Nucleosides and Nucleotides. 95. Improved Synthesis of 1-(2-Azido-2-deoxy-β-D-arabinofuranosyl)cytosine (Cytarazid) and -thymine. Inhibitory Spectrum of Cytarazid on the Growth of Various Human Tumor Cells in Vitro[1]

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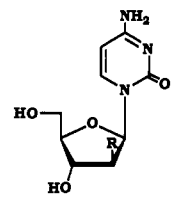
Reaction of N^3-benzoyl-1-[3,5-*O*-(tetraisopropyldisiloxan-1,3-diyl)-β-D-ribofuranosyl]thymine (**4a**) with diphenyl phosphorazidate, diethyl azodicarboxylate, and triphenylphosphine in tetrahydrofuran afforded N^3-benzoyl-1-[2-azido-2-deoxy-3,5-*O*-(tetraisopropyldisiloxan-1,3-diyl)-β-D-arabinofuranosyl]thymine (**5a**) in good yield. After the sequence of deblocking of **5a** gave 1-(2-azido-2-deoxy-β-D-arabinofuranosyl)thymine (**7**), it was heated in *N,N*-dimethylformamide to produce 6,2′-imino-1-(2-deoxy-β-D-arabinofuranosyl)thymine (**8**). This reaction disclosed the *arabino* configuration for **5a**. Similarly the N^3-benzoyluracil derivative **4b** was transformed to the corresponding 2′-"up"-azidouridine derivative **5b**, which was further converted to 1-(2-azido-2-deoxy-1-β-D-arabinofuranosyl)cytosine (**1**, cytarazid). The antineoplastic activity of **1** was compared with that of *ara*-C against various human cancer cell lines in vitro.

1-(2-Azido-2-deoxy-β-D-arabinofuranosyl)cytosine (cytarazid, **1**) was introduced as a cytidine deaminase resistant 1-(β-D-arabinofuranosyl)cytosine (*ara*-C) analogue by Bobek.[2,3] Although *ara*-C (**2**, Chart I) is one of the most effective drugs for the treatment of human acute myeloblastic leukemia,[4-6] its usefulness is limited by several drawbacks: a short half-life time in plasma due, in part, to deamination to inactive 1-β-D-arabinofuranosyluracil by cytidine deaminase, development of resistance, and ineffectiveness on solid tumors.[7-9] To overcome these problems, a large number of prodrugs[10] of *ara*-C have been synthesized and other substituents introduced into the 2′-*arabino* position in place of the hydrogen atom of 2′-deoxycytidine. A product of the latter approach, cytarazid (**1**), is a potent inhibitor of mouse leukemic L1210, human T-cell acute lymphoblastic leukemia Molt 4F, and HeLa cells in vitro and is resistant to cytidine deaminase.[2,3,11] Also **1** is a potent antileukemic agent against L1210 in vivo when administered at 40 mg/kg twice daily for 2 days; long-term survivors (over 120 days) were observed. The original method for the synthesis of **1**, however, involved a multistep synthesis of anomeric 3-*O*-acetyl-5-*O*-benzoyl-2-azido-2-deoxy-D-arabinofuranosyl chlorides from D-glucose to be condensed with a silylated cytosine.[2,12,13] The desired β-nucleoside was obtained in only 38.7% yield by this method, along with the formation of the α-anomer (9.4%) in the condensation step. Moreover, an overall yield of **1** from 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose was 0.04%. Therefore, further biological evaluation of **1** depends on the availability of the compound.

It seems worthwhile to develop a synthetic method from readily accessible pyrimidine nucleosides such as uridine or cytidine. However, it has been generally recognized that the intramolecular nucleophilic attack of the 2-carbonyl group of the pyrimidine base on the 2′-position having a leaving group in such a pyrimidine nucleoside is predominant, rather than the intermolecular nucleophilic substitution. For example, treatment of 1-[3,5-*O*-(tetraisopropyldisiloxan-1,3-diyl)-β-D-ribofuranosyl]uracil (**3b**) with triflic anhydride afforded the 2,2′-anhydrouridine derivative exclusively as an isolable product.[14] Loibner and Zbiral reported that 1-(3-azido-3-deoxy-β-D-xylofuranosyl)uracil was obtained when 2′,5′-di-*O*-trityluridine was treated with a combination of hydrogen azide, triphenylphosphine, and diethyl azodicarboxylate, without formation of the 2,3′-anhydrouridine derivative.[15] However when this procedure was applied to 3′,5′-di-*O*-trityluridine, the product was the 2,2′-anhydrouridine derivative, and the desired 2′-azido-2′-deoxy-β-D-arabinofuranosyluracil derivative was not obtained at all.

If the nucleophilicity of the 2-carbonyl oxygen could be reduced, the direct S_N2 reaction at the 2′-position of uridine derivatives would be realized. We have found a

Chart I



1; R = N_3
2; R = OH

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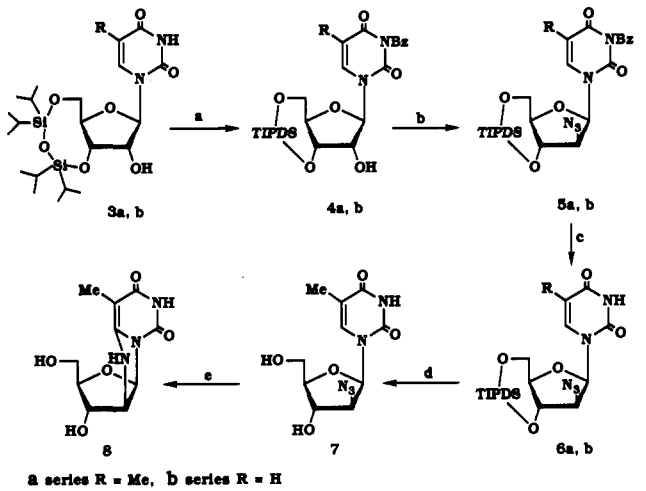
Scheme I[a]

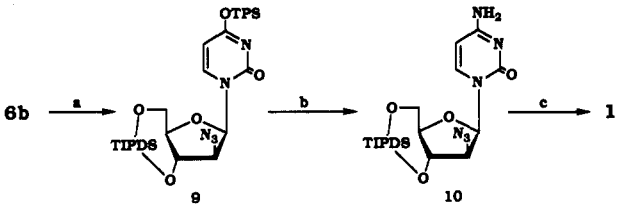


[a] (a) BzCl, Et_3N in CH_2Cl_2 for **4a**, BzCl, $Et_4N^+Br^-$ in aqueous Na_2CO_3/CH_2Cl_2 for **4b**; (b) DPPA, Ph_3P, diethyl azodicarboxylate, THF or HN_3, Ph_3P, diethyl azodicarboxylate, THF; (c) concentrated NH_4OH, MeOH; (d) TBAF, THF; (e) Δ, DMF.

benzoyl group to be an effective choice for N^3-protection, which would reduce the nucleophilicity of the 2-carbonyl group.[17] In this paper, we describe a new improved method for the introduction of an azido group to the 2′-"up"-position of uridine and 5-methyluridine derivatives and its conversion to **1**. We also include inhibitory effects of **1**, compared with that of *ara*-C, on the growth of various tumor cell lines in vitro.

Our starting material for this investigation, N^3-benzoyl-1-(3,5-*O*-TIPDS-β-D-ribofuranosyl)thymine (**4a**, TIPDS = tetraisopropyldisiloxan-1,3-diyl) was prepared from the corresponding TIPDS-5-methyluridine **3a** (Scheme I). Treatment of **3a** with benzoyl chloride in a mixture of triethylamine in dichloromethane afforded **4a** in 61% yield. When **4a** was treated with hydrogen azide, triphenylphosphine, and diethyl azodicarboxylate in tetrahydrofuran at room temperature, one nucleosidic product (**5a**), which shows an azide stretching at 2120 cm^{-1} in its infrared spectrum (IR), was obtained in 60% yield after purification by silica gel column chromatography. Its mass spectral data has a molecular ion peak at m/z 629. The ^1H NMR spectrum of this nucleoside showed a 1′-proton at 6.26 ppm as a doublet ($J_{1',2'}$ = 6.3 Hz) while the 1′-proton of **4a** appeared at 5.75 ppm as a narrow doublet ($J_{1',2'}$ = 0.7 Hz), and a lower field shift of the 2′-proton in **5a** was also observed. Although this implies that an azide group was introduced into the 2′-position, the configuration of the 2′-position could not be identified at this stage. If this azido group is introduced into the 2′-"up"-position via intermolecular nucleophilic displacement, this could cyclize to the C-6 position of the base moiety to form a 6,2′-imino-bridged nucleoside through an intermediacy of an 8-azapurine derivative.[17,18] Debenzoylation of **5a** with concentrated NH_4OH in MeOH gave a crystalline product (**6a**), which was then treated with tetrabutylammonium fluoride (TBAF) in THF to furnish 1-(2-azido-2-deoxy-β-D-arabinofuranosyl)thymine (**7**)[19] in 90% from **5a** as colorless crystals. When compound **7** was heated with NaN_3 in *N*,*N*-dimethylformamide (DMF) at reflux temperature,[17] the product obtained was identified as 6,2′-imino-1-(2-deoxy-β-D-arabinofuranosyl)thymine (**8**)[19] on the basis of the following data. The ^1H NMR spectrum of **8** showed the absence of H-6, indicating substitution at C-6 of the thymine aglycon. The overall ^1H NMR spectral pattern is akin to that of *N*-methyl-6,2′-imino-1-(2-deoxy-β-D-arabinofuranosyl)uracil.[20] These data, together with elemental analyses and mass spectroscopic data, are consistent with the *N*-6,2′-imino-bridged structure. Thus, the structure of azide nucleoside **7** is established as 1-(2-azido-2-deoxy-β-D-arabinofuranosyl)thymine. It is obvious that the reaction of N^3-benzoylthymine nucleoside **4a** with hydrogen azide brought about the S_N2 manner at the 2′-position and the N^3-benzoyl group could prevent the intramolecular nucleophilic attack of the 2-carbonyl group to the 2′-position to form the 2,2′-anhydro linkage. This is the first example of introduction of a nucleophile by intermolecular nucleophilic substitution to the 2′-"up"-position of pyrimidine nucleosides bearing a 2-carbonyl group.

Scheme II[a]



[a] (a) TPSCl, $Et_4N^+Br^-$ in Aqueous $NaHCO_3/CH_2Cl_2$; (b) concentrated NH_4OH, dioxane; (c) TBAF, THF.

Instead of using the highly toxic and explosive hydrogen azide, diphenyl phosphorazidate (DPPA) could replace it as an azide source. When **4a** was treated with DPPA, triphenylphosphine, and diethyl azodicarboxylate in THF at room temperature, **5a** was similarly obtained in 70% yield.

To synthesize **1** (Scheme II), we started from N^3-benzoyl-1-(3,5-*O*-TIPDS-β-D-ribofuranosyl)uracil (**4b**), which was prepared from 1-(3,5-*O*-TIPDS-β-D-ribofuranosyl)uracil (**3b**) by benzoyl chloride under the phase-transfer conditions.[21] Treatment of **4b** with DPPA, triphenylphosphine, and diethyl azodicaboxylate gave the desired 2′-"up"-azidouridine derivative **5b** in 74% yield. After debenzoylation of **5b** with NH_4OH in MeOH, **6b** was obtained as colorless crystals in 94% yield. Compound **6b** was converted into O^4-triisopropylbenzenesulfonate derivative **9** under phase-transfer conditions[21] and this was, without purification, treated with NH_4OH in dioxane to afford cytosine derivative **10** in 83% yield from **6b**. Deblocking of **10** with TBAF in THF furnished **1** in quantitative yield as a HCl salt. Thus, cytarazid was synthesized in 42% overall yield in six steps from uridine and this method is superior to the previous condensation procedure.

We compared the in vitro cytotoxic spectrum of cytarazid with that of *ara*-C in the various human tumor cell lines including eight osteosarcomas, three lung adenocarcinomas, three lung carcinomas, three stomach adenocarcinomas, a bladder transitional-cell carcinoma, a renal cell carcinoma, a malignant fibrous histiocytoma, and a fibrosarcoma. As summarized in Table I, cytarazid was

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Table I. Inhibitory Effects of Cytarazid and *ara*-C on the Growth of Various Human Tumor Cell Lines in Vitro[a]

human cell line	IC_{50},[b] μg/mL	
	cytarazid (1)	*ara*-C (2)
MG-63[c]	30.0	29.5%[m]
OST[c]	6.4	48.0%[m]
HOS[c]	9.4	45.4%[m]
KHOS-321H[c]	0.62	0.27
SK-ES-1[c]	1.1	0.09
MNNG-HOS[c]	1.5	2.6
U20-S[c]	14.0	43.9%[m]
SAOS-2[c]	2.8	2.7
PC-3[d]	18.0	46.2%[m]
PC-8[d]	1.2	0.28
PC-9[d]	1.0	1.6
PC-13[e]	18.0	40.0%[m]
QG-56[f]	5.4	24.4%[m]
PC-6[g]	18.0	50.0
ST-KM[h]	1.1	48.6%[m]
NUGC-4[h]	1.4	49.9%[m]
MKN-28[h]	2.2	4.5
T-24[i]	0.56	0.5
NCC-nu[j]	1.8	0.13
MFH-ST[k]	14.0	47.9%[m]
HT-1080[l]	1.6	0.13

[a] Antineoplastic activity assay in vitro was determined by using human tumor cells. Roswell Park Memorial Institute medium 1640 supplemented with 10% heat-inactivated fetal bovine serum and 50 μg/mL of kanamycin was used as the cell cultured medium. Tumor cells (1×10^4 cells/mL) were cultured in a CO_2 gas incubator at 37 °C for 72 h in 1 mL of medium containing various concentrations of test compound. Their viability, estimated by use of a variation of a colorimetric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay,[22] was compared to that of control cells incubated in the identical medium without the compound and OD (570 nm) was measured. Percent inhibition was calculated as follows: % inhibition = [1 − OD(570 nm) of sample well/OD(570 nm) of control well] × 100. The results are representative of three separate experiments. [b] IC_{50} values were given as the concentration in μg/mL required for 50% inhibition of cell growth. [c] Human osteosarcoma. [d] Human lung adenocarcinoma. [e] Human lung large-cell carcinoma. [f] Human lung squamous-cell carcinoma. [g] Human lung small-cell carcinoma. [h] Human stomach adenocarcinoma. [i] Human bladder transitional-cell carcinoma. [j] Human renal cell carcinoma. [k] Human malignant fibrous histiocytoma. [l] Human fibrosarcoma. [m] Growth inhibition rate (%) at 100 μg/mL.

cytotoxic to all 21 tumor cell lines; it had potent cytotoxicity in 12 cell lines with the concentrations required for 50% inhibition of cell growth (IC_{50} values) from 0.62 to 2.8 μg/mL and moderately cytotoxicity to nine cell lines having IC_{50} values from 5.4 to 30 μg/mL. On the other hand, *ara*-C had good cytotoxicity to only 10 of the cell lines (0.09–4.5 μg/mL) tested in this study. It is important to note that cytarazid is a potent inhibitor of growth of human stomach adenocarcinomas, the incidence of which is high in Japan. Cytarazid is an interesting and promising agent which should be considered for further detailed preclinical evaluation.

Cytarazid has proved resistant to deamination[2,11] by human cytidine deaminase, but it serves as a substrate for human deoxycytidine kinase.[11] Cytarazid 5′-triphosphate inhibits α- and β-DNA polymerases from HeLa cells at rather lower concentrations than *ara*-C 5′-triphosphate.[11] Therefore, it was proposed that the balance between susceptibility to deamination and the rate of phosphorylation probably contributes to the differential action of these nucleosides and the inhibition of DNA synthesis due to interference with DNA polymerase is the primary effect of cytarazid action. The different spectrum of cytotoxicity against various human solid tumor cells between those nucleosides might be also related to susceptibility to deamination. Our new method for synthesizing cytarazid could warrant further biochemical studies to elucidate these differences.

Experimental Section

Melting points were measured on a Yanagimoto MP-3 micro melting point apparatus and are uncorrected. The ^1H NMR spectra were recorded on a JEOL FT100FT or FX-270FT spectrometer and tetramethylsilane as an internal standard. Chemical shifts are reported in parts per million (δ), and signals are expressed as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), or br (broad). All exchangeable protons were detected by addition of D_2O. UV absorption spectra were recorded with a Shimadzu UV-240 spectrophotometer. Mass spectra (MS) were measured on a JEOL JMX-DX303 spectrometer. TLC was done on Merck Kieselgel F254 precoated plates. Silica gel used for column chromatography was YMC gel 60A (70–230 mesh).

N^3-Benzoyl-1-(3,5-*O*-TIPDS-β-D-ribofuranosyl)thymine (4a). Benzoyl chloride (0.64 mL, 5.5 mmol) was added to a solution of **3a** (2.5 g, 5 mmol) and triethylamine (0.9 mL, 6.5 mmol) in dry CH_2Cl_2 (40 mL) at 0 °C under Ar. The mixture was stirred overnight at room temperature and was diluted with $CHCl_3$, which was successively washed with 10 mL each of 0.01 N HCl, H_2O, saturated aqueous $NaHCO_3$, and H_2O. The separated organic phase was dried (Na_2SO_4) and the solvent was removed under reduced pressure. The residue was purified over a silica gel column (2 × 23 cm) eluted with hexane–EtOAc (5:1 then 2:1), giving **4a** (1.84 g, 61%, as a foam). Elution of the column with EtOAc recovered the starting material **3a** (500 mg, 20%). EI-MS: *m/z* 605 (M^+ + H), 561 (M^+ − *i*Pr). ^1H NMR ($CDCl_3$): δ 1.05–1.11 (m, 28 H, *i*Pr), 1.96 (d, 3 H, 5-Me, $J_{Me,6}$ = 1.2 Hz), 2.83 (br s, 1 H, 2′-OH), 4.06–4.23 (m, 5 H, 2′,3′,4′,5′,5″-H), 5.75 (d, 1 H, 1′-H, $J_{1',2'}$ = 0.7 Hz), 7.48–7.97 (m, 6 H, benzoyl + 6-H).

N^3-Benzoyl-1-(2-azido-2-deoxy-3,5-*O*-TIPDS-β-D-arabinofuranosyl)thymine (5a). (a) A solution of diethyl azodicarboxylate (2.3 mL, 15 mmol) in THF (5 mL) was added dropwise to a solution of **4a** (1.21 g, 2 mmol) and triphenylphosphine (786 mg, 3 mmol) in THF (10 mL) at 0 °C. A benzene solution of NH_3 [2.4 mL, prepared from concentrated H_2SO_4 (2.8 mL) and NaN_3 (6.5 g, 0.1 mol) in a mixture of H_2O (6 mL) and benzene (40 mL)] was then added to the above solution. The mixture was stirred for 15 h at room temperature. After addition of EtOH (2 mL), the mixture was concentrated in vacuo and the residue was purified over a silica gel column (3 × 37 cm) eluted with hexane–EtOAc (5:1) to afford **5a** (753 mg, 60%, crystallized from Et_2O–hexane). (b) A mixture of diethyl azodicarboxylate (2.3 mL, 15 mmol) and diphenyl phosphorazidate (3.23 mL, 15 mmol) in THF (15 mL) was added dropwise over 30 min to a solution of **4a** (3 g, 5 mmol) and triphenylphosphine (3.9 g, 15 mmol) in THF (60 mL) at 0 °C under Ar. The mixture was stirred for 4 h at room temperature. After addition of EtOH (3 mL), the solvent was concentrated to dryness in vacuo. The residue was purified over a silica gel column (4.5 × 23 cm) eluted with benzene–EtOAc (60:1 then 40:1) to afford **5a** (2.2 g, 70%, crystallized from Et_2O–hexane). Mp: 124–126 °C (eff). EI-MS: *m/z* 629 (M^+), 601 ($M^+ - N_2$), 588 ($M^+ - N_2$ − *i*Pr). IR (Nujol): 2120 cm^{-1} (N_3). ^1H NMR ($CDCl_3$): δ 1.11 (m, 28 H, *i*Pr), 1.97 (d, 3 H, 5-Me, $J_{Me,6}$ = 1.0 Hz), 4.11–4.31 (m, 5 H, 2′,3′,4′,5′,5″-H), 6.26 (d, 1 H, 1′-H), $J_{1',2'}$ = 6.3 Hz), 7.40–7.99 (m, 6 H, benzoyl + 6-H).

1-(2-Azido-2-deoxy-3,5-*O*-TIPDS-β-D-arabinofuranosyl)thymine (6a). Concentrated NH_4OH (12 mL) was added to a solution of **5a** (1.76 g, 2.79 mmol) in MeOH (30 mL) at 0 °C. The mixture was stirred for 2 h at room temperature, then the solvent was removed in vacuo. The residue was purified over a silica gel column, eluted with 2% EtOH in $CHCl_3$, to afford **6a** (1.31 g, 90%, crystallized from aqueous MeOH). Mp: 142–143 °C (eff). EI-MS: *m/z* 526 (M^+ + H), 497 ($M^+ - N_2$), 482 (M^+ − *i*Pr), 454 ($M^+ - N_2$ − *i*Pr). IR (Nujol): 2180 cm^{-1} (N_3). ^1H NMR (DMSO-d_6): δ 1.04 (m, 28 H, *i*Pr), 1.77 (br s, 3 H, 5-Me), 3.89–4.19 (m, 4 H, 3′,4′,5′,5″-H), 4.71 (m, 1 H, 2′-H), 6.19 (d, 1 H, 1′-H, $J_{1',2'}$ = 6.3 Hz), 7.21 (br d, 1 H, 6-H), 11.28 (br s, 1 H, NH). Anal. ($C_{22}H_{39}N_5O_6Si_2$) C, H, N.

1-(2-Azido-2-deoxy-β-D-arabinofuranosyl)thymine (7). A THF solution of TBAF (1 M, 10 mL) was added to a solution of **6a** (2.15 g, 4.1 mmol) in THF (50 mL) at 0 °C. The mixture was stirred for 2 h at room temperature, then the solvent was removed in vacuo. The residue was purified over a silica gel column (3.2

× 12 cm), eluted with 10–20% EtOH–$CHCl_3$, to afford **7** (1.19 g, quant. crystallized from aqueous EtOH). Mp: 172.5–174 °C (eff). IR (Nujol): 2100 cm^{-1} (N_3). EI-MS: m/z 255 ($M^+ - N_2$), 240 ($M^+ - HN_3$). ^1H NMR (DMSO-d_6): δ 1.77 (d, 3 H, 5-Me, $J_{Me,6}$ = 1.2 Hz), 3.66 (m, 3 H, 4′,5′,5″-H), 4.05 (br q, 1 H, 3′-H), 4.42 (dd, 1 H, 2′-H, J = 6.6 Hz, and 7.1 Hz), 5.22 (br t, 1 H, 5′-OH), 5.91 (d, 1 H, 3′-OH), 6.14 (d, 1 H, 1′-H, $J_{1',2'}$ = 6.4 Hz), 7.74 (d, 1 H, 6-H, $J_{6,Me}$ = 1.2 Hz), 11.38 (br s, 1 H, NH). Anal. ($C_{10}H_{13}N_5O_5$) C, H, N.

6,2′-Imino-1-(2-deoxy-β-D-arabinofuranosyl)thymine (8). A mixture of **7** (90 mg, 0.31 mmol) and NaN_3 (40 mg, 0.6 mmol) in DMF (8 mL) was heated under reflux for 1 h. The solvent was removed in vacuo and the residue was purified over a silica gel column (1.7 × 10 cm), eluted with 20–40% EtOH in $CHCl_3$, to afford **8** (61 mg, 77%, crystallized from MeOH). Mp: 222–223 °C, EI-MS: m/z 255 (M^+). ^1H NMR (DMSO-d_6): δ 1.06 (s, 3 H, 5-Me), 3.24 (m, 2 H, 5′,5″-H), 3.94–4.26 (m, 3 H, 2′,3′,4′-H), 4.85 (br t, 1 H, 5′-OH), 5.55 (d, 1 H, 3′-OH), 6.13 (d, 1 H, 1′-H, $J_{1',2'}$ = 5.9 Hz), 8.02 (br s, 1 H, NH), 10.30 (br s, 1 H, NH). Anal. ($C_{10}H_{13}N_3O_5 \cdot H_2O$) C, H, N.

N^3-Benzoyl-(3,5-O-TIPDS-β-D-ribofuranosyl)uracil (4b). Benzoyl chloride (6.2 mL, 53.3 mmol) was added dropwise over 60 min to a mixture of **3b** (20 g, 41 mmol) and tetrabutylammonium bromide (560 mg) in CH_2Cl_2 (800 mL) and 0.2 M aqueous Na_2CO_3 solution (1600 mL) with vigorous stirring at room temperature. The mixture was further stirred for 15 h. The separated organic phase was washed with H_2O, dried (Na_2SO_4), then concentrated to dryness. The residue was dissolved in 1,2-dichloroethane (400 mL) and the mixture was heated at 60 °C for 20 min. The solvent was removed in vacuo and the residue was purified over a silica gel column (5.3 × 24 cm), eluted with benzene–EtOAc (20:1), to afford N^3-$O^{2'}$-dibenzoyl-3′,5′-O-TIPDS-uridine (3.9 g, 16%, foam): EI-MS: mz 694 (M^+), ^1H NMR ($CDCl_3$): δ 1.07 (m, 28 H, iPr), 4.08–4.55 (m, 4 H, 3′,4′,5′,5″-H), 5.66 (d, 1 H, 2′-H, $J_{2',3'}$ = 5.4 Hz), 5.76 (d, 1 H, 5-H, $J_{5,6}$ = 8.1 Hz), 6.02 (s, 1 H, 1′-H), 7.34–8.07 (m, 11 H, 6-H + benzoyl). Elution of the column with benzene–EtOAc (1:1) afforded **4b** (19.5 g, 81%, as a foam). EI-MS: m/z 590 (M^+), 547 ($M^+ - i$Pr). ^1H NMR ($CDCl_3$): δ 1.07 (m, 28 H, iPr), 3.94 (br s, 1 H, 2′-OH), 4.06–4.36 (m, 5 H, 2′,3′,4′,5′,5″-H), 5.76 (s, 1 H, 1′-H), 5.80 (d, 1 H, 5-H, $J_{5,6}$ = 8.1 Hz), 7.94–7.97 (m, 5 H, benzoyl), 7.79 (d, 1 H, 6-H).

N^3-Benzoyl-1-(2-azido-2-deoxy-3,5-O-TIPDS-β-D-arabinofuranosyl)uracil (5b). A mixture of diethyl azodicarboxylate (0.59 mL, 3.8 mmol) and diphenyl phosphorazidate (0.83 mL, 3.8 mmol) in THF (5 mL) was added dropwise over 10 min to a solution of **4b** (600 mg, 1 mmol) and triphenylphosphine (1 g, 3.8 mmol) in THF (10 mL) at 0 °C. The mixture was stirred for 3 h at room temperature and the solvent was removed in vacuo. The residue was purified over a silica gel column (2 × 18 cm), eluted with benzene–EtOAc (60:1 to 40:1) to afford **5b** (454 mg, 74%, crystallized from hexane–EtOAc). Mp: 98–100 °C. EI-MS: m/z 615 (M^+), 587 ($M^+ - N_2$), 544 ($M^+ - N_2 - i$Pr). IR (Nujol): 2100 cm^{-1} (N_3). ^1H NMR ($CDCl_3$): δ 1.07 (m, 28 H, iPr), 3.76–4.51 (m, 5 H, 2′,3′,4′,5′,5″-H), 5.80 (d, 1 H, 5-H, $J_{5,6}$ = 9.3 Hz), 6.24 (d, 1 H, 1′-H, $J_{1',2'}$ = 6.9 Hz), 7.51 (d, 1 H, 6-H, $J_{6,5}$ = 9.3 Hz), 7.40–8.00 (m, 5 H, benzoyl). Anal. ($C_{28}H_{41}N_5O_7Si_2$) C, H, N.

1-(2-Azido-2-deoxy-3,5-O-TIPDS-β-D-arabinofuranosyl)-uracil (6b). Concentrated NH_4OH (20 mL) was added to a solution of **5b** (2.5 g, 4.1 mmol) in MeOH (60 mL) at 0 °C. The mixture was stirred for 1.5 h at room temperature, then the solvent was removed in vacuo. The residue was purified over a silica gel column (2.3 × 20 cm), eluted with hexane/EtOAc (1:1), to afford **6b** (1.97 g, 94%, crystallized from hexane–EtOAc). Mp: 156–157 °C. EI-MS: m/z 511 (M^+). IR (Nujol): 2100 cm^{-1} (N_3). ^1H NMR ($CDCl_3$): δ 1.07 (m, 28 H, iPr), 3.83–4.32 (m, 5 H, 2′,3′,4′,5′,5″-H), 5.71 (d, 1 H, 5-H, $J_{5,6}$ = 8.1 Hz), 6.25 (d, 1 H, 1′-H, $J_{1',2'}$ = 6.1 Hz), 7.63 (d, 1 H, 6-H, $J_{6,5}$ = 8.1 Hz), 8.36 (br s, 1 H, NH). The analytical sample was slightly hygroscopic, as the presence of H_2O was detected by ^1H NMR. Anal. ($C_{21}H_{37}N_5O_6Si_2 \cdot 0.5H_2O$) C, H, N.

1-(2-Azido-2-deoxy-3,5-O-TIPDS-β-D-arabinofuranosyl)-cytosine (10). 2,4,6-Triisopropylbenzenesulfonyl chloride (3 g, 9.8 mmol) was added to a mixture of **6b** (2 g, 3.9 mmol) and tetrabutylammonium bromide (200 mg) in CH_2Cl_2 (150 mL) and 0.2 M aqueous $NaHCO_3$ (150 mL). The mixture was stirred vigorously for 6.5 h at room temperature. The separated organic phase was washed with H_2O, dried (Na_2SO_4), and concentrated to dryness in vacuo. The residue containing **9** dissolved in dioxane (100 mL) was treated with concentrated NH_4OH (30 mL) overnight at room temperature. The solvent was removed by evaporation and the residue was purified over a silica gel column (2.3 × 13 cm) eluted with 15–20% EtOH in $CHCl_3$ to afford **10** (1.64 g, 83%, crystallized from aqueous EtOH). Mp: 178 °C. EI-MS: m/z 510 (M^+), 482 ($M^+ - N_2$), 467 ($M^+ - i$Pr), 439 ($M^+ - N_2 - i$Pr). IR (Nujol): 2100 cm^{-1} (N_3). ^1H NMR (DMSO-d_6): 1.05 (m, 28 H, iPr), 3.88–4.16 (m, 4 H, 3′,4′,5′,5″-H), 4.63 (m, 1 H, 2′-H), 5.70 (d, 1 H, 5-H, $J_{5,6}$ = 7.6 Hz), 6.21 (d, 1 H, 1′-H, $J_{1',2'}$ = 6.8 Hz), 7.21 (br s, 2 H, 4-NH_2), 7.46 (d, 1 H, 6-H, $J_{6,5}$ = 7.1 Hz). Anal. ($C_{21}H_{38}N_6O_5Si_2$) C, H, N.

1-(2-Azido-2-deoxy-β-D-arabinofuranosyl)cytosine Hydrochloride (1, Cytarazid). TBAF (1 M THF solution, 0.8 mL) was added to a solution of **10** (216 mg, 0.42 mmol) in THF (10 mL) at 0 °C. The mixture was stirred for 45 min at 0 °C and the solvent was removed in vacuo. The residue was purified over a silica gel column (2 × 7 cm), eluted from 15–20% EtOH in $CHCl_3$, to afford **1** (114 mg, quant.), which was dissolved in 3% HCl in EtOH (3 mL) and after several coevaporations with EtOH gave the HCl salt of **1**. Mp: 165 °C. FAB-MS: m/z 269 (M^+). ^1H NMR (DMSO-d_6): δ 3.64–3.87 (m, 3 H, 4′,5′,5″-H), 4.15 [m, 1 H, 3′-H, after addition of D_2O: 3.97 (dd, $J_{2',3'}$ = 5.6 Hz, $J_{3',4'}$ = 5.9 Hz)], 4.31 (dd, 1 H, 2′-H, $J_{2',1'}$ = 5.9 Hz, $J_{2',3'}$ = 5.6 Hz), 5.06 (br s, 1 H, 5′-OH), 5.74 (d, 1 H, 5-H, $J_{5,6}$ = 7.6 Hz), 5.82 (br d, 1 H, 3′-OH), 6.14 (d, 1 H, 1′-H, $J_{1',2'}$ = 5.9 Hz), 7.18 (br s, 2 H, 4-NH_2), 7.71 (d, 1 H, 6-H, $J_{6,5}$ = 7.3 Hz). Anal. ($C_9H_{12}N_6O_4 \cdot HCl$) C, H, N.

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