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Synthesis, structure, and biological evaluation of C-2 sulfonamido pyrimidine nucleosides

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Abstract—The C-2 sulfonamido pyrimidine nucleosides were prepared by opening the 2,2′- or 2,3′-bond in anhydronucleosides under nucleophilic attack of sulfonamide anions. Reaction of the sodium salt of *p*-toluenesulfonamide or 2-(aminosulfonyl)-*N*,*N*-dimethylnicotinamide with 2,2′-anhydro-1-(β-D-arabinofuranosyl)cytosine gave the C-2 sulfonamido derivatives in excellent yields. Ring opening of the less reactive 2,2′-anhydrouridine and 2,3′-anhydrothymidine could be accomplished with DBU/CH_3CN activation of *p*-toluenesulfonamide, giving moderate yields for C-2 sulfonamido derivatives. The action of acetic acid or $ZnBr_2/CH_2Cl_2$ on 5-methyl-N^2-tosyl-1-(2-deoxy-5-*O*-trityl-β-D-*threo*-pentofuranosyl)isocytosine led to the cleavage of both the protection group and the nucleoside bond, yielding 5-methyl-N^2-tosylisocytosine as the major product. Structures of the prepared C-2 sulfonamido nucleosides were confirmed by the 1D and 2D NMR experiments, and X-ray structural analysis of 4-imino-N^2-tosylamino-1-(β-D-arabinofuranosyl)pyrimidine. Both methods confirmed β-configuration and *anti*-conformation of the 2-sulfonamido nucleosides. The investigated compounds displayed moderate inhibition of tumor cell growth in vitro, as determined by the MTT assay using six different human tumor cell lines.

1. Introduction

Modified nucleosides and nucleic acid bases have been the subject of many studies due to their potential activity as enzyme inhibitors resulting in antitumor[1] and antiviral[2] activity. The sulfonamide group $R^1SO_2-NHR^2$ is a common pharmacophore found in various biologically active molecules, enzyme inhibitors and receptor antagonists.[3] Recently, we reported the synthesis of a series of pyrimidine nucleic base derivatives **I** possessing a sulfonamide pharmacophore,[4] and showed that these types of nucleic base derivatives exhibit strong in vitro antitumor activity.[5] These types of nucleic base derivatives were found to inhibit DNA, RNA and protein synthesis and to induce apoptosis in human tumor cells.[5] Also, the *N*-sulfonyl derivatives of purine bases **II** and sulfonamido derivatives of purine nucleosides **III** showed moderate in vitro antitumor activity (Fig. 1).[6]

With respect to the found biological activity, it appeared of interest to synthesize the C-2-sulfonamido derivatives of pyrimidine nucleosides and to evaluate their antitumor activity. It is known that 1-(β-D-arabinofuranosyl)cytosine (ara-C)[7] was found effective in the treatment of acute myeloblastic leukemia,[8] and that the 2,2′-anhydro-1-(β-D-arabinofuranosyl)cytosine is a highly effective antitumor agent as well.[9] In this work, we report on the synthesis, NMR and X-ray structural studies of C-2-sulfonamido pyrimidine nucleosides, and present the results of their in vitro antitumor activity against various human tumor cell lines.

2. Results and discussion

2.1. Synthesis

Anhydronucleosides are important modifications of natural nucleosides and have been used as intermediates in the chemical synthesis of arabinonucleosides. 2,2′-Anhydro-1-(β-D-arabinofuranosyl)cytosine has been shown to be a useful intermediate for the synthesis of 1-(β-D-arabinofuranosyl)cytosine (ara-C).[7] Nucleophilic opening of the anhydronucleosides represents a classical method for the preparation of sugar modified nucleoside derivatives or C-2 substituted derivatives.[10] Hence, for the preparation of C-2 sulfonamido pyrimidine nucleosides **3**, **5**, and **7**, we considered the approach through ring opening of the

Keywords: anhydronucleosides; C-2 sulfonamido pyrimidine nucleosides; X-ray structure; in vitro antitumor activity.

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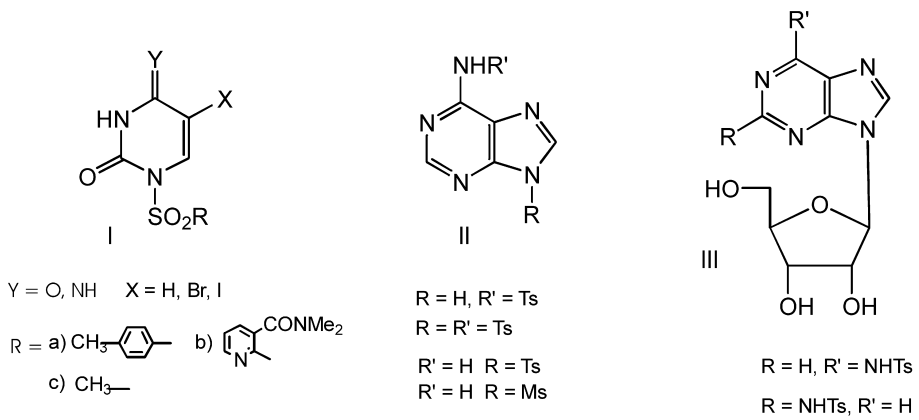
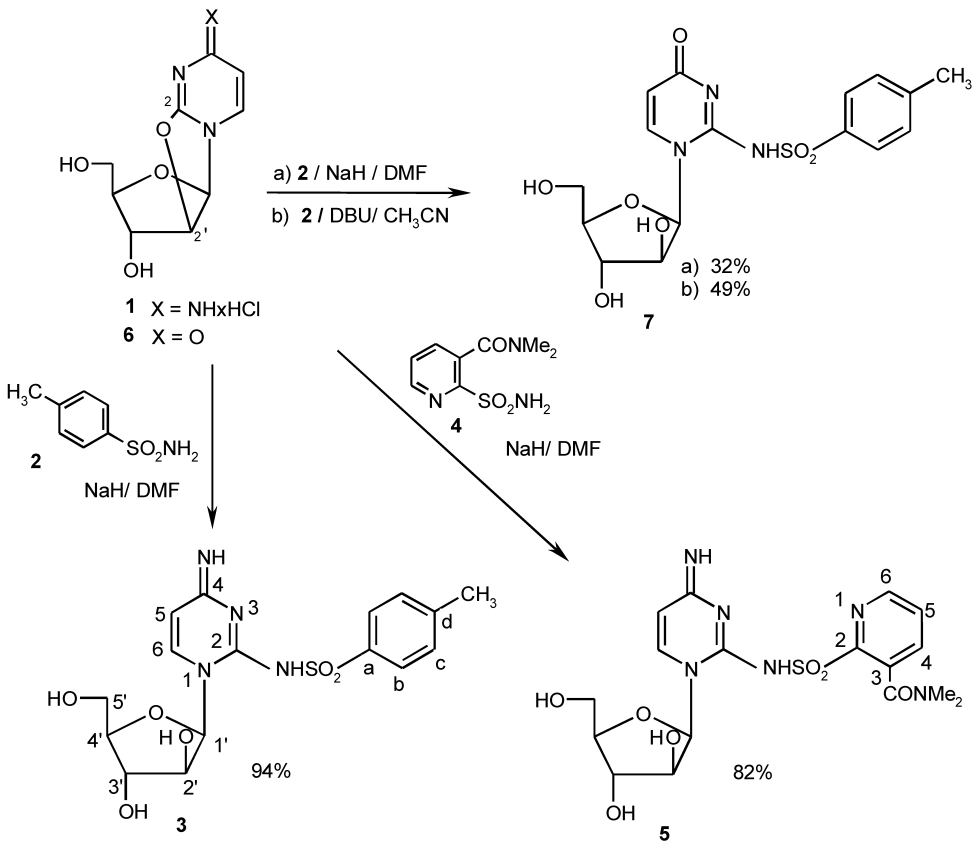


Figure 1. Recently reported pyrimidine and purine derivatives possessing sulfonamide pharmacophore.

2,2′- and 2,3′-anhydronucleosides by nucleophilic attack of the selected sulfonamide anion. Novotný et al.[11] found that 2,2′-anhydro-1-(β-D-arabinofuranosyl)cytosine reacted with 2 equiv. of 4-aminobenzenesulfonamide sodium salt giving the 2-sulfonamido derivative in 70% yield and that the less reactive 2,2′-anhydro-1-(β-D-arabinofuranosyl)-uracil gave only 16% yield of the corresponding 2-sulfonamido derivative. Following the reported procedure,[11] we found that the 2,2′-anhydrocytidine hydrochloride **1**,[12a] in reaction with 2 equiv. of the sodium salt of *p*-toluenesulfonamide **2** (dry DMF, room temperature), gave 4-imino-N^2-tosylamino-1-(β-D-arabinofuranosyl)pyrimidine **3** in 94% yield (Scheme 1). The sulfonamido derivative **5** was synthesized in the same way (82% yield) using the sodium salt of 2-(aminosulfonyl)-*N*,*N*-dimethylnicotinamide **4** as a nucleophile. The starting sulfonamide **4** was prepared by the reaction of 2-chlorosulfonyl-3-(*N*,*N*-dimethylamino-carbonyl)pyridine and methanolic ammonia.[13]

Introduction of the sulfonamido group into the less reactive 2,2′-anhydrouridine **6** needed vigorous conditions. Following the procedure of Reese,[14] the starting 2,2′-anhydro-1-(β-D-arabinofuranosyl)uracil **6** was obtained in quantitative yield. In the reaction of **6** with a two-fold excess of *p*-toluenesulfonylamide **2**, activated with 1 equiv. of NaH in dry DMF (reflux for 3 h), the N^2-tosylisocytidine **7** (32% yield) was isolated by preparative chromatography on a funnel (Scheme 1). However, when the suspension of



Scheme 1.

p-toluenesulfonylamide **2** (2 equiv.) was treated with 2 equiv. of 1,8-diazabicyclo[5.4.0] undec-7-ene (DBU) in acetonitrile, and then refluxed with 2,2′-anhydro derivative **6**, the yield of **7** increased to 49%. The latter procedure was also found more suitable for a larger scale preparation of **7**. However, 2,2′-anhydro compound **6** failed to react with 2-(aminosulfonyl)-*N*,*N*-dimethylnicotinamide **4** under the same reaction conditions, giving mostly unreacted material.

Compounds **3**, **5**, and **7** were found to be stable in crystalline forms. Novotný et al.[11] reported on the quantitative conversion of 2-(*p*-aminobenzenesulfonylamino) derivatives of ara-C and ara-U into cyclocytidine and cyclouridine, respectively, in 50% acetic acid. In contrast, the C-2 sulfonamido derivatives **3**, **5**, and **7** were stable in 50% acetic acid at room temperature for 15 days.

The starting materials for the introduction of sulfonamido group into the C-2 position of 2,3′-anhydrothymidine: 5′-*O*-trityl-2,3′-anhydrothymidine **8**,[15] 2,3′-anhydrothymidine **12**,[16] and 5′-*O*-TBDMS-2,3′-anhydrothymidine **13**,[17] were prepared by the known methods. The reaction of sulfonamide **2** with the 5′-*O*-trityl-2,3′-anhydrothymidine **8** was investigated first (Scheme 2). When the DMF solution of **2** was treated with sodium hydride and 5′-*O*-Tr-2,3′-anhydrothymidine **8** at 110°C, for 72 h, the 5-methyl-N^2-tosyl-1-(2-deoxy-5-*O*-trityl-β-D-*threo*-pentofuranosyl)isocytosine **9** was obtained in only 9% yield. However, introduction of the sulfonamido group into the C-2 position of 5′-*O*-trityl-2,3′-anhydrothymidine **8** can be accomplished with DBU/CH_3CN activation of sulfonamide **2**, in a relatively high yield (67%). On the other hand, deprotection of 5-methyl-N^2-tosyl-1-(2-deoxy-5-*O*-trityl-β-D-*threo*-pentofuranosyl)-isocytosine **9** with 80% acetic acid or under aprotic neutral conditions, $ZnBr_2/CH_2Cl_2$,[18] leads to the cleavage of both the protection group and the nucleosidic bond. Thus, treatment of the 5-methyl-N^2-tosyl-1-(2-deoxy-5-*O*-trityl-β-D-*threo*-pentofuranosyl)isocytosine **9** with $ZnBr_2/CH_2Cl_2$ gave 5-methyl-N^2-tosylisocytosine **10** in 49% yield as the major product, and 5-methyl-N^2-tosyl-1-(2-deoxy-β-D-*threo*-pentofuranosyl)isocytosine **11** in only 17% yield, as a minor product isolated by chromatographic purification. This observation was not quite unexpected in view of the known liability of the glycosidic linkage in 2′-deoxynucleosides toward acid conditions.[19]

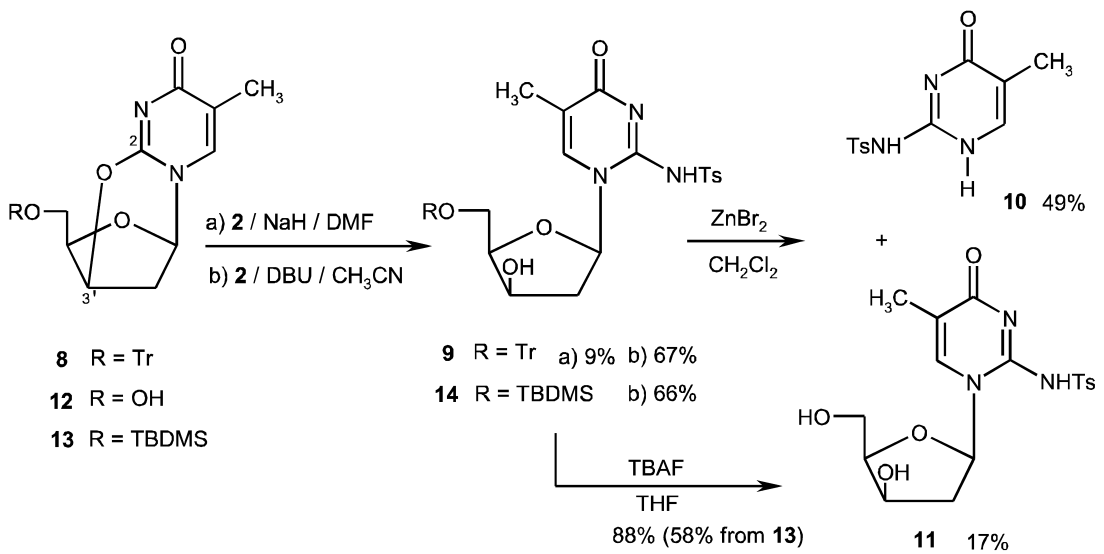
When sulfonamide **2** was treated with DBU/CH_3CN in acetonitrile and reacted with 2,3′-anhydrothymidine **12** possessing a free 5′-hydroxyl group, the C-2 sulfonamido derivative **11** was isolated in only 15% yield, due to the low solubility of **12** in this solvent. Then, we turned to the reaction of 5′-*O*-TBDMS-protected 2,3′-anhydrothymidine **13** under the same conditions. Compound **13** was heated with sulfonamide **2** activated with DBU in acetonitrile, and 5-methyl-N^2-tosyl-1-(5-*O*-*t*-butyldimethylsilyl-2-deoxy-β-D-*threo*-pentofuranosyl)isocytosine **14** was isolated in 66% yield. Deprotection of *t*-butyldimethylsilyl group in **14** was performed with tetrabutylammonium fluoride in THF to give the desired 5-methyl-N^2-tosyl-1-(2-deoxy-β-D-*threo*-pentofuranosyl)isocytosine **11** in 88% yield (58% overall yield from **13**).

The 2,3′-anhydro compounds **8**, **12**, and **13** failed to react with 2-(aminosulfonyl)-*N*,*N*-dimethylnicotinamide **4** under the same reaction conditions.

2.2. NMR studies

Structures of the prepared C-2 sulfonamido derivatives **3**, **5**, and **7** and **9**, **11**, and **14** were confirmed by 1D and 2D (COSY, HETCOR, NOESY) NMR experiments, and X-ray analysis of **3**.

NOESY maps of the C-2 sulfonamido derivatives **3**, **5**, and **7** (Fig. 2) show strong NOE interactions between H-1′ and H-2′ in accord with the β-anomeric configuration; the latter is also supported by weak NOE effects between H-1′ and OH-2′ in **3**, and H-1′ and H-4′ in **7**. The NOE interactions between H-6 and H-3′, H-6 and H-5′ observed for C-2 sulfonamido derivatives **3**, **5**, and **7** provide clear-cut evidence for their *anti*-conformations. A weak NOE interaction between H-6 and OH-2′ in **7** also supports the arabino configuration.



Scheme 2.

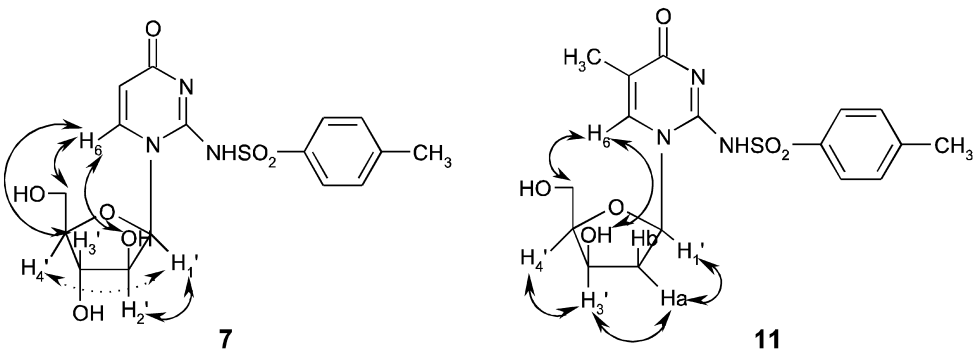


Figure 2. NOESY experiment of compounds **7** and **11** in DMSO-d_6.

In the ^{1}H NMR spectra 2,3′-anhydro compounds **8**, **12**, and **13**, the H-2′$_\alpha$ appeared in a higher field (δ 2.42–2.43 ppm) than H-2′$_\beta$ (δ 2.52–2.54 ppm). The opposite trend was observed for C-2 sulfonamido compounds **9**, **11**, and **14**. The H-2′$_\alpha$ appeared in a lower field (δ 2.45–2.49 ppm) compared to H-2′$_\beta$ (δ 1.75–1.88 ppm). In the NOESY maps of C-2 sulfonamido compounds **9**, **11**, and **14**, the NOE interactions between H-6 and OH-3′, and H-6 and H-5′ clearly confirm their *anti*-conformations (Fig. 2). The strong NOE effects observed for H-1′ and H-2′$_\alpha$, and H-3′ and H-2′$_\alpha$ in all compounds, together with weaker NOE interactions for H-4′ and H-1′ in the case of **14**, are in accord with the β-anomeric configurations of **9**, **11**, and **14**.

2.3. Molecular structure of 4-imino-*N*^{2}-tosylamino-1-(β-D-arabino-furanosyl)pyrimidine 3

The pentose ring of **3** reveals C2′-*endo* (^2E) puckering with C2′ being located 0.580(4) Å above the least squares plane calculated through the remaining four ring atoms as previously reported for related structures.[20] Deviation of the remaining four atoms C1′, C3′, C4′ and O1′ from their ls plane are −0.010(4), 0.008(4), −0.015(4) and 0.016(2) Å, respectively. The CP puckering parameters[21] of the pentose ring are: Q_2=0.370(4) Å and ϕ_2=77.5(5)°. Compound **3** is characterized by the *R* absolute configuration at C4′ and at C1′, whereas the remaining chiral C atoms, C2′ and C3′, reveal the *S* absolute configuration. The *R* absolute configuration at the anomeric C atom (C1′) defines the direction of the glycosidic bond, placing its substituent (the pyrimidine base) above the plane of the sugar ring, thus defining the β-conformation of the nucleoside. The torsion angle about the glycosidic bond (O1′–C1′–N1–C6) reveals the value of 9.5(4)° defining the *anti* orientation of the pyrimidine ring with respect to the sugar unit. This is the preferred orientation in most nucleosides and

Table 1. Hydrogen bonds in the crystal of **3**

	D–H···*A* (Å)	D–H (Å)	H···*A* (Å)	D–H···*A* (°)	Sym. operation on *A*
N15–H15A···O2	1.05(4)	2.06(4)	3.072(3)	160(3)	$y, -1+x, 1/3-z$
N15–H15B···O23	0.99(5)	2.23(5)	3.174(4)	158(4)	$y, -1+x, 1/3-z$
O22–H···O24	1.41(5)	1.24(5)	2.647(3)	177(5)	x, y, z
O23–H···O2	0.85(4)	2.05(3)	2.886(3)	167(4)	$x, x-y, 1/6-z$

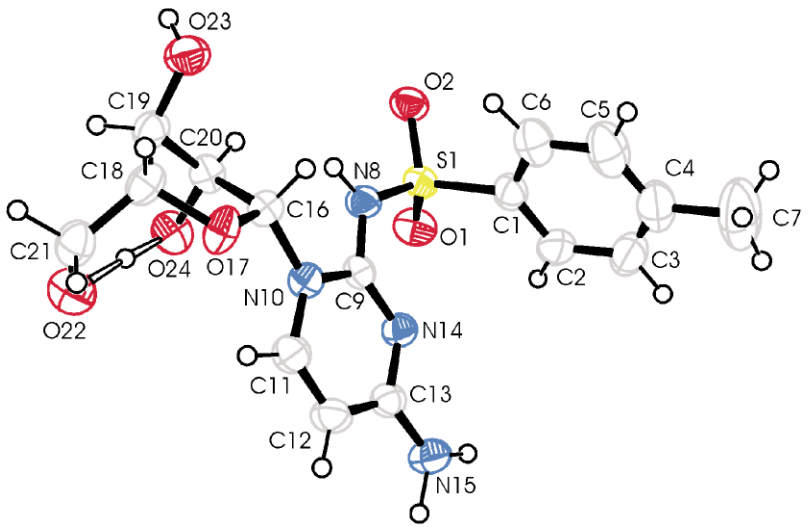


Figure 3. The ORTEP drawing of the nucleoside molecule with atom numbering. Thermal ellipsoids are scaled to the 50% probability, and hydrogen atoms are excluded for clarity. The atom numbering is arbitrary and has nothing to do with the IUPAC nomenclature.

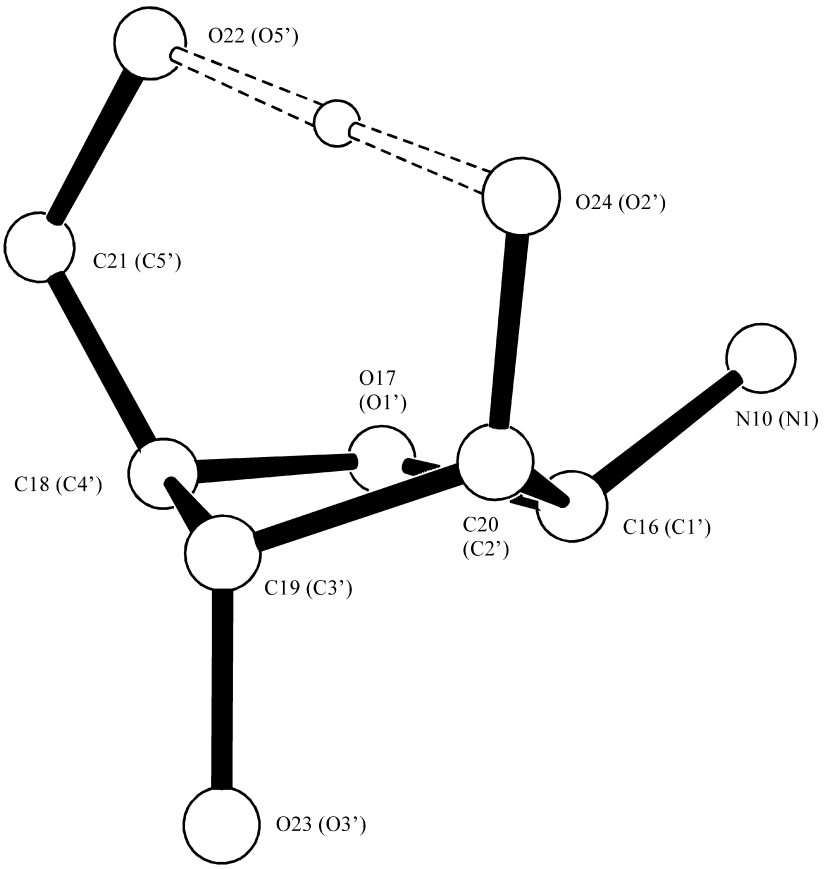


Figure 4. PLUTON drawing of the arabinofuranose unit with N10 from pyrimidine, with the intramolecular asymmetric short hydrogen bond O5′–H···O2′ indicated. The atom numbering according to the IUPAC nomenclature for nucleosides is given in the parentheses.

nucleotides.[22] Torsion angles O1′–C4′–C5′–O5′ and C3′–C4′–C5′–O5 reveal values of −65.2(3) and 53.5(4)° and define the so-called *gauche*, *gauche* orientation of the C4′–C5′ bond.

O2′ is above the plane of the pentose ring, whereas O3′ is below it, with the torsion angle O2′–C2′–C3′–O3′, −162.4(2)°. Such an orientation of O2′ combined with the *gauche*, *gauche* orientation of the hydroxymethyl group at

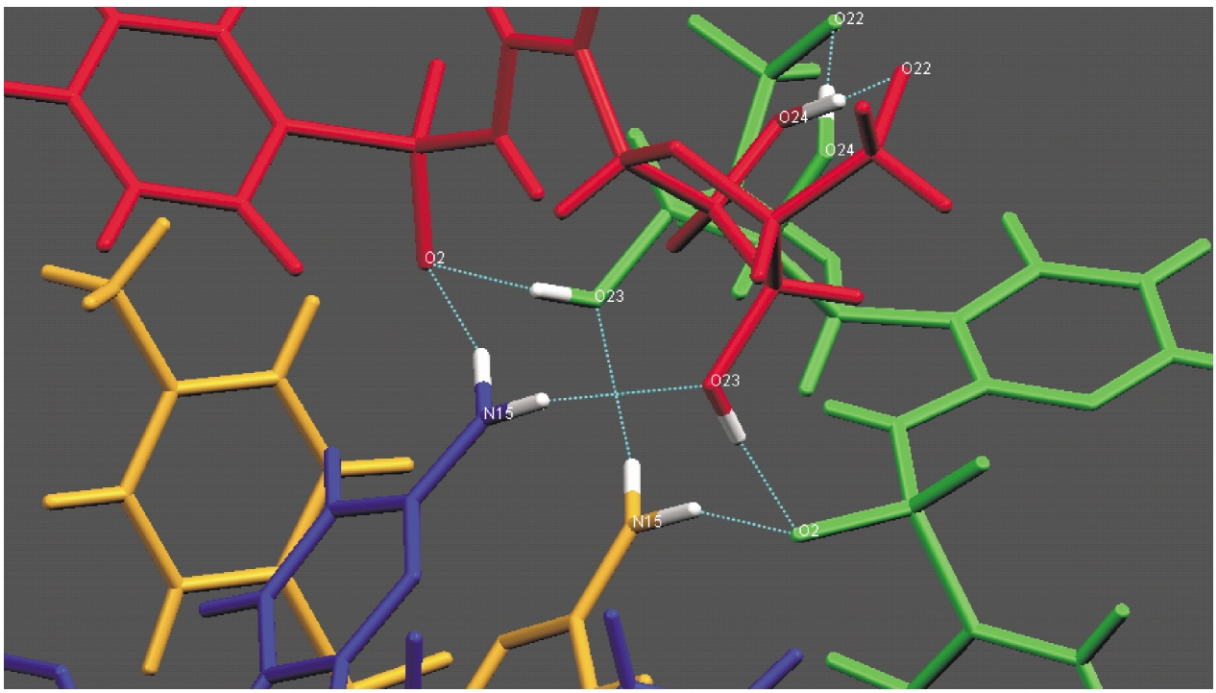


Figure 5. The intermolecular hydrogen bonding pattern in the crystal structure of **3**.

C5′ enables the formation of a short asymmetric hydrogen bond O5′–H···O2′.[23,24] O5′ and O2′ are only 2.647(5) Å apart, with the H atom being slightly closer to the O2′ (Table 1, Figs. 3 and 4).

2.4. Crystal packing of 3 (Table 1)

N15 is involved in two intermolecular hydrogen bonds via its hydrogen atoms H15A and H15B. These hydrogen bonds connect N15 with O2 and O23 (O3′, according to the arabinose nomenclature) from the neighboring molecule (y, $-1+x$, $1/3-z$). O23 (O3′) acts at the same time as an acceptor in the above described contact and as a donor in the hydrogen bond O23–H···O2 (x, $x-y$, $1/6-z$), thus playing a role of a double acceptor to O2 and forming a 12-membered crown-like macrocycle based on hydrogen bonding with four neighboring nucleoside molecules involved (Fig. 5).

2.5. Cell growth inhibition

Influence of the C-2 sulfonamido pyrimidine derivatives on human tumor cells growth is shown in Table 2. Moderate (20–50% inhibition) to weak (0–20% inhibition) potency of cytotoxicities was found in all investigated compounds. Their growth inhibitory effects on different cultured cells varied from one type of cells to the next and also depended on the concentration applied. Application of cytidine derivatives **3** and **5** showed similar effects on tumor cell growth in comparison with uridine derivative **7**. The cell lines most sensitive to investigated compounds were human colon carcinoma (HT-29) and human breast adenocarcinoma (MCF-7), followed by human hepatocarcinoma (Hep2). As expected, the most resistant cells were poorly differentiated cells from lymph node metastases of colon carcinoma (SW 620).

All five compounds **3**, **5**, **7**, **10**, and **11** were also evaluated for cytotoxicity in different human leukemia cell lines. Details of those results will be reported elsewhere.

Table 2. Growth inhibitory effects of C-2 sulfonamido pyrimidine derivatives on human tumor cells

Cell line[a]	Concentration (M)[b]	Compounds				
		3	**5**	**10**	**7**	**11**
MiaPaCa2	10^{-6}	–	–	4.6	3.4	3.6
	10^{-5}	–	–	–	24.8	6.8
	10^{-4}	–	4.3	–	2	4.1
	10^{-3}	41.3	24.4	13.3	45.1	13.6
HeLa2	10^{-6}	–	8	2.3	11.5	–
	10^{-5}	3.8	3.3	10.4	7.1	3.8
	10^{-4}	9.2	30.4	23	6.1	–
	10^{-3}	22.6	56.3	36.3	26.5	6.7
Hep2	10^{-6}	2.4	4.9	12.5	2.9	2.1
	10^{-5}	3.2	11.2	14.8	3.9	5.4
	10^{-4}	10.1	9.3	30.9	16.8	3.6
	10^{-3}	24	38.1	26.2	44.8	12.4
CaCo2	10^{-6}	–	–	–	–	–
	10^{-5}	–	–	–	–	1.5
	10^{-4}	41.5	31.5	–	–	–
	10^{-3}	45.2	61.6	43.3	2.9	4.7
HT-29	10^{-6}	11.9	28.5	18.32	5.8	–
	10^{-5}	10.3	9.2	15.7	5.7	–
	10^{-4}	22.2	26.3	41.6	5.3	–
	10^{-3}	39.5	50	44.5	46.8	11.8
SW-620	10^{-6}	–	–	3.7	5.3	–
	10^{-5}	–	–	–	–	–
	10^{-4}	–	–	–	–	–
	10^{-3}	23.5	43.2	12.6	4.7	–
MCF-7	10^{-6}	26.8	–	7.3	1	–
	10^{-5}	32.9	12.5	22	20.9	–
	10^{-4}	17.2	9.7	22.7	25.9	1.6
	10^{-3}	43.7	56.1	42.2	39.6	8.3

The treated tumor cells growth inhibition was calculated relative to growth of untreated (control) cells and shown as percent (%), '–'=without inhib. activity.

[a] Cell lines: pancreatic carcinoma (MiaPaCa2), colon carcinomas (CaCo2, HT-29), cervical carcinoma (HeLa2), breast adenocarcinoma (MCF-7), hepatocellular carcinoma cells (Hep2), poorly differentiated cells from lymph node metastasis of colon carcinoma (SW 620).

[b] Exponentially growing cells were treated with different concentration of investigated compounds during 72-hours period. Cytotoxicity was analysed with MTT survival assay. All experiments were performed at least three times.

3. Conclusion

Introduction of the sulfonamido group into the C-2 position of pyrimidine nucleosides was achieved by ring opening of the 2,2′- and 2,3′-anhydronucleosides with selected sulfonamide anion as a nucleophile. The ring opening of the less reactive 2,2′-anhydrouridine and 2,3′-anhydrothymidine can be accomplished with DBU/CH_3CN activation of *p*-toluenesulfonamide giving acceptable yields (50–70%) of tosylated products. All of the prepared 2-sulfonamido nucleosides displayed moderate to weak concentration-dependent inhibition of human tumor cell growth, as determined by the MTT assay using seven different tumor cell lines. The results of previous and this study reveal that the C-2 sulfonamido pyrimidine nucleoside derivatives represent a new group of compounds with a promising anticancer activity that merits further synthetic and biological investigations directed toward finding new anticancer drugs.

4. Experimental

4.1. General

Solvents were distilled from appropriate drying agents shortly before use. TLC was carried out on DC-plastikfolien Kieselgel 60 F_{254} and preparative thick layer (2 mm) chromatography was done on Merck 60 F_{254}. Flash column chromatography was performed on silica gel Merck 0.040–0.063 mm. Melting points were determined on a Kofler hot-stage apparatus and were uncorrected. UV Spectra [λ_{max}/nm, log ε/dm^3 mol^{-1} cm^{-1}] were taken on a Philips PU8700 UV/VIS spectrophotometer. IR spectra were obtained for KBr pellets on a Perkin–Elmer 297 spectrophotometer.

The ^{1}H and ^{13}C NMR spectra were recorded on a Varian Gemini 300 spectrometer, operating at 75.46 MHz for the ^{13}C nucleus. The samples were dissolved in DMSO-d_6

and measured at 20°C in 5 mm NMR tubes. Sample concentrations were 0.1 M for ^{1}H and 0.2 M for ^{13}C measurements. Chemical shifts (δ/ppm) were referred to TMS. Digital resolution was 0.3 Hz per point in ^{1}H and 0.5 Hz per point in ^{13}C NMR one-dimensional spectra. The applied techniques were standard ^{1}H and ^{13}C with broadband proton decoupling, ^{13}C gated decoupling, COSY, NOESY and HETCOR. For proton decoupling, the Waltz-16 modulation was used. The COSY spectra were recorded in the magnitude mode with 1024 points in F2 dimension and 256 increments in F1 dimension, zero-filled to 1024 points. Increments were measured with 16 scans, 4500 Hz spectral width and a relaxation delay of 0.8 s. The corresponding digital resolution was 8.9 Hz per point and 17.6 Hz per point in F2 and F1 dimensions, respectively. The NOESY spectra were recorded in a phase-sensitive mode and at mixing times of 0.45–0.80 s. All other measurement parameters were as for the COSY spectra. The HETCOR spectra were recorded with 2048 points in F2 dimension and 256 increments in F1 dimension, zero-filled to 512 points. Each increment was recorded by 96 scans with a relaxation delay of 1.0 s. Spectral widths were 19000 Hz in F2 and 4500 Hz in F1 dimensions, and the corresponding digital resolutions were 18.6 Hz per point and 17.6 Hz per point, respectively.

4.2. X-Ray structure analysis

$C_{16}H_{20}N_4O_6S$, M_r=396.42 g mol^{-1}, hexagonal, space group: $P6_122$, a=15.3746(8) Å, c=27.683(4) Å, V=5667.0(9) Å^3, ρ=1.394 g cm^{-3}, μ=1.893 mm^{-1}. The crystal was prismatic and colorless, dimensions 0.2×0.2×0.2 mm^3. Three intensity control reflections measured every 60 min did not show any significant loss of intensity during the data collection, which was performed on an Enraf Nonius CAD4 diffractometer, using a graphite monochromated Cu Kα (1.54179 Å) radiation at room temperature [293(2) K]. The WinGX standard procedure was applied for data reduction.[25] Absorption correction based on eight Ψ-scan reflexions was performed.[26] There were 3978 unique reflections collected, out of which 3151 were observed [$I>2\sigma(I)$], R_{int}=0.0343, R_σ=0.0398. The structure was solved with SIR97,[27] and refined with SHELXL97.[28] Molecular geometry calculations were performed with PLATON,[29] and molecular graphics were prepared using ORTEP-3,[30] and CCDC-Mercury.[31] The model was refined using the full matrix least squares refinement to the final R_1=0.0374, wR_2 (all)=0.0985, S=1.076, $\Delta\rho_{max}$=0.18 e Å^{-3}, $\Delta\rho_{min}$=−0.36 e Å^{-3}. The final value of the Flack parameter, x=0.00(2), confirmed the right choice of the absolute configuration. The atomic scattering factors were those included in SHELXL97.[28] Hydrogen atoms were refined as the riding entities, except for those included in hydrogen bonds that were located in the Fourier map and freely refined. In the final steps of refinement, the proposed weighting scheme was applied.

CCDC-198722 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via http://www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033; or deposit@ccdc.cam.ac.uk)

4.3. Biological studies

4.3.1. Cell cultures. Seven human cell lines were used for this biological investigation: pancreatic carcinoma (Mia-PaCa2), colon carcinoma (CaCo2, HT-29), cervical carcinoma (HeLa2), breast adenocarcinoma (MCF-7), hepatocellular carcinoma cells (Hep2), and poorly differentiated cells from lymph node metastasis of colon carcinoma (SW 620). The cells were grown in monolayer at 37°C in a humidified atmosphere with 5% CO_2 in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (v/v) fetal bovine serum, 2 mM glutamine, 100 U penicillin and 100 μg/mL streptomycin.

4.3.2. Cytotoxicity assay. The cytotoxic effects of C-2 sulfonamido pyrimidine derivatives on cell growth and viability were determined using a validated colorimetric MTT assay as described by Horiuchi et al.[32] Briefly, 2×10^4 cells/mL were plated in 96-microwell Plate and allowed to attach overnight. After 24 h, different concentrations (1×10^{-6}–1×10^{-3} M) of tested compounds were added to each well. Control cells were grown under the same conditions without addition of test compounds. The cells were incubated at 37°C for 3 days, whereupon 20 μL of MTT (5 mg MTT/mL PBS) was added to each well. After 4 h of incubation, the medium was removed, the wells were washed with PBS, and then 30 μL DMSO was added to each well to solubilize the precipitation. The Plate were transferred to an Elisa reader (Stat fax 2100, Pharmacia Biotech) to measure the extracted dye at 570 nm. All experiments were performed at least three times, with six wells for each concentration of test compounds. The percentage of treated tumor cells growth inhibition was calculated relative to the growth of untreated (control) cells. Variation between the experiments was less than 10%. Background absorbency from the control wells (same media, no cells) was subtracted.

4.4. Synthesis

4.4.1. 2,2′-Anhydro-1-(β-D-arabinofuranosyl)cytosine hydrochloride 1. The starting material 2-(hydroxymethyl)-6-imino-2,3,3a,9a-tetrahydro-6*H*-furo[2,3-*d*]pyrimido[2,1-*b*][1,3]oxazol-3-ol hydrochloride (**1**) was isolated in 60% yield, following the Kondo and Inoue Procedure.[12a] The resulting white crystals have: mp 266–267°C (lit.[12a] 238–244°C; lit.[12b] 266–267°C).

4.4.2. 4-Imino-*N*^{2}-tosylamino-1-(β-D-arabinofuranosyl)-pyrimidine 3. To a mixture of *p*-toluensulfonamide **2** (1.96 g, 11.5 mmol) and dimethylformamide (30 mL), sodium hydride (500 mg, 55% in oil, 11.5 mmol) and 2,2′-anhydro-1-(β-D-arabinofuranosyl)cytosine hydrochloride **1** (1.50 g, 5.7 mmol) were added under stirring. The mixture was stirred at room temperature for 1 h and evaporated. The residue was chromatographed on a short column of silica gel in the system (CH_2Cl_2/MeOH 9:1, 3:1) yielding *N*1-{1-[3,4-dihydroxy-5-(hydroxymethyl)tetrahydro-2-furanyl]-4-imino-1,4-dihydro-2-pyrimidinyl}-4-methyl-1-benzensulfonamide (**3**), 2.13 g (94%) as a colorless crystals: mp=201°C; R_f=0.35 (CH_2Cl_2/MeOH 5:1); UV (EtOH) λ_{max}/nm: 222, 245, and 285 (sh), log ε/dm^3 mol^{-1} cm^{-1}: 4.45, 4.56, and 3.92; IR (KBr) ν_{max}/cm^{-1}: 3400 (s),

3300 (s), 3210 (s), 1640 (s), 1620 (s), 1540 (s), 1450 (s), 1400 (m), 1300 (m), 1265 (s), 1205 (m); ^{1}H NMR (DMSO-d_6) δ/ppm: 7.82 (d, 2H, J=8.0 Hz, Ts-b), 7.71 (brd, 2H, J=7.3 Hz, H-6 and NH), 7.57 (brs, 1H, NH), 7.18 (d, 2H, J=8.0 Hz, Ts-c), 6.21 (d, 1H, J=3.3 Hz, H-1′), 5.88 (d, 1H, J=7.3 Hz, H-5), 5.51 (brd, 2H, J=3.3 Hz, OH-2′and OH-3′), 5.05 (t, 1H, J=5 Hz, OH-5′), 4.02 (brs, 1H, H-2′), 3.88 (brs, 1H, H-3′), 3.78 (brs, 1H, H-4′), 3.57 (brt, 2H, J=4.7 Hz, H-5′$_a$ and H-5′$_b$) 2.30 (s, 3H, CH_3-Ts); ^{13}C NMR (DMSO-d_6) δ/ppm: 162.85 (s, C-4), 153.20 (s, C-2), 143.04 (d, C-6), 141.27 (s, Ts-d), 140.49 (s, Ts-a), 128.30 (d, Ts-c), 127.98 (d, Ts-b), 95.18 (d, C-5), 87.49 (d, C-1′), 86.03 (d, C-4′), 76.59 (d, C-3′), 74.37 (d, C-2′), 61.28 (t, C-5′), 21.06 (q, CH_3–Ts); Anal. calcd for $C_{16}H_{20}N_4O_6S$ (M_r=396.41): C 48.36, H 5.25, N 14.08%; found: C 48.48, H 5.09, N 14.13%.

Crystals for X-ray. The compound **3** sample was dissolved in hot methanol, and after storage at 18°C, for 48 h, gave colorless crystals (prisms).

4.4.3. 4-Imino-N^2-[({3-[(dimethylamino)carbonyl]-2-pyridinyl(sulfonyl)amino]-1-(β-D-arabinofuranosyl)pyrimidine 5. To a mixture of 2-aminosulfonyl-3-(*N*,*N*-dimethylaminocarbonyl)pyridine[13] **4** (175 mg, 0.8 mmol) and dimethylformamide (3 mL), sodium hydride (35 mg, 55% in oil, 0.8 mmol) and 2,2′-anhydro-1-(β-D-arabinofuranosyl)-cytosine hydrochloride **1** (105 mg, 0.4 mmol) were added under stirring. The mixture was stirred at room temperature for 2 h and evaporated. The residue was purified by preparative chromatography (CH_2Cl_2/MeOH 3:1) yielding 131 mg (82%) of *N*3,*N*3-dimethyl-2-[({1-[3,4-dihydroxy-5-(hydroxymethyl)tetrahydro-2-furanyl]-4-imino-1,4-dihydro-2-pyrimidinyl}amino)sulfonyl]nicotinamide (**5**), as white crystals: mp=190°C; R_f=0.59 (CH_2Cl_2/MeOH 3:1); UV (MeOH) λ_{max}/nm: 232 and 270 (sh), log ε/dm^3 mol^{-1} cm^{-1}: 4.32 and 3.89; IR (KBr) ν_{max}/cm^{-1}: 3340 (s, br), 3200 (s), 2910 (m), 1641 (s, br), 1550 (m), 1480 (s), 1410 (m), 1380 (m), 1160 (m), 1112 (s); ^{1}H NMR (DMSO-d_6) δ/ppm: 8.53 (d, 1H, J=4.3 Hz, H-6–Py), 7.75 (d, 1H, J=7.7 Hz, H-6), 7.70 (d, 1H, J=7.0 Hz, H-4–Py), 7.50 (dd, 1H, J=4.3, 7.0 Hz H-5–Py), 7.36 (brs, 1H, NH), 7.15 (brs, 1H, NH), 6.21 (t, 1H, J=4.3 Hz H-1′), 5.87 (d, 1H, J=7.7 Hz, H-5), 5.52 (brd, 2H, J=3.3 Hz, OH-2′and OH-3′), 5.04 (brs, 1H, OH-5′), 4.06 (brs, 1H, H-2′), 3.89 (brs, 1H, H-3′), 3.79 (brs, 1H, H-4′), 3.58 (brs, 2H, 2H-5′) 2.93 (s, 3H, CH_3–N), 2.73 (s, 3H, CH_3–N); ^{13}C NMR (DMSO-d_6) δ/ppm: 168.03 (s, C=O–Py), 162.61 (s, C-4), 156.26 (s, C-2–Py), 153.64 (s, C-2), 148.87 (d, C-6–Py), 142.90 (d, C-6), 136.34 (d, C-4–Py), 131.41 (s, C-3–Py), 125.45 (d, C-5–Py), 95.41 (d, C-5), 87.37 (d, C-1′), 85.84 (d, C-4′), 76.27 (d, C-3′), 74.76 (d, C-2′), 61.08 (t, C-5′), 38.48 (q, CH_3–N), 34.47 (q, CH_3–N). Anal. calcd for $C_{17}H_{22}N_6O_7S$ (M_r=454.47): C 36.58, H 5.47,N 15.14%; found: C 36.86, H 5.54, N 15.18%.

4.4.4. 2,2′-Anhydro-1-(β-D-arabinofuranosyl)uracil 6. The product 3-hydroxy-2-(hydroxymethyl)-2,3,3a,9a-tetrahydro-6*H*-furo(2,3-*d*(pyrimido(2,1-*b*(oxazol-6-one (**6**) was isolated in quantitative yield.[14] The resulting white solid had mp=234°C (lit.[14] mp=234–237°C).

4.4.5. N^2-Tosylarabinoisocytidine 7. (a) 2,2′-Anhydro-1-(β-D-arabinofuranosyl)uracil **6** (362 mg, 1.6 mmol) was added to a stirred solution of *p*-toluensulfonamide **2** (549 mg, 3.2 mmol) and sodium hydride (70 mg, 55% in oil, 1.6 mmol) in dimethylformamide (7 mL). The mixture was stirred at 110°C for 3 h, and evaporated to dryness. The residue was purified by preparative chromatography (EtOAc/acetone/EtOH/H_2O 15:3:2:2) yielding 204 mg (32%) of N^2-tosylarabinoisocytidine **7**, as solid foam.

(b) To a stirred solution of *p*-toluensulfonamide **2** (2.84 g, 16.6 mmol) and DBU (2.5 mL, 16.6 mmol) in dry acetonitrile (11 mL), 2,2′-anhydro-1-(β-D-arabinofuranosyl)uracil **6** (1.88 g, 8.3 mmol) was added. The reaction mixture was refluxed for 72 h, cooled and treated with a small amount of methanol. The starting material was filtered off, and the filtrate was evaporated. The residue was purified by preparative chromatography on a funnel (CH_2Cl_2/MeOH 9:1), yielding 1.62 g (49%) of *N*1-{1-[3,4-dihydroxy-5-(hydroxymethyl)tetrahydro-2-furanyl]-4-oxo-1,4-dihydro-2-pyrimidinyl}-4-methyl-1-benzenesulfonamide (**7**), in the form solid foam: R_f=0.81 (EtOAc/acetone/EtOH/H_2O 4:1:1:1); UV (MeOH) λ_{max}/nm: 227, 243, and 265 (sh), log ε/dm^3 mol^{-1} cm^{-1}: 4.08, 4.08, and 3.83; IR (KBr) ν_{max}/cm^{-1}: 3582 (s), 3511 (s), 3286 (s), 2921 (m), 1690 (s), 1598 (s), 1468 (m), 1365 (m), 1296 (s), 1121 (s), 1101 (s), 1064 (s), 1009 (s); ^{1}H NMR (DMSO-d_6) δ/ppm: 10.63 (brs, 1H, NH), 7.79 (d, 2H, J=8 Hz, Ts-b), 7.76 (d, 1H, J=8.3 Hz, H-6), 7.36 (d, 2H, J=8.0 Hz, Ts-c), 6.01 (d, 1H, J=3.7 Hz H-1′), 5.89 (d, 1H, J=8.3 Hz, H-5), 5.58 (brs, 1H, OH-2′), 5.00 (brs, 1H, OH-3′), 5.00 (br s, 1H, OH-5′), 3.97 (brs, 1H, H-2′), 3.86 (brs, 1H, H-3′), 3.78 (d, 1H, J=2.9 Hz, H-4′), 3.57 (pd, 2H, J=5.3 Hz, H-5′$_a$ and H-5′$_b$) 2.36 (s, 3H, CH_3–Ts); ^{13}C NMR (DMSO-d_6) δ/ppm: 159.28 (s, C-4), 148.58 (s, C-2), 143.09 (s, Ts-d), 142.95 (d, C-6), 139.49 (s, Ts-a), 129.75 (d, Ts-c), 125.86 (d, Ts-b), 102.53 (d, C-5), 87.61 (d, C-1′), 86.43 (d, C-4′), 75.80 (d, C-3′), 74.95 (d, C-2′), 60.89 (t, C-5′), 21.09 (q, CH_3–Ts). Anal. calcd for $C_{16}H_{19}N_3O_7S$ x $2H_2O$ (M_r=433.45): C 44.34, H 5.23, N 9.69%; found: C 44.02, H 4.77, N 9.61%.

4.4.6. 5′-*O*-Trityl-2,3′-anhydro-1-(2-deoxy-β-D-*threo*-pentofuranosyl)thymine 8. Following the reported procedure for the synthesis of 2,3′-anhydro-1-(2-deoxy-5-*O*-trityl-β-D-*threo*-pentofuranosyl)-6-methyluracil,[15b] tritylation and subsequent mesylation afforded 3′-mesyl-5′-*O*-trityl-thymidine (70%), which was converted into 2,3′-anhydro derivative **8** by treatment with DBU/CH_3CN (82%). The product 4-methyl-10-[(trityloxy)methyl]-8,11-dioxa-2,6-diazatricyclo[7.2.1.0^{2,7}]dodeca-3,6-dien-5-one (**8**) was isolated as a white solid: m.p.=229–230°C (lit.[15c] mp=226–227°C).

4.4.7. 2,3′-Anhydro-1-(2-deoxy-β-D-*threo*-pentofuranosyl)thymine 12. The starting material 10-(hydroxymethyl)-4-methyl-8,11-dioxa-2,6-diazatricyclo(7.2.1.0^{2,7}-(dodeca-3,6-dien-5-one (**12**) was prepared by treatment of 3′-mesylthymidine with DBU/CH_3CN in 65% yield; mp=230–231°C (lit.[16b] 235–237°C).

4.4.8. 5′-*O*-*t*-Butyldimethylsilyl-2,3′-anhydro-1-(2-deoxy-β-D-*threo*-pentofuranosyl)thymine 13. Synthesis of 10-({[1-(*tert*-butyl)-1,1-dimethylsilyl]oxy}methyl)-4-methyl-8,11-dioxa-2,6-diazatricyclo[7.2.1.0^{2,7}]dodeca-3,6-dien-5-one (**13**) was achieved by treatment of 3′-mesyl-5′-TBDMS

thymidine[17a] (5 g, 11.5 mmol) with DBU (2.3 mL, 15.4 mmol), in acetonitrile (80 mL). The residue was purified by preparative chromatography on a funnel (CH_2Cl_2/MeOH 9:1), yielding product **13** (3.7 g, 95%): mp=174–175°C (lit.[17b] 175–176°C).

4.4.9. 5-Methyl-N^2-tosyl-1-(2-deoxy-5-*O*-trityl-β-D-*threo*-pentofuranosyl)isocytosine 9. (a) 5′-*O*-Trityl-2,3′-anhydro-1-(2-deoxy-(-D-*threo*-pentofuranosyl)thymine **8** (444 mg, 0.95 mmol) was added to a stirred solution of *p*-toluensulfonamide **2** (325 mg, 1.9 mmol) and sodium hydride (42 mg, 55% in oil, 0.95 mmol) in dimethylformamide (5 mL). The mixture was stirred at 110°C for 72 h, and evaporated to dryness. The residue was purified by preparative chromatography (CH_2Cl_2/MeOH 9:1) to give 340 mg of starting material **8** and 55 mg (9%) of *N*1-[1-({4-hydroxy-5-[(trityloxy)methyl]tetrahydro-2-furanyl}-5-methyl-4-oxo-1,4-dihydro-2-pyrimidinyl]-4-methyl-1-benzenesulfonamide (**9**), as white crystals.

(b) To a stirred solution of *p*-toluensulfonamide **2** (736 mg, 4.3 mmol) and DBU (329 μL, 2.2 mmol) in dry acetonitrile (15 mL), 5′-*O*-trityl-2,3′-anhydro-1-(2-deoxy-β-D-*threo*-pentofuranosyl)thymine **8** (1.03 g, 2.2 mmol) was added. The reaction mixture was refluxed for 70 h, cooled and treated with dichloromethane and hexane. The main part of product **9** (560 mg) was crystallized from solution and filtered off. Purification of mother liquors by flash chromatography on silica gel (CH_2Cl_2/MeOH 20:1) gave a further 380 mg (total yield: 940 mg, 67%) of *N*1-[1-({4-hydroxy-5-[(trityloxy)methyl]tetrahydro-2-furanyl}-5-methyl-4-oxo-1,4-dihydro-2-pyrimidinyl]-4-methyl-1-benzenesulfonamide (**9**), as white crystals: mp=110–111°C; R_f=0.82 (CH_2Cl_2/MeOH 9:1); UV (MeOH) λ_{max}/nm: 249 and 275 (sh), log ε/dm^3 mol^{-1} cm^{-1}: 4.29 and 4.08; IR (KBr) ν_{max}/cm^{-1}: 3440 (s, br), 3210 (m), 3040 (m), 2910 (s), 1670 (m), 1650 (m), 1580 (s), 1350 (m), 1268 (w, br), 1060 (m); ^{1}H NMR (DMSO-d_6) δ/ppm: 10.69 (brs, 1H, NH), 7.79 (d, 2H, J=8.3 Hz, Ts-b), 7.61 (s, 1H, H-6), 7.43–7.24 (m, 17H, Ts-c and Tr), 6.10 (d, 1H, J=7 Hz H-1′), 5.09 (d, 1H, J=2.7 Hz, OH-3′), 4.20 (d, 1H, J=8.0 Hz, H-4′), 4.15 (brs, 1H, H-3′), 3.39 (m, 1H, H-5′$_a$) 3.20 (m, 1H, H-5′$_b$) 2.45 (m, 1H, H-2′$_\alpha$), 2.36 (s, 3H, CH_3–Ts), 1.88 (brd, 1H, H-2′$_\beta$), 1.67 (s, 3H, 5-CH_3); ^{13}C NMR (DMSO-d_6) δ/ppm: 160.22 (s, C-4), 147.83 (s, C-2), 143.68 (s, Tr-a), 142.88 (s, Ts-d), 139.84 (s, Ts-a), 137.12 (d, C-6), 129.70 (d, Ts-c), 128.41 (d, Tr-c), 128.05 (d, Tr-b), 127.18 (d, Tr-d), 125.82 (d, Ts-b), 111.21 (s, C-5), 87.27 (d, C-1′), 86.23 (s, C(Tr)), 84.87 (d, C-4′), 68.73 (d, C-3′), 63.08 (t, C-5′), 41.23 (t, C-2′), 21.08 (q, CH_3–Ts), 12.75 (q, 5-CH_3); MS (EI) exact mass calcd for $C_{36}H_{35}N_3O_6S$+Na^+: *m/e* 660.21388 ([M+Na]$^+$); found 660.218300.

4.4.10. 5-Methyl-N^2-tosyl-1-(5-*O*-*t*-butyldimethylsilyl-2-deoxy-β-D-*threo*-pentofuranosyl)isocytosine 14. To a stirred solution of *p*-toluensulfonamide **2** (513 mg, 3 mmol) and DBU (448 μL, 3 mmol) in dry acetonitrile (10 mL), 5′-*O*-*t*-butyldimethylsilyl-2,3′-anhydro-1-(2-deoxy-β-D-*threo*-pentofuranosyl)thymine **13** (500 mg, 1.5 mmol) was added. The reaction mixture was refluxed for 72 h, cooled and evaporated. The residue was purified on a short silica gel column in the system CH_2Cl_2/MeOH 9:1, yielding 505 mg (66%) of *N*1-{1-[5-({[1-(*tert*-butyl)-1,1-dimethylsilyl]oxy}methyl)4-hydroxy-tetrahydro-2-furanyl]-5-methyl-4-oxo-1,4-dihydro-2-pyrimidinyl}-4-methyl-1-benzenesulfonamide (**14**), as foam; R_f=0.80 (CH_2Cl_2/MeOH 9:1); UV (MeOH) λ_{max}/nm: 249 and 275 (sh), log ε/dm^3 mol^{-1} cm^{-1}: 4.27 and 4.05; IR (KBr) ν_{max}/cm^{-1}: 3448 (m), 2954 (m), 2929 (m), 1670 (s), 1654 (m), 1474 (s), 1363 (m), 1257 (m), 1141 (m), 1071 (s); ^{1}H NMR (DMSO-d_6) δ/ppm: 10.75 (brs, 1H, NH), 7.67 (d, 2H, J=8 Hz, Ts-b), 7.60 (s, 1H, H-6), 7.17 (d, 2H, J=8 Hz, Ts-c), 6.15 (d, 1H, J=7 Hz, H-1′), 4.17 (brs, 1H, H-3′), 3.90 (m, 1H, H-5′$_a$), 3.77 (m, 3H, H-4′, H-5′$_b$, OH-3′), 2.45 (m, 1H, H-2′$_\alpha$), 2.27 (s, 3H, CH_3–Ts), 1.75 (brd, 1H, H-2′$_\beta$), 1.67 (s, 3H, 5-CH_3), 0.82 (s, 9H, *t*-Bu), 0.00 (s, 6H, 2CH_3Si); ^{13}C NMR (DMSO-d_6) δ/ppm: 163.10 (s, C-4), 152.01 (s, C-2), 142.27 (s, Ts-d), 140.35 (s, Ts-a), 135.73 (d, C-6), 128.59 (d, Ts-c), 126.21 (d, Ts-b), 111.32 (s, C-5), 85.39 (d, C-1′), 84.45 (d, C-4′), 68.65 (d, C-3′), 61.87 (t, C-5′), 41.27 (t, C-2′), 25.83 (q, *t*-Bu–CH_3), 20.84 (q, CH_3–Ts), 18.03 (s, *t*-Bu–C), 13.47 (q, 5-CH_3), −5.5 (q, CH_3–Si), −5.4 (q, CH_3–Si). Anal. calcd for $C_{23}H_{35}N_3O_6SSi$ (M_r=509.68): C 54.20, H 6.92, N 8.24%; found: C 54.12, H 6.75, N 8.12%.

4.4.11. 5-Methyl-N^2-tosylisocytosine 10 and 5-methyl-N^2-tosyl-1-(2-deoxy-β-D-*threo*-pentofuranosyl)isocytosine 11. (a) *Detritylation*. To a solution of 5-methyl-N^2-tosyl-1-(2-deoxy-5-*O*-trityl-β-D-*threo*-pentofuranosyl)isocytosine **9** (200 mg, 0.3 mmol) in dry dichloromethane (2 mL), under argon, $ZnBr_2$ (705 mg, 3.1 mmol) was added. The mixture was stirred at room temperature for 12 h, diluted with CH_2Cl_2 (40 mL), and treated with aq. Na_2HPO_4. The organic phase was separated and dried over anhydrous Na_2SO_4. The solution was evaporated and treated with a small amount of methanol. The crystals of 5-methyl-N^2-tosylisocytosine **10** (43 mg, 49% yield) were filtered off, and the filtrate was evaporated. Final purification of the residue by preparative chromatography (CH_2Cl_2/MeOH 9:1) afforded 21 mg (17%) of 5-methyl-N^2-tosyl-1-(2-deoxy-β-D-*threo*-pentofuranosyl)isocytosine **11**, as foam.

(b) *Ring opening of 2,3′-anhydrothymidine **10** with DBU/CH_3CN*. To a stirred solution of *p*-toluensulfonamide **2** (640 mg, 3 mmol) and DBU (553 μL, 3.7 mmol) in dry acetonitrile (20 mL), 2,3′-anhydro-1-(2-deoxy-β-D-*threo*-pentofuranosyl)thymine **12** (426 mg, 1.9 mmol) was added. The suspension was refluxed for 72 h, cooled and treated with a small amount of methanol. The starting material was filtered off, and the filtrate was evaporated. The residue was purified on a short silica gel column in the system CH_2Cl_2/MeOH 9:1, yielding 113 mg (15%) of 5-methyl-N^2-tosyl-1-(2-deoxy-β-D-*threo*-pentofuranosyl)isocytosine **11**, as foam.

(c) *Deprotection of **14***. To a solution of 5-methyl-N^2-tosyl-1-(5-*O*-*t*-butyldimethylsilyl-2-deoxy-β-D-*threo*-pentofuranosyl)isocytosine **14** (500 mg, 0.98 mmol) in dry THF (4 mL), 1 M solution of tetrabutylammonium fluoride (1.08 mL) in THF was added, and the mixture was stirred at room temperature for 15 min. After the solvent was removed under reduced pressure, the residue was purified over short silica gel column chromatography (CH_2Cl_2/MeOH 9:1), giving 341 mg (88%) of 5-methyl-N^2-tosyl-1-

(2-deoxy-β-D-*threo*-pentofuranosyl)isocytosine **11**, as foam.

4.4.12. 5-Methyl-*N*^{2}-tosylisocytosine 10. 4-Methyl-*N*-(5-methyl-4-oxo-1,4-dihydro-2-pyrimidinyl)-1-benzenesulfonamide (**10**); mp=278°C; R_f=0.56 (CH_2Cl_2/MeOH 9:1); UV (MeOH) λ_{max}/nm: 226, 246, and 270 (sh), log ε/dm^3 mol^{-1} cm^{-1}: 4.19, 4.27, and 4.12; IR (KBr) ν_{max}/cm^{-1}: 3210 (s), 1683 (s), 1630 (s), 1479 (m), 1395 (m), 1386 (m), 1268 (s), 1130 (s), 1080 (s); ^{1}H NMR (DMSO-d_6) δ/ppm: 11.71 (brs, 1H, NH), 11.31 (brs, 1H, NH), 7.73 (d, 2H, J=8.0 Hz, Ts-b), 7.33 (brd, 3H, J=8 Hz, Ts-c and H-6), 2.33 (s, 3H, CH_3–Ts), 1.75 (s, 3H, 5-CH_3); ^{13}C NMR (DMSO-d_6) δ/ppm: 162.14 (s, C-4), 149.75 (s, C-2), 142.46 (s, Ts-d), 140.20 (s, Ts-a), 137.19 (d, C-6), 129.53 (d, Ts-c), 125.81 (d, Ts-b), 112.84 (s, C-5), 21.03 (q, CH_3–Ts), 11.94 (q, 5-CH_3). Anal. calcd for $C_{12}H_{13}N_3O_3S$ (M_r=279.3): C 51.60, H 4.69, N 15.04%; found: C 51.35, H 4.47, N 14.9; MS (EI) exact mass calcd for $C_{12}H_{13}N_3O_3S+H^+$: *m/e* 280.07504 ([M+H]$^+$); found 280.084961.

4.4.13. 5-Methyl-*N*^{2}-tosyl-1-(2-deoxy-β-D-*threo*-pentofuranosyl)isocytosine 11. *N*-{1-[4-dihydroxy-5-(hydroxymethyl)tetrahydro-2-furanyl]-5-methyl-4-oxo-1,4-dihydro-2-pyrimidinyl}-4-methyl-1-benzenesulfonamide (**11**); R_f=0.37 (CH_2Cl_2/MeOH 9:1); UV (MeOH) λ_{max}/nm: 224, 249, and 274 (sh), log ε/dm^3 mol^{-1} cm^{-1}: 4.15, 4.25, and 4.06; IR (KBr) ν_{max}/cm^{-1}: 3448 (m), 2925 (m), 1686 (s), 1590 (s), 1477 (m), 1355 (m), 1284 (m), 1073 (s); ^{1}H NMR (DMSO-d_6) δ/ppm: 10.65 (brs, 1H, NH), 7.86 (s, 1H, H-6), 7.75 (d, 2H, J=8.0 Hz, Ts-b), 7.34 (d, 2H, J=8.0 Hz, Ts-c), 6.03 (d, 1H, J=7 Hz, H-1′), 5.16 (d, 1H, J=3.0 Hz, OH-3′), 4.74 (t, 1H, J=5.3 Hz, OH-5′), 4.17 (d, 1H, J=3.3 Hz, H-3′), 3.85 (m, 1H, H-4′), 3.68 (m, 2H, 2H-5′) 2.49 (m, 1H, H-2′$_\alpha$), 2.35 (s, 3H, CH_3–Ts), 1.88 (brd, 1H, H-2′$_\beta$), 1.80 (s, 3H, 5-CH_3); ^{13}C NMR (DMSO-d_6) δ/ppm: 160.70 (s, C-4), 148.17 (s, C-2), 142.75 (s, Ts-d), 139.98 (s, Ts-a), 137.50 (d, C-6), 129.63 (d, Ts-c), 125.84 (d, Ts-b), 111.55 (s, C-5), 86.35 (d, C-1′), 86.13 (d, C-4′), 68.37 (d, C-3′), 59.43 (t, C-5′), 41.18 (t, C-2′), 21.06 (q, CH_3–Ts), 12.72 (q, 5-CH_3); MS (EI) exact mass calcd for $C_{17}H_{19}N_5O_6S+K^+$: *m/e* 434.079366 ([M−H+K]$^+$); found 434.002251.

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