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Original article

In search of *Flavivirus* inhibitors part 2: Tritylated, diphenylmethylated and other alkylated nucleoside analogues

Milind Saudi [a], Joanna Zmurko [b], Suzanne Kaptein [b], Jef Rozenski [a], Johan Neyts [b], Arthur Van Aerschot [a,*]

[a] Medicinal Chemistry, Rega Institute for Medical Research, KU Leuven, Minderbroedersstraat 10, BE-3000 Leuven, Belgium
[b] Virology and Chemotherapy, Rega Institute for Medical Research, KU Leuven, Minderbroedersstraat 10, BE-3000 Leuven, Belgium

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ABSTRACT

Several flaviviruses, such as the yellow fever virus and the dengue virus cause severe and potentially lethal infection in man. Following up on our initial hit 3′,5′-bistritylated uridine **1**, a series of alkylated nucleoside analogues were synthesized and evaluated for their *in vitro* antiviral activities against dengue fever virus and yellow fever virus. Hereto, alkyl and aryl groups were attached at various positions of the sugar ring combined with subtle variation of the heterocyclic base. Among the new series of derivatives, 3′,5′-di-*O*-trityl-5-fluoro-2′-deoxyuridine (**39**) was the most efficient in this series and inhibited both yellow fever virus and dengue virus replication with a 50% effective concentration (EC_{50}) of ~1 μg/mL without considerable cytotoxicity. The other fluorinated derivatives proved more toxic. Almost all diphenylmethylated pyrimidine nucleosides with 3′,5′-di-*O*-benzhydryl-2′-deoxyuridine (**50**) as the example were endowed with strong cytotoxic effects down to 1 μg/mL.

1. Introduction

The genus *Flavivirus* comprises several important human pathogens including the tick-borne encephalitis virus, the West Nile virus, the dengue fever virus (DENV) and the yellow fever virus (YFV) [1,2]. Most *flaviviruses* are transmitted to humans by mosquitoes. Each year an estimated 360 million people living in tropical and subtropical regions become infected with the DENV of which ~96 million develop disease [3]. Approximately 500,000 of the infected individuals progress to dengue hemorrhagic fever, leading each year to 22,000 deaths [4–7]. With increasing levels of international travel, urbanization and population growth, incidences of the DENV illness have increased by 30-fold in the past 50 years. Even though dengue is the fastest progressing vector-borne disease worldwide, there is neither vaccine nor an antiviral drug available till date. There are an estimated 200,000 cases of yellow fever, of which 30,000 lead to death, worldwide each year. The virus is endemic in tropical areas of Africa and Latin America, with a combined population of over 900 million people [8,9]. Although a highly effective and safe vaccine (17D) is available [10], many people do not get vaccinated, primarily because a cold chain is needed for this live- attenuated vaccine. An antiviral drug would prove useful for treating the severe infections that may occur in the non-vaccinated, especially during epidemics. A most common strategy for developing an antiviral drug is the high-throughput screening of large compound libraries *in vitro* to identify some hit molecules. This is further followed by a hit-optimization process. Previous efforts in our group lead to discovery of 3′,5′-bistritylated uridine **1** (Fig. 1) as a potential inhibitor of *Flavivirus* replication [11,12]. The finding of this lipophilic structure being endowed with high antiviral activity for flaviviruses stimulated the interest for further structure-activity research. In view of previous results and the lack of activity for tritylated purines and for cytidine derivatives, only some uracil analogues were considered for further evaluation, being the 5-methyl, N^3,5-dimethyl, and 5-fluorouracil derivatives, with 5-chlorouracil having been studied before [11]. In addition, substitution of the trityl moieties was investigated, as for drug development purposes a less lipophilic structure is warranted. We therefore synthesized and tested a series of compounds which were less hydrophobic than the initial lead. Hereto, a series of close analogues were prepared in which different groups were substituted at the 2′-, 3′- and 5′- position of nucleosides in an effort to set a structure–activity relationship (SAR). We here report on this series of nucleoside analogues of which some have shown to

* Corresponding author.
E-mail address: arthur.vanaerschot@rega.kuleuven.be (A. Van Aerschot).

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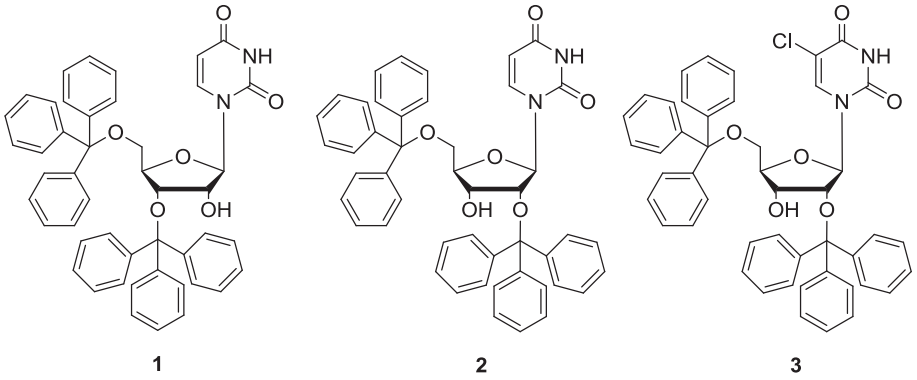


Fig. 1. Structure of the lead compound **1** and its antiviral active analogues **2** and **3**.

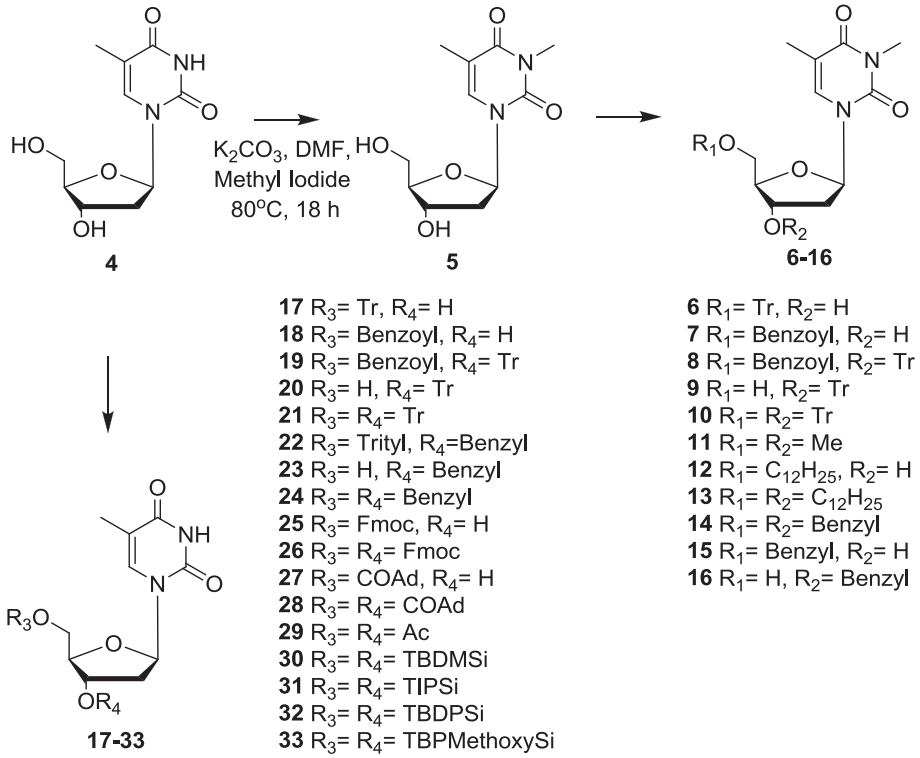
provide weak inhibition for *in vitro* DENV and/or YFV replication. Unfortunately we have not been able to find suitable substitutions for the trityl moieties without causing toxicity or without serious loss of the inhibitory activity.

2. Results and discussion

2.1. Synthetic aspects

Considerable attention in the past has been directed towards the synthesis of nucleoside analogues inhibiting the DNA or RNA polymerization process in the hope of discovering new selective and more effective antivirals. Following up on the lead compound **1** [11], an additional series of tritylated as well as diphenylmethylated uridine and thymidine derivatives was envisaged. Likewise, in an effort to substitute for the large trityl moieties in the lead compound, a series of thymidine analogues carrying different lipophilic moieties at their 3′- and 5′-hydroxyl position were prepared in an effort to further delineate the SAR properties. The chemistry in general is straightforward and the reaction sequences for assembly of the target compounds are illustrated in Figs. 2–4.

In a first strategy, N^3-methylation of thymidine has been carried out using methyl iodide and potassium carbonate in dry DMF to obtain intermediate **5** in 81% yield [13]. Subsequent mono or bis-tritylation provided the compounds **6**, **9** and **10** (see Fig. 2 for reaction conditions). Analogously, thymidine was tritylated affording **17**, **20** and **21**, respectively (Fig. 2), and 5-fluoro-2′-deoxyuridine and 5-fluorouridine gave the congeners **38**–**39** and **41**–**43**,



17 R_3= Tr, R_4= H
18 R_3= Benzoyl, R_4= H
19 R_3= Benzoyl, R_4= Tr
20 R_3= H, R_4= Tr
21 R_3= R_4= Tr
22 R_3= Trityl, R_4=Benzyl
23 R_3= H, R_4= Benzyl
24 R_3= R_4= Benzyl
25 R_3= Fmoc, R_4= H
26 R_3= R_4= Fmoc
27 R_3= COAd, R_4= H
28 R_3= R_4= COAd
29 R_3= R_4= Ac
30 R_3= R_4= TBDMSi
31 R_3= R_4= TIPSi
32 R_3= R_4= TBDPSi
33 R_3= R_4= TBPMethoxySi

6 R_1= Tr, R_2= H
7 R_1= Benzoyl, R_2= H
8 R_1= Benzoyl, R_2= Tr
9 R_1= H, R_2= Tr
10 R_1= R_2= Tr
11 R_1= R_2= Me
12 R_1= $C_{12}H_{25}$, R_2= H
13 R_1= R_2= $C_{12}H_{25}$
14 R_1= R_2= Benzyl
15 R_1= Benzyl, R_2= H
16 R_1= H, R_2= Benzyl

Fig. 2. Synthesis of different 3′ or 5′ modified or bis-modified thymidine analogues. Reaction conditions: i) either 1.2 or 2.5 eq of TrCl, pyridine, 85 °C, 16–18 h; ii) 1.2 eq BzCl, pyridine, rt, 2 h; iii) LiOH, MeOH:H_2O (1:1), rt, 18 h; iv) NaH, MeI, dry DMF, rt, 45 min; v) NaH, $C_{12}H_{25}Br$, either 1.1 or 2.2 eq, dry DMF, rt, 2 h; vi) 2.2 eq of NaH, 2.5 eq BnBr, dry DMF, rt, 2 h; vii) NaH, BnBr, 1.1 eq each, dry DMF, 0 °C, 4 h; viii) 2.2 eq NaH, 2.5 eq BnBr, dry THF, 50 °C; ix) 80% aq AcOH, 50 °C, 1 h; x) either 1.2 or 2.5 eq of FmocCl, pyridine, rt, 18 h; xi) 2.2 eq AdamantylCl, pyridine, 70 °C, 18 h; xii) 2.2 eq of AcCl, ACN, TEA, DMAP, 5 h; xiii) 3 eq of t-butyldimethylsilyl chloride (TBDMSiCl), imidazole, dry DMF, rt, 18 h; xiv) 3 eq triisopropylsilyl chloride (TIPSiCl), imidazole, dry DMF, rt, 18 h; xv) 3 eq t-butyldiphenylsilyl chloride (TBDPSiCl), imidazole, dry DMF, rt, 18 h; xvi) 3 eq t-butylphenylmethoxysilyl bromide (TBPMethoxySiBr), imidazole, dry DMF, rt, 18 h. Synthesis starting from **5**: for **6**: i using 1.2 eq; **7**: ii; **8**: ii, i; **9**: ii, i, iii; **10**: i using 2.5 eq; **11**: iv; **12**: v; **13**: v; **14**: vi; **15** and **16**: vii. Synthesis starting from **4**: **17**: i; **18**: ii; **19**: ii, i; **20**: ii, i, iii; **21**: i; **22**: i, viii; **23**: i, viii, ix; **24**: viii; **25** and **26**: x; **27** and **28**: xi; **29**: xii; **30**: xiii; **31**: xiv; **32**: xv; **33**: xvi.

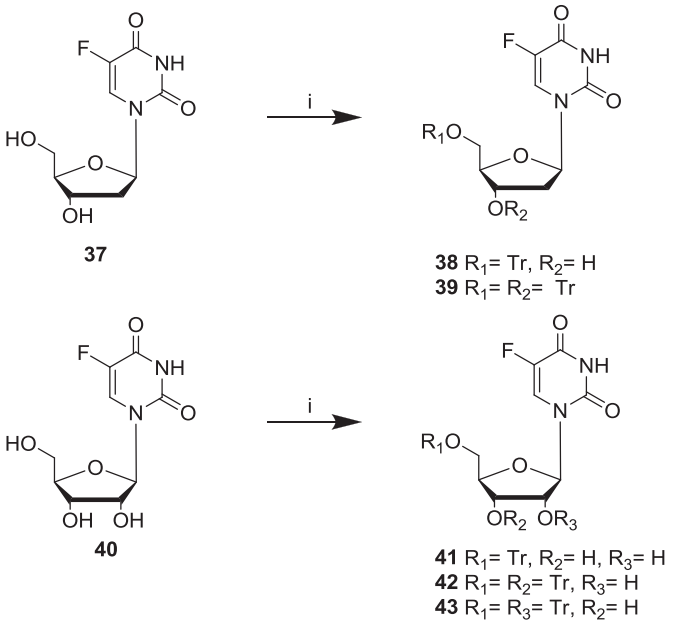


Fig. 3. Synthesis of tritylated derivatives of 5-fluoro-2′-deoxyuridine and 5-fluorouridine. Reaction conditions: i) either 1.2 or 2.5 eq of triphenylmethyl chloride, pyridine, 85 °C, 16 h.

respectively following the reaction conditions as shown in Fig. 3. Preparation of these respective tritylated derivatives allowed evaluation of the antiflavivirus activity and examination of the role of the heterocyclic moiety herein, using thymine, N^3-methyl thymine or 5-fluorouracil as the heterocyclic base while preserving the hydrophobic tritylated sugar part. Exploring further the 3D space around the base moiety, the necessity for the lactam moiety was verified. Hereto, a triazolo intermediate was used to obtain the O^4-methylated analogue **35** instead of the N^3-methyl thymidine (**5**) which is obtained by direct alkylation (Fig. 4). Prior trimethylsilyl protection was accomplished with TMS chloride and triethylamine in acetonitrile at room temperature, followed by phosphorus oxychloride promoted introduction of the triazole moiety [14]. This method of choice has become very widely used in nucleoside research. Acetic acid-methanol (1:4 v/v) was added to the residue, and the resulting solution was allowed to stand at room temperature for 4.5 h to accomplish trimethylsilyl deprotection. The intermediate **34** was allowed to react with sodium methoxide in methanol at ambient temperature to afford unprotected O^4-methyl thymidine (**35**) [14]. Some starting thymidine however was recovered indicating the labiality of the methoxy substituent. The methylated product was purified by column chromatography in 60% yield and was expected to be a valuable intermediate in the preparation of many key target compounds. However, upon tritylation at elevated temperatures, the O^4-methyl moiety was cleaved resulting into formation of the known 3′,5′ di-*O*-tritylthymidine (**21**) (Fig. 4).

Furthermore, the hydroxyl groups in compounds **4** and **5** were alkylated under different conditions depending on the moiety to be introduced. Small groups like methyl, benzyl and acetyl were easily introduced, but likewise bulky groupings like different trialkylsilyl, fluorenylmethyloxycarbonyl (Fmoc), adamantyl and long chain alkyl moieties were attached at the 3′- and 5′-positions following standard procedures to obtain either mono- or di-substituted thymidine derivatives **6**–**33** (for reaction conditions see Fig. 2).

Finally, the trityl moieties could be substituted by the less crowded diphenylmethyl group, which however is less straightforward to introduce. During this work, a convenient and effective method for synthesis of the diphenylmethyl ethers of several nucleosides has been developed with the aid of palladium (II) chloride as the catalyst [15]. Hereto, we allowed the respective nucleoside to react with diphenylmethanol (DPM-OH) in dichloroethane (DCE) in presence of 0.2 equivalents of $PdCl_2$ at 85 °C overnight (Fig. 5).

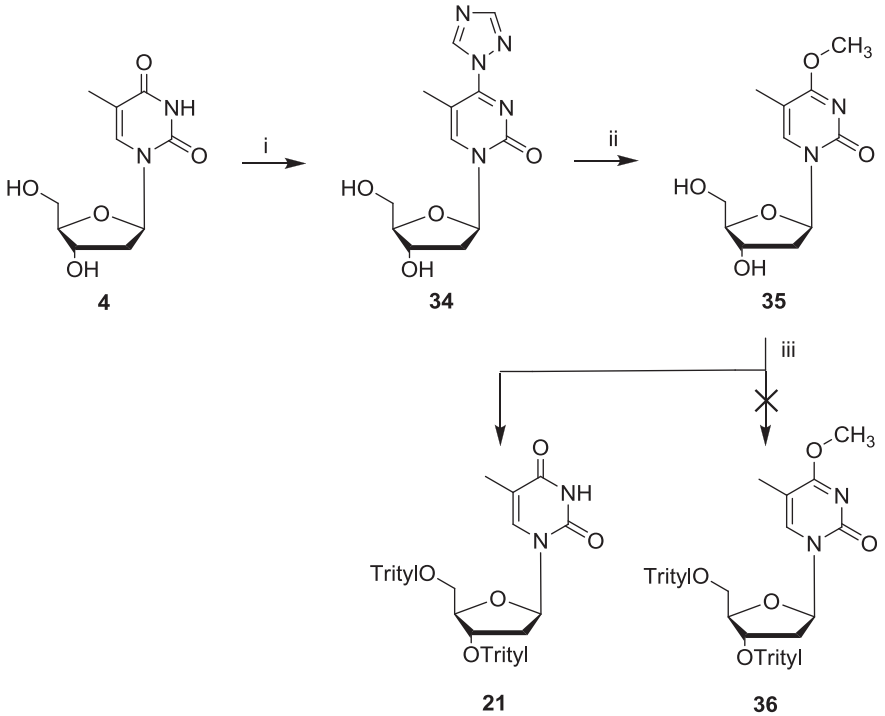


Fig. 4. Attempted synthesis of O^4-methyl -3′,5′-bistritylthymidine. Reaction conditions: i) a) TEA, TMSCl, ACN, rt, 1.5 h; b) 1H-triazole, $POCl_3$, 0 °C, 5 h; ii) NaOMe, MeOH, rt, 30 min; iii) 2.5 eq TrCl, pyridine, 85 °C, 18 h.

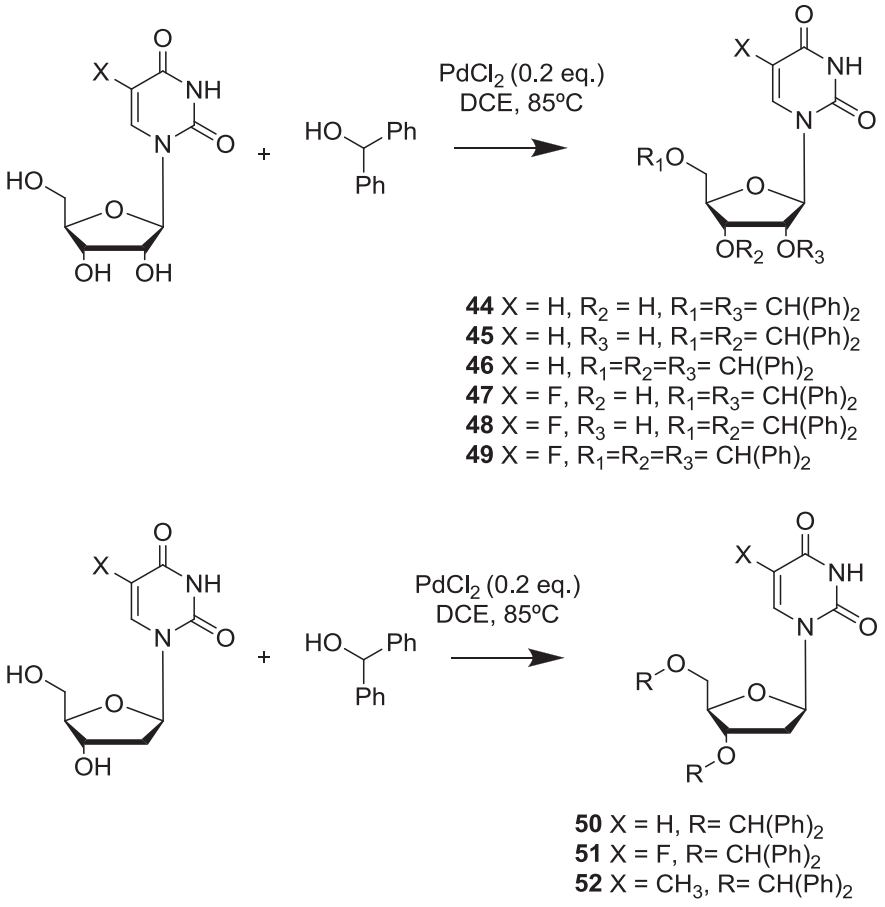


Fig. 5. Introduction of benzhydryl moieties on some selected ribonucleosides and 2′-deoxyribonucleosides.

2.2. Assessment of the antiviral activity

The previously reported lipophilic uridine analogues 3′,5′-bistrityl uridine **1** and 2′,5′-bistrityl uridine **2** exert selective inhibition of *in vitro* DENV and YFV replication [11,12]. Compound **2** inhibited YFV replication with an EC_{50} of 1.2 μg/mL (1.65 μM) with calculated selectivity index (SI) of around 80. However, the molecule proved less potent against DENV (SI of ~3). The regioisomer **1** proved to be endowed with more potent activity against both viruses with EC_{50} of 1.0 μg/mL (1.37 μM) and SI of >85 for YFV inhibition. This molecule also proved less potent against DENV (SI of ~5). The most active analogue in this series 2′,5′-bistrityl-5-chlorouridine **3** had EC_{50} of 0.75 μg/mL (1.0 μM) resulting in SI of >90. On the other hand, the monotritylated analogue proved markedly more toxic. Our efforts therefore were directed towards synthesising a series of nucleoside derivatives with either substitutions of the base moiety, or substituting for the large and very hydrophobic triphenylmethyl groups at the sugar part.

Our efforts to understand the structure–activity relationship of the lead molecules started with substitution of hydrogen at the 5-position of the base moiety with fluorine. The unique properties of fluorine have led to its widespread application in drug design as an isostere for hydrogen. Early applications of the exchange of H by F focused on the region-specific deployment of fluorine to interfere with metabolic processes, a tactic that relies on the powerful electron withdrawing properties of F and the strength of the C–F. The latter is also known to be chemically inert under most biological conditions [16–20].

Table 1 reports on the DENV and YFV antiviral activities and cellular toxicities of respectively tritylated, diphenylmethylated and some benzylated nucleoside analogues. The results for some tritylated analogues were reported before and are included here for comparative purposes [11,12]. Upon analysis one notices that the results for the tritylated 5-fluorouridine congeners largely correspond with those for uridine with strong YFV inhibition for the 2′,5′-bistrityl derivative **42** (EC_{50} 1.1 μg/mL) and its 3′,5′ congener **43** (EC_{50} 0.43 μg/mL; CC_{50} 4.4 μg/mL). Despite the strong antiviral effect, the enhanced cellular toxicity renders both compounds with a reduced selectivity index (SI). The bistritylated 5-fluoro-2′-deoxyuridine **39** however retains analogous anti-YFV activity and cellular toxicity (EC_{50} 1.05 μg/mL; CC_{50} > 50 μg/mL) compared to its 2′-deoxyuridine congener, preserving a strong SI of >47. 5-Fluorination of uracil therefore does not provide an advantage, keeping in mind the potential *in vivo* cleavage of glycosidic bonds, yielding the anticancer thymidylate synthase inhibitor 5-fluorouracil [21]. In analogy with the uridine series, the 2′,5′-bistrityl derivative **42** did not display inhibitory properties against proliferation of DENV, while the 3′,5′-bistrityl congeners **39** and **43** displayed EC_{50} values in the micromolar range with a SI of >41 for the 5-fluoro-2′-deoxyuridine analogue **39**. The bistritylated N^3-methyl thymidine analogue **10** however lost all of its antiviral and toxic activity, except for the toxic effect of the 5′-monotritylated analogue **6** displaying a CC_{50} of 5 μg/mL. The obtained inhibitory activities should be placed in perspective with recent results obtained for some reference compounds as highlighted in Table 1. Herein, the cytidine analogue 2′-*C*-methylcytidine in our hands performs very consistently and inhibits DENV replication with an EC_{50} of around 10 μM. Valopicitabine, a prodrug of this anti-HCV reagent was first described by Gosselin et al. [22] and was reported being active as well against flaviviruses. Favipiravir (T705)

Table 1
Antiviral activity assessment of some tritylated, diphenylmethylated and benzylated nucleoside analogues versus Dengue virus and yellow fever virus, determined as described in the Materials and Methods section (values expressed in microgram/mL unless indicated). Bnh is used as abbreviation for the benzhydryl (diphenylmethyl) group. Activities for some compounds were reported before, see Ref. [11].

	DENV			YFV		
	EC_{50}	CC_{50}	SI	EC_{50}	CC_{50}	SI
Uridine analogues						
5′-trityl[11]	6	10	1.67	4	18	4.5
2′,5′-trityl[11] (**2**)	30	>100	>3	1.2	>100	>80
2′,5′-Bnh (**44**)	>50	3.7	0.07	>50	2.4	–
3′,5′-trityl[11] (**1**)	1.75	>10	>6	1	>85	>85
3′,5′-Bnh (**45**)	1.23	1.85	1.5	>50	>50	1.0
2′,3′,5′-Bnh (**46**)	>50	33.3	0.67	>50	2.4	–
5-Fluorouridine derivatives						
5′-trityl (**41**)	>50	8.15 (±2.05)	<0.16	>50	9.0 (±0.9)	>0.18
2′,5′-trityl (**42**)	>50	9.6	<0.2	1.1	29.0	27
2′,5′-Bnh (**47**)	>50	1.23	0.02	1.15	1.16	1.0
3′,5′-trityl (**43**)	3.4	6.7	2	0.425 (±0.1)	4.38 (±1.7)	10.3
3′,5′-Bnh (**48**)	1.23	3.7	3	>50	2.8	–
2′,3′,5′-Bnh (**49**)	11.1	5.56	0.5	4	>50	>12.5
2′-deoxyuridine congeners						
5′-trityl[11]	ND	8	–	3	5	1.7
3′,5′-trityl[11]	3.7	>65	>17	0.9	>65	>70
3′,5′-Bnh (**50**)	>50	1.23	0.02	>50	1.07	–
5-Fluoro 2′-deoxyuridine derivatives						
5′-trityl (**38**)	>50	4.6	<0.1	>50	6.9 (±3.9)	<0.14
3′,5′-trityl (**39**)	1.2	>50	>41	1.05 (±0.42)	>50	>47
3′,5′-Bnh (**51**)	>50	3.7		>50	1.85	–
Thymidine series						
5′-trityl (**17**)	4	4	1	ND	8	
5′-benzyl (**23**)	>100	>100	1	>100	>100	–
3′,5′-trityl (**21**)	ND	35		10	70	7
3′,5′-Bnh (**52**)	>50	1.23	0.02	>50	1.5	–
3′,5′-benzyl (**24**)	>100	18.75	<0.19	>100	37.5	<0.37
N^3-methyl thymidine analogues						
5′-trityl (**6**)	ND	5		5	5	1
5′-benzyl (**15**)	ND	60		ND	70	
3′,5′-trityl (**10**)	>100	>100	1	>100	>100	1
3′,5′-benzyl (**14**)	ND	8		ND	12	
Reference compounds						
2CMC	2.57 (±0.5) (10 μM)	>15 (>50 μM)	>5	ND	ND	
T705	ND	ND		25 (157 μM)	>385 (>2.4 mM)	>15
Ivermectin	>1 μM	3.8 μM		±5 nanoM	3.5 μM	±700

on the other hand is weakly inhibitory for YFV [23] while the helicase inhibitor ivermectin is a very potent inhibitor of *Flavivirus* replication especially YFV [24], but performs weaker for *in vitro* inhibition of DENV.

In an effort to reduce the lipophilicity, we substituted both trityl moieties in **1** and **2** with the benzhydryl group. We also prepared the 2′,3′,5′- trisbenzhydryl derivative which will have analogous lipophilicity as the bistrityl derivatives but the orientation of the 6 phenyl rings in space being different. In parallel other nucleosides with a 5-fluoro or a 5-methyl substituent at the base moiety were alkylated with benzhydryl group and their activities were determined against YFV. Remarkably, most of these diphenylmethylated analogues (**44**–**52**) are devoid of antiviral effects, but display strong cellular toxicity as compared to the trityl derivatives (Table 1). Toxicities (CC_{50} values) in general are around 1–2 μg/mL and deserve further attention. Two notable exceptions are found in the 5-fluorouridine series where the 2′,5′-bisalkylated analogue **47** displays an equal antiviral effect (EC_{50} 1.15 μg/mL or 2 μM) and the peralkylated derivative **49** surprisingly is much less toxic rendering the compound with a SI of 12.5 (EC_{50} 4 μg/mL; CC_{50} > 50 μg/mL).

Finally, for the perbenzylated compounds we did find marginal cellular toxicity (compounds **14** and **24**), while all other evaluated compounds proved devoid of antiviral activity and of cellular toxicity (not shown). The mixed antiviral results obtained so far, render a clear structure–activity relationship difficult and compromise further study of the tritylated nucleoside analogues as anti-YFV compounds, unless a mutant resistant to one of the antiviral active tritylated analogues could be grown. In spite of numerous efforts, up to now such mutants could not be generated.

3. Conclusion

A large series of either alkylated, silylated or acylated pyrimidine nucleoside analogues was synthesized and evaluated, primarily for their anti-YFV activity. Fluorination of the base moiety as in 2′,5′ (**42**) and 3′,5′-bistritylated 5-fluorouridine (**43**) preserved the antiviral potential but rendered the compounds more toxic compared to their uridine congeners, thus reducing the SI. Only with bistritylated 5′-fluoro-2′-deoxyuridine (**39**) the cellular toxicity was reduced and with an EC50 for YFV of 1.05 μg/mL (1.45 μM), an SI of around or above 50 was obtained. Pyrimidine nucleosides decorated with diphenylmethyl moieties proved surprisingly toxic to cells and in general displayed CC_{50} values of around 1–2 μg/mL, worthwhile to study further. All other synthesized analogues proved devoid of activity.

4. Materials and methods

4.1. General synthetic procedures

Starting nucleosides were obtained from ACROS. All other chemicals were provided by Aldrich or ACROS and were of the

highest quality. ^{1}H and ^{13}C NMR spectra were determined with a 300 MHz Varian Gemini apparatus with tetramethylsilane as internal standard for the ^{1}H NMR spectra (s = singlet, d = doublet, dd = double doublet, t = triplet, br. s = broad signal, br. d = broad doublet, m = multiplet) and the solvent signal – DMSO-d_6 (δ = 39.6 ppm) or CDCl3 (δ = 76.9 ppm) – for the ^{13}C NMR spectra. Exact mass measurements were performed with a quadrupole/orthogonal acceleration time-of-flight tandem mass spectrometer (qTOF2, Micromass, Manchester, UK) fitted with a standard electrospray ionization (ESI) interface. All solvents were carefully dried or bought as such. Detailed procedures can be found in the supplementary file.

4.2. Alkylated thymidine analogues

4.2.1. N^3-methyl-thymidine (**5**) [25,26]

Thymidine (**4**, 500 mg, 2.06 mmol) was dissolved in dry DMF (10 mL) in a 50 ml round bottom flask to give a colourless solution under argon atmosphere. Potassium carbonate (856 mg, 6.19 mmol) and methyl iodide (321 μL, 5.16 mmol) were added to the reaction mixture. The reaction was sonicated for 2 h at 55 °C and was monitored by TLC in 10% MeOH/CH_2Cl_2. After 2 h the DMF was evaporated and diluted with 20 mL CH_2Cl_2. The crude product was purified by column chromatography and was eluted with CH_2Cl_2 followed by 10% MeOH/CH_2Cl_2 to give 430 mg (81%) of the desired compound **5**. ^{1}H NMR (MeOD): δ 7.76 (s, 1H), 6.19 (t, 1H), 5.26 (s, 1H), 5.05 (s, 1H), 4.23 (s, 1H), 3.77 (s, 1H), 3.39–3.62 (m, 2H), 3.16 (s, 3H), 2.08–2.10 (m, 2H), 1.81 (s, 3H). ^{13}C NMR (75 MHz, MeOD) δ 163.0, 150.7, 134.6, 108.4, 87.5, 85.0, 70.4, 61.4, 39.8, 27.6, 13.1. HRMS (EI+): m/z for $[C_{11}H_{16}N_2O_5Na]^+$ calcd. 279.0952; found 279.0957.

4.2.2. N^3-methyl-5′-O-trityl-thymidine (**6**)

N-methyl-thymidine (**5**, 100 mg, 0.39 mmol) was evaporated 3 times with 5 mL of anhydrous pyridine and dissolved in 2 mL of dry pyridine. Trityl chloride (131 mg, 0.468 mmol) was added and a red colour was formed. The mixture was stirred at 80 °C under argon atmosphere and the reaction progress was monitored using TLC. After 18 h, the mixture was cooled to room temperature, quenched with 1.5 mL of MeOH and stirred for 30 min. The mixture was evaporated to dryness. The obtained crude compound was purified by column chromatography (CH_2Cl_2/MeOH, 95:5) to obtain 165 mg (84%) of **6**. TLC (CH_2Cl_2/MeOH, 9:1): R_f = 0.90. ^{1}H NMR (500 MHz, MeOD): δ 7.71 (s, 1H), 7.44 (d, 6H, J = 7.40 Hz), 7.33–7.25 (m, 9H), 6.34 (t, 1H, J = 6.71 Hz, H-1′), 4.56–4.51 (m, 1H, H-3′), 4.04–3.99 (m, 1H, H-4′), 3.44–3.34 (m, 2H, H-5′, H-5″), 3.29 (s, 3H, N–CH_3), 2.35 (t, 2H, J = 5.50 Hz, H-2′, H-2″), 1.46 (s, 3H, 5-CH_3). ^{13}C NMR (125 MHz, MeOD) δ 165.6 (C-4), 152.5 (C-2), 145.0, 135.8 (C-6), 129.9, 129.0, 124.5, 110.6 (C-5), 88.6, 87.8 (C-4′), 87.2 (C-1′), 72.5 (C-3′), 65.0 (C-5′), 41.6 (C-2′), 28.2 (N–CH_3), 12.8 (5-CH_3). HRMS (EI+): m/z for $[C_{30}H_{30}N_2O_5Na]^+$ calcd. 521.2053; found 521.2043.

4.2.3. 5′-O-benzoyl-N^3-methyl-thymidine (**7**)

N-methyl-thymidine (**5**, 100 mg, 0.41 mmol) was evaporated 3 times with 5 mL of anhydrous pyridine and was dissolved in 2 mL of anhydrous pyridine. Reaction was cooled at 0 °C and benzoyl chloride (51 μL, 0.43 mmol) was added. The mixture was stirred for 2 h under argon atmosphere. Reaction progress was monitored using TLC. The mixture was evaporated to dryness. The obtained crude compound was purified by column chromatography (CH_2Cl_2/MeOH, 95:5) to obtain 125 mg (85%) of **7**. TLC (CH_2Cl_2/MeOH, 9:1): R_f = 0.60. ^{1}H NMR (500 MHz, MeOD): δ 8.04–8.02 (m, 2H), 7.64–7.61 (m, 1H), 7.51–7.47 (m, 2H), 7.47–7.44 (m, 1H), 6.30–6.28 (t, 1H, J = Hz, H-1′), 4.67–4.64 (dd, 1H), 4.54–4.49 (m, 1H), 4.23–4.20 (m, 1H), 3.30–3.26 (s, 3H, 5-CH_3), 2.41–2.37 (m, 1H), 2.31–2.25 (m, 1H), 1.67 (s, 3H, 5-CH_3), 1.78–1.73 (m, 2H, H-2′, H-2″). ^{13}C NMR (125 MHz, MeOD): δ 168.5, 166.4, 153.3, 136.4, 135.5, 132.0, 131.5, 130.7, 111.6, 88.5, 87.0, 73.2, 66.3, 49.9, 42.0, 29.0, 13.9.

4.2.4. 5′-O-benzoyl-N^3-methyl-3′-O-trityl-thymidine (**8**)

In a 25 ml round bottom flask, compound **7** (200 mg, 0.55 mmol) was evaporated 3 times with 5 mL of anhydrous pyridine and dissolved in 2 mL of anhydrous pyridine. Trityl chloride (340.4 mg, 1.22 mmol) was added under argon atmosphere. The mixture was stirred at 85 °C and reaction progress was monitored using TLC. After 18 h, the mixture was cooled to room temperature, quenched with 1.5 mL of MeOH and stirred for 30 min. The mixture was evaporated to dryness. The obtained crude compound was purified by column chromatography to obtain 220 mg (66%) of **8**. TLC (ethyl acetate/hexane, 1:1): R_f = 0.90. ^{1}H NMR (500 MHz, MeOD): δ 7.49–7.11 (m, 20H), 7.09–7.08 (d, 2H), 6.50–6.49 (t, 1H, H-1′), 4.43–4.41 (d, 1H), 4.29–4.24 (m, 1H) 4.06–4.05 (d, 1H), 3.81–3.76 (m, 1H), 3.32 (s, 3H, N–CH_3), 2.31–2.24 (m, 1H), 1.82–1.71 (m, 1H), 1.59 (s, 3H, 5-CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 165.6, 163.1, 150.7, 143.6, 133.2, 132.2, 129.1, 128.5, 128.3, 127.9, 127.3, 109.8, 87.8, 85.7, 83.5, 74.2, 63.9, 39.6, 27.5, 12.5. HRMS (EI+): m/z for $[C_{37}H_{35}N_2O_6]^+$ calcd. 603.2489; found 603.2501.

4.2.5. N^3-methyl-3′-O-trityl-thymidine (**9**)

An amount of 200 mg (0.33 mmol) of **8** was dissolved in 8.0 mL of absolute MeOH and was stirred at room temperature while LiOH (80 mg, 1.90 mmol) in H_2O (8 mL) was added to the reaction mixture. After 18 h TLC indicated product formation. The reaction mixture was extracted with CH_2Cl_2 (10 mL × 2). The organic layer was dried over $MgSO_4$, filtered and the solvent was removed by rotary evaporation. The crude product was purified by column chromatography to obtain 110 mg (66%) of pure **9**. TLC (CH_2Cl_2/MeOH, 9:1): R_f = 0.40. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.49–7.44 (m, 6H), 7.34–7.29 (m, 6H), 7.28–7.24 (m, 3H), 7.19 (s, 1H), 6.14 (dd, 1H, J = 5.50 Hz, H-1′), 4.42–4.38 (m, 1H, H-3′), 3.95–3.91 (m, 1H, H-4′), 3.64–3.58 (m, 1H, H-5′/H-5″), 3.26 (s, 3H, N–CH_3), 3.27–3.22 (m, 1H, H-5′/H-5″), 2.05–1.98 (m, 1H, H-2′/H-2″), 1.89 (s, 3H, 5-CH_3), 1.79–1.73 (m, 1H, H-2′/H-2″). ^{13}C NMR (125 MHz, $CDCl_3$): δ 163.5 (C-4), 151.1 (C-2), 144.2, 135.1 (C-6), 128.8, 128.1, 127.4, 110.1 (C-5), 89.0 (C-1′), 87.8, 86.6 (C-4′), 74.6 (C-3′), 62.6 (C-5′), 38.5 (C-2′), 27.8 (N–CH_3), 13.2 (5-CH_3). HRMS (EI+): m/z for $[C_{30}H_{30}N_2O_5Na]^+$ calcd. 521.2052; found 521.2048.

4.2.6. N^3-methyl-3′,5′-di-O-trityl-thymidine (**10**)

In a 25 ml round bottom flask 100 mg (0.39 mmol) of **5** was evaporated 3 times with 5 mL of anhydrous pyridine and dissolved in 3 mL of anhydrous pyridine. Trityl chloride (544 mg, 1.95 mmol) was added to the reaction mixture. The mixture was heated at 85 °C for 18 h under argon atmosphere. After 18 h, the mixture was cooled to room temperature, quenched with 3 mL of MeOH and stirred for 30 min. The mixture was evaporated to dryness. The obtained crude compound was purified by column chromatography to obtain 220 mg (76%) of **10**. TLC (ethyl acetate): R_f = 0.80. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.50 (s, 1H), 7.38–7.35 (m, 6H), 7.28–7.10 (m, 24H), 6.52–6.40 (dd, 1H, J = 5.50 Hz, H-1′), 4.39–4.37 (m, 1H, H-3′), 3.81–3.80 (m, 1H, H-4′), 3.34 (s, 3H, N–CH_3), 3.23–3.19 (m, 1H, H-5′/H-5″), 2.84–2.81 (m, 1H, H-5′/H-5″), 2.03–1.98 (m, 1H, H-2′/H-2″), 1.95–1.90 (m, 1H, H-2′/H-2″), 1.89 (s, 3H, 5-CH_3). ^{13}C NMR (125 MHz, $CDCl_3$): δ 163.7 (C-4), 151.2 (C-2), 144.0, 143.3, 133.6 (C-6), 128.7, 128.5, 128.0, 127.9, 127.3, 127.2, 110.0 (C-5), 87.8, 87.3, 85.8 (C-4′), 85.3 (C-1′), 75.4 (C-3′), 63.8 (C-5′), 39.9 (C-2′), 27.9 (N–CH_3), 12.5 (5-CH_3). HRMS (EI+): m/z for $[C_{49}H_{45}N_2O_5]^+$ calcd. 741.3323; found 741.3327.

4.2.7. N^3-methyl-3′,5′-di-O-methyl-thymidine (**11**)

To a suspension of sodium hydride (11.2 mg, 0.46 mmol) in anhydrous DMF (0.5 mL) at 0 °C under argon was added dropwise compound **5** (50 mg, 0.20 mmol) dissolved in DMF (0.5 mL). The mixture was stirred for 20 min at room temperature. Methyl iodide (29.2 μL, 0.46 mmol) was added to the solution at room temperature, and the reaction mixture was sonicated for 45 min. The solvent was removed *in vacuo*, and purification by flash chromatography (elution with 60–90% ethyl acetate in hexane) afforded compound **11** (30 mg, 52%) as a white foam. TLC (ethyl acetate): $R_f = 0.70$. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.61 (s, 1H), 6.35–6.32 (t, 1H, H-1′), 4.20–4.10 (m, 1H), 4.10–4.00 (m, 1H), 3.70–3.66 (m, 1H), 3.59–3.56 (m, 1H), 3.44 (s, 3H), 3.35 (s, 3H), 3.34 (s, 3H), 2.44–2.40 (m, 1H, H-2′/H-2″), 2.07–2.00 (m, 1H, H-2′/H-2″), 1.94 (s, 3H, 5-CH_3). ^{13}C NMR (125 MHz, $CDCl_3$): δ 163.7, 133.8, 109.7, 85.9, 83.7, 81.1, 73.0, 59.1, 56.9, 37.5, 27.8, 13.5. HRMS (EI+): m/z for $[C_{13}H_{20}N_2O_5Na]^+$ calcd. 307.1264; found 307.1263.

4.2.8. 5′-O-dodecyl-N^3-methyl-thymidine (**12**)

To a suspension of sodium hydride (60% dispersion in oil, 10 mg, 0.45 mmol) in anhydrous DMF (0.5 mL) was added compound **5** (50 mg, 0.39 mmol) in DMF (0.5 mL) at 0 °C under argon atmosphere. The mixture was stirred for 20 min at 0 °C. The 1-bromododecane (103.2 μL, 0.43 mmol) was added to the solution and the reaction mixture was allowed to stir at room temperature for 2 h. The solvent was removed *in vacuo*, and purification by flash chromatography (elution with 5% methanol in CH_2Cl_2) afforded compound **12** (20 mg, 23%) as a colourless oil. TLC (CH_2Cl_2/MeOH, 9:1): $R_f = 0.80$. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.62 (s, 1H), 6.46–6.42 (m, 1H, H-1′), 4.53–4.51 (m, 1H), 4.11–4.09 (m, 1H), 3.76–3.71 (m, 1H), 3.65–3.60 (m, 1H), 3.56–3.46 (m, 2H), 3.35 (m, 3H), 2.41–2.33 (m, 1H, H-2′/H-2″), 2.32 (bs, 1H, OH), 2.24–2.15 (m, 1H, H-2′/H-2″), 1.95 (s, 3H, 5-CH_3), 1.69–1.57 (m, 2H), 1.34–1.20 (m, 18H), 0.89–0.87 (m, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 163.7, 133.7, 109.8, 85.8, 85.7, 72.6, 71.9, 70.6, 41.1, 31.9, 29.7, 29.6, 29.6, 29.5, 29.4, 29.3, 27.8, 26.2, 22.7, 14.1, 13.4; HRMS (EI+): m/z for $[C_{23}H_{41}N_2O_5]^+$ calcd. 425.3000; found 425.2992.

4.2.9. 3′,5′-Di-O-dodecyl-N^3-methyl-thymidine (**13**)

To a suspension of sodium hydride (23.4 mg, 0.98 mmol) in anhydrous DMF (2 mL) at 0 °C under argon was added dropwise compound **5** (100 mg, 0.41 mmol) dissolved in DMF (2 mL). The mixture was stirred for 15 min at room temperature. An amount of 1-bromododecane (281.3 μL, 1.17 mmol) was added to the solution at room temperature. The reaction mixture was sonicated for 45 min and was allowed to stir at room temperature for 2 h. The solvent was removed *in vacuo*, and purification by flash chromatography (elution with 10–20% ethyl acetate in hexane) afforded **13** (155 mg, 68%) as a colourless oil. TLC (ethyl acetate/hexane 1:1): $R_f = 0.80$. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.63 (s, 1H), 6.36–6.34 (m, 1H, H-1′), 4.13–4.11 (m, 1H), 4.08–4.06 (m, 1H), 3.74–3.72 (m, 1H), 3.57–3.53 (m, 2H), 3.48–3.43 (m, 2H), 3.40–3.37 (m, 1H), 3.34 (s, 3H), 2.41–2.38 (m, 1H, H-2′/H-2″), 2.06–2.03 (m, 1H, H-2′/H-2″), 1.94 (s, 3H, 5-CH_3), 1.63–1.55 (m, 4H), 1.40–1.20 (m, 36H), 0.89–0.87 (m, 6H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 163.7, 151.1, 133.7, 109.6, 85.9, 84.0, 79.5, 71.8, 70.9, 69.6, 38.1, 31.9, 29.7, 29.6, 29.5, 29.4, 29.3, 27.8, 26.2, 26.1, 22.7, 14.1, 13.4; HRMS (EI+): m/z for $[C_{35}H_{65}N_2O_5]^+$, calcd. 593.4887; found 593.4883.

4.2.10. 3′,5′-Di-O-benzyl-N^3-methyl-thymidine (**14**)

Compound **5** (100 mg, 0.41 mmol) in DMF (2 mL) was added dropwise to suspension of sodium hydride (23.4 mg, 0.98 mmol) in anhydrous DMF (2 mL) at 0 °C under argon atmosphere. The mixture was stirred for 15 min at room temperature. Benzyl bromide (140.0 μL, 1.17 mmol) was added to the solution at room temperature. The reaction mixture was allowed to stir at room temperature for 2 h. The solvent was removed *in vacuo*, and purification by flash chromatography (elution with 40–70% ethyl acetate in hexane) afforded 153 mg, (86%) of **14** as a white solid. TLC (ethyl acetate/hexane 2:1): $R_f = 0.80$. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.57 (s, 1H), 7.38–7.28 (m, 10H) 6.45–6.41 (m, 1H, H-1′), 4.63–4.49 (m, 4H, $2CH_2$), 4.30–4.26 (m, 2H, H-3′ and H-4′), 3.86–3.81 (m, 1H, H-5′), 3.68–3.64 (m, 1H, H-5″), 3.34 (s, 3H, N–CH_3), 2.53–2.48 (m, 1H, H-2′/H-2″), 2.17–2.07 (m, 1H, H-2′/H-2″), 1.69 (s, 3H, 5-CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 163.6, 151.0, 137.5, 137.4, 133.6, 128.5, 128.0, 127.9, 127.6, 127.4, 109.8, 85.8, 83.8, 78.9, 73.5, 71.4, 70.2, 38.0, 27.7, 12.9; HRMS (EI+): m/z for $[C_{25}H_{29}N_2O_5]^+$ calcd. 437.2071; found 437.2088.

4.2.11. 5′-O-benzyl-N^3-methyl-thymidine (**15**) and 3′-O-benzyl-N^3-methyl-thymidine (**16**)

To a suspension of sodium hydride (60% dispersion in oil, 10 mg, 0.45 mmol) in anhydrous DMF (0.5 mL) was added dropwise compound **5** (50 mg, 0.39 mmol) in DMF (0.5 mL) at 0 °C under argon atmosphere. The mixture was stirred for 20 min. Benzyl bromide (51 μL, 0.43 mmol) was added to the solution at and was allowed to stir at 0 °C for 4 h. The solvent was removed *in vacuo*, and purification was done by flash chromatography (elution with 5% Methanol in CH_2Cl_2) affording **15** (18 mg, 26%) and **16** (16 mg, 23%) as a white foam.

5′-O-benzyl-N^3-methyl-thymidine (**15**): TLC (CH_2Cl_2/MeOH, 9:1): $R_f = 0.60$. ^{1}H NMR (500 MHz, MeOD): δ 7.56 (s, 1H), 7.37–7.30 (m, 5H), 6.43 (t, 1H, $J = 6.6$ Hz, H-1′), 4.59 (s, 2H), 4.55–4.54 (m, 1H, H-3′), 4.11–4.10 (m, 1H, H-4′), 3.82–3.79 (m, 1H, H-5′), 3.72–3.70 (m, 1H, H-5″), 3.31 (s, 3H, N–CH_3), 2.59 (bs, 1H, OH), 2.38–2.34 (m, 1H, H-2′), 2.24–2.19 (m, 1H, H-2″), 1.68 (s, 3H, 5-CH_3). ^{13}C NMR (125 MHz, MeOD): δ 163.7 (C-4), 151.1 (C-2), 137.4, 133.7 (C-6), 128.6, 128.0, 127.5, 110.0 (C-5), 85.8 (C-1′), 73.6 (C-4′), 72.4 (C-3′), 70.1, 62.9 (C-5′), 41.0 (C-2′), 27.8 (N–CH_3), 13.0 (5-CH_3). HRMS (EI+): m/z for $[C_{18}H_{23}N_2O_5]^+$ calcd. 347.1601; found 347.1608.

3′-O-benzyl-N^3-methyl-thymidine (**16**): TLC (CH_2Cl_2/MeOH, 9:1): $R_f = 0.70$. ^{1}H NMR (500 MHz, MeOD): δ 7.39–7.29 (m, 6H), 6.12 (t, 1H, $J = 7.44$ Hz, H-1′), 4.59–4.52 (m, 2H), 4.32–4.30 (m, 1H, H-3′), 4.18–4.16 (m, 1H, H-4′), 3.94–3.92 (m, 1H, H-5′), 3.77–3.75 (m, 1H, H-5″), 3.33 (s, 3H, N–CH_3), 2.71 (bs, 1H, OH), 2.42–2.36 (m, 2H, H-2′, H-2″), 1.93 (s, 3H, 5-CH_3). ^{13}C NMR (125 MHz, MeOD): δ 163.5 (C-4), 151.0 (C-2), 137.4, 135.0 (C-6), 128.5, 128.0, 127.7, 110.1 (C-5), 88.6 (C-1′), 85.2 (C-4′), 78.7 (C-3′), 71.6, 62.9 (C-5′), 37.1 (C-2′), 27.8 (N–CH_3), 13.3 (5-CH_3). HRMS (ES+): m/z for $[C_{18}H_{23}N_2O_5]^+$, calcd. 347.1601; found 347.1609.

4.2.12. 5′-O-trityl-thymidine (**17**) [27]

In a 25 ml round bottom flask 100 mg (0.39 mmol) of **4** was evaporated 3 times with 3 mL of dry pyridine and dissolved in 3 mL of dry pyridine. Trityl chloride (120.0 mg, 0.43 mmol) was added and the reaction mixture was heated at 65 °C for 16 h under argon atmosphere. After 16 h, the mixture was cooled to room temperature, quenched with 3 mL of MeOH and stirred for 30 min. The mixture was evaporated to dryness. The obtained crude compound was purified by column chromatography to obtain 92 mg (48%) of **17**. TLC (CH_2Cl_2/MeOH, 9:1): $R_f = 0.70$. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.61 (bs, 1H), 7.56 (s, 1H), 7.42–7.26 (m, 15H), 6.42–6.40 (m, 1H, H-1′), 4.58 (s, 1H), 4.20–4.10 (m, 1H), 3.49–3.46 (m, 1H), 3.40–3.37 (m, 1H), 2.44–2.40 (m, 1H, H-2′/H-2″), 2.34–2.30 (m, 1H, H-2′/H-2″), 1.48 (s, 3H, 5-CH_3). ^{13}C NMR (125 MHz, $CDCl_3$): δ 163.6, 150.3, 143.3, 135.5, 128.6, 128.2, 128.0, 127.5, 111.3, 87.5, 86.0, 84.6, 72.4, 70.7, 63.7, 40.9, 11.9; HRMS (EI+): m/z for $[C_{42}H_{48}N_4O_{10}Na]^+$, calcd. 507.1890; found 507.1907.

4.2.13. 5′-O-benzoyl-thymidine (**18**) [28]

Thymidine **4** (100 mg, 0.41 mmol) was evaporated 3 times with 5 mL of anhydrous pyridine and then dissolved in 2 mL of

anhydrous pyridine. Reaction was cooled at 0 °C and benzoyl chloride (51 μL, 0.43 mmol) was added to the reaction mixture. The mixture was stirred for 2 h at 0 °C under argon atmosphere. Reaction progress was monitored using TLC. After completion the mixture was evaporated to dryness. The obtained crude compound was purified by column chromatography (CH_2Cl_2/MeOH, 95:5) to obtain 120 mg (84%) of **18**. TLC (CH_2Cl_2/MeOH, 9:1): $R_f = 0.60$. ^{1}H NMR (300 MHz, $CDCl_3$): 11.32 (s, 1H, OH), 8.01–7.98 (m, 2H), 7.71–7.66 (m, 1H), 7.55 (t, 2H, J = 7.65 Hz), 7.40 (2, 1H), 6.22 (t, 1H, J = 6.9 Hz), 5.49 (d, 1H, J = 4.5 Hz), 4.58–4.39 (m, 3H), 4.08–4.05 (m, 1H), 2.30–2.09 (m, 2H), 1.60 (s, 3H, 5-CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 165.7, 163.7, 150.5, 135.8, 133.7, 129.5129.3, 129.0, 109.9, 83.9, 83.8, 70.4, 64.5, 12.0. HRMS (EI+): m/z for $[C_{17}H_{18}N_2O_6Na]^+$ calcd. 369.1057; found 369.1056.

4.2.14. 5′-O-benzoyl-3′-O-trityl-thymidine (**19**)

In a 25 ml round bottom flask 108 mg (0.31 mmol) of **18** was evaporated 3 times with 2 mL of anhydrous pyridine and dissolved in 0.5 mL of anhydrous pyridine. Trityl chloride (191.4 mg, 0.69 mmol) was added. The mixture was stirred at 85 °C under argon atmosphere and reaction progress was monitored using TLC. After 18 h, the mixture was cooled to room temperature, quenched with 1.5 mL of MeOH and stirred for 30 min. The mixture was evaporated to dryness. The crude compound was purified by column chromatography to obtain 75 mg (40%) of **19**. TLC (CH_2Cl_2/MeOH, 9:1): $R_f = 0.80$. ^{1}H NMR (500 MHz, MeOD): δ 7.88 (d, 1H), 7.65–6.27 (m, 20H), 4.49–4.47 (m, 1H), 4.32–4.27 (m, 1H) 4.06–4.00 (m, 1H), 2.06–2.02 (m, 1H), 1.80–1.56 (m, 1H), 1.55 (s, 3H, 5-CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 165.6, 163.0, 149.8, 143.6, 134.4, 133.2, 129.1, 128.5, 128.3, 127.9, 127.3, 110.8, 87.8, 85.0, 83.5, 74.2, 64.0, 39.5, 11.7. HRMS (EI-): m/z for $[C_{36}H_{31}N_2O_6]^-$ calcd. 587.2187; found 587.2181.

4.2.15. 3′-O-trityl-thymidine (**20**) [29]

The product **19** (50 mg, 0.09 mmol) was dissolved in 1 mL of absolute MeOH and was stirred at rt. LiOH (17.9 mg, 0.43 mmol) in H_2O (2 mL) was added to the reaction mixture. After 5 h TLC indicates product formation. The reaction mixture was extracted with CH_2Cl_2 (10 mL × 2). The organic layer was washed with water (5 mL × 1), dried over $MgSO_4$, filtered and the solvent was removed by rotary evaporation. The crude product was purified by column chromatography to obtain 25 mg (61%) of pure **20**. TLC (ethyl acetate/hexane, 1:1): $R_f = 0.20$. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.68 (s, 1H), 7.51–7.46 (m, 6H), 7.37–7.31 (m, 6H), 7.30–7.25 (m, 3H), 6.31 (t, 1H, J = 5.50 Hz, H-1′), 4.42–4.35 (m, 1H, H-3′), 3.85–3.78 (m, 1H, H-4′), 3.48–3.42 (m, 1H, H-5′/H-5″), 3.27–3.22 (m, 1H, H-5′/H-5″), 1.81 (s, 3H, 5-CH_3), 1.77–1.75 (m, 2H, H-2′, H-2″). ^{13}C NMR (125 MHz, MeOD): δ 166.4 (C-4), 152.5 (C-2), 145.8, 138.0 (C-6), 130.1, 129.1, 128.5, 111.7 (C-5), 89.2, 88.1 (C-4′), 86.4 (C-1′), 76.5 (C-3′), 63.0 (C-5′), 40.4 (C-2′), 12.4 (5-CH_3); HRMS (EI+): m/z for $[C_{29}H_{28}N_2O_5Na]^+$ calcd. 507.1890; found 507.1884.

4.2.16. 3′,5′-di-O-trityl-thymidine (**21**) [29]

In a 25 ml round bottom flask 100 mg (0.41 mmol) of **4** was co-evaporated 3 times with 2 mL of dry pyridine and dissolved in 2 mL of dry pyridine. Trityl chloride (287.5 mg, 1.03 mmol) was added under argon atmosphere. The mixture was heated at 65 °C for 16 h. After 16 h, the mixture was cooled to room temperature, quenched with 3 mL of MeOH and stirred for 20 min. The mixture was evaporated to dryness. The obtained crude compound was purified by column chromatography to obtain 251 mg (84%) of **21** as white foam. TLC (ethyl acetate/hexane, 1:1): $R_f = 0.60$. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.61 (bs, 1H), 7.56 (s, 1H), 7.42–7.26 (m, 15H), 6.42–6.40 (m, 1H, H-1′), 4.58 (s, 1H), 4.20–4.10 (m, 1H), 3.49–3.46 (m, 1H), 3.40–3.37 (m, 1H), 2.44–2.40 (m, 1H, H-2′/H-2″), 2.34–2.30 (m, 1H, H-2′/H-2″), 1.48 (s, 3H, 5-CH_3). ^{13}C NMR (125 MHz, $CDCl_3$): δ 163.6, 150.3, 143.3, 135.5, 128.6, 128.2, 128.0, 127.5, 111.3, 87.5, 86.0, 84.6, 72.4, 70.7, 63.7, 40.9, 11.9; HRMS (EI+): m/z for $[C_{48}H_{42}N_2O_5Na]^+$ calcd. 749.2986; found 749.2991.

4.2.17. 3′-O-benzyl-5′-O-trityl-thymidine (**22**)

To a suspension of 18.2 mg of sodium hydride (0.45 mmol) in anhydrous THF (1 mL) was added dropwise compound **17** (200 mg, 0.41 mmol) in THF (1 mL) at room temperature under argon atmosphere. The mixture was stirred at 50 °C for 1 h. An amount of benzyl bromide (54 μL, 0.45 mmol) was added to the solution and stirring was continued for 3 h. Reaction was monitored by TLC in 100% ethyl acetate. The solvent was removed *in vacuo*, and purification was done by flash chromatography (elution with 80–90% ethyl acetate in hexane) afforded 190 mg (80%) of **22**. TLC (ethyl acetate): $R_f = 0.70$. ^{1}H NMR (500 MHz, MeOD): δ 7.52–7.48 (m, 3H), 7.41–7.39 (m, 5H), 7.32–7.24 (m, 15H), 6.43–6.40 (m, 1H, H-1′), 5.56–5.54 (m, 1H), 4.02–4.00 (m, 1H), 3.48–3.45 (m, 1H), 3.39–3.36 (m, 1H), 2.41–2.37 (m, 1H), 2.32–2.26 (m, 1H), 1.53 (s, 3H, 5-CH_3). ^{13}C NMR (125 MHz, MeOD): δ 163.4, 151.0, 143.3, 136.9, 133.6, 129.3, 128.6, 128.4, 128.0, 127.4, 110.5, 87.5, 85.7, 85.3, 72.3, 63.6, 44.5, 41.0, 12.7; HRMS (EI+): m/z for $[C_{36}H_{34}N_2O_5Na]^+$ calcd. 597.2360; found 597.2361.

4.2.18. 3′-O-benzyl-thymidine (**23**) [30]

The compound **22** (150 mg, 0.26 mmol) was dissolved in 80% aqueous acetic acid (10 mL) and heated at 50 °C for 1 h. Solvent was removed *in vacuo*, and the crude product thus obtained was purified on silica gel column using MeOH/CH_2Cl_2 (8:92, v/v) as eluent to give compound **23** (76 mg, 88%) as a solid. TLC (MeOH/CH_2Cl_2, 1:9): $R_f = 0.50$. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.87 (s, 1H), 7.35–7.23 (m, 5H), 6.33–6.30 (m, 1H, H-1′), 5.10 (s, 2H), 4.41–4.38 (m, 1H), 3.92–3.90 (m, 1H), 3.82–3.79 (m, 1H), 3.74–3.71 (m, 1H), 2.30–2.25 (m, 1H), 2.23–2.18 (m, 1H), 1.92 (s, 3H, 5-CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 162.9, 150.7, 136.5, 134.5, 128.9, 128.0, 127.3, 87.3, 86.4, 71.3, 62.1, 44.2, 39.8, 13.00; HRMS (EI+): m/z for $[C_{17}H_{21}N_2O_5]^+$ calcd. 333.1444; found 333.1436.

4.2.19. 3′,5′-di-O-benzyl-thymidine (**24**) [31]

To a suspension of sodium hydride (7.2 mg, 0.18 mmol) in anhydrous THF (0.5 mL) was added **23** (50 mg, 0.15 mmol) in THF (0.5 mL) dropwise at room temperature under argon atmosphere. The mixture was stirred at 50 °C for 1 h. An amount of benzyl bromide (21 μL, 0.18 mmol) was added to the solution and stirring was continued for 5 h. Reaction was monitored by TLC. The solvent was removed *in vacuo*, and purification was done by flash chromatography (elution with 30–50% ethyl acetate/hexane) afforded 34 mg (53%) of **24**. TLC (ethyl acetate/hexane, 1:1): $R_f = 0.60$. ^{1}H NMR (500 MHz, MeOD): δ 7.67 (s, 1H), 7.35–7.27 (m, 10H), 6.31–6.28 (m, 1H, H-1′), 4.61–4.53 (m, 4H), 4.32–4.30 (m, 1H), 4.24–4.22 (m, 1H), 3.81–3.78 (m, 1H), 3.68–3.66 (m, 1H), 2.45–2.40 (m, 1H), 2.23–2.17 (m, 1H), 1.55 (s, 3H, 5-CH_3). ^{13}C NMR (125 MHz, MeOD): δ 166.3, 152.3, 139.5, 137.8, 129.6, 129.5, 129.0, 128.8, 111.6, 86.5, 85.4, 80.6, 74.5, 72.2, 71.7, 31.7, 12.3; HRMS (EI+): m/z for $[C_{24}H_{26}N_2O_5Na]^+$ calcd. 445.1734; found 445.1736.

4.2.20. 5′-O-fmoc-thymidine (**25**) [32]

Thymidine (**4**, 50 mg, 0.21 mmol) was dissolved and co-evaporated 3 times with 2 mL of dry pyridine and dissolved in 0.5 mL of dry pyridine. Fmoc chloride (64 mg, 0.25 mmol) was added to the reaction mixture. The mixture was allowed to stir at room temperature for 18 h under argon atmosphere. After 18 h, mixture was evaporated to dryness. The obtained crude compound was purified by column chromatography to obtain 50 mg (52%) of **25**. TLC (CH_2Cl_2/MeOH, 9:1): $R_f = 0.70$. ^{1}H NMR (500 MHz, $CDCl_3$):

δ 7.89 (s, 1H), 7.79–7.76 (m, 2H), 7.60–7.58 (m, 2H), 7.43–7.40 (m, 1H), 7.40–7.35 (m, 2H), 7.30–7.26 (m, 2H), 6.30–6.22 (m, 1H, H-1′), 4.65–4.62 (m, 1H, H-3′), 4.50–3.47 (m, 1H), 4.44–4.41 (m, 1H), 4.31–4.23 (m, 3H), 4.07–4.04 (m, 1H), 2.25–2.20 (m, 1H, H-2′/H-2″), 2.03–1.97 (m, 1H, H-2′/H-2″), 1.67 (s, 3H, 5-CH_3). ^{13}C NMR (125 MHz, $CDCl_3$): δ 166.3, 156.4, 152.2, 144.7, 144.6, 142.7, 142.6, 137.4, 129.0, 128.9, 128.2, 128.1, 126.0, 125.9, 121.1, 111.7, 86.4, 86.0, 72.2, 70.7, 68.4, 40.9, 12.6; HRMS (EI+): m/z for $[C_{25}H_{24}N_2O_7Na]^+$ calcd. 487.1475; found 487.1470.

4.2.21. 3′,5′-bis-O-fmoc-thymidine (**26**)

Thymidine **4** (50 mg, 0.21 mmol) was dissolved and co-evaporated 3 times with 2 mL of dry pyridine and dissolved in 1 mL of dry pyridine. Fmoc chloride (128 mg, 0.50 mmol) was added to the reaction mixture. The mixture was allowed to stir at room temperature for 18 h under argon atmosphere. After 18 h, mixture was evaporated to dryness. The obtained crude compound was purified by column chromatography to obtain 88 mg (62%) of **26**. TLC (ethyl acetate): $R_f = 0.90$. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.40 (bs, 1H, NH), 7.80–7.74 (m, 4H), 7.63–7.54 (m, 4H), 7.45–7.38 (m, 4H), 7.37–7.27 (m, 5H), 6.41–6.38 (m, 1H, H-1′), 5.15–5.13 (m, 1H), 4.63–4.59 (m, 1H, H-3′), 4.51–4.44 (m, 4H), 4.42–4.39 (m, 1H), 4.30–4.24 (m, 3H), 2.54–2.50 (m, 1H, H-2′/H-2″), 2.16–2.10 (m, 1H, H-2′/H-2″), 1.78 (s, 3H, 5-CH_3). ^{13}C NMR (125 MHz, $CDCl_3$): δ 163.2, 154.6, 154.4, 150.1, 143.0, 142.9, 141.4, 134.7, 128.1, 128.0, 127.2, 127.1, 125.1, 124.9, 124.8, 120.2, 111.7, 84.7, 82.0, 77.8, 70.3, 70.1, 67.2, 46.8, 46.7, 37.3, 12.6; HRMS (EI+): m/z for $[C_{40}H_{35}N_2O_9]^+$ calcd. 687.2336; found 687.2335.

4.2.22. 5′-O-adamantyl-thymidine (**27**) and 3′,5′-bis-O-adamantyl-thymidine (**28**)

Thymidine **4** (300 mg, 1.24 mmol) was co-evaporated 3 times with 5 mL of dry pyridine and dissolved in 2 mL of dry pyridine under argon atmosphere. A solution of adamantyl chloride in CH_2Cl_2 (2.3 mL, 2.27 mmol) was added dropwise to the reaction mixture. The mixture was stirred at 70 °C and reaction progress was monitored using TLC. After 18 h, the mixture was cooled to room temperature. The mixture was evaporated to dryness. The obtained crude compound was purified by column chromatography (CH_2Cl_2/MeOH, 95:5) to obtain the title compounds **28** (180 mg, 26%) and **27** (75 mg, 15%). TLC (ethyl acetate/hexane, 1:1): $R_f = 0.70$ and 0.60.

5′-O-adamantyl-thymidine (**27**) ^{1}H NMR (500 MHz, MeOD): δ 8.79 (bs, 1H), 7.26 (s, 1H), 6.31–6.28 (m, 1H), 4.45–4.42 (m, 1H), 4.37–4.35 (m, 1H), 4.30–4.27 (m, 1H), 4.25–4.22 (m, 1H), 4.18–4.16 (m, 1H), 2.71 (bs, 1H), 2.49–2.44 (m, 1H), 2.12–2.07 (m, 1H), 2.04 (bs, H), 1.94 (s, 3H, 5-CH_3), 1.91–1.88 (m, 6H), 1.77–1.71 (m, 9H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 177.6, 163.5, 150.2, 134.9, 101.2, 84.9, 85.0, 71.5, 63.5, 41.0, 40.6, 39.0, 36.3, 27.8, 12.5; HRMS (EI+): m/z for $[C_{21}H_{28}N_2O_6Na]^+$ calcd. 427.1840; found 427.1835.

3′,5′-bis-O-adamantyl-thymidine (**28**) ^{1}H NMR (500 MHz, MeOD): δ 7.30 (s, 1H), 6.30–6.27 (m, 1H), 5.19–5.17 (m, 1H), 4.46–4.42 (m, 1H), 4.30–4.27 (m, 1H), 4.20–4.18 (m, 1H), 2.50–2.46 (m, 1H), 2.13–2.04 (m, 1H), 2.03 (bs, 6H), 1.94 (s, 3H, 5-CH_3), 1.90–1.84 (m, 12H), 1.77–1.67 (m, 12H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 177.2, 177.0, 150.0, 134.5, 111.4, 84.8, 82.8, 73.8, 63.7, 40.6, 39.1, 38.6, 37.9, 36.3, 29.7, 27.8, 12.5; HRMS (EI+): m/z for $[C_{32}H_{42}N_2O_7Na]^+$ calcd. 589.2884; found 589.2886.

4.2.23. 3′,5-di-O-acetyl-thymidine (**29**) [33]

A suspension of compound **4** (300 mg, 1.24 mmol) in acetonitrile (5 mL) was treated with triethylamine (0.692 mL, 4.95 mmol) and acetyl chloride (0.213 mL, 2.85 mmol) in the presence of a catalytic amount of 4-(dimethylamino)pyridine (DMAP) (15 mg, 0.12 mmol). The resulting mixture was stirred at room temperature for 5 h and was then diluted with CH_2Cl_2 and water (1:1). The organic phase was separated, washed with water (3 × 50 mL) and dried over Na_2SO_4, filtered and concentrated *in vacuo*. The residue was purified by silica gel column chromatography to afford 380 mg (94%) of **29**. TLC (ethyl acetate/hexane, 1:1): $R_f = 0.70$. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.81 (bs, 1H), 7.27 (s, 1H), 6.35–6.30 (m, 1H, H-1′), 5.23–5.21 (m, 1H), 4.41–4.24 (m, 3H), 2.50–2.35 (m, 1H), 2.27–2.24 (m, 1H), 2.13 (s, 3H), 2.12 (s, 3H), 1.68 (s, 3H, 5-CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.4, 170.2, 163.5, 150.3, 134.5, 111.6, 84.8, 82.1, 74.1, 63.8, 37.5, 20.9, 20.8, 12.7; HRMS (EI+): m/z for $[C_{14}H_{18}N_2O_7K]^+$ calcd. 365.0751; found 365.0748.

4.2.24. 3′,5′-di-O-(tert-butyldimethylsilyl)-thymidine (**30**) [34]

Thymidine **4** (200 mg, 0.826 mmol) was dissolved in dry DMF (4 mL). Tert-butyldimethylsilyl chloride (871 mg, 5.78 mmol) and imidazole (562 mg, 8.26 mmol) were added to the reaction mixture. The clear solution was allowed to stir at room temperature for 18 h. Water (40 mL) was added, the aqueous layer was extracted with ethyl acetate (3 × 50 mL), and the combined organic layers were washed with brine (3 × 50 mL), dried over Na_2SO_4, and concentrated to dryness. The oily residue was purified by silica gel column chromatography to give pure compound **30** (360 g, 92%) as a white foam. TLC (ethyl acetate/hexane, 1:1): $R_f = 0.70$. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.81 (bs, 1H), 7.45 (s, 1H), 6.35–6.33 (m, 1H, H-1′), 4.42–4.40 (m, 1H), 3.94–3.93 (m, 1H), 3.89–3.86 (m, 1H), 3.78–375 (m, 1H), 2.27–2.24 (m, 1H), 2.03–1.98 (m, 1H), 1.91 (s, 3H, 5-CH_3); ^{13}C NMR (125 MHz, MeOD): δ 163.8, 150.3, 135.5, 110.8, 87.8, 84.8, 72.2, 63.0, 41.4, 29.7, 25.9, 25.7, 12.5, −4.7, −4.9, −5.4, −5.5; HRMS (EI+): m/z for $[C_{22}H_{42}N_2O_5Si_2K]^+$ calcd. 509.2269; found 509.2263.

4.2.25. 3′,5′-di-O-(triisopropylsilyl)-thymidine (**31**) [35]

Thymidine **4** (200 mg, 0.826 mmol) was dissolved in dry DMF (4 mL). Triisopropylsilyl chloride (1.24 mL, 5.78 mmol) and imidazole (562 mg, 8.26 mmol) were added to the reaction mixture at room temperature. The obtained clear solution was allowed to stir at room temperature for 18 h. Water (40 mL) was added and the aqueous layer was extracted with ethyl acetate (3 × 50 mL). The combined organic layers were washed with brine (3 × 50 mL), dried over Na_2SO_4, and concentrated to dryness. The oily residue was purified by silica gel column chromatography to get 430 mg (94%) of pure compound **31** as white foam. TLC (ethyl acetate/hexane, 1:1): $R_f = 0.70$. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.46 (bs, 1H), 7.46 (s, 1H), 6.38–6.35 (m, 1H, H-1′), 4.63–4.61 (m, 1H), 4.03–4.02 (m, 1H), 3.97–3.94 (m, 1H), 3.90–3.88 (m, 1H), 2.33–2.39 (m, 1H), 2.06–2.02 (m, 1H), 1.91 (s, 3H, 5-CH_3), 1.17–1.13 (m, 3H), 1.10–1.05 (m, 45H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 163.6, 150.1, 135.4, 110.7, 88.4, 84.8, 72.7, 63.6, 41.8, 18.0, 17.9, 17.9, 17.7, 12.3, 12.3, 12.0, 11.8; HRMS (EI+): m/z for $[C_{28}H_{55}N_2O_5Si_2]^+$ calcd. 555.3644; found 555.3656.

4.2.26. 3′,5′-di-O-(tert-butyldiphenylsilyl)-thymidine (**32**)

Tert-butyldiphenylsilyl chloride (1.5 mL, 5.78 mmol) and imidazole (562 mg, 8.26 mmol) was added to a solution of 200 mg (0.826 mmol) of **4** in dry DMF (4 mL). The clear solution was stirred at room temperature for 18 h. Reaction was quenched by adding 40 mL of water. The aqueous layer was extracted with ethyl acetate (3 × 50 mL). The combined organic layers were washed with brine (3 × 50 mL), dried over Na_2SO_4, and concentrated to dryness. The oily residue was purified by silica gel column chromatography to obtain compound 510 mg (87%) of **32** as white foam. TLC (ethyl acetate/hexane, 1:1): $R_f = 0.60$. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.02 (bs, 1H), 7.65–7.63 (m, 2H), 7.59–7.58 (m, 2H), 7.53–7.53 (m, 2H), 7.48–7.45 (m, 3H), 7.42–7.40 (m, 6H), 7.34–7.28 (m, 6H), 6.53–6.51 (m, 1H, H-1′), 4.56–4.55 (d, 1H), 4.00–3.99 (m, 1H), 3.76–3.74 (m, 1H), 3.31–3.29 (m, 1H), 2.35–2.32 (m, 1H), 2.00–1.96 (m, 1H), 1.49 (s, 3H, 5-CH_3), 1.09 (s, 9H), 0.94 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 163.3, 150.1, 135.7, 135.7, 135.4, 135.4, 135.1, 133.2, 133.1, 133.0,

132.1, 130.0, 130.0, 130.0, 127.9, 127.9, 111.0, 87.8, 84.4, 74.0, 64.0, 41.3, 26.9, 19.3, 19.0, 11.9; HRMS (EI+): m/z for $[C_{42}H_{51}N_2O_5Si_2]^+$ calcd. 719.3330; found 719.3328.

*4.2.27. 3′,5′-di-O-(tert-butylphenylmethoxysilyl)-thymidine (**33**)*

To a solution of **4** (200 mg, 0.826 mmol) in dry DMF (4 mL), tert-butylphenylmethoxysilyl bromide (0.46 mL, 2.06 mmol) and imidazole (281 mg, 4.13 mmol) were added to obtain a clear solution. The reaction mixture was allowed to stir at room temperature for 18 h. Water (40 mL) was added to the reaction mixture. The aqueous layer was extracted with ethyl acetate (3 × 50 mL), and the combined organic layers were washed with brine (3 × 50 mL), dried over Na_2SO_4, and concentrated to dryness. The oily residue was purified by silica gel column chromatography to give pure compound **33** (450 mg, 87%) as a white foam. TLC (ethyl acetate/hexane, 1:1): $R_f = 0.60$. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.58 (bs, 1H), 7.65–7.33 (m, 10H), 6.52–6.45 (m, 1H, H-1′), 4.86–4.76 (m, 1H), 4.18–4.15 (m, 1H), 4.13–3.87 (m, 2H), 3.70–3.50 (m, 6H), 2.50–2.44 (m, 1H), 2.17–2.06 (m, 1H), 1.26 (s, 3H), 1.00–0.97 (m, 9H), 0.92–0.90 (m, 9H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 163.1, 150.2, 135.2, 134.9, 131.2, 130.4, 130.3, 130.2, 128.0, 127.9, 111.1, 87.7, 87.6, 84.8, 84.4, 72.7, 72.4, 63.7, 63.0, 51.8, 51.7, 51.4, 41.5, 41.4, 41.3, 29.7, 26.1, 26.1, 18.7, 18.7, 12.3, 12.2, 11.7; HRMS (EI+): m/z for $[C_{32}H_{46}N_2O_7Si_2Na]^+$ calcd. 649.2735; found 649.2711.

*4.2.28. 1-((2R,4S,5R)-4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-5-methyl-4-(1H-1,2,4-triazol-1-yl)pyrimidin-2(1H)-one (**34**) [14]*

Triethylamine (10.5 mL, 75.3 mmol) and chlorotrimethylsilane (3.2 mL, 25.2 mmol) were added to a stirred suspension of thymidine **4** (1.22 g, 5.04 mmol) in acetonitrile (40 mL) at room temperature. After 1.5 h, the reaction mixture was cooled (ice water bath), and 1,2,4-1H-triazole (3.12 g, 45.2 mmol) and phosphorus oxychloride (0.95 mL, 10.2 mmol) were added with continued stirring. After a period of 5 h, the product was poured into saturated aqueous sodium hydrogen carbonate (250 mL), and the resulting mixture was extracted with dichloromethane (2 × 25 mL). The combined organic extracts were dried (Na_2SO_4) and evaporated under reduced pressure. Acetic acid-methanol (1:4 v/v, 15 mL) was added to the residue, and the resulting solution was allowed to stir at room temperature. After 4.5 h, diethyl ether (30 mL) was added dropwise, with stirring, to this solution over a period of 30 min. After a further period of 2 h, colourless crystals of the target compound **34** (1.30 g, 88%) were collected by filtration. ^{1}H NMR (300 MHz, DMSO): δ 9.23 (s, 1H), 8.62 (s, 1H), 8.38 (s, 1H), 6.13 (t, 1H, $J = 6$ Hz), 5.31 (bs, 1H), 5.22 (bs, 1H), 4.28–4.25 (m, 1H), 3.92–3.60 (m, 3H), 3.12–3.05 (m, 1H), 2.42–2.11 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 157.9, 153.5, 153.2, 148.0, 145.4, 104.5, 88.2, 87.0, 69.4, 60.5, 45.7, 41.1, 16.3, 8.7; HRMS (EI+): m/z for $[C_{12}H_{16}N_5O_4]^+$ calcd. 294.1197; found 294.1201.

*4.2.29. O^4-methyl thymidine **(35)** [14]*

In a 25 ml round bottom flask the triazolide **34** (590 mg, 2.0 mmol) was dissolved in anhydrous methanol. Sodium methoxide in methanol (0.5 ml, 30% w) was added to the reaction mixture which was stirred at room temperature. After 15 min, the reaction mixture was evaporated under reduced pressure and the residue was redissolved in methanol (0.5 mL). The suspension was purified by column chromatography (methanol/DCM, 1:9) to get a white solid **35** (300 mg, 60%). ^{1}H NMR (300 MHz, CD_3OD): δ 8.28 (bs, 1H), 8.02 (s, 1H), 6.15 (t, 1H, $J = 6.6$ Hz), 4.26–4.21 (m, 1H), 3.85 (s, 3H, CH_3), 3.83–3.80 (m, 1H), 3.67–3.64 (m, 2H), 2.24–2.16 (m, 1H), 2.02–1.96 (m, 1H), 1.99 (s, 3H, CH_3); ^{13}C NMR (75 MHz, CD_3OD) δ: 170.1, 154.8, 141.0, 103.0, 87.7, 85.6, 70.1, 61.1, 54.2, 40.7, 12.0; HRMS (EI+): m/z for $[C_{11}H_{16}N_2O_5Na]^+$ calcd. 279.0952; found 279.0948.

*4.2.30. 5-fluoro-5′-O-trityl-2′-deoxyuridine (**38**) [36]*

In a 25 ml round bottom flask an amount of 100 mg (0.41 mmol) of **37** was co-evaporated 3 times with 5 mL of dry pyridine and dissolved in 3 mL of dry pyridine. Trityl chloride (136 mg, 0.49 mmol) was added to the reaction mixture. The mixture was heated at 50 °C for 18 h. After 18 h, the mixture was cooled to room temperature, quenched with 3 mL of MeOH and stirred for 30 min. The mixture was evaporated to dryness. The obtained crude compound was purified by column chromatography to obtain 140 mg (70%) of **38**. TLC ($MeOH/CH_2Cl_2$, 1:9): $R_f = 0.80$. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.70 (s, 1H), 7.82 (d, 1H), 7.46–7.43 (m, 6H), 7.37–7.28 (m, 11H), 6.32–6.28 (m, 1H, H-1′), 4.57–4.55 (m, 1H), 4.08–4.06 (m, 1H), 3.48–3.46 (m, 2H, H-5′/H-5″), 2.54–2.46 (m, 1H, H-2′/H-2″), 2.32–2.19 (m, 1H, H-2′/H-2″); ^{13}C NMR (75 MHz, $CDCl_3$): δ 149.5, 148.5, 138.9, 128.5, 128.1, 127.5, 124.2, 123.8, 87.8, 86.1, 85.4, 71.7, 63.2, 41.1; HRMS (EI+): m/z for $[C_{28}H_{25}FN_2O_5Na]^+$ calcd. 511.1639; found 511.1638.

*4.2.31. 5-fluoro-3′,5′-di-O-trityl-2′-deoxyuridine (**39**)*

In a 25 ml round bottom flask an amount of 100 mg (0.41 mmol) of **37** was evaporated 3 times with 5 mL of dry pyridine and dissolved in 3 mL of dry pyridine. Trityl chloride (340 mg, 1.22 mmol) was added to the reaction mixture. The mixture was heated at 85 °C for 18 h under argon atmosphere. After 18 h, the mixture was cooled to room temperature, quenched with 3 mL of MeOH and stirred for 30 min. The mixture was evaporated to dryness. The obtained crude compound was purified by column chromatography to obtain 200 mg (67%) **39**. TLC (ethyl acetate/hexane 1:1): $R_f = 0.80$. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.10 (bs, 1H), 7.73 (d, 1H), 7.43–7.40 (m, 6H), 7.29–7.25 (m, 25H), 6.37–6.33 (m, 1H, H-1′), 3.89 (s, 1H), 3.19–3.15 (m, 1H, H-5′/H-5″), 3.04–3.00 (m, 1H, H-5′/H-5″), 2.07–1.97 (m, 1H, H-2′/H-2″), 1.90–1.84 (m, 1H, H-2′/H-2″); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 148.2, 143.6, 142.8, 141.6, 138.5, 128.4, 127.8, 127.7, 127.1, 127.0, 124.1, 123.7, 87.6, 87.3, 85.5, 85.3, 74.9, 63.4; HRMS (EI+): m/z for $[C_{47}H_{39}FN_2O_5Na]^+$ calcd. 753.2735; found 753.2725.

*4.2.32. 5-fluoro-5′-O-trityl-uridine (**41**) [37]*

An amount of 100 mg (0.38 mmol) of **40** was co-evaporated 3 times with 5 mL of dry pyridine and dissolved in 3 mL of dry pyridine in a 25 ml round bottom. Trityl chloride (128 mg, 0.46 mmol) was added to the reaction mixture. The mixture was heated at 50 °C for 18 h under argon atmosphere. After 18 h, the mixture was cooled to room temperature, quenched with 3 mL of MeOH and was allowed to stir for 30 min. The mixture was evaporated to dryness. The obtained crude compound was purified by column chromatography to obtain 122 mg (63%) of **41**. TLC ($MeOH/CH_2Cl_2$, 1:9): $R_f = 0.80$. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.86 (d, 1H), 7.40–7.37 (m, 6H), 7.31–7.22 (m, 11H), 5.79–5.77 (m, 1H, H-1′), 4.33–4.31 (m, 1H), 4.24–4.21 (m, 1H), 4.14–4.12 (m, 1H), 3.45–2.42 (m, 2H, H-5′/H-5″); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 149.4, 143.0, 142.0, 138.8, 128.4, 127.8, 127.2, 124.2, 123.7, 89.5, 87.5, 83.6, 74.9, 69.8, 62.6; HRMS (EI+): m/z for $[C_{28}H_{25}FN_2O_6Na]^+$ calcd. 527.1589; found 527.1585.

*4.2.33. 5-fluoro-3′,5′-di-O-trityl-uridine (**42**) and 5-fluoro-2′,5′-di-O-trityl-uridine (**43**)*

In a 25 ml round bottom flask an amount of 100 mg (0.38 mmol) of **40** was co-evaporated 3 times with 5 mL of dry pyridine and then dissolved in 4 mL of dry pyridine. Trityl chloride (319 mg, 1.14 mmol) was added to the reaction mixture. The mixture was heated at 80 °C for 18 h under argon atmosphere. After 18 h, the mixture was cooled to room temperature, quenched with 4 mL of MeOH and stirred for 30 min. The mixture was evaporated to dryness. The obtained crude compound was purified by column chromatography to obtain 10 mg of **42** and 10 mg of **43**.

5-fluoro-3′,5′-di-O-trityl-uridine (**42**) [38]: TLC (ethyl acetate/hexane 1:1): $R_f = 0.70$. ^{1}H NMR (500 MHz, $CDCl_3$): δ 9.07 (bs, 1H), 7.79 (d, 1H), 7.37–7.35 (m, 6H), 7.27–7.21 (m, 25H), 6.10 (d, 1H, H-1′), 4.30–4.28 (m, 1H, H-3′), 4.08–4.05 (m, 1H, H-2′), 3.41 (s, 1H, H-4′), 3.21–3.18 (m, 1H, H-5′/H-5″ and OH), 2.84–2.82 (m, 1H, H-5′/H-5″). ^{13}C NMR (125 MHz, $CDCl_3$): δ 156.7 (CO), 156.6 (CO), 149.3, 143.4, 143.0, 128.7, 128.5, 128.2, 128.0, 127.7, 127.4, 89.3 (C1′), 88.3 (quat. C), 88.1 (quat. C), 83.3 (C4′), 75.1 (C2′), 73.6 (C3′), 63.3 (C5′). HRMS (EI+): m/z for $[C_{47}H_{39}FN_2O_6Na]^+$ calcd. 769.2684; found 769.2677.

5-fluoro-2′,5′-di-O-trityl-uridine (**43**) [38]: TLC (ethyl acetate/hexane 1:1): $R_f = 0.80$. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.84 (bs, 1H), 7.77 (d, 1H), 7.54–7.52 (m, 6H), 7.33–7.15 (m, 25H), 6.54–6.52 (m, 1H, H-1′), 4.55–4.52 (m, 1H, H-2′), 4.01 (s, 1H), 3.21–3.18 (m, 1H, H-5′/H-5″), 3.08–3.06 (m, 1H, H-5′/H-5″), 2.88 (d, 1H, H-3′). ^{13}C NMR (125 MHz, $CDCl_3$): δ 149.3, 143.1 (CO), 142.7 (CO), 138.9, 128.6, 128.4, 128.3, 128.0, 127.9, 127.5, 88.1 (quat. C), 88.0 (quat. C), 86.7 (C1′), 84.5 (C4′), 77.7 (C2′), 70.9 (C3′), 64.2 (C5′). HRMS (EI+): m/z for $[C_{47}H_{39}FN_2O_6Na]^+$ calcd. 769.2684; found 769.2693.

4.3. General procedure for introducing benzhydryl moieties

To a solution of the respective nucleoside (100 mg) and diphenylmethanol in dichloroethane (5 mL/mmol) was added palladium chloride (0.2 eq). The reaction mixture was heated at 85 °C under Argon atmosphere for 16 h or until disappearance of the starting materials. The reaction was monitored by TLC. The solvent was removed *in vacuo* and the crude mixture obtained was purified by column chromatography to afford the desired compound. Analytical details for the different compounds obtained can be found in Ref. [15].

4.4. Antiviral and toxicity assay for DENV

Green monkey kidney cells [Vero-B ECACC] were grown in minimum essential medium MEM Rega-3 (Gibco, Merelbeke, Belgium) supplemented with 10% foetal calf serum (FCS), 1% L-glutamine and 1% sodium bicarbonate. Vero-B cells were seeded at a density 7×10^3 cells/well in 100 μl assay medium and allowed to adhere overnight. Antiviral assays were performed in medium supplemented with 2% FCS, 1% L-glutamine and 1% sodium bicarbonate. After washing cells twice with 2% FCS medium, serial compound dilutions (1:2) were added to each well (starting concentration 100 μg/mL), followed by adding 100 μl of 2% PBS culture medium containing 100 μL 50% cell culture infectious doses (i.e., $CCID_{50}$) of virus.

After 7 days of incubation, the FCS culture medium was discarded and cells were fixed with ethanol and stained with 1% methylene blue, and EC_{50} and CC_{50} were determined visually. The 50% effective concentration (EC_{50}) is defined as the compound concentration that is required to inhibit the virus-induced CPE by 50%, and 50% cytotoxic concentration (CC_{50}) is defined as the compound concentration that is required to inhibit the cell growth by 50%. 3′,5′ di-*O*-trityluridine (initial hit) and ribavirin were included as reference compounds.

4.5. Antiviral and toxicity assay for YF17D

Green monkey kidney cells [Vero-B ATCC CCL-81] were grown in minimum essential medium MEM Rega-3 (Gibco, Merelbeke, Belgium) supplemented with 10% foetal calf serum (FCS), 1% L-glutamine and 1% sodium bicarbonate. Vero-B cells were seeded at a density of 2×10^4 cells/well in 100 μl assay medium and allowed to adhere overnight. Antiviral assays were performed in medium supplemented with 2% FCS, 1% L-glutamine and 1% sodium bicarbonate. After washing cells twice with 2% FCS medium, serial compound dilutions (1:2) were added to each well (starting concentration 50 μg/mL), followed by adding 100 μl of 2% PBS culture medium containing 100 μL 50% cell culture infectious doses (i.e., $CCID_{50}$) of virus. After 7 days of incubation, 2% FCS culture medium was discarded and cells were fixed with ethanol and stained with 1% methylene blue, and EC_{50} and CC_{50} were determined visually as for DENV.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.02.011.

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