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Synthesis, structural and conformational studies of *Z*- and *E*-isomers of fluorinated C-6 isobutenyl *N*-methyl thymine derivatives

Svjetlana Krištafor [a,1], Andrijana Meščić [a,1], Mario Cetina [b,2], Silvija Korunda [a,1], Damjan Makuc [c,d,3], Janez Plavec [c,d,e,3], Silvana Raić-Malić [a,1,*]

[a] Department of Organic Chemistry, Faculty of Chemical Engineering and Technology, University of Zagreb, Marulićev trg 20, HR-10000 Zagreb, Croatia
[b] Department of Applied Chemistry, Faculty of Textile Technology, University of Zagreb, Prilaz baruna Filipovića 28a, HR-10000 Zagreb, Croatia
[c] Slovenian NMR Centre, National Institute of Chemistry, Hajdrihova 19, SI-1000 Ljubljana, Slovenia
[d] EN-FIST Centre of Excellence, Dunajska 156, SI-1000 Ljubljana, Slovenia
[e] Faculty of Chemistry and Chemical Technology, University of Ljubljana, Aškerčeva cesta 5, SI-1000 Ljubljana, Slovenia

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ABSTRACT

A new series of conformationally restricted pyrimidine derivatives bearing C-6 isobutenyl side-chain (**2**–**9**) has been prepared. The novel fluoroalkenyl pyrimidine nucleoside mimetic **3** as model compound for development of tracer molecule in positron emission tomography (PET) was synthesized by fluorination reaction of methoxytritylated pyrimidine derivative using diethylaminosulfur trifluoride (DAST). Conversion of one hydroxyl group to methoxytritylated, fluorinated, mesylated and acetylated pyrimidine derivatives (**2**, **3**, **5**–**7** and **9**) afforded a mixture of *Z*- and *E*-isomers in which *Z*-isomers were predominant. Conformational study of **1**, and its fluorinated structural congeners **3** and **4** by the use of NOE experiments revealed predominant conformation of compounds where vinyl H-1′ proton is spatially close to N-1 methyl and H-3′b methylene protons and on the other hand H-3′a methylene protons are close to C-5 methyl protons. The stereostructure of 1,3-dihydroxyisobutenyl *N*-methyl thymine **1** was unambiguously confirmed by X-ray crystal structure analysis.

1. Introduction

An interest in acyclic nucleoside analogs began in mid-1970s when acyclovir was first reported as a potent anti-herpes drug. The expressed selectivity of acyclovir as an antiviral drug and the understanding of its mechanism of action towards viral enzymes provided the impetus for further synthesis of related compounds and their screening as potential antiherpetic drugs [1–3]. Among these acyclonucleosides, ganciclovir [4] and penciclovir [5,6] arose and became the therapeutic compounds of choice to interfere with severe herpes virus infections. Fluoro derivatives of ganciclovir and penciclovir were evaluated as tracers for non-invasive positron emission tomography (PET) imaging of herpes simplex virus type 1 thymidine kinase (HSV-1 TK) gene expression [7,8]. Moreover, in the area of medicinal chemistry, incorporation of fluorine has played a significant role in the development of new anti-cancer and anti-viral agents. The presence of fluorine in a molecule can increase molecules' binding affinity to a target protein and enhance its metabolic stability or modulate its physicochemical properties, such as its lipophilicity, acidity or basicity [9]. For this reason, the development of synthetic methods for fluorine-containing heterocyclic compounds has been an important field in both organofluorine chemistry and organic synthesis.

In an effort to develop more potent and selective antiviral agents, structural modifications of the heterocyclic bases and/or modifications on the sugar moiety of natural nucleosides can be attempted. Among these transformations, the introduction of alkenyl moieties into pyrimidines and pyrimidine nucleosides is of great interest in view of their biological activities [10–12]. Thus, it was found that introduction of a double bond gave a slight rigidity to the unsaturated nucleoside analogs [12,13] which stabilized these compounds in the best conformation to improve their interactions with phosphorylating enzymes.

Recently, we have reported the synthesis and biological results of a new type of C-6 fluoroalkylated and fluorophenylalkylated pyrimidine derivatives as model compounds for development of tracer molecules in positron emission tomography (PET) [14–18]. Besides, findings from the molecular docking of unsaturated C-6 substituted 1,3-dihidroxyisobutenyl *N*-methyl thymine into the active site of HSV-1 TK indicated the same interactions of this compound as for natural substrate thymidine [19].

* Corresponding author. Tel.: +385 1 4597 213; fax: +385 1 4597 224.
E-mail addresses: mario.cetina@ttf.hr (M. Cetina), janez.plavec@ki.si (J. Plavec), sraic@fkit.hr (S. Raić-Malić).
[1] Tel.: +385 1 4597 213; fax: +385 1 4597 224.
[2] Tel.: +385 1 3712 590; fax: +385 1 3712 599.
[3] Tel.: +386 1 4760 353; fax: +386 1 4760 300.

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In this work, we report the synthesis and structural studies of C-6 substituted isobutenyl thymine derivatives (**2**–**9**), a thymine-based acyclic C–C nucleosides in which the unsaturated acyclic sugar moiety is attached at C-6 position of the pyrimidine ring rather than at N-1 position. In addition, X-ray crystal structural study of C-6 substituted dihydroxyisobutenyl *N*-methyl thymine **1** is also presented. Since intermolecular interactions can provide better solubility of pharmacologically active compounds in water, we particularly pay attention on supramolecular assembling of **1** whose fluorinated structural analog **3** can be used as model compound for development of tracer molecule in PET.

2. Results and discussion

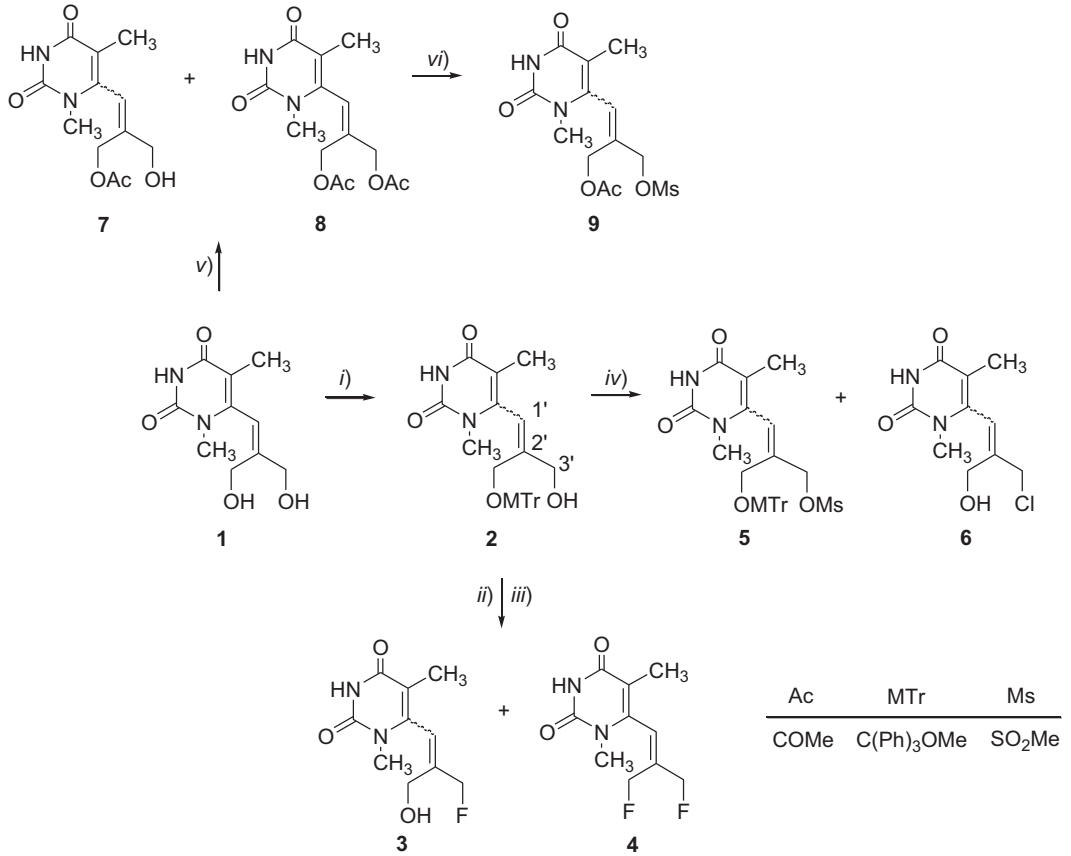
2.1. Chemistry

6-(1,3-Dihydroxyisobutenyl) *N*-methyl pyrimidine derivative (**1**) [19] was synthesized according to a multistep procedure, which included lithiation reaction of appropriate pyrimidine derivative and nucleophilic addition to 1,3-dibenzyloxy-2-propanone [20,21], followed by various transformations both of the acyclic side-chain at C-6 and of the heterocyclic base. In the next step, one primary hydroxyl group in **1** was selectively converted to methoxytritylated group (Scheme 1).

Thus, reaction of compound **1** and *p*-anisylchlorodiphenylmethane (MTrCl) in the presence of 4-DMAP gave C-6 isobutenyl substituted pyrimidine derivative **2** in 52% yield, as a mixture of *Z*- and *E*-isomers in which *Z*-isomer prevails. The ratio of *Z*- and *E*-isomer is 5 to 2. Interestingly, the methoxytritylation reaction of the saturated analog of **1**, 6-[3-hydroxy-2-(hydroxymethyl)propyl]-1,5-dimethylpyrimidin-2,4-dione (*N*-MeDHBT) gave, under the same experimental conditions, both mono- and dimethoxytritylated derivatives. The treatment of compound **2** with diethylaminosulphur trifluoride (DAST) and subsequent hydrolysis, gave monofluorinated **3** (as a mixture of *Z*- and *E*-isomers) and difluorinated derivative **4** in 24% and 36% yield, respectively. In comparison to a parent compound **2**, the content of *E*-isomer in a mixture of **3** is higher. Thus, the ratio of *Z*- and *E*-isomer is 5 to 4. The formation of difluorinated compound **4** in this reaction could be explained by hydrolysis of methoxytrityl protection, followed by fluorination of two hydroxyl groups. Transformation of hydroxyl group in **2** using mesyl chloride (MsCl) in pyridine gave compound **5** containing 6-[2-(*p*-anisyldiphenylmethoxy)methyl-3-mesylpropenyl] side-chain. Besides, chlorinated pyrimidine derivative **6** was also obtained in this reaction. Similar to fluorination reaction, mesylation of **2** afforded mesylated **5** and chlorinated **6** as mixtures of *Z*- and *E*-isomers in which the contents of *E*-isomers are higher (**5** and **6**; *Z*/*E* = 3/2) than that in starting compound **2**. Compound **1** was submitted to acetylation reaction with acetic anhydride in the presence of 4-DMAP and triethylamine to afford monoacetylated **7** (*Z*/*E* = 2/1) and diacetylated **8**. Monoacetylated compound **7** was subsequently converted to *N*-methylpyrimidine **9** with 6-(2-acetoxymethyl-3-mesylpropenyl) side-chain. The ratio of *Z*- and *E*-isomers in **9** is also 5 to 4 that is in accord to fluorinated pyrimidine derivative **3**.

2.2. NMR spectroscopic analysis

The chemical identities of compounds **2**–**9** were confirmed by ^{1}H, ^{13}C and ^{19}F NMR measurements. Proton, carbon and fluorine NMR chemical shifts of **3**–**5** are reported in Table 1, whereas proton and carbon NMR chemical shifts of **2**, **5**–**9** are given in



Scheme 1. Synthesis of C-6 isobutenyl *N*-methyl thymine derivatives (**2**–**9**). *Reagents and conditions*: (i) MTrCl, 4-DMAP, DMF, room temperature, 3 h; (ii) DAST, CH_2Cl_2, −75 °C, 1 h, rt, 2 h; (iii) 5% methanolic HCl, reflux, 15 min; (iv) MsCl, pyridine, −8 to 0 °C, 3 h; (v) Ac_2O, 4-DMAP, CH_3CN, room temperature, 45 min; (vi) MsCl, pyridine, −5 °C, 4 h.

Table 1
^{1}H, ^{13}C and ^{19}F NMR chemical shifts (δ in ppm) and coupling constants (J in Hz) for monofluorinated (**3**) and difluorinated (**4**) pyrimidine derivatives.

Compd.	NH	H-1′	H-3′a	H-3′b	N-1 Me	C-5 Me	OH-a	OH-b
3-(E)	11.28 (1H, *s*)/ 11.30 (1H, *s*)[a]	6.27 (1H, *s*)	5.10 (1H, *dd*, J = 12.2, J_{HF} = 46.8) 5.14 (1H, *dd*, J = 12.2, J_{HF} = 46.8)	3.87–3.95 (2H, *m*)	3.13 (3H, *s*)	1.65 (3H, *s*)	–	5.04 (1H, *t*, J = 5.1)
3-(Z)	11.28 (1H, *s*)/ 11.30 (1H, *s*)[a]	6.37 (1H, *s*)	4.84 (1H, *dd*, J = 11.4, J_{HF} = 46.8) 4.87 (1H, *dd*, J = 11.4, J_{HF} = 46.8)	4.16–4.20 (2H, *m*)	3.10 (3H, *s*)	1.65 (3H, *s*)	–	5.31 (1H, *t*, J = 5.3)
4	11.35 (1H, *s*)	6.58 (1H, *s*)	4.83–5.07 (2H, *m*)	5.17 (2H, *d*, J_{HF} = 46.4)	3.11 (3H, *s*)	1.65 (3H, *s*)	–	–

Compd.	C-5 Me	N-1 Me	C-2	C-4	C-5	C-6	C-1′	C-2′	C-3′a	C-3′b
3-(E)[b]	11.5	31.7	151.0	163.4	107.0	147.1 (*d*, J_{CF} = 1.5)/147.0 (*s*)[a]	118.1 (*d*, J_{CF} = 12.2)	143.2 (*d*, J_{CF} = 13.0)	82.7 (*d*, J_{CF} = 166.3)	57.4
3-(Z)[b]	11.6	31.8	151.0	163.3	107.0	147.1 (*d*, J_{CF} = 1.5)/147.0 (*s*)[a]	118.7 (*d*, J_{CF} = 8.4)	143.4 (*d*, J_{CF} = 14.5)	79.6 (*d*, J_{CF} = 160.2)	60.7
4	11.5	31.7	150.9	163.3	107.3	145.7 (*m*)	123.0 (*dd*, J_{CF} = 11.4, 7.6)	138.0 (*dd*, J_{CF} = 15.1, 14.0)	79.0 (*dd*, J_{CF} = 161.0, 2.7)	82.1 (*dd*, J_{CF} = 166.3, 3.4)

Compd.	C-3′aF	C-3′bF
3-(E)[c]	−219.3 (*m*, J = 46.8, 2.9)	–
3-(Z)[c]	−221.0 (*m*, J = 46.8, 2.1)	–
4	−222.2 (*m*, J = 46.4, 2.1)	−218.5 (*m*, J = 46.4, 2.8)

Compounds were recorded in Me_2SO-d_6.
[a] δ of isomers could not be unambiguously assigned.
[b] 200 MHz.
[c] 282 MHz.

supplementary data. Proton-decoupled ^{13}C NMR spectra showed C–F coupling constants that enabled straightforward identification of fluorinated carbon atoms and their neighbors.

NMR spectra of compounds **2**, **3**, **5**–**7** and **9** exhibited two set of signals which were attributed to *Z*- and *E*-isomers. Individual resonances in isomeric pairs were assigned by NOE measurements, which in addition revealed isomers' conformational preferences. The saturation of hydroxymethyl group H-3′b in pyrimidine derivative **3-(Z)** with 6-(3-fluoromethyl-2-hydroxypropenyl) side-chain gave moderate NOE enhancement at H-1′ (4.8%, Fig. 1a).

In contrast, NOE interaction between fluoromethyl group H-3′b and H-1′ proton was observed in **3-(E)** isomer (5.5%, Fig. 1b). Besides, NOE between hydroxymethyl group H-3′b and C-5 methyl group (3.2%) was in agreement with *E*-configuration.

The saturation of H-1′ proton in difluorinated pyrimidine derivative **4** resulted in strong NOE enhancement at N-1 methyl (6.2%) and H-3′b methylene (5.4%) protons, whereas weak NOE was observed at C-5 methyl protons (2.0%, Fig. 1c). Furthermore, saturation of H-3′b methylene protons showed strong NOE enhancement at H-1′ (7.5%), whereas saturation of H-3′a methylene protons resulted in moderate NOE at C-5 methyl protons. These results suggested predominant conformation of compound **4**, where H-1′ is spatially closer to N-1 methyl group.

In addition, *Z*- and *E*-isomers were observed for compounds **2**, **5**–**7** and **9** with the ratio of 5:2, 3:2, 3:2, 2:1 and 5:4, respectively. Conformational properties of compounds **1**, **3** and **4** are assessed with the use of 1D difference NOE enhancements (Table 2).

2.3. X-ray crystal structure of 1

1,3-Dihydroxyisobutenyl *N*-methyl thymine **1** crystallized in triclinic space group $P\bar{1}$ as a hydrate (Fig. 2). The bond lengths in **1** present no unexpected features and those in pyrimidin-2,4-dione ring are in agreement with equivalent ones in 1-methylthymine [22,23] and 5-(2-acetoxyethyl)-6-methylpyrimidin-2,4-dione [24]. Furthermore, the bond lengths are also within the range in pyrimidin-2,4-diones we published recently [25–27], with the exception of C(5)–C(6) bond in uracil- and 5-fluorouracyl *N*-phthalimide protected 4-amino-2-butenyl derivatives [26] which is *ca.* 0.04–0.05 Å shorter. It should be also noted that a survey of Cambridge Structural Database [28] revealed that this is the first structure comprising 1,3-dihydroxyisobutenyl chain. The C9/C10/

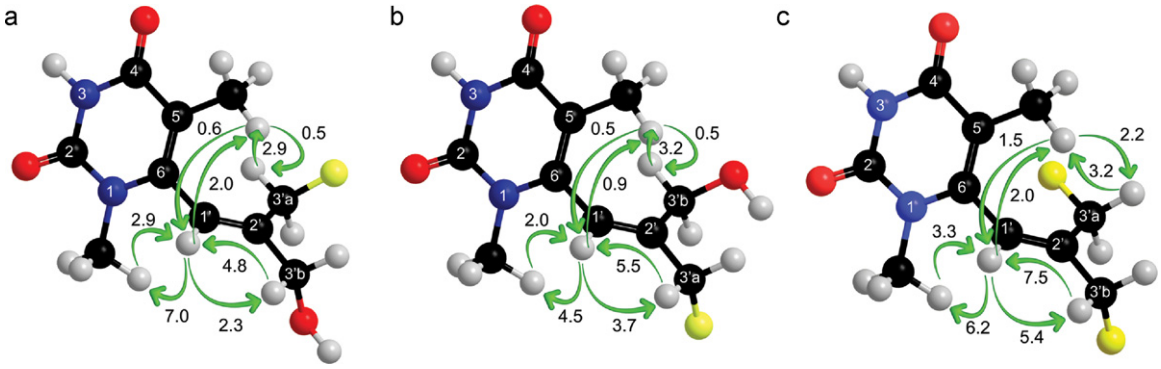


Fig. 1. Predominant conformations of fluorinated pyrimidine derivatives: isomeric pair **3-(Z)**/**3-(E)** (a and b) and **4** (c) with key NOE enhancements (in %).

Table 2
Key NOE enhancements for compound **1** and its monofluorinated (**3**) and difluorinated (**4**) pyrimidine derivatives (in %).

Saturated	C-5 Me			H-1′				N-1 Me	H-3′a		H-3′b	
Enhanced	H-1′	H-3′a	H-3′b	C-5 Me	H-3′a	H-3′b	N-1 Me	H-1′	C-5 Me	H-1′	C-5 Me	H-1′
1	1.3	1.3	0.1	2.7	0.6	3.0	7.2	3.3	2.9	0.5	0.5	4.3
3-(E)	0.5	0.0	0.5	0.9	3.7	0.0	4.5	2.0	0.0	5.5	3.2	0.0
3-(Z)	0.6	0.5	0.0	2.0	0.0	2.3	7.0	2.9	2.9[a]	0.0	0.0	4.8
4	1.5	2.2	0.0	2.0	0.0	5.4	6.2	3.3	3.2[a]	0.0	0.0	7.5[b]

[a] High-field part of the multiplet was saturated.
[b] Low-field part of the multiplet was saturated.

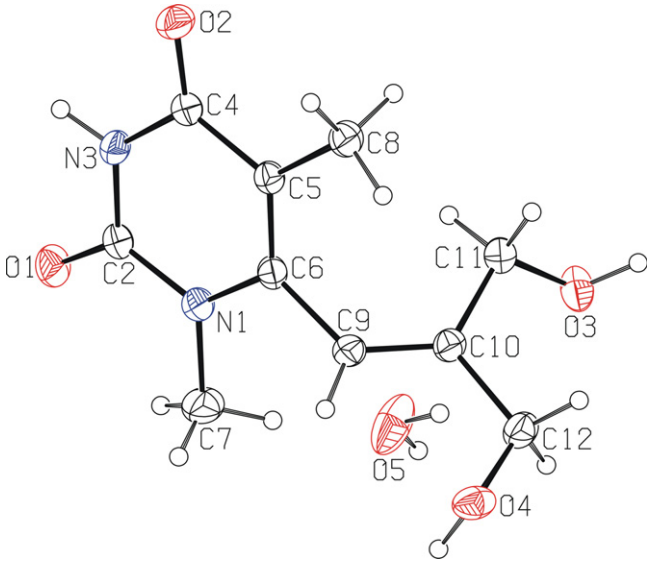


Fig. 2. A molecular structure of compound **1**, with the atom-numbering scheme. Displacement ellipsoids for nonhydrogen atoms are drawn at the 30% probability level.

C11/C12 atoms of the 1,3-dihydroxyisobutenyl moiety form an angle of 63.66(8)° with respect to the ring. Such orientation could be also described by the C5–C6–C9–C10 torsion angle of −65.75(19)°. The planarity of ethenyl moiety is further confirmed by the C6–C9–C10–C11 and C6–C9–C10–C12 torsion angles of 0.7(2)° and 179.58(12)°, respectively. Two hydroxyl oxygen atoms have different conformation with respect to the C9 atom of the ethenyl moiety, *anticlinal* and *synperiplanar* [C9–C10–C11–O3 = −138.42(13)°; C9–C10–C12–O4 = −10.83(19)°].

2.4. Antitumoral activities

The novel compounds were evaluated for their cytostatic activities against malignant human tumor cell lines: cervical carcinoma (HeLa), breast carcinoma (MCF-7), laryngeal carcinoma (HepG2), colon carcinoma (SW 620), pancreatic carcinoma (MiaPaCa-2) as well as human fibroblast cells (WI38). Of all the compounds, C-6 isobutenyl pyrimidine derivative **2** containing methoxytrityl moiety exhibited moderate activities (IC_{50} in the range 20.41–44.83 μM) against all evaluated cell lines including normal human fibroblasts. Other compounds did not display antiproliferative activity on the panel tumor cell lines and normal human fibroblasts WI38.

3. Conclusion

The syntheses of novel C-6 substituted isobutenyl *N*-methyl thymine derivatives (**2**–**9**) are reported. Among them, monofluorinated thymine derivative **3** was prepared as model compound for development of tracer molecule in PET, while compounds **5** and **9** with protected hydroxyl functionalities can be used as precursor molecules for the synthesis of ^{18}F labeled *N*-methyl thymine with 3-(^{18}F)fluoromethyl-2-hydroxypropenyl at C-6 of pyrimidine moiety. The methoxytritylation, fluorination, mesylation and acetylation reactions of unsaturated pyrimidine derivatives gave compounds **2**, **3**, **5**–**7** and **9** as mixtures of *Z*- and *E*-isomers in which *Z*-isomers prevailed. Conformational study of pyrimidine derivative **3** with 6-(3-fluoromethyl-2-hydroxypropenyl) side-chain showed NOE enhancement between C-5 methyl and fluoromethyl group H-3′a and moderate NOE enhancement between H-1′ and hydroxymethyl protons H-3′b in **3-(Z)** that is in agreement with *Z*-configuration. On the contrary, **3-(E)** showed strong NOE between H-1′ and fluoromethyl group H-3′a and moderate NOE between C-5 methyl and hydroxymethyl group H-3′b. Furthermore, NOE experiments of **1**, and its fluorinated structural congeners **3** and **4** revealed predominant conformation of compounds where vinyl H-1′ proton is spatially close to N-1 methyl and H-3′b methylene protons and on the other hand H-3′a methylene protons are close to C-5 methyl protons. The stereostructure of 1,3-dihydroxyisobutenyl *N*-methyl thymine **1** showed that two hydroxyl oxygen atoms in side-chain of **1** have anticlinal and synperiplanar conformation with respect to the C9 atom of ethenyl moiety.

4. Experimental

4.1. General

Melting points (uncorrected) were determined with *Kofler* micro hot-stage (*Reichert*, Wien). Precoated *Merck* silica gel 60F-254 plates were used for thin layer chromatography and the spots were detected under UV light (254 nm). Column chromatography was performed using silica gel (0.063–0.2 mm) *Fluka*; glass column was slurry-packed under gravity. ^1H and ^{13}C NMR spectra were acquired on *Varian Unity Inova* 300 MHz, *Varian NMR System* 800 MHz and *Bruker* 300 MHz NMR spectrometer (300 MHz for ^1H, 75.47 and 200 MHz for ^{13}C using TMS as internal standard). All data were recorded in Me_2SO-d_6 and $CDCl_3$ at 298 K. Chemical shifts were referenced to the residual solvent signal of Me_2SO at δ 2.50 ppm for ^1H and δ 39.50 ppm for ^{13}C, and $CDCl_3$ at δ 7.24 ppm for ^1H and δ 77.23 ppm for ^{13}C. ^{19}F chemical shifts were referenced externally with respect to CCl_3F (δ 0.0 ppm). Individual resonances were assigned on the basis of their chemical shifts, signal intensities, multiplicity of resonances and H–H coupling constants. Mass spectra were recorded on an *Agilent 6410* instrument equipped with electrospray interface and triple quadrupole analyzer (LC/MS/MS). High performance liquid chromatography was performed on an *Agilent 1100* series system with UV detection (photodiode array detector) using Zorbax C18 reverse-phase analytical column (2.1 mm × 30 mm, 3.5 μm). All compounds used for biological evaluation showed >95% purity in this HPLC system.

4.2. Procedures for the preparation of compounds

4.2.1. 6-[2-(p-Anisyldiphenylmethoxy)methyl-3-hydroxypropenyl]-1,5-dimethylpyrimidin-2,4-dione (2)

To a cold solution of compound **1** (53 mg, 0.23 mmol) in DMF (2 mL) and triethylamine (0.1 mL), 4-DMAP (0.52 mg) was added

under Ar. The mixture was stirred for 15 min at 0 °C and MTrCl (158.5 mg, 0.51 mmol) was added. The reaction mixture was stirred for 3 h at room temperature, quenched by addition of methanol (10 mL) and evaporated to dryness. The residue was purified using column chromatography (CH_2Cl_2:MeOH = 10:1) to obtain compound **2** (60 mg, 52%) as white crystals; mp 172–174 °C; Anal. Calcd for $C_{30}H_{30}N_2O_5$: C, 72.27; H, 6.06. Found: C, 72.49; H, 6.05; ESI-MS: *m*/*z* 499 $[MH]^+$.

4.2.2. 6-[3-Fluoromethyl-2-hydroxypropenyl]-1,5-dimethylpyirimidin-2,4-dione (3) and 6-[3-fluoro-2-(fluoromethyl)propenyl]-1,5-dimethylpyrimidin-2,4-dione (4)

The solution of compound **2** (60 mg, 0.12 mmol) in dry CH_2Cl_2 (25 mL) was cooled to −75 °C and DAST (0.08 mL, 0.61 mmol) was added under Ar. The cooling bath was removed and the reaction mixture was stirred at room temperature for 2 h. Methanol (5 mL) was added to the reaction mixture and the solution was evaporated to dryness. The residue was dissolved in 5% methanolic HCl (4 mL) and refluxed for 15 min. After evaporation to dryness, the residue was purified by column chromatography (CH_2Cl_2:MeOH = 20:1) to yield compounds **3** (6.5 mg, 24%) and **4** (9.2 mg, 36%) as crystals.

3: mp 149–155 °C; Anal. Calcd for $C_{10}H_{13}N_2O_3F$: C, 52.63; H, 5.74. Found: C, 52.79; H, 5.76; ESI-MS: *m*/*z* 229 $[MH]^+$.

4: mp 230–235 °C; Anal. Calcd for $C_{10}H_{12}N_2O_2F_2$: C, 52.17; H, 5.25. Found: C, 52.01; H, 5.27; ESI-MS: *m*/*z* 231 $[MH]^+$.

4.2.3. 6-[2-(p-Anisyldiphenylmethoxy)methyl-3-mesylpropenyl]-1,5-dimethylpyrimidin-2,4-dione (5) and 6-[3-chloro-2-(hydroxymethyl)propenyl]-1,5-dimethylpyrimidin-2,4-dione (6)

To a cold solution of compound **2** (10 mg, 0.02 mmol) in dry pyridine (0.3 ml) mesyl chloride (0.05 ml, 0.65 mmol) was added under Ar. The reaction mixture was stirred for 1 h at −8 °C. The stirring was continued for 2 h at 0 °C, followed by addition of methanol and water. The solvents were evaporated to dryness and after purification by column chromatography (CH_2Cl_2:MeOH = 20:1) compounds **5** (3 mg, 26%) and **6** (3 mg, 50%) were isolated as oils.

5: Anal. Calcd for $C_{31}H_{32}N_2O_7S$: C, 64.57; H, 5.59. Found: C, 64.70; H, 5.61; ESI-MS: *m*/*z* 577 $[MH]^+$.

6: Anal. Calcd for $C_{10}H_{13}N_2O_3Cl$: C, 49.09; H, 5.36. Found: C, 49.24; H, 5.35; ESI-MS: *m*/*z* 245 $[MH]^+$, 247 $[MH+2]^+$.

4.2.4. 6-[2-Acetoxyomethyl-3-hydroxypropenyl]-1,5-dimethylpyirimidin-2,4-dione (7) and 6-[3-acetoxy-2-(acetoxymethyl)propenyl]-1,5-dimethylpyrimidin-2,4-dione (8)

To a stirred suspension of compound **1** (76 mg, 0.33 mmol) in dry acetonitrile (5 mL), 4-DMAP (3 mg, 0.03 mmol), triethylamine (0.1 ml, 0.65 mmol) and acetic anhydride (0.1 mL, 1.07 mmol) were added. The reaction mixture was stirred for 45 min at room temperature, quenched by addition of water and evaporated to dryness. Chromatography on silica gel column (CH_2Cl_2:MeOH = 10:1) yielded compounds **7** (6 mg, 47%) and **8** (4 mg, 26%) as oily products.

7: Anal. Calcd for $C_{12}H_{16}N_2O_5$: C, 53.73; H, 6.01. Found: C, 53.89; H, 6.00; ESI-MS: *m*/*z* 269 $[MH]^+$.

8: Anal. Calcd for $C_{14}H_{18}N_2O_6$: C, 54.19; H, 5.85. Found: C, 54.03; H, 5.83; ESI-MS: *m*/*z* 311 $[MH]^+$.

4.2.5. 6-(2-Acetoxymethyl-3-mesylpropenyl)-1,5-dimethylpyrimidin-2,4-dione (9)

To a cold solution of compound **7** (11 mg, 0.04 mmol) in dry pyridine (0.3 ml) mesyl chloride (0.15 ml, 1.94 mmol) was added under argon atmosphere. The reaction mixture was stirred for 4 h at −5 °C, quenched by addition of methanol and water and evaporated to dryness. After column chromatography (CH_2Cl_2:MeOH = 20:1) compound **9** (3 mg, 55%) was obtained as an oil. **9-(Z)**: Anal. Calcd for $C_{13}H_{18}N_2O_7S$: C, 45.08; H, 5.24. Found: C, 45.22; H, 5.22; ESI-MS: *m*/*z* 347 $[MH]^+$.

4.3. Crystal structure determination

The crystals suitable for X-ray single crystal structure study were grown by slow evaporation from ethanol solution (96%). The intensities were collected on an Oxford Diffraction Xcalibur2 diffractometer with a Sapphire 3 CCD detector using graphite-monochromated CuK_α radiation (λ = 1.54184 Å) and ω scan-mode. *CrysAlis* programs [29] were used for data collection and processing. The intensities were corrected for absorption using the multi-scan absorption correction method [29]. The crystal structure was solved by direct methods [30] and all non-hydrogen atoms were refined anisotropically by full-matrix least-squares calculations [30] based on F^2 using the programs integrated in *WinGX* [31] program package. The hydrogen atoms attached to the N3, O3, O4 and O5 atoms have been found in a difference Fourier map and have been refined freely. All other hydrogen atoms were treated using appropriate riding models, with *SHELXL97* [30] defaults. *PLATON* [32] program was used for structure analysis and drawings preparation. CCDC 808738 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Crystal data for **1**: crystal dimension 0.30 mm × 0.30 mm × 0.30 mm; $C_{10}H_{16}N_2O_5$, M_r = 244.25, triclinic space group $P\bar{1}$ (No. 2); a = 7.6949(2), b = 8.4584(3), c = 9.0650(3) Å, α = 81.867(3), β = 84.643(2), γ = 86.134(3)°; V = 580.64(3) Å^3; Z = 2; dx = 1.397 g cm^{-3}; T = 295 K; 5406 reflections measured, R/wR = 0.0434/0.1253 for 176 parameters and 2096 reflections with $I \geq 2\sigma(I)$, R/wR = 0.0440/0.1259 for all 2141 unique reflections measured in the range 11.56°–2θ–139.98°; S = 1.082.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jfluchem.2011.06.002.

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