Research paper

Extension of furopyrimidine nucleosides with 5-alkynyl substituent: Synthesis, high fluorescence, and antiviral effect in the absence of free ribose hydroxyl groups

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ABSTRACT

A novel methodology to access alkynyl nucleoside analogues is elaborated. Highly fluorescent 5-alkynylfuropyrimidines were synthesized (97-46%) and their antiviral properties investigated *in vitro*. Regiochemistry of the functionalization was achieved with the aid of 5-*endo-dig* electrophilic halocyclization of acetyl 5-*p*-tolyl- or 5-*p*-pentylphenyl-2′-deoxyuridine. Structure of one of the resulting nucleosides, 6-*p*-tolyl-5-iodo-2′-deoxyribofuranosyl-furo[2,3-*d*]pyrimidin-2-one, was confirmed by X-ray crystallography, and its conformation was compared to related nucleosides. Diverse alkynyl substituents were introduced at the heterobicyclic base C-5 position via Sonogashira coupling of 5-iodo-2′-deoxyribofuranosyl-furo[2,3-*d*]pyrimidin-2-ones. The resulting compounds had fluorescence emissions of 452−481 nm. High quantum yields of 0.53−0.60 were observed for 9-ethynyl-9-fluorenol and propargyl alcohol/methyl ether-modified furopyrimidines. These modified nucleosides, designed in the form of ribose acetyl esters, are potential tools for fluorescent tagging, studying nucleoside metabolism, 2′-deoxyribonucleoside kinase activity, and antiviral activity. Antiviral assays against a broad spectrum of DNA and RNA viruses showed that in human embryonic lung (HEL) cell cultures some of the compounds posess antiviral activity (EC$_{50}$ 1.3−13.2 μM) against varicella-zoster virus (VZV). The alkynyl furopyrimidine with two *p*-pentylphenyl substituents emerged as the best compound with reasonable and selective anti-VZV activity, confirming *p*-pentylphenyl potency as a pharmacophore.

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1. Introduction

Nucleoside analogues are a cornerstone for the treatment of cancer and viral diseases [1−8]. Among the most active analogues, furopyrimidine nucleosides stand out as potent and selective antiviral agents with a high specific activity against varicella-zoster virus (VZV) [9−11]. *p*-Alkylphenyl, particularly *p*-pentylphenyl, substituents attached to C-6 give rise to the most effective structures (**1a,b**; Fig. 1) [12−14]. In parallel, significant biological activity

can be imposed by appending an alkyne, an example includes 5-alkynyluridines [15−17]. Alkynyl modifications also provide a synthetic handle for further functionalization and modification of nucleosides [18−21].

Intrigued by the combination of two active units, a synthesis of alkynyl-substituted furopyrimidines was envisioned. Despite extensive modification efforts, synthetic chemists have rarely described a furopyrimidine ring with directly attached alkynes. To this point, one report has described a C-4 attached alkyne, introduced to the pyrimidine ring before cycloisomerization to furopyrimidine [22]. C-6 alkynyl analogues have been prepared by direct bromination of the furopyrimidine ring and subsequent coupling with terminal alkynes [23−26]. However, introduction of an alkyne at C-5 lacks a general method thus far. Formerly,

potent pharmacophore

1a, R = *n*-C₅H₁₁, R' = H
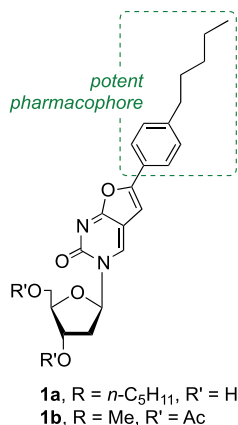**1b**, R = Me, R' = Ac

Fig. 1. Structures of selected 6-(*p*-alkylphenyl) furopyrimidine nucleosides **1**.

compounds containing an alkyne group attached at the C-5 position were detected. These byproducts were isolated with meager yields (3−9%) in reactions that targeted other structures [27−29]. Recently, an interesting synthetic approach towards triazole conjugates of alkynyl-modified furopyrimidine base has been reported [30]. This methodology leads to compounds with desirable biological activity but synthetically renders mixtures containing alkyne-free furopyrimidine. Additionally, it lacks diversity, since both, C-6 substituent and C-5 alkyne endgroup, originate from the same terminal alkyne reaction component.

An electrophilic cyclization reaction leading to the formation of β-halofuran rings offers a significant synthetic opportunity [31,32]. An analogous protocol leads to the formation of 5-halofuropyrimidine nucleosides, which introduces the halogen in a fully regioselective manner [33]. Addressing this so far unresolved synthetic shortcoming in nucleoside chemistry, the extension of the heterocyclic base conjugated system by appending an alkyne at C-5 was sought by combining iodofuropyrimidines with various terminal acetylenes. Considering the antiviral activity of furopyrimidine nucleosides **1**, synthesis of a series of derivatives containing a *p*-alkylphenyl group positioned at C-6 scaffold was attempted.

Interestingly, acyl-substituted nucleosides are becoming effective prodrugs due to increased lipophilicity. The isobutyryl group is installed at the 5′-hydroxyl end of *N*-4-hydroxycytidine derivative EIDD-2801, which is an oral experimental antiviral drug [34]. This synthetic nucleoside is one of only two highly effective active compounds against coronaviruses to come out of 6 years of screening [35]. Other groups, including 5′-L-valyl ester, were investigated to increase oral bioavailability [36,37]. We decided to investigate a series of compounds with the acetyl group installed on the ribose unit as its 3′- and 5′-ester.

2. Results and discussion

Synthesis. Starting compounds containing 4-methylphenyl (*p*-tolyl) and 4-pentylphenyl substituents at C-6 were selected. *p*-Alkylphenyl substituents are participants of the most potent antiviral active structures [12]. Preparative syntheses of a starting material 2′-deoxy-5-alkynyluridines **2** and **3** were carried out from 2′-deoxy-5-iodouridine (**Ac-IdU**) and the appropriate terminal alkyne (Pd- and Cu-catalyzed Sonogashira coupling, Scheme 1) [38]. The next steps combine cycloisomerization and halofunctionalization reactions. Halogens such as iodine, bromine, and chlorine can be introduced via electrophilic cyclization of 5-alkynyluridines [33,39]. Among the halogens, iodo derivatives are most convenient to prepare, since they are more prone to

crystallize, which often satisfies the isolation protocol. Additionally, iodo derivatives are expected to be the most reactive toward functionalization. 5-*Endo-dig* electrophilic cyclization of 5-alkynyl-2′-deoxyuridine (**2** or **3**) with *N*-iodosuccinimide in acetone at 23 °C furnished corresponding 5-iodofuropyrimidines (**4** or **5**). Following this procedure iodo-functionalized nucleosides **4** and **5** were obtained with a good yield (92% and 76%, respectively; Scheme 1). Structure of the acylated nucleoside **4** and **5** were confirmed by ¹H and ¹³C NMR, IR spectroscopy, and HRMS. The ¹³C NMR spectra feature characteristic iodofuro resonances C(5)-I at 53.88 and 53.95 ppm (CDCl₃), which differ from non-iodinated derivatives [40].

To explore the structure-reactivity relationship for the appended alkyne fragment, novel nucleosides were prepared (Scheme 1). Alkynyl substituents include an oxypropynyl unit (propargyl alcohol, **6a/7a**, R' = CH₂OH; 76/81% yield), its one carbon extended homologue (**6b/7b**, R' = CH₂CH₂OH; 95/81%), tertiary aryl alcohol (**6c**, R' = 9-fluorenol-9-yl; 83%), methyl propargyl ether (**6d**, R' = CH₂OMe; 66%), propargyl acetate (**6e**, R' = CH₂OAc; 46%), *N*-(3-butynyl)phthalimide and *N*-(5-hexynyl) phthalimide (**6f/6g**, R' = (CH₂)₂-phthalimide/(CH₂)₄-phthalimide; 93/82%). The protected amine linkers (phthalimide) offer an opportunity for further functionalization, for example to *N*-phosphoryl amino acids [41]. Finally, four structures containing combinations of 4-methylphenyl (*p*-tolyl) and 4-pentylphenyl (antiviral active substituents, cf. Fig. 1) were also obtained (**6h/7h**, R' = C₆H₄-*p*-CH₃ and **6i/7i**, R' = C₆H₄-*p*-*n*-C₅H₁₁ 70/64% and 97/72% yield). The protocol involved Sonogashira coupling in the presence of tetrakis(triphenylphosphine)-palladium(0), copper(I) iodide, and triethylamine (DMF, 23 °C). The structures of alkynyl nucleosides **6** and **7** were confirmed by ¹H and ¹³C NMR, as well as IR spectroscopy. ¹H NMR spectra exhibited one signal in the region of the H-6, as expected (8.51−8.25 ppm). Two signals characteristic for an alkyne were observed in ¹³C NMR for **6** and **7** (99.19−94.50 and 95.40−94.28 ppm). In the ¹³C NMR spectrum for **6c**, an excess of five signals was observed beyond the number of expected equivalent carbons. It is likely that the conformation with C-2 carbonyl group *anti* with respect to the ribose, alike the one observed in the crystal structure of **4** (*vide infra*), exists in solution. The resulting hindered rotation of fluorenol, enhanced by potential interactions of the quaternary and O5′ acetyloxy group, may lead to a magnetic nonequivalency. This effect was observed for most (five out of six per ring) of the fluorenol aromatic carbons. Similarly, in the ¹H NMR propargyl protons of CH₂OH groups in compounds **6a** and **7a** appeared as nonequivalent dAB (coupling with OH proton). A DEPT spectrum for the phthalimide-derived compound **6g**, which was applied to the entire series of ¹³C NMR assignments, is provided in the Supplementary Materials. HRMS spectra for both **6** and **7** exhibited an *m/z* peak with an accurate mass [M + H]⁺ for the molecular ion.

Crystallography. To gain more molecular information, crystallographical analysis of furopyrimidine was attempted. Despite the persistent synthetic pursuit of furopyrimidine nucleosides, so far only a few and only C-6 substituted have been crystallographically characterized [20,25,38,40,42−46]. Although nucleosides are often resistant to crystallization, efforts to obtain diffraction quality crystals were successful for compound **4**, using methanol at room temperature. X-ray crystallography confirmed the structure of the 3′,5′-di-*O*-acetyl-5-iodo-6-(*p*-tolyl)furopyrimidine (**4**, Fig. 2) [47].

In the solid state, the C2 carbonyl group of planar furanopyrimidine ring **4** adopted an *anti* orientation toward the ribose, with a glycosidic bond torsion angle χ (C2−N3−C1′−O4′) of −167.5(2)°, a conformation similar to previously crystallographically characterized furanopyrimidine nucleosides (−120.8(5) to −172.81(18), average −155.8; Table 1). In the molecule of **4**, the N-glycosidic
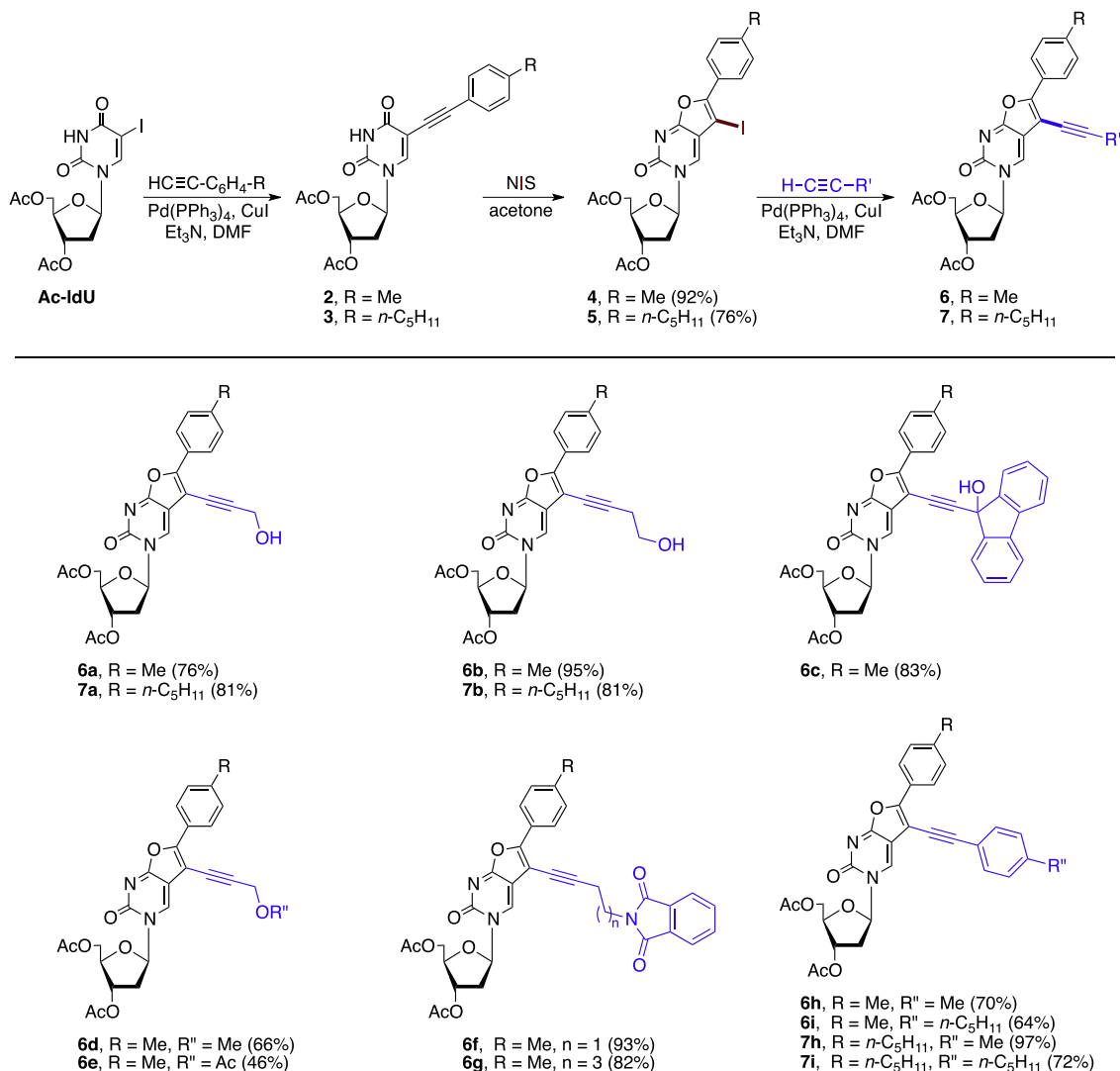
Scheme 1. Synthesis and structures of 5-iodo- and 5-alkynylfuropyrimidine nucleosides **4,5** and **6,7**. For summary of yields see Table S1 in the Supplementary Materials (p. S2).

bond (ribose−base distance), C1′−N3 1.488(3)Å, was comparable to previously crystallographically characterized furanopyrimidine nucleosides (1.468(7) to 1.502(6), average 1.487; Table 1). Ribose geometrical parameters, such as the pseudorotation phase angle (P) were determined for **4** to be C3′-exo with P = 209.73° (puckering amplitude of 27.38). The same parameters calculated for other crystallographically characterized furopyrimidines indicate the presence of both C2′-endo and C3′-exo conformation (Table 1). Results of this analysis indicates that appending an alkyne at the C-5 position did not meaningfully affect the base conformational positioning, which is consistent across these characterized compounds; this effect was not correlated to the ribose ring puckering since it varies in all of the above reviewed furopyrimidine structures in the solid state, and is likely determined by packing forces.

In addition, X-ray crystallographic analysis determined the iodine atom position at C-5 in the furopyrimidine framework, confirming the regiochemistry of the iodocyclization reaction. If a cycloisomerization were to precede the halogenation, then other available sp^2 carbons in the bicyclic core could potentially compete for the iodination reagent.

Fluorescence. The fact that furopyrimidine nucleosides display a strong optical response has been known for a while now; these bicyclic structures have been heralded as "fluorescent", featured in publication titles [48,49]. Surprisingly, we were able to find in the literature only one quantitative measurements of photophysical properties of furopyrimidine nucleosides [50], beyond the furopyrimidine base [51]. In contrast, a number of (empirical and theoretical) optical parameters studies of pyrrolopyrimidines have been reported [50,52,53].

Considering the importance of fluorescent nucleic acid components [54−57], quantitative optical properties of selected furopyrimidine nucleosides (**6a,c,d,f,h** and **7a,h**, as well as **4**) were determined in methanol (UV−visible and emission spectra; Figs. 3 and 4, respectively). Almost identical UV−vis and fluorescence traces were observed for **6h** and **7h** homologues.

An examination of UV−visible spectra indicates molar absorptivity ε ranging from 8700 to 19,600 $M^{-1}cm^{-1}$ (Table 2). The pentyl substituent slightly increases ε, as compared to the corresponding methyl-substituted homologues (**7a** vs **6a**, and **7h** vs **6h**). By comparison, furopyrimidine without an alkyne substituent (reference compound **1b**) absorbs at λ_{max} 280 and 352 nm (ε 23,200 and 17,300 $M^{-1}cm^{-1}$, respectively). Emission wavelength (λ_{em}) values were observed for alkynyl furopyrimidines **6a,c,d,f,h** and **7a,h** in the range of 453−481 nm (λ_{ex} = 360 nm, Table 2), and were red-
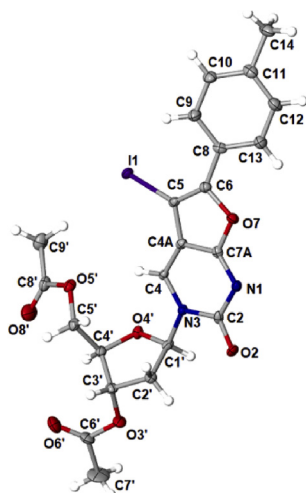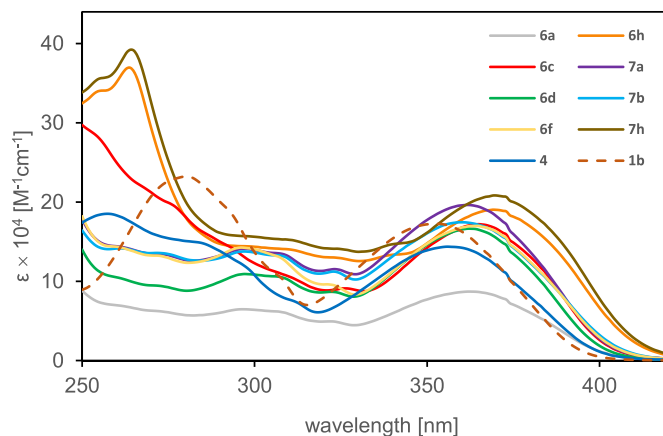
Fig. 2. An ORTEP view of the **4** with the crystallographical atom-labeling scheme. Thermal ellipsoids are drawn at the 50% probability level. Selected interatomic distances [Å]: C(5)–I(1) 2.072(3); C(4a)-C(5) 1.441(4); C(5)–C(6) 1.360(4); C(6)–O(7) 1.416(4); C(7a)-O(7) 1.361(3); C(4a)-C(71) 1.397(4). Key angles [deg]: N3–C1′-O4′ 109.3(2); C4A-C5-I1 121.67(19); C6–C5–I1 130.9(2).

Fig. 3. UV−visible spectra of selected furopyrimidine nucleosides (methanol, 24 °C).

shifted compared to the alkyne-free **1b** (λ_{em} = 434 nm). Slightly lower values were determined for the iododerivative **4** (λ_{em} = 445 nm).

The nucleosides' fluorescence quantum yields Φ showed trends towards a slight increase with the presence of pentyl vs a methyl substituent (**7a** vs **6a**, and **7h** vs **6h**, Table 2). The highest quantum yields were observed for the fluorenol containing, propargyl alcohol containing, and propargyl methyl ether containing nucleosides **6c**, **7a** and **6d** (Φ = 0.60, 0.59, and 0.53, respectively). The iodine attached to the furopyrimidine core quenched the emission of fluorescence almost completely for iodonucleoside **4** (Φ = 0.04), in line with identified halogen effect [58]. Overall, the quantum yields of alkynyl furopyrimidines are competitive with those of recently reported alkynyl fluorescent nucleosides [59,60], and are even comparable with values for nucleosides labeled with auxiliary chromophores [61].

Biological activity. Considering the antiviral activity of furopyrimidine nucleosides, the biological activity of compounds **6** and **7** was investigated. Antiviral properties were determined using *in vitro* assays against several DNA and RNA viruses, including herpes simplex virus 1 (HSV-1), herpes simplex virus 2 (HSV-2), thymidine kinase deficient (TK⁻) HSV-1, adenovirus-2, human coronavirus (229E), human cytomegalovirus (AD-169 and Davis strains), varicella-zoster virus (VZV, TK⁺ Oka strain and TK⁻ 07-1 strain), vesicular stomatitis virus, respiratory syncytial virus, parainfluenza-3 virus, reovirus-1, sindbis virus, coxsackie virus B4, punta toro virus, yellow fever virus, influenza A virus (H1N1 and H3N2 subtypes), and influenza B virus. Results from these *in vitro* assays indicate that some of the derivatives showed activity exclusively against VZV in human embryonic lung (HEL) fibroblasts (Table 3).

In particular, compounds **6c**, **6g**, **6h**, **7a**, **7b**, and **7i** were inhibitory against VZV/TK-competent (wild-type; EC_{50} in the range of 1.295−7.575 μM) as well as VZV/TK-deficient (mutant; EC_{50} in the range of 7.61−13 μM). Although these compounds did not alter cell morphology up to a concentration of 100 μM, they affected HEL cells growth, except for **7i**. Hence, the selectivity indices (SI) [ratio CC_{50} (50% cytostatic concentration for HEL cells)/EC_{50} (50% effective antiviral concentration)] were ≤1 for **6c**, **6g**, **6h**, **7a** and **7b**. In contrast, specific antiviral effects (i.e. SI's of 67 and 11, respectively for VZV TK⁺ and TK⁻ strains) were observed for **7i**, the compound with duplicate *p*-pentylphenyl pharmacophore. Even though **7i** proved active against the wild-type and TK-VZV viruses, the activity was 6-fold lower against the mutant virus than the wild-type virus.

Table 1
Comparison of selected conformational parameters for crystallographically characterized furopyrimidine nucleosides and **4**.

CCDC deposition number	C-6 substituent	χ [°] (C2−N3−C1′−O4′)	ribose−base distance C1′−N3 [Å]	ring puckering	P − phase angle, amplitude[a]	ref.
175815	ferrocene	−148.0(2)	1.478(3)	C2′-endo	164.2, 36.60	42
		−155.4(2)	1.479(2)	C3′-exo	184.4, 36.10	
249882	*p*-tolyl[b]	−161.86(19)	1.483(3)	C2′-endo[b]	150.6, 26.36	38
		−162.2(2)	1.484(3)	C2′-endo	150.5, 26.26	
683563	propyl	−153.26(13)	1.4850(19)	C2′-endo	170.8, 34.12	40
683603	*p*-Bu-phenyl	−153.8(3)	1.481(4)	C2′-endo	168.0, 33.62	25
683604	hexyl-pyridine	−154.9(2)	1.498(3)	C2′-endo	171.1, 33.62	25
715301	deoxyuridine[b]	−172.81(18)	1.497(2)	C3′-exo[bc]	208.8, 33.62	20
785464	thiophene-pyrrole	−160.0(3)	1.502(6)	C3′-exo	182.1, 35.41	43
943139	propanol	−120.8(5)	1.468(7)	C2′-endo	159.0, 37.25	44
1435280	Ph(CH₂)₂[c]	−159.62(17)	1.485(3)	C2′-endo[c]	173.2, 37.25	45
1864088	*p*-tolyl[b,d]	−167.5(2)	1.488(3)	C3′-exo[b,d]	209.7, 27.38	this work[d]

[a] Calculated using pucker.py script for PyMOL, written by S. M. Law (https://pymolwiki.org/index.php/Pucker).
[b] Di-*O*-acetyl derivative.
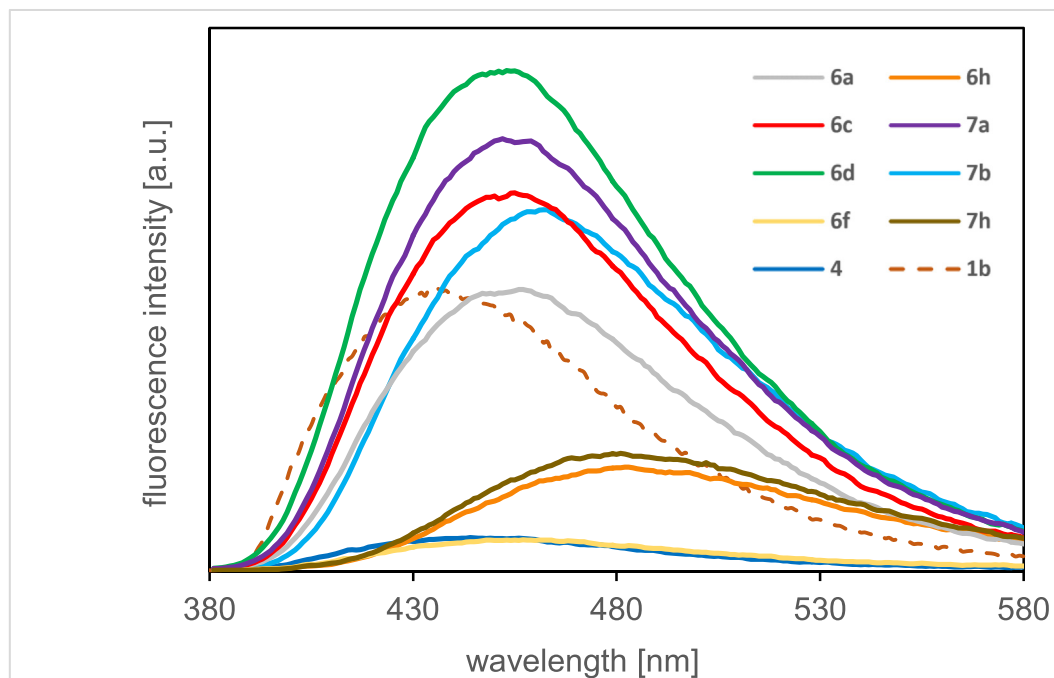[c] Arabino derivative.
[d] 5-Iodo derivative **4**.

Fig. 4. Emission (λ_{ex} = 360 nm) spectra of selected furopyrimidine nucleosides (methanol, 24 °C).

Conclusions. The methodology to install alkynes at C-5 of the furopyrimidine ring was elaborated. Novel 5-alkynylfuropyrimidine derivatives, with biologically active substituents at C-6 (*p*-tolyl and *p*-pentylphenyl), were synthesized. Diverse substituents at the propargyl carbon atom, including alkyl, aryl, hydroxy, methoxy, and acetoxy groups, were introduced. It has been demonstrated that the presence of an alkyne significantly increases the fluorescence quantum yield compared to the iodo precursor. Also, X-ray crystallographic analysis of the iododerivative **4** determined the nucleoside's conformation (sugar puckering and base positioning) in the solid state (*anti* and C3′-*exo*). Accordingly, the characterization of the formed fused furan ring and the regiochemistry of the iodocyclization reaction were confirmed. Synthesis of corresponding nucleosides with free hydroxy groups is currently in progress.

The *in vitro* assays against several DNA and RNA viruses indicate that some of the compounds showed antiviral activity only against VZV despite protected ribose hydroxyl groups. However, inhibition for the growth of HEL cells was also observed, and overall, the investigated nucleosides exhibited cytotoxicity, except for the compound with a duplicate *p*-pentylphenyl pharmacophore (**7i**). Compound **7i** with two *p*-pentylphenyl groups exhibited reasonable anti-VZV activity and was not inhibitory to HEL cells growth up

to a concentration of 100 μM.

3. Materials and methods (experimental)

General Instrumentation. NMR measurements were carried out on a Bruker Avance III 500 spectrometer, operating at 500.13 MHz for ^{1}H and 125.75 MHz for ^{13}C, at 20 °C. Mass spectra were recorded on an Agilent 6520 Q-TOF LCMS (HRMS). FT-IR spectra were recorded on Varian 3100 Excalibur, ThermoScientific Nicolet 6700 ATR, and Bruker Alpha-P ATR spectrometers. All products were stored in a refrigerator (4 °C).

2′-Deoxy-3′,5′-di-*O*-acetyl-5-iodouridine (**Ac-IdU**) [38], 2′-deoxy-3′,5′-di-*O*-acetyl-5-*p*-tolylethynyluridine (**2**) [40,62], and 2′-deoxy-3′,5′-di-*O*-acetyl-5-(4-pentylphenyl)ethynyluridine (**3**) [19] were prepared by literature protocols [63].

3.1. Iodocyclization of 2′-deoxy-3′,5′-di-O-acetyl-5-p-alkylphenylethynyluridines 2 and 3. General procedure

A round bottom flask was charged with 2′-deoxy-3′,5′-di-*O*-acetyl-5-*p*-alkylphenylethynyluridine **2** or **3** (1.88 mmol), *N*-iodosuccinimide (0.508 g, 2.26 mmol), and acetone (15 mL). The reaction mixture was stirred at room temperature for 2 h under argon.

Table 2
Optical properties of selected furopyrimidine nucleosides **6**,**7**, and **4** (λ_{ex} = 360 nm).

nucleoside	R′	λ_{max} [nm]	molar absorptivity [$M^{-1}cm^{-1}$]	λ_{em} [nm]	Φ
6a	CH_2OH	362	8700	456	0.49
7a	CH_2OH	362	19,600	452	0.59
7b	CH_2CH_2OH	360	17,400	461	0.50
6c	9-fluorenol	365	22,900	454	0.60
6d	CH_2OMe	362	16,600	453	0.53
6f	$(CH_2)_2$-phthalimide	364	17,000	457	0.04
6h	*p*-C_6H_4Me	369	19,000	481	0.19
7h	*p*-C_6H_4Me	370	20,800	481	0.21
4	n/a	356	14,400	445	0.05

Table 3
Antiviral activity of furopyrimidines **6** and **7** against varicella-zoster virus (VZV) in human embryonic lung (HEL) cells.[a]

Compound	Antiviral activity EC$_{50}$ [μM][b]		Cytotoxicity [μM]	
	TK$^+$ VZV strain	TK$^-$ VZV strain	Cell morphology	Cell growth
	OKA	07−1	(MCC)[c]	(CC$_{50}$)[d]
6a	66.87	>100	>100	nd[e]
7a	7.56 ± 0.74	12.99 ± 7.0	100	3.8 ± 1.1
6b	76.47	>100	>100	nd[e]
7b	4.02 ± 2.8	12.99 ± 7.0	100	2.66 ± 0.30
6c	1.3 ± 0.5	7.61 ± 0.0	100	2.36 ± 0.39
6d	20	44.72	>100	nd[e]
6e	>100	100	>100	nd[e]
6f	31.02	38.07	100	nd[e]
6g	7.23 ± 1.71	13.2 ± 2.7	100	6.22 ± 0.73
6h	6.84 ± 0.0	10.92 ± 1.98	60	8.77 ± 0.48
7h	7.61	>20	100	nd[e]
6i	>4	>4	20	nd[e]
7i	1.5 ± 0.7	8.94 ± 0.0	100	>100
acyclovir	0.49	23.22	>440	>300
brivudin	0.026	12.01	>300	>300

[a] For detailed Tables see Supporting Materials.
[b] Effective concentration required to reduce virus plaque formation by 50%. Virus input was 20 plaque forming units (PFU).
[c] Minimum cytotoxic concentration that causes a microscopically detectable alteration of cell morphology.
[d] Cytotoxic concentration required to reduce cell growth by 50%.
[e] Not determined.

Solvent was removed and the residue was dissolved in chloroform (10 mL). Silica gel column chromatography (230−400 mesh, chloroform) gave a colorless fraction. The product was dried by oil pump vacuum for 2 h.

3.2. 3-(3′,5′-di-O-acetyl-2′-deoxy-β-ᴅ-ribofuranosyl)-5-iodo-6-(4-methylphenyl)-2,3-dihydrofuro-[2,3-d]pyrimidin-2-one (4)

From 2′-deoxy-3′,5′-di-O-acetyl-5-(4-tolyl)ethynyluridine (**2**, 0.800 g). White solid of **4** (0.955 g, 1.73 mmol, 92%), mp 60-63 °C. NMR (CDCl$_3$, δ): ^{1}H 8.11 (s, 1H, H-4), 7.97 (d, *J* = 8.1 Hz, 2H, C$_6$H$_4$), 7.28 (d, *J* = 8.1 Hz, 2H, C$_6$H$_4$), 6.36 (dd, *J* = 7.6, 6.0 Hz, 1H, H-1′), 5.26 (td, *J* = 6.2, 2.0 Hz, 1H, H-3′), 4.48 (dd, *J* = 12.0, 2.7 Hz, 1H, H-5′), 4.45 (dd, *J* = 5.4, 2.6 Hz, 1H, H-4′), 4.41 (dd, *J* = 11.4, 3.3 Hz, 1H, H-5′), 2.97 (ddd, *J* = 14.5, 5.7, 1.3 Hz, 1H, H-2″), 2.40 (s, 3H, CH$_3$), 2.13 (s, 3H, Ac), 2.12 (s, 3H, Ac), 2.11 (partially obscured ddd, *J* = 7.7, 7.5 Hz, 1H, H-2′); ^{13}C 170.54, 170.48, 170.24, 154.87, 153.27, 140.93, 136.33, 129.47 (2C), 127.22 (2C), 125.17, 112.67, 88.72, 83.56, 74.48, 63.82, 53.88, 39.55, 21.61, 21.25, 20.96. NMR (DMSO-d$_6$, δ): ^{1}H 8.16 (s, 1H, H-4), 7.91 (d, *J* = 7.7 Hz, 2H, C$_6$H$_4$), 7.36 (d, *J* = 7.3 Hz, 2H, C$_6$H$_4$), 6.19 (t, *J* = 6.5 Hz, 1H, H-1′), 5.25−5.21 (m, 1H, H-3′), 4.47−4.42 (m, 1H, H-4′), 4.37 (dd, *J* = 12.1, 3.0 Hz, 1H, H-5′), 4.28 (dd, *J* = 12.2, 5.2 Hz, 1H, H-5′), 2.62 (dd, *J* = 14.2, 5.4 Hz, 1H, H-2′), 2.42 (ddd, *J* = 14.5, 7.2, 7.2 Hz, 1H, H-2″), 2.36 (s, 3H, CH$_3$), 2.12 (s, 3H, CH$_3$), 2.08 (s, 3H, CH$_3$). IR (ν, cm^{-1}, ATR) 3089 w, 3028 w, 3089 w, 2951 w, 1738 s, 1665 vs, 1590 s, 1375 m, 1220 vs, 1184 s, 1014 m. UV−vis (MeOH, 21.7 × 10^{-6} M) λ$_{max}$ 257 (ε 18,500), 356 nm (ε 14,400 M^{-1}cm^{-1}). Fluorescence (MeOH, ca. 3.65 × 10^{-6} M) λ$_{ex}$ 360 nm, λ$_{em}$ 444 nm, Φ = 0.05. HRMS (ESI-TOF) [M + H]$^+$ calcd for C$_{22}$H$_{22}$IN$_2$O$_7$ 553.0466, found: 553.0466.

3.3. 3-(3′,5′-di-O-acetyl-2′-deoxy-β-ᴅ-ribofuranosyl)-5-iodo-6-(4-pentylphenyl)-2,3-dihydrofuro-[2,3-d]pyrimidin-2-one (5).

From 2′-deoxy-3′,5′-di-O-acetyl-5-(4-pentylphenyl)ethynyluridine (**3**, 0.906 g). White solid of **5** (0.869 g, 1.43 mmol, 76%). NMR

(CDCl$_3$, δ): ^{1}H 8.10 (s, 1H, H-4), 7.97 (d, *J* = 8.4 Hz, 2H, C$_6$H$_4$), 7.26 (d, *J* = 8.1 Hz, 2H, C$_6$H$_4$), 6.35 (dd, *J* = 7.8, 5.8 Hz, 1H, H-1′), 5.24 (td, *J* = 6.1, 1.9 Hz, 1H, H-3′), 4.47 (dd, *J* = 11.9, 2.9 Hz, 1H, H-5′), 4.44 (dd, *J* = 5.0, 2.3 Hz, 1H, H-4′), 4.39 (dd, *J* = 11.5, 3.0 Hz, 1H, H-5′), 2.95 (ddd, *J* = 14.6, 5.6, 1.3 Hz, 1H, H-2″), 2.61 (t, 2H, *J* = 7.8 Hz, CH$_2$), 2.14−2.06 (partially obscured m, 1H, H-2′), 2.12 (s, 3H, Ac), 2.10 (s, 3H, Ac), 1.60 (p, *J* = 7.4 Hz, 2H, CH$_2$), 1.33−1.25 (m, 4H, 2CH$_2$), 0.86 (t, *J* = 6.7 Hz, 3H, CH$_3$); ^{13}C 170.57, 170.47, 170.33, 154.88, 153.15, 145.90, 136.39, 128.83 (2C), 127.16 (2C), 125.29, 112.69, 88.72, 83.54, 74.56, 63.88, 53.95, 39.51, 35.93, 31.48, 30.95, 22.59, 21.35, 21.04, 14.14. IR (cm^{-1}, ATR) 2953 w, 2923 w, 2850 w, 1734 s, 1650 vs, 1590 s, 1381 m, 1210 m, 1181 s, 1010 s. HRMS (ESI-TOF) [M + H]$^+$ calcd for C$_{26}$H$_{30}$IN$_2$O$_7$ 609.1093, found: 609.1117.

3.4. 5-Alkynyl-6-arylfuropyrimidine nucleosides 6a-h or 7a,b,h,i. General procedure

A round bottom flask (25 mL) was charged with iodofuropyrimidine nucleoside (**4**, 0.276 g, 0.500 mmol; or **5**, 0.304 g, 0.500 mmol), Pd(PPh$_3$)$_4$ (0.035 g, 0.030 mmol), CuI (0.006 g, 0.03 mmol), DMF (5 mL), Et$_3$N (0.174 mL, 1.25 mmol), and the respective terminal alkynes. The reaction mixture was stirred at room temperature for 22 h. Solvent was removed (by oil pump vacuum) and the residue was dissolved in chloroform (10 mL), and subjected to silica gel column chromatography (230−400 mesh, chloroform). The product was dried by oil pump vacuum for 2 h to give **6a-i** or **7a,b,h,i**.

3.5. 3-(3′,5′-di-O-acetyl-2′-deoxy-β-ᴅ-ribofuranosyl)-5-(3-hydroxyprop-1-yn-1-yl)-6-(4-methylphenyl)-2,3-dihydrofuro[2,3-d]pyrimidin-2-one (6a)

From 2-propyn-1-ol (propargyl alcohol; 0.072 mL, 1.2 mmol). Light yellow solid: 0.182 g, 0.379 mmol, 76%. NMR (CDCl$_3$, δ): ^{1}H 8.26 (s, 1H, H-4), 8.00 (d, *J* = 8.4 Hz, 2H, C$_6$H$_4$), 7.27 (d, *J* = 8.8 Hz, 2H, C$_6$H$_4$), 6.34 (dd, *J* = 8.1, 5.5 Hz, 1H, H-1′), 5.27 (td, *J* = 6.5, 2.1 Hz, 1H, H-3′), 4.71 (dd, *J* = 11.8, 1.9 Hz, 1H, H-5′), 4.61 and 4.58 (dAB, *J* = 16.0, 5.6 Hz, 2H, CH$_2$O), 4.47−4.44 (m, 1H, H-4′), 4.42 (dd, *J* = 11.9, 4.6 Hz, 1H, H-5′), 3.46 (t, *J* = 6.3 Hz, 1H, OH), 3.01 (ddd, *J* = 14.5, 5.5, 1.5 Hz, 1H, H-2″), 2.41 (s, 3H, CH$_3$), 2.14 (s, 3H, Ac), 2.13 (s, 3H, Ac), 2.03 (ddd, *J* = 14.5, 7.9, 6.6 Hz, 1H, H-2′); ^{13}C 172.02, 170.60, 170.25, 156.08, 154.62, 141.02, 134.39, 129.63 (2C), 125.99 (2C), 125.28, 109.47, 97.53, 94.53, 89.02, 84.06, 74.62, 74.51, 63.96, 51.55, 39.56, 21.68, 21.19, 20.95. NMR (DMSO-d$_6$, δ): ^{1}H 8.43 (s, 1H, H-4), 8.01 (d, *J* = 8.2 Hz, 2H, C$_6$H$_4$), 7.37 (d, *J* = 8.2 Hz, 2H, C$_6$H$_4$), 6.21 (t, *J* = 7.2 Hz, 1H, H-1′), 5.53−5.49 (m, 1H, H-3′), 5.24−5.19 (m, 1H, H-4′), 4.45−4.39 (m, 3H, CH$_2$, H-5′), 4.38−4.29 (m, 2H, H-5″, OH), 2.63−2.57 (m, 1H, H-2′), 2.46−2.40 (m, 1H, H-2″), 2.38 (s, 3H, CH$_3$), 2.09 (s, 3H, CH$_3$), 2.08 (s, 3H, CH$_3$). IR (ν, cm^{-1}, ATR) 2938 vw, 2925 vw, 1739 s, 1664 vs, 1599 s, 1573 s, 1395 m, 1220 vs, 1018 vs. UV−vis (MeOH, 23.5 × 10^{-6} M) λ$_{max}$ 297 (ε 6470), 322 (ε 4940), 362 nm (ε 8700 M^{-1}cm^{-1}). Fluorescence (MeOH, ca. 5.39 × 10^{-6} M) λ$_{ex}$ 360 nm, λ$_{em}$ 456 nm, Φ = 0.49. HRMS (ESI-TOF) [M + H]$^+$ calcd for C$_{25}$H$_{25}$N$_2$O$_8$ 481.1605, found: 481.1605.

3.6. 3-(3′,5′-di-O-acetyl-2′-deoxy-β-ᴅ-ribofuranosyl)-5-(4-hydroxybut-1-yn-1-yl)-6-(4-methylphenyl)-2,3-dihydrofuro[2,3-d]pyrimidin-2-one (6b)

From 3-butyn-1-ol (0.095 mL, 1.25 mmol). Light yellow solid: 0.235 g, 0.476 mmol, 95%. NMR (CDCl$_3$, δ): ^{1}H 8.27 (s, 1H, H-4), 8.02 (d, *J* = 8.3 Hz, 2H, C$_6$H$_4$), 7.24 (d, *J* = 8.3 Hz, 2H, C$_6$H$_4$), 6.31 (dd, *J* = 7.9, 5.6 Hz, 1H, H-1′), 5.24 (td, *J* = 6.4, 2.1 Hz, 1H, H-3′), 4.57 (dd,

$J = 11.9, 2.5$ Hz, 1H, H-5′), 4.43−4.41 (m, 1H, H-4′), 4.39 (dd, $J = 11.5$, 4.0 Hz, 1H, H-5′), 3.90 (br t, $J = 5.8$ Hz, 2H, CH$_2$O), 2.97 (ddd, $J = 14.4$, 5.6, 1.8 Hz, 1H, H-2″), 2.97 (signal partially obscured, 1H, OH), 2.81 (t, $J = 6.0$ Hz, 2H, CH$_2$), 2.40 (s, 3H, CH$_3$), 2.12 (s, 3H, Ac), 2.08 (s, 3H, Ac), 2.05 (ddd, $J = 14.6$, 7.8, 6.7 Hz, 1H, H-2′); ^{13}C 171.08, 170.54, 170.16, 156.21, 154.62, 140.68, 134.68, 129.53 (2C), 125.88 (2C), 125.53, 109.39, 97.26, 95.36, 88.86, 83.79, 74.52, 71.18, 63.85, 60.74, 39.48, 24.45, 21.63, 20.96 (2C). IR (ν, cm^{-1}, ATR) 3391 br w, 2933 w, 1740 s, 1664 vs, 1641 m, 1574 m, 1396 m, 1367 m, 1216 vs, 1188 s, 1100 m, 1032 s, 1015 s. HRMS (ESI-TOF) [M + H]$^+$ calcd for C$_{26}$H$_{27}$N$_2$O$_8$ 495.1762, found: 495.1762.

3.7. 3-(3′,5′-di-O-acetyl-2′-deoxy-β-D-ribofuranosyl)-5-((9-hydroxy-9H-fluoren-9-yl)ethynyl)-6-(4-methylphenyl)-2,3-dihydrofuro[2,3-d]pyrimidin-2-one (**6c**)

From 9-ethynyl-9-fluorenol (0.259 mg, 1.25 mmol). Yellow solid: 0.261 g, 0.415 mmol, 83%. NMR (CDCl$_3$, δ): ^{1}H 8.37 (s, 1H, H-4), 7.82 (d, $J = 8.3$ Hz, 2H, p-C$_6$H$_4$), 7.81 (dd, $J = 11.5$, 7.4 Hz, 2H, o-C$_6$H$_4$), 7.69 (dd, $J = 7.5$, 3.3 Hz, 2H, o-C$_6$H$_4$), 7.46 (virtual quartet, $J = 7.4$ Hz, 2H, o-C$_6$H$_4$), 7.41 (virtual triplet, $J = 7.4$ Hz, 2H, o-C$_6$H$_4$), 7.07 (d, $J = 8.4$ Hz, 2H, p-C$_6$H$_4$), 6.36 (dd, $J = 7.6$, 5.5 Hz, 1H, H-1′), 5.28 (td, $J = 6.4$, 2.0 Hz, 1H, H-3′), 4.66 (dd, $J = 12.2$, 2.5 Hz, 1H, H-5′), 4.55 (dd, $J = 12.2$, 4.4 Hz, 1H, H-5′), 4.51−4.48 (m, 1H, H-4′), 4.32 (s, 1H, OH), 3.02 (ddd, $J = 14.6$, 5.6, 1.7 Hz, 1H, H-2″), 2.35 (s, 3H, CH$_3$), 2.15 (s, 3H, Ac), 2.11 (s, 3H, Ac), 2.09 (ddd, $J = 14.9$, 7.4, 7.4 Hz, 1H, H-2′); ^{13}C 171.85, 170.52, 170.18, 157.07, 154.54, 146.64, 146.34, 140.93, 139.27, 139.21, 134.48, 129.89, 129.83, 129.37 (2C), 128.61, 128.52, 125.88 (2C), 125.03, 124.29, 124.20, 120.48 (2C), 109.01, 99.19, 94.28, 89.06, 84.06, 75.04, 74.52, 72.13, 63.97, 39.51, 21.58, 21.11, 20.92. IR (ν, cm^{-1}, CH$_2$Cl$_2$) 2361 w, 2225 w, 2152 w, 1745 m, 1674 vs, 1603 m, 1576 m, 1235 s, 1187 m. UV−vis (MeOH, 18.9×10^{-6} M) λ_{max} 365 nm (ε 17,200 M^{-1}cm^{-1}). Fluorescence (MeOH, ca. 3.67×10^{-6} M) λ_{ex} 360 nm, λ_{em} 453 nm, Φ = 0.60. HRMS (ESI-TOF) [M + H]$^+$ calcd for C$_{37}$H$_{31}$N$_2$O$_8$ 631.2075, found: 631.2075.

3.8. 3-(3′,5′-di-O-acetyl-2′-deoxy-β-D-ribofuranosyl)-5-(3-methoxyprop-1-yn-1-yl)-6-(4-methylphenyl)-2,3-dihydrofuro[2,3-d]pyrimidin-2-one (**6d**)

From methyl propargyl ether (0.105 mL, 1.25 mmol). Light yellow solid: 0.141 g, 0.285 mmol, 66%. NMR (CDCl$_3$, δ): ^{1}H 8.31 (s, 1H, H-4), 8.03 (d, $J = 8.3$ Hz, 2H, C$_6$H$_4$), 7.27 (d, $J = 7.9$ Hz, 2H, C$_6$H$_4$), 6.36 (dd, $J = 7.7$, 5.9 Hz, 1H, H-1′), 5.25 (td, $J = 6.5$, 2.2 Hz, 1H, H-3′), 4.47 (dd, $J = 11.6$, 2.5 Hz, 1H, H-5′), 4.44−4.41 (m, 1H, H-4′), 4.41 (s, 2H, CH$_2$), 4.39 (dd, $J = 11.5$, 3.5 Hz, 1H, H-5′), 3.46 (s, 3H, CH$_3$), 2.94 (ddd, $J = 14.5$, 5.6, 2.1 Hz, 1H, H-2″), 2.40 (s, 3H, CH$_3$), 2.16−2.10 (obscured ddd, $J = 3.6$, 3.6, Hz, 1H, H-2′), 2.11 (s, 3H, Ac), 2.10 (s, 3H, Ac); ^{13}C 170.47, 170.21, 170.17, 157.06, 154.54, 141.09, 134.80, 129.61 (2C), 126.11 (2C), 125.32, 109.07, 94.50, 94.43, 88.62, 83.47, 75.66, 74.34, 63.69, 60.53, 58.01, 39.47, 21.66, 20.94, 20.82. IR (ν, cm^{-1}, CH$_2$Cl$_2$) 2937 w, 1749 s, 1674 vs, 1576 m, 1234 m, 1109 w. UV−vis (MeOH, 24.1×10^{-6} M) λ_{max} 362 nm (ε 16,600 M^{-1}cm^{-1}). Fluorescence (MeOH, ca. 3.61×10^{-6} M) λ_{ex} 360 nm, λ_{em} 453 nm, Φ = 0.53. HRMS (ESI-TOF) [M + H]$^+$ calcd for C$_{26}$H$_{27}$N$_2$O$_8$ 495.1762, found: 495.1762.

3.9. 5-(3-Acetoxyprop-1-yn-1-yl)-3-(3′,5′-di-O-acetyl-2′-deoxy-β-D-ribofuranosyl)-6-(4-methylphenyl)-2,3-dihydrofuro[2,3-d]pyrimidin-2-one (**6e**)

From propargyl acetate (0.124 mL, 1.25 mmol). Light yellow solid: 0.206 g, 0.395 mmol, 79%. NMR (CDCl$_3$, δ): ^{1}H 8.34 (s, 1H, H-

4), 8.04 (d, $J = 8.2$ Hz, 2H, C$_6$H$_4$), 7.30 (d, $J = 8.1$ Hz, 2H, C$_6$H$_4$), 6.38 (dd, $J = 7.7$, 5.8 Hz, 1H, H-1′), 5.27 (td, $J = 6.4$, 2.1 Hz, 1H, H-3′), 4.99 (s, 2H, CH$_2$), 4.51 (dd, $J = 11.9$, 2.7 Hz, 1H, H-5′), 4.45 (dd, $J = 5.7$, 2.7 Hz, 1H, H-4′), 4.43 (dd, $J = 11.9$, 3.6 Hz, 1H, H-5′), 2.96 (ddd, $J = 14.5$, 5.7, 2.1 Hz, 1H, H-2″), 2.42 (s, 3H, CH$_3$), 2.17−2.10 (m, 10H, 3Ac, H-2′); ^{13}C 172.37, 172.08, 172.06, 159.50, 156.42, 143.20, 136.78, 131.54 (2C), 128.09 (2C), 127.04, 110.76, 95.87, 94.41, 90.61, 85.43, 77.79, 76.28, 65.62, 54.55, 49.08, 41.37, 23.57, 22.84, 22.74, 22.66. IR (ν, cm^{-1}, CH$_2$Cl$_2$) 2930, 1749, 1675, 1603, 1575. HRMS (ESI-TOF) [M + H]$^+$ calcd for C$_{27}$H$_{27}$N$_2$O$_9$ 523.1711, found: 523.1712.

3.10. 3-(3′,5′-di-O-acetyl-2′-deoxy-β-D-ribofuranosyl)-5-(4-(1,3-dioxoisoindolin-2-yl)but-1-yn-1-yl)-6-(4-methylphenyl)-2,3-dihydrofuro[2,3-d]pyrimidin-2-one (**6f**)

From N-(3-butynyl)phthalimide (0.249 mg, 1.25 mmol). Light yellow solid: 0.290 g, 0.465 mmol, 93%. NMR (CDCl$_3$, δ): ^{1}H 8.51 (s, 1H, H-4), 7.92 (d, $J = 8.3$ Hz, 2H, p-C$_6$H$_4$), 7.81 (AA'XX', $J = 5.5$, 3.1 Hz, 2H, o-C$_6$H$_4$), 7.69 (AA'XX', $J = 5.5$, 3.1 Hz, 2H, o-C$_6$H$_4$), 7.15 (d, $J = 8.3$ Hz, 2H, p-C$_6$H$_4$), 6.42 (dd, $J = 7.7$, 5.8 Hz, 1H, H-1′), 5.31 (td, $J = 6.6$, 2.1 Hz, 1H, H-3′), 4.57 (dd, $J = 11.3$, 2.8 Hz, 1H, H-5′), 4.48−4.46 (m, 1H, H-4′), 4.44 (dd, $J = 11.5$, 4.0 Hz, 1H, H-5′), 4.01 (dp, $J = 10.3$, 4.9 Hz, 2H, CH$_2$), 3.03−2.91 (m, 1H, H-2″), 2.98 and 2.96 (AB, $J = 5.8$ Hz, 2H, CH$_2$), 2.35 (s, 3H, CH$_3$), 2.26 (ddd, $J = 14.5$, 7.8, 6.8 Hz, 1H, H-2′), 2.12 (s, 3H, Ac), 2.09 (s, 3H, Ac); ^{13}C 170.48, 170.34, 170.12, 168.18 (2C), 156.16, 154.68, 140.58, 135.57, 134.21 (2C), 131.89 (2C), 129.46 (2C), 125.83 (2C), 125.42, 123.48 (2C), 109.32, 95.66, 95.04, 88.70, 83.51, 74.64, 71.84, 63.96, 39.43, 36.44, 21.62, 20.98, 20.91, 20.03. IR (ν, cm^{-1}, CH$_2$Cl$_2$) 2272 vw, 2168 vw, 2139 vw, 1745 m, 1719 vs, 1673 vs, 1603 m, 1575 m, 1397 m, 1237 m, 1113 w. UV−vis (MeOH, 17.3×10^{-6} M) λ_{max} 364 nm (ε 17,000 M^{-1}cm^{-1}). Fluorescence (MeOH, ca. 3.52×10^{-6} M) λ_{ex} 360 nm, λ_{em} 457 nm, Φ = 0.04. HRMS (ESI-TOF) [M + H]$^+$ calcd for C$_{34}$H$_{30}$N$_3$O$_9$ 624.1977, found: 624.1977.

3.11. 3-(3′,5′-di-O-acetyl-2′-deoxy-β-D-ribofuranosyl)-5-(4-(1,3-dioxoisoindolin-2-yl)hex-1-yn-1-yl)-6-(4-methylphenyl)-2,3-dihydrofuro[2,3-d]pyrimidin-2-one (**6g**)

From N-(5-hexynyl)phthalimide (0.284 mg, 1.25 mmol). Light brown oil: 0.267 g, 0.410 mmol, 82%. NMR (CDCl$_3$, δ): ^{1}H 8.25 (s, 1H, H-4), 7.97 (d, $J = 8.2$ Hz, 2H, p-C$_6$H$_4$), 7.78 (AA'XX', $J = 5.4$, 3.2 Hz, 2H, o-C$_6$H$_4$), 7.67 (AA'XX', $J = 5.5$, 3.2 Hz, 2H, o-C$_6$H$_4$), 7.21 (d, $J = 8.2$ Hz, 2H, p-C$_6$H$_4$), 6.35 (dd, $J = 7.7$, 5.7 Hz, 1H, H-1′), 5.24 (td, $J = 6.6$, 2.1 Hz, 1H, H-3′), 4.46 (dd, $J = 11.9$, 3.1 Hz, 1H, H-5′), 4.40 (dd, $J = 5.9$, 3.0 Hz, 1H, H-4′), 4.36 (dd, $J = 11.9$, 3.7 Hz, 1H, H-5′), 3.73 (t, $J = 7.1$ Hz, 2H, CH$_2$), 2.91 (ddd, $J = 14.5$, 5.7, 2.0 Hz, 1H, H-2″), 2.58 (t, $J = 7.1$ Hz, 2H, CH$_2$), 2.34 (s, 3H, CH$_3$), 2.15 (ddd, $J = 14.5$, 7.7, 6.8 Hz, 1H, H-2′), 2.09 (s, 3H, Ac), 2.06 (s, 3H, Ac), 1.87 (p, $J = 7.4$ Hz, 2H, CH$_2$), 1.70 (p, $J = 7.4$ Hz, 2H, CH$_2$); ^{13}C (includes DEPT) 170.30, 170.01, 169.95, 168.24 (2C), 155.86, 154.46, 140.35, 134.61(CH), 133.85(2CH), 131.89 (2C), 129.31(2CH), 125.65(2CH), 125.42, 123.05(2CH), 109.25, 98.95, 95.34, 88.51(CH), 83.30(CH), 74.35(CH), 70.30, 63.60(CH$_2$), 39.22(CH$_2$), 37.20(CH$_2$), 27.72(CH$_2$), 25.52(CH$_2$), 21.46(CH$_3$), 20.80(CH$_3$), 20.64(CH$_3$), 19.35(CH$_2$). IR (ν, cm^{-1}, ATR) 3000 s, 2972 s, 2940 s, 1742 vs, 1670 m, 1370 s, 1230 s, 1217 s, 1100 w, 1013 w. HRMS (ESI-TOF) [M + H]$^+$ calcd for C$_{36}$H$_{34}$N$_3$O$_9$ 652.2290, found: 652.2303.

3.12. 3-(3′,5′-di-O-acetyl-2′-deoxy-β-D-ribofuranosyl)-6-(4-methylphenyl)-5-((4-methylphenyl)ethynyl)-2,3-dihydrofuro[2,3-d]pyrimidin-2-one (**6h**)

From 4-ethynyltoluene (0.158 mL, 1.25 mmol). Light brown solid: 0.189 g, 0.350 mmol, 70%. NMR (CDCl$_3$, δ): ^{1}H 8.37 (s, 1H, H-4), 8.07 (d, J = 8.3 Hz, 2H, C$_6$H$_4$), 7.43 (d, J = 8.1 Hz, 2H, C$_6$H$_4$), 7.25 (d, J = 9.0 Hz, 2H, C$_6$H$_4$), 7.19 (d, J = 7.8 Hz, 2H, C$_6$H$_4$), 6.38 (dd, J = 7.6, 5.7 Hz, 1H, H-1′), 5.25 (td, J = 6.4, 2.1 Hz, 1H, H-3′), 4.49 (dd, J = 14.1, 4.8 Hz, 1H, H-5′), 4.43−4.37 (m, 2H, H-4′,5′), 2.93 (ddd, J = 14.5, 5.7, 2.1 Hz, 1H, H-2″), 2.38 (s, 6H, CH$_3$), 2.15 (ddd, J = 14.5, 7.6, 6.8 Hz, 1H, H-2′), 2.10 (s, 3H, Ac), 2.00 (s, 3H, Ac); ^{13}C 170.31, 170.09 (2C), 156.09, 154.42, 140.63, 139.42 (2C), 134.74, 131.31 (2C), 129.31 (2C), 129.28 (2C), 125.90 (2C), 125.44, 118.99, 108.86, 98.30, 95.23, 88.42, 83.32, 74.16, 63.52, 39.29, 21.49 (2C), 20.78, 20.60. IR (ν, cm^{-1}, CH$_2$Cl$_2$) 3016 w, 2970 w, 2946 w, 2210, 1749, 1674. UV−vis (MeOH, 24.4 × 10^{-6} M) λ$_{max}$ 264 (ε 36,900), 369 nm (ε 19,000 M^{-1}cm^{-1}). Fluorescence (MeOH, ca. 3.57 × 10^{-6} M) λ$_{ex}$ 360 nm, λ$_{em}$ 481 nm, Φ = 0.19. HRMS (ESI-TOF) [M + H]$^+$ calcd for C$_{31}$H$_{29}$N$_2$O$_7$ 541.1969, found: 541.1969.

3.13. 3-(3′,5′-di-O-acetyl-2′-deoxy-β-D-ribofuranosyl)-6-(4-methylphenyl)-5-((4-pentylphenyl)ethynyl)-2,3-dihydrofuro[2,3-d]pyrimidin-2-one (**6i**)

From 4-n-pentylphenylacetylene (0.243 mL, 1.25 mmol). Yield: 0.191 g, 0.320 mmol (64%, oil). NMR (CDCl$_3$, δ): ^{1}H 8.39 (s, 1H, H-4), 8.08 (d, J = 8.3 Hz, 2H, C$_6$H$_4$), 7.46 (d, J = 8.0 Hz, 2H, C$_6$H$_4$), 7.26 (d, J = 8.2 Hz, 2H, C$_6$H$_4$), 7.21 (d, J = 8.0 Hz, 2H, C$_6$H$_4$), 6.39 (dd, J = 7.6, 5.8 Hz, 1H, H-1′), 5.25 (td, J = 6.4, 2.0 Hz, 1H, H-3′), 4.50 (dd, J = 14.7, 5.1 Hz, 1H, H-5′), 4.44−4.38 (m, 2H, H-4′,5′), 2.94 (ddd, J = 14.5, 5.6, 2.1 Hz, 1H, H-2″), 2.63 (t, J = 7.8 Hz, 2H, CH$_2$), 2.39 (s, 3H, CH$_3$), 2.16 (ddd, J = 14.4, 7.5, 6.9 Hz, 1H, H-2′), 2.11 (s, 3H, Ac), 2.00 (s, 3H, Ac), 1.62 (p, J = 7.5 Hz, 2H, CH$_2$), 1.36−1.28 (m, 4H, 2CH$_2$), 0.89 (t, J = 6.8 Hz, 3H, CH$_3$); ^{13}C 170.48, 170.26 (2C), 156.28, 154.59, 144.62, 140.79, 134.90, 131.51 (2C), 129.58 (2C), 128.80 (2C), 126.08 (2C), 125.61, 119.35, 109.04, 98.52, 95.40, 88.58, 83.48, 77.97, 74.31, 63.68, 39.46, 35.96, 31.46, 30.94, 22.54, 21.66, 20.94, 20.76, 14.06. IR (cm^{-1}, ATR) 2958 m, 2924 m, 1740 s, 1671 vs, 1572 m, 1402 m, 1226 vs, 1058 vs. HRMS (ESI-TOF) [M + H]$^+$ calcd for C$_{35}$H$_{37}$N$_2$O$_7$ 597.2596, found: 597.2599.

3.14. 3-(3′,5′-di-O-acetyl-2′-deoxy-β-D-ribofuranosyl)-5-(3-hydroxyprop-1-yn-1-yl)-6-(4-pentylphenyl)-2,3-dihydrofuro[2,3-d]pyrimidin-2-one (**7a**)

From 2-propyn-1-ol (propargyl alcohol; 0.072 mL, 1.2 mmol). Yellow solid: 0.217 g, 0.405 mmol, 81%. NMR (CDCl$_3$, δ): ^{1}H 8.26 (s, 1H, H-4), 8.02 (d, J = 8.3 Hz, 2H, C$_6$H$_4$), 7.27 (d, J = 9.1 Hz, 2H, C$_6$H$_4$), 6.34 (dd, J = 8.0, 5.5 Hz, 1H, H-1′), 5.27 (td, J = 6.6, 2.0 Hz, 1H, H-3′), 4.70 (dd, J = 11.9, 2.0 Hz, 1H, H-5′), 4.61 and 4.57 (dAB, J = 16.3, 5.7 Hz, 2H, CH$_2$O), 4.46−4.43 (m, 1H, H-4′), 4.41 (dd, J = 11.3, 4.3 Hz, 1H, H-5′), 3.50 (t, J = 6.3 Hz, 1H, OH), 3.00 (ddd, J = 14.6, 5.6, 1.5 Hz, 1H, H-2″), 2.64 (t, J = 7.8 Hz, 2H, CH$_2$), 2.13 (s, 3H, Ac), 2.12 (s, 3H, Ac), 2.04 (ddd, J = 14.6, 7.9, 6.6 Hz, 1H, H-2′), 1.63 (p, J = 7.5 Hz, 2H, CH$_2$), 1.37−1.29 (m, 4H, 2CH$_2$), 0.89 (t, J = 6.9 Hz, 3H, CH$_3$); ^{13}C 171.98, 170.60, 170.25, 156.14, 154.63, 146.05, 134.41, 129.00 (2C), 126.02 (2C), 125.45, 109.48, 97.54, 94.52, 89.02, 84.05, 74.61, 74.51, 63.95, 51.53, 39.54, 35.98, 31.44, 30.89, 22.55, 21.18, 20.95, 14.05. IR (ν, cm^{-1}, ATR) 3350 br w, 2968 m, 2928 m, 1739 s, 1665 vs, 1599 s, 1219 vs, 1066 vs. UV−vis (MeOH, 22.2 × 10^{-6} M) λ$_{max}$ 362 nm (ε 19,600 M^{-1}cm^{-1}). Fluorescence (MeOH, ca. 3.73 × 10^{-6} M) λ$_{ex}$ 360 nm, λ$_{em}$ 452 nm, Φ = 0.59. HRMS (ESI-TOF) [M + H]$^+$ calcd for C$_{29}$H$_{33}$N$_2$O$_8$ 537.2231, found: 537.2231.

3.15. 3-(3′,5′-di-O-acetyl-2′-deoxy-β-D-ribofuranosyl)-5-(4-hydroxybut-1-yn-1-yl)-6-(4-pentylphenyl)-2,3-dihydrofuro[2,3-d]pyrimidin-2-one (**7b**)

From 3-butyn-1-ol (0.095 mL, 1.25 mmol). Light yellow solid: 0.223 g, 0.405 mmol, 81%. NMR (CDCl$_3$, δ): ^{1}H 8.27 (s, 1H, H-4), 8.04 (d, J = 8.2 Hz, 2H, C$_6$H$_4$), 7.25 (d, J = 8.0 Hz, 2H, C$_6$H$_4$), 6.31 (dd, J = 7.4, 5.9 Hz, 1H, H-1′), 5.24 (td, J = 6.6, 1.5 Hz, 1H, H-3′), 4.57 (dd, J = 11.9, 2.2 Hz, 1H, H-5′), 4.44−4.41 (m, 1H, H-4′), 4.38 (dd, J = 11.9, 4.2 Hz, 1H, H-5′), 3.89 (AB, J = 6.0 Hz, 2H, CH$_2$), 3.03−3.00 (br s, 1H, OH), 2.97 (ddd, J = 14.9, 5.8, 1.0 Hz, 1H, H-2″), 2.81 (t, J = 6.0 Hz, 2H, CH$_2$), 2.62 (t, J = 7.8 Hz, 2H, CH$_2$), 2.12 (s, 3H, Ac), 2.08 (s, 3H, Ac), 2.06 (ddd, J = 14.5, 7.8, 6.5 Hz, 1H, H-2′), 1.61 (p, J = 7.4 Hz, 2H, CH$_2$), 1.36−1.28 (m, 4H, 2CH$_2$), 0.88 (t, J = 7.0 Hz, 3H, CH$_3$); ^{13}C 171.08, 170.54, 170.16, 156.25, 154.63, 145.72, 134.69, 128.90 (2C), 125.90 (2C), 125.71, 109.41, 97.24, 95.34, 88.88, 83.79, 74.53, 71.21, 63.86, 60.75, 39.48, 35.95, 31.45, 30.89, 20.45, 22.54, 20.95 (2C), 14.04. IR (ν, cm^{-1}, ATR) 3380 br w, 2958 m, 2927 m, 1739 s, 1665 vs, 1572 m, 1399 m, 1219 vs, 1044 vs. UV−vis (MeOH, 21.6 × 10^{-6} M) λ$_{max}$ 360 nm (ε 17,400 M^{-1}cm^{-1}). Fluorescence (MeOH, ca. 3.63 × 10^{-6} M) λ$_{ex}$ 360 nm, λ$_{em}$ 461, Φ = 0.50. HRMS (ESI-TOF) [M + H]$^+$ calcd for C$_{30}$H$_{35}$N$_2$O$_8$ 551.2388, found: 551.2388.

3.16. 3-(3′,5′-di-O-acetyl-2′-deoxy-β-D-ribofuranosyl)-5-(4-methylphenyl)-6-(4-pentylphenyl)-2,3-dihydrofuro[2,3-d]pyrimidin-2-one (**7h**)

From 4-ethynyltoluene (0.158 mL, 1.25 mmol). Light brown solid: 0.289 g, 0.485 mmol, 97%. NMR (CDCl$_3$, δ): ^{1}H 8.41 (s, 1H, H-4), 8.15 (d, J = 8.4 Hz, 2H, C$_6$H$_4$), 7.47 (d, J = 8.0 Hz, 2H, C$_6$H$_4$), 7.30 (d, J = 8.4 Hz, 2H, C$_6$H$_4$), 7.22 (d, J = 7.9 Hz, 2H, C$_6$H$_4$), 6.41 (dd, J = 7.6, 5.7 Hz, 1H, H-1′), 5.27 (td, J = 6.6, 2.0 Hz, 1H, H-3′), 4.51 (dd, J = 11.7, 2.4 Hz, 1H, H-5′), 4.46−4.43 (m, 1H, H-4′), 4.41 (dd, J = 11.8, 3.6 Hz, 1H, H-5′), 2.95 (ddd, J = 14.5, 5.8, 2.2 Hz, 1H, H-2″), 2.66 (t, J = 7.8 Hz, 2H, CH$_2$), 2.41 (s, 3H, CH$_3$), 2.16 (ddd, J = 14.4, 7.6, 6.8 Hz, 1H, H-2′), 2.13 (s, 3H, Ac), 2.01 (s, 3H, Ac), 1.64 (p, J = 7.5 Hz, 2H, CH$_2$), 1.37−1.31 (m, 4H, 2CH$_2$), 0.89 (t, J = 7.0 Hz, 3H, CH$_3$); ^{13}C 170.51, 170.32, 170.26, 156.41, 154.63, 145.91, 139.63, 134.84, 131.52 (2C), 129.46 (2C), 128.99 (2C), 126.16 (2C), 125.80, 119.17, 109.13, 98.46, 95.35, 88.57, 83.48, 77.99, 74.28, 63.69, 39.52, 36.00, 31.47, 30.92, 22.56, 21.67, 20.96, 20.75, 14.05. IR (ν, cm^{-1}, ATR) 2975 m, 2927 m, 1740 s, 1671 vs, 1403 s, 1226 vs, 1058 vs. UV−vis (MeOH, 19.9 × 10^{-6} M) λ$_{max}$ 370 nm (ε 20,800 M^{-1}cm^{-1}). Fluorescence (MeOH, ca. 3.55 × 10^{-6} M) λ$_{ex}$ 360 nm, λ$_{em}$ 481, Φ = 0.21. HRMS (ESI-TOF) [M + H]$^+$ calcd for C$_{35}$H$_{37}$N$_2$O$_7$ 597.2595, found: 597.2595.

3.17. 3-(3′,5′-di-O-acetyl-2′-deoxy-β-D-ribofuranosyl)-5-(4-pentylphenyl)-6-(4-pentylphenyl)-2,3-dihydrofuro[2,3-d]pyrimidin-2-one (**7i**)

From 4-n-pentylphenylacetylene (0.243 mL, 1.25 mmol). Light brown oil: 0.235 g, 0.360 mmol, 72%. NMR (CDCl$_3$, δ): ^{1}H 8.40 (s, 1H, H-4), 8.12 (d, J = 7.8 Hz, 2H, C$_6$H$_4$), 7.47 (d, J = 8.0 Hz, 2H, C$_6$H$_4$), 7.28 (d, J = 8.2 Hz, 2H, C$_6$H$_4$), 7.21 (d, J = 8.2 Hz, 2H, C$_6$H$_4$), 6.39 (dd, J = 7.3, 6.1 Hz, 1H, H-1′), 5.27 (td, J = 6.6, 2.0 Hz, 1H, H-3′), 4.50 (dd, J = 13.1, 3.7 Hz, 1H, H-4′), 4.44−4.38 (m, 2H, H-5′), 2.94 (ddd, J = 14.6, 5.7, 1.9 Hz, 1H, H-2″), 2.64 (t, J = 7.5 Hz, 2H, CH$_2$), 2.63 (t, J = 7.6 Hz, 2H, CH$_2$), 2.16 (ddd, J = 14.4, 7.2, 7.2 Hz, 1H, H-2′), 2.11 (s, 3H, Ac), 2.00 (s, 3H, Ac), 1.63 (p, J = 7.3 Hz, 4H, 2CH$_2$), 1.36−1.29 (m, 8H, 4CH$_2$), 0.89 (t, J = 6.6 Hz, 3H, CH$_3$) 0.88 (t, J = 6.6 Hz, 3H, CH$_3$); ^{13}C 170.48, 170.26 (2C), 156.34, 154.60, 145.84, 144.62, 134.90, 131.53 (2C), 128.96 (2C), 128.80 (2C), 126.12 (2C), 125.80, 119.36, 109.08, 98.53, 95.38, 88.59, 83.48, 77.99, 74.30, 63.68, 39.48, 35.97 (2C), 31.46 (2C), 30.94, 30.90, 22.55 (2C), 20.94, 20.75, 14.06 (2C). HRMS

(ESI-TOF) $[M + H]^+$ calcd for $C_{39}H_{45}N_2O_7$ 653.3221, found: 653.3221.

X-ray Crystallography. See Supporting Materials.

Fluorescence. Absorbance was measured using a Cary-100 UV−vis spectrophotometer (Agilent, USA) in double-beam mode (sample and solvent blank) using a 10 mm path quartz cuvette (Starna 23-Q-10 cell with a Teflon stopper). Fluorescence quantum yields were determined according to the relative method [64]. Corrected fluorescence spectra were collected on a Fluorolog 3 fluorometer (Horiba Jobin-Yvon, Japan) equipped with an R928 photomultiplier tube (Hamamatsu, USA). Each nucleoside's fluorescent properties were determined using five freshly prepared samples of varying concentration in methanol (HPLC grade) whose absorbance at the excitation wavelength was spread between 0.1 and 0.05 absorbance units. In the same manner, five freshly prepared standard samples of 9,10-dimethylanthracene (Aldrich) in ethanol (spectrophotometric or reagent grade) were used as a reference for calculation of the quantum yield $\Phi_D = 0.82$ [65]. Sample preparation and measurement were conducted at 24 °C. Solvent correction was carried out using refractive index values [66].

Biological Studies. The antiviral assays were based on inhibition of virus-induced cytopathicity or plaque formation in HEL [herpes simplex virus 1 (HSV-1) (KOS), HSV-2 (G), vaccinia virus, vesicular stomatitis virus, human cytomegalovirus (HCMV), varicella-zoster virus (VZV), adenovirus-2 and human corona virus (299E)], Vero (parainfluenza-3, reovirus-1, Sindbis virus and Coxsackie B4), HeLa (vesicular stomatitis virus, Coxsackie virus B4, and respiratory syncytial virus) or MDCK [influenza A (H1N1, H3N2) and influenza B] cell cultures. Confluent cell cultures (or nearly confluent for MDCK cells) in microtiter 96-well plates were inoculated with 100 $CCID_{50}$ of virus (1 CCID50 being the virus dose to infect 50% of the cell cultures) or with 20 plaque forming units (PFU). After 1−2 h virus adsorption period, residual virus was removed, and the cell cultures were incubated in the presence of varying concentrations of the test compounds in duplicate. Viral cytopathicity was recorded as soon as it reached completion in the control virus-infected cell cultures that were not treated with the test compounds. Antiviral activity was expressed as the EC_{50} or the concentration required for reducing virus-induced cytopathogenicity or viral (VZV) plaque formation by 50%. The minimal cytotoxic concentration (MCC) of the compounds was defined as the compound concentration that caused a microscopically visible alteration of cell morphology. Alternatively, cytotoxicity of the test compounds was measured based on inhibition of cell growth. HEL cells were seeded at a rate of 5×10^3 cells/well into 96-well microtiter plates and allowed to proliferate for 24 h. Then, the medium containing different concentrations of the test compounds was added. After 3 days of incubation at 37 °C, the cell number was determined with a Coulter counter. The cytostatic concentration was calculated as the CC_{50}, or the compound concentration required for reducing cell proliferation by 50% relative to the number of cells in the untreated controls.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejmech.2020.112884.
Contains: Preparative procedures for compounds **Ac-IdU**, **2**, and **3**, summary of yields table for compounds **6** and **7**. ^{1}H and ^{13}C NMR spectra for compounds **4**, **5**, **6**, and **7**. UV-vis and fluorescence spectra for compounds **4**, **6a,c,d,f,h**, and **7a,b,h**. Antiviral activity data for compounds **6** and **7**, and comparison with other furopyrimidines.

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