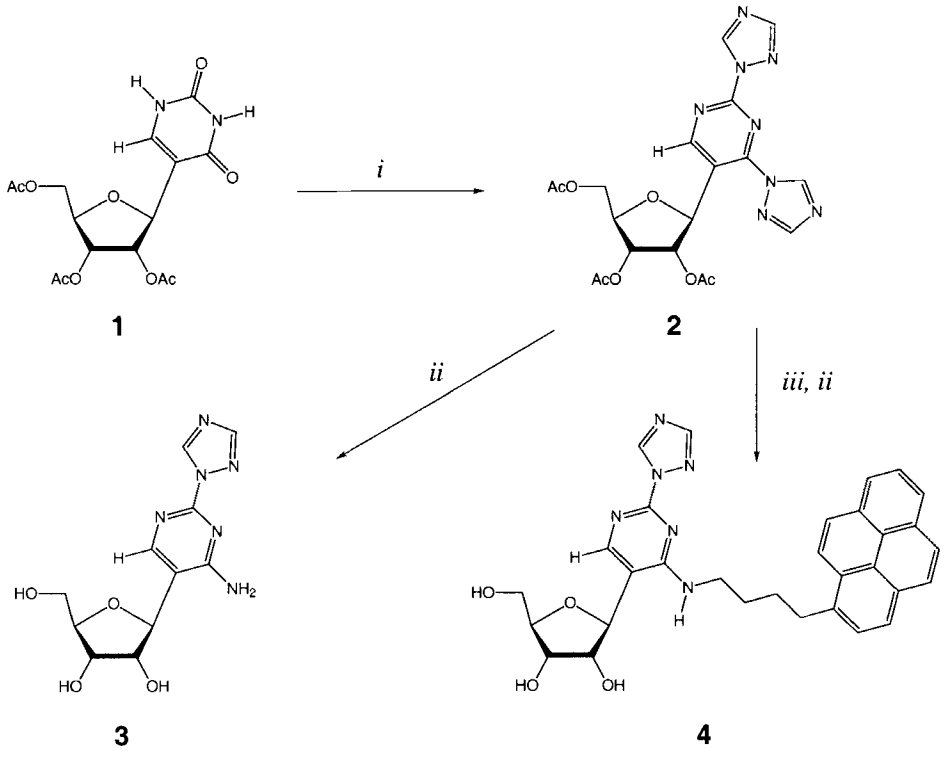


FIGURE 1



i; $POCl_3$ (6 eq.), 1,2,4-triazole (30 eq.), Et_3N, CH_3CN, r.t., 6h; *ii*; NH_4OH-MeOH (1:1 v/v), 65 °C, 4h; *iii*; 4-(pyren-1-yl)butylamine (4 eq.), pyridine-water (9:1 v/v), 70 °C, 48h

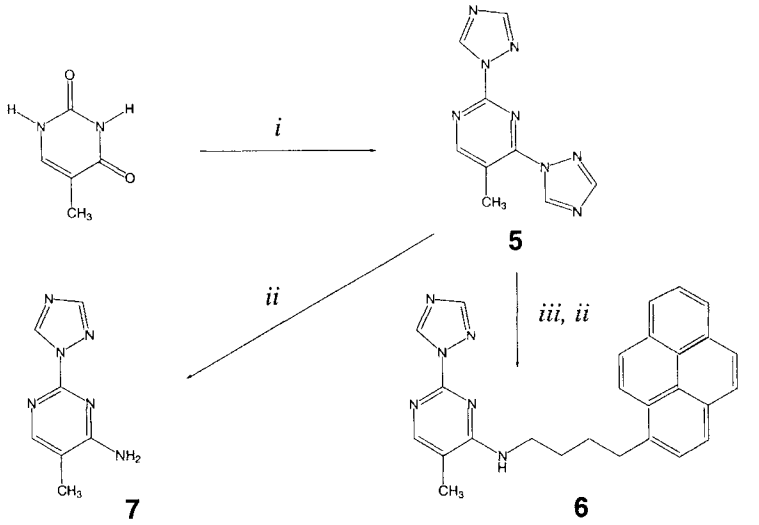
SCHEME 1

It was found that **1** was converted into disubstituted product **2** which was isolated in 70% yield, while the same treatment of N-nucleosides leads to only a mono-substituted product.[1] These two triazolyl groups in **2** exhibited different reactivity towards nucleophilic substitution. Thus, upon treatment with ammonia, **2** gave mono-triazolyl derivative **3** as evidenced by FAB-mass and ^{1}H-NMR-spectroscopic analyses. However, these data did not offer unequivocal assignment of the site of substitution.

In an attempt to establish the regiochemistry of the nucleophilic substitution, reaction of **2** with 4-(pyren-1-yl)butylamine,[4] was performed. 4-(Pyren-1-yl)butylamine was chosen because of the rising interest in nucleosides bearing intercalating groups as starting materials for synthesis of modified oligonucleotides. Again, only one of the two triazolyl groups participated in the reaction. The presence of an intact triazolyl group in product **4** was proven by FAB-mass and ^{1}H-NMR spectrometry. In the ^{1}H-NMR spectrum, the H-6 signal appears at 8.09 (a), exocyclic NH at 7.66 (b), H-1' at 4.60 (c), H-2' and H-3' at 4.10 (d), and neighboring CH_2-groups (e) at 3.68 ppm. When (b) was irradiated and NOE Difference Spectra (NOEDS) was recorded, NOE was observed for (c) –7.5%, (d) –8.65%, and (e) –9.8%. Irradiation of H-1' (c) exerted NOE (a), (b), and (d) by –17.8%; -4.4%, and –5.7%, respectively. These data clearly show the close proximity of the pyrenylbutylamino side chain and sugar ring protons, establishing the site of substitution at C4. Similarly, the reaction of **2** with ammonia led to regioselective introduction of an amino group at C4, thus allowing us to assign the structure of **3** as presented in SCHEME 1.

We anticipated that the discovered difference in reactivity between the two triazolyl groups in the pyrimidine ring of C-nucleoside is conserved and not influenced by modifications of the sugar moiety. In order to prove it, we chose thymine (which can be viewed as an analogue of ψ-uridine, where β-D-ribofuranosyl residue is replaced with methyl group) and performed the same sequence of reactions (SCHEME 2).

Triazolylation of thymine afforded, as expected, disubstituted product **5** which, upon treatment with 4-(pyren-1-yl)butylamine, was converted into a single product **6** containing one triazolyl and one 4-(pyren-1-yl)butylamino group according to FAB-mass and ^{1}H-NMR spectra. In the ^{1}H-NMR spectrum, the H-6 signal appears at 7.96 (a), exocyclic NH at 7.17 (b), the methylene groups at C4 of the pyrenylbutylamine and C5-methyl group appear at 3.60 (c) and 2.03 (d), respectively. In order to determine the position of pyrenylbutylamino substituent, NOEDS analyses were undertaken. Irradiation of (b) caused NOE on (c) and (d) by –10.4% and –13.0%, respectively. Irradiation of CH_3-signal (d) exerted NOE on (a) and (b) by –4.1% and –3.7%, respectively.These



i, ii, iii as in SCHEME 1

SCHEME 2

experiments firmly established the position of substitution at C4 of the pyrimidine ring.

When ammonia was used as the nucleophilic agent, again, only mono-substitution occurred in high yield. The product can be safely assigned to 4-amino-5-methyl-2-(1,2,4-triazol-1-yl)pyrimidine **7**.

In conclusion, triazolylation of thymine and ψ-uridine afforded the 2,4-disubstituted product in high yield. The triazolyl group on C4 is susceptible to nucleophilic substitution but the triazolyl group at C2 is resistant. Thus, selective modification at the C4 of pyrimidine *C*-nucleoside can be achieved *via* triazolylation.

EXPERIMENTAL

^{1}H-NMR spectra were recorded on a JEOL Eclipse 270 spectrometer, using $Si(CH_3)_4$ as the internal standard. Chemical shifts are reported in ppm (δ), and signals are described as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), and bs (broad singlet). Values given for coupling constants are first order if not mentioned otherwise.

FAB-mass spectra were obtained by M-SCAN, Inc. (Pennsylvania). Elemental analyses were performed by Galbraith Laboratories, Inc. (Knoxville, Tennessee). UV spectra were recorded on a Hewlett Packard 8452A spectrophotometer. Anhydrous solvents and silica gel (200-400 mesh 60A) for column chromatography were purchased from Aldrich. 4-(Pyren-1-yl)butylamine was prepared as described.[4]

Triazolylation. To an ice-chilled solution of 1,2,4-triazole (8.29 g, 120 mmol) in a mixture of CH_3CN (150 mL) and Et_3N (18.39 mL, 132 mmol) was added $POCl_3$ (3.10 mL, 33 mmol) dropwise with stirring. The mixture was left at room temperature for 40 min, then either a suspension of thymine (630 mg, 5 mmol) or a solution of nucleoside **1** (1.85 g, 5 mmol) in 20 mL of CH_3CN was added. The mixture was kept stirring for 10 h at room temperature, and filtered from insoluble materials which were washed with CH_3CN (3 x 10 mL). The combined filtrate and washings were concentrated *in vacuo*, and the residue was partitioned between saturated aqueous $NaHCO_3$ (50 ML) and EtOAc (30 mL). The aqueous layer was extracted with EtOAc (6 x 30 mL). The organic layers were combined, washed with brine (2 x 15 mL), dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography on silica gel. The column was eluted with EtOAc-hexane for **2** or wet EtOAc for **5**, and the appropriate fractions were combined, evaporated, and freeze-dried from benzene. This protocol was used to prepare the following compounds:

2,4-Di-(1,2,4-triazol-1-yl)-5-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-pyrimidine (2) was obtained as a white foam in 70% yield (1.65 g). UV (MeOH) λ_{max} (nm) 236, 282 (pH 7); 282 (pH 1); 246, 268 (sh) (pH 12). MS: m/z 473 ($M + H^+$). ^{1}H-NMR ($CDCl_3$): δ 9.35 (s, 1H, triazole), 9.34 (d, 1H, H-6), 9.26, 8.20, and 8.00 (3s, 1H each, triazole); 6.10 (d, 1H, H-1'), 5.50 (dd, 1H, H-2'), 5.12 (m, 1H, H-3'), 4.48-4.21 (m, 3H, H-4', H-5',5"), 2.18, 2.11, 2.08 (3s, 3H each, Ac). *Anal.* Calcd for $C_{19}H_{20}N_8O_7 \cdot 0.8$ EtOAc: C, 49.11; H, 4.91; N, 20.64. Found: C, 49.04; H, 4.97; N, 20.75. The presence of a small amount of EtOAc in the analytical sample was detected by ^{1}H-NMR.

2,4-Di-(1,2,4-triazol-1-yl)-5-methylpyrimidine (5) was obtained as a yellowish foarm in 57% yield (650 mg). The analytical sample was prepared by dissolving the foam in dioxane, and the solution filtered and lyophilized. UV (MeOH-H_2O) λ_{max} (nm): 236, 286 (pH 7); 236, 286 (pH 1); 214, 246, 266 (sh) (pH 12). MS: m/z 229 ($M + H^+$). ^{1}H-

NMR (DMSO-d_6): δ 9.78 and 9.75 (2s, 1H each, triazole); 9.02 (s, 1H, H-6); 8.47 and 8.36 (2s, 1H each, triazole); 2.65 (s, 3H, CH_3). *Anal.* Calcd for $C_9H_8N_8$0.3dioxane: C, 48.00; H, 4.11; N, 44.03. Found: C, 47.99; H, 4.17; N, 44.23.

Ammonolysis of ditriazolides. To a solution of triazolide **2** or **5** (0.5 mmol) in MeOH (3 mL) was added conc. NH_4OH (3 mL), and the mixture was heated in a pressure tube for 4 h at 65 °C. After cooling, the mixture was concentrated and the residue purified by chromatography on a silica gel column which was washed with $CHCl_3$-MeOH. The appropriate fractions were combined, evaporated, and freeze-dried from benzene for **3** or dioxane for **7** yielding the 4-amino derivative. The following compounds were prepared according to this protocol:

2-(1,2,4-Triazol-1-yl)-4-amino-5-(β-D-ribofuranosyl)pyrimidine (3) was obtained in 75% yield (108 mg) as a white foam. UV (MeOH-H_2O) λ_{max} (nm): 246, 268 (pH 1,7, 12). MS: m/z 295 (M + H^+). ^{1}H-NMR (DMSO-d_6): δ 9.23 and 8.21 (2s, 1H each, triazole); 8.15 (s, 1H, H-6); 4.55 (d, 1H, H-1', $J_{1',2'}$ = 7.5 Hz); 4.09-3.98 (m, 2H, H-3',4'); 3.90 (m, 1H, H-2'); 3.68-3.53 (m, 2H, H-5',5"). *Anal.* Calcd for $C_{12}H_{18}N_6O_4$0.8MeOH: C, 45.77; H, 6.36; N, 25.01. Found: C, 45.93; H, 6.12; N, 24.83.

2-(1,2,4-Triazol-1-yl)-4-amino-5-methylpyrimidine (7) was prepared in 87% yield (76 mg) as a white solid. UV (MeOH-H_2O) λ_{max} (nm): 222, 250, 286 (pH 7); 238, 260 (sh) (pH 1); 224, 250, 284 (pH 12). MS: m/z 177 (M + H^+). ^{1}H-NMR (DMSO-d_6): δ 9.18 and 8.19 (2s, 1H each, triazole); 8.01 (s, 1H, H-6); 7.30 (bs, 2H, NH_2); 2.03 (s, 3H, CH_3). The analytical sample was obtained by dissolving the product in dioxane, and the solution was filtered and lyophilyzed. *Anal.* Calcd for $C_7H_8N_6$0.6dioxane: C, 49.29; H, 5.65; N, 36.69. Found: C, 49.75; H, 5.54; N, 37.12.

2-(1,2,4-Triazol-1-yl)-4-[4-(pyren-1-yl)butylamino]-5-methylpyrimidine (6). To a suspension of ditriazolide **5** (114 mg, 0.5 mmol) and aminobutylpyrene (550 mg, 2 mmol) in 5 mL of pyridine was added H_2O (0.5 mL) to obtain a clear solution which was placed in a pressure tube and heated for 48 h at 70 °C. After cooling, the mixture was concentrated and the residue purified by chromatography on a silica gel column. The column was eluted with $CHCl_3$-MeOH (97:3 v/v), and the appropriate fractions were

combined, evaporated, and freeze-dried from benzene yielding **6** (166 mg, 79%) as a yellowish oil. UV (MeOH) λ_{max} (nm): 242, 254, 264, 276, 312, 326, 342. MS: m/z 433 ($M + H^+$). ^{1}H-NMR ($CDCl_3$): δ 9.09 (s, 1H, triazole); 8.23-7.81 (m, 11H, H-6, pyrene, and triazole); 4.75 (t, 1H, $N\underline{H}C_4H_8$); 3.58 (m, 2H, $NHC\underline{H}_2C_3H_6$); 3.39 (t, 2H, $NHC_3H_6C\underline{H}_2$); 2.02-1.91 (m, 5H, $NHC_2H_4C\underline{H}_2CH_2$ and CH_3); 1.87-1.71 (m, 2H, $NHCH_2C\underline{H}_2C_2H_4$). *Anal.* Calcd for $C_{27}H_{24}N_6 \cdot 0.25MeOH$: C, 74.29; H, 5.72; N, 19.07. Found: 74.03; H, 5.86; N, 18.93.

2-(1,2,4-Triazol-1-yl)-4-[4-(pyren-1-yl)butylamino]-5-(β-D-ribofuranosyl)-pyrimidine (4). Nucleoside **2** (0.5 mmol) was treated as described in the above protocol for **6**. After separation by column chromatography using EtOAc-hexane, the appropriate fractions were evaporated yielding a yellowish oil. MS: m/z 677 ($M + H^+$). *Anal.* Calcd for $C_{37}H_{36}N_6O_7 \cdot 2.0EtOAc$: C, 63.36; H, 6.14; N, 9.85. Found: C, 63.19; H, 6.21; N, 9.86. The oil was dissolved in a mixture of 3 mL each of MeOH and conc. NH_4OH, and the solution was heated in a pressure tube for 4 h at 65 °C. After cooling, the mixture was evaporated and the residue purified by flash chromatography on a silica gel column ($CHCl_3$-MeOH) to give **4** (154 mg, 73%) as a yellowish foam. UV (MeOH) λ_{max} (nm): 242, 254, 264, 276, 312, 326, 342. MS: m/z 551 ($M + H^+$). ^{1}H-NMR ($CDCl_3$): δ 9.28 (s, 1H, triazole); 8.35-7.92 (m, 11H, H-6, pyrene, and triazole); 4.75 (t, 1H, $N\underline{H}C_4H_8$); 4.60 (d, 1H, H-1', $J_{1',2'} = 7.8$ Hz); 4.10-4.03 (m, 2H, H-2',3'); 3.94 (m, 1H, H-4'); 3.72 –3.54 (m, 4H, H-5',5" and $NHC\underline{H}_2C_3H_6$); 3.39 (t, 2H, $NHC_2H_4CH_2C\underline{H}_2$); 2.96-1.76 (m, 4H, $NHCH_2C_2\underline{H}_4CH_2$).

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