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5-Bromo- and 5–Iodo-*N*-1-sulfonylated Cytosine Derivatives. Exclusive Formation of Keto-Imino Tautomers

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N-1-Sulfonylated cytosine **5** and 5-halogeno-*N*-1-sulfonylated cytosine derivatives **1** and **2** were synthesized by condensation of silylated cytosine or 5-halogenocytosine bases with tosylchloride in acetonitrile, or by the reaction of cytosine or 5-halogenocytosine bases with tosylchloride in pyridine. The NMR evidences are presented, showing that **1** and **2** form exclusively rare keto-imino tautomers in DMSO-d_6 solution, while *N*-1-sulfonylated cytosine **5** appears as a common keto-amino tautomer.

Key words: synthesis, *N*-1-sulfonylated cytosine derivatives, 5-bromo and 5-iodocytosine derivatives, NMR study, keto-imino and keto-amino tautomers

INTRODUCTION

Various cytidine derivatives modified at the base or the ribose are known to exhibit antiviral or antitumor activity.[1–4] On the other hand, cytosine (C) is a very important nucleic base with respect to DNA structure; in its protonated form (C^+) it is involved in formation of $C{\cdot}C^+$ DNA duplexes[5,6] and $C{\cdot}G{\cdot}C^+$ triplexes.[7] The keto-imino tautomer of cytosine is a mutagenic base analogue capable of pairing with adenine.[8] Although three tautomers

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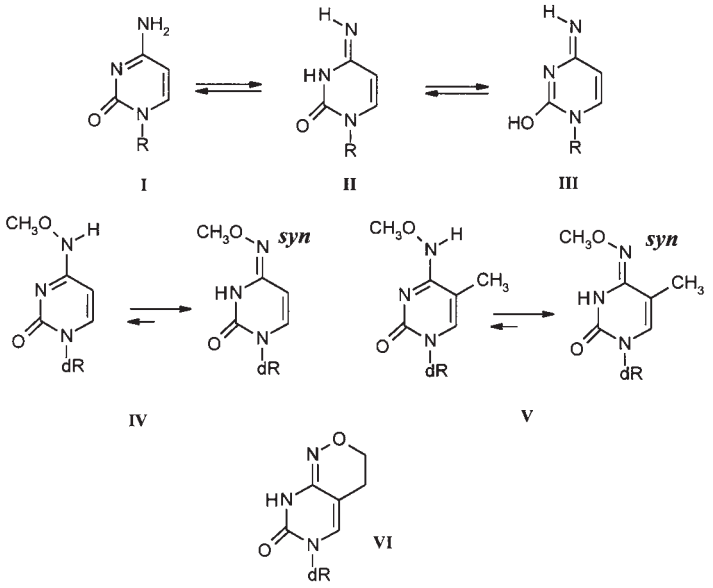


Figure 1. Tautomers of cytosine derivatives.

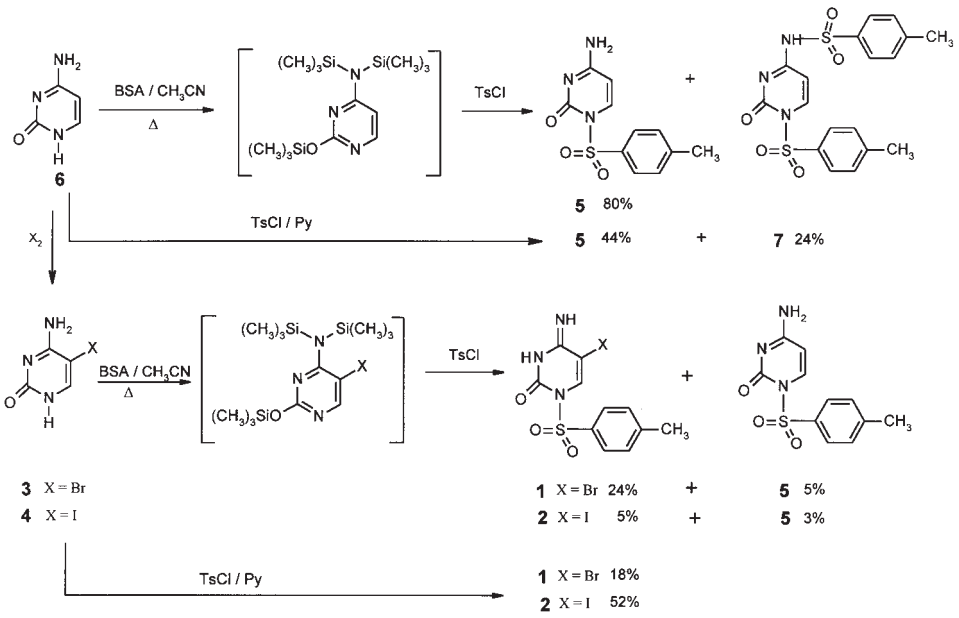
(**I**, **II** and **III**, Figure 1) are possible for *N*-1 substituted cytosine, the lactam-amine structure **I** has been found to be the dominant tautomer in aqueous and DMSO solutions.[9–12] However, some substituted cytosines, for example, *N*-4-methoxy (**IV**)[13] or *N*-4-methoxy-5-methyldeoxycytidine (**V**),[14] give the equilibrium with predominant keto-imino tautomer, possessing *syn* arrangement of *N*-4-methoxy groups (Figure 1). Incorporation of such cytidine derivatives in DNA considerably destabilizes the duplex since *syn*-conformation is unfavorable for normal Watson-Crick pairing with guanine.[15] In contrast, derivative **VI** with its fixed *anti*-configuration of imino fragment forms base pairs with A and G of comparable stabilities.[16] These results emphasize the influence of cytosine tautomers on the stability of DNA structure.

Recently, we have described the synthesis and *in vitro* antitumor activity of a series of novel 1-sulfonyluracil and 1-sulfonylcytosine derivatives.[17,18] In the next step of our research, we report here on the synthesis and ^{1}H NMR data of 5-bromo-1-*p*-toluensulfonylcytosine (**1**) and 5-iodo-1-*p*-toluensulfonylcytosine (**2**) derivatives as new candidates for compounds with antitumor activity. The ^{1}H NMR study revealed that both cytosine derivatives in DMSO form exclusively keto-imino tautomers with *anti*-configuration of the imino fragment. This opens a new possibility of preparing *N*-4--unsubstituted cytidine analogues, which should preferably form keto-imino

tautomers with an *anti*-configuration favoring efficient pairing with G and A. Such cytidine tautomers, if incorporated in DNA, could exhibit interesting effects on the DNA structure and duplex stability.

RESULTS AND DISCUSSION

5-Halogeno-*N*-1-sulfonylated cytosine derivatives **1** and **2** have been prepared from silylated 5-bromocytosine (**3**)[19] or 5-iodocytosine (**4**)[20] and tosylchloride in acetonitrile or by the reaction of cytosine derivatives **3** or **4** with tosylchloride in pyridine (Scheme 1). Both methods gave **1** and **2** in a relatively low yield. The C-5 dehalogenation was also observed by the former method, giving *N*-1-sulfonylated cytosine **5** as by-product in 3–5% yield. In



Scheme 1.

comparison, *N*-1 sulfonylation of silylated cytosine (**6**) gave 1-tosylcytosine **5** in 80% yield, while the reaction of **6** and tosylchloride in pyridine, besides the mono-sulfonylated product **5** (44%), gave also bis-sulfonylated product **7** in 24% yield. The sulfonylation of both 5-bromo (**3**) and 5-iodocytosine (**4**) is less efficient than that of cytosine (**6**). Apparently, the electron donating effect of 5-halogeno substituents dominates over the electron withdrawing one, diminishing the acidity of *N*-1 hydrogen. This may result in a relatively

low effective concentration of *N*-1 anion and hence low yields of sulfonylated products.

^{1}H NMR spectra (all measured in DMSO-d_6) of **1** and **2** differ from that of *N*-1-sulfonylated cytosine **5**, with respect to chemical shifts and multiplicity (Figure 2a). The most striking difference refers to *N*-protons. In the spectrum of **5**, two amino protons at *N*-4, readily exchangeable for deuterium by addition of D_2O, appear as a singlet at δ 7.95 ppm. This is in accord with the existence of the keto-amino tautomer, known to be the most stable form of unsubstituted cytosine. In contrast, two slightly broadened one-proton singlets at δ 8.61 and 7.72 ppm and δ 9.18 and 8.82 ppm, all exchangeable for deuterium, can be observed for **1** and **2**, respectively. The large chemical shift difference between these protons, $\Delta\delta$ of 0.89 ppm for **1** and of 0.36 ppm for **2**, strongly suggests the formation of the keto-imino tautomer. This large chemical shift difference can hardly be explained by the restricted rotation around C(4)NH_2 bond in keto-amino tautomers containing 5-halogeno substituents. Besides, if this were the case, a geminal coupling between two non-equivalent amino protons of the keto-amino tautomer should exist, which should give an additional doublet at both signals. However, no apparent splitting was detected at either of the two signals in 1D spectra of **1** and **2**. There is a possibility that the magnitude of geminal coupling is smaller than the signal width. If this happens, the splitting is not observable in 1D spectrum, but it should be visible in 2D spectra as cross-peaks between the corresponding signals. However, an inspection of COSY spectrum (Figure 2b) showed no cross-peaks between two non-equivalent *N*-protons, completely ruling out the geminal spin-spin interaction. Therefore, the observed chemical non-equivalency of *N*-protons is due to their bonding to different N-atoms, *i.e.* the existence of keto-imino tautomers of **1** and **2**. The configuration of the imino fragment in **1** and **2** can be *syn*- or *anti*- with respect to lactam NH. In the *syn*-configuration the free electron pair of imino nitrogen would be in close proximity to 5-bromo or 5-iodo substituents, as appears from the examination of CPK models. Thus, *syn*-configuration should be less stable than *anti*-configuration due to electron repulsion in the former. Consequently, keto-imino tautomers of **1** and **2** with *anti*-configuration of the imino fragment are much more likely to form. This is supported by NOESY spectra, where no cross-peaks between lactam NH and imino NH protons could be observed, in agreement with the large spatial separation of these protons in *anti*-configuration.

CONCLUSIONS

5-Bromo-1-*p*-toluensulfonylcytosine (**1**) and 5-iodo-1-*p*-toluensulfonylcytosine (**2**) have been prepared by condensation of the corresponding silylated

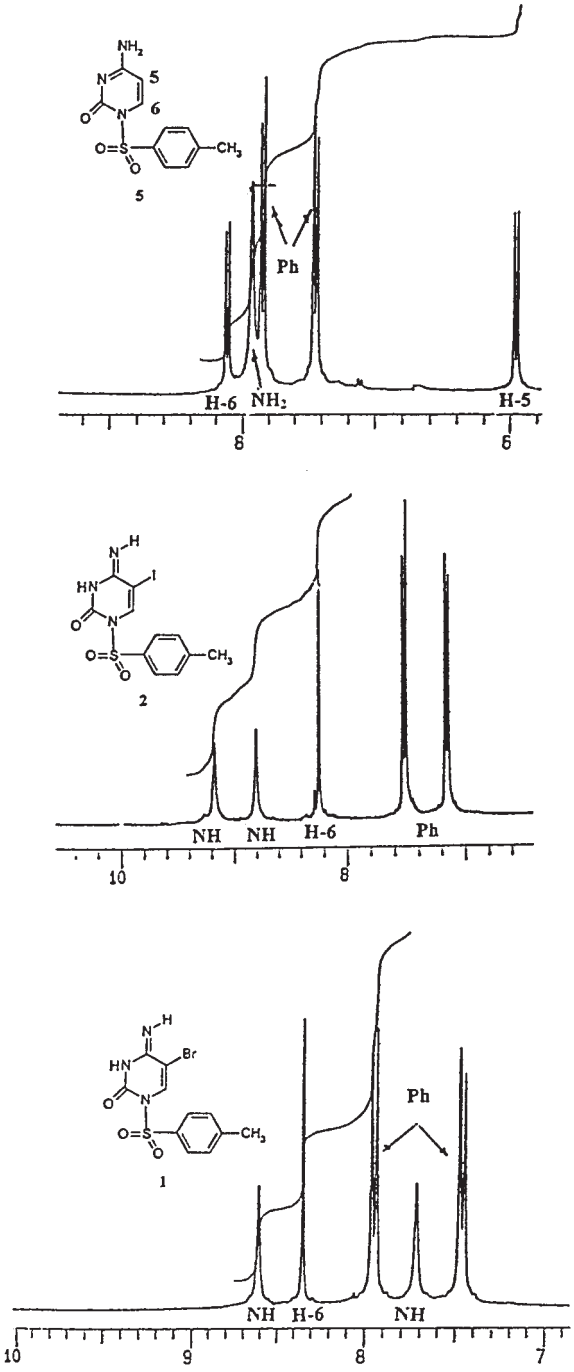


Figure 2a. ^{1}H NMR Spectra (DMSO-d_6) of *N*-1-sulfonylated cytosine derivatives.

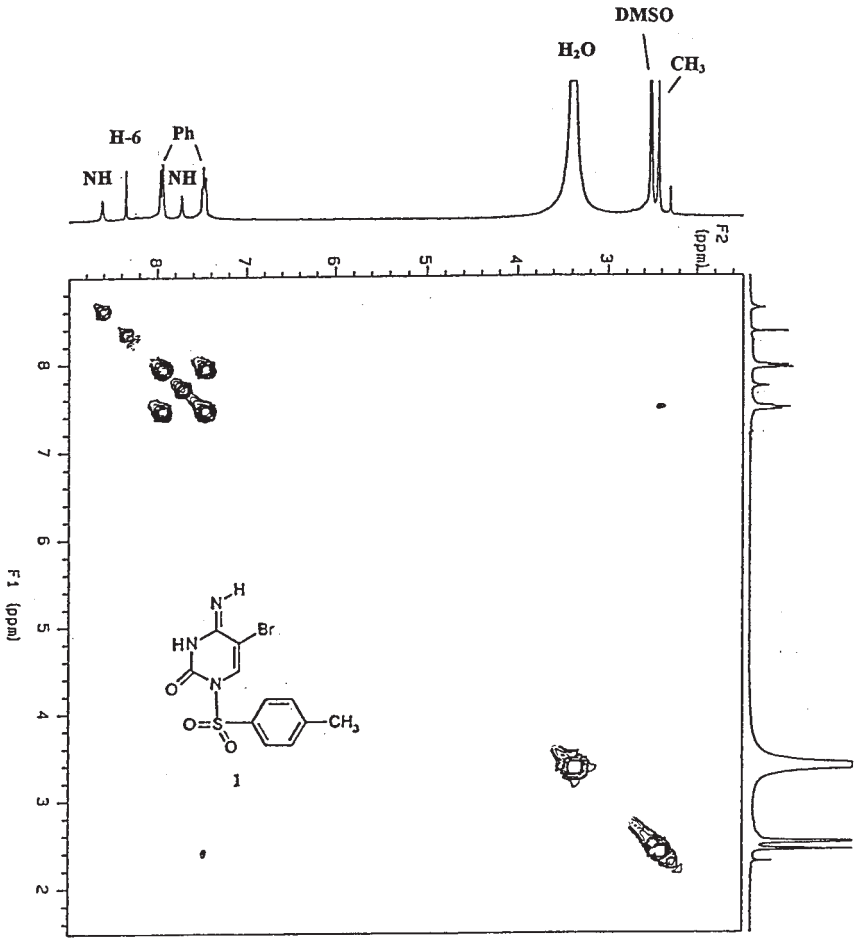
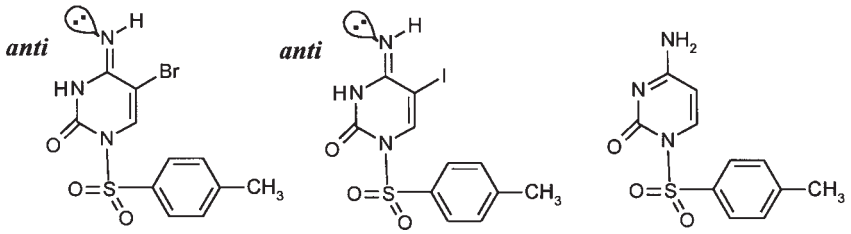


Figure 2b. COSY Spectrum of 5-bromo-*N*-1-sulfonylated cytosine.

5-halocytosine and tosylchloride in acetonitrile or by the reaction of 5-halocytosine and tosylchloride in pyridine. ^{1}H NMR spectra of **1** and **2** taken in DMSO-d_6 revealed that in contrast to 1-*p*-toluensulfonylcytosine (**5**), which in the same solvent forms keto-amino tautomer, both halogenocytosine derivatives form exclusively keto-imino tautomers. It was also found that the keto-imino tautomers of **1** and **2** possess *anti*-configuration of the imino fragment (Figure 3). These results open new possibilities for preparation of rare *anti*-keto-imino cytosine and cytidine tautomers and the use of the latter for incorporation into DNA. Due to favorable pairing of such tautomers with both G and A, interesting effects on DNA stability and structure might be expected.



1 2 5

Figure 3. Tautomers of *N*-1-sulfonylated cytosine derivatives.

EXPERIMENTAL

General: Solvents were distilled from appropriate drying agents shortly before use. TLC was carried out on Merck precoated glass plates or DC-plastikfolien Kieselgel 60 F_{254} and preparative thick layer (2 mm) chromatography 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 are 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. Concentrations of samples were 0.1 M for ^{1}H and 0.2 M for ^{13}C measurements. Chemical shifts (δ/ppm) are 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 mixing times 0.45–0.80 s. All other measurement parameters were as for 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.

1-p-*Toluenesulfonylcytosine (**5**)*

A) A mixture of cytosine (**6**) (222 mg, 2.00 mmol) and bis(trimethylsilyl)acetamide (BSA) (1.48 mL, 6.00 mmol) was heated under reflux in dry acetonitrile (7 mL) for 30 minutes. The obtained colorless solution was cooled to 0 °C and *p*-toluenesu-

lfonylchloride (TsCl) (457 mg, 2.40 mmol) was added. After heating under reflux for 45 minutes, the solvent was evaporated. Ethanol was added into the residue and the obtained solid was filtered off and recrystallized from hot ethanol, yielding product **5** as white crystals: 421 mg (80%); m.p. = 216 C; R_f = 0.66 (CH_2Cl_2/MeOH 3:1); UV (MeOH) λ_{max} = 234.5 and 248.0, log ε = 3.18 and 3.57; IR (KBr) ν_{max}/cm^{-1}: 3360 (s), 3100 (s), 1660 (s, br), 1520 (s), 1490 (s), 1380 (s), 1375 (s), 1350 (s), 1285 (s), 1185 (s), 1175 (s); ^{1}H NMR (DMSO-d_6) δ/ppm: 8.14 (d, 1H, H-6, $J_{6,5}$=7.8 Hz), 7.95 (s, 2H, NH_2), 7.87 (d, 2H, Ph, J=8.1 Hz), 7.46 (d, 2H, Ph, J=8.1 Hz), 5.98 (d, 1H, H-5, $J_{5,6}$=7.8 Hz), 2.42 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6) δ/ppm: 166.27 (s, C-4), 151.22 (s, C-2), 145.61 (s, Ph), 139.73 (d, C-6), 134.47 (s, Ph), 129.80 (d, Ph), 129.02 (d, Ph), 97.50 (d, C-5), 21.20 (q, CH_3).

Anal. Calcd. for $C_{11}H_{11}N_3O_3S$ (M_r = 265.30): C 49.80, H 4.18, N 15.84%; found: C 50.09, H 4.02, N 15.89%.

B) TsCl (343 mg, 1.8 mmol) was added to a suspension of cytosine (**6**) (100 mg, 0.9 mmol) in dry pyridine (9.5 mL). After stirring at room temperature for 48 hours, the solvent was evaporated under pressure. The remaining oil contained two compounds that were separated by chromatography on silica gel (CH_2Cl_2/MeOH 15:1). Fractions containing the individual products were pooled and concentrated to dryness to give 106 mg (44%) of 1-*p*-toluenesulfonylcytosine (**5**) and 92 mg (24%) of less polar product, bis(*p*-toluenesulfonyl)cytosine **7**.

4-N-p-*Toluenesulfonyl-1*-p-*toluenesulfonylcytosine (**7**) white crystals:*

m.p. = 182 C; R_f = 0.80 (CH_2Cl_2/MeOH 9:1); UV (MeOH) λ_{max} = 227.2 and 283.0, log ε = 4.39 and 4.45; IR (KBr) ν_{max}/cm^{-1}: 3200 (w), 3090 (w), 3030 (w), 2920 (w), 2850 (w), 1730–1715 (s, doublet), 1630 (s), 1570 (s, doublet), 1450 (m, doublet), 1400 (s, doublet), 1370 (m), 1290 (s), 1260 (m), 1175 (s), 1155 (s); ^{1}H NMR (DMSO-d_6) δ/ppm: 12.10 (s, br, 1H, NH-4), 8.26 (d, 1H, H-6, $J_{6,5}$=8.4 Hz), 7.93 (d, 2H, Ph, J=8.1 Hz), 7.73 (d, 2H, Ph, J=8.1 Hz), 7.49 (d, 2H, Ph, J=8.1 Hz), 7.37 (d, 2H, Ph, J=8.1 Hz), 6.76 (d, 1H, H-5, $J_{5,6}$=8.4 Hz), 2.43 (s, 3H, CH_3), 2.38 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6) δ/ppm: 159.79 (s, C-4), 146.79 (s, Ph), 146.42 (s, C-2), 142.94 (s, Ph), 139.52 (d, C-6), 132.91 (s, Ph), 132.90 (s, Ph), 130.06 (d, Ph), 129.63 (d, Ph), 129.38 (d, Ph), 126.47 (d, Ph), 98.78 (d, C-5), 21.21 (q, CH_3), 20.95 (q, CH_3).

Anal. Calcd. for $C_{18}H_{17}N_3O_5S_2$ (M_r = 419.48): C 51.54, H 4.09, N 10.02%; found: C 51.26, H 4.20, N 10.00%.

5-Bromo-1-p-*toluenesulfonylcytosine (**1**)*

A) A mixture of 5-bromocytosine (**3**) (110 mg, 0.58 mmol) and BSA (0.43 mL, 1.74 mmol) in dry acetonitrile (2 mL) was heated under reflux for 45 minutes. The colorless solution was cooled to 0 °C and TsCl (133 mg, 0.70 mmol) was added. After heating under reflux for 30 minutes, the solvent was evaporated under pressure. Ethanol was added into the residue and the obtained solid of 5-bromocytosine was filtered off. The filtrate was evaporated, and the residue was separated by preparative chromatography (CH_2Cl_2/MeOH 9:1) yielding product **1**, 48 mg (24%) and 1-*p*-toluenesulfonylcytosine (**5**), 8 mg (5%).

B) TsCl (300 mg, 1.58 mmol) was added to a suspension of 5-bromocytosine (**3**) (150 mg, 0.79 mmol) in dry pyridine (8 mL). After stirring at room temperature over-

night, the solvent was evaporated under pressure. Ethanol was added and the obtained solid of 5-bromocytosine was filtered off. The filtrate was evaporated and the residue was purified by preparative chromatography (CH_2Cl_2/MeOH 9:1). After recrystallization from methanol, product 1, 50 mg (18%) was obtained: m.p. = 213 C; R_f = 0.44 (CH_2Cl_2/MeOH 9:1); UV (MeOH) λ_{max} = 234.7 and 254.0; log ε = 3.98 and 3.95; IR (KBr) ν_{max}/cm^{-1}: 3450 (s), 3070 (m), 2960 (w), 2920 (w), 1680 (s), 1640 (s), 1605 (m), 1490–1475 (s, dublet), 1370 (s), 1170 (s); ^{1}H NMR (DMSO-d_6) δ/ppm: 8.61 (s, 1H, NH), 8.35 (s, 1H, H-6), 7.95 (d, 2H, Ph, J=8.1 Hz), 7.72 (s, 1H, NH), 7.46 (d, 2H, Ph, J=8.1 Hz), 2.42 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6) δ/ppm: 159.10 (s, C-4), 149.00 (s, C-2), 148.08 (d, C-6), 145.19 (s, Ph), 138.73 (s, Ph), 128.70 (d, Ph), 125.91 (d, Ph), 85.27 (s, C-5), 21.03 (q, CH_3).

Anal. Calcd. for $C_{11}H_{10}N_3O_3SBr$ (M_r = 344.19): C 38.39, H 2.93, N 12.21%; found: C 38.19, H 2.94, N 12.44%.

*5-Iodo-1-*p*-toluenesulfonylcytosine (**2**)*

A) A mixture of 5-iodocytosine (**4**) (250 mg, 1.06 mmol) and BSA (0.78 mL, 3.17 mmol) in dry acetonitrile (4 mL) was heated under reflux for 45 minutes. TsCl (242 mg, 1.27 mmol) was added into the obtained red solution, cooled to 0 °C. After heating under reflux for 45 minutes, the solvent was evaporated under pressure. Ethanol was added into the residue and the obtained solid of product 2 was filtered off and recrystallized from ethanol. The filtrate was evaporated and the residue was separated by preparative chromatography (CH_2Cl_2/MeOH 9:1), yielding 10 mg of product 2 (overall yield 5%) and 1-*p*-toluenesulfonylcytosine (**5**), 8 mg (3%).

B) TsCl (322 mg, 1.69 mmol) was added to a suspension of 5-iodocytosine (**4**) (200 mg, 0.84 mmol) in dry pyridine (9 mL). After stirring at room temperature overnight, the solvent was evaporated under pressure. Ethanol was added and the obtained solid of product **2** was filtered off. After recrystallization from ethanol, product **2** was obtained: 171 mg (52%); m.p. = 210 C; R_f = 0.80 (CH_2Cl_2/MeOH 9:1); UV λ_{max} = 228.9 and 289.4, log ε = 4.15 and 3.67; IR (KBr) ν_{max}/cm^{-1}: 3480 (m), 3310 (m), 3200 (w), 2920 (vw), 2850 (vw), 1640 (s), 1560 (s), 1525 (m), 1390 (s), 1370 (s), 1190 (s), 1170 (s); ^{1}H NMR (DMSO d_6) δ/ppm: 9.18 (s, 1H, NH), 8.82 (s, 1H, NH), 8.25 (s, 1H, H-6), 7.53 (d, 2H, Ph, J=8.0 Hz), 7.16 (d, 2H, Ph, J=8.0 Hz), 2.31 (s, 3H, CH_3); ^{13}C NMR (DMSO d_6) δ/ppm: 160.46 (s, C-4), 153.49 (d, C-6), 147.38 (s, C-2), 145.32 (s, Ph), 138.36 (s, Ph), 128.48 (d, Ph), 125.76 (d, Ph), 55.08 (s, C-5), 20.88 (q, CH_3).

Anal. Calcd. for $C_{11}H_{10}N_3O_3SI$ (M_r = 391.19): C 33.77, H 2.58, N 10.74%; found: C 33.94, H 2.75, N 10.71%

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SAŽETAK

5-Brom- i 5–jod-*N*-1-sulfonilirani derivati citozina. Isključivo nastajanje keto-imino-tautomera

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Sintetizirani su *N*-1-sulfonilirani derivat citozina **5** i 5-halogen-*N*-1-sulfonilirani derivati citozina **1** i **2** i to kondenzacijom silililiranog citozina ili 5-halogencitozina s tosil-kloridom u acetonitrilu, ili reakcijom citozina ili 5-halogen citozina s tosil-kloridom u piridinu. NMR eksperimenti pokazuju isključivo nastajanje keto-imino-tautomera **1** i **2** u DMSO-d_6 otopinama, dok se *N*-1-sulfonilirani derivat citozina **5** pojavljuje u uobičajenom keto-amino-obliku.