Anti-flavivirus Activity of Different Tritylated Pyrimidine and Purine Nucleoside Analogues

Christopher McGuigan,*[a] Michaela Serpi,[a] Magdalena Slusarczyk,[a] Valentina Ferrari,[a] Fabrizio Pertusati,[a] Silvia Meneghesso,[a] Marco Derudas,[a] Laura Farleigh,[b] Paola Zanetta,[b] and Joachim Bugert*[b]

Introduction

Medicinal chemists have directed significant efforts to the synthesis of nucleoside and nucleotide analogues endowed with antiviral and anticancer activities.[1] Their synthesis is not always straightforward and often it is complicated by several issues, such as their poor solubility in organic solvents, the presence of several reactive functional groups, and the susceptibility of the glycosidic bond to undergo hydrolytic cleavage. To overcome all these problems, different chemical strategies have been elaborated. Among them, chemical manipulation by means of protecting groups remains a fundamental synthetic approach to prepare nucleoside analogues. Different protecting groups are commonly employed in nucleoside and nucleotide chemistry including both base and acid-labile. The triphenyl methyl group, most commonly indicated as the trityl group is typically used to protect selectively primary, over secondary hydroxyl functions.[2] The positive charge on the alpha carbon, stabilized by the resonance effect of three aromatic rings makes trityl ethers acid-labile. Since its discovery one hundred years ago, the trityl group has become a key protective group, widely used in nucleoside, oligonucleotide, peptide, carbohydrate chemistry, and indeed in almost all other fields of organic and bioorganic chemistry.[3] Typically, the trityl group is introduced prior to manipulation of the structure and then removed when appropriate during the synthesis. However some recent studies indicated that trityl-containing compounds themselves possess a certain biological activity. To some degree, such discoveries may have been serendipitous. To the best of our knowledge the first account reporting on a trityl compound biologically active was in 2002 from Hernandez et al.[4] In their studies, 5’-O-tritylthymidine (1, Figure 1) emerges as an novel inhibitor of human mitochondrial thymidine kinase. After that, several other studies described the biological potential of nucleoside analogues bearing the trityl group.[5,6]

Figure 1. Examples of tritylated nucleosides reported as flavivirus inhibitors.

[a] Prof. C. McGuigan, Dr. M. Serpi, Dr. M. Slusarczyk, V. Ferrari, Dr. F. Pertusati, Dr. S. Meneghesso, Dr. M. Derudas School of Pharmacy and Pharmaceutical Sciences, Cardiff University King Edward VII Avenue, Cardiff CF10 3NB (United Kingdom) E-mail: cmcgugian@cardiff.ac.uk

[b] L. Farleigh, P. Zanetta, Dr. J. Bugert Medical Microbiology and Infectious Diseases, School of Medicine Cardiff University, Heath Park, Cardiff CF14 4XN (United Kingdom)

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During the screening of a large compound library aimed at recognizing hit molecules, identified 2',5'-bis-O-tritylated uridine (2) as a flavivirus inhibitor. As a consequence, several other trityl uridine nucleosides, including different regioisomers, were prepared and evaluated in vitro against two important members of Flaviviridae family, yellow fever (YFV) and dengue (DENV) viruses. Among the different compounds 3',5'-bis-O-tritylated uridine (3) was endowed with the best inhibitory properties (YFV IC₅₀ = 1.0 µM; DENV IC₅₀ = 1.75 µM). The finding of this lipophilic structure being endowed with high antiviral activity for flaviviruses, encouraged the same authors to undertake further research. However, modifications of the trityl groups, combined with minor variations of the heterocyclic base generally led only to weak in vitro flavivirus inhibition and in some cases to marked in vitro cytotoxicity. From these studies, only 3',5'-bis-O-tritylated 5-fluoro-2'-deoxycytidine (4) showed no cellular toxicity up to a concentration of 25 µM, while having EC₅₀ values for YFV and DENV respectively of 1.05 µg mL⁻¹ and 1.2 µg mL⁻¹.

In the attempt to explain the mode of action of this class of compounds, De Burghgraeve et al. undertook further investigations. Their preliminary data suggested that the antiviral activity of this class of compounds might be due to the inhibition of the viral RNA-dependent RNA polymerase rather than to a mechanism interfering during an early or a late stage of the viral life cycle such as virus entry, assembly, or release.

Despite being under partial control, yellow fever case numbers are now increasing globally, posing a serious risk of local epidemic outbreaks. The global incidence of dengue has also grown dramatically in recent decades. Both viruses are now considered global threats for the entire world population. Although a safe and effective vaccine against YFV exists, there is currently no vaccine available for dengue virus and its development has proven to be very difficult because of the existence of multiple serotypes. With no therapeutic agent available to treat and/or prevent severe epidemics of either yellow fever or dengue virus infections, prevention is currently limited to vector (mosquitoes) control measures. Therefore, there is an obvious and urgent need for the development of effective anti-flavivirus drugs.

Based on these considerations and the interesting data reported in the literature for the tritylated nucleosides, we became interested in assessing the impact of the trityl group on several pyrimidine nucleosides not included in the previous studies against both YFV and DENV.

Taking into account that in different studies, hindered nucleoside analogues (NAs) including 3',5'-O-trityl derivatives of adenine nucleoside analogues were found to elicit anti-flavivirus activity to some extent, we decided to include also a few selected purine analogues in our investigations. The structures of the pyrimidine and purine nucleosides used in our studies are shown in Figure 2.

Results and Discussion

Chemistry

Tritylation of nucleoside analogues has always been a relatively straightforward procedure. Generally, it is sufficient to stir the selected nucleosides with an excess of trityl chloride at an elevated temperature (110 °C). However some reports have shown that the temperature can be as low as 80–90 °C. For our synthesis, we followed the milder approach reported by Van Aerschot. Briefly, the selected nucleoside was treated in anhydrous pyridine, with an excess of trityl chloride (2.8 equiv), and the resulting mixture heated at 80 °C for 18 h. After aqueous work-up, the desired tritylated compounds were isolated by column chromatography on silica gel. In order to avoid decomposition of the trityl moiety, 0.5% of triethylamine was added to the chromatographic eluent in order to neutralize the silica gel acidity. Although this was not strictly necessary for trityl-bearing derivatives A–D (provided that their purification was performed in a reasonable time), we found its use imperative during the purification of 4,4’-dimethoxytrityl derivatives (E–H). The reaction always returned compounds with a diverse degree of tritylation (5’, 3’, and 2’) depending upon the reactivity of the nucleoside. The different regioisomers were separated by column chromatography. The general synthetic procedure for the preparation of the trityl ethers of these compounds is summarized in Scheme 1, and complete compound information can be found in Table 1.

In order to expand the structure–activity relationship (SAR), and considering that flavivirus inhibition profile has been linked to the critical requirement of bulkiness in the region between the 3’ and 5’ positions, we decided to synthesize also the 4,4’-dimethoxytrityl derivatives (5–20E–H, Scheme 2, Table 2) of nucleosides 5–20.

5’-2’-O-Bis tritylated regioisomers were distinguished from those 5’-3’-O-bis tritylated analogues using 2D NMR experiments. Correlation spectroscopy (COSY) analysis allowed the correct assignment of each proton for all the compounds. Coupling of the OH signal to either the signal of 2’ or 3’ indicated which position is unalkylated.

![Scheme 1](image-url)
Biological evaluation

The antiviral effect of all the parent nucleosides and their corresponding tritylated and 4,4'-dimethoxytritylated analogues was assessed using a cell viability assay in which Vero cells were infected with either yellow fever (YFV) or dengue viruses (DENV, serotype D2) and further treated with the tested compounds at three different concentrations (5, 10, and 20 μM). Cell viability was determined by measurement of the level of ATP presents even days post infection when compared to vehicle only (DMSO) controls. The results are reported as percentage of viable cells left after the assay. Tables 3 and 4 gathered only compounds exhibiting an antiviral activity and include also their CC50 values, defined as the concentration required to decrease cell growth by 50%. The toxicity of all the tested compounds on uninfected cells was also determined in parallel.

As expected, the parent nucleosides did not show any antiviral activity versus both the flaviviruses, considered in this study.

In the series of the tritylated nucleoside analogues two compounds: the 5'-O-tritylated 5-FUR derivative (6D) and the 5'-O-tritylated thymidine derivative (1) showed a similar protective
Table 1. Summary of all synthesized tritylated compounds.

| Cmpd | Nucleoside | 5’ | 3’ | 2’ |
|------|------------|----|----|----|
| 5A   | RBV (5)    | OTr | OTr | OTr |
| 5B   | RBV (5)    | OTr | OTr | OTr |
| 5C   | RBV (5)    | OTr | OH  | OTr |
| 6B   | 5-FUR (6)  | OTr | OTr | OTr |
| 6C   | 5-FUR (6)  | OTr | OH  | OTr |
| 6D   | 5-FUR (6)  | OTr | OH  | OTr |
| 7B   | 5-BrU (7)  | OTr | OTr | OTr |
| 7C   | 5-BrU (7)  | OTr | OH  | OTr |
| 8B   | 4’-AzaU (8)| OTr | OTr | OTr |
| 8C   | 4’-AzaU (8)| OTr | OH  | OTr |
| 8D   | 4’-AzaU (8)| OTr | OH  | OTr |
| 9    | Uridine (9)| OTr | OTr | OH  |
| 10B  | AraU (10)  | OTr | OTr | OTr |
| 10C  | AraU (10)  | OTr | OH  | OTr |
| 10D  | AraU (10)  | OTr | OH  | OTr |
| 11D-NHTr| 3TC (11)   | OTr | –   | –   |
| 12D  | N-acetyl-3TC (12)| OTr | –   | –   |
| 13D  | d4T (13)   | OTr | –   | –   |
| 14D  | 5-idU (14) | OTr | OH  | –   |
| 4    | 5-FUDR (15)| OTr | OTr | –   |
| 15D  | 5-FUDR (15)| OTr | OH  | –   |
| 16B  | 2-FdU (16) | OTr | OTr | –   |
| 16D  | 2-FdU (16) | OTr | OH  | –   |
| 17D  | 5-AzaC (17)| OTr | OH  | OH  |
| 18D  | AZT (18)   | OTr | –   | –   |
| 19B  | 5-MeU (19) | OTr | OTr | OH  |
| 19C  | 5-MeU (19) | OTr | OH  | OTr |
| 19D  | 5-MeU (19) | OTr | OH  | OTr |
| 20B  | Fludarabine (20)| OTr | OTr | OH  |
| 20D-NHTr| Fludarabine(20)| OTr | OH  | OH  |
| 21B  | Thymidine (21)| OTr | OTr | –   |
| 1    | Thymidine (21)| OTr | OH  | –   |
| 22B-NHTr| PCV (22)   | OTr | OTr | –   |
| 22B  | PCV (22)   | OTr | OTr | –   |
| 23C  | 3’-dA (23) | OTr | –   | OTr |
| 24B  | BCNA (24)  | OTr | OTr | –   |
| 24D  | BCNA (24)  | OTr | OH  | –   |

RBV: ribavirin, 5-FUR: 5-fluorouridine, 5-Bfu: 5-bromouridine, 4’-AzaU: 4’-azidouridine, AraU: 2’-3’-O-arabinouridine, 3TC: lamivudine, 4’-O-tritylated 2’-3’-deoxyuridine: YFV inhibition [% Cells alive] D2 inhibition [% Cells alive] Toxicity CC50 [μM]

Table 2. Summary of all synthesized 4,4’-dimethoxytritylated compounds.

| Cmpd | Nucleoside | 5’ | 3’ | 2’ |
|------|------------|----|----|----|
| 5E   | RBV (5)    | ODMTr | ODMTr | ODMTr |
| 5G   | RBV (5)    | ODMTr | ODMTr | ODMTr |
| 6F   | 5-FUR (6)  | ODMTr | ODMTr | ODMTr |
| 6G   | 5-FUR (6)  | ODMTr | ODMTr | ODMTr |
| 6H   | 5-FUR (6)  | ODMTr | ODMTr | ODMTr |
| 8G   | 4’-AzaU (8)| ODMTr | ODMTr | ODMTr |
| 9G   | Uridine (9)| ODMTr | ODMTr | ODMTr |
| 9H   | Uridine (9)| ODMTr | ODMTr | ODMTr |
| 10E  | AraU (10)  | ODMTr | ODMTr | ODMTr |
| 10F  | AraU (10)  | ODMTr | ODMTr | ODMTr |
| 12H  | N-acetyl-3TC (12)| ODMTr | ODMTr | ODMTr |
| 15F  | 5-FUDR (15)| ODMTr | ODMTr | ODMTr |
| 15H  | 5-FUDR (15)| ODMTr | ODMTr | ODMTr |
| 16F  | 2-FdU (16) | ODMTr | ODMTr | ODMTr |
| 16H  | 2-FdU (16) | ODMTr | ODMTr | ODMTr |
| 20H  | Fludarabine (20)| ODMTr | ODMTr | ODMTr |

Table 3. Antiviral activity and toxicity of selected tritylated compounds.

| Cmpd | Nucleoside | 5’ | 3’ | 2’ | 5 μM | 10 μM | 20 μM | 5 μM | 10 μM | 20 μM | Toxicity CC50 [μM] |
|------|------------|----|----|----|------|-------|-------|------|-------|-------|-------------------|
| 5B   | RBV (5)    | OTr | OTr | OTr | 0    | 0     | 0     | 0    | 0     | 16.85 ± 1.51 | > 20              |
| 6D   | 5-FUR (6)  | OTr | OTr | OH  | 0    | 0     | 0     | 31.24 ± 2.34 | 0     | 0     | 2.47 ± 0.06     | > 20              |
| 14D  | 5-idU (14) | OTr | OH  | –   | 0    | 0     | 0     | 0    | 0     | 10.88 ± 1.44 | > 20              |
| 16D  | 2-FdU (16) | OTr | OH  | –   | 0    | 0     | 0     | 0    | 0     | 8.59 ± 0.05  | > 20              |
| 19D  | 5-MeU (19) | OTr | OH  | OTr | 0    | 0     | 0     | 0    | 0     | 0     | 0     | > 20              |
| 20D-NHTr| Fludarabine (20)| OTr | OH  | OTr | 0.28 | 0.84  | 71.98 ± 5.75 | 100 ± 1.48 | 100 ± 7.83 | > 20              |
| 21B  | Thymidine (21)| OTr | OTr | –   | 0    | 0     | 0     | 0    | 0     | 0     | 0     | > 20              |
| 1    | Thymidine (21)| OTr | OH  | –   | 0.12 ± 0.03 | 25.77 ± 1.03 | 0     | 0    | 0     | 0.04 ± 0.04 | > 20              |
| 22B-NHTr| PCV (22)   | OTr | OTr | –   | 0    | 0     | 0     | 0    | 0     | 0     | 0     | > 20              |

[a] Determined as an inhibition percentage of yellow fever (YFV, 17D strain of ASIBI) and dengue viruses (DENV, serotype D2). Values are given as means ± SD for n = 3. RBV: ribavirin, 5-FUR: 5-fluorouridine, 5-idU: 5-iodo-2’-deoxyuridine, 2’-FdU: 2’-fluoro-2’-deoxyuridine, 5-MeU: 5-methyluridine, PCV: penciclovir, OTr: O-trityl.
DENV. Despite being the analogue of an anticancer agent, compound 20D showed no cytotoxicity in our assays, up to 20 μM.

This noncytotoxic profile was observed also for all the other analogues considered in this work with no evidence of toxicity at concentration up to 20 μM.

Next, the antiviral effect of dimethoxytritylation of selected nucleoside analogues was evaluated similarly to the tritylated congeners against YF and DENV viruses. Table 4 collects the result for the compounds of this series showing some antiviral activity. In particular the 5′-O-DMT-FUR derivative (6H), and, the 5′-O-DMT-FU derivative (15H) emerged as the most active compounds against YFV with 100% inhibition at 20 μM. Compound 15H was found to retain high inhibition also at 10 μM with 95% of viable cells counted. Differently from the 5′-O-tritylated-2′-deoxy-2′-fluorouridine analogues 16D, its 5′-O-dimethoxytrityl congener 16H had pronounced inhibitory activity but surprisingly only against YFV (78% at 20 μM).

Intriguing results were also found for the 5′-O-dimethoxytritylated-uridine analogue 9H, which showed inhibitory activity only against YFV with an inhibition of 1% at 5 μM; 7% at 10 μM; and 100% at 20 μM.

All 4,4′-dimethoxytritylated compounds tested were devoid of anti-DENV activity and were found to be not toxic at concentrations up to 20 μM.

As mentioned already, compounds 3 and 2 have been previously reported as relatively potent anti-YFV and anti DENV agents. In contrast, our assays revealed no potency either as anti-YFV or anti-DENV agents. Explanation of the differing results from those reported could be found in the differing detection methods used. We assayed the metabolic activity of cells, quantitating ATP, which is rapidly degraded in dying cells. Using such an assay indicating active intracellular processes, it is possible to dissect toxic and viral effects that lead to cells with intact membranes but no metabolic activity; that latter method (methylene blue dye exclusion) was used in the prior reports. The ATP assay used here is more sensitive, indicates both toxic and viral effects leading to the loss of metabolic activity, and is the assay of choice to identify compounds, which lead to increased cell survival.

### Conclusion

We herein report the synthesis of tritylated and 4,4′-dimethoxytritylated pyrimidine and purine nucleoside analogues and the evaluation of their inhibitory activity against YF and DENV viruses. The activity was reported as % of viable cells resulting from treatment with our compounds after exposure to the viruses. In the series of 38 tritylated analogues, two compounds, 6D and 1, showed selective and moderate YFV inhibition (31% and 26%, respectively) at 20 μM concentration. In the DENV inhibition assay, the tritylated fludarabine derivative 20D-NHTr displayed pronounced anti-DENV inhibitory activity, reaching 100% of virus inhibition at a concentration of 10 μM. Within the panel of 16 4,4′-dimethoxytritylated compounds, five of the monosubstituted derivatives (6H, 9H, 12H, 15H, and 16H) revealed selective anti-YFV inhibitory activity ranging from 7 to 95% at 10 μM and from 70 to 100% at 20 μM. All the tested compounds proved to be noncytotoxic at concentrations up to 20 μM.

In conclusion, our preliminary biological data support an antiflavivirus activity for tritylated/4,4′-dimethoxytritylated nucleoside analogues. Previous studies have suggested that these drugs might act as potential inhibitors of the DENV viral RNA-dependent RNA polymerase (RdRp). However, evidence to unravel their mechanism of action have not been provided yet. In fact, their structure–activity relationship remains unclear and requires additional studies and therefore it is not possible for us here to speculate on a possible mechanism. It might be however that the prior identified lead impacted on cellular metabolic processes rather than a viral target.

### Experimental Section

#### General experimental

All solvents and reagents were used as obtained from commercial sources unless otherwise indicated. All reactions were performed under an argon atmosphere. The 1H and 13C NMR spectra were recorded on a Bruker spectrometer (Billerica, MA, USA) operating at 500 MHz for 1H and 125 MHz for 13C. CDCl3 was used as the solvent for NMR experiments, unless otherwise stated. 1H chemical shift values (δ) are referenced to the residual nondeuterated components of the NMR solvents (δ = 7.26 ppm for CHCl3, etc.). The 13C chemical shifts (δ) are referenced to CDC13 (central peak, δ = 77.0 ppm). Fluorine chemical shifts are referenced to CFCl3.

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**Table 4. Antiviral activity and toxicity of selected 4,4′-dimethoxytritylated compounds.[a]**

| Cmpd | Nucleoside | 5′ | 3′ | 2′ | 5 μM | 10 μM | 20 μM | 5 μM | 10 μM | 20 μM |
|------|------------|----|----|----|------|-------|-------|------|-------|-------|
| 6H   | S-FUR (6)  | ODMTr | OH | OH | 9.22 ± 0.46 | 30.89 ± 1.85 | 100 ± 8.68 | 0   | 0     | >20   |
| 9H   | Uridine (9) | ODMTr | OH | OH | 1.06 ± 0.27 | 7.00 ± 0.98  | 100 ± 5.23  | 0   | 0     | >20   |
| 12H  | N-acetyl-3TC (12) | ODMTr | –  | –  | 0     | 70.45 ± 18.31 | 0   | 0     | >20   |
| 15H  | S-FUDR (15) | ODMTr | OH | –  | 0     | 95.17 ± 10.4 | 100 ± 5.4 | 0   | 0     | >20   |
| 16H  | 2′-FdU (16) | ODMTr | OH | –  | 0     | 77.99 ± 14.81 | 0   | 0     | >20   |

[a] Determined as an inhibition percentage of yellow fever (YFV, 17D strain of ASIIB) and dengue viruses (DENV, serotype D2). Values are given as means ± SD for n = 3. S-FUR: 5-fluorouridine, 3TC: lamivudine, 5-FUDR: 5-fluoro-2′-deoxyuridine, 2′-FdU: 2′-fluoro-2′-deoxyuridine.
spectra (MS) were measured in positive-mode electrospray ionization (ESI). Thin-layer chromatography (TLC) was performed on silica gel 60 F254 plastic sheets. Column chromatography was performed using silica gel (35–75 mesh) or on an Isolera Biotage system (Uppsala, Sweden). Purity of prepared compounds was determined to be >95% by high-performance liquid chromatography (HPLC)–UV analysis (Thermo HPLC connected with UV detector; Varian Pursuit XS, 46 mm×150 mm, 5.0 μm, Palo Alto, CA, USA).

**Chemistry**

**General Procedure 1.** A mixture of a nucleoside (1.0 eq mol⁻¹), an appropriate trityl chloride (2.2 eq mol⁻¹), and 4-dimethylaminopyridine (DMAP, 2.8 mol eq⁻¹, unless stated otherwise) in anhydrous pyridine (45 mL mmol⁻¹) was heated at 80°C under an argon atmosphere for 18 h. The reaction was quenched by addition of MeOH (2 mL mmol⁻¹) at rt, and kept stirring at rt for 30 min. The solution was then concentrated and diluted in CH₂Cl₂. The organic solution was washed with saturated solution of NaHCO₃ (3× 20 mL), and the combined aqueous layers were extracted with CH₂Cl₂. Combined organic layers were dried over Na₂SO₄, filtered, and concentrated under vacuum. The residue was purified by column chromatography on silica gel (eluent system gradient MeOH in CH₂Cl₂=1–3% containing 0.5% triethylamine).

Experimental data for all active compounds are reported below and for inactive compounds in the Supporting Information.

Tritylated derivatives of ribavirin (5) were prepared according to the general procedure 1 from 5 (0.500 g, 2.05 mmol) and trityl chloride (1.6g, 5.74 mmol). Column purification with a gradient of MeOH/triethylamine (1.05% to 2.05%) in CH₂Cl₂ as an eluent yielded 5,3’,2’-tri-O-tritylribavirin (5A), 5,3’,3’-bis-O-tritylribavirin (5B) and 5,2’-bis-O-tritylribavirin (5C).

5,3’-Bis-O-tritylribavirin (5B) was obtained as a white solid (0.268 g, 18%); H NMR (500 MHz, 25°C, CDCl₃), δ = 8.21 (s, 1H, H-3), 7.43–7.38 (m, 6H, H-Ph), 7.34–7.18 (m, 24H, H-Ph), 5.64 (br s, 1H, H-NH₂), 5.89 (d, J₇,₆ = 3.08 Hz, 1H, H-1'), 5.66 (br s, 1H, NH₂), 4.43–4.39 (m, 1H, H-3'), 4.19–4.14 (m, 1H, H-4'), 3.61–3.56 (m, 1H, H-2'), 3.47–3.43 (m, 1H, H-5'), 3.00 (dd, J = 10.9, 5.5 Hz, 1H, H-5'), 2.85 ppm (d, J = 4.5 Hz, 1H, OH-2'); ¹³C NMR (125 MHz, 25°C, CDCl₃), δ = 160.4 ppm (C=O), 156.9 ppm (C=O), 144.1 ppm (C=O), 143.5 ppm, 143.3 ppm (C-Ph), 128.7, 128.6, 128.3, 127.8, 127.1 (C-Ph), 92.4 ppm (C-Ph), 88.1, 87.1 ppm (CPh₂), 83.5 ppm (C-4), 74.3 ppm (C-1'), 74.1 ppm, 64.0 ppm (C-5'), MS (ES⁻) found: m/z 725.1 [M−Na]⁻, calc'd for [C₁₅H₁₃NO₄]⁻; m/z 728.83 [M]; Reverse-phase HPLC (H₂O/CH₃CN from 90:10 to 100:0 to 30 min), flow = 1 mL min⁻¹, λ = 245 nm, t₁/₂ = 28.10 min.

Tritylated derivatives of 5-fluorouridine (6) were prepared according to the general procedure 1 from 6 (0.30 g, 1.14 mmol) and trityl chloride (1.02 g, 3.66 mmol) in pyridine (6 mL). Column chromatography purification using a gradient of MeOH/triethylamine (1.05% to 2.05%) in CH₂Cl₂ as an eluent yielded 5,3’-bis-O-trityl-5-fluorouridine (6B), 5,2’-bis-O-trityl-5-fluorouridine (6C), and 5’-O-trityl-5-fluorouridine (6D).

5’-O-Trityl-5-fluorouridine (6D) was obtained as a white solid (0.183 g, 32%); H NMR (500 MHz, 25°C, CDCl₃), δ = 7.42 (d, J₇,₆ = 6.0 Hz, 1H, H-6), 7.22–7.20 (m, 6H, H-Ph), 7.05–7.02 (m, 6H, H-Ph), 6.89–6.85 (m, 6H, H-Ph), 5.72 (dd, J = 4.5, 1.5 Hz, 1H, H-1'), 4.04 ppm (t, J = 5.0 Hz, 1H, H-3'), 3.96 ppm (t, J = 5.0 Hz, 1H, H-2'), 3.90–3.87 (m, 1H, H-4'), 3.30 ppm (dd, J = 11.0, 2.5 Hz, 1H, H-5'), 2.35 ppm (dd, J = 11.0, 2.5 Hz, 1H, H-5'); ¹³C NMR (125 MHz, 25°C, CDCl₃), δ = 161.0 ppm (C, CDCl₃) δ = 156.3 ppm (C=O, CDCl₃).
as an eluent yielded 12H as a white solid (0.52 g, 17 %); 1H NMR (500 MHz, 25 °C, CDCl3): δ = 9.73 (bs, 1H, NH), 8.43 (d, J = 7.5 Hz, 1H, H-6), 7.49–7.47 (m, 2H, H-Ph), 7.38–7.29 (m, 7H, H-Ph), 7.21 (d, J = 7.5 Hz, H-5), 6.90–6.88 (m, 4H, H-Ph), 6.37 (dd, J = 5.5, 2.0 Hz, 1H, H-1), 5.34 (t, J = 5.0 Hz, 1H, H-4), 3.84 (s, 6H, OCH3), 3.68 (dd, J = 12.5, 2.5 Hz, 1H, H-5a), 3.64–3.60 (m, 2H, H-5b and H-2a), 3.29 (d, J = 12.5 Hz, H-2b), 2.27 ppm (s, 3H, CH3); 13C NMR (125 MHz, 25 °C, CDCl3): δ = 170.5 (COCH3), 163.0 (C-2), 158.7 (CH2O-C-Ph), 154.9 (C-4), 145.7 (C-5), 145.3 (C-Ph), 135.3, 131.5 (C-Ph), 128.4, 128.1, 113.7 (CH-Ph), 87.9 (C-5), 87.6 (CH-1), 87.3 (CPh3), 63.5 (CH3-S), 55.3 (OCH3), 39.7 (CH2-2), 24.9 ppm (COCH3); MS (ES+): found: m/z 596.2 [M + Na]+, calcd for [C19H19NO5]+ m/z 573.974 [M] = 254 nm, flow = 1 mL min⁻¹, λ = 254 nm, tR = 21.59 min.

5'-O-Trityl-5-ido-2'-deoxyuridine (14D) was prepared according to the general procedure 1 from 5'-ido-2'-deoxyuridine (14) (0.5 g, 1.41 mmol), trityl chloride (1.26 g, 4.52 mmol), and DMAP (0.172 g, 1.41 mmol) in anhydrou pyridine (10 mL). Column chromatography eluting with a gradient of MeOH (1% to 4%) in CH2Cl2 gave 5'-O-trityl-5-ido-2'-deoxyuridine (14D) as a white solid (0.28 g, 55%); 1H NMR (500 MHz, 25 °C, CDCl3): δ = 0.94 (3H, 1H, NH), 8.16 (s, 1H, H-6), 7.47–7.46 (m, 6H, H-Ph), 7.36–7.33 (m, 6H, H-Ph), 7.29–7.26 (m, 3H, H-Ph), 6.34 (dd, J = 6.0, 8.0 Hz, 1H, H-1), 4.58–4.57 (m, 1H, H-1), 4.15–4.13 (m, 1H, H-4), 3.45–3.39 (m, 2H, H-5), 2.56–2.52 (m, 1H, H-2a), 2.32–2.28 ppm (m, 1H, H-2b); 13C NMR (125 MHz, 25 °C, CDCl3): δ = 160.0 (C-2), 149.7 (C-4), 144.2 (C-6), 143.3 (C-Ph), 128.6, 128.1, 127.4 (CH-Ph), 88.3 (C-5), 87.7 (CPh3), 86.3 (C-4), 85.5 (C-1), 73.2 (C-3), 63.6 (C-5), 49.5 ppm (C-2); MS (ES+): found: m/z 596.4130 [M]+; Reverse-phase HPLC (H2O/CH3CN from 80:20 to 0:100 in 30 min), flow = 1 mL min⁻¹, λ = 254 nm, tR = 20.71 min.

Dimethoxytritylated derivatives of 5'-fluoro-2'-deoxyuridine (16) were prepared according to the general procedure 1 from 15 (0.30 g, 1.22 mmol) and 4,4'-dimethoxytrityl chloride (0.90 g, 2.68 mmol) in pyridine (6 mL). Column chromatography purification using gradient of MeOH/triethylamine (1:0.5% to 2.5%) in CH2Cl2 as an eluent followed by preparative TLC purification with MeOH/triethylamine (2.05%) in CH2Cl2 yielded 5,3'-bis-O-dimethoxytrityl-5'-fluoro-2'-deoxyuridine (15F) and 5'-O-dimethoxytrityl-5'-fluoro-2'-deoxyuridine (15H) (10.5% to 2.5% in CH2Cl2) to give 5,3'-bis-O-trityl-2'-fluoro-2'-deoxyuridine (16D) and 5'-O-trityl-2'-fluoro-2'-deoxyuridine (16).
Tritylated derivatives of fludarabine (20) were prepared according to the standard procedure 1 from 20 (0.30 g, 1.05 mmol) and trityl chloride (0.64 g, 2.31 mmol) in pyridine (6 mL). Column chromatography purification was performed using a gradient of MeOH/triethylamine (1.0:5% to 2.05%) in CH₂Cl₂ as eluent to yield two products which were further purified by preparative TLC with MeOH/triethylamine (2.05%) in CH₂Cl₂ to yield 5’, 3’-bis-O-trityl-fludarabine (20B) and 5’,O4',O6-tri-O-trityl-fludarabine (20D-NHTR).

Biological assays

Tritylated derivatives of thymidine (21) were prepared according to the general procedure 1 from 21 (0.30 g, 1.24 mmol) and trityl chloride (0.75 g, 2.72 mmol) in pyridine (6 mL). Column chromatography purification was performed using a gradient of MeOH/triethylamine (1.0:5% to 3.05%) in CH₂Cl₂ as eluent to yield 5’,3’-bis-O-trityl-thymidine (21B) and 5’-O-trityl-thymidine (1).

Virology

Tritylated derivatives of penciclovir (22) were prepared according to general procedure 1 from 22 (0.57 g, 2.25 mmol) and trityl chloride (1.86 g, 6.75 mmol) in anhydrous pyridine (10 mL). Column chromatography elution was using a gradient of MeOH/triethylamine (1.0:5% to 3.05%) in CH₂Cl₂, yielded 5’,3’-bis-O-trityl-penciclovir (22B) and 2,N,5’,3’-O-tri-trityl penciclovir (22B-NHTR).

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