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Article

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Novel antiviral activity of L-dideoxy bicyclic nucleoside analogues versus vaccinia and measles viruses *in vitro*

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Abstract

Dideoxy bicyclic pyrimidine nucleoside analogues (ddBCNAs) with [D] chirality have previously been described by us to inhibit replication of human cytomegalovirus. We herein report for the first time that activity against vaccinia virus (VACV) was achieved using novel [L] analogues. A structure activity relationship was established: Antiviral activity versus VACV was highest with an ether side chain with an optimum of n-C_9H_{18}-O-n-C_5H_{11}. This gave an IC_{50} of 190 nM, a 60 fold enhancement over the FDA approved antiviral cidofovir. Interestingly, L-ddBCNAs also inhibit wild type measles virus syncytia formation with a $TCID_{50}$ of 7.5 μM for the lead compound. We propose that L-ddBCNAs represent significant innovative antiviral candidates versus measles and poxviruses and we suggest a mechanism of action versus one or more cellular targets that are essential for viral replication.

Introduction

Small pox – variola was declared eradicated in 1979. However, threats of bioterrorism have re-ignited interest in finding suitable treatments that could be used in case of a small pox outbreak.[1] At present, the only drug that has been approved for treatment of poxviruses is cidofovir.[2] This drug is not ideal as it has severe side effects. Vaccinia Virus (VACV) is a convenient surrogate poxvirus for the development of new antipoxviral agents *in vitro* VACV strains that are resistant to cidofovir have also been described.[3] Alternative treatments would also be useful for related infections with Molluscum contagiosum virus[4] and human monkey pox virus.[5]

A number of new potential antipoxviral agents have recently been reported. These include ST-246, a molecule that targets the F13L protein of the virus,[6] although resistance to this drug has already been described,[7] and terameprocol, which reduces the spread of the virus by inhibiting the formation of actin tails at the surface of infected cells (cellular target).[8]

Human measles is usually a mild childhood infection but is a known cause of severe pneumonia in developing countries with over 1 million fatalities worldwide in 2005. The live measles vaccine is not given to infants under 12 months in age and maternal measles virus antibodies are lost at 4-9 months, so individuals are susceptible to the virus before the vaccination is given.[9] The ideal antiviral therapy for measles would be a small molecule inhibitor that is safe, effective, inexpensive to reproduce and stable. Having a host target may offer the additional benefit of reducing the emergence of rapid resistance. Ribavirin inhibits measles virus *in vitro* but only at supra-physiological levels (IC_{50} of

1160 μM), but is not effective for the treatment of measles virus infections.[10] At present there are no measles antiviral drugs on the market.

Previously, we have reported novel potent and selective inhibitors of human cytomegalovirus based on D-dideoxy bicyclic furanopyrimidine nucleoside analogues (D-ddBCNAs) bearing alkyl side chains at the C5-position, with action suggested early in the virus lifecycle.[11] Further potency of D-ddBCNAs versus VACV[12] suggested a non virus specific mechanism of action and possibly a cellular target.

In order to further increase potency and explore the structure activity relationships (SARs), we were interested in other unusual sugars and particularly in unnatural L-configuration nucleosides. Indeed, several L-nucleosides have emerged as potent antiviral agents,[13,14] which include β-L-[2-(hydroxymethyl)-1,3-oxathiolan-4-yl]cytosine (Lamivudine, 3TC),[15] 5-fluoro-β-L-2′,3′-dideoxy-3′-thiacytidine (Emtricitabine, FTC),[16] 5-fluoro-β-L-2′,3′-dideoxycytidine (L-FddC),[17] β-L-2′-fluoro-5-methylarabinofuranosyl uracil (clevudine, L-FMAU),[18] 2′,3′-dideoxy-2′,3′-didehydro-β-L-5-fluorocytidine (Elvucitabine, β-L-Fd4C)[19] and carbocyclic nucleoside L-Cd4U.[20] Moreover, it has been reported that some of them have more potent antiviral activity than their D-enantiomers with reduced toxicity.[21,22]

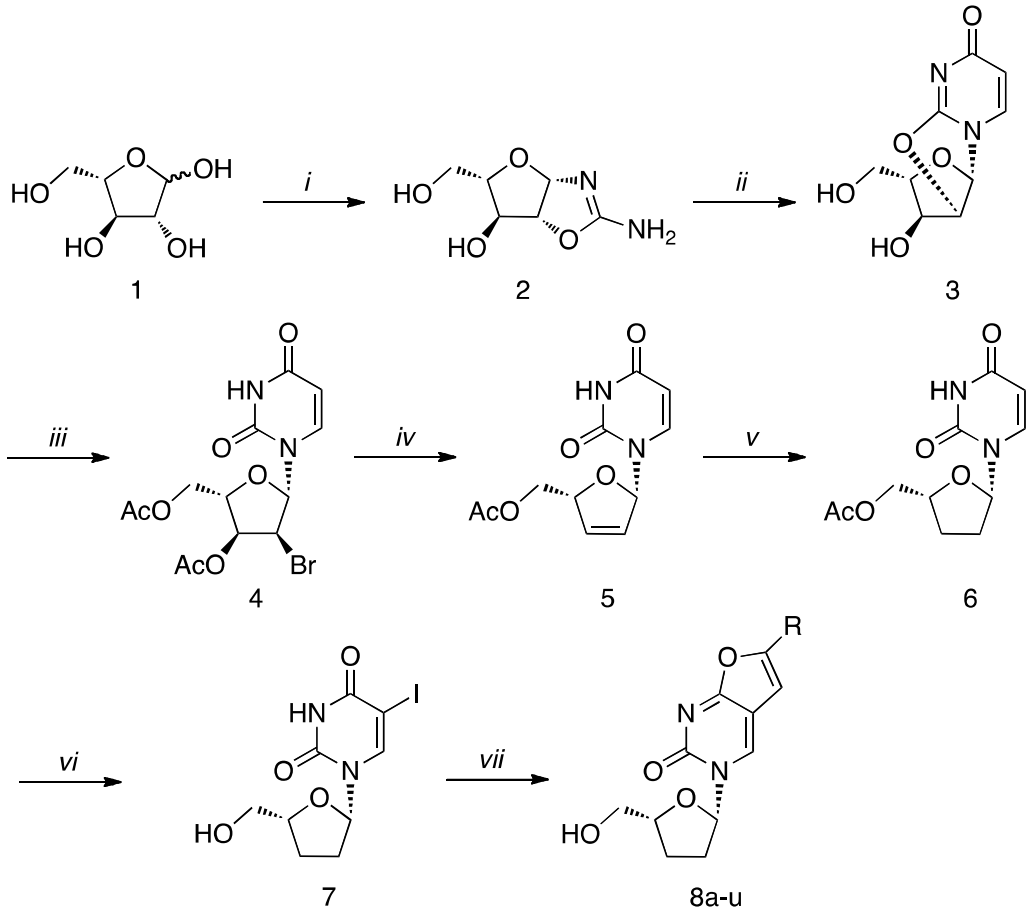
Therefore, it was of particular interest to synthesise and study the antiviral activity of L-dideoxy bicyclic furanopyrimidine nucleoside analogues (L-ddBCNAs) with different side chains.

Results and discussion

Chemistry. We previously presented the successful synthesis of different D-BCNAs.[11,23-26] For the D-dideoxy family, the bicyclic nucleosides were obtained from the key synthon

D-5-iodo-2',3'-dideoxyuridine (D-IddU) *via* Sonogashira reaction with different acetylenes followed by an intramolecular cyclisation.[11] The described formation of the D-IddU started from the D-2'-deoxyuridine. We wanted to apply our methodology for the synthesis of the L-ddBCNAs. However, the L-2'-deoxyuridine is not commercially available. Thus, we sought a new route, illustrated in Scheme 1, starting from L-arabinose **1**.

Scheme 1. Synthetic route to target L-ddBCNAs.



(*i*) NC-NH_2, NH_4OH, MeOH, rt, 3 days (70%); (*ii*) Methyl propiolate, EtOH, reflux, 3 h (69%); (*iii*) AcBr, CH_3CN, reflux, 2 h (75%); (*iv*) Zn, glacial AcOH, EtOH, reflux, 2 h (53%); (*v*) H_2, Pd/C, MeOH, rt, 18 h (97%); (*vi*) 1) I_2, CAN, CH_3CN, reflux, 6 h; 2)

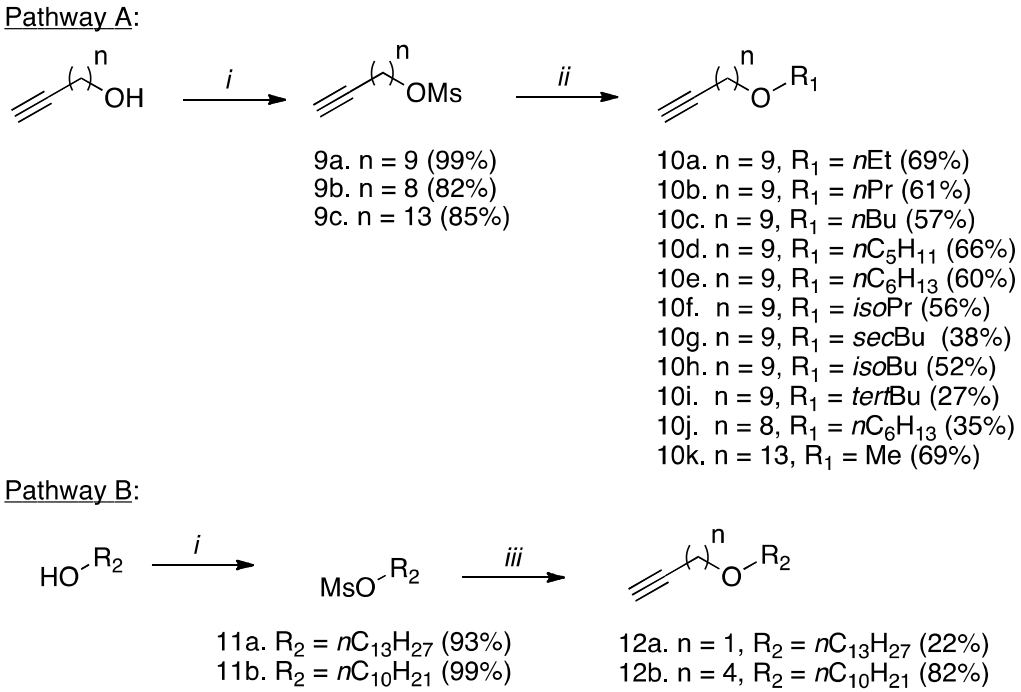
MeONa, MeOH, rt, 18 h (60% over 2 steps); (*vii*) acetylenes, $Pd(PPh_3)_4$, CuI, DIPEA, DMF, rt, 18 h and then addition of Et_3N, CuI, 80 ^oC, 8 h (7-53%).

First, reaction of the carbohydrate **1** with cyanamide, according to the methodology reported by Ladurée *et al*.,[27] afforded the corresponding bicyclic oxazoline **2**. Treatment with methyl propiolate in methanol yielded the 2,2'-anhydro-nucleoside **3** which *via* a bromoacetylation reaction[28] with acetyl bromide in acetonitrile gave the nucleoside **4** in good yield. Reductive β-elimination with activated zinc powder in the presence of glacial acetic acid[29] afforded the 5'-O-acetyl-2',3'-didehydro-2',3'-dideoxyuridine **5** which gave almost quantitatively the dideoxy nucleoside **6** upon catalytic hydrogenation. The L-5-iodo-2',3'-dideoxyuridine **7** was obtained after first iodination at the C5-position of the base with iodine in the presence of ammonium cerium(IV) nitrate (CAN)[30] and then deacetylation using sodium methoxide, in 60% yield over two steps. Palladium-catalysed Sonogashira coupling of the key synthon **7** with a series of acetylenes gave the corresponding 5-alkynyl nucleosides. Addition of copper (I) iodide *in situ* with heating of the reaction resulted in their intramolecular cyclisation to afford the desired furanopyrimidine compounds **8a-u** with alkyl, aryl or ether side chains. Indeed, we have reported in the case of D-deoxy BCNAs that aryl and ether groups decrease the lipophilicity of nucleosides and improve their activity.[24,26]

The alkyl and aryl acetylenes needed for the Sonogashira reaction were commercially available. However, the ether alkynes were synthesised following two different pathways depending on the target ethers (Scheme 2). The first method (pathway A) started with the mesylation of alkyn-1-ols to obtain the intermediates **9a-c**, which were then coupled with different alcohols to give the ethers **10a-j**. In the second method (pathway B), the starting

materials were alcohols, which were first transformed into the mesylated compounds **11a** and **11b** and then reacted with selected alkynols to obtain the ethers **12a** and **12b**.

Scheme 2. Synthetic routes to alkynyl ethers **10a-j** and **12a-b**.



(*i*) MsCl, Et_3N, THF, rt, 18 h; (*ii*) Alcohol, NaH, THF, 70 ^oC, 24 h; (iii) Alkynol, NaH, THF, 60 ^oC, 36 h.

The structure and the purity of all L-ddBCNAs were confirmed by spectroscopic, chromotographic and analytical data before they were tested for inhibition of viral infections.

Antiviral activity. The L-ddBCNAs **8a-u** were evaluated by luciferase assay for their ability to inhibit the early stages of VACV replication from entry to early gene transcription in RK 13 cells (Table 1), following the initial notion of an effect early in the viral lifecycle.[11]

Table 1. L-ddBCNA structures, clogP and luciferase reporter assay versus VAVC.

Cpds	R	clogP[a]	Luciferase reporter[b] (%)
8a	*n*C_6H_{13}	2.60	70.7
8b	*n*C_7H_{15}	3.13	43.2
8c	*n*C_8H_{17}	3.68	41.9
8d	*n*C_9H_{19}	4.19	38.1
8e	*n*$C_{10}H_{21}$	4.72	45.7
8f	*n*$C_{12}H_{25}$	5.77	96.4
8g	Ph-*n*Bu	3.64	43.7
8h	Ph-*n*C_5H_{11}	4.17	73.6
8i	*n*C_9H_{18}-O-*n*Et	3.30	79.9
8j	*n*C_9H_{18}-O-*n*Pr	3.83	46.8
8k	*n*C_9H_{18}-O-*n*Bu	4.36	27.5
8l	*n*C_9H_{18}-O-*n*C_5H_{11}	4.89	27.7
8m	*n*C_9H_{18}-O-*n*C_6H_{13}	5.42	35.7
8n	*n*C_9H_{18}-O-*iso*Pr	3.61	79.9
8o	*n*C_9H_{18}-O-*sec*Bu	4.14	69.9
8p	*n*C_9H_{18}-O-*iso*Bu	4.23	64.3
8q	*n*C_9H_{18}-O-*ter*Bu	4.01	70.7
8r	*n*C_8H_{16}-O-*n*C_6H_{13}	4.89	37.2
8s	*n*C_4H_8-O-*n*$C_{10}H_{21}$	4.89	26.1
8t	CH_2-O-*n*$C_{13}H_{27}$	5.46	43.9
8u	*n*$C_{13}H_{26}$-O-Me	5.03	60.5
Cidofovir		-2.39	94.6

[a] *clogP values calculated with Chemdraw ultra 12.0.* [b] *Luciferase reporter activity as percent of no drug control (100%) in RK13 cells.*

Luciferase inhibition was observed in compounds **8a**-**h** bearing alkyl and aryl chains, with an optimum at the analogue **8d** (nC$_9$H$_{19}$ chain). The apparent reduction in activity for longer alkyl chain derivatives **8e** (nC$_{10}$H$_{21}$ chain) and **8f** (nC$_{12}$H$_{25}$ chain) may be caused by their low water solubility (calculated logP data of 4.72 and 5.77). This led to the synthesis of ether compounds with a nonyl chain prior to oxygen (**8i-q**). The best reduction of luciferase values was then identified among the L-ddBCNAs **8j-m** with linear ether side chains and a SAR regarding the length of the chain emerged; best activity was found for compounds **8k** and **8l** with butyl and pentyl termini. Using the length of side chain in **8l** alteration of the oxygen atom position toward the base (analogues **8r-u**) did not improve the activity. However the good luciferase values of derivatives **8e** (nC$_8$H$_{16}$-O-nC$_6$H$_{13}$ chain) and **8f** (nC$_4$H$_8$-O-nC$_{10}$H$_{21}$ chain) in spite of high calculated logP data (of 4.89) shows that lipophilicity of compounds is only one parameter in their mechanism of action.

These results were followed up for the most potent ether compounds by plaque assay and determination of IC_{50} in BSC-1 cells with data shown in Table 2. The effect of L-ddBCNAs on cell viability (CC_{50}) was tested measuring ATP levels in live BSC-1 cells (CellTiter-Glo® Luminescent Cell Viability Assay).

Table 2. IC_{50} and CC_{50} data versus VACV of selected ether L-BCNAs.

Cpds	R	IC_{50}[a] (μM)	CC_{50}[a] (μM)	CC_{50}[b] (μM)
8i	nC$_9$H$_{18}$-O-nEt	12.9	79.4	>100
8j	nC$_9$H$_{18}$-O-nPr	1.9	89.1	>100
8k	nC$_9$H$_{18}$-O-nBu	1.8	>100	>100

8l	nC$_9$H$_{18}$-O-nC$_5$H$_{11}$	0.19	>100	>100
8m	nC$_9$H$_{18}$-O-nC$_6$H$_{13}$	0.19	74.1	>100
8r	nC$_8$H$_{16}$-O-nC$_6$H$_{13}$	1.7	-	-
8s	nC$_4$H$_8$-O-nC$_{10}$H$_{21}$	2.2	-	-
8t	CH$_2$-O-nC$_{13}$H$_{27}$	3.4	-	-
Cidofovir		11.5	>100	>100

[a] *in BSC-1 cells.* [b] *in HFFF cells.*

The IC_{50} values from 4 day plaque assays of ethers with increasing chains (compounds **8i-m**) followed a similar SAR to that identified for the 2 hour luciferase activity data. Both assays showed the same trend perhaps implying the same mechanism of action. The 2 hour luciferase assay was used as a predictor of compound efficacy. Moreover, all L-ddBCNAs with the exception of **8i** performed better than the cidofovir control. The IC_{50} of nucleosides **8l** and **8m** of 0.19 μM is a 60 fold improvement of activity.

The ether series L-ddBCNAs have a mild effect on BSC-1 cell viability with a selectivity index of >500 for the lead compound **8l** (IC_{50} = 0.19 μM and CC_{50} >100 μM). BSC-1 cells did not show visible cytotoxicity in phase contrast microscopy after 2 hours in the luciferase reporter assays or after 4 days at CC_{50} in the plaque reduction assays. The mild toxicity observed in BSC-1 cells for some compounds does not follow the SAR of the ether series L-ddBCNAs and is not predictive of antiviral activity. No cytotoxic effects of L-ddBCNAs **8i-m** were observed in HFFF cells up to 100 μM.

The lead bicyclic nucleosides **8l** was then evaluated by $TCID_{50}$ assay for its ability to inhibit formation of cytopathogenic effect of adenovirus, herpesvirus (type 1 and 2) and vaccine and wild type measles viruses (Table 3).

Table 3. $TCID_{50}$ (μM) of compound **8l** versus other viruses.

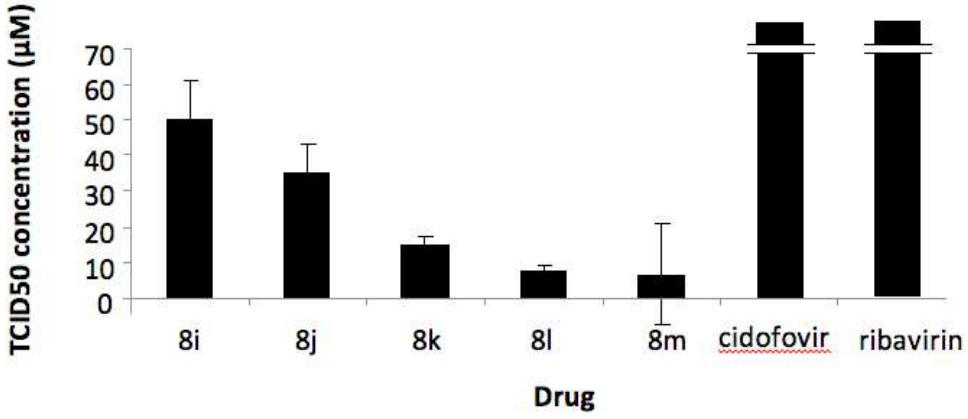
	HFFF	BSC-1	B95a
Adenovirus 5	>50	>50	nd
HSV 1 NCPV 17+	>50	>50	nd
HSV 2 NCPV 132349	>50	>50	nd
Measles virus Edmonston (vaccine)	nd [a]	>50	>50
Measles virus wild type *mWTFb*	nd [a]	nd [a]	7.5

[a] *cells not susceptible. nd: not determined.*

Nucleoside **8l** was unable to suppress the cytopathogenic effects of adeno, herpes simplex and measles vaccine viruses in BSC-1 and HFFF cells. However, it inhibits wild type measles virus syncytia formation by 50% at 7.5 μM. This is a 150 fold improvement over ribavirin *in vitro* (IC_{50} of 1160 μM;[10] Figure 1). When L-ddBCNAs **8i-m** of the ether series were tested versus wild type measles virus in B95a cells (Figure 1), the SAR was found to follow closely the one found previously for VACV in RK13 (luciferase assay) and BSC-1 (plaque assay) cells. Cidofovir, a DNA polymerase inhibitor, active against VACV, shows as expected, no effect in this assay. The ribavirin IC_{50} (1.2 mM, data not shown) is out of the range of this figure. Wortmannin, a furanosteroid inhibitor of PI3

like kinases,[31] inhibited measles wild type virus in this assay with an IC_{50} of 75 μM. (data not shown).

Figure 1. SAR of ether series of L-ddBCNAs versus wild type measles virus.



The SAR observed versus both vaccinia and wild type measles viruses is highly suggestive of a drug interaction with a specific target. While measles wild type viruses are only able to utilize the CD150 receptor found on human and simian B cells, the vaccine strain of measles virus uses the ubiquitous CD46 receptor. CD150 and CD46 receptors are located in different membrane microdomains, so a differential effect of L-ddBCNAs is possible. Membrane microdomains are involved in both measles and vaccinia virus replication.[32, 33]

Is it possible that the reduction of VACV plaque size herein observed could be due to an effect on the dissemination of VACV in cell culture 'conveyor belt'.[34]

The phenolic antioxidant terameprocol[8] shows a similar activity profile to L-ddBCNAs, where the target of the antiviral activity is the actin-cytoskeleton, clearly a cellular target. The main effect is a quantifiable reduction in plaque size, just as observed with the L-

ddBCNAs. With a cellular target of this nature, viral infection in the host would not be completely shut down, but rather the dissemination of the virus would be affected. Inhibiting specific aspects of a virus lifecycle induces the virus to develop resistance to the drug by altering the target of the drug. Targeting cellular processes means that there may be a possibility of increased toxicity, but reduces the opportunity for resistance. Antivirals versus cellular targets are not subject to viral resistance, which may develop quickly with all virus-targeting drugs.

Conclusion

In summary we have described the successful synthesis of substituted L-dideoxy bicyclic furanopyrimidine nucleoside analogues in 8 steps starting from L-arabinose.

A series of ether-substituted compounds has been shown to induce suppression of orthopox- and measles virus replication *in vitro*. Actually, early (2 hours p.i.) luciferase reporter assays against VACV and plaque/syncytium reduction assays against VACV and measles virus highlighted a SAR regarding the length of the chain. Notably the lead L-ddBCNA **8l** with nC$_9$H$_{18}$-O-nC$_5$H$_{11}$ side chain possessed an IC_{50} value of 0.19 μM against VACV, with good selectivity index, and a $TCID_{50}$ value of 7.5 μM against measles virus.

We propose that interactions between L-ddBCNAs and membrane microdomains are involved in the inhibition of virus replication. This kind of host targeting action would prevent drug resistance issues. Further studies about the mechanism of action of L-ddBCNAs are currently under investigation in our laboratory.

Experimental section

Chemistry. The numbering of the bicyclic ring follows the recommended IUPAC nomenclature guidelines. The naming of the compounds follows IUPAC nomenclature and/or standard accepted nomenclature for nucleoside chemistry.

All solvents used were anhydrous and used as supplied by Aldrich. All nucleosides and solid reagents were dried for several hours under high vacuum over potassium hydroxide. All glassware was oven dried at 130 °C for several hours or overnight and allowed to cool in a desiccator or under a stream of dry nitrogen. TLC was performed on precoated aluminum-backed plates (60 F-54, 0.2 mm thickness; supplied by E. Merck AG, Darmstadt, Germany) and spots visualized by UV (at 254 or 336 nm depending on the compound). Column chromatography was performed with silica gel supplied by Fisher (60A, 35-70 μm). ^{1}H and ^{13}C NMR spectra were recorded in a Bruker spectrometer (500 MHz and 125 MHz, respectively) and auto-calibrated to the deuterated solvent reference peak. All ^{13}C NMR spectra were proton-decoupled. Coupling constants (*J*) are measured in Hertz. The following abbreviations are used in the assignment of NMR signals: br (broad), s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), dd (doublet of doublet), ddd (doublet of doublet of doublet), dt (doublet of triplet). Low resolution and accurate mass analysis were performed by the service at the Department of Chemistry, University of Wales, Cardiff, UK, with electrospray (ES). The ≥95% purity of all the final compounds was confirmed using analytical HPLC analysis. HPLC experiments were done on a Thermo Fisher Scientific Spectra System SCM1000 provided with a System Controller SN4000, a pump Spectra System P4000 and a Spectra UV2000 detector set (detection at 254 nm) and using a 5 μM Hypersil GOLD (150 × 4.6 mm)

reverse phase column eluting with the indicated mobile phase with a flow rate of 1 mL/min.

2-amino-[1,2-*d*]arabinosyl-oxazole (2). A suspension of L-arabinose **1** (17 g, 113 mmol), cyanamide (10 g, 238 mmol, 2.1 eq.) in anhydrous MeOH (30 mL) and 6M NH_4OH (5 mL) were stirred at room temperature for 65 h. The solvents were then evaporated from the resulting suspension under reduced pressure. The crude was triturated with MeOH to give a white solid (13.16 g, 66%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 6.27 (2H, br s, NH_2), 5.67 (1H, d, J = 5.5 Hz, H-1'), 5.39 (1H, br s, OH-3'), 4.68 (1H, bs, OH-5'), 4.53 (1H, d, J = 5.5 Hz, H-2'), 4.02-3.99 (1H, m, H-3'), 3.67-3.62 (1H, m, H-4'), 3.31-3.23 (2H, m, H-5'); ^{13}C NMR (125 MHz, DMSO-d_6): δ 162.18 (C-2), 99.95 (C-1'), 88.04 (C-2'), 84.58 (C-4'), 75.62 (C-3'), 6.55 (C-5').

2,2'-Anhydrouridine (3). A solution of 2-amino-[1,2-*d*]arabinosyl-oxazole **2** (24.0 g, 137.81 mmol) and methyl propiolate (23 mL, 275.62 mmol, 2 eq.) in 50% aqueous ethanol solution (350 mL) were refluxed for 2 hours. The solvent was removed under reduced pressure to obtain a residue that was dissolved in acetone (400 mL) and left at 0 °C overnight. The resulting precipitate was filtered and washed with acetone to obtain a white solid (23.06 g, 74%); ^{1}H NMR (500 MHz, DMSO-d_6): δ 7.83 (1H, d, J = 7.4 Hz, H-5), 6.31 (1H, d, J = 5.8 Hz, H-1'), 5.87 (1H, d, J = 4.3 Hz, OH-3'), 5.84 (1H, d, J = 7.4 Hz, H-6), 5.20 (1H, d, J = 5.5 Hz, H-2'), 4.97 (1H, t, J = 5.0 Hz, OH-5'), 4.41-4.37 (1H, m, H-3'), 4.08 (1H, t, J = 4.1 Hz, H-4'), 3.31-3.25 (1H, m, H-5'), 3.23-3.16 (1H, m, H-5'); ^{13}C NMR (125 MHz, DMSO-d_6): δ 171.10 (C-4), 159.77 (C-2), 136.77 (C-5), 108.59 (C-6), 89.97 (C-1'), 89.16 (C-4'), 88.71 (C-2'), 74.71 (C-3'), 60.81 (C-5').

5',3'-Diacetyl-2'-bromo-2'-deoxyuridine (4). 2,2'-Anhydrouridine **3** (22.0 g, 97.26 mmol) was suspended in CH_3CN (400 mL) and heated under reflux. Acetyl bromide (64.7 mL, 875.37 mmol, 9 eq.) was added dropwise. The mixture was stirred at reflux for 2 hours. The solvent was removed under reduced pressure and the residue obtained was dissolved in dichloromethane (400 mL), washed with a solution, dried over $MgSO_4$ and evaporated to obtain the title compound as a brown oil (33.0 g, 87%); ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.99 (1H, s, NH), 7.45 (1H, d, *J* = 8.4 Hz, H-6), 6.22 (1H, d, *J* = 5.21 Hz, H-1'), 5.82 (1H, dd, *J* = 7.4 Hz, 1.57 Hz, H-5), 5.16 (1H, t, *J* = 5.04 Hz, H-3'), 4.61 (1H, t, *J* = 5.6 Hz, H-2'), 4.46-4.41 (1H, m, H-4'), 4.42-4.36 (1H, m, H-5'), 3.31-3.25 (1H, m, H-5'), 2.22 (3H, s, CH_3), 2.16 (3H, s, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 171.04 (CO), 169.69 (CO), 162.50 (C-4), 149.93 (C-2), 138.92 (C-5), 103.29 (C-6), 91.01 (C-1'), 80.11 (C-4'), 70.76 (C-3'), 62.55 (C-5'), 48.41 (C-2'), 20.78 (CH_3), 20.61 (CH_3)**;** MS (ES^+) m/z 413-415 ($M+Na^+$).

5'-Acetyl-2', 3 -dideoxy-2',3'-dehydro-uridine (5). Glacial acetic acid (1.5 mL, 26.20 mmol, 1.6 eq.) was added dropwise at 0 ^{0}C to a stirred suspension of zinc (4.20 g, 64.23 mmol, 4 eq.) and 5',3'-diacetyl-2'-bromo-2'-deoxyuridine **4** (6.2 g, 15.85 mmol) in ethanol (195 mL). The resulting mixture was stirred at room temperature until the TLC (3% methanol in ethyl acetate) showed that the starting material had completely reacted. The mixture was filtered through a sintered funnel packed with celite to remove zinc and triethylamine (1.4 mL) was added to the filtrate. The solvent was removed under reduced pressure and the residue obtained was extracted with ethyl acetate (250 mL). Removal of solvent under reduced pressure gave a brown oil which was purified by column chromatography (1% methanol in ethyl acetate) to afford the title compound as a yellow

oil (2.24 g, 53%); ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.37 (1H, br s, NH), 7.44 (1H, d, J = 8.1 Hz, H-6), 6.79 (1H, m, H-1'), 6.42 (1H, dt, J = 6.1 Hz, 1.7 Hz, H-3'), 6.00 (1H, ddd, J = 6.1 Hz, 2.3 Hz, 1.3 Hz, H-2'), 5.66 (1H, d, J = 8.1 Hz, H-5), 4.97 (1H, m, H-4'), 4.17 (2H, d, J = 3.4 Hz, H-5'), 2.00 (3H, s, CH_3)**;** ^{13}C NMR (125 MHz, DMSO-d_6): δ 170.07 (CO), 163.09 (C-4), 150.72 (C-2), 140.52 (C-6), 133.85 (C-3'), 126.37 (C-2'), 101.90 (C-5), 89.34 (C-1'), 83.80 (C-4'), 64.48 (C-5'), 20.56 (CH_3)**;** MS (ES^+) m/z 275 ($M+Na^+$).

5'-Acetyl-2',3'-dideoxyuridine (6). To a solution of 5'-Acetyl-2',3-dideoxy-2',3'-dehydro-uridine **5** (4.88 g, 19.34 mmol) in anhydrous methanol (80 mL) was added 10% Pd on carbon (0.23 g, 5%w). The mixture was stirred under a hydrogen atmosphere for 6 h. The reaction was monitored by NMR analysis. The mixture was then filtered through a Celite pad. The solvent was removed under reduced pressure to afford the title compound as a pale brown oil (5.0 g, 99%); ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.27 (1H, br s, NH), 7.66 (1H, d, J = 8.0 Hz, H-6), 5.98 (1H, dd, J = 7.2 Hz, 3.8 Hz, H-1'), 5.64 (1H, d, J = 8.0 Hz, H-5), 4.21-4.18 (3H, m, H-5', H-4'), 2.34-2.22 (1H, m, H-2'), 2.06-1.95 (5H, m, H-2', H-3', CH_3), 1.81-1.72 (1H, m, H-3'); ^{13}C NMR (125 MHz, DMSO-d_6): δ 170.21 (CO), 163.08 (C-4), 150.48 (C-2), 140.43 (C-6), 101.51 (C-5), 85.24 (C-1'), 77.95 (C-4'), 64.88 (C-5'), 30.8 (C-2'), 25.56 (C-3'), 20.60 (CH_3); MS (ES^+) m/z 277 ($M+Na^+$).

L-5-iodo-2',3'-dideoxyuridine (7). To a solution of 5'-Acetyl-2',3'-dideoxyuridine **6** (2.00 g, 7.86 mmol) in anhydrous acetonitrile (8 mL) were added I_2 (1.20 g, 4.72 mmol, 0.6 eq.) and CAN (2.16 g, 3.94 mmol, 0.5 eq.). The mixture was stirred at reflux for 3 h (progress of the reaction was monitored by TLC using 1% methanol in ethyl acetate as the eluent). The solvent was removed under reduced pressure and the residue was

dissolved in dichloromethane (300 mL) and washed with a saturated solution of sodium bicarbonate. The two phases were separated and the organic layer was dried over sodium sulphate and evaporated under reduced pressure to obtain the 5'-Acetyl-2',3'-dideoxy-5-iodo-uridine intermediate as a brown solid. This residue was dissolved in MeOH (60 mL) and MeONa (0.63 g, 11.66 mmol, 1.5 eq.) was added. The solution was stirred for 21 h. As the reaction was not complete, an additional amount of MeONa (0.21 g, 3.88 mmol, 0.5 eq.) was added and the resulting mixture was stirred for further 1 h. Amberlite IR 120 was then added until the pH of the solution became 6.0 and the mixture was filtered. The solution was evaporated and the solid was washed with diethyl ether to yield the title compound (off-white solid, 1.57 g, 60%); ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.27 (1H, br s, NH), 8.57 (1H, s, H-6), 5.87 (1H, dd, J = 6.6 Hz, 2.8 Hz, H-1'), 5.22 (1H, t, J = 5.0 Hz, OH), 4.05 (1H, m, H-4'), 4.72-4.53 (2H, m, H-5'), 2.26-1.83 (4H, m, H-2', H3'); ^{13}C NMR (125 MHz, DMSO-d_6): δ 160.59 (C-4), 150.05 (C-2), 145.19 (C-6), 85.83 (C-1'), 82.10 (C-4'), 68.11 (C-5), 61.20 (C-5'), 32.45 (C-2'), 23.93 (C-3'); Reverse HPLC eluting with H_2O/MeOH (gradient from 90:10 to 0:100 in 30 min), t_R = 08.96 min.

General procedure A: formation of mesylates (9a-c and 11a-b).

To a stirred solution of alcohol in THF (0.7 mL/mmol) was added triethylamine (1.3 eq.) and methanesulphonyl chloride (1.3 eq.) dropwise at 0 ^{o}C under a nitrogen atmosphere. The reaction was left stirring for 18 h at room temperature. The solvent was evaporated; water was added to the reaction mixture and then extracted with dichloromethane. The organic layer was dried over sodium sulphate, filtered and evaporated under reduced pressure to obtain the desired product.

10-Undecynyl mesylate (9a). The procedure A was carried out using 10-undecyn-1-ol (10 g, 59.42 mmol) to give the desired compound as a yellow oil (15.5 g, 100%); ^{1}H

NMR (500 MHz, $CDCl_3$): δ 4.26 (2H, t, J = 7.1 Hz, OCH_2), 3.05 (3H, s, CH_3), 2.20 (2H, dt, J = 7.1 Hz, 2.6 Hz, αCH_2), 1.97 (H, t, J = 2.5 Hz, HC≡C), 1.60-1.28 (14H, m, 7 x CH_2).

9-Decynyl mesylate (9b). The procedure A was carried out using 9-decyn-1-ol (2.50 g, 16.21 mmol) to give the desired compound as a yellow oil (3.10 g, 82%); ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.24 (2H, t, J = 6.6 Hz, OCH_2), 3.02 (3H, s, CH_3), 2.01 (2H, dt, J = 7.1 Hz, J = 2.6 Hz, αCH_2), 1.96 (1H, t, J = 2.6 Hz, HC≡C), 1.58-1.31 (12H, m, 6 x CH_2); ^{13}C NMR (125 MHz, $CDCl_3$): δ 84.64 (HC≡C), 70.13 (OCH_2), 68.19 (HC≡C), 37.39 (CH_3), 29.13, 2 x 28.89 (double intensity), 28.57, 28.39, 25.38 (6 x CH_2), 18.38 (αCH_2).

14-pentadecynyl mesylate (9c). The procedure A was carried out using 14-pentadecyn-1-ol[30] (1.80 g, 8.022 mmol) to give the desired compound as a pale brown oil (2.07 g, 85%); ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.24 (2H, t, J = 6.1 Hz, OCH_2), 3.01 (3H, s, CH_3), 2.20 (2H, dt, J = 7.1 Hz, 2.7 Hz, HC≡CCH_2), 1.95 (1H, t, J = 2.7 Hz, HC≡C), 1.76 (2H, m, CH_2), 1.56 (2H, m, CH_2), 1.43 (2H, m, CH_2), 1.40-1.23 (16H, m, 8 x CH_2); ^{13}C NMR (125 MHz, $CDCl_3$): δ 84.79 (HC≡C), 70.18 (OCH_2), 68.03 (HC≡C), 37.65 (CH_3), 29.59, 29.55, 29.47, 29.40, 29.13, 29.09, 28.89, 28.85, 28.74, 28.49, 25.73 (11 x CH_2), 18.63 (C≡CCH_2).

Tridecyl mesylate (11a). The procedure A was carried out using 1-tridecanol (5.00 g, 24.95 mmol) to give the desired compound as a pale brown oil (6.39 g, 93%); ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.25 (2H, t, J = 6.6 Hz, OCH_2), 3.03 (3H, s, CH_3), 1.76 (2H, m, OCH_2CH_2), 1.47-1.23 (20H, m, 10 x CH_2), 0.91 (3H, t, J = 7.0 Hz, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 70.19 (OCH_2), 37.38 (CH_3), 31.91, 29.64, 29.62, 29.60, 29.51, 29.41, 29.34, 29.13, 29.03, 25.42 (10 x CH_2), 22.68 (CH_2CH_3), 14.10 (CH_3).

Decyl mesylate (11b). The procedure A was carried out using 1-decanol (8.6 mL, 45 mmol) to give the desired compound as a pale brown oil (11.00 g, 100%); ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.24 (2H, t, J = 6.6 Hz, OCH_2), 3.03 (3H, s, CH_3), 1.78 (2H, m, $OCH_2C\underline{H}_2$), 1.46-1.23 (14H, m, 7 x CH_2), 0.91 (3H, t, J = 7.0 Hz, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 70.19 (OCH_2), 37.38 (CH_3), 31.85, 29.46, 29.41, 29.25, 29.13, 29.02, 25.42 (7 x CH_2), 22.66 ($\underline{C}H_2CH_3$), 14.08 (CH_3).

General procedure B: synthesis of alkyloxy-alkyne (10a-j and 12a-b).

To a stirred solution of alcohol (1.2 eq.) in THF (2.5 mL/mmol) at 0 ^{o}C under a nitrogen atmosphere was added slowly NaH (dispersion, 60%, 1.25 eq). After 5 min, the mesylate was added and the reaction was heated at 70 ^{o}C for 19 h. Then the solvent was removed under reduced pressure and the residue was dissolved in dichloromethane (100 mL) and washed with water (2 x 100 mL). The organic layer was dried over sodium sulphate, evaporated to dryness and purified by column chromatography to obtain the desired compound.

11-ethyloxy-1-undecyne (10a). The procedure B was carried out using ethanol (0.448 g, 9.735 mmol) and 10-undecynyl mesylate **9a** (2.04 g, 8.28 mmol). The crude was purified by column chromatography (5% ethyl acetate in hexane) to give the product **10a** as a colourless oil (0.87 g, 69%); ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.54-3.36 (4H, m, OCH_2), 2.20 (2H, m, αCH_2), 1.95 (1H, ddd, J = 3.1 Hz, 2.2 Hz, 1.0 Hz, HC≡C), 1.64-1.27 (8H, m, 4 x CH_2), 1.21 (3H, dt, J = 7.4 Hz, 1.1 Hz, CH_3)**;** ^{13}C NMR (125 MHz, $CDCl_3$): δ 84.76 (CH-$\underline{C}$), 70.76 (OCH_2), 68.03 ($\underline{C}$H-C), 66.04 ($O\underline{C}H_2CH_3$), 29.81, 29.77, 29.43, 29.03, 28.72, 28.48, 28.25 (7 x CH_2), 18.39 (αCH_2), 15.24 (CH_3).

11-propyloxy-1-undecyne (10b). The procedure B was carried out using propanol (0.75 mL, 9.936 mmol) and 10-undecynyl mesylate **9a** (2.04 g, 8.28 mmol). The crude was purified by column chromatography (5% ethyl acetate in hexane) to give the product **10b** as a colourless oil (1.06 g, 61%); ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.50-3.36 (4H, m, 2 x OCH_2), 2.21 (2H, m, αCH_2), 1.95 (1H, ddd, *J* = 3.1 Hz, 2.2 Hz, 1.0 Hz, HC≡C), 1.65-1.48 (8H, m, 4 x CH_2), 1.46-1.20 (8H, m, 4 x CH_2), 0.94 (3H, dt, *J* = 7.4 Hz, 1.1 Hz, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 84.75 (CH-C), 72.55 (OCH_2), 70.94 (OCH_2), 68.01 (CH-C), 29.78, 29.69, 29.42, 29.03, 28.72, 28.48, 26.17, 22.94 (8 x CH_2), 18.38 (αCH_2), 10.57 (CH_3).

11-butyloxy-1-undecyne (10c). The procedure (B) was carried out using butanol (0.77mL, 8.44 mmol) and 10-undecynyl mesylate **9a** (1.60g, 6.49 mmol). The crude was purified by column chromatography (5% ethyl acetate in hexane) to give the product **10c** as a colourless oil (0.83 g, 57%); ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.47-3.38 (4H, m, 2 x OCH_2), 2.20 (2H, m, αCH_2), 1.95 (1H, t, *J* = 2.6 Hz, HC≡C), 1.65-1.26 (18H, m, 9 x CH_2), 0.94 (3H, t, *J* = 6.5 Hz, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 84.75 (HC≡C), 70.93 (OCH_2), 70.63 (OCH_2), 68.01 (HC≡C), 31.88, 29.77, 2 x 29.42 (double intensity), 29.03, 28.72, 28.48, 26.17, 19.37 (9 x CH_2), 18.38 (αCH_2), 13.91 (CH_3).

11-pentyloxy-1-undecyne (10d). The procedure B was carried out using pentanol (0.84 mL, 7.79 mmol) and 10-undecynyl mesylate **9a** (1.60 g, 6.49 mmol). The crude was purified by column chromatography (5% ethyl acetate in hexane) to give the product **10d** as a colourless oil (1.00 g, 66%); ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.48 (4H, m, 2 x OCH_2), 2.20 (2H, dt, *J* = 7.1 Hz, 2.7 Hz, αCH_2), 1.95 (1H, t, *J* = 2.7 Hz, HC≡C), 1.67-1.22 (20H, m, 10 x CH_2), 0.92 (3H, t, *J* = 7.3 Hz, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 84.75

(HC≡C), 70.96 (OCH_2), 70.94 (OCH_2), 68.01 (HC≡C), 31.59, 29.78, 29.49, 29.36, 29.04, 28.73, 28.49, 28.39, 26.17, 22.65 (10 x CH_2) 18.39 (αCH_2), 14.03 (CH_3).

11-hexyloxy-1-undecyne (10e). The procedure B was carried out using hexanol (0.98 mL, 7.79 mmol) and 10-undecynyl mesylate **9a** (1.60 g, 6.49 mmol). The crude was purified by column chromatography (5% ethyl acetate in hexane) to give the product **10e** as a colourless oil (0.98 g, 60%); ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.41 (4H, t, J = 6.7 Hz, 2 x OCH_2), 2.19 (2H, dt, J = 7.1 Hz, 2.6 Hz, αCH_2), 1.95 (1H, t, J = 2.7 Hz, HC≡C), 1.65-1.24 (22H, m, 11 x CH_2), 0.92 (3H, t, J = 7.2 Hz, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 84.75 (HC≡C), 70.97 (OCH_2), 70.93 (OCH_2), 68.02 (HC≡C), 31.73, 29.78, 2 x 29.76 (double intensity), 29.43, 29.03, 28.73, 28.48, 26.18, 25.88, 22.63 (11 x CH_2), 18.39 (αCH_2), 14.03 (CH_3).

11-(*iso*-propyloxy)-1-undecyne (10f). The procedure B was carried out using *iso*-propanol (0.372 mL, 4.86 mmol) and 10-undecynyl mesylate **9a** (1.00 g, 4.06 mmol). The mixture was heated for total of 24 h at 70 °C, with further 0.75 eq. of NaH added after the first 7 h. After treatment, the crude was purified by column chromatography (5% ethyl acetate in hexane) to give the product **10f** as a colourless oil (0.484 g, 56%); ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.56 (1H, m, J = 6.1 Hz, CH), 3.41 (2H, t, J = 6.8 Hz, OCH_2), 2.20 (2H, dt, J = 7.1 Hz, 2.7 Hz, αCH_2), 1.96 (1H, t, J = 2.7 Hz, HC≡C), 1.58-1.52 (4H, m, 2 x CH_2), 1.44-1.29 (10H, m, 5 x CH_2), 1.18 (3H, s, CH_3), 1.17 (3H, s, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 84.81 (HC≡C), 71.23 (OCH), 68.23 (OCH_2), 68.02 (HC≡C), 30.18, 2 x 29.44 (double intensity), 29.04, 28.73, 28.48, 26.18 (7 x CH_2), 22.16 (double intensity, 2 x CH_3), 18.39 (αCH_2).

11-(*sec*-butyloxy)-1-undecyne (10g). The procedure B was carried out using *sec*-butanol (0.447 mL, 4.87 mmol) and 10-undecynyl mesylate **9a** (1.00 g, 4.06 mmol). The crude was purified by column chromatography (5% ethyl acetate in hexane) to give the product **10g** as a colourless oil (0.372 g, 38%); ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.50-3.29 (3H, m, CH_2OCH), 2.20 (2H, dt, J = 7.1 Hz, 2.6 Hz, αCH_2), 1.96 (1H, t, J = 2.7 Hz, HC≡C), 1.62-1.27 (16H, m, 8 x CH_2), 1.14 (3H, d, J = 6.1 Hz, CHC$\underline{H}_3$), 0.92 (3H, t, J = 7.4 Hz, $CH_2C\underline{H}_3$); ^{13}C NMR (125 MHz, $CDCl_3$): δ 84.79 (HC≡$\underline{C}$), 76.59 (OCH), 68.47 (OCH_2), 68.01 (H$\underline{C}$≡C), 30.21, 29.44 (double intensity), 29.26, 29.04, 28.74, 28.49, 26.18 (8 x CH_2), 19.30 (CH$\underline{C}H_3$), 18.39 (αCH_2), 9.86 ($CH_2\underline{C}H_3$).

11-(*iso*-butyloxy)-1-undecyne (10h). The procedure B was carried out using *iso*-butanol (0.450 mL, 4.87 mmol) and 10-undecynyl mesylate **9a** (1.00 g, 4.06 mmol). The mixture was heated for total of 28 h at 70 °C, with further 0.75 eq. of NaH and 0.6 eq. of *iso*-butanol added after the first 18 h. After the treatment, the crude was purified by column chromatography (5% ethyl acetate in hexane) to give the product **10h** as a light yellow oil (0.480 g, 52%); ^{1}H NMR (500 MHz; $CDCl_3$): δ 3.41 (2H, t, J = 6.7 Hz, $CH_2C\underline{H}_2O$), 3.19 (2H, d, J = 6.7 Hz, $OC\underline{H}_2CH$), 2.20 (2H, dt, J = 7.1 Hz, 2.6 Hz, αCH_2), 1.96 (1H, t, J = 2.7 Hz, HC≡C), 1.92-1.85 (1H, m, CH), 1.59-1.53 (4H, m, 2 x CH_2), 1.36-1.28 (13H, m, 2 x CH_2), 0.93 (3H, s, CH_3), 0.91 (3H, s, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 84.80 (HC≡$\underline{C}$), 77.86 ($O\underline{C}H_2CH$), 71.07 ($O\underline{C}H_2CH_2$), 68.03 (H$\underline{C}$≡C), 29.76, 2 x 29.44 (double intensity), 29.05, 28.75, 28.50, 28.45, 26.15, 25.89, 22.63 (10 x CH_2), 18.39 (αCH_2), 14.04 (CH_3).

11-(*tert*-butyloxy)-1-undecyne (10i). The procedure B was carried out using *tert*-butanol (0.925 mL, 9.74 mmol) and 10-undecynyl mesylate **9a** (2.00 g, 8.12 mmol). The mixture

was heated for total of 28 h at 70 °C, with further 0.75 eq. of NaH added after the first 18 h. After treatment, the crude was purified by column chromatography (5% ethyl acetate in hexane) to give the product **10i** as a colourless oil (0.478 g, 26%); ^{1}H NMR (500 MHz; $CDCl_3$): δ 3.41 (2H, t, J = 6.7 Hz, OCH_2), 2.21 (2H, dt, J = 7.1 Hz, 2.7 Hz, αCH_2), 1.96 (1H, t, J = 2.7 Hz, HC≡C), 1.58-1.52 (4H, m, 2 x CH_2), 1.41-1.28 (10H, m, 5 x CH_2), 1.21 (9H, s, 3 x CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 84.81 (HC≡C), 72.97 ($OC(CH_3)_3$), 70.97 (OCH_2), 68.05 (HC≡C), 29.79, 2 x 29.45 (double intensity), 29.06, 28.76, 28.50, (6 x CH_2), 27.61 (3 x CH_3), 26.20, 18.42 (2 x CH_2).

10-hexyloxy-1-decyne (10j). The procedure B was carried out using hexanol (1.2mL, 9.29 mmol) and 9-decynyl mesylate **9b** (1.8 g, 7.74 mmol). The mixture was heated for a total of 55 h at 70 °C, with further 1.2 eq. of NaH added after the first 19 h and again 0.5 eq. of NaH and 0.5 eq. of 9-decynyl mesylate **9b** added after 40 h. After treatment, the crude was purified by column chromatography (5% ethyl acetate in hexane) to obtain the product **10j** as a pale brown oil (0.572 g, 35%); ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.41 (4H, t, J = 6.6 Hz, 2 x OCH_2), 2.20 (2H, dt, J = 7.1 Hz, 2.6 Hz, αCH_2), 1.96 (1H, t, J = 2.7 Hz, HC≡C), 1.70-1.26 (20H, m, 10 x CH_2), 0.92 (3H, t, J = 7.2 Hz, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 84.75 (HC≡C), 70.98 (OCH_2), 70.91 (OCH_2), 68.04 (HC≡C), 31.73, 2 x 29.76 (double intensity), 29.34, 29.05, 28.70, 28.47, 26.15, 25.89, 22.63 (10 x CH_2), 18.39 (αCH_2), 14.04 (CH_3).

15-methoxy-1-pentadecyne (10k). The procedure B was carried out using methanol (0.36mL, 8.76 mmol) and 14-pentadecynyl mesylate **9c** (2.04 g, 6.74 mmol) at 60 ^{o}C for 4 h. Some methanol (1.3 eq.) and NaH (1.35 eq.) were then added and the mixture was heated further 3 h at 60 ^{o}C. After treatment, the crude was purified by column

chromatography (5% ethyl acetate in hexane) to obtain the product **10k** as a colourless oil (1.11 g, 69%); ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.40 (2H, *J* = 6.7 Hz, OCH_2), 3.35 (3H, s, CH_3), 2.20 (2H, dt, *J* = 7.1 Hz, 2.7 Hz, $CH_2C{\equiv}C$), 1.95 (1H, t, *J* = 2.7 Hz, $HC{\equiv}C$), 1.64-1.50 (6H, m, 3 x CH_2), 1.46-1.23 (16H, m, 8 x CH_2); ^{13}C NMR (125 MHz, $CDCl_3$): δ 84.79 ($HC{\equiv}\underline{C}$), 72.98 (OCH_2), 68.01 ($H\underline{C}{\equiv}C$), 58.51 (CH_3), 31.58, 2 x 29.66 (double intensity), 29.61, 29.58, 29.50, 29.11, 28.76, 28.50, 26.14, 22.65 (11 x CH_2), 18.40 ($\underline{C}H_2C{\equiv}C$).

3-tridecyloxy-1-propyne (12a). The procedure B was carried out using 2-propyn-1-ol (0.56 g, 10.00 mmol) and tridecyl mesylate **11a** (4.2 g, 15.0 mmol)**.** The mixture was heated for a total of 36 h at 60 °C with a further 1.0 eq. of NaH added after the first 24 h. After treatment, the crude was purified by column chromatography (5% ethyl acetate in hexane) to give the desired compound as a pale brown oil (0.90 g, 22%); ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.17 (2H, d, *J* = 2.6 Hz, $OCH_2C{\equiv}C$), 3.54 (2H, t, *J* = 7.1 Hz, $OC\underline{H}_2CH_2$), 2.43 (1H, t, *J* = 2.6 Hz, $HC{\equiv}C$), 1.62 (2H, m, $OCH_2C\underline{H}_2$), 1.62-1.22 (20H, m, 10 x CH_2), 0.91 (3H, t, *J* = 7.1 Hz, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 80.09 ($HC{\equiv}\underline{C}$), 73.97 ($H\underline{C}{\equiv}C$), 70.32 ($O\underline{C}H_2CH_2$), 57.99 ($O\underline{C}H_2C{\equiv}C$), 31.92, 29.67, 29.65, 29.60, 29.57, 29.52, 29.44, 29.35, 26.21, 26 09 (10 × CH_2), 22.68 (αCH_2), 14.10 (CH_3).

6-decyloxy-1-hexyne (12b). The procedure B was carried out using hexyn-1-ol (0.66 mL, 6.11 mmol) and decyl mesylate **11b** (2.17 g, 9.17 mmol, 1.5 eq.). The mixture was heated for a total of 90 h at 60 °C, with a further 1 eq. of NaH added after the first 24 h and another 1 eq. of NaH and 1 eq. of decyl mesylate added after 48 h. After treatment, the crude was purified by column chromatography (5% ethyl acetate in hexane) to obtain the desired compound as a brown oil (1.2 g, 82%); ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.47-

3.36 (4H, m, 2 x OCH_2), 2.24 (2H, dt, J = 7.0 Hz, J = 2.6 Hz, CH_2), 1.96 (1H, t, J = 2.6 Hz, HC≡C), 1.76-1.53 (6H, m, 3 x CH_2), 1.38-1.21 (14H, m, 7 x CH_2), 0.89 (3H, t, J = 7.0 Hz, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 84.36 (HC≡C), 71.01 (OCH_2), 70.17 (OCH_2), 68.29 (HC≡C), 31.90, 29.80, 29.32, 29.11, 28.81, 26.20, 25.28, 24.85, 22.67, 22.64 (10 × CH_2), 18.23 (αCH_2), 14.08 (CH_3).

General procedure C: synthesis of 3-(2',3'-dideoxy-ribo-β-L-furanosyl)-6-substituted-furo[2,3-d]pyrimidin-2(3*H*)-one analogues (8a-u).

To a solution of L-5-iodo-2',3'-dideoxyuridine **7** in DMF (5.5 mL/mmol) was added the alkyne (3 eq.), Tetrakis (triphenylphosphine) Pd(0) (0.1 eq.), CuI (0.2 eq.) and DIPEA (2 eq.). The mixture was stirred at room temperature for 16 h and further CuI (0.2 eq.) and triethylamine (5.5 mL/mmol) were added. The mixture was stirred at 80 °C for 8 h. The solvent was removed under high vacuum and the residue was purified by flash column chromatography.

3-(2',3'-dideoxy-ribo-β-L-furanosyl)-6-hexylfuro[2,3-*d*]pyrimidin-2(3*H*)-one (8a).

The procedure C was carried out using 1-octyne (0.29 g, 2.66 mmol), which gave the desired alkyne intermediate (0.130 g, 0.374 mmol, 34%). This compound was dissolved in DMF (5 mL) and to this solution Et_3N (5 mL) and CuI (0.014 g, 0.075 mmol, 0.2 eq) were added. The resulting solution was heated at 80 ^{0}C for 8h. Solvent was removed under high vacuum. The product **8a** was then obtained as an off-white solid (0.026 g, 22%) after purification by preparative thin layer chromatography using 20% chloroform in ethyl acetate as the eluent; ^{1}H NMR (500 MHz, $CDCl_3$): δ 876 (1H, s, H-4), 6.20 (1H, m, H-1'), 6.15 (1H, s, H-5), 4.31 (1H, m, H-4'), 4.16 (1H, ddd, J = 11.9 Hz, 4.2 Hz, 2.9 Hz, H-5'), 3.89 (1H, ddd, J = 11.9 Hz, 3.0 Hz, H-5'), 2.69-2.54 (3H, m, CH_2, H-2'), 2.25-

2.16 (1H, m, H-2'), 1.99-1.90 (2H, m, H-3'), 1.72 (2H, m, J = 7.1 Hz, CH_2), 1.41-1.22 (6H, m, 3 x CH_2), 0.90 (3H, t, J = 6.3 Hz, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 171.74 (C-7a), 159.47 (C-6), 155.05 (C-2), 135.98 (C-4), 107.25 (C-4a), 99.10 (C-5), 89.00 (C-1'), 82.93 (C-4'), 62.81 (C-5'), 33.79 (C-2'), 31.43, 28.69, 28.25 (3 x CH_2), 26.78 (C-3'), 23.89, 22.50 (2 x CH_2), 14.01 (CH_3); MS (ES^+) m/z 321 ($M+H^+$). Accurate mass: $C_{17}H_{25}N_2O_4$ requires 321.1814; found 321.1824. Reverse HPLC eluting with H_2O/MeOH (gradient from 100:0 to 0:100 in 25 min), t_R = 21.81 min.

3-(2',3'-dideoxy-ribo-β-L-furanosyl)-6-septylfuro[2,3-*d*]pyrimidin-2(3*H*)-one (8b). The procedure C was carried out using 1-nonyne (0.275 g, 2.22 mmol), which gave, after purification by flash column chromatography (5% methanol in ethyl acetate), the product **8b** as an off-white solid (0.128 g, 53%); ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.84 (1H, s, H-4), 6.20 (1H, dd, J = 6.7 Hz, 2.5 Hz, H-1'), 6.16 (1H, s, H-5), 4.30 (1H, m, H-4'), 4.18 (1H, m, H-5'), 3.91 (1H, m, H-5'), 3.16 (1H, t, J = 5.4 Hz, OH), 2.70-2.54 (3H, m, αCH_2, H-2'), 2.21 (1H, m, H-2'), 1.97 (2H, m, H-3'), 1.69 (2H, m, J = 7.1 Hz, CH_2), 1.44-1.22 (8H, m, 4 x CH_2), 0.90 (3H, t, J = 6.5 Hz, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 171.72 (C-7a), 159.43 (C-6), 155.06 (C-2), 136.12 (C-4), 107.25 (C-4a), 99.15 (C-5), 89.00 (C-1'), 83.01(C-4'), 62.74 (C-5'), 33.81 (C-2'), 31.68, 28.98, 28.91, 28.25 (4 x CH_2), 26.83 (C-3'), 23.87, 22.59 (2 x CH_2), 14.04 (CH_3); MS (ES^+) m/z 335 (100%, $M+H^+$), 373 (9%, $M+K^+$), 398 (81%, $M+CH_3CN+Na^+$). Accurate mass: $C_{18}H_{27}N_2O_4$ requires 335.1971; found 335.1958. Reverse HPLC eluting with H_2O/MeOH (gradient from 100:0 to 10:90 in 15 min and with 10:90 for 10 min), t_R = 17.91 min.

3-(2',3'-dideoxy-ribo-β-L-furanosyl)-6-octylfuro[2,3-*d*]pyrimidin-2(3*H*)-one (8c). The procedure C was carried out using 1-decyne (0.31 g, 2.22 mmol). After purification by

flash column chromatography (2% methanol in ethyl acetate) to obtain a pale brown oil and trituration with ethyl acetate, the product **8c** was obtained as an off-white solid (0.060 g; 23%); ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.83 (1H, s, H-4), 6.42 (1H, s, H-5), 5.97 (1H, m, H-1'), 5.15 (1H, t, J = 5.1 Hz, OH), 4.14 (1H, m, H-4'), 3.80 (1H, m, H-5'), 3.60 (1H, m, H-5'), 2.61 (2H, t, J = 7.2 Hz, αCH$_2$), 2.42 (1H, m, H-2'), 1.97 (1H, m, H-2'), 1.88-1.56 (2H, m, H-3'), 1.6 (2H, m, J = 7.5 Hz, CH$_2$), 0.84 (3H, t, J = 6.7 Hz, CH$_3$); ^{13}C NMR (125 MHz, DMSO-d_6): δ 170.99 (C-7a), 158.01 (C-6), 153.76 (C-2), 137.00 (C-4), 107.33 (C-4a), 99.72 (C-5), 88.02 (C-1'), 83.00 (C-4'), 61.33 (C-5'), 33.03 (C-2'), 31.16, 28.55, 28.50, 28.29, 27.29 (5 x CH$_2$), 26.35 (C-3'), 23.61, 22.00 (2 x CH$_2$), 13.88 (CH$_3$); MS (ES$^+$) m/z 349 (M+H$^+$). Accurate mass: $C_{19}H_{29}N_2O_4$ requires 349.2127; found 349.2141. Reverse HPLC eluting with H$_2$O/MeOH (gradient from 100:0 to 0:100 in 25 min and then at 0:100 for 5 min), t_R = 26.04 min.

3-(2',3'-dideoxy-ribo-β-L-furanosyl)-6-nonylfuro[2,3-*d*]pyrimidin-2(3*H*)-one (8d). The procedure C was carried out using 1-undecyne (0.405 g, 2.66 mmol), which gave, after purification by flash column chromatography (40% dichloromethane in ethyl acetate), the product **8d** as an off-white solid (0.150 g, 47%); ^{1}H NMR (500 MHz, CDCl$_3$): δ 8.76 (1H, s, H-4), 6.20 (1H, dd, J = 6.8 Hz, J = 2.6 Hz, H-1'), 6.15 (1H, s, H-5), 4.31 (1H, m, H-4'), 4.17 (1H, m, H-5'), 3.91 (1H, m, H-5'), 2.83 (1H, t, J = 5.5 Hz, OH), 2.70-2.55 (3H, m, αCH$_2$, H-2'), 2.24 (1H, m, H-2'), 1.95 (2H, m, H-3'), 1.70 (2H, m, J = 7.2 Hz, CH$_2$), 1.45-1.21 (12H, m, 6 x CH$_2$), 0.90 (3H, t , J = 6.7 Hz, CH$_3$); ^{13}C NMR (125 MHz, CDCl$_3$): δ 171.75 (C-7a), 159.49 (C-6), 155.02 (C-2), 135.89 (C-4), 107.22 (C-4a), 99.07(C-5), 88.99 (C-1'), 82.88 (C-4'), 62.87 (C-5'), 33.78 (C-2'), 31.84, 29.45, 29.26, 29.04, 28.26 (5 x CH$_2$), 26.83 (C-3'), 23.91, 22.64 (2 x CH$_2$), 14.08 (CH$_3$);

MS (ES^+) m/z 363 (53%, $M+H^+$), 426 (80%, $M+CH_3CN+Na^+$). Accurate mass: $C_{20}H_{31}N_2O_4$ requires 363.2284; found 363.2289. Reverse HPLC eluting with H_2O/MeOH (gradient from 100:0 to 10:90 in 15 min and then at 10:90 for 10 min), t_R = 21.57 min.

3-(2',3'-dideoxy-ribo-β-L-furanosyl)-6-decylfuro[2,3-*d*]pyrimidin-2(3*H*)-one (8e). The procedure C was carried out using 1-dodecyne (0.516 g, 3.11 mmol), which gave, after purification by flash column chromatography (40% dichloromethane in ethyl acetate), the product **8e** as an off-white solid (0.100 g, 33%); ^1H NMR (500 MHz, $CDCl_3$): δ 8.75 (1H, s, H-4), 6.21 (1H, m, H-1'), 6.15 (1H, s, H-5), 4.32 (1H, m, H-4'), 4.17 (1H, m, H-5'), 3.90 (1H, m, H-5'), 2.86 (1H, t, *J* = 5.4 Hz, OH), 2.68-2.57 (3H, m, αCH_2, H-2'), 2.21 (1H, m, H-2'), 1.96 (2H, m, H-3'), 1.72 (2H, m, *J* = 7.0 Hz, CH_2), 1.44-1.21 (14H, m, 7 x CH_2), 9.00 (3H, t, *J* = 6.4 Hz, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 171.75 (C-7a), 159.49 (C-6), 155.03 (C-2), 135.90 (C-4), 107.23 (C-4a), 99.07 (C-5), 88.99 (C-1'), 82.88 (C-4'), 62.86 (C-5'), 33.78 (C-2'), 31.87, 29.56, 29.50, 29.29, 29.27, 29.05, 28.26 (7 x CH_2), 26.83 (C-3'), 23.91, 22.66 (2 x CH_2), 14.09 (CH_3); MS (ES^+) m/z 377 (16%, $M+H^+$), 399 (6%, $M+Na^+$), 415 (8%, $M+K^+$), 440 (100%, $M+CH_3CN+Na^+$). Accurate mass: $C_{21}H_{33}N_2O_4$ requires 377.2440; found 377.2426. Reverse HPLC eluting with H_2O/MeOH (gradient from 100:0 to 0:100 in 30 min and then at 0:100 for 5 min), t_R = 33.00 min.

3-(2',3'-dideoxy-ribo-β-L-furanosyl)-6-dodecylfuro[2,3-*d*]pyrimidin-2(3*H*)-one (8f). The procedure C was carried out using 1-tetradecyne (0.345 g, 1.77 mmol), which gave, after purification by flash column chromatography (40% dichloromethane in ethyl acetate), the product **8f** as an off-white solid (0.100 g, 42%); ^1H NMR (500 MHz, $CDCl_3$): δ 8.77 (1H, s, H-4), 6.23 (1H, dd, *J* = 6.6 Hz, 2.5 Hz, H-1'), 6.16 (1H, s, H-5),

4.32 (1H, m, H-4'), 4.18 (1H, m, H-5'), 3.90 (1H, m, H-5'), 2.90 (1H, br s, OH), 2.67-2.56 (3H, m, αCH$_2$, H-2'), 2.21 (1H, m, H-2'), 1.94 (2H, m, H-3'), 1.69 (2H, m, *J* = 7.5 Hz, CH$_2$), 1.43-1.20 (18H, m, 9 x CH$_2$), 9.00 (3H, t, *J* = 6.5 Hz, CH$_3$); ^{13}C NMR (125 MHz, CDCl$_3$): δ 171.74 (C-7a), 159.48 (C-6), 155.03 (C-2), 135.95 (C-4), 107.24 (C-4a), 99.08 (C-5), 88.99 (C-1'), 82.91 (C-4'), 62.83 (C-5'), 33.79 (C-2'), 31.90, 29.64, 29.61, 29.51, 29.33, 29.28, 29.06, 28.26 (9 x CH$_2$), 26.84 (C-3'), 23.90, 22.67 (2 x CH$_2$), 14.10 (CH$_3$); MS (ES$^+$) m/z 405 (M+H$^+$). Accurate mass: $C_{23}H_{37}N_2O_4$ requires 405.2753; found 405.2739. Reverse HPLC eluting with H$_2$O/MeOH (gradient from 100:0 to 10:90 in 15 min, then at 10:90 for 10 min and another gradient from 10:90 to 0:100 in 5 min), t_R = 28.99 min.

3-(2',3'-dideoxy-ribo-β-L-furanosyl)-6-(4-*n*-butylphenyl)-furo[2,3-*d*]pyrimidin-2(3*H*)-one (8g). The procedure C was carried out using 4-*n*-butylphenylacetylene (0.351 g, 0.39 mL, 2.22 mmol). The obtained residue was purified by column chromatography (5% methanol in dichloromethane) to give the product **8g** as an off-white solid (0.110 g, 40%); ^{1}H NMR (500 MHz, CDCl$_3$): δ 8.90 (1H, s, H-4), 7.63 (2H, d, *J* = 8.2 Hz, *Ph*), 7.22 (2H, d, *J* = 8.3 Hz, *Ph*), 6.71 (1H, s, H-5), 6.25 (1H, dd, *J* = 6.7 Hz, 2.3 Hz, H-1'), 4.32 (1H, m, H-4'), 4.2 (1H, m, H-5'), 3.92 (1H, m, H-5'), 2.92 (1H, t, *J* = 5.0 Hz, OH), 2.70-2.59 (3H, m, αCH$_2$, H-2'), 2.25 (1H, m, H-2'), 2.00 (2H, m, H-3'), 1.65 (2H, m, CH$_2$), 1.39 (2H, m, CH$_2$), 0.98 (3H, t, *J* = 6.6 Hz, CH$_3$); ^{13}C NMR (125 MHz, CDCl$_3$): δ 171.58 (C-7a), 155.58 (C-6), 155.00 (C-2), 144.80 (C-*para Ph*), 136.79 (C-4), 128.96 (*Ph*), 125.98 (C-*ipso Ph*), 124.82 (*Ph*), 107.81 (C-4a), 97.22 (C-5), 89.11 (C-1'), 83.03 (C-4'), 62.79 (C-5'), 35.53 (CH$_2$), 33.85 (C-2'), 33.36 (CH$_2$), 23.83 (C-3'), 22.32 (CH$_2$), 13.91 (CH$_3$); MS (ES$^+$) m/z 369 (44%, M+H$^+$), 407 (70%, M+K$^+$), 432 (50%,

M+CH_3CN+Na^+). Accurate mass: $C_{21}H_{25}N_2O_4$ requires 369.1814; found 369.1830. Reverse HPLC eluting with H_2O/MeOH (gradient from 100:0 to 0:100 in 35 min), t_R = 33.29 min.

3-(2',3'-dideoxy-ribo-β-L-furanosyl)-6-(4-*n*-pentylphenyl)-furo[2,3-*d*]pyrimidin-2(3*H*)-one (8h). The procedure C was carried out using 4-*n*-pentylphenylacetylene (0.382 g, 0.43 mL, 2.18 mmol). The obtained residue was purified by column chromatography (5% methanol in dichloromethane) to give the product **8h** as an off-white solid (0.102 g, 36%); ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.80 (1H, s, H-4), 7.68 (2H, d, *J* = 8.2 Hz, *Ph*), 7.26 (2H, d, *J* = 8.3 Hz, *Ph*), 6.70 (1H, s, H-5), 6.26 (1H, dd, *J* = 6.67 Hz, 2.26 Hz, H-1'), 4.35 (1H, m, H-4'), 4.20 (1H, m, H-5'), 3.90 (1H, m, H-5'), 2.65 (3H, m, αCH_2, H-2'), 2.30-2.18 (2H, m, H-2', OH), 1.95 (2H, m, H-3'), 1.61 (2H, m, CH_2), 1.42-1.29 (4H, m, 2 x CH_2), 0.91 (3H, t, *J* = 6.5 Hz, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.10 (C-7a), 156.00 (C-6), 155.50 (C-2), 144.91 (C-*para Ph*), 136.36 (C-4), 128.99 (*Ph*), 126.00 (C-*ipso Ph*), 124.87 (*Ph*), 125.98, 106.60 (C-4a), 97.04 (C-5), 89.10 (C-1'), 82.76 (C-4'), 63.04 (C-5'), 35.83 (C-2'), 33.78, 31.45, 30.92 (3 x CH_2), 23.91 (C-3'), 22.51 (CH_2), 13.99 (CH_3); MS (ES^+) m/z 383 (14%, M+H^+), 405 (3%, M+Na^+), 421 (7%, M+K^+), 446 (62%, M+CH_3CN+Na^+). Accurate mass: $C_{22}H_{27}N_2O_4$ requires 383.1971; found 383.1988. Reverse HPLC eluting with H_2O/MeOH (gradient from 100:0 to 10:90 in 15 min and then at 10:90 for 10 min), t_R = 21.00 min.

3-(2',3'-dideoxy-ribo-β-L-furanosyl)-6-ethyloxynonylfuro[2,3-*d*]pyrimidin-2(3*H*)-one (8i). The procedure C was carried out using 11-ethyloxy-1-undecyne **10a** (0.348 g, 1.775 mmol). The obtained residue was purified by column chromatography (4% methanol in ethyl acetate) to give the product **8i** as an off-white solid (0.102 g, 42%); ^{1}H NMR (500

MHz, $CDCl_3$): δ 8.71 (s, 1H, H-4), 6.21 (1H, dd, *J* = 6.7 Hz, 2.2 Hz, H-1'), 6.13 (1H, s, H-5), 4.33-4.28 (1H, m, H-4'), 4.18-4.43 (1H, m, H-5'), 3.86 (1H, dt, *J* = 11.9 Hz, 3.8 Hz, H-5'), 3.49 (2H, q, *J* = 7.0 Hz, $CH_3C\underline{H_2}O$), 3.42 (2H, t, *J* = 6.8 Hz, $CH_2C\underline{H_2}O$), 2.65 (2H, t, *J* = 7.4 Hz, αCH_2), 2.63-2.58 (1H, m, H-2'), 2.53 (1H, t, *J* = 4.7 Hz, OH), 2.26-2.19 (1H, m, H-2'), 1.99-1.90 (2H, m, H-3'), 1.72-1.65 (2H, m, βCH_2), 1.62-1.54 (2H, m, $C\underline{H_2}CH_2O$), 1.40-1.25 (10H, m, 5 x CH_2), 1.22 (3H, t, *J* = 7.0 Hz, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 171.79 (C-7a), 159.47 (C-6), 154.98 (C-2), 135.70 (C-4), 107.15 (C-4a), 99.02 (C-5), 88.98 (C-1'), 82.76 (C-4'), 70.77 ($CH_2\underline{C}H_2O$), 66.06 ($CH_3\underline{C}H_2O$), 62.93 (C-5') 33.76 (C-2'), 29.69, 29.39, 29.35, 29.09, 28.88, 28.21, 26.73, 26.15 (8 x CH_2), 23.91(C-3'), 15.24 (CH_3); MS (ES^+) m/z 429 ($M+Na^+$). Accurate mass: $C_{22}H_{34}N_2O_5Na$ requires 429.2365; found 429.2365. Reverse HPLC eluting with H_2O/MeOH (gradient from 100:0 to 0:100 in 35 min and then at 0:100 for 10 min), t_R = 33.25 min.

3-(2',3'-dideoxy-ribo-β-L-furanosyl)-6-propyloxynonylfuro[2,3-*d*]pyrimidin-2(3*H*)-one (8j). The procedure C was carried out using 11-propyloxy-1-undecyne **10b** (0.466 g, 2.218 mmol). The obtained residue was purified by column chromatography (4% methanol in ethyl acetate) to give the product **8j** as an off-white solid (0.105 g, 34%); ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.84 (1H, s, H-4), 6.18 (1H, dd, *J* = 6.5 Hz, 2.0 Hz, H-1'), 6.15 (1H, s, H-5), 4.31-4.26 (1H, m, H-4'), 4.18-4.10 (1H, m, H-5'), 3.87 (1H, dt, *J* = 12.0 Hz, 3.0 Hz, H-5'), 3.50 (1H, m, OH), 3.40 (2H, t, *J* = 6.7 Hz, OCH_2), 3.40 (2H, t, *J* = 6.8 Hz, OCH_2), 2.63-2.54 (3H, m, αCH_2, H-2'), 2.21-2.16 (1H, m, H-2'), 2.02-1.89 (2H, m, H-3'), 1.69-1.50 (6H, m, 3 x CH_2), 1.40-1.21 (10H, m, 5 x CH_2), 0.92 (3H, t, *J* = 7.4 Hz, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 171.68 (C-7a), 159.33 (C-6), 155.10 (C-2), 136.32 (C-4), 107.24 (C-4a), 99.21 (C-5), 89.00 (C-1'), 83.10 (C-4'), 72.55 (OCH_2),

70.88 (OCH_2), 62.57 (C-5'), 33.82 (C-2'), 29.74, 29.39 (double intensity), 29.13, 28.93, 28.20, 26.76, 26.13 (8 x CH_2), 23.83 (C-3'), 22.91 (CH_2), 10.56 (CH_3); MS (ES^+) m/z 443 ($M+Na^+$). Accurate mass: $C_{23}H_{36}N_2O_5$ requires 443.2522; found 443.2539. Reverse HPLC eluting with H_2O/MeOH (gradient from 100:0 to 0:100 in 35 min and then at 0:100 for 10 min), t_R = 34.04 min.

3-(2',3'-dideoxy-ribo-β-L-furanosyl)-6-butyloxynonylfuro[2,3-*d*]pyrimidin-2(3*H*)-one (8k). The procedure C was carried out using 11-butyloxy-1-undecyne **10c** (0.497 g, 2.217 mmol). The obtained residue was purified by column chromatography (4% methanol in ethyl acetate) to give the product **8k** as an off-white solid (0.090 g, 28%); ^1H NMR (500 MHz, $CDCl_3$): δ 8.76 (1H, s, H-4), 6.20 (1H, dd, *J* = 6.7 Hz, 2.4 Hz, H-1'), 6.14 (1H, s, H-5), 4.34-4.28 (1H, m, H-4'), 4.20-4.12 (1H, m, H-5'), 3.88 (1H, dt, *J* = 11.9 Hz, 3.5 Hz, H-5'), 3.41 (4H, q, *J* = 6.1 Hz, 2 x OCH_2), 2.88 (1H, t, *J* = 4.5 Hz, OH), 2.64 (2H, t, *J* = 7.3 Hz, αCH_2), 2.60 (1H, m, H-2'), 2.24-2.18 (1H, m, H-2'), 1.99-1.90 (2H, m, H-3'),1.71-1.61 (2H, m, βCH_2), 1.61-1.50 (4H, m, 2 x CH_2), 1.44-1.24 (12H, m, 6 x CH_2), 0.93 (3H, t, *J* = 7.4 Hz, CH_3); ^{13}C NMR ($CDCl_3$): δ 171.75 (C-7a), 159.42 (C-6), 155.02 (C-2), 135.94 (C-4), 107.18 (C-4a), 99.09 (C-5), 88.99 (C-1'), 82.90 (C-4'), 70.94 (OCH_2), 70.66 (OCH_2), 62.80 (C-5'), 33.79 (C-2'), 31.85, 29.76, 29.39, 29.37, 29.12, 28.92, 28.21, 26.75, 26.14 (9 x CH_2), 23.87 (C-3'), 19.36 (CH_2), 13.93 (CH_3); MS (ES^+) m/z 457 ($M+Na^+$). Accurate mass: $C_{24}H_{39}N_2O_5$ requires 435.2859; found 435.2839. Reverse HPLC eluting with H_2O/MeOH (gradient from 100:0 to 0:100 in 35 min and then at 0:100 for 10 min), t_R = 35.15 min.

3-(2',3'-dideoxy-ribo-β-L-furanosyl)-6-pentyloxynonylfuro[2,3-*d*]pyrimidin-2(3*H*)-one (8l). The procedure C was carried out using 11-pentyloxy-1-undecyne **10d** (0.465 g,

1.952 mmol). The obtained residue was purified by column chromatography (4% methanol in ethyl acetate) to give the product **8l** as an off-white solid (0.102 g, 35%); ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.76 (1H, s, H-4), 6.20 (1H, dd, *J* = 6.7 Hz, 2.5 Hz, H-1'), 6.14 (1H, s, H-5), 4.33-4.29 (1H, m, H-4'), 4.18-4.13 (1H, m, H-5'), 3.88 (1H, dt, *J* = 12.0 Hz, 3.9 Hz, H-5'), 3.41 (4H, dt, *J* = 6.7 Hz, 0.9 Hz, 2 x OCH_2), 2.90 (1H, br s, OH), 2.64 (2H, t, *J* = 7.5 Hz, αCH_2), 2.63-2.56 (1H, m, H-2'), 2.25-2.18 (1H, m, H-2'), 2.00-1.91 (2H, m, H-3'), 1.72-1.63 (2H, m, βCH_2), 1.61-1.55 (4H, m, 2 x CH_2), 1.35-1.28 (14H, m, 7 x CH_2), 0.91 (3H, t, *J* = 6.9 Hz, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 171.75 (C-7a), 159.41 (C-6), 155.01 (C-2), 135.96 (C-4), 107.18 (C-4a), 99.09 (C-5), 88.99 (C-1'), 82.91 (C-4'), 70.98 (OCH_2), 70.94 (OCH_2), 62.79 (C-5'), 33.79 (C-2'), 29.76, 29.45, 29.39, 29.38, 29.12, 28.92, 28.36, 28.21, 26.75, 26.14 (10 x CH_2), 23.87 (C-3'), 22.54 (CH_2), 14.04 (CH_3); MS (ES^+) m/z 449 (11%, $M+H^+$), 471 ($M+Na^+$). Accurate mass: $C_{25}H_{41}N_2O_5$ requires 449.3015; found 449.3033. Reverse HPLC eluting with H_2O/MeOH (gradient from 100:0 to 0:100 in 35 min), t_R = 26.13 min.

3-(2',3'-dideoxy-ribo-β-L-furanosyl)-6-hexyloxynonylfuro[2,3-*d*]pyrimidin-2(3*H*)-one (8m). The procedure C was carried out using 11-hexyloxy-1-undecyne **10e** (0.448 g, 1.775 mmol). The obtained residue was purified by column chromatography (4% methanol in ethyl acetate) to give the product **8m** as an off-white solid (0.120 g, 44%); ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.76 (1H, s, H-4), 6.21 (1H, dd, *J* = 6.7 Hz, 2.3 Hz, H-1'), 6.14 (1H, s, H-5), 4.33-4.29 (1H, m, H-4'), 4.19-4.13 (1H, m, H-5'), 3.87 (1H, dt, *J* = 12.0 Hz, 3.9 Hz, H-5'), 3.41 (4H, dt, *J* = 6.7 Hz, 1.3 Hz, 2 x OCH_2), 2.87 (1H, t, *J* = 4.9 Hz, OH), 2.64 (2H, t, *J* = 7.3 Hz, αCH_2), 2.63-2.57 (1H, m, H-2'), 2.24-2.19 (1H, m, H-2'), 1.98-1.92 (2H, m, H-3'), 1.71-1.65 (2H, m, βCH_2), 1.69-1.53 (4H, m, 2 x CH_2), 1.38-

1.26 (16H, m, 8 x CH_2), 0.90 (3H, t, J = 6.9 Hz, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 171.75 (C-7a), 159.41 (C-6), 155.01 (C-2), 135.93 (C-4), 107.17 (C-4a), 99.08 (C-5), 88.98 (C-1'), 82.89 (C-4'), 71.00 (OCH_2), 70.94 (OCH_2), 62.80 (C-5'), 33.79 (C-2'), 31.71, 29.76, 29.73, 29.39, 29.38, 29.12, 28.92, 28.21, 26.75, 26.15, 25.86 (11 x CH_2), 23.87 (C3'), 22.62 (CH_2), 14.03 (CH_3); MS (ES^+) m/z 463 (5%, $M+H^+$), 485 (100%, $M+Na^+$), 501 (8%, $M+K^+$). Accurate mass: $C_{26}H_{43}N_2O_5$ requires 463.3172; found 463.3163. Reverse HPLC eluting with H_2O/MeOH (gradient from 100:0 to 10:90 in 15 min, then at 10:90 for 10 min and another gradient from 10:90 to 0:100 in 5 min), t_R = 26.91 min.

3-(2',3'-dideoxy-ribo-β-L-furanosyl)-6-*iso*propyloxynonylfuro[2,3-*d*]pyrimidin-2(3*H*)-one (8n). The procedure C was carried out using 11-*iso*propyloxy-1-undecyne **10f** (0.280 g, 1.330 mmol). The obtained residue was purified by column chromatography (4% methanol in ethyl acetate) to give the product **8n** as an off-white solid (0.019 g, 10%); ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.80 (1H, s, H-4), 6.20 (1H, dd, J = 6.6 Hz, 2.2 Hz, H-1'), 6.14 (1H, s, H-5), 4.30 (1H, ddt, J = 9.2 Hz, 6.0 Hz, 3.0 Hz, H-4'), 4.16 (1H, d, J = 11.8 Hz, H-5'), 3.88 (1H, dd, J = 12.0 Hz, 3.2 Hz, H-5'), 3.55 (1H, m, J = 6.1 Hz, OCH), 3.40 (2H, t, J = 6.7 Hz, OCH_2), 3.16 (1H, br s, OH), 2.63 (2H, t, J = 7.5 Hz, αCH_2), 2.60-2.57 (1H, m, H-2'), 2.23-2.18 (1H, m, H-2'), 1.97-1.93 (2H, m, H-3'), 1.67 (2H, m, J = 7.2 Hz, βCH_2), 1.55 (2H, m, J = 7.0 Hz, $OCH_2C\underline{H_2}$), 1.38-1.26 (10H, m, 5 x CH_2), 1.17 (3H, s, CH_3), 1.15 (3H, s, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 171.72 (C-7a), 159.38 (C-6), 155.05 (C-2), 136.10 (C-4), 107.21 (C-4a), 99.15 (C-5), 89.00 (C-1'), 83.00 (C-4'), 71.29 (OCH), 68.24 (OCH_2), 62.68 (C-5'), 33.83 (C-2'), 30.16, 29.41, 29.39, 29.13, 28.92, 28.21, 26.75, 26.19 (8 x CH_2), 23.85 (C-3'), 22.19 (double intensity, 2 x CH_3); MS

(ES$^+$) m/z 443 (100%, M+Na$^+$), 863 (30%, MM+Na$^+$). Reverse HPLC eluting with H_2O/CH_3CN (elution with mixture 0:100 for 35 min, then gradient from 0:100 to 90:10 in 2 min and at 90:10 for 3 min), t_R = 36.18 min.

3-(2',3'-dideoxy-ribo-β-L-furanosyl)-6-*sec*butyloxynonylfuro[2,3-*d*]pyrimidin-2(3*H*)-one (8o). The procedure C was carried out using 11-*sec*butyloxy-1-undecyne **10g** (0.197 g, 0.877 mmol). The obtained residue was purified by column chromatography (4% methanol in ethyl acetate) to give the product **8o** as an off-white solid (0.032 g, 36%); ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.84 (1H, s, H-4), 6.19 (1H, dd, *J* = 6.6 Hz, 2.3 Hz, H-1'), 6.14 (1H, s, H-5), 4.33-4.29 (1H, m, H-4'), 4.16 (1H, d, *J* = 11.0 Hz, H-5'), 3.87 (1H, dt, *J* = 12.0 Hz, 3.9 Hz, H-5'), 3.49-3.28 (4H, m, OCH_2, OH, OCH), 2.63 (2H, t, *J* = 7.6 Hz, αCH_2), 2.59-2.56 (1H, m, H-2'), 2.23-2.18 (1H, m, H-2'), 2.02-1.90 (2H, m, H-3'), 1.69-1.63 (2H, m, βCH_2), 1.59-1.25 (14H, m, 7 x CH_2), 1.12 (3H, d, *J* = 6.1 Hz, CH_3), 0.09 (3H, t, *J* = 7.4 Hz, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 171.70 (C-7a), 159.33 (C-6), 155.08 (C-2), 136.28 (C-4), 107.21 (C-4a), 99.19 (C-5), 89.00 (C-1'), 83.09 (C-4'), 76.64 (OCH), 68.48 (OCH_2), 62.58 (C-5'), 33.86 (C-2'), 30.19, 29.41 (double intensity), 29.25, 29.12, 28.94, 28.22, 26.77, 26.22 (9 x CH_2), 23.82 (C-3'), 19.31 (CH_3), 09.87 (CH_3); MS (ES$^+$) m/z 457 (100%, M+Na$^+$), 891 (51%, MM+Na$^+$). Reverse HPLC eluting with H_2O/CH_3CN (mixture 0:100 for 35 min, then gradient from 0:100 to 90:10 in 2 min and with 90:10 for 3 min), t_R = 36.71 min.

3-(2',3'-dideoxy-ribo-β-L-furanosyl)-6-*iso*butyloxynonylfuro[2,3-*d*]pyrimidin-2(3*H*)-one (8p). The procedure C was carried out using 11-*iso*butyloxy-1-undecyne **10h** (0.297 g, 1.319 mmol). The obtained residue was purified by column chromatography (4% methanol in ethyl acetate) to give the product **8p** as an off-white solid (0.047 g, 41%); ^{1}H

NMR (500 MHz, $CDCl_3$): δ 8.69 (1H, s, H-4), 6.22 (1H, dd, *J* = 6.7 Hz, 2.5 Hz, H-1'), 6.13 (1H, s, H-5), 4.34-4.29 (1H, m, H-4'), 4.16 (1H, d, *J* = 11.5 Hz, H-5'), 3.88 (1H, d, *J* = 12.0 Hz, H-5'), 3.41 (2H, t, *J* = 6.7 Hz, OCH_2), 3.19 (2H, d, *J* = 6.7 Hz, OCH_2), 2.65 (2H, t, *J* = 7.6 Hz, αCH_2), 2.63-2.59 (1H, m, H-2'), 2.42 (1H, s, OH), 2.25-2.20 (1H, m, H-2'), 1.97-1.92 (2H, m, H-3'), 1.87 (1H, m, CH), 1.69 (2H, m, βCH_2), 1.58 (2H, m, $OCH_2C\underline{H_2}$), 1.36-1.31 (10H, m, 5 x CH_2), 0.92 (3H, s, CH_3), 0.91 (3H, s, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 171.79 (C-7a), 159.51 (C-6), 154.97 (C-2), 135.67 (C-4), 107.18 (C-4a), 99.01 (C-5), 88.99 (C-1'), 82.75 (C-4'), 77.87 (OCH_2), 71.07 (OCH_2), 62.98 (C-5'), 33.76 (C-2'), 29.74, 29.40 (double intensity), 29.14, 28.93 (5 x CH_2), 28.43 (CH), 28.24, 26.77, 26.16 (3 x CH_2), 23.93 (C-3'), 19.31 (double intensity, 2 x CH_3); Reverse HPLC eluting with H_2O/MeOH (gradient from 90:10 to 0:100 in 40 min and then at 0:100 for 5 min), t_R = 37.72 min.

3-(2',3'-dideoxy-ribo-β-L-furanosyl)-6-*tert*butyloxynonylfuro[2,3-*d*]pyrimidin-2(3*H*)-one (8q). The procedure C was carried out using 11-*tert*butyloxy-1-undecyne **10i** (0.296 g, 1.319 mmol). The obtained residue was purified by column chromatography (4% methanol in ethyl acetate) to give the product **8q** as an off-white solid (0.011 g, 7%); ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.67 (1H, s, H-4), 6.22 (1H, dd, *J* = 6.7 Hz, 2.5 Hz, H-1'), 6.13 (1H, t, *J* = 1.1 Hz, H-5), 4.34-4.29 (1H, m, H-4'), 4.16 (1H, d, *J* = 12 Hz, H-5'), 3.88 (1H, dt, *J* = 11.9 Hz, 3.5 Hz, H-5'), 3.35 (2H, t, *J* = 6.8 Hz, OCH_2), 2.66 (2H, t, *J* = 7.5 Hz, αCH_2), 2.64-2.59 (1H, m, H-2'), 2.27-2.21 (1H, m, H-2', OH), 1.99-1.89 (2H, m, H-3'), 1.72-1.66 (2H, m, βCH_2), 1.55-1.50 (2H, m, $OCH_2C\underline{H_2}$), 1.39-1.28 (10H, m, 5 x CH_2), 1.21 (9H, s, 3 x CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 171.81 (C-7a), 159.53 (C-6), 154.94 (C-2), 135.54 (C-4), 107.16 (C-4a), 98.98 (C-5), 88.99 (C-1'), 82.68 (C-4'),

72.48 (O<u>C</u>$(CH_3)_3$), 63.04 (C-5'), 61.69 (OCH_2), 33.75 (C-2'), 30.72, 29.44, 29.39, 29.11, 28.89, 28.22 (6 x CH_2), 27.61 (3 x CH_3), 26.73, 26.22 (2 x CH_2), 23.96 (C-3'); MS (ES^+) m/z 457 (100%, $M+Na^+$), 891 (13%, $MM+Na^+$). Reverse HPLC eluting with H_2O/MeOH (gradient from 90:10 to 0:100 in 40 min and with 0:100 for 5 min), t_R = 36.23 min.

3-(2',3'-dideoxy-ribo-β-L-furanosyl)-6-hexyloxyoctylfuro[2,3-*d*]pyrimidin-2(3*H*)-one (8r). The procedure C was carried out using 10-hexyloxy-1-decyne **10j** (0.352 g, 1.48 mmol, 2.5 eq.). The obtained residue was purified by column chromatography (4% methanol in ethyl acetate) to give the product **8r** as an off-white solid (0.080 g, 30%); ^1H NMR (500 MHz, $CDCl_3$): δ 8.73 (1H, s, H-4), 6.21 (1H, dd, *J* = 6.1 Hz, 1.8 Hz, H-1'), 6.13 (1H, s, H-5), 4.34-4.27 (1H, m, H-4'), 4.19-4.12(1H, m, H-5'), 3.88 (1H, dt, *J* = 7.6 Hz, 4.0 Hz, H-5'), 3.41 (4H, t, *J* = 6.1 Hz, 2 x OCH_2), 2.71 (1H, t, *J* = 4.7 Hz, OH), 2.67-2.56 (3H, m, αCH_2, H-2'), 2.26-2.18 (1H, m, H-2'), 2.00-1.91 (2H, m, H-3'), 1.77-1.64 (2H, m, βCH_2), 1.62-1.52 (4H, m, 2 x CH_2), 1.42-1.23 (14H, m, 7 x CH_2), 0.90 (3H, t, *J* = 6.8 Hz, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 171.76 (C-7a), 159.44 (C-6), 154.99 (C-2), 135.83 (C-4), 107.17 (C-4a), 99.05 (C-5), 88.99 (C-1'), 82.83 (C-4'), 71.00 (OCH_2), 70.89 (OCH_2), 62.88 (C-5'), 33.77 (C-2'), 31.71, 29.73, 29.32, 29.19 (double intensity), 28.94, 28.23, 26.78, 26.14, 25.86 (10 x CH_2), 23.90 (C-3'), 22.62 (CH_2), 14.04 (CH_3); MS (ES^+) m/z 449 (11%, $M+H^+$), 471 (64%, $M+Na^+$), 487 (100%, $M+K^+$). Accurate mass: $C_{25}H_{41}N_2O_5$ requires 449.3015; found 449.3033. Reverse HPLC eluting with H_2O/MeOH (gradient from 100:0 to 0:100 in 35 min and then at 0:100 for 10 min), t_R = 38.17 min.

3-(2',3'-dideoxy-ribo-β-L-furanosyl)-6-methyloxytridecylfuro[2,3-*d*]pyrimidin-2(3*H*)-one (8s). The procedure C was carried out using 15-methoxy-1-pentadecyne **10k**

(0.423 g, 1.774 mmol). The obtained residue was purified by column chromatography (3% methanol in ethyl acetate) to give the product **8s** as an off-white solid (0.050 g, 19%); ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.75 (1H, s, H-4), 6.20 (1H, dd, *J* = 6.7 Hz, 2.4 Hz, H-1'), 6.15 (1H, s, H-5), 4.35-4.27 (1H, m, H-4'), 4.19-4.13 (1H, m, H-5'), 3.88 (1H, dt, *J* = 12.0 Hz, 4.0 Hz, H-5'), 3.38 (2H, t, *J* = 6.7 Hz, OCH_2), 2.35 (3H, s, OCH_3), 2.84 (1H, *J* = 4.8 Hz, OH), 2.64 (2H, t, *J* = 7.5, αCH_2), 2.62-2.57 (1H, m, H-2'), 2.24-2.15 (1H, m, H-2'), 1.98-1.92 (1H, m, H-3'), 1.71-1.65 (2H, m, βCH_2), 1.61-1.54 (2H, m, γCH_2), 1.40-1.23 (18H, m, 9 x CH_2); ^{13}C NMR (125 MHz, $CDCl_3$): δ 171.75 (C-7a), 159.47 (C-6), 155.02 (C-2), 135.90 (C-4), 107.22 (C-4a), 99.08 (C-5), 88.99 (C-1'), 82.88 (C-4'), 72.99 (OCH_2), 62.83 (C-5'), 58.52 (OCH_3), 33.77 (C-2'), 29.63, 29.56, 29.54, 29.48 (double intensity), 29.45 (double intensity), 29.23, 29.01, 28.25, 26.81, 26.12 (12 x CH_2), 23.91 (C-3'); MS (ES^+) m/z 449 (100%, $M+H^+$), 471 (68%, $M+Na^+$), 487 (84%, $M+K^+$). Accurate mass: $C_{25}H_{40}N_2O_5$ requires 449.3015; found 449.2997. Reverse HPLC eluting with H_2O/MeOH (gradient from 100:0 to 10:90 in 15 min, then at 10:90 for 5 min and another gradient from 10:90 to 0:100 in 5 min), t_R = 24.29 min.

3-(2',3'-dideoxy-ribo-β-L-furanosyl)-6-tridecyloxymethylfuro[2,3-*d*]pyrimidin-2(3*H*)-one (8t). The procedure C was carried out using 3-tridecyloxy-1-propyne **12a** (0.352 g, 1.776 mmol). The obtained residue was purified by column chromatography (4% methanol in ethyl acetate) to give the product **8t** as an off-white solid (0.024 g, 10%); ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.87 (1H, s, H-4), 6.45 (1H, s, H-5), 6.21 (1H, dd, *J* = 6.8 Hz, 2.0 Hz, H-1'), 4.48 (2H, s, C$\underline{H_2}$OCH_2CH_2), 4.34-4.29 (1H, m, H-4'), 4.20-4.15 (1H, m, H-5'), 3.90-3.87 (1H, m, H-5'), 3.56 (2H, t, *J* = 6.65 Hz, OC$\underline{H_2}CH_2$), 2.68-2.57 (1H, m, H-2'), 2.28-2.18 (1H, m, H-2'), 1.98-1.89 (2H, m, H-3'), 1.67-1.58 (2H, m,

J = 6.9 Hz, $OCH_2C\underline{H}_2$), 1.40-1.22 (20H, m, 10 x CH_2), 0.90 (3H, t, *J* = 6.7 Hz, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 171.78 (C-7a), 159.36 (C-6), 154.75 (C-2), 137.60 (C-4), 107.62 (C-4a), 102.36 (C-5), 88.13 (C-1'), 82.92 (C-4'), 71.49 ($O\underline{C}H_2CH_2$), 64.95 ($\underline{C}H_2OCH_2CH_2$), 62.79 (C-5'), 33.79 (C-2'), 31.91 (double intensity), 29.65, 29.61, 29.58 (double intensity), 29.47, 29.35, 26.06 (double intensity) (10 x CH_2), 23.74 (C-3'), 22.68 (CH_2), 14.10 (CH_3); MS (ES^+) m/z 449 (24%, $M+H^+$), 471 (36%, $M+Na^+$), 512 (100%, $M+CH_3CN+Na^+$). Accurate mass: $C_{25}H_{41}N_2O_5$ requires 449.3015; found 449.3024. Reverse HPLC eluting with H_2O/MeOH (gradient from 100:0 to 10:90 in 15 min, then at 10:90 for 5 min and another gradient from 10:90 to 0:100 in 5 min), t_R = 24.87 min.

3-(2',3'-dideoxy-ribo-β-L-furanosyl)-6-decyloxybutylfuro[2,3-*d*]pyrimidin-2(3*H*)-one (8u). The procedure C was carried out using 6-decyloxy-1-hexyne **12b** (0.352 g, 1.776 mmol). The obtained residue was purified by column chromatography (4% methanol in ethyl acetate) to give the title compound as an off-white solid (0.070 g, 26%); ^1H NMR (500 MHz, $CDCl_3$): δ 8.72 (1H, s, H-4), 6.21 (1H, br d, *J* = 6.1 Hz, H-1'), 6.14 (1H, s, H-5), 4.34-4.28 (1H, m, H-4'), 4.19-4.12 (1H, m, H-5'), 3.91-3.86 (1H, m, H-5'), 3.45 (2H, t, *J* = 6.3 Hz, 2.1 Hz, OCH_2), 3.41 (2H, t, *J* = 6.7 Hz, 2.1 Hz, OCH_2), 2.69 (2H, t, *J* = 7.4 Hz, αCH_2), 2.64-2.57 (2H, m, H-2', OH), 2.23-2.19 (1H, m, H-2'), 1.98-1.92 (2H, m, H-3'), 1.81-1.73 (2H, m, βCH_2), 1.69-1.62 (2H, m, CH_2), 1.60-1.54 (2H, m, CH_2), 1.35-1.24 (12H, m, 6 x CH_2), 0.90 (3H, t, *J* = 6.5 Hz, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 171.75 (C-7a), 159.11 (C-6), 154.97 (C-2), 135.87 (C-4), 107.10 (C-4a), 99.23 (C-5), 89.00 (C-1'), 82.81 (C-4'), 71.15(OCH_2), 70.23 (OCH_2), 62.92 (C-5'), 33.77 (C-2'), 29.74, 29.60, 29.57, 29.49, 29.31, 29.12 (6 x CH_2) 28.08 (αCH_2), 26.18 (CH_2), 23.90 (C-3'), 23.68 (βCH_2), 22.67 (CH_2), 14.10 (CH_3); MS (ES^+) m/z 449 (17%, $M+H^+$), 471 (6%, $M+Na^+$),

487 (4%, $M+K^+$), 512 (100%, $M+CH_3CN+Na^+$). Accurate mass: $C_{25}H_{41}N_2O_5$ requires 449.3015; found 449.3028. Reverse HPLC eluting with H_2O/MeOH (gradient from 100:0 to 10:90 in 15 min, then at 10:90 for 5 min, another gradient from 10:90 to 0:100 in 5 min and then at 0:100 for 10 min), t_R = 26.45 min.

Antiviral assays. L-ddBCNAs were then prepared as 10 mM stock solutions in dimethyl sulfoxide (DMSO) to do the antiviral assays. Wortmannin (W3144; Sigma–Aldrich) was obtained as 10 mM 0.2 μm filtered ready made solution in DMSO. Ribavirin (R9644; Sigma–Aldrich) and Cidofovir (C5874; Sigma–Aldrich) were prepared as 100 mM stock solution in water.

Cell culture. Hela cells (ATCC CCL-2) were used for VACV stock production. BSC-1 cells (ATCC CCL-26), RK13 cells (ATCC CCL-37), HFFF (gift of G Wilkinson) and B95a simian B cells (IZSBS–Istituto Zooprofilattico Sperimentale (Brescia, Italy) were used for antiviral and proliferation assays. All cells were grown in Dulbecco's Modified Eagle Medium (DMEM; Invitrogen, Life technologies, Paisley, UK) with 4.5g/L glucose and L-glutamine without sodium pyruvate 1X (Gibco, Life technologies, Paisley, UK), supplemented with 10% (*v/v*) heat inactivated foetal bovine serum (FBS; Gibco; lot:41G6401K) was used for the culture of all cells. Cells were cultured in the absence of antibiotics prior to assay, when penicillin/streptomycin was added (Gibco). Visibly contaminated cultures were discarded, mycoplasma contamination was ruled out by mycoplasma PCR (Geneflow, UK).

Virus culture. Vaccinia viruses were propagated in adherent HeLa cells, and mature intracellular virus (MV) was prepared using the method described by Townsley and coworkers[35] Vaccinia virus WR (VACV-WR) is a kind gift of Dr Bernhard Moss, NIH,

NIAID, LVD, to Joachim J. Bugert. VACV-WR luciferase reporter virus v3 was constructed to express firefly luciferase under the control of the synthetic – optimized early/late poxviral promoter using the pRB21 based donor plasmid p240.[36] The VACV-WR gfp reporter virus v300 was constructed to express enhanced green fluorescent protein (EGFP) under the control of the synthetic – optimized early/late poxviral promoter using the donor plasmid p300.[37]

Wild type measles viruses were propagated and titered in B95a cells and prepared as described by Hashimoto and coworkers[38] Wild type measles virus mWTFb is a kind gift of Jürgen Schneider Schaulies (Universität Würzburg, Germany). Wild type measles virus mIC323 expressing green fluorescent protein (GFP) is a kind gift of Yusuke Yanagi (NIID, Tokyo, Japan).

Measles virus vaccine strain Edmonston was purchased from ATCC (ATCC- VR24).

Measles virus Edmonston, Adenovirus 5 (ATCC VR-5), Herpes simplex viruses type 1 (NCPV 17+) and type 2 (NCPV 132349) were propagated in Vero cells.[39]

VACV luciferase assays. RK13 cells in 96 well plates were pre-treated for 30 minutes with each of the drugs at 10 μM and a DMSO control. Luciferase producing vaccinia virus (v3) was then added to the wells at an moi of 0.2. The cells were collected 2 hours pi. 100 μL of 1x passive lysis buffer (Promega, Madison, Wisconsin, USA) per well was added to each well and the plate was agitated on a Bellydancer for 15 minutes. The plate was then frozen at -20 ^{o}C. After thawing, 20 μL of each of the samples was transferred to a black 96 well plate (NUNC, Roskilde, Denmark) and processed using the luciferase assay substrate and buffer II (Promega, Madison, Wisconsin, USA). The luminescence was measured in a 96 well luminescence plate reader (FLUOstar OPTIMA FL, BMG

Labtech). The percentages of the treated versus the no drug control readings were calculated.

VACV plaque reduction assays (IC_{50}). BSC-1 cells grown to confluency in 24 well plates were pre-treated with a series of dilutions of drug (50, 5, 0.5, 0.1, 0.05 μM and no drug control) in quadruplicate. These were then infected with 30 plaque forming units (pfu) of vaccinia virus WR per well. The virus was left to adsorb onto the cells for one hour, before the drug and the virus was removed and replaced with 1 mL of avicel overlay (50% avicel, 50% DMEM containing 1% P/S and drug to the required concentration). The plates were incubated for 4 days and were then rinsed with PBS and stained with crystal violet stain for 4-6 hours. The plaque sizes were analysed by scanning the plates at 600 dpi and measurement of the area of each of the plaques using Image J software. IC_{50} values were defined as the concentration of antiviral that reduced the size of plaques by 50% in comparison to cells infected in the absence of antiviral. The final IC_{50} values for all virus/antiviral combinations are each a mean from three experiments. The IC_{50} values were calculated for each drug using the GraphPad Prism software (GraphPad Software, Inc., USA).

$TCID_{50}$ assays. Tenfold dilutions of HSV-1/2, adenovirus 5, and measles viruses were used to infect HFFF, BSC-1 cells and B95a cells in 96 well plates (10 dilutions, two mock, 8 times repeated) and evaluated 3 days post infection for cytopathic effects in comparison to mock infected wells. Cells were grown in 96 well plates (NUNC) until 80% confluent. Cells were pretreated with medium containing the appropriate amount of antivirals for 30 minutes prior to infection. Each well was infected with 100 pfu of HSV-1/2 and Adenovirus 5, or 100 syncytia forming units (sfu) of measles virus WTFb and

incubated in DMEM without FBS or antibiotics containing no antivirals or antivirals at concentrations ranging from 1 to 100 μM for 3 days.

Cytopathogenic effects were assessed by phase contrast microscopy. $TCID_{50}$ was defined as the concentration of antivirals that reduced the CPE by 50% in comparison to cells infected in the absence of antivirals as an average of 3 experiments. Controls consisting of virus and cell only (no drug), and cell only (no virus/no virus and no drug) were included in each experiment. $TCID_{50}$ values were calculated using the method of Reed and Muench.[40]

Cell viability. L-ddBCNAs were tested for effects on cell viability over a period of three days in BSC-1 cells using a range of drug concentrations according to the manufacturer's instructions (G7571 CellTiter-Glo® Luminescent Cell Viability Assay; Promega, Madison, Wisconsin, USA). The assay measures the number of viable cells in culture based on quantitation of the ATP present, which signals the presence of metabolically active cells. CC_{50} values were calculated for each drug versus no drug using the GraphPad Prism software (GraphPad Software, Inc., USA).

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Abbreviations used

B95a, simian B lymphocyte strain 95 adherent; BCNA, bicyclic nucleoside analogue; BSC-1, grivet monkey kidney cell line; ddBCNA, dideoxy bicyclic nucleoside analogue; DIPEA, diisopropylethylamine; DMEM, Dulbeccos Modified Eagle's medium; FBS, Fetal Bovine serum; HeLa, human cervical cancer cells taken from Henrietta Lacks; HFFF, human fetal foreskin fibroblast cell; IddU, 5-iodo-2',3'-dideoxyuridine; MV, mature intracellular virus; nd, not done; pfu, plaque forming unit; p.i., post infection; RK13, rabbit kidney epithelial cell line 13; sfu, syncytia forming unit; TCID50, tissue culture inhibitory concentration 50%; VACV, vaccinia virus; Vero, kidney epithelial cells from an African green monkey; WR, Western Reserve strain of vaccinia virus.

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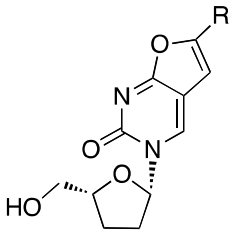

L-ddBCNAs
R = alkyl, aryl, ether
Activity against vaccinia and measles viruses