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Synthesis and biological evaluation of nucleobase-modified analogs of the anticancer compounds 3′-*C*-ethynyluridine (EUrd) and 3′-*C*-ethynylcytidine (ECyd)

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Abstract—A series of nucleobase-modified analogs of the anticancer compounds 3′-*C*-ethynyluridine (EUrd) and 3′-*C*-ethynylcytidine (ECyd) were designed to overcome the strict substrate specificity of the activating uridine–cytidine kinase. EUrd, ECyd and target nucleosides were obtained using a short convergent synthetic route utilizing diacetone-α-D-glucose as starting material. 5-Iodo-substituted EUrd was the most potent inhibitor among the novel nucleobase-modified analogs in in vitro assays against human adenocarcinoma breast and prostate cancer cells with IC_{50} values down to 35 nM.

1. Introduction

3′-*C*-Ethynylcytidine (ECyd, **1**) (Fig. 1) displays excellent broad-spectrum antitumor activity in nanomolar range in vitro as well as in tumor models.[1] ECyd is phosphorylated by uridine–cytidine kinase (UCK, EC 2.7.1.48) to give the corresponding 5′-monophosphate that upon further phosphorylation by nucleotide kinases gives the biologically active triphosphate (ECTP). ECTP competitively inhibits DNA templated RNA synthesis catalyzed by human RNA polymerases I, II, or III, which leads to cell apoptosis by a mechanism, which is not yet fully elucidated.[2–4] ECyd is distributed very selectively into tumor tissue,[5,6] accounting for the absence of severe toxicities.[2,7] These properties render ECyd as a highly promising agent in solid human cancer therapy and ECyd is, therefore, currently evaluated in Phase I clinical trials.

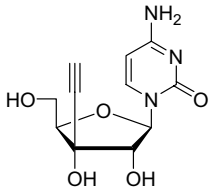

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Figure 1. Anticancer compound ECyd **1**.

Structure–activity relationship studies have revealed the configurational requirements at C2′ and C3′[8] and have clarified the importance of the C3′-substituent,[8–10] O4′-functionality[11] and the positioning of the ethynyl group.[8,12] Furthermore, the effect of introduction of additional branching at C4′ and C5′ and conformational restriction of the furanose ring has been studied.[13] These SAR studies strongly indicate that *ribo*-configuration of the furanose moiety is essential for UCK-catalyzed activation and that an ethynyl group placed at the C3′(*β*)-position is optimal for anticancer activity. Moreover, UCK has strict substrate specificity, offering limited possibilities for introduction of sugar modifications for improvement of activity. Most of the synthesized analogs to date have, therefore, been insufficiently phosphorylated to exert a potential pharmacological action. UCK2, one of at least two subtypes of UCK present in human cells,[14,15] has been proposed to be the more

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important UCK-subtype for activation of ECyd.[4] A variety of nucleobase-modified ribonucleosides are also phosphorylated by UCK2, including 3-deazauridine, 4-thiouridine, 5-halouridines, 5-hydroxyuridine, 6-azauridine and 2-thiocytidine.[14] In light of the aforementioned reasons, it is, therefore, surprising that merely the natural nucleobases (uracil, thymine, cytosine, adenine, and guanine), 5-fluorouracil, 5-fluorocytosine, and 4-amino-5-oxopyrido[2,3-*d*]pyrimidine have been attached to the pharmacologically relevant 3′-*C*-ethynyl-*β*-D-*ribo*-pentofuranosyl skeleton.[1,16] Uracil (EUrd **17**), 5-fluorouracil, and 5-fluorocytosine derivatives display significant inhibitory effects against leukemia cell lines, albeit being up to 150 times less active than ECyd.[1]

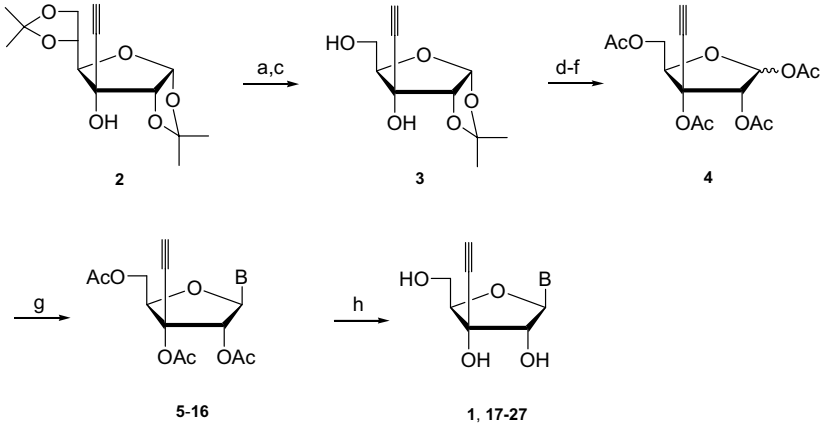
Herein we describe the synthesis of ECyd **1**, EUrd **17** and a series of nucleobase-modified analogs **18**–**27** hereof (Scheme 1 and Table 1), which were anticipated to overcome the crucial activation step catalyzed by UCK and to be further converted to potentially pharmacologically active triphosphates.

2. Chemistry

2.1. Synthesis of nucleobase-modified ECyd-analogs

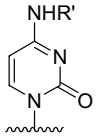
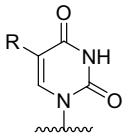
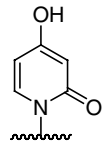
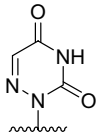
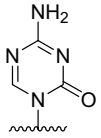
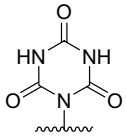
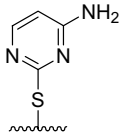
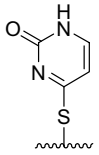
A desirable synthetic route towards EUrd, ECyd, and nucleobase-modified analogs hereof, would proceed via a convergent strategy, where a protected 3-*C*-ethynyl-D-*ribo*-pentofuranose as glycosyl donor is reacted with a series of persilylated nucleobases. Of three synthetic routes reported to date leading to ECyd,[1,17,18] two proceed via convergent strategies. Both synthetic routes attract by excellent yields, but require many chromatographic purification steps[1] or employ non-standard protecting groups, which were selected for optimized large-scale production of ECyd and EUrd.[17] We, therefore, chose a slightly different convergent strategy in which peracetylated furanose **4** was identified as the key intermediate (Scheme 1). Known furanose **2**,[19] easily obtained from inexpensive diacetone-α-D-glucose, was used as starting material. Chemoselective cleavage of the 5,6-*O*-isopropylidene group of furanose **2** with aqueous acetic acid, followed by oxidative cleavage of the vicinal diol and sodium borohydride reduction of the resulting aldehyde gave diol **3** in 84% yield over three steps. Diacetylation of diol **3**, followed by isopropylidene cleavage with aqueous trifluoroacetic acid and peracetylation of the resulting anomeric diol, gave glycosyl donor **4** in 74% yield over three steps.

Glycosylation of furanose **4** with a series of persilylated nucleobases (generated in situ from the corresponding nucleobase and *N*,*O*-bis(trimethylsilyl)acetamide, BSA) and trimethylsilyl triflate (TMSOTf)[20] in anhydrous acetonitrile at elevated temperatures (50–60 °C) for 1–3 days, gave *β*-configured nucleosides **5**–**12** in good yields (63–94%, see Table 1 for yields and structures) via anchimeric assistance of the O2-acetyl group. In contrast, 5-azacytidine and cyanuric acid derivatives **13** and **14** were only obtained in 50% and 30% yield, respectively. Reaction of glycosyl donor **4** with persilylated 2-thiocytosine or 4-thiouracil under these conditions unexpectedly furnished S-linked ribosides **15** and **16** in 74% and 83% yield, respectively. A similar glycosylation has only been observed during synthesis of *S*-*β*-D-glucuronides.[21,22] Deprotection of nucleosides **5**–**12** and **14** with saturated methanolic ammonia proceeded smoothly to afford target nucleosides **1**, **17**–**23** and **25** in modest yields (46–83%, see Table 1 for yields and structures), which primarily reflect loss of compound during column chromatography or crystallization. Since 5-azacytidine derivatives are known to be very labile in the presence of strong nucleophiles,[23] a different deprotection protocol was chosen for 5-azacytidine derivative **13**. Subjecting nucleoside **13** to classical Zemplén conditions (catalytic sodium methoxide in anhydrous MeOH) resulted in full decomposition to very polar products (results not shown), whereas deprotection with dilute methanolic ammonia successfully afforded nucleoside **24** in 63% yield. Deprotection of *S*-ribosides **15** and **16** with methanolic ammonia gave **26** and **27** in 72% and 70% yield, respectively.



Scheme 1. Reagents and conditions: (a) 80% aq AcOH, rt; (b) $NaIO_4$, MeOH/H_2O (3:1, v/v), 0 °C to rt; (c) $NaBH_4$, MeOH, 0 °C to rt, 84% over three steps; (d) Ac_2O, DMAP, pyridine, rt; (e) 80% aq TFA, 0 °C to rt; (f) Ac_2O, DMAP, pyridine, rt, 74% over three steps; (g) nucleobase, BSA, TMSOTf, CH_3CN (yields see Table 1); (h) NH_3/MeOH (yields see Table 1).

Table 1. Structures of nucleobase-modifications and yields of glycosylations and deacylations

B=		Product glycosylation	Yield (%)	Product deacetylation	Yield (%)
		**5** R′ = Bz	71	**1** R′ = H	74
	R = H	**6**	90	**17**	77
	R = OH	**7**	64	**18**	46
	R = Cl	**8**	78	**19**	83
	R = Br	**9**	94	**20**	68
	R = I	**10**	80	**21**	62
		**11**	79	**22**	63
		**12**	63	**23**	82
		**13**	50	**24**	63
		**14**	30	**25**	65
		**15**	74	**26**	72
		**16**	~83	**27**	70

2.2. Structure analysis

The identity of all new compounds was established on the basis of 1D- and 2D-NMR techniques, UV-measurements, high resolution MALDI-MS, and/or elemental analysis. ECyd **1** and EUrd **17** were identified by comparison with published spectral data.[1] Differentiation between N1-, N3-, N4-, O- or S-linked species, that is the regiochemical constitution of compounds **18-27** and their intermediates, was ascertained by comparison of ^{1}H NMR, ^{13}C NMR, and/or UV data of related compounds. Key observations are shown in Table 2 and discussed below.

Comparison of UV and ^{13}C NMR data of nucleoside **18** and 5-hydroxyuridine,[24,25] along with the appearance of the 5-OH group as an exchangeable singlet at 8.71 ppm in ^{1}H NMR, excluded the possibility of an O-linked product and indicated the N1-linked structure of nucleoside **18**. Assignments of 5-halonucleosides **19**–**21** were verified by a characteristic downfield signal of H-6 in ^{1}H NMR (~8.5 ppm) and absence of bathochromic shifts in their UV spectra in alkaline medium, since bathochromic shifts are a known characteristic of N3-nucleosides.[26] The regiochemical constitution of nucleoside **22** was indicated by comparison of ^{1}H NMR data of **22** and 3-deazauridine.[27] In particular, H-3 was observed as an exchangeable doublet (2.6 Hz coupling to H-5), characteristic of the dynamic keto–enol tautomeric equilibrium of the nucleobase moiety.[28] Nucleosides **23** and **24** were identified by comparison of ^{13}C NMR data with 6-azauridine[29] and 5-azacytidine,[25]

Table 2. Selected NMR and UV data for nucleosides **18**–**27** and related compounds

Compound	NMR (ppm)[a]	UV λ_{max} (nm)			Ref.
		pH 1	pH 7	pH 11	
18	160.4, 149.6, 132.5, 119.9	279	279	305	—
5-Hydroxyuridine	161.5, 150.8, 133.4, 120.1	280[b]	281	303[c]	24,25
19	—	276	275	276	—
20	—	278	278	278	—
21	—	287	287	278	—
22	7.77, 6.05, 5.91, 5.54[d]	—	—	—	—
3-Deazauridine	7.78, 5.98, 5.95, 5.56[d]	—	—	—	27
23	156.7, 149.1, 136.5	—	—	256	—
6-Azauridine	158.5, 149.5, 137.3	—	—	257[e]	29,40
24	165.6, 156.6, 153.2	—	—	—	—
5-Azacytidine	166.6, 157.3, 154.4	—	—	—	25
25	150.6, 150.6, 150.1	—	—	—	—
1-(*β*-D-*ribo*-Pentofuranosyl)cyanuric acid	150.1, 150.1, 149.1	—	—	—	25
26	168.6, 163.1, 154.8, 101.6	239	219/282	—	—
2-Thiocytidine	180.6, 160.8, 142.4, 98.8			—	31
S-Methyl-2-thiocytosine	—	241[f]	224/285	—	32
27	176.2, 154.0, 144.2, 101.9	263/322	262/298	300	—
4-Thiouridine	190.2, 148.0, 136.0, 112.6	—	—	—	33
3-(*β*-D-*ribo*-Pentofuranosyl)-4-thiouracil	193.7, 149.0, 136.5, 114.2	—	—	—	25
S-Methyl-4-thiouracil	177.5, 154.3, 143.1, 101.8	270/325	270/300	300	34,35

[a] ^{13}C NMR chemical shift values of nucleobase carbons, unless otherwise mentioned.
[b] pH 2.
[c] pH 12.
[d] ^{1}H NMR chemical shift values of H-6, H-1′, H-5 and H-3, respectively.
[e] 0.2 M NaOH.
[f] pH 0.

respectively. In accordance with previous observations on 1-(*β*-D-*ribo*-pentofuranosyl)cyanuric acid,[25] ^{13}C NMR signals of C-2 and C-6 of the non-UV active nucleoside **25** merged due to the symmetrical base moiety, while C-4 appeared slightly upfield. Furthermore, a downfield shift of H-2′ (~0.8 ppm) and upfield shift of 5′-OH (~0.7 ppm), compared to the corresponding signals of EUrd **17**, were observed in the ^{1}H NMR spectrum of nucleoside **25**, in agreement with similar observations on 1-(*β*-D-*ribo*-pentofuranosyl)cyanuric acid.[30] ^{13}C NMR signals of the nucleobase carbons of **26** and 2-thiocytidine[31] were very different implying a different regiochemical constitution of the nucleobase moiety of **26**. Comparison of the UV spectra of **26** and *S*-methyl-2-thiocytosine[32] allowed identification of **26** as the *S*-linked riboside. ^{13}C NMR signals of the nucleobase carbons of **27**, deviated significantly from 4-thiouridine[33] and 3-(*β*-D-*ribo*-pentofuranosyl)-4-thiouracil,[25] while corresponding well with data for *S*-methyl-4-thiouracil.[34] Furthermore, the UV spectra of **27** and *S*-methyl-4-thiouracil[35] exhibited similar characteristics supporting further proof for the proposed assignment.

The furanose ring of ECyd is known to adopt a South[36] conformation in both solid state and solution.[1,11] Substitution of the cytosine moiety with the different nucleobases, does not perturb the conformation of the furanose ring significantly, as indicated by the relatively large[37] $J_{H\text{-}1',H\text{-}2'}$ values (6.1–8.3 Hz) observed in the ^{1}H NMR of target nucleosides **18**–**27**.

3. Biological evaluation

Compounds **1** and **5**–**27** were evaluated for antiviral activity against HIV-1 in MT-4 cells as previously described.[38] All compounds were inactive against HIV-1 at the highest tested concentration of 100 μM. Acylated nucleosides **5**, **6**, **9**, **10**, and **12** and unprotected nucleosides **20**, **21**, **23**, **24** displayed moderate cytotoxicity (CD_{50}: 0.53–9.7 μM) against MT-4 cells, whereas ECyd and EUrd were strongly cytotoxic with CD_{50} values of 0.02 μM and 0.03 μM, respectively (Table 3).

Next, novel nucleosides **18**–**27** were evaluated against human adenocarcinoma breast cancer (MCF-7) and prostate cancer (PC-3) cell lines along with the reference compounds (Table 3). ECyd displayed very potent anticancer activity with IC_{50} values of 2.2 nM and 0.15 nM against MCF-7 and PC-3 cells, respectively. In agreement with previous results,[1] EUrd was 1.1–11 times less active than ECyd. 5-Iodouridine derivative **21**, the most potent inhibitor of tumor cell proliferation among the novel nucleobase-modified nucleosides, displayed IC_{50} values of 35 nM (MCF-7) and 160 nM (PC-3). Decreasing the size of the 5-substituent by introduction of a bromine or chlorine atom as in nucleosides **19** and **20** resulted in a systematic drop in activity. Interestingly, the corresponding 5-fluoro-EUrd analog, carrying the smallest halogen, is known to be only 19–48 fold less active than EUrd against L1210 and KB cells.[1] Furthermore, the substrate preference of UCK2 is known to decrease in the order: 5-fluorouridine > 5-bromouridine > 5-iodouridine.[14] The very weak anticancer activ-

Table 3. Cytotoxicity of 3′-*C*-ethynyl-*β*-D-*ribo*-pentofuranosyl nucleosides on MT-4 cells and inhibition of tumor cell proliferation in vitro[a]

Compound	CD_{50} (μM) MT-4	IC_{50} (μM) MCF-7	IC_{50} (μM) PC-3
5	1.29	nd	nd
6	0.53	nd	nd
7	>100	nd	nd
8	>100	nd	nd
9	4.1	nd	nd
10	0.57	nd	nd
11	> 100	nd	nd
12	9.7	nd	nd
13	>100	nd	nd
14	>100	nd	nd
15	>100	nd	nd
16	>100	nd	nd
18	>100	>25	>25
19	>100	>25	>25
20	4.5	0.73	19
21	0.46	0.035	0.16
22	>100	> 25	>25
23	3.8	35	43
24	4.1	4.7	7.2
25	>100	>25	>25
26	>100	>25	>25
27	>30	>25	>25
EUrd **17**	0.03	0.0025	0.0017
ECyd **1**	0.02	0.0022	0.00015

nd = Not determined.

[a] CD_{50} is the cytotoxic dose of compound required to reduce proliferation of normal uninfected MT-4 cells by 50%. IC_{50} represents the concentration at which cell growth was inhibited by 50% as compared to a negative control experiment.

ity of 6-azauridine derivative **23** compared to 5-iodouridine derivative **21**, and the lack of activity of 5-hydroxyuridine derivative **18** and 3-deazauridine derivative **22**, is also interesting considering that the corresponding nucleosides without the C3′-ethynyl modification are significantly better substrates for UCK2 than 5-iodouridine.[14] These observations lead us to speculate that: (1) UCK2 may have a different substrate preference for nucleobase-modified 3′-*C*-ethynyl-D-*ribo*-pentofuranosyl nucleosides than for the corresponding nucleobase-modified ribonucleosides without sugar modifications and/or (2) nucleotide kinases and RNA polymerases may have different substrate preferences leading to either insufficient phosphorylation or to lack of recognition by RNA polymerase of the corresponding triphosphates. However, other factors (lipophilicity, pK_a, etc.) may influence the biological activity of nucleosides **18**–**27** studied in our whole cell assays.

4. Experimental

General experimental section: All solvents and reagents were obtained from commercial suppliers and used without further purification unless stated otherwise. All solvents used for chromatography were of technical grade and used without further purification except CH_2Cl_2, which was distilled prior to use. Petroleum ether of the distillation range 60–80 °C was used. Solvents for use in reactions were of analytical grade. Anhydrous pyridine was used directly as obtained from commercial suppliers. Acetonitrile was dried through storage over activated 3 Å molecular sieves. Reactions were conducted under an atmosphere of argon when anhydrous solvents were used. Reactions were monitored by thin-layer chromatography (TLC) using silica gel coated plates with fluorescence indicator (SiO_2-60, F-254), which were visualized (1) under UV light, (2) by dipping in 5% concd sulfuric acid in anhydrous ethanol (v/v) followed by heating, or (3) by dipping in a solution of molybdato-phosphoric acid (12.5 g/L) and cerium(IV) sulfate (5 g/L) in 3% concd sulfuric acid in water (v/v) followed by heating. Silica gel column chromatography using moderate pressure (pressure ball) was performed with Silica gel 60 (particle size 0.040–0.063 mm, Merck). Evaporation of solvents was carried out under reduced pressure with temperatures not exceeding 45 °C. After column chromatography, appropriate fractions were pooled, evaporated and dried at high vacuum for at least 12 h to give the obtained products in high purity (>95%) unless stated otherwise. In absence of elemental analysis, ^{1}H NMR and/or ^{13}C NMR ascertained sample purity. No corrections in yields were made for solvent of crystallization. ^{1}H NMR and ^{13}C NMR spectra were recorded at 300 MHz and 75.5 MHz, respectively. Chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane or deuterated solvent as the internal standard (δ_H: $CDCl_3$ 7.26 ppm, DMSO-d_6 2.50 ppm; δ_C: $CDCl_3$ 77.00 ppm, DMSO-d_6 39.43 ppm). Exchangeable (ex) protons were detected by disappearance of peaks on D_2O addition. Assignments of NMR spectra were based on 2D spectra (HETCOR, COSY) and follow standard carbohydrate/nucleoside nomenclature. Quaternary carbons were not assigned in ^{13}C NMR. Traces of solvents in NMR spectra were identified by reference to published data.[39] MALDI-HRMS were recorded in positive ion mode on a IonSpec Fourier transform mass spectrometer. UV spectra were recorded at room temperature on a Shimadzu UV-160A spectrophotometer in the range 230–500 nm, using a quartz cell with a 1 cm path length. The pH was adjusted by addition of concd aq HCl or concd aq NaOH. Elemental analyses were obtained from the Microanalytical Department, University of Copenhagen.

4.1. 3-*C*-Ethynyl-1,2-*O*-isopropylidene-α-D-*ribo*-pentofuranose (3)

Known furanose **2**[19] (11.30 g, 39.8 mmol) was dissolved in 80% aqueous acetic acid (250 mL). After stirring at rt for 24 h, the solvent was evaporated and the residue co-evaporated with toluene (2 × 100 mL) and anhydrous EtOH (100 mL) to furnish the crude triol, which was directly dissolved in ice-cold MeOH/H_2O (300 mL, 3:1 v/v). To this was added sodium periodate (10.20 g, 47.7 mmol) and the reaction mixture warmed to rt and stirred for 15 h. Solids were filtered off, washed (MeOH), and the solution concentrated to 1/4 volume, saturated with NaCl (s) and extracted with CH_2Cl_2 (3 × 100 mL). The combined organic phase was evaporated to dryness to leave the crude hydroxyaldehyde as a yellow foam (8.5 g), which was directly dissolved in

ice-cold MeOH (175 mL). Sodium borohydride (4.51 g, 0.12 mol) was added portionwise over 15 min. The reaction mixture was warmed to rt and after stirring for 6.5 h, the reaction mixture was neutralized by addition of 10% aqueous acetic acid and evaporated to near dryness. The resulting residue was partitioned between EtOAc (125 mL) and brine (50 mL). The phases were separated and the aqueous phase extracted with CH_2Cl_2 (2×125 mL) and EtOAc (4×125 mL). The combined organic phase was evaporated to dryness and the resulting residue was purified by silica gel column chromatography (0–7% MeOH in CH_2Cl_2, v/v) to give diol **3** (7.17 g, 84% over three steps) as a white solid material. $R_f = 0.3$ (10% MeOH in $CHCl_3$, v/v); MALDI-HRMS *m/z* 237.0736 ($[M+Na]^+$, $C_{10}H_{14}O_5{\cdot}Na^+$ calcd 237.0733); ^1H NMR (DMSO-d_6) δ 5.94 (s, 1H, ex, 3-OH), 5.74 (d, 1H, $J =$ 3.7 Hz, H-1), 4.76 (t, 1H, ex, $J = 5.7$ Hz, 5-OH), 4.39 (d, 1H, $J = 3.7$ Hz, H-2), 3.82 (dd, 1H, $J = 8.2$ Hz, 2.0 Hz, H-4), 3.49–3.69 (m, 3H, HC≡C, H-5), 1.45 (s, 3H, CH_3), 1.28 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6) δ 111.8, 103.4 (C-1), 83.6 (C-2), 82.2, 81.8 (C-4), 77.4, 73.6, 60.9 (C-5), 26.4 (CH_3). Anal. Calcd for $C_{10}H_{14}O_5{\cdot}1/16\ H_2O$: C, 55.78; H, 6.61. Found: C, 55.54; H, 6.67.

4.2. 1,2,3,5-Tetra-*O*-acetyl-3-*C*-ethynyl-α,β-D-*ribo*-pentofuranose (4)

Diol **3** (4.91 g, 22.9 mmol) was dried by co-evaporation with anhydrous pyridine (75 mL) and dissolved in anhydrous pyridine (50 mL). To this, Ac_2O (8.7 mL, 91.9 mmol) and DMAP (0.28 g, 2.29 mmol) were added and the reaction mixture was stirred for 5 h at rt whereupon analytical TLC showed full conversion of starting material to one product with high mobility ($R_f = 0.8$, 10% MeOH in $CHCl_3$, v/v). Crushed ice (20 mL) was added and the reaction mixture was evaporated to near dryness and co-evaporated with toluene (30 mL). The residue was taken up in EtOAc (150 mL), and the organic phase washed with satd aq $NaHCO_3$ (2×50 mL). The organic phase was evaporated to near dryness and co-evaporated with toluene (150 mL) to afford a crude white solid material (6.6 g) which was dissolved in ice-cold 80% aqueous TFA (50 mL). After stirring the reaction mixture at rt for 6 h analytical TLC showed full conversion to two more polar compounds ($R_f = 0.2$ and 0.4, respectively, 10% MeOH in $CHCl_3$, v/v). Solvents were evaporated and the residue coevaporated with toluene (2×50 mL) and anhydrous pyridine (25 mL). Subsequently, the crude anomeric alcohol was dissolved in anhydrous pyridine (50 mL) and Ac_2O (8.7 mL, 91.9 mmol) and DMAP (0.28 g, 2.29 mmol) were added. The reaction mixture was stirred for 40 h whereupon crushed ice (20 mL) was added. The reaction mixture was diluted with EtOAc (200 mL), the phases separated and the organic phase washed with satd aq $NaHCO_3$ (2×75 mL). The organic phase was evaporated to dryness and the resulting residue co-evaporated with toluene (3×50 mL) and purified by silica gel column chromatography (0–3% MeOH in CH_2Cl_2, v/v) to give an anomeric mixture (ratio ≈ 4:5 by ^1H NMR) of glycosyl donor **4** (5.84 g, 74% over three steps) as a transparent yellow oil. Physical data for anomeric mixture: $R_f = 0.6$ (10% MeOH in $CHCl_3$, v/v); MALDI-HRMS *m/z* 365.0825 ($[M+Na]^+$, $C_{15}H_{18}O_9{\cdot}Na^+$ calcd 365.0843); ^{13}C NMR ($CDCl_3$) δ 170.60, 170.58, 169.2, 169.1, 168.8, 168.6, 168.5, 168.4, 98.6, 93.7, 82.7, 81.5, 78.7, 78.5, 77.9, 76.4, 75.9, 75.6, 75.2, 75.0, 64.4, 63.6, 21.1, 21.0, 20.94, 20.89, 20.87, 20.6, 20.4. Anal. Calcd for $C_{15}H_{18}O_9$: C, 52.63; H, 5.30. Found: C, 52.62; H, 5.19.

4.3. General procedure for glycosylations to give protected nucleosides 5–16

Glycosyl donor **4** and nucleobase (quantities of substrates and reagents given in corresponding paragraph) were dried by co-evaporation with anhydrous CH_3CN (25 mL) and resuspended in anhydrous CH_3CN (10 mL). *N*,*O*-Bis(trimethylsilyl)acetamide (BSA) was added in one portion and the mixture refluxed until a homogenous solution was obtained (less than 1 h). After cooling to rt, trimethylsilyl triflate (TMSOTf) was added in one portion and the reaction mixture stirred under heating. In some cases (see below) additional TMSOTf was added. In a typical workup procedure (0.5 g scale), the reaction mixture was poured into satd aq $NaHCO_3$ (20 mL) and diluted with EtOAc (30 mL). The phases were separated and the aqueous phase extracted with EtOAc (3×30 mL). The combined organic phase was evaporated to dryness and the resulting residue purified by silica gel column chromatography.

4.3.1. 1-[2,3,5-Tri-*O*-acetyl-3-*C*-ethynyl-β-D-*ribo*-pentofuranosyl]-4-*N*-benzoylcytosine (5). After stirring glycosyl donor **4** (1.02 g, 2.98 mmol), 4-*N*-benzoylcytosine (1.28 g, 5.96 mmol), BSA (3.0 mL, 11.9 mmol) and TMSOTf (1.6 mL, 8.94 mmol) for 24 h at 60 °C, the reaction mixture was worked up. Purification by silica gel column chromatography (0–4% MeOH in CH_2Cl_2, v/v) gave protected nucleoside **5** (1.04 g, 71%) as a white solid material. $R_f = 0.4$ (5% MeOH in CH_2Cl_2, v/v); UV λ_{max} pH 1, 260 nm, 314 nm, λ_{max} H_2O, 261 nm, 302 nm, λ_{max} pH 11, 316 nm; MALDI-HRMS *m/z* 520.1308 ($[M+Na]^+$, $C_{24}H_{23}N_3O_9{\cdot}Na^+$ calcd 520.1327); ^1H NMR (DMSO-d_6) δ 11.33 (br s, 1H, ex, NH), 8.25 (d, 1H, $J = 7.3$ Hz, H-6), 7.98–8.02 (m, 2H, Ph), 7.43–7.67 (m, 4H, H-5, Ph), 6.09 (d, 1H, $J = 4.8$ Hz, H-1′), 5.66 (d, 1H, $J = 4.8$ Hz, H-2′), 4.48–4.61 (m, 3H, H-4′, H-5′), 4.06 (s, 1H, HC≡C), 2.14 (s, 3H, CH_3), 2.11 (s, 3H, CH_3), 2.08 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6) δ 169.9, 168.2, 168.1, 167.3, 163.5, 154.1, 144.7 (C-6), 132.9 (Ph), 132.7 (Ph), 128.41 (Ph), 128.36 (Ph), 96.9 (C-5), 87.3 (C-1′), 81.8, 80.8 (C-4′), 76.9 (C-2′), 76.1, 74.5, 62.9 (C-5′), 20.6 (CH_3), 20.5 (CH_3), 20.2 (CH_3). Anal. Calcd for $C_{24}H_{23}N_3O_9{\cdot}1/16\ CH_2Cl_2$: C, 57.48; H, 4.64; N, 8.36. Found: C, 57.16; H, 4.55; N, 8.23.

4.3.2. 1-[2,3,5-Tri-*O*-acetyl-3-*C*-ethynyl-β-D-*ribo*-pentofuranosyl]uracil (6). After stirring peracetylated furanose **4** (0.93 g, 2.72 mmol), uracil (0.61 g, 5.43 mmol), BSA (2.7 mL, 10.9 mmol) and TMSOTf (1.5 mL, 8.15 mmol) for 21 h at 60 °C, the reaction mixture was worked up. Purification by silica gel column chromatography (0–4% MeOH in $CHCl_3$, v/v) gave protected nucleoside **6** (0.96 g, 90%) as a white solid material. $R_f = 0.3$ (10%

MeOH in $CHCl_3$, v/v); UV λ_{max} pH 1, 259 nm, λ_{max} H_2O, 259 nm, λ_{max} pH 11, 261 nm; MALDI-HRMS *m/z* 417.0889 ($[M + Na]^+$, $C_{17}H_{18}N_2O_9{\cdot}Na^+$ calcd 417.0905); ^1H NMR (DMSO-d_6) δ 11.50 (br s, 1H, ex, NH), 7.74 (d, 1H, $J = 8.1$ Hz, H-6), 6.01 (d, 1H, $J = 6.0$ Hz, H-1′), 5.78 (d, 1H, $J = 8.1$ Hz, H-5), 5.54 (d, 1H, $J = 6.0$ Hz, H-2′), 4.41–4.55 (m, 3H, H-4′, H-5′), 4.04 (s, 1H, HC≡C), 2.15 (s, 3H, CH_3), 2.09 (s, 3H, CH_3), 2.06 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6) δ 169.8, 168.8, 168.3, 162.6, 150.2, 139.6 (C-6), 102.9 (C-5), 85.0 (C-1′), 81.1, 80.1 (C-4′), 76.3, 76.1, 74.6, 62.9 (C-5′), 20.7 (CH_3), 20.5 (CH_3), 20.1 (CH_3). Anal. Calcd for $C_{17}H_{18}N_2O_9{\cdot}1/4\ CHCl_3$: C, 48.84; H, 4,34; N, 6.60. Found: C, 48.64; H, 4,24; N, 6.54.

4.3.3. 1-[2,3,5-Tri-*O*-acetyl-3-*C*-ethynyl-β-D-*ribo*-pentofuranosyl]-5-hydroxyuracil (7). Glycosyl donor **4** (0.50 g, 1.46 mmol), 5-hydroxyuracil (0.37 mg, 2.92 mmol), BSA (1.5 mL, 5.84 mmol) and TMSOTf (0.79 mL, 4.38 mmol) were stirred at 50 °C for 44 h, whereupon the reaction mixture was cooled to rt and a second portion of TMSOTf (0.40 mL, 2.21 mmol) was added. Stirring at 50 °C for additional 21 h resulted in complete consumption of starting material and the reaction mixture was worked up. Purification by silica gel column chromatography (0–4% MeOH in CH_2Cl_2, v/v) gave protected nucleoside **7** (0.38 g, 64%) as a white solid material. $R_f = 0.5$ (10% MeOH in $CHCl_3$, v/v); UV λ_{max} pH 1, 278 nm, λ_{max} H_2O, 279 nm, λ_{max} pH 11, 306 nm; MALDI-HRMS *m/z* 433.0841 ($[M+Na]^+$, $C_{17}H_{18}N_2O_{10}{\cdot}Na^+$ calcd 433.0854); ^1H NMR (DMSO-d_6) δ 11.64 (br s, 1H, ex, NH), 9.05 (br s, 1H, ex, 5-OH), 7.20 (s, 1H, H-6), 6.02 (d, 1H, $J = 6.0$ Hz, H-1′), 5.48 (d, 1H, $J = 6.0$ Hz, H-2′), 4.41–4.49 (m, 3H, H-4′, H-5′), 4.07 (s, 1H, HC≡C), 2.15 (s, 3H, CH_3), 2.07–2.08 (2s, $2 \times 3H$, $2 \times CH_3$); ^{13}C NMR (DMSO-d_6) δ 169.8, 168.8, 168.4, 160.1, 149.0, 133.3, 117.9 (C-6), 85.0 (C-1′), 81.1, 79.6 (C-4′), 76.5, 75.8 (C-2′), 74.5, 62.8 (C-5′), 20.6 (CH_3), 20.5 (CH_3), 20.1 (CH_3). Anal. Calcd for $C_{17}H_{18}N_2O_{10}{\cdot}1/4\ CH_2Cl_2$: C, 48.01; H, 4.32; N, 6.49. Found: C, 48.06; H, 4.24; N, 6.44.

4.3.4. 1-[2,3,5-Tri-*O*-acetyl-3-*C*-ethynyl-β-D-*ribo*-pentofuranosyl]-5-chlorouracil (8). Peracetylated furanose **4** (0.50 g, 1.46 mmol), 5-chlorouracil (0.43 g, 2.92 mmol), BSA (1.5 mL, 5.84 mmol) and TMSOTf (0.79 mL, 4.38 mmol) were stirred at 50 °C for 29 h, whereupon the reaction mixture was worked up. Purification by silica gel column chromatography (0–3% MeOH in CH_2Cl_2, v/v) gave protected nucleoside **8** (0.49 g, 78%) as a white solid material. $R_f = 0.6$ (10% MeOH in $CHCl_3$, v/v); UV λ_{max} pH 1, 274 nm, λ_{max} H_2O, 275 nm, λ_{max} pH 11, 274 nm; MALDI-HRMS *m/z* 451.0522 ($[M+Na]^+$, $C_{17}H_{17}ClN_2O_9{\cdot}Na^+$ calcd 451.0515); ^1H NMR (DMSO-d_6) δ 12.04 (br s, 1H, ex, NH), 8.17 (s, 1H, H-6), 6.01 (d, 1H, $J = 6.4$ Hz, H-1′), 5.63 (d, 1H, $J = 6.4$ Hz, H-2′), 4.57 (t, 1H, $J = 4.8$ Hz, H-4′), 4.48 (d, 2H, $J = 4.8$ Hz, H-5′), 4.08 (s, 1H, HC≡C), 2.14 (s, 3H, CH_3), 2.09 (s, 3H, CH_3), 2.07 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6) δ 169.7, 168.9, 168.4, 158.6, 149.4, 136.9 (C-6), 108.4, 84.8 (C-1′), 81.2, 80.5 (C-4′), 76.3, 76.1, 74.6, 63.0 (C-5′), 20.8 (CH_3), 20.4 (CH_3), 20.2 (CH_3). Anal. Calcd for $C_{17}H_{17}ClN_2O_9$: C, 47.62; H, 4.00; N, 6.53. Found: C, 47.70; H, 3.96; N, 6.35.

4.3.5. 1-[2,3,5-Tri-*O*-acetyl-3-*C*-ethynyl-β-D-*ribo*-pentofuranosyl]-5-bromouracil (9). Peracetylated furanose **4** (0.50 g, 1.46 mmol), 5-bromouracil (0.56 g, 2.92 mmol), BSA (1.5 mL, 5.84 mmol) and TMSOTf (0.79 mL, 4.38 mmol) were stirred at 50 °C for 23 h whereupon the reaction mixture was worked up. Purification by silica gel column chromatography (0–3% MeOH in CH_2Cl_2, v/v) gave protected nucleoside **9** (0.65 g, 94%) as a white solid material. $R_f = 0.6$ (10% MeOH in $CHCl_3$, v/v); UV λ_{max} pH 1, 277 nm, λ_{max} H_2O, 276 nm, λ_{max} pH 11, 276 nm; MALDI-HRMS *m/z* 495.0008 ($[M+Na]^+$, $C_{17}H_{17}BrN_2O_9{\cdot}Na^+$ calcd 495.0010); ^1H NMR (DMSO-d_6) δ 12.01 (br s, 1H, ex, NH), 8.22 (s, 1H, H-6), 6.01 (d, 1H, $J = 6.4$ Hz, H-1′), 5.63 (d, 1H, $J = 6.4$ Hz, H-2′), 4.46–4.60 (m, 3H, H-4′, H-5′), 4.08 (s, 1H, HC≡C), 2.14 (s, 3H, CH_3), 2.08–2.10 (2s, $2 \times 3H$, $2 \times CH_3$); ^{13}C NMR (DMSO-d_6) δ 169.7, 168.9, 168.4, 158.7, 149.6, 139.2 (C-6), 97.0, 84.8 (C-1′), 81.2, 80.5 (C-4′), 76.3, 76.1, 74.6, 63.0 (C-5′), 20.8 (CH_3), 20.5 (CH_3), 20.2 (CH_3). Anal. Calcd for $C_{17}H_{17}BrN_2O_9$: C, 43.15; H, 3.62; N, 5.92. Found: C, 43.13; H, 3.52; N, 5.82.

4.3.6. 1-[2,3,5-Tri-*O*-acetyl-3-*C*-ethynyl-β-D-*ribo*-pentofuranosyl]-5-iodouracil (10). Glycosyl donor **4** (0.50 g, 1.46 mmol), 5-iodouracil (0.70 g, 2.92 mmol), BSA (1.5 mL, 5.84 mmol) and TMSOTf (0.79 mL, 4.38 mmol) were stirred at 50 °C for 29 h whereupon the reaction mixture was worked up. Purification by silica gel column chromatography (0–4% MeOH in CH_2Cl_2, v/v) gave protected nucleoside **10** (0.61 g, 80%) as a white solid material. $R_f = 0.8$ (10% MeOH in $CHCl_3$, v/v); UV λ_{max} pH 1, 286 nm, λ_{max} H_2O, 286 nm, λ_{max} pH 11, 279 nm; MALDI-HRMS *m/z* 542.9890 ($[M+Na]^+$, $C_{17}H_{17}IN_2O_9{\cdot}Na^+$ calcd 542.9871); ^1H NMR (DMSO-d_6) δ 11.87 (br s, 1H, ex, NH), 8.19 (s, 1H, H-6), 6.00 (d, 1H, $J = 6.2$ Hz, H-1′), 5.59 (d, 1H, $J = 6.2$ Hz, H-2′), 4.45–4.58 (m, 3H, H-4′, H-5′), 4.10 (s, 1H, HC≡C), 2.14 (s, 3H, CH_3), 2.09–2.10 (2s, $2 \times 3H$, $2 \times CH_3$); ^{13}C NMR (DMSO-d_6) δ 169.7, 168.9, 168.4, 160.1, 149.9, 143.8 (C-6), 84.7 (C-1′), 81.1, 80.4 (C-4′), 76.3, 76.1, 74.6, 71.0, 62.9 (C-5′), 20.7 (CH_3), 20.5 (CH_3), 20.1 (CH_3).

4.3.7. 1-[2,3,5-Tri-*O*-acetyl-3-*C*-ethynyl-β-D-*ribo*-pentofuranosyl]-3-deazauracil (11). Peracetylated furanose **4** (0.50 g, 1.46 mmol), 3-deazauracil (0.32 g, 2.92 mmol), BSA (1.5 mL, 5.84 mmol) and TMSOTf (0.79 mL, 4.38 mmol) were stirred at 50 °C for 24 h, whereupon the reaction mixture was cooled to rt and a second portion of TMSOTf (0.30 mL, 1.65 mmol) added. After stirring at 50 °C for additional 16 h a third portion of TMSOTf (0.10 mL, 0.56 mmol) was added. After stirring for additional 26 h at 50 °C, the reaction mixture was worked up. Purification by silica gel column chromatography (0–4% MeOH in CH_2Cl_2, v/v) gave protected nucleoside **11** (0.51 g, 79%) as a white solid material. $R_f = 0.5$ (10% MeOH in CH_2Cl_2, v/v); UV λ_{max} pH 1, 278 nm, λ_{max} H_2O, 282 nm, $_{max}$ pH 11, 256 nm;

MALDI-HRMS *m/z* 416.0965 ([M+Na]$^+$, $C_{18}H_{19}NO_9$·Na$^+$ calcd 416.0952); ^{1}H NMR (DMSO-d_6) δ 10.91 (br s, 1H, ex, OH), 7.58 (d, 1H, J = 7.9 Hz, H-6), 6.26 (d, 1H, J = 5.3 Hz, H-1′), 6.02 (dd, 1H, J = 7.9 Hz, 2.6 Hz, H-5), 5.55 (d, 1H, ex, J = 2.6 Hz, H-3), 5.51 (d, 1H, J = 5.3 Hz, H-2′), 4.41–4.50 (m, 3H, H-4′, H-5′), 4.03 (s, 1H, HC≡C), 2.14 (s, 3H, CH_3), 2.06–2.07 (2s, 2 × 3H, 2 × CH_3); ^{13}C NMR (DMSO-d_6) δ 169.9, 168.3, 168.2, 166.8, 162.4, 133.9 (C-6), 101.4 (C-5), 97.6 (C-3), 84.7 (C-1′), 81.1, 79.9 (C-4′), 76.9, 76.6, 74.5, 62.8 (C-5′), 20.6 (CH_3), 20.4 (CH_3), 20.1 (CH_3). Anal. Calcd for $C_{18}H_{19}NO_9$·1/16 CH_2Cl_2: C, 54.42; H, 4.84; N, 3.51. Found: C, 54.25; H, 4.82; N, 3.52.

4.3.8. 1-[2,3,5-Tri-*O*-acetyl-3-*C*-ethynyl-*β*-D-*ribo*-pentofuranosyl]-6-azauracil (12). Peracetylated furanose **4** (0.50 g, 1.46 mmol), 6-azauracil (0.33 g, 2.92 mmol), BSA (1.5 mL, 5.84 mmol) and TMSOTf (0.79 mL, 4.38 mmol) were stirred at 50 °C for 48 h. The reaction mixture was cooled to rt and a second portion TMSOTf (0.40 mL, 2.21 mmol) added. After heating at 50 °C for additional 19 h the reaction mixture was worked up. Purification by silica gel column chromatography (0–2% MeOH in CH_2Cl_2, v/v) afforded protected nucleoside **12** (0.37 g, 63%) as a slightly yellow solid material. R_f = 0.5 (5% MeOH in CH_2Cl_2, v/v); UV λ_{max} pH 1, 258 nm, λ_{max} H_2O, 257 nm, λ_{max} pH 11, 255 nm; MALDI-HRMS *m/z* 418.0865 ([M+Na]$^+$, $C_{16}H_{17}N_3O_9$·Na$^+$ calcd 418.0857); ^{1}H NMR (DMSO-d_6) δ 12.40 (br s, 1H, ex, NH), 7.66 (s, 1H, H-5), 6.08 (d, 1H, J = 6.3 Hz, H-1′), 5.88 (d, 1H, J = 6.3 Hz, H-2′), 4.45–4.52 (m, 2H, H-4′, H-5$_A$′), 4.17 (dd, 1H, J = 12.5 Hz, 7.3 Hz, H-5′$_B$), 3.91 (s, 1H, HC≡C), 2.15 (s, 3H, CH_3), 2.05–2.07 (2s, 2 × 3H, 2 × CH_3); ^{13}C NMR (DMSO-d_6) δ 169.9, 168.7, 168.3, 156.1, 148.1, 137.1 (C-5), 85.9 (C-1′), 80.4, 80.0, 76.4, 74.7, 74.4, 62.8 (C-5′), 20.7, 20.5, 20.2. Anal. Calcd for $C_{16}H_{17}N_3O_9$: C, 48.61; H, 4.33; N, 10.63; Found: C, 48.41; H, 4.31; N, 10.31.

4.3.9. 1-[2,3,5-Tri-*O*-acetyl-3-*C*-ethynyl-*β*-D-*ribo*-pentofuranosyl]-5-azacytosine (13). Glycosyl donor **4** (0.41 g, 1.20 mmol), 5-azacytosine (0.27 g, 2.41 mmol), BSA (1.2 mL, 4.81 mmol) and TMSOTf (0.65 mL, 3.61 mmol) were stirred at 50 °C for 24 h. The reaction mixture was cooled to rt and a second portion of TMSOTf (0.20 mL, 1.11 mmol) added. The reaction mixture was stirred for additional 6 h at 50 °C whereupon it was worked up. To prevent decomposition of the nucleobase moiety, the organic phase was washed quickly with ice-cold satd aq $NaHCO_3$ (25 mL). The phases were separated and the aqueous phase extracted with EtOAc (3 × 25 mL). The combined organic phase was evaporated to dryness and the resulting residue was purified by silica gel column chromatography (0–3% MeOH in EtOAc, v/v) to afford nucleoside **13** (0.24 g, 50%) as a white solid material. R_f = 0.4 (10% MeOH in EtOAc, v/v); UV λ_{max} pH 1, 255 nm, λ_{max} H_2O, 243 nm, λ_{max} pH 11, 251 nm; MALDI-HRMS *m/z* 417.1006 ([M+Na]$^+$, $C_{16}H_{18}N_4O_8$·Na$^+$ calcd 417.1017); ^{1}H NMR (DMSO-d_6) δ 8.43 (s, 1H, H-6), 7.75 (s, 1H, ex, NH_2), 7.73 (s, 1H, ex, NH_2), 5.91 (d, 1H, J = 5.7 Hz, H-1′), 5.77 (d, 1H, J = 5.7 Hz, H-2′), 4.44–4.55 (m, 3H, H-4′, H-5′), 4.06 (s, 1H, HC≡C), 2.13 (s, 3H, CH_3), 2.09 (s, 3H, CH_3), 2.07 (s, 3H, CH_3); (DMSO-d_6) δ 169.9, 168.5, 168.2, 165.4, 155.8 (C-6), 152.3, 86.1 (C-1′), 81.4, 80.5 (C-4′), 76.3 (C-2′), 74.7, 63.0 (C-5′), 20.7 (CH_3), 20.5 (CH_3), 20.2 (CH_3).

4.3.10. 1-[2,3,5-Tri-*O*-acetyl-3-*C*-ethynyl-*β*-D-*ribo*-pentofuranosyl]cyanuric acid (14). Glycosyl donor **4** (0.40 g, 1.27 mmol), cyanuric acid (0.30 g, 2.34 mmol), BSA (1.7 mL, 7.04 mmol) and TMSOTf (0.63 mL, 3.51 mmol) were stirred at 55 °C for 21 h, whereupon analytical TLC showed approx. 70% conversion of starting material into several non-UV active compounds. The reaction mixture was cooled to rt and a second portion of TMSOTf (0.20 mL, 1.11 mmol) added. After stirring for additional 24 h the reaction mixture was worked up. Purification by silica gel column chromatography (0–2% MeOH in $CHCl_3$, v/v) afforded nucleoside **14** (145 mg, 30%) as a yellow solid material, which was used in the next step without further purification. R_f = 0.5 (10% MeOH in CH_2Cl_2, v/v); MALDI-HRMS *m/z* 434.0785 ([M+Na]$^+$, $C_{16}H_{17}N_3O_{10}$·Na$^+$ calcd 434.0806); ^{1}H NMR (DMSO-d_6) δ 6.28 (d, 1H, J = 7.5 Hz), 6.07 (d, 1H, J = 7.5 Hz), 4.49–4.55 (m, 2H), 4.21 (dd, 1H, J = 12.1 Hz, J = 8.8 Hz), 3.93 (s, 1H, HC≡C), 2.14 (s, 3H, CH_3), 2.07 (s, 3H, CH_3), 2.02 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6) δ 169.9, 169.1, 168.4, 148.4, 147.1, 83.7, 80.4, 80.3, 76.6, 75.2, 73.0, 63.6, 20.9, 20.5, 20.2. Acetamide was identified as a trace impurity.

4.3.11. *S*-[2,3,5-Tri-*O*-acetyl-3-*C*-ethynyl-*β*-D-*ribo*-pentofuranosyl]-2-thiocytosine (15). Glycosyl donor **4** (0.40 g, 1.17 mmol), 2-thiocytosine (0.30 g, 2.34 mmol), BSA (1.2 mL, 4.67 mmol) and TMSOTf (0.63 mL, 3.51 mmol) were stirred at 50 °C for 40 h, whereupon the reaction mixture was worked up. Purification by silica gel column chromatography (0–2% MeOH in CH_2Cl_2, v/v) gave protected nucleoside **15** (0.35 g, 74%) as a white solid material. R_f = 0.5 (10% MeOH in $CHCl_3$, v/v); UV λ_{max} pH 1, 237 nm, λ_{max} H_2O, 281 nm, λ_{max} pH 11, 281 nm; MALDI-HRMS *m/z* 432.0839 ([M+Na]$^+$, $C_{17}H_{19}N_3O_7S$·Na$^+$ calcd 432.0836); ^{1}H NMR (DMSO-d_6) δ 7.90 (d, 1H, J = 5.9 Hz, H-6), 7.07 (br s, 2H, 2 ex, NH_2), 6.22 (d, 1H, J = 3.3 Hz, H-1′), 6.19 (d, 1H, J = 5.9 Hz, H-5), 5.68 (d, 1H, J = 3.3 Hz, H-2′), 4.32–4.44 (m, 2H, H-4′, H-5′$_A$), 4.16–4.24 (dd, 1H, J = 11.4 Hz, 6.6 Hz, H-5′$_B$), 4.09 (s, 1H, HC≡C), 2.12 (s, 3H, CH_3), 2.11 (s, 3H, CH_3), 2.05 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6) δ 169.9, 168.5, 168.2, 166.8, 163.2, 154.9 (C-6), 102.0 (C-5), 85.3 (C-1′), 81.9, 80.9, 78.3 (C-2′), 75.9, 75.2, 63.3 (C-5′), 20.5 (CH_3), 20.4 (CH_3), 20.2 (CH_3). Anal. Calcd for $C_{17}H_{19}N_3O_7S$: C, 49.87; H, 4.68; N, 10.26; S, 7.83. Found: C, 49.69; H, 4.55; N, 10.11; S, 7.83.

4.3.12. *S*-[2,3,5-Tri-*O*-acetyl-3-*C*-ethynyl-*β*-D-*ribo*-pentofuranosyl]-4-thiouracil (16). Peracetylated furanose **4** (0.50 g, 1.46 mmol), 4-thiouracil (0.37 g, 2.85 mmol), BSA (1.5 mL, 5.84 mmol) and TMSOTf (0.79 mL, 4.38 mmol) were stirred at 50 °C for 28 h whereupon the reaction mixture was worked up. Purification by silica gel column chromatography (0–4% MeOH in

CH_2Cl_2, v/v) afforded pure nucleoside **16** (0.17 g) as a yellow solid material. Furthermore, **16** (0.33 g, ≈83% combined yield) contaminated with traces of 4-thiouracil was isolated. The two fractions were combined and used in the next step without further purification. Physical data for pure fraction: R_f = 0.4 (5% MeOH in CH_2Cl_2, v/v); UV λ_{max} pH 1, 261 nm, 302 nm, λ_{max} H_2O, 258 nm, 297 nm, λ_{max} pH 11, 301 nm; MALDI-HRMS *m/z* 433.0662 ($[M + Na]^+$, $C_{17}H_{18}N_2O_8S{\cdot}Na^+$ calcd 433.0676); ^1H NMR (DMSO-d_6) δ 11.73 (br s, 1H, ex), 7.75 (d, 1H, J = 6.6 Hz, H-6), 6.35 (d, 1H, J = 6.6 Hz, H-5), 6.27 (d, 1H, J = 2.9 Hz, H-1′), 5.67 (d, 1H, J = 2.9 Hz, H-2′), 4.38–4.44 (m, 2H, H-4′, H-5′$_A$), 4.18–4.26 (dd, 1H, J = 12.5 Hz, 7.7 Hz, H-5′$_B$), 4.13 (s, 1H, HC≡C), 2.13 (s, 3H, CH_3), 2.12 (s, 3H, CH_3), 2.06 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6) δ 173.6, 169.9, 168.7, 168.2, 153.8, 144.9 (C-6), 101.7 (C-5), 83.1 (C-1′), 82.1, 81.2 (C-4′), 78.3 (C-2′), 75.7, 75.0, 63.1 (C-5′), 20.5 (CH_3), 20.4 (CH_3), 20.2 (CH_3). Anal. Calcd for $C_{17}H_{18}N_2O_8S$: C, 49.75; H, 4.42; N, 6.83; S, 7.81. Found: C, 49.36; H, 4.31; N, 6.78; S, 7.91.

4.4. General procedure for deacylations to give nucleosides 1, 17–23, 25–27

The protected nucleoside (quantity of substrate given in the corresponding paragraph) was dissolved in saturated methanolic ammonia (20 mL for 0.3 g of protected nucleoside) and stirred in a sealed container for 24–48 h (exact time indicated in corresponding paragraph) at rt. The reaction mixture was evaporated to dryness and co-evaporated several times with absolute EtOH to afford a residue, which was purified by either washing with an appropriate solvent, silica gel column chromatography or crystallization.

4.4.1. 1-[3-*C*-Ethynyl-β-D-*ribo*-pentofuranosyl]cytosine (ECyd, 1). Nucleoside **5** (0.51 g, 1.03 mmol) was stirred 46 h. The residue resulting after workup was washed with ice-cold EtOH to afford the known nucleoside **1** (0.21 g, 74%) as a white solid material. R_f = 0.1 (20% MeOH in CH_2Cl_2, v/v); Selected data ^1H NMR (DMSO-d_6 + 1 drop D_2O) δ 5.83 (d, 1H, J = 6.6 Hz, H-1′), 4.11 (d, 1H, J = 6.6 Hz, H-2′). ^1H NMR (DMSO-d_6), ^{13}C NMR (DMSO-d_6) and UV data were identical to previously published data.[1,18]

4.4.2. 1-[3-*C*-Ethynyl-β-D-*ribo*-pentofuranosyl]uracil (EUrd, 17). Nucleoside **6** (0.66 g, 1.68 mmol) was stirred 46 h. The residue resulting after workup was adsorbed on Kieselguhr and purified by silica gel column chromatography (0–40% MeOH in CH_2Cl_2, v/v) to afford the known nucleoside **17** (0.35 g, 77%) as a white solid material. R_f = 0.3 (20% MeOH in CH_2Cl_2, v/v); UV λ_{max} pH 1, 261 nm, λ_{max} H_2O, 261 nm, λ_{max} pH 11, 261 nm; MALDI-HRMS *m/z* 291.0597 ($[M+Na]^+$, $C_{11}H_{12}N_2O_6{\cdot}Na^+$ calcd 291.0587); Selected data ^1H NMR (DMSO-d_6 + 1 drop D_2O) δ 5.82 (d, 1H, J = 7.3 Hz, H-1′), 4.17 (d, 1H, J = 7.3 Hz, H-2′); ^{13}C NMR (DMSO-d_6) δ 162.9, 150.8, 140.8 (C-6), 102.1 (C-5), 86.5 (C-4′), 85.9 (C-1′), 82.6, 77.7 (C-2′), 77.0, 72.6, 61.3 (C-6′). ^1H NMR (DMSO-d_6) data were identical to previously published data.[1]

4.4.3. 1-[3-*C*-Ethynyl-β-D-*ribo*-pentofuranosyl]-5-hydroxyuracil (18). Nucleoside **7** (0.30 g, 0.74 mmol) was stirred for 50 h. The residue resulting after workup was purified by silica gel column chromatography (0–15% MeOH in CH_2Cl_2, v/v) to afford nucleoside **18** (98 mg, 46%). R_f = 0.6 (40% MeOH in CH_2Cl_2, v/v); UV λ_{max} pH 1, 279 nm, λ_{max} H_2O, 279 nm, λ_{max} pH 11, 305 nm; MALDI-HRMS *m/z* 307.0534 ($[M+Na]^+$, $C_{11}H_{12}N_2O_7{\cdot}Na^+$ calcd 307.0537); ^1H NMR (DMSO-d_6) δ 11.47 (s, 1H, ex, NH), 8.71, (s, 1H, ex, 5-OH), 7.52 (s, 1H, H-6), 5.85 (s, 1H, ex, 3′-OH), 5.83 (d, 1H, J = 7.3 Hz, H-1′), 5.79 (d, 1H, ex, J = 6.6 Hz, 2′-OH), 5.12 (t, 1H, ex, J = 4.2 Hz, 5′-OH), 4.15 (br t, 1H, H-2′), 3.85 (br t, 1H, H-4′), 3.60–3.75 (m, 2H, H-5′), 3.54 (s, 1H, HC≡C); ^{13}C NMR (DMSO-d_6) δ 160.4, 149.6, 132.5, 119.9 (C-6), 86.2 (C-4′), 85.8 (C-1′), 82.9, 77.3 (C-2′), 76.9, 72.6, 61.3 (C-5′). Anal. Calcd for $C_{11}H_{12}N_2O_7{\cdot}3/16$ MeOH: C, 46.30; H, 4.43; N, 9.65. Found: C, 45.90; H, 4.37; N, 9.76.

4.4.4. 1-[3-*C*-Ethynyl-β-D-*ribo*-pentofuranosyl]-5-chlorouracil (19). Nucleoside **8** (0.31 g, 0.72 mmol) was stirred for 48 h. The residue resulting after workup was purified by crystallization (MeOH and minimal H_2O), which after washing with cold MeOH afforded unprotected nucleoside **19** (146 mg) as white needles. Repeated crystallization of the mother liquor afforded additional **19** (combined yield 180 mg, 83%). R_f = 0.3 (20% MeOH in CH_2Cl_2, v/v); mp (MeOH/H_2O) 133–136 °C; UV λ_{max} pH 1, 276 nm, λ_{max} H_2O, 275 nm, λ_{max} pH 11, 276 nm; MALDI-HRMS *m/z* 325.0213 ($[M+Na]^+$, $C_{11}H_{11}ClN_2O_6{\cdot}Na^+$ calcd 325.0198); ^1H NMR (DMSO-d_6) δ 11.92 (br s, 1H, ex, NH), 8.44 (s, 1H, H-6), 5.96 (s, 1H, ex, 3′-OH), 5.81–5.86 (m, 2H, 1 ex, 2′-OH, H-1′), 5.31 (t, 1H, ex, J = 3.9 Hz, 5′-OH), 4.21 (br t, 1H, H-2′), 3.93 (t, 1H, J = 2.9 Hz, H-4′), 3.72–3.80 (m, 1H, H-5′$_A$), 3.63–3.69 (m, 1H, H-5′$_B$), 3.57 (s, 1H, HC≡C); Selected data ^1H NMR (DMSO-d_6 + 1 drop D_2O) 5.82 (d, 1H, J = 7.3 Hz, H-1′), 4.20 (d, 1H, J = 7.3 Hz, H-2′); ^{13}C NMR (DMSO-d_6) δ 158.8, 149.9, 138.0 (C-6), 107.6, 86.8 (C-4′), 86.3 (C-1′), 82.5, 78.0 (C-2′), 77.1, 72.7, 61.2 (C-5′). Anal. Calcd for $C_{11}H_{11}ClN_2O_6{\cdot}7/8$ MeOH: C, 43.27; H, 4.13; N, 8.54. Found: C, 42.87; H, 4.33; N, 8.31.

4.4.5. 1-[3-*C*-Ethynyl-β-D-*ribo*-pentofuranosyl]-5-bromouracil (20). Nucleoside **9** (0.34 g, 0.72 mmol) was stirred for 48 h. The residue resulting after workup was purified by crystallization (MeOH), which after washing with cold CH_2Cl_2 afforded the unprotected nucleoside **20** (150 mg) as white needles. Repeated crystallization of the mother liquor afforded additional **20** (combined yield 171 mg, 68%). R_f = 0.4 (20% MeOH in CH_2Cl_2, v/v); mp (MeOH) 125–128 °C; UV λ_{max} pH 1, 278 nm, λ_{max} H_2O, 278 nm, λ_{max} pH 11, 278 nm; MALDI-HRMS *m/z* 368.9708 ($[M+Na]^+$, $C_{11}H_{11}BrN_2O_6{\cdot}Na^+$ calcd 368.9693); ^1H NMR (DMSO-d_6) δ 11.87 (br s, 1H, ex, NH), 8.52 (s, 1H, H-6), 5.96 (s, 1H, ex, 3′-OH), 5.81–5.86 (m, 2H, 1 ex, 2′-OH, H-1′), 5.31 (t, 1H, ex, J = 4.0 Hz, 5′-OH), 4.21 (br t, 1H, H-2′), 3.93 (t, 1H, J = 2.9 Hz, H-4′), 3.72–3.80 (m, 1H, H-5′$_A$), 3.62–3.70 (m, 1H, H-5′$_B$), 3.56 (s, 1H, HC≡C); Selected

data ^{1}H NMR (DMSO-d_6 + 1 drop D_2O) 5.82 (d, 1H, J = 7.2 Hz, H-1′), 4.20 (d, 1H, J = 7.2 Hz, H-2′); ^{13}C NMR (DMSO-d_6) δ 158.7, 150.2, 140.5 (C-6), 96.2, 86.8 (C-4′), 86.2 (C-1′), 82.5, 78.0 (C-2′), 77.1, 72.8, 61.1 (C-5′). Anal. Calcd for $C_{11}H_{11}BrN_2O_6$·7/16 MeOH: C, 38.09; H, 3.44; N, 7.77. Found: C, 38.18; H, 3.69; N, 7.37.

4.4.6. 1-[3-*C*-Ethynyl-*β*-D-*ribo*-pentofuranosyl]-5-iodouracil (21). Nucleoside **10** (0.33 g, 0.64 mmol) was stirred for 21 h. The residue resulting after workup was purified by silica gel column chromatography (0–100% EtOAc in petroleum ether, v/v, then 0–3% MeOH in EtOAc, v/v) to afford nucleoside **21** (156 mg, 62%). R_f = 0.4 (20% MeOH in CH_2Cl_2, v/v); UV λ_{max} pH 1, 287 nm, λ_{max} H_2O, 287 nm, λ_{max} pH 11, 278 nm; MALDI-HRMS *m/z* 416.9574 ([M+Na]$^+$, $C_{11}H_{11}IN_2O_6$·Na$^+$ calcd 416.9554); ^{1}H NMR (DMSO-d_6) δ 11.72 (br s, 1H, ex, NH), 8.53 (s, 1H, H-6), 5.93 (s, 1H, ex, 3′-OH), 5.84 (d, 1H, ex, J = 6.6 Hz, 2′-OH), 5.80 (d, 1H, J = 7.0 Hz, H-1′), 5.29 (t, 1H, ex, J = 3.8 Hz, 5′-OH), 4.20 (br t, 1H, H-2′), 3.92 (t, 1H, J = 2.9 Hz, H-4′), 3.71–3.79 (m, 1H, H-5′$_A$), 3.62–3.69 (m, 1H, H-5′$_B$), 3.56 (s, 1H, HC≡C); ^{13}C NMR (DMSO-d_6) δ 160.3, 150.5, 145.3 (C-6), 86.7 (C-4′), 86.0 (C-1′), 82.5, 78.0 (C-2′), 77.0, 72.7, 69.9, 61.1 (C-5′). Anal. Calcd for $C_{11}H_{11}IN_2O_6$·1/2 MeOH: C, 33.68; H, 3.19; N, 6.83. Found: C, 34.08; H, 3.35; N, 6.54.

4.4.7. 1-[3-*C*-Ethynyl-*β*-D-*ribo*-pentofuranosyl]-3-deazauracil (22). Nucleoside **11** (0.29 g, 0.73 mmol) was stirred for 23 h. The residue resulting after workup was purified by silica gel column chromatography (0–12% MeOH in $CHCl_3$, v/v) to afford nucleoside **22** (123 mg, 63%) as a white solid material. R_f = 0.4 (20% MeOH in CH_2Cl_2, v/v); UV λ_{max} pH 1, 235 nm, 266 nm, λ_{max} H_2O, 279 nm, λ_{max} pH 11, 256 nm; MALDI-HRMS *m/z* 290.0626 ([M+Na]$^+$, $C_{12}H_{13}NO_6$·Na$^+$ calcd 290.0635); ^{1}H NMR (DMSO-d_6) δ 10.66 (br s, 1H, ex, OH), 7.77 (d, 1H, J = 7.7 Hz, H-6), 6.05 (d, 1H, J = 6.6 Hz, H-1′), 5.91 (dd, 1H, J = 7.7 Hz, 2.6 Hz, H-5), 5.80 (br s, 1H, ex, 3-OH), 5.74 (br d, 1H, ex, J = 6.2 Hz, 2′-OH) 5.54 (d, 1H, ex, J = 2.6 Hz, H-3), 5.05 (br t, 1H, ex, 5′-OH), 4.14 (br t, 1H, H-2′), 3.88 (t, 1H, J = 3.7 Hz, H-4′), 3.63–3.74 (m, 2H, H-5′), 3.51 (s, 1H, HC≡C); ^{13}C NMR (DMSO-d_6) δ 166.5, 163.2, 135.4 (C-6), 100.4 (C-5), 97.8 (C-3), 86.2 (C-1′/C-4′), 86.0 (C-1′/C-4′), 83.0, 78.9 (C-2′), 77.0, 72.6, 61.5 (C-5′).

4.4.8. 1-[3-*C*-Ethynyl-*β*-D-*ribo*-pentofuranosyl]-6-azauracil (23). Nucleoside **12** (0.27 g, 0.69 mmol) was stirred for 24 h. The residue resulting after workup was adsorbed on silica gel and purified by silica gel column chromatography (0–30% MeOH in CH_2Cl_2, v/v) to afford nucleoside **23** (152 mg, 82%) as a pale yellow solid material. R_f = 0.6 (40% MeOH in CH_2Cl_2, v/v); UV λ_{max} pH 1, 261 nm, λ_{max} H_2O, 261 nm, λ_{max} pH 11, 256 nm; MALDI-HRMS *m/z* 292.0538 ([M+Na]$^+$, $C_{10}H_{11}N_3O_6$·Na$^+$ calcd 292.0540); ^{1}H NMR (DMSO-d_6) δ 12.29 (br s, 1H, ex, NH), 7.61 (s, 1H, H-5), 5.91 (br s, 1H, ex, 3′-OH), 5.84 (d, 1H, J = 7.3 Hz, H-1′), 5.76–5.81 (m, 1H, ex, 2′-OH), 4.54 (br t, 1H, ex, J = 7.3 Hz, 5′-OH), 4.45 (m, 1H, H-2′), 3.84 (dd, 1H, J = 7.3 Hz, 3.3 Hz, H-4′), 3.59–3.67 (m, 1H, H-5′$_A$), 3.51 (s, 1H, HC≡C), 3.41–3.49 (m, 1H, H-5′$_B$); ^{13}C NMR (DMSO-d_6) δ 156.7, 149.1, 136.5 (C-5), 87.3 (C-1′), 87.2 (C-4′), 82.8, 76.6, 76.0 (C-2′), 71.9, 62.4 (C-5′). Anal. Calcd for $C_{10}H_{11}N_3O_6$·1/4 H_2O: C, 43.88; H, 4.23; N, 15.35. Found: C, 44.06; H, 4.14; N, 15.01.

4.4.9. 1-[3-*C*-Ethynyl-*β*-D-*ribo*-pentofuranosyl]-5-azacytosine (24). To an ice-cold solution of protected nucleoside **13** (174.4 mg, 0.44 mmol) in anhydrous MeOH (15 mL) was added saturated methanolic ammonia (1.00 mL). The reaction mixture was warmed to rt and stirred for 27 h, whereupon it was evaporated to dryness and coevaporated with anhydrous EtOH (2 × 10 mL). The resulting residue was purified by silica gel column chromatography (0–20% MeOH in CH_2Cl_2, v/v) to afford nucleoside **24** (75 mg, 63%) as a white solid material. R_f = 0.3 (40% MeOH in $CHCl_3$, v/v); UV λ_{max} pH 1, 257 nm, λ_{max} H_2O, 242 nm, λ_{max} pH 11, 249 nm; MALDI-HRMS *m/z* 291.0692 ([M+Na]$^+$, C_{10} $H_{12}N_4O_5$·Na$^+$ calcd 291.0700); ^{1}H NMR (DMSO-d_6) δ 8.54 (s, 1H, H-6), 7.63 (br s, 1H, ex, NH_2), 7.61 (br s, 1H, ex, NH_2), 5.93 (s, 1H, ex, 3′-OH), 5.86 (d, 1H, ex, J = 6.6 Hz, 2′-OH), 5.74 (d, 1H, J = 6.6 Hz, H-1′), 5.10 (t, 1H, ex, J = 4.8 Hz, 5′-OH), 4.29 (t, 1H, J = 6.6 Hz, H-2′), 3.92 (br t, 1H, H-4′), 3.65–3.73 (m, 2H, H-5′), 3.57 (s, 1H, HC≡C); ^{13}C NMR (DMSO-d_6) δ 165.6, 156.6 (C-6), 153.2, 87.3 (C-1′), 86.7 (C-4′), 82.6, 78.1 (C-2′), 77.4, 72.5, 61.3 (C-5′).

4.4.10. 1-[3-*C*-Ethynyl-*β*-D-*ribo*-pentofuranosyl]cyanuric acid (25). Protected nucleoside **14** (115 mg, 0.28 mol) was stirred for 40 h. The residue resulting after workup was purified using silica gel column chromatography (0–30% MeOH in $CHCl_3$, v/v) to afford nucleoside **25** (51 mg, 65%) as a white solid material. R_f = 0.2 (20% MeOH in $CHCl_3$, v/v); MALDI-HRMS *m/z* 308.0481 ([M+Na]$^+$, $C_{10}H_{11}N_3O_7$·Na$^+$ calcd 308.0489); ^{1}H NMR (DMSO-d_6) δ 5.91 (d, 1H, J = 8.3 Hz, H-1′), 5.83 (s, 1H, ex, 3′-OH), 5.74 (d, 1H, ex, J = 6.6 Hz, 2′-OH), 4.94 (dd, 1H, J = 8.3 Hz, 6.6 Hz, H-2′), 4.44 (t, 1H, ex, J = 5.7 Hz, 5′-OH), 3.85 (t, 1H, J = 4.9 Hz, H-4′), 3.59–3.65 (m, 2H, H-5′), 3.49 (s, 1H, HC≡C); ^{13}C NMR (DMSO-d_6) δ 150.6, 150.2, 87.1, 85.7, 83.1, 76.3, 73.4, 72.3, 62.2.

4.4.11. *S*-[3-*C*-Ethynyl-*β*-D-*ribo*-pentofuranosyl]-2-thiocytosine (26). Protected nucleoside **15** (0.26 g, 0.63 mmol) was stirred for 40 h. The residue resulting after workup was crystallized (EtOH and minimal H_2O), which after washing with cold EtOH afforded nucleoside **26** (108 mg) as a white solid material. Crystallization from the mother liquor afforded additional nucleoside **26** (combined yield 128 mg, 72%). R_f = 0.4 (20% MeOH in CH_2Cl_2, v/v); mp (EtOH/H_2O) 176–180 °C; UV λ_{max} pH 1, 239 nm, λ_{max} H_2O, 219 nm, 282 nm, λ_{max} pH 11, 282 nm; MALDI-HRMS *m/z* 306.0524 ([M+Na]$^+$, $C_{11}H_{13}N_3O_4S$·Na$^+$ calcd 306.0519); ^{1}H NMR (DMSO-d_6) δ 7.89 (d, 1H, J = 5.9 Hz, H-6), 6.97 (br s, 2H, 2 ex, NH_2), 6.15 (d, 1H, J = 5.9 Hz, H-5), 5.87 (d, 1H, J = 6.6 Hz, H-1′), 5.84 (d, 1H, ex, J = 6.6 Hz, 2′-OH), 5.67 (br s, 1H, ex,

3-OH), 4.73 (br t, 1H, ex, $J = 5.3$ Hz, 5′-OH), 4.03 (t, 1H, $J = 6.6$ Hz, H-2′), 3.77 (dd, 1H, $J = 6.2$ Hz, 2.8 Hz, H-4′), 3.49–3.64 (m, 3H, H-5′, HC≡C); ^{13}C NMR (DMSO-d_6) δ 168.6, 163.1, 154.8 (C-6), 101.6 (C-5), 86.7 (C-4′), 85.7 (C-1′), 83.1, 79.9 (C-2′), 77.0, 72.6, 61.9 (C-5′). Anal. Calcd for $C_{11}H_{13}N_3O_4S$: C, 46.63; H, 4.63; N, 14.83; S, 11.32. Found: C, 46.52; H, 4.61; N, 14.72; S, 11.22.

4.4.12. *S*-[3-*C*-Ethynyl-β-D-*ribo*-pentofuranosyl]-4-thiouracil (27). Protected nucleoside **16** (0.40 g, 0.98 mmol with traces of 4-thiouracil), was stirred for 41 h. The residue resulting after workup was adsorbed on silica gel and purified by silica gel column chromatography (0–12% EtOH in $CHCl_3$, v/v) to furnish nucleoside **27** (195 mg, 70%). $R_f = 0.2$ (20% EtOH in CH_2Cl_2, v/v); UV λ_{max} pH 1, 263 nm, 322 nm, λ_{max} H_2O, 262 nm, 298 nm, λ_{max} pH 11, 300 nm; MALDI-HRMS *m/z* 307.0356 ($[M+Na]^+$, $C_{11}H_{12}N_2O_5S{\cdot}Na^+$ calcd 307.0359); ^{1}H NMR (DMSO-d_6) δ 11.57 (br s, 1H, ex), 7.68 (d, 1H, $J = 6.6$ Hz, H-6), 6.28 (d, 1H, $J = 6.6$ Hz, H-5), 5.89–5.94 (m, 2H, 1 ex, H-1′, 2′-OH), 5.82 (br s, 1H, ex, 3-OH), 4.79 (t, 1H, ex, $J = 5.3$ Hz, 5′-OH), 4.12 (br t, 1H, H-2′), 3.85 (dd, 1H, $J = 5.5$ Hz, 2.9 Hz, H-4′), 3.40–3.64 (m, 3H, H-5′, HC≡C); Selected data ^{1}H NMR (DMSO-d_6 + 1 drop D_2O) δ 5.90 (d, 1H, $J = 6.1$ Hz, H-1′), 4.12 (d, 1H, $J = 6.1$ Hz, H-2′); ^{13}C NMR (DMSO-d_6) δ 176.2, 154.0, 144.2 (C-6), 101.9 (C-5), 87.3 (C-4′), 84.2 (C-1′), 82.8, 80.0 (C-2′), 77.3, 72.3, 61.7 (C-5′). Traces of EtOH were observed.

5. Anticancer assay

Human adenocarcinoma breast cancer cell line MCF-7 was originally obtained from the Human Cell Culture Bank, Mason Research Institute (Rockville, MD). MCF-7 cells were routinely propagated in growth medium composed of Dulbecco′s Modified Eagle Medium: Ham′s Nutrient Mixture F-12, 1:1 mix (Invitrogen, Denmark, catalog number 21041–025), supplemented with 2.5 mM Glutamax (Invitrogen, Denmark), 6 ng/mL insulin (Novo Nordic, Denmark) and 1% fetal calf serum (FCS).[41] Human adenocarcinoma prostate cancer cell line PC-3 was purchased from ATCC-LGC Promochem (United Kingdom, ATCC catalog number CRL-1435) and grown in ATCC complete growth medium, ATCC catalog number 30–2004 (Ham′s F12K medium with 2 mM L-glutamine, 1.5 g/L sodium bicarbonate, also known as Kaighn′s modification of Ham′s F-12 medium) supplemented with 10% FCS. MCF-7 cells and PC-3 cells were propagated at 37 °C in a humidified 5% CO_2 incubator. In all experiments 250 U/mL penicillin and 31.25 μg/mL streptomycin sulfate were added to prevent bacterial infections.

Growth experiments were performed in 24 multi well dishes (Nunc, Roskilde, Denmark), which were seeded with 1.5×10^4 MCF-7 cells per well or 2.6×10^4 PC-3 cell per well. Two days after seeding, the medium was changed to experimental medium containing the tested compounds, which were dissolved in sterile water. After three additional days the experimental medium was renewed. Five days after initial addition of experimental medium, the cell cultures were washed once in PBS buffer (8 g/L NaCl, 0.2 g/L KCl, 1.44 g/L $Na_2HPO_4{\cdot}2H_2O$, 0.24 g/L KH_2PO_4, pH 7.4) and then cells were fixed and stained for 10 min with a solution of 0,5% (w/v) crystal violet (Sigma–Aldrich) and 25% (v/v) methanol. Unbound crystal violet was removed by gentle wash in tap water. After drying, the crystal violet stained cells were re-dissolved in a solution 0.1 M sodium citrate/50% (v/v) ethanol solution. The optical density measured at 570 nm was a relative measure for the cell number. Growth inhibition of cancer cells was expressed as percent of control, that is untreated cells, and data points were mean of triplicate samples. This assay was applied for practical reasons and yields the same results as directly counting the number of cells.[42]

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Supplementary data

^{1}H NMR spectra of **27** and ^{13}C NMR spectra of **1**, **10**, **13**–**14**, **17**–**18**, and **24**–**25**. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2004.11.054.

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