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Full Paper

Antiviral and Cytostatic Evaluation of 5-(1-Halo-2-sulfonylvinyl)- and 5-(2-Furyl)uracil Nucleosides

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Transition metal-catalyzed halosulfonylation of 5-ethynyl uracil nucleosides provided (*E*)-5-(1-chloro-2-tosylvinyl)uridines. Tetrabutylammonium fluoride-mediated direct C–H arylation of 5-iodouracil nucleosides with furan or 2-heptylfuran gave 5-furyl-substituted nucleosides without the necessity of using the organometallic substrates. These two classes of 5-substituted uracil nucleosides as well their corresponding ester derivatives were tested against a broad range of DNA and RNA viruses and the human immunodeficiency virus (HIV). The 3′,5′-di-*O*-acetyl-5-(*E*)-(1-chloro-2-tosylvinyl)-2′-deoxyuridine (**24**) inhibited the growth of L1210, CEM and HeLa cancer cells in the lower micromolar range. The (β-chloro)-vinyl sulfone **24** and 5-(5-heptylfur-2-yl)-2′-deoxyuridine (**10**) displayed micromolar activity against varicella zoster virus (VZV). The 5-(5-heptylfur-2-yl) analog **10** and its 3′,5′-di-*O*-acetyl-protected derivative showed similar activity against the cytomegalovirus (CMV). The 5-(fur-2-yl) derivatives of 2′-deoxyuridine and *arabino*-uridine inhibited the replication of herpes simplex virus (HSV) TK^+ strains while the 5-(5-heptylfur-2-yl) derivative **10** displayed antiviral activity against the parainfluenza virus.

Keywords: Anticancer agents / Antiviral agents / Prodrugs / Pyrimidine nucleosides / Vinyl sulfones

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Introduction

Pyrimidine nucleosides with C5 modified bases have been extensively studied and many analogs serve as potent antiviral and anticancer agents [1, 2]. Highly potent and selective antiviral drugs of this class include (*E*)-5-(2-bromovinyl)-2′-deoxyuridine (BVDU) [3] and (*E*)-5-(2-iodovinyl) araU whose ^{125}I radioisotope has been used as uptake marker for thymidine kinase positive herpes viral infections [4]. The bicyclic furanopyrimidine-2-one nucleoside analogs display remarkable antiviral potency against the varicella zoster virus (VZV) [5, 6]. Uracil and uracil nucleosides substituted with aryl groups at positions 5 and/or 6 also display a wide range of biological activities [7–9]. Herdewijn et al. found that 5-(5-bromo or 5-chlorothien-2-yl)-2′-deoxyuridine was equipotent to BVDU in the inhibition of herpes simplex virus (HSV) type I replication [10] and showed high affinity for HSV TK [11]. Moreover, Seley-Radke et al. reported that carbocyclic 5′-nor nucleosides with 5-(thien-2-yl)uracil base had significant cytotoxic activity against several tumor cell lines [12, 13]. Recently, it was also demonstrated that acyclic 5-(fur-2-yl)uracil nucleosides are more potent than the reference drug 5-fluorouracil against MCF-7 cancer cells [14].

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Nucleoside analogs are phosphorylated to their corresponding triphosphates which act either as inhibitors of viral and/or cellular DNA and RNA polymerases, or as chain terminators following incorporation into a growing DNA or RNA strand [15]. Prodrug approaches have been utilized to improve cellular permeability and absorption of the nucleoside analogs by either increasing lipophilicity of the parent drug or targeting transporters [16–19]. For example, a variety of alkyl/aromatic ester, phosphoramidate, and amino acid ester prodrugs of 5-fluoro-2′-deoxyuridine have been examined as potential alternatives to floxuridine [20, 21]. Recently, it was also showed that 3′,5′-di-*O*-trityl-5-fluoro-2′-deoxyuridine efficiently inhibited both yellow fever virus and dengue virus replication [22].

Introduction of reactive groups at the C5 position of pyrimidine nucleobases such as alkyne [23–26], azide [27–29], vinyl sulfonamide [30], or ene/diene [31–33], among others [34], have been explored for bioconjugation and cellular imaging. We recently developed the synthesis of several C5-substituted uracil nucleoside analogs [35–37]. One group of these C5 modified nucleosides possesses a reactive 1-chloro(or bromo or iodo)-2-sulfonylvinyl scaffold in which a halogen atom can undergo efficient replacement with thiols or amines via addition–elimination mechanism, and therefore, can be explored as convenient substrates for bioconjugation with the amino acid or protein residues [37]. The second group contains the aryl or heteroaryl (2-furyl, 2-thienyl, or 2-pyrrolyl) substituent at C5 [36]. The latter C5 modified analogs were prepared by direct C–H arylation of 5-iodouracil nucleosides with arenes and heteroarenes which avoids usage of the organometallic precursors and proceeds without necessities of adding any ligands [10, 12, 13, 38–41]. Herein, we report the antiviral and cytostatic evaluation of the uracil nucleosides substituted at C5 with 1-halo-2-sulfonylvinyl or heteroaren-2-yl scaffolds as well as their derivatives with a long lipophilic alkyl chain in the heteroarene ring and/or alkyl ester prodrugs.

Results and discussion

Chemistry

$FeCl_3$-catalyzed halovinylsulfonylation of 5-ethynyl-2′-deoxyuridine **1** provided (*E*)-5-(1-chloro-2-tosylvinyl)-2′-deoxyuridine **2** (90%; Scheme 1) [37]. Esterification of **2** with undecanoic anhydride (1.2 equiv) yielded (*E*)-5′-*O*-undecanoyl ester **3** (45%) and the 3′,5′-di-*O*-undecanoyl diester **4** (10%). Increasing the ratio of undecanoic anhydride to **2** from 1.2 to 3.0 yielded **4** in higher yield (75%).

Tetrabutylammonium fluoride (TBAF)-mediated direct C–H arylation of 5-iodouracil nucleosides (**5** or **7**) with furan yielded 5-(fur-2-yl)-2′-deoxyuridine **8** (73%) [36] or 2′,3′,5′-tri-*O*-acetyl-5-(fur-2-yl)uridine **9** (67%, Scheme 2). Treatment of 5-iodo-2′-deoxyuridine **5** with 2-heptylfuran gave 2′-deoxy-5-(5-heptylfur-2-yl)uridine **10** (61%) as a single isomer. Analogous cross-coupling of the protected 2′-deoxyuridine **6** or uridine **7** with 2-heptylfuran provided regioselectively acetyl protected 5-(5-heptylfur-2-yl) derivatives **11** (60%) and **12** (55%). Deacetylation of **12** with methanolic ammonia afforded 5-(5-heptylfur-2-yl)uridine (**13**; 81%). Esterification of **8** with undecanoic anhydride yielded 5′-*O*-undecanoyl- and 3′,5′-di-*O*-undecanoyl-5-(fur-2-yl)-2′-deoxyuridine **14** and **15**. Analogously **10** was converted to 5-(5-heptylfur-2-yl) esters **16** and **17**.

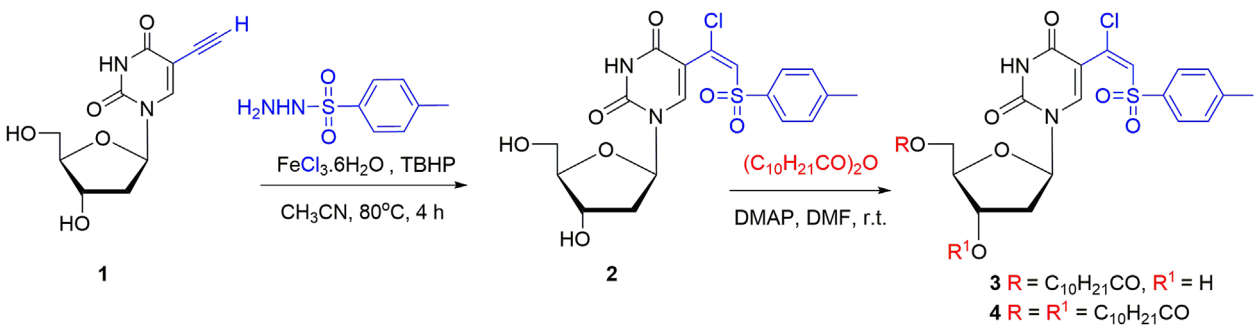
For the synthesis of 5′-monoesters of the 5-furyl substituted uridines (e.g., **21** and **22**), we have developed a three step protocol starting from 2′,3′-*O*-isopropylideneuridine **18** (Scheme 3). Thus, treatment of **18** with undecanoic acid in the presence of DCC gave 2′,3′-*O*-isopropylidene-5′-*O*-undecanoyluridine (**19**) in 90% yield. Iodination of **19** with ICl in CH_2Cl_2 yielded 5′-*O*-undecanoyl-5-iodouridine (**20**). Direct C–H cross-coupling of **20** with furan or 2-heptylfuran in the presence of TBAF [36] gave 5′-*O*-undecanoyl-5-(fur-2-yl)uridine (**21**, 63%) or 5′-*O*-undecanoyl-5-(5-heptylfur-2-yl)uridine (**22**, 44%), respectively.

Other uracil nucleoside analogs modified at C5 with 5-(1-substituted-2-tosylvinyl) [37] and 5-(2-heteroaryl) [36] scaffolds have been synthesized according to the literature protocols and are listed in Fig. 1.

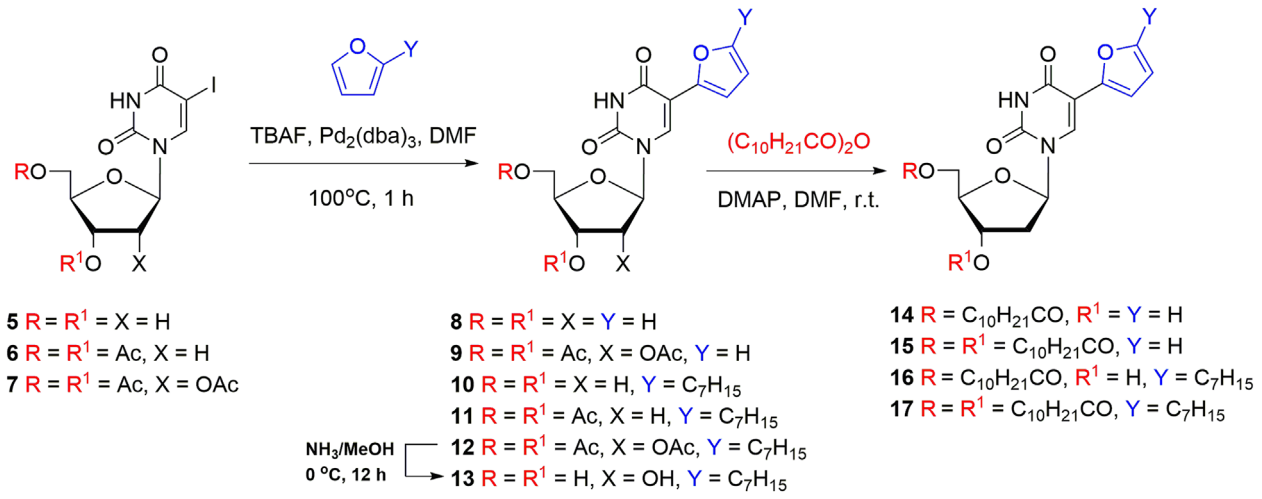
Pharmacology

Inhibition of cell proliferation

The C5 substituted pyrimidine nucleosides (**2–4**, **8–17**, **21–35**) were first examined for their antiproliferative activity in murine leukemia (L1210), human leukemia (CEM), and human cervical carcinoma (HeLa) cells. From the (β-halo)vinyl sulfones



Scheme 1. Synthesis of 5-(1-chloro-2-tosylvinyl)-2′-deoxyuridine and its esters.

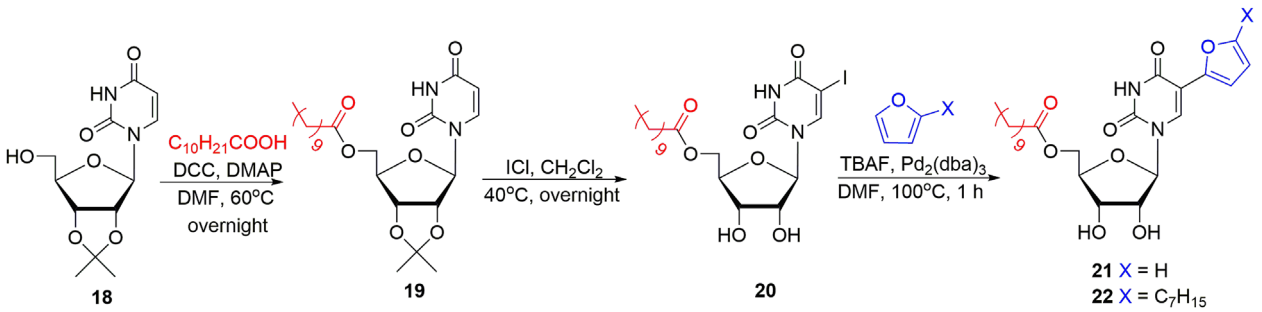


5 R = R^1 = X = H
6 R = R^1 = Ac, X = H
7 R = R^1 = Ac, X = OAc

8 R = R^1 = X = Y = H
9 R = R^1 = Ac, X = OAc, Y = H
10 R = R^1 = X = H, Y = C_7H_{15}
11 R = R^1 = Ac, X = H, Y = C_7H_{15}
12 R = R^1 = Ac, X = OAc, Y = C_7H_{15}
13 R = R^1 = H, X = OH, Y = C_7H_{15}

14 R = $C_{10}H_{21}CO$, R^1 = Y = H
15 R = R^1 = $C_{10}H_{21}CO$, Y = H
16 R = $C_{10}H_{21}CO$, R^1 = H, Y = C_7H_{15}
17 R = R^1 = $C_{10}H_{21}CO$, Y = C_7H_{15}

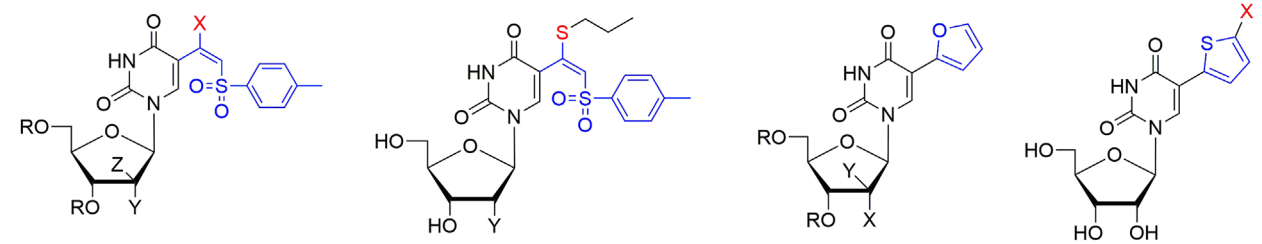
Scheme 2. Synthesis of 5-(fur-2-yl)- or 5-(5-heptylfur-2-yl)uracil nucleosides and their esters.



18 **19** **20**

21 X = H
22 X = C_7H_{15}

Scheme 3. Synthesis of 5-(fur-2-yl)- or 5-(5-heptylfur-2-yl)uridine and their 5′-esters.



23 R = Y = Z = H, X = Br
24 R = Ac, Y = Z = H, X = Cl
25 R = Z = H, Y = OH, X = Cl
26 R = Z = H, Y = OH, X = Br
27 R = Y = H, Z = OH, X = Cl

28 Y = H
29 Y = OH

30 R = Ac, X = Y = H
31 R = H, X = OH, Y = H
32 R = X = H, Y = OH
33 R = Ac, X = H, Y = OAc

34 X = H
35 X = Me

Figure 1. Other 5-(1-substituted-2-tosylvinyl)uracil nucleosides **23–29** [37] and 5-(2-heteroaryl)uracil nucleosides **30–35** [36] tested.

tested (**2–4**, **23–27**), only the acetyl protected 2′-deoxyuridine (β-chloro)vinyl sulfone **24** inhibited the growth of these cell lines in the lower μM range (Table 1). Interestingly (β-halo)vinyl sulfone derivatives with a larger acyl protected group **3** and **4** did not show improved activity. From the 5-(fur-2-yl) analogs tested (**8–17**, **21**, **22**, **30–33**), the 2′-deoxy-5-(5-heptylfur-2-yl)uridine **10** inhibited the growth of CEM cell lines in the μM range.

Table 1. Inhibitory effects on the proliferation of murine leukemia cells (L1210), human T-lymphocyte cells (CEM), and human cervix carcinoma cells (HeLa).

Compound	IC_{50}[a] (μM)		
	L1210	CEM	HeL
8	>100	>100	32 ± 1
9	>100	80 ± 8	>100
10	48 ± 6	16 ± 4	>100
11	65 ± 10	36 ± 3	>100
12	88 ± 17	53 ± 1	>100
13	95 ± 8	42 ± 2	>100
16	>100	93 ± 4	>100
17	>100	78 ± 16	>100
21	43 ± 8	70 ± 2	64 ± 5
24	5.6 ± 4.7	11 ± 10	23 ± 8
27	>100	>100	>100
29	>100	>100	93 ± 14
31	>100	60 ± 27	83 ± 25
32	>100	>100	>100
33	40 ± 10	63 ± 3	>100
34	>100	86 ± 6	>100
35	>100	>100	>100

[a] Fifty percentage inhibitory concentration.

Antiviral activity

The antiviral activity of all compounds was tested against a broad range of DNA and RNA viruses and the human immunodeficiency (HIV) virus. Some of the compounds proved active against herpesviruses though they were less potent than the reference anti-herpesvirus drugs (Table 2). The 5-(5-heptylfur-2-yl) **(10)** and 3′,5′-di-*O*-acetyl-5-(5-heptylfur-2-yl) **(11)** derivatives inhibited the replication of human cytomegalovirus (HCMV) and varicella zoster virus (VZV) bearing a wild-type thymidine kinase (TK^+) with 50% effective concentrations (EC_{50}'s) in the range of 10–20 μM. Compound **10** was equally active against TK^+ and TK-deficient (TK^-) VZV strains while compound **11** failed to inhibit a TK^- VZV mutant virus. Neither compound **10** nor **11** were able to decrease herpes simplex virus 1 (HSV-1) and 2 (HSV-2) induced cytopathic effect. In contrast, the 5-(fur-2-yl)uracil nucleoside **8** emerged among the compounds synthesized as the most potent inhibitor of the HSV-1 TK^+ strain Kos with an EC_{50} of 4 μM. Compound **8** was less active against HCMV, HSV-2, and the VZV TK^+ Oka strain than against HSV-1 while it lacked activity against TK^- HSV-1 and VZV. The spectrum of activity of compound **32** only included HSV-1 and VZV TK^+ strains. The (β-chloro)vinyl sulfone **24 s**howed an EC_{50} of 4 μM for the Oka strain (VZV TK^+) and marginal activity against HCMV. Except for compound **10** that displayed antiviral activity against parainfluenza virus (Table 3), none of the compounds showed activity against the other tested viruses.

Conclusion

We have developed methods to synthesize two classes of C5-substituted uracil nucleosides. One group contains a reactive 1-chloro(or bromo)-2-sulfonylvinyl scaffold prepared by halovinyl sulfonylation of 5-ethynyluracils. The second group of compounds encompasses heteroaryl (2-furyl, 5-heptyl-2-furyl, or 2-thienyl) modification at C5 which were prepared by direct C–H arylation of 5-iodouracil nucleosides with heteroarenes. The acetyl protected (β-chloro)vinyl sulfone **24** inhibited the growth of the L1210, CEM, and HeLa cancer cells in the lower μM range. The 5-(5-heptylfur-2-yl)-2′-deoxyuridine **10** showed micromolar activity against VZV, CMV, and parainfluenza virus. The 5-(fur-2-yl) derivatives

Table 2. Anti-herpesvirus activity of the test compounds in HEL (human embryonic lung) fibroblasts.

Compound	Cytotoxicity (μM)	EC_{50} (μM)[a]						
	MCC[b]	HSV-1 (KOS)	HSV-2 (G)	HSV-1 TK^- (KOS ACV^r)	HCMV (AD-169)	HCMV (Davis)	VZV TK^+ (Oka)	VZV TK^- (07-1)
8	>100	4 ± 0	47 ± 37	>100	45	20	32	>100
10	100	>100	>100	>100	10	20	13 ± 2	12 ± 5
11	100	>100	>100	>100	10 ± 2	12 ± 4	20	>20
24	100	>100	>100	>100	>20	20	4	>20
32	>100	14 ± 8	>100	>100	>100	>100	25	>100
Acyclovir	>440	0.4 ± 0.1	0.3 ± 0.1	110 ± 104	ND	ND	0.7 ± 0.1	44 ± 7
Brivudin	>300	0.04 ± 0	188 ± 88	27 ± 32	ND	ND	0.02 ± 0.01	29 ± 10
Ganciclovir	>350	0.06 ± 0.04	0.07 ± 0.03	4.4 ± 3.4	7.9 ± 2.4	4.3 ± 3.6	ND	ND
Cidofovir	>350	2.7 ± 1.0	1.5 ± 0.7	1.4 ± 0.9	0.9 ± 0.6	0.8 ± 0.6	ND	ND

[a] Required to reduce virus-induced cytopathogenicity by 50%.
[b] Minimum cytotoxic concentration (MCC) required to cause a microscopically detectable alteration of normal cell morphology.

Table 3. Activity against parainfluenza virus.

Compound	Cytotoxicity (μM) MCC[b]	EC_{50} (μM)[a] Parainfluenza-3 virus
10	>100	14 ± 8

[a] Required to reduce virus-induced cytopathogenicity by 50%.
[b] Minimum cytotoxic concentration (MCC) required to cause a microscopically detectable alteration of normal cell morphology.

of 2′-deoxyuridine and *arabino*-uridine inhibited the replication of HSV TK^+ strains.

Experimental

Chemistry

General

^1H (400 MHz) and ^{13}C (100.6 MHz) NMR spectra were recorded in solutions of $CDCl_3$ unless otherwise noted. Reaction progress was monitored by TLC on Merck Kieselgel 60-F_{254} sheets with product detection by 254-nm light. Products were purified by column chromatography using Merck Kiselgel 60 (230–400 mesh) or by automated flash chromatography using a CombiFlash system. Reagent grade chemicals were used and solvents were dried by reflux and distillation from CaH_2 under N_2 unless otherwise specified. All reactions were carried out under the N_2 atmosphere.

The InChI codes of the investigated compounds together with some biological activity data are provided as Supporting Information.

Synthesis of the 5-vinyl sulfone and 5-heteroarene analogs

The 5-vinyl sulfone analogs:(*E*)-5-(1-Bromo-2-tosylvinyl)-2′-deoxyuridine (**23**), (*E*)-3′,5′-di-*O*-acetyl-5-(1-chloro-2-tosylvinyl)-2′-deoxyuridine (**24**), (*E*)-5-(1-chloro-2-tosylvinyl)uridine (**25**), (*E*)-5-(1-bromo-2-tosylvinyl)uridine (**26**), (*E*)-1-(β-D-arabinofuranosyl)-5-(1-chloro-2-tosylvinyl)uracil (**27**), (*E*)-5-1-propylthio-2-tosylvinyl)-2′-deoxyuridine (**28**), and (*E*)-5-(1-propylthio-2-tosylvinyl)uridine (**29**) were prepared as reported [37]. The 5-heteroarene analogs: 3′,5′-di-*O*-acetyl-5-(fur-2-yl)-2′-deoxyuridine (**30**), 5-(fur-2-yl)uridine (**31**), 1-(β-D-arabinofuranosyl)-5-(fur-2-yl)uracil (**32**), 1-(2,3,5-tri-*O*-acetyl-β-D-arabinofuranosyl)-5-(fur-2-yl)uracil (**33**), 5-(thiophen-2-yl)uridine (**34**), and 5-(5-methylthiophen-2-yl)uridine (**35**) were prepared as reported [36] (Fig. 1).

*(E)-5′-O-Undecanoyl-5-(1-chloro-2-tosylvinyl)-2′-deoxyuridine (**3**)*

Procedure A: Undecanoic anhydride (24 mg, 0.067 mmol) was added to a stirred solution of **2** [37] (25 mg, 0.056 mmol) in DMF (1 mL) containing DMAP (6.0 mg, 0.05 mmol) at ambient temperature. After 2 h, the volatiles were evaporated and the residue was partitioned between CH_2Cl_2 and 0.1 M HCl/H_2O. The organic layer was washed with $NaHCO_3/H_2O$ and brine and was column chromatographed (hexane/EtOAc, 90:10 → 60:40) to give **3** (15 mg, 45%) and **4** (4.4 mg, 10%) in addition to the unchanged **2** (~25%; TLC). Compound **3** had: ^1H NMR δ 0.88 (t, J = 6.6 Hz, 3H, CH_3), 1.24–1.39 (m, 14H, 7 × CH_2), 1.62 ("quin", J = 7.4 Hz, 2H, CH_2), 2.10–2.18 (m, 1H, H2′), 2.34 (t, J = 7.7 Hz, 2H, CH_2), 2.42 (s, 3H, CH_3), 2.49 (ddd, J = 13.8, 6.3, 4.0 Hz, 1H, H2″), 3.00 (br s, 1H, 3′-OH), 4.17–4.40 (m, 4H, H3′,4′,5′,5″), 6.26 (t, J = 6.5 Hz, 1H, H1′), 6.85 (s, 1H, CH), 7.35 (d, J = 8.1 Hz, 2H, Ar), 7.74 (d, J = 8.1 Hz, 2H, Ar), 8.33 (s, 1H, H6), 8.44 (s, 1H, NH); ^{13}C NMR δ 14.2, 21.9 (CH_3), 22.0, 25.0, 29.2, 29.4, 29.5, 29.7, 29.8, 32.2, 34.4 (CH_2), 40.8 (C2′), 63.7 (C5′), 71.6 (C3′), 84.7 (C4′), 85.8 (C1′), 108.2 (C5), 128.0 (Ar), 130.3 (Ar), 134.0 (Ar), 136.0 (=CH), 140.8 (Ar), 143.0 (=CCl), 146.0 (C6), 149.3 (C2), 159.0 (C4), 174.0 (C=O); HRMS calcd. for $C_{29}H_{39}{}^{35}ClN_2NaO_8S$ $[M+Na]^+$ 633.2013, found 633.2018.

*(E)-3′,5′-Di-O-undecanoyl-5-(1-chloro-2-tosylvinyl)-2′-deoxyuridine (**4**)*

Treatment of **2** [37] (25 mg, 0.056 mmol) with undecanoic anhydride (62 mg, 0.17 mmol) for 6 h by Procedure A (column chromatography; hexane/EtOAc, 100:0 → 80:20) gave **4** (32 mg, 75%): ^1H NMR δ 0.84–0.90 (m, 6H, 2 × CH_3), 1.20–1.37 (m, 28H, 14 × CH_2), 1.60–1.68 (m, 4H, 2 × CH_2), 2.35 ("t", J = 8.2 Hz, 4H, 2 × CH_2), 2.38–2.42 (m, 1H, H2′), 2.46 (s, 3H, CH_3), 2.57 (ddd, J = 12.8, 5.4, 2.1 Hz, 1H, H2″), 4.29–4.32 (m, 1H, H4′), 4.42 (dd, J = 12.1, 2.4 Hz, 1H, H5′), 4.50 (dd, J = 12.1, 2.3 Hz, 1H, H5″), 5.23–5.27 (m, 1H, H3′), 6.35 (dd, J = 8.5, 5.5 Hz, 1H, H1′), 6.88 (s, 1H, vinyl), 7.34 (d, J = 8.1 Hz, 2H, Ar), 7.70 (d, J = 8.1 Hz, 2H, Ar), 7.90 (s, 1H, H6), 8.34 (s, 1H, NH); ^{13}C NMR δ 14.2, 21.8 (CH_3), 22.8, 24.9, 25.0, 29.3, 29.37, 29.43, 29.5, 29.58, 29.63, 29.69, 29.72, 32.0, 34.2, 34.3 (CH_2), 38.2 (C2′), 63.8 (C5′), 74.2 (C3′), 83.3 (C4′), 85.8 (C1′), 109.1 (C5), 128.0 (Ar), 130.2 (Ar), 134.9 (CH), 136.9 (Ar), 139.9 (Ar), 141.6 (C6), 145.6 (=CCl), 149.1 (C2), 158.6 (C4), 173.3, 173.4 (C=O); HRMS calcd. for $C_{40}H_{59}{}^{35}ClN_2NaO_9S$ $[M+Na]^+$ 801.3527, found 801.3505.

*2′,3′,5′-Tri-O-acetyl-5-(fur-2-yl)uridine (**9**)*

Procedure B: Furan (0.7 mL, 680 mg, 10 mmol) and TBAF (1 M/THF, 3.5 mL, 3.5 mmol) were added to a stirred solution of **7** (248 mg, 0.5 mmol) in DMF (5 mL) containing tris-(dibenzylideneacetone)dipalladium (22.9 mg, 0.025 mmol) at ambient temerature. The resulting suspension was stirred for 1 h at 100°C. The volatiles were evaporated under reduced pressure and the residue was dissolved in EtOAc and washed with saturated $NaHCO_3/H_2O$ and brine and the organic layer was dried over anhydrous Na_2SO_4. The residue was column chromatographed ($CHCl_3$/MeOH, 95:5) to give **9** (146 mg, 67%): ^1H NMR δ 2.10 (s, 3H, CH_3), 2.15 (s, 3H, CH_3), 2.22 (s, 3H, CH_3), 4.38–4.40 (m, 3H, H4′,5′,5″), 5.38–5.42 (m, 2H, H2′,3′), 6.24–6.28 (m, 1H, H1′), 6.47 (dd, J = 3.3, 1.8 Hz, 1H, furan), 7.09 (d, J = 3.4, 1H, furan), 7.33 (d, J = 1.4, 1H, furan), 7.91 (s, 1H, H6), 9.20 (s, 1H, NH); ^{13}C NMR δ 20.5, 20.7, 20.8, 64.6, 70.9,

73.0, 80.6, 86.8, 108.4, 110.2, 112.3, 132.5, 141.3, 145.6, 149.6, 157.8, 169.77, 169.83, 170.4; HRMS calcd. for $C_{19}H_{21}N_2O_{10}$ $[M+H]^+$ 437.1191, found 437.1178.

*5-(5-Heptylfur-2-yl)-2′-deoxyuridine (**10**)*
Treatment of 5-iodo-2′-deoxyuridine **5** (53 mg, 0.15 mmol) with 2-heptylfuran (0.29 mL, 249 mg, 1.5 mmol) as described by Procedure B (column chromatography; hexane/EtOAc, 20:80) gave **10** (35 mg, 61%): UV (MeOH) λ_{max} 256, 326 nm (ε 14250, 11300), λ_{min} 287 nm (ε 4000); ^{1}H NMR (DMSO-d_6) δ 0.86 (t, J = 6.7 Hz, 3H, CH_3), 1.24–1.31 (m, 8H, 4 × CH_2), 1.60 (quin, J = 6.7 Hz, 2H, heptyl), 2.17 ("dd", J = 6.6, 4.9 Hz, 2H, H2′,2″), 2.60 (t, J = 7.4 Hz, 2H, CH_2), 3.60–3.62 (m, 2H, H5′,5″), 3.83 (q, J = 3.3 Hz, 1H, H4′), 4.29 (quin, J = 4.2, 1H, H3′), 5.05 (t, J = 5.0 Hz, 1H, 5′-OH), 5.28 (d, J = 4.1 Hz, 1H, 3′-OH), 6.11 (d, J = 3.1 Hz, 1H, furan), 6.21 (t, J = 6.6 Hz, 1H, H1′), 6.72 (d, J = 3.1 Hz, 1H, furan), 8.27 (s, 1H, H6), 11.58 (s, 1H, NH); ^{13}C NMR δ 14.2, 22.1, 27.0, 28.6, 31.1, 39.4, 60.8, 70.3, 84.5, 87.4, 105.7, 106.8, 108.6, 133.6, 144.5, 149.6, 154.8, 160.0; HRMS calcd. for $C_{20}H_{29}N_2O_6$ $[M+H]^+$ 393.2020, found 393.2023.

*3′,5′-Di-O-acetyl-5-(5-heptylfur-2-yl)-2′-deoxyuridine (**11**)*
Treatment of **6** [42] (150 mg, 0.34 mmol) with 2-heptylfuran (0.6 mL, 565 mg, 3.4 mmol) as described by Procedure B (column chromatography; hexane/EtOAc, 80:20 → 60:40) gave **11** (86 mg, 60%): ^{1}H NMR δ 0.87 (t, J = 7.1 Hz, 3H, CH_3), 1.22–1.37 (m, 8H, 4 × CH_2), 1.61 (q, J = 7.4 Hz, 2H, CH_2), 2.11 (s, 3H, CH_3), 2.12 (s, 3H, CH_3), 2.25 ("ddd", J = 16.6, 8.7, 2.2 Hz, 1H, H2′), 2.50–2.57 (m, 1H, H2″), 2.59 (t, J = 7.5 Hz, 2H, CH_2), 4.30–4.34 (m, 1H, H4′), 4.38–4.42 (m, 2H, H5′,5″), 5.28 ("dt", J = 6.4, 1.6 Hz, 1H, H3′), 6.05 (d, J = 3.3 Hz, 1H, furan), 6.40 (dd, J = 8.6, 5.5 Hz, 1H, H1′), 6.98 (d, J = 3.3 Hz, 1H, furan), 7.85 (s, 1H, H6), 9.30 (s, 1H, NH); ^{13}C NMR δ 14.2 (CH_3), 20.9, 21.1 (Ac), 22.8, 28.1, 28.2, 29.2, 29.3, 31.9 (CH_2), 38.1 (C2′), 61.2 (C5′), 74.7 (C3′), 82.7 (C4′), 85.7 (C1′), 107.4 (furan), 108.2 (C5), 111.0 (furan), 131.3 (C6), 143.8, 149.4 (furan), 156.2 (C2), 159.9 (C4), 170.3, 170.5 (Ac); HRMS calcd. for $C_{24}H_{32}N_2NaO_8$ $[M+Na]^+$ 499.2056, found 499.2078.

*2′,3′,5′-Tri-O-acetyl-5-(5-heptylfur-2-yl)uridine (**12**)*
Treatment of 2′,3′,5′-tri-*O*-acetyl-5-iodouridine **7** (400 mg, 0.8 mmol) with 2-heptylfurane (1.5 mL, 665 mg, 4.0 mmol) by Procedure B (column chromatography; hexane/EtOAc, 50:50) gave **12** (236 mg, 55%): ^{1}H NMR δ 0.88 (t, J = 6.7 Hz, 3H, CH_3), 1.25–1.34 (m, 8H, 4 × CH_2), 1.62 (quin, J = 7.8 Hz, 2H, CH_2), 2.10 (s, 3H, Ac), 2.15 (s, 3H, Ac), 2.18 (s, 3H, Ac), 2.59 (t, J = 7.8 Hz, 2H, CH_2), 4.38–4.40 (m, 3H, H4′,5′,5″), 5.40–5.44 (m, 2H, H2′,3′), 6.05 (d, J = 3.6 Hz, 1H, furan), 6.21 (d, J = 5.5 Hz, 1H, H1′), 6.99 (d, J = 3.3 Hz, 1H, furan), 7.75 (s, 1H, H6), 9.03 (s, 1H, NH); ^{13}C NMR δ 14.0 (CH_3), 20.4, 20.6, 20.8 (Ac), 22.6, 28.0, 28.1, 29.0, 29.2, 31.7 (CH_2), 63.4 (C5′), 70.8 (C3′), 72.7 (C2′), 80.4 (C4′), 87.0 (C1′), 107.3 (furan), 108.5 (C5), 111.2 (furan), 131.2 (C6), 143.5, 149.4 (furan), 156.1 (C2), 160.0 (C4), 169.7, 169.7, 170.1 (Ac); HRMS calcd. for $C_{26}H_{35}N_2O_{10}$ $[M+H]^+$ 535.2286, found 535.2288.

*5-(5-Heptylfur-2-yl)uridine (**13**)*
Methanolic ammonia (6.4 mL) was added to **12** (100 mg, 0.19 mmol) in 1.6 mL MeOH and the resulting mixture was stirred at 0°C → r.t for 12 h. Volatiles were evaporated and the residue was column chromatographed (EtOAc/MeOH, 95:5) to give **13** (62 mg, 81%): UV (MeOH) λ_{max} 254, 326 nm (ε 13600, 10950), λ_{min} 287 nm (ε 3850); ^{1}H NMR (DMSO-d_6) δ 0.86 (t, J = 6.6 Hz, 3H, CH_3), 1.24–1.31 (m, 8H, 4 × CH_2), 1.60 (quin, J = 6.9, 2H, CH_2), 2.60 (t, J = 7.3 Hz, 2H, CH_2), 3.60 (ddd, J = 12.0, 4.8, 3.1 Hz, 1H, H5′), 3.68 (ddd, J = 12.0, 4.8, 2.9 Hz, 1H, H5″), 3.89–3.91 (m, 1H, H4′), 4.02 (q, J = 4.8 Hz, 1H, H3′), 4.10 (q, J = 5.0 Hz, 1H, H2′), 5.11 (d, J = 5.2 Hz, 1H, 3′-OH), 5.15 (t, J = 4.8 Hz, 1H, 5′-OH), 5.44 (d, J = 5.6 Hz, 1H, 2′-OH), 5.86 (d, J = 4.8 Hz, 1H, H1′), 6.11 (d, J = 3.1 Hz, 1H, furan), 6.73 (d, J = 3.1 Hz, 1H, furan), 8.36 (s, 1H, H6), 11.60 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 13.9, 22.0, 27.2, 27.3, 28.3, 28.5, 31.2 (heptyl), 60.6 (C5′), 69.9 (C3′), 74.0 (C2′), 84.9 (C1′), 88.3 (C4′), 105.9 (C5), 106.7 (furan), 108.7 (furan), 133.9 (C6), 144.5 (furan), 149.6 (furan), 154.7 (C2), 160.1 (C4); HRMS calcd. for $C_{20}H_{29}N_2O_7$ $[M+H]^+$ 409.1969, found 409.1982.

*5′-O-Undecanoyl-5-(fur-2-yl)-2′-deoxyuridine (**14**)*
Treatment of **8** [36] (25 mg, 0.08 mmol) with undecanoic anhydride by Procedure A (column chromatography; hexane/EtOAc, 100:0 → 70:30) gave **14** (18 mg, 52%) and **15** (5 mg, 10%) in addition to unchanged **8** (∼15%; TLC). Compound **14** had: ^{1}H NMR δ 0.88 (t, J = 7.1 Hz, 3H, CH_3), 1.22–1.40 (m, 14H, 7 × CH_2), 1.60 ("quin", J = 7.3 Hz, 2H, CH_2), 2.10–2.17 (m, 1H, H2′), 2.35 (t, J = 7.6 Hz, 2H, CH_2), 2.50 (ddd, J = 13.7, 6.3, 3.9 Hz, 1H, H2″), 3.05 (br s, 1H, 3′-OH), 4.15 (q, J = 3.9, 1H, H4′), 4.25 (dd, J = 12.1, 3.3 Hz, 1H, H5′), 4.36–4.42 (m, 2H, H3′,5″), 6.26 (t, J = 6.3 Hz, 1H, H1′), 6.60 (dd, J = 3.3, 1.8 Hz, 1H, furan), 7.05 (d, J = 3.5 Hz, 1H, furan), 7.38 (d, J = 1.2 Hz, 1H, furan), 8.25 (s, 1H, H6), 8.38 (s, 1H, NH); HRMS calcd. for $C_{24}H_{34}N_2NaO_7$ $[M+Na]^+$ 485.2264; found 485.2271.

*3′,5′-Di-O-undecanoyl-5-(fur-2-yl)-2′-deoxyuridine (**15**)*
Treatment of **8** [36] (25 mg, 0.08 mmol) with undecanoic anhydride (84 mg, 0.24 mmol) by Procedure A (6 h) gave **15** (38.5 mg, 77%): ^{1}H NMR δ 0.82–0.91 (m, 6H, 2 × CH_3), 1.20–1.40 (m, 28H, 14 × CH_2), 1.60–1.68 (m, 4H, 2 × CH_2), 2.24 (ddd, J = 14.6, 8.6, 6.5 Hz, 1H, H2′), 2.33–2.40 (m, 4H, 2 × CH_2), 2.54 (ddd, J = 14.1, 5.6, 1.3 Hz, 1H, H2″), 4.30 ("q", J = 2.7 Hz, 1H, H4′), 4.36 (dd, J = 12.2, 2.8 Hz, 1H, H5′), 4.45 (dd, J =11.8, 3.5 Hz, 1H, H5″), 5.27 ("dt", J = 6.4, 1.6 Hz, 1H, H3′), 6.40 (dd, J = 8.8, 6.1 Hz, 1H, H1′), 6.47 (dd, J = 3.3, 1.8 Hz, 1H, furan), 7.05 (d, J = 3.4 Hz, 1H, furan), 7.33 (d, J = 1.6 Hz, 1H, furan), 8.00 (s, 1H, H6), 8.95 (s, 1H, NH); ^{13}C NMR δ 14.5, 23.0, 25.1, 25.2, 29.3, 29.4, 29.6, 29.7, 29.9, 32.3, 34.1, 34.3, 34.5, 39.0 (C2′), 64.0 (C5′), 74.6 (C3′), 83.2 (C4′), 86.0 (C1′), 108.0 (C5), 110.0 (furan), 112.5 (furan), 133.0 (C6), 142.0 (furan), 146.0 (furan), 149.5 (C2), 160.2 (C4), 173.2, 173.4 (C=O); HRMS calcd. for $C_{35}H_{54}N_2NaO_8$ $[M+Na]^+$ 653.3778, found 653.3778.

*5′-O-Undecanoyl-5-(5-heptylfur-2-yl)-2′-deoxyuridine (**16**)*
Treatment of **10** (25 mg, 0.064 mmol) with undecanoic anhydride by Procedure A (hexane/EtOAc, 100:0 → 80:20) gave **16** (17 mg, 48%; TLC ($CHCl_3$/MeOH, 95:5), $R_f = 0.50$), **17** (4.7 mg, 10%; $R_f = 0.90$) and unchanged **10** (~15%, TLC; $R_f = 0.10$). Compound **16** had: ^{1}H NMR δ 0.85–0.90 (m, 6H, 2 × CH_3), 1.28–1.31 (m, 22H, 11 × CH_2), 1.54–1.64 (m, 4H, 2 × CH_2), 2.13–2.16 (m, 1H, H2′), 2.27–2.32 (m, 2H, CH_2), 2.46 (ddd, $J = 13.9, 6.4, 4.3$ Hz, 1H, H2″), 2.56 (t, $J = 7.4$ Hz, 2H, CH_2), 2.98 (s, 1H, 3′-OH), 4.19 (q, $J = 3.5$, 1H, H4′), 4.28 (dd, $J = 12.3, 3.4$ Hz, 1H, H5′), 4.33–4.41 (m, 2H, H3′,5″), 6.05 (d, $J = 3.2$ Hz, 1H-furan), 6.28 (t, $J = 6.4$ Hz, 1H, H1′), 6.90 (d, $J = 3.2$ Hz, 1H-furan), 8.10 (s, 1H, H6), 8.44 (s,1H, NH); ^{13}C NMR δ 14.2, 22.9 (CH_3), 24.9, 28.1, 28.2, 29.2, 29.2, 29.3, 29.4, 29.5, 29.6, 29.7, 31.9, 32.1, 34.2, 34.3, 34.4 (CH_2), 40.7 (C2′), 63.6 (C5′), 71.8 (C3′), 84.4 (C4′), 85.5 (C1′), 108.2 (C5), 110.6 (furan), 113.0 (furan), 129.0 (furan), 133.0 (C6), 149 (C2), 156.0 (furan), 159.6 (C4), 174.0 (C=O); HRMS calcd. for $C_{31}H_{48}N_2NaO_7$ $[M+Na]^+$ 583.3359, found 583.3375.

Note: Also isolated from column chromatography was a product (2 mg, 5%; TLC, $R_f = 0.55$) whose structure was tentatively assigned as 3′-*O*-undecanoyl-5-(5-heptylfur-2-yl)-2′-deoxyuridine (^{1}H NMR δ 5.39 ("dt", $J = 8.1, 1.6$ Hz, 1H, H3′)).

*3′,5′-Di-O-undecanoyl-5-(5-heptylfur-2-yl)-2′-deoxyuridine (**17**)*
Treatment of **10** (25 mg, 0.064 mmol) with undecanoic anhydride (65 mg, 0.19 mmol) by Procedure A (6 h) gave **17** (36 mg, 80%). Compound **17** had: ^{1}H NMR δ 0.83–0.92 (m, 9H, 3 × CH_3), 1.22–1.38 (m, 36H, 18 × CH_2), 1.55–1.70 (m, 6H, 3 × CH_2), 2.25 (ddd, $J = 14.7, 8.5, 6.6$ Hz, 1H, H2′), 2.30–2.40 (m, 4H, 2 × CH_2), 2.53–2.57 (m, 1H, H2″), 2.60 (t, $J = 7.6$ Hz, 2H, CH_2), 4.31 (q, $J = 3.0$ 1H, H4′), 4.38 (dd, $J = 12.2, 2.8$ Hz, 1H, H5′), 4.42 (dd, $J = 11.3, 3.8$ Hz, 1H, H5″), 5.26 ("dt", $J = 6.8, 1.6$ Hz, 1H, H3′), 6.05 (d, $J = 3.2$ Hz, 1H, furan), 6.37 (dd, $J = 8.8, 6.1$ Hz, 1H, H1′), 6.95 (d, $J = 3.3$ Hz, 1H, furan), 7.88 (s, 1H, H6), 9.00 (s, 1H, NH); HRMS calcd. for $C_{42}H_{68}N_2NaO_8$ $[M+Na]^+$ 751.4873, found 751.4851.

*5′-O-Undecanoyl-5-(fur-2-yl)uridine (**21**)*
Step a: DDC (516 mg, 1.25 mmol) was added to a stirred solution of 2′,3′-*O*-isopropylideneuridine **18** (142 mg, 0.5 mmol), undecanoic acid (163 mg, 0.875 mmol), and 4-dimethylaminopyridine (91.6 mg, 0.375 mmol) in DMF (2 mL) at r.t. The resulting mixture was stirred at 60°C overnight. Volatiles were evaporated and the residue was partitioned between EtOAc and 0.1 M HCl solution. The organic layer was washed with saturated solutions of $NaHCO_3$ and brine, and then was column chromatographed (hexane/EtOAc, 50:50) to give 2′,3′-*O*-isopropylidene-5′-*O*-undecanoyluridine (**19**; 203 mg, 90%) of sufficient purity to be used in next step: ^{1}H NMR δ 0.88 (t, $J = 7.1$ Hz, 3H, CH_3), 1.22–1.65 (m, 22H, 8 × CH_2, 2 × CH_3), 2.31 (t, $J = 7.5$ Hz, 2H, CH_2), 4.26–4.38 (m, 3H, H4′,5′,5″), 4.80 (dd, $J = 5.7, 3.8$ Hz, 1H, H3′), 4.98 (dd, $J = 6.2, 1.4$ Hz, 1H, H2′), 5.65 (d, $J = 1.3$ Hz, 1H, H1′), 5.72 (d, $J = 8.2$ Hz, 1H, H5), 7.28 (d, $J = 8.2$ Hz, 1H, H6), 8.98 (s, 1H, NH). *Step b:* ICl (1 M/CH_2Cl_2; 0.75 mL, 0.75 mmol) was added to a stirred solution of **19** (224 mg, 0.5 mmol) in CH_2Cl_2 (4.3 mL) at ambient temperature and the resulting mixture was stirred at 40°C (oil-bath) overnight. The reaction solution was washed with 2% $NaHSO_3$ until the color turned into light yellow. The organic layer was washed with saturated solutions of $NaHCO_3$ and brine and then was column chromatographed (hexane/EtOAc, 60:40 → 10:90) to give 5′-*O*-undecanoyl-5-iodouridine (**20**; 66 mg, 25%): ^{1}H NMR δ 0.87 (t, $J = 6.6$ Hz, 3H, CH_3), 1.25–1.31 (m, 14H, 7 × CH_2), 1.67 (quin, $J = 7.0$ Hz, 2H, CH_2), 2.42–2.57 (m, 2H, CH_2), 4.22–4.48 (m, 5H, H2′,3′,4′,5′,5″), 5.91 (d, $J = 2.9$ Hz, 1H, H1′), 8.02 (s, 1H, H6), 10.69 (s, 1H, NH). *Step c:* Treatment of **20** (27 mg, 0.05 mmol) with furan by Procedure B (column chromatography; hexane/EtOAc, 20:80) gave **21** (15 mg, 63%); ^{1}H NMR δ 0.87 (t, $J = 6.7$ Hz, 3H, CH_3), 1.23–1.25 (m, 14H, 7 × CH_2), 1.59 (quin, $J = 6.8$, 2H, CH_2), 2.35 (t, $J = 7.5$ Hz, 2H, CH_2), 4.24 ("t", $J = 4.9$ Hz, 1H, H3′), 4.35–4.40 (m, 4H, H2′,4′,5′,5″), 5.95 (d, $J = 4.1$ Hz, 1H, H1′), 6.40 (dd, $J = 3.3, 1.8$ Hz, 1H, furan), 6.97 (d, $J = 3.2$ Hz, 1H, furan), 7.27 ("s", 1H, furan), 7.96 (s, 1H, H6), 9.89 (s, 1H, NH); HRMS calcd. for $C_{24}H_{35}N_2O_8$ $[M+H]^+$ 479.2388, found 479.2397.

*5′-O-Undecanoyl-5-(5-heptylfur-2-yl)uridine (**22**)*
Treatment of **20** (27 mg, 0.05 mmol) with 2-heptylfurane (96 μL, 83 mg, 0.5 mmol) by Procedure B (column chromatography; hexane/EtOAc, 30:70) gave **22** (13 mg, 44%): ^{1}H NMR (DMSO-d_6) δ 0.82–0.87 (m, 6H, 2 × CH_3), 1.18–1.30 (m, 22H, 11 × CH_2), 1.48 (quin, $J = 6.9$, 2H, CH_2), 1.58 (quin, $J = 7.2$, 2H, CH_2), 2.22–2.37 (m, 2H, CH_2), 2.58 (t, $J = 7.5$ Hz, 2H, CH_2), 3.95 (q, $J = 5.0$ Hz, 1H, H3′), 4.08–4.13 (m, 2H, H2′,4′), 4.22 (dd, $J = 12.5, 2.2$ Hz, 1H, H5′), 4.30 (dd, $J = 12.5, 5.6$ Hz, 1H, H5″), 5.32 (d, $J = 5.9$ Hz, 1H, 3′-OH), 5.54 (d, $J = 5.0$ Hz, 1H, 2′-OH), 5.82 (d, $J = 5.3$ Hz, 1H, H1′), 6.14 (d, $J = 3.4$ Hz, 1H, furan), 6.77 (d, $J = 3.7$ Hz, 1H, furan), 7.78 (s, 1H, H6), 11.68 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ 13.87 (CH_3), 13.90 (CH_3), 22.0, 24.4, 27.3, 27.4, 28.35, 28.37, 28.58, 28.61, 28.8, 28.9, 31.18, 31.23, 33.2 (CH_2), 63.4 (C5′), 69.9 (C3′), 73.3 (C2′), 81.5 (C4′), 88.9 (C1′), 101.1 (C5), 106.9 (furan), 109.2 (furan), 132.8 (C6), 144.3 (furan), 149.5 (furan), 154.7 (C2), 160.0 (C4), 172.6 (C=O); HRMS calcd. for $C_{31}H_{49}N_2O_8$ $[M+H]^+$ 577.3483, found 577.3509.

Pharmacology

Proliferation assays
Human cervical carcinoma (HeLa) cells were seeded in 96-well plates at 15,000 cells/well in the presence of fivefold dilutions of the compounds. After 4 days of incubation, the cells were trypsinized and counted by means of a Coulter counter (Analis, Belgium). Suspension cells (mouse leukemia L1210 and human lymphoid CEM cells) were seeded in 96-well plates at 60000 cells/well in the presence of the compounds. L1210 and CEM cells were allowed to proliferate for 48 or 96 h, respectively, and then counted. The 50% inhibitory concentration (IC_{50}) was defined as the compound concentration required to reduce cell proliferation by 50%.

Antiviral assays

The compounds were evaluated against the following viruses: herpes simplex virus type 1 (HSV-1) strain KOS, thymidine kinase-deficient (TK^-) HSV-1 KOS strain resistant to ACV (ACV^r), herpes simplex virus type 2 (HSV-2) strain G, varicella zoster virus (VZV) strain Oka, TK^- VZV strain 07-1, human cytomegalovirus (HCMV) strains AD-169 and Davis, vaccinia virus Lederle strain, respiratory syncytial virus (RSV) strain Long, vesicular stomatitis virus (VSV), Coxsackie B4, parainfluenza 3, influenza virus A (subtypes H1N1, H3N2), influenza virus B, Sindbis, reovirus-1, Punta Toro, human immunodeficiency virus type 1 strain IIIB, and human immunodeficiency virus type 2 strain ROD. The antiviral, other than anti-HIV, assays were based on inhibition of virus-induced cytopathicity or plaque formation in human embryonic lung (HEL) fibroblasts, African green monkey cells (Vero), human epithelial cells (HeLa), or Madin-Darby canine kidney cells (MDCK). Confluent cell cultures in microtiter 96-well plates were inoculated with 100 $CCID_{50}$ of virus (1 $CCID_{50}$ being the virus dose to infect 50% of the cell cultures) or with 20 or 100 plaque forming units (PFU) (VZV or HCMV) in the presence of varying concentrations of the test compounds. Viral cytopathicity or plaque formation 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 compound concentration required to reduce virus-induced cytopathogenicity or viral plaque formation by 50%. Cytotoxicity of the test compounds was expressed as the minimum cytotoxic concentration (MCC) or the compound concentration that caused a microscopically detectable alteration of cell morphology.

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