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Synthesis and in vitro growth inhibitory activity of novel silyl- and trityl-modified nucleosides

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ABSTRACT

Seventeen silyl- and trityl-modified (5′-*O*- and 3′,5′-di-*O*-) nucleosides were synthesized with the aim of investigating the in vitro antiproliferative activities of these nucleoside derivatives. A subset of the compounds was evaluated at a fixed concentration of 100 μM against a small panel of tumor cell lines (HL-60, K-562, Jurkat, Caco-2 and HT-29). The entire set was also tested at varying concentrations against two human glioma lines (U373 and Hs683) to obtain GI_{50} values, with the best results being values of ~25 μM.

1. Introduction

In recent years, nucleoside derivatives and analogues have found significant application in the field of oncology.[1] Of interest is that ~10 nucleoside base-derived compounds are currently approved for clinical use[2] and there is still much interest in the development of newer generations of nucleoside compounds with anticancer properties.[3,4] Both purine and pyrimidine nucleoside analogues are currently in clinical use as antimetabolites, with examples including the pyrimidine analogues cytarabine[5] (myelogenous leukemia, multiple myeloma, Hodgkin's and non-Hodgkin's lymphomas)[6] and gemcitabine[7] (pancreatic cancer,[8] metastatic bladder cancer,[9] non-small cell lung cancer,[10] breast,[11] ovarian and neck cancers[12]), the purine analogues fludarabine (low-grade lymphomas and chronic lymphocytic leukemia[13]) and cladribine (low-grade lymphomas,[14] chronic lymphocytic leukemia,[15] hairy cell leukemia[15] and non-Hodgkin's lymphoma[16]) and the fluoropyrimidines, for example, 5′-fluorouracil (gastrointestinal, pancreatic, head and neck, renal, skin, prostate, breast cancers)[17] and its prodrug capecitabine[17] (metastatic colorectal cancer,[18] metastatic breast cancer[19]).

In 2006, Herczegh and co-workers noted that silylated leinamycin antibiotic nucleoside analogues, such as compound **1** shown in Figure 1, had significantly higher in vitro growth inhibitory activity toward human cancer cells than their 'free' non-protected derivatives.[20] These authors postulated that the effect was due to differences in lipophilic character imparted by the *tert*-butyldimethylsilyl (TBDMS) groups used. In a more recent set of studies, Peterson and colleagues disclosed the synthesis and antiproliferative activities of a number of *N*-6,5′-bis-ureidoadenosine derivatives containing one or two TBDMS groups—see for example, compound **2**, as depicted in Figure 1.[21] These researchers further developed these scaffolds[22–25] and also

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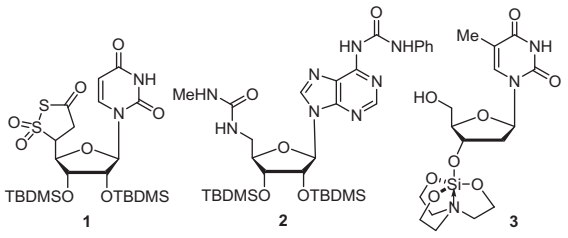


Figure 1. Examples of cytotoxic leinamycin nucleoside analogue **1**,[20] a bis-sila-substituted *N*-6,5′-bis-ureidoadenosine derivative **2**[21] and 3′-*O*-silatranylthymidine **3**.[27]

investigated their mode of action and possible targets.[23,26] In another earlier, but related example, Fenlon and co-workers published details of nucleoside-derivatives that contained carbon-oxygen-silicon bonds in the form of silatrane functional groups.[27] The researchers tested the in vitro anti-cancer activity of 3′-*O*-silatranylthymidines, such as compound **3** (Fig. 1), against human breast (MDA-MB-435) and central nervous system (SNB-19 and U251) cancer cell lines at a fixed concentration (100 μM) and found them to exhibit modest activities in the high micromolar ranges (for a review on silatranes and their applications see Ref. 28). It is important to note that another class of silyl-containing *tert*-butyldimethylsilyl-spiroaminooxathioledioxide (TSAO) compounds have been found to be effective non-nucleoside reverse transcriptase inhibitors (for examples, see review[29] and recent Refs. 30,31).

In terms of some final nucleoside examples, during a study involving triorganosilyl derivatives of some biologically active heterocyclic bases, Lukevits and co-workers[32] showed that, in contrast to uridine, 5′-*O*-*tert*-butyldimethylsilyluridine **4d** (Fig. 2) exhibited some anti-tumor activity. These researchers were also able to show that 5′-*O*-*tert*-butyldimethylsilyluridine **4d** suppressed the growth of HT-1080 (fibrosarcoma in human lungs—96% inhibition at 279 μM) and NiH 3T3 cells (fibroblasts in mice—95% inhibition at 279 μM) in culture. As expected, under the same conditions, uridine showed a complete absence of any in vitro activity, as it is a normal product of nucleoside synthesis. The researchers also commented that, in addition to the lipophilic properties imparted by the TBDMS groups, the oxygen–silicon bond in these compounds appeared to be reasonably stable to hydrolysis.[32] Note should also be taken here of research performed by the Schmalz group, in which they demonstrated that nucleosides with unsaturated carbocyclic portions,[33,34] as well as metal derivatives of the same motif,[35–37] benefited from having organosilyl fragments as part of their structure (mainly on the 5′ position, structures not shown).

As shown by the examples briefly described in the introduction, observations that silyl derivatives of nucleosides afford compounds with interesting antiproliferative activities, provided a motivation for this present study. Other researchers,[38–43] and ourselves,[44] have recently also noted that the introduction of bulky silyl groups has resulted in increased antiproliferative activity of a variety of scaffolds – in fact, the introduction of silyl groups into pharmaceutically relevant molecules has become an active area of research.[45–47]

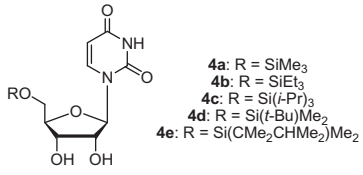


Figure 2. Trialkylsilyl derivatives of uridine **4a**–**e**, synthesized by Lukevits and co-workers.[32]

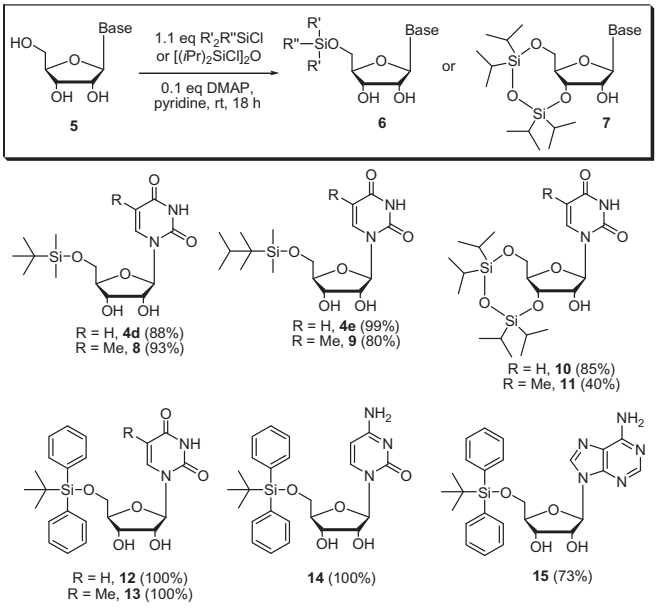
With this in mind, and based on a recent disclosure from our research collaborations that these types of compounds show unusual in vitro antiproliferative effects,[48] it was decided to report on the synthesis of a broader set of different nucleosides including purine and pyrimidines, each in turn modified with a wider range of silyl-protecting groups at the 5′- or 3′,5′-(di)hydroxyl groups. In addition, the utilization of another lipophilic protecting group for the 5′-position, namely the trityl group, would also be investigated. Due to our continued interest in the design and synthesis of novel anticancer entities,[44,49–54] the aim of this study was thus to provide additional compounds for the study of in vitro growth inhibitory effects displayed by silyl-derivatized nucleosides.

2. Results and discussion

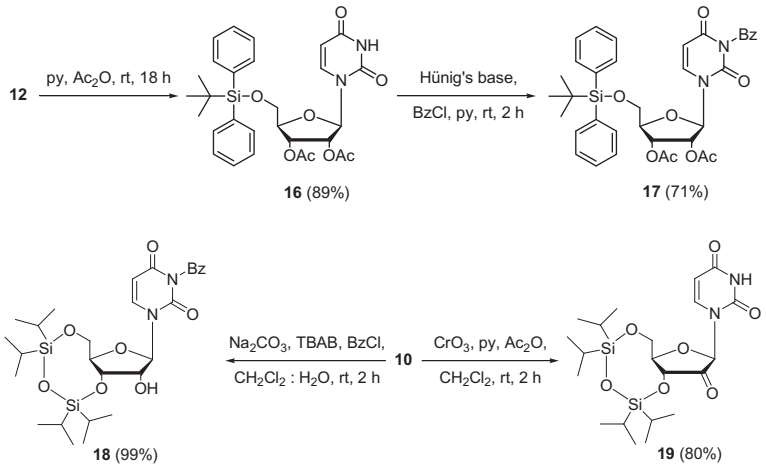
2.1. Chemistry

The main set of compounds targeted, comprised of a number of nucleosides with different silyl substituents on the 5′-*O* position. All of these compounds were readily synthesized by the reaction of the purchased nucleosides **5** with the various silyl chlorides, to afford **6** or **7** as described in the boxed generalized reaction in Scheme 1. As such, the nucleosides uridine and 5-methyluridine were converted into their TBDMS- **4d** and **8**, ThexylDMS- **4e** and **9**, and TBDPS-derivatives **12** and **13**, respectively. In addition, the di-sila derivatives **10** and **11** were also generated from the same nucleosides by reaction with the commercially available 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane. Finally, the TBDPS derivatives of cytidine (**14**) and adenosine (**15**) were also prepared, so that the library would contain representative members of both the purine and pyrimidine families.

It was also of interest to see whether the masking of any other functional groups present in the nucleosides would affect their ability to act as in vitro growth inhibitory agents (for a detailed discussion on the molecular mechanisms of the nucleoside family, see the following review[55]). To this end, the free alcohols of the TBDPS-uridine derivative **12** were protected as their acetyl derivatives to give **16**, followed by the protection of the base NH as the benzoyl derivative **17** (Scheme 2). In a similar manner, disila-**10** was readily converted into the benzoyl derivative **18**, while treatment of **10**



Scheme 1. Synthesis of the silyl derivatives of a number of nucleosides.



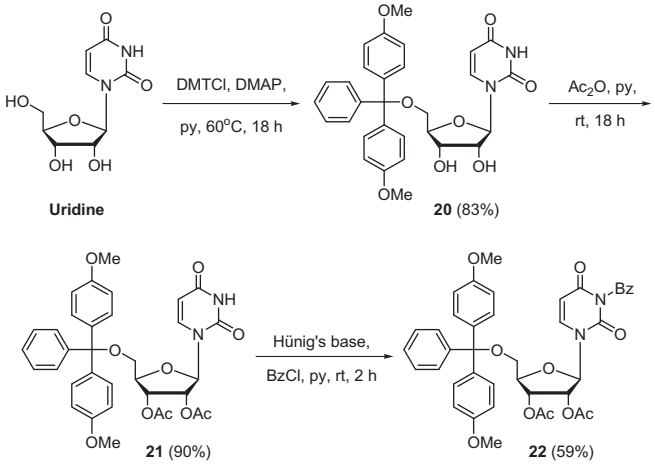
Scheme 2. Synthesis of additional uridine derivatives.

with chromium(VI)oxide/pyridine/acetic anhydride (1:2:1) also afforded the keto-uridine derivative **19**.

Having synthesized a number of silyl derivatives, it was decided to also utilize a dimethoxytrityl-protecting group as this would also result in nucleosides attached to another very bulky non-polar group on the 5′-*O* position. With this in mind, uridine was treated with dimethoxytrityl chloride (DMTCl) in the presence of base to readily afford the uridine-derivative **20**. To afford additional compounds with modulated lipophilicity, this compound was converted into the diacetyl derivative **21** and subsequently into the benzoyl-uridine derivative **22** (Scheme 3).

2.2. Bio-evaluation

An initial screen utilizing a number of cell viability assays was performed on a small group of synthetic compounds at a single concentration, in order to obtain information for the activity of a subset of the compounds synthesized. For this study, five human cancer cell lines were used: three leukemic cell lines [HL-60 (promyelocytic leukemia), K-562 (myelogenous leukemia) and Jurkat (T-cell leukemia)] and two colorectal adenocarcinoma cell lines (HT-29 and Caco-2). Human leucocytes were used as a normal control and as an indicator for toxicity. In addition, the synthetic compounds were assayed against two established human glioma lines (U373 and Hs683) to determine GI_{50} values for the entire library of modified nucleosides.



Scheme 3. Use of trityl-protecting group to generate nucleoside derivatives.

2.2.1. Effect of synthetic compounds on growth of cancer cell lines and white blood cells

The effect of the synthetic compounds on cell population growth was evaluated by exposing a cell population to compounds **12**, **14**, **15**, **16**, **20** and **21** and then monitoring the mitochondrial enzymatic reduction of MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] to formazan crystals. The cancer cells were initially exposed to the synthetic compounds (final concentration of 100 μM) at 37 °C for 24 h. The MTT assay was then used to quantify cell viability after exposure (Table 1). A compound was considered active if it reduced the growth of the cells by at least 50% (the GI_{50} concentration), after exposure of the cells for 24 or 72 h with the compound of interest, as compared to the control arbitrarily normalized at 100%.

The results from the initial screen were promising, with the TBDPS-containing compounds **12**, **14** and **15** showing activity. Of the three, 5′-*O*-(*tert*-butyldiphenylsilyl)uridine **12** was the best, inhibiting the proliferation of the suspension cell lines (leukemia) to below 10%, while the adherent colorectal Caco-2 and HT-29 cells showed between 60% and 75% inhibition (Table 1). Of interest, was that the 5′-*O*-(4,4′-dimethoxytrityl)uridine **20** displayed similar activity values to the TBDPS-uridine derivative **12** (Table 1, entries 1 and 5). The activity of the compounds in which the 2′- and 3′-alcohols had been acetylated was lower for TBDPS-protected compound **16** (versus diol **12**), while the acetylated dimethoxytrityl derivative **21** appeared to lose all potency when compared to the diol **20** (Table 1, entries 4 vs 1 and 6 vs 5).

Freshly isolated leucocytes were also exposed to the same set of synthetic derivatives (compounds **12**, **14**, **15**, **16**, **20** and **21**) and camptothecin, at a final concentration of 100 μM, for a period of 24 h. Cell viability was then determined by the MTT assay (Table 1). All the synthetic derivatives screened showed reduced in vitro growth inhibitory effects toward the leucocytes when compared to 100 μM camptothecin, suggesting that the synthetic derivatives prepared for this study may be sparing to non-cancerous cells. Finally, from Table 1 it could be seen that compounds **12** and **20** show selective activity toward the leukemic cells, which is promising.

2.2.2. Effect of synthetic compounds on cancer cell population growth and determination of GI_{50} values

Having identified that certain compounds possessed interesting activity in terms of the inhibition of cancer cell population growth, it was decided to test an extended library of seventeen synthetic compounds against two adherent human glioma lines (U373 and Hs683), across a range of concentrations in order to determine

Table 1
Percentage of cell viability of the cell line panel and normal, un-stimulated leucocytes (white blood cells, WBC), treated with 100 μM of the synthetic compounds or camptothecin for 24 h

Entry	Compound	Cell viability (% surviving at 100 μM as compared to control (100%)					
		HL-60	Jurkat	K-562	Caco-2	HT-29	WBC
1	**12**	5.6 ± 0.3	6.3 ± 0.6	9.4 ± 0.1	37.4 ± 1.0	29.4 ± 0.6	61.5 ± 4.5
2	**14**	26.0 ± 0.6	16.0 ± 1.3	35.6 ± 0.9	37.2 ± 0.4	25.6 ± 0.7	NI
3	**15**	27.9 ± 0.5	18.3 ± 1.2	45.5 ± 0.7	NA	NA	59.3 ± 2.0
4	**16**	11.2 ± 0.5	NA	35.5 ± 2.4	NA	NA	NI
5	**20**	6.5 ± 0.5	5.2 ± 1.0	8.3 ± 0.3	26.1 ± 0.2	27.8 ± 1.2	82.7 ± 2.1
6	**21**	NA	NA	NA	NA	NA	NI
7	Camptothecin	23.4 ± 0.7	8.9 ± 0.7	39.6 ± 0.9	12.8 ± 0.2	11.4 ± 0.2	34.6 ± 2.0

NA = not active, NI = no inhibition (cell viability >50% for cell lines, cell viability 100% for WBC).

GI_{50} values. In terms of a positive control for this study, narciclasine was selected, as this particular compound has been shown by Kiss and co-workers to potently inhibit growth of these cell lines.[56,57] In this series of experiments, GI_{50} concentrations were determined after having cultured the glioma cells for 72 h in the presence of the compound of interest (as compared to the controls, i.e. untreated cells).

Regarding comparison with results available in the literature, the first compounds to be evaluated were the TBDMS-protected uridine derivatives **4d** (R = H) and **8** (R = Me). Compound **4d** was poorly active in the human glioma lines U373 and Hs683, with GI_{50} values of 71 ± 2 μM and 84 ± 3 μM respectively, and compound **8** did not fare better with GI_{50} values of >100 μM for U373 and 77 ± 1 μM for Hs683 (as a reference point, when cisplatin was tested against the same two cell lines, GI_{50} values of 5 μM and 0.4 μM were obtained for U373 and Hs683, respectively[58]). In a similar fashion, the thexyl-protected compound **4e** showed poor inhibition of glioma cell population growth (Table 2, entry 3). Of interest, was that the related 5′-*O*-(dimethylthexylsilyl)-5-methyluridine **9** showed GI_{50} values of 68 ± 1 μM and 64 ± 2 μM for the U373 and Hs683 cells respectively, indicating a slight increase in efficacy. In this particular case, the addition of a methyl group to compound **4e**, resulting in **9**, does therefore appear to improve the compound's in vitro growth inhibitory activity.

Next, the set of TBDPS-derivatives were evaluated, and the results showed that the compounds containing the TBDPS group showed increased growth inhibitory activity ranging between 25 and 46 μM, irrespective of the uridine **12**, 5-methyluridine **13**, cytidine **14** or adenosine **15** base utilized. It can be tentatively concluded that it is the lipophilic TBDPS group that has a positive effect on the inhibition of cancer cell population growth. Two other derivatives based on the TBDPS-containing uridine scaffold were also tested, namely **16** and **17**. To this end, it was found that the GI_{50} values against U373 and Hs683 cells were 32 ± 1 and 29 ± 1 μM respectively for 2′,3′-*O*-diacetyl-5′-*O*-(*tert*-butyldiphenylsilyl)uridine **16**, which were similar to those values obtained for the unprotected compound **12**. In addition, when the amine on this particular base was protected with a benzoyl group to afford compound **17**, the mean growth inhibitory results were comparable in potency to compounds **12** and **16**.

The next group of compounds investigated in our study were those containing the 1,3-dihydroxy-1,1,3,3-tetraisopropyldisiloxane protecting group, which to our knowledge have not been tested before in this manner. The simplest of these was the disilyl-compound **10**, for which GI_{50} values of 81 ± 4 and 83 ± 2 μM were determined for the U373 and Hs683 glioma cells, respectively. The methyluridine derivative **11** had similar potency, and while the benzoyl-protected derivative **18** had better activity (29 ± 2 μM for both cell lines), the 2′-keto-compound **19** was again in the same region of activity (71 ± 2 and 70 ± 4 μM, for U373 and Hs683 glioma cells respectively).

Finally, a series of compounds where the *tert*-butyldiphenylsilyl protecting group on the 5′-oxygen was exchanged for a 4,4′-dimethoxytrityl protecting group was evaluated. The GI_{50} values were found to be in the 28–44 μM range for all the 4,4′-dimethoxytrityl derivatives, including the simple 5′-*O*-(4,4′-dimethoxytrityl) uridine **20**, the diacylated compound **21** and the 3-*N*-benzoyl-2′,3′-*O*-diacetyl-protected uridine derivative **22**.

Finally, the lipophilicity (*A*Log*P*) values were calculated for each of the nucleoside analogues. Although no obvious trend in the relationship between the GI_{50} values and the *A*Log*P* values was observed, it should be noted that the more water-soluble compounds (e.g. compounds **4d/e** and **8**) tended to be less active, while those less water-soluble tended to have improved (i.e. decreasing) GI_{50} values (e.g. compounds **17** and **18**). This observation could imply that the bulkier non-polar silyl groups could be assisting in the compounds being able to cross cell membranes or being responsible for bioactivity in terms of disrupting cellular membranes. It should be noted that in previous papers (for example[20,29,32]) mention has been made of the ability of lipophilic silyl groups on nucleoside cores to facilitate the crossing of cell membrane barriers. In addition, it has also been noted that the efficacy of the silyl-containing compounds is related to the rate of hydrolysis,[32] explaining why sterically hindered groups like TBDPS and thexyl-DMS could be considered potentially important structural elements in the design of novel growth inhibitory compounds, at least in vitro.

Table 2
In vitro compound-induced anti-growth effects on various cancer cell lines expressed as GI_{50} values

Entry	Compound	GI_{50} (μM) [72 h culture with compounds]		*A*Log*P*
		U373	Hs683	
1	**4d**	71 ± 2	84 ± 3	1.4
2	**8**	>100	77 ± 1	1.8
3	**4e**	>100	>100	2.1
4	**9**	68 ± 1	64 ± 2	2.6
5	**10**	81 ± 4	83 ± 2	6.5
6	**11**	87 ± 2	91 ± 3	7.0
7	**12**	36 ± 1	35 ± 1	4.6
8	**13**	25 ± 1	27 ± 1	5.0
9	**14**	28 ± 1	40 ± 2	4.6
10	**15**	44 ± 1	46 ± 3	5.1
11	**16**	32 ± 1	29 ± 1	5.3
12	**17**	26 ± 1	35 ± 2	7.0
13	**18**	29 ± 2	29 ± 2	8.2
14	**19**	71 ± 2	70 ± 4	8.5
15	**20**	40 ± 3	39 ± 2	2.6
16	**21**	40 ± 3	28 ± 5	3.3
17	**22**	39 ± 1	44 ± 2	5.0
18	Narciclasine	0.029	0.040	ND

Note: ND = not determined.

3. Conclusions

Seventeen novel nucleoside derivatives were synthesized and tested against a range of cancer cell lines. The majority of the compounds comprised of 5′-*O*-silyl- and trityl-protected nucleoside derivatives and a number of previously unknown modifications of the nucleoside base or ribose portion were also achieved. For the cell viability assay at 100 μM, compounds **12** and **20** reduced cell viability by 65–95%, while for the GI_{50} determinations against two human glioma lines (U373 and Hs683), compounds **13**–**18**, **20**, **21** gave GI_{50} values in the range of 25–40 μM. From the results it appeared that in general, the nucleoside derivatives with the more lipophilic silyl/trityl groups had improved GI_{50} values.

4. Experimental procedures

4.1. Chemistry

4.1.1. General chemistry methods

All solvents and reagents were used as obtained from commercial sources unless otherwise indicated. All reactions were performed under an Ar atmosphere unless otherwise stated. The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Advance-300 spectrometer at 300.13 MHz for ^{1}H and 75 MHz for ^{13}C using standard pulse sequences. Deuterated chloroform or deuterated dimethylsulfoxide were used as solvents for NMR spectroscopy experiments. ^{1}H chemical shift values (δ) are referenced to tetramethylsilane (δ = 0.00 ppm for $CDCl_3$) or the residual nondeuterated components of the NMR solvents (δ = 2.50 ppm for d_6-DMSO). The ^{13}C chemical shifts (δ) are referenced to $CDCl_3$ (central peak, δ = 77.0 ppm) or d_6-DMSO (δ = 39.5 ppm) as the internal standard. Mass spectra were measured in positive mode electrospray ionization (ESI) and the data was obtained on a VG7-SEQ Double Focusing Mass Spectrometer at 70 eV and 200 μA. All melting points were obtained on a Reichert hot-stage microscope and are uncorrected. TLC was performed using aluminum-backed Macherey-Nagel ALUGRAM Sil G/UV_{254} plates, pre-coated with 0.25 mm silica gel 60. Column chromatography was performed using Macherey-Nagel Silica gel 60 (particle size 0.063–0.200 mm). Basified silica gel was used for the column chromatography of modified nucleosides, where specified in the text, and was prepared as follows: the silica required for the column was shaken with a 10% NEt_3/hexane (*v*/*v*) solution, used in the ratio of 1 cm^3 solution/1 g silica, for 15 min at rt. Thereafter, the silica was dried by removing the solvent in vacuo.

4.1.2. General procedure for the silylation of the 5′-OH of uridine and 5-methyluridine

The glassware and attachments were filled with a 5% TMSCl: hexane solution and were allowed to stand overnight. After this time, the glassware was oven-dried, placed under high vacuum and allowed to cool under an Ar atmosphere. To a stirred solution of uridine/5-methyluridine (1.0 equiv) and DMAP (0.1 equiv) in distilled pyridine (10 mL:1 g starting material) was added the corresponding silyl chloride (1.1 equiv). The reaction mixture was stirred overnight and then quenched by the addition of distilled H_2O (5 mL:1 g starting material) with vigorous stirring. The mixture was dissolved in excess EtOAc and concentrated under reduced pressure. The residue obtained was partitioned between EtOAc (20 mL:1 g starting material) and furthermore washed with distilled H_2O (3 × 10 mL:1 g starting material). The organic fraction was dried over anhydrous Na_2SO_4, filtered and the solvent was removed in vacuo. No further purification was required.

4.1.2.1. 5′-*O*-(*tert*-Butyldimethylsilyl)uridine (4d). Yield: 88% (white foam); Spectroscopic data correlated well to that previously reported by Debarge and co-workers.[59] ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.53 (br s, 1H), 8.08 (d, *J* = 8.1 Hz, 1H), 5.91 (d, *J* = 2.1 Hz, 1H), 5.65 (d, *J* = 8.1 Hz, 1H), 5.52 (br s, 1H), 4.26–4.23 (m, 2H), 4.14 (d, *J* = 5.3 Hz, 1H), 4.03 and 3.85 (2 × d, *J* = 11.3 Hz, 2H), 3.66 (br s, 1H), 0.92 (s, 9H), 0.12 (s, 6H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 164.0, 151.3, 140.5, 102.0, 90.2, 84.8, 75.6, 69.2, 61.8, 25.9, 18.4, −5.6; HRMS (ESI) *m*/*z* calculated for $C_{15}H_{27}N_2O_6Si$ $(M+H)^+$ 359.16329, found 359.16336.

4.1.2.2. 5′-*O*-(*tert*-Butyldimethylsilyl)-5-methyluridine (8). Yield: 93% (white powder); Spectroscopic data correlated well to that previously reported by Debarge and co-workers.[59] ^{1}H NMR (300 MHz, $CDCl_3$/MeOD, signals for NH and OH peaks not observed): δ 7.57 (s, 1H), 5.96 (d, *J* = 5.0 Hz, 1H), 4.15–4.07 (m, 3H), 3.95 and 3.86 (2 × dd, *J* = 2.1, 11.6 Hz, 2H), 1.91 (s, 3H), 0.96 (s, 9H), 0.15 (s, 3H), 0.16 (s, 3H); ^{13}C NMR (50 MHz, DMSO): δ 163.6, 150.7, 135.6, 109.7, 87.1, 84.3, 73.1, 70.0, 63.1, 25.8, 18.0, 12.1, −5.3; HRMS (ESI) *m*/*z* calculated for $C_{16}H_{29}N_2O_6Si$ $(M+H)^+$ 373.17894, found 373.17903.

4.1.2.3. 5′-*O*-(Dimethylthexylsilyl)uridine (4e). Yield: 99% (white foam); ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.50 (br s, 1H), 8.02 (d, *J* = 8.1 Hz, 1H), 5.90 (d, *J* = 2.6 Hz, 1H), 5.65 (d, *J* = 8.1 Hz, 1H), 4.26–4.11 (m, 2H), 4.20 (d, *J* = 5.4 Hz, 1H), 3.88 and 3.71 (2 × d, *J* = 11.4 Hz, 2H), 3.49 (br s, 1H), 2.04 (br s, 1H), 1.63 (sept, *J* = 6.6 Hz, 1H), 0.88 (br s, 12H), 0.15 (s, 6H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 164.0, 151.3, 140.4, 102.0, 90.2, 84.9, 75.6, 69.3, 61.7, 34.1, 25.4, 20.2, 18.4, −3.5; HRMS (ESI) *m*/*z* calculated for $C_{17}H_{31}N_2O_6Si$ $(M+H)^+$ 387.19459, found 387.19505.

4.1.2.4. 5′-*O*-(Dimethylthexylsilyl)-5-methyluridine (9). Yield: 80% (white solid); ^{1}H NMR (300 MHz, $CDCl_3$/d_6-DMSO): δ 12.13 (br s, 1H), 8.22 (s, 1H), 6.73 (d, *J* = 5.2 Hz, 1H), 6.14 (d, *J* = 5.2 Hz, 1H), 5.83 (d, *J* = 3.7 Hz, 1H), 4.85–4.84 (m, 2H), 4.79–4.77 (m, 1H), 4.67 and 4.62 (2 × dd, *J* = 2.6, 11.5 Hz, 2H), 4.20 (s, 3H), 1.49 (sept, *J* = 6.7 Hz, 1H), 1.75 (br s, 12H), 1.00 (s, 6H); ^{13}C NMR (50 MHz, DMSO): δ 163.6, 150.7, 135.6, 109.5, 87.0, 84.3, 72.9, 69.9, 63.0, 33.6, 24.6, 20.1, 18.4, 12.0, −3.5; HRMS (ESI) *m*/*z* calculated for $C_{18}H_{33}N_2O_6Si$ $(M+H)^+$ 401.21024, found 401.20987.

4.1.3. 1-[(6a*R*,8*R*,9*R*,9a*S*)-9-Hydroxy-2,2,4,4-tetraisopropyltetrahydro-6*H*-furo[3,2-*f*][1,3,5,2,4]trioxadisilocin-8-yl]pyrimidine-2,4(1*H*,3*H*)-dione (10)

A solution of uridine (1.68 g, 6.87 mmol) in distilled pyridine (20.5 mL) was prepared and cooled to 0 °C in an ice-water bath, before the addition of the 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (2.3 mL, 7.2 mmol). The reaction mixture was stirred overnight and subsequently quenched by the addition of distilled H_2O (21 mL). The solvents were removed under reduced pressure and the residue obtained was partitioned between EtOAc (70 mL) and distilled H_2O (2 × mL). The organic fraction was dried over anhydrous Na_2SO_4, filtered and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel, using a gradient of EtOAc–hexane (10–50%) for elution. Product **10** (2.80 g) was obtained as a white foam, yield: 85%. ^{1}H and ^{13}C NMR spectra obtained correlated well to those reported by Matsuda and co-workers.[60] ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.64 (br s, 1H), 7.77 (d, *J* = 8.1 Hz, 1H), 5.75 (d, *J* = 2.1 Hz, 1H), 5.73–5.69 (m, 1H), 4.32 (dd, *J* = 4.7, 8.8 Hz, 1H), 4.25–4.14 (m, 3H), 4.00 (dd, *J* = 2.5, 13.2 Hz, 1H), 3.62 (br s, 1H), 1.10–1.02 (m, 28H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 163.5, 150.2, 139.9, 102.0, 90.9, 81.8, 75.1, 68.7, 60.1, 17.4, 17.33, 17.25, 17.2, 17.0, 16.91, 16.88, 16.8, 13.3, 12.93, 12.88, 12.4; HRMS (ESI) *m*/*z* calculated for $C_{21}H_{39}N_2O_7Si_2$ $(M+H)^+$ 487.22903, found 487.22848.

4.1.4. 1-[(6a*R*,8*R*,9*S*,9a*R*)-Tetrahydro-9-hydroxy-2,2,4,4-tetraisopropyl-6*H*-furo[3,2-*f*]-[1,3,5,2,4]trioxadisilocin-8-yl](5-methylpyrimidine)-2,4(1*H*,3*H*)-dione (11)

Protocol the same as for the preparation of **10**, starting from 5-methyluridine. Product **11** (0.78 g) was obtained as a white foam, yield: 40%. Proton NMR spectroscopic data correlated well to that previously reported by Turkman and co-workers.[61] ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.47 (br s, 1H), 7.46 (s, 1H), 5.72 (s, 1H), 4.37–4.33 (m, 1H), 4.23–4.14 (m, 2H), 4.12–3.98 (m, 2H), 3.68 (d, J = 1.4 Hz, 1H), 1.91 (s, 3H), 1.10–1.04 (m, 28H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 164.0, 150.2, 135.6, 110.6, 91.1, 81.8, 75.0, 68.9, 60.2, 17.4, 17.3, 17.2, 17.2, 17.0, 17.0, 16.9, 16.8, 12.9, 12.7, 12.6, 12.5; HRMS (ESI) m/z calculated for $C_{22}H_{41}N_2O_7Si_2$ $(M+H)^+$ 501.24468, found 501.24409.

4.1.5. General procedure for the silylation of the 5′-OH of nucleosides using *tert*-butydiphenylsilyl chloride

The nucleoside (1.0 equiv) and DMAP (0.1 equiv) were stirred with distilled pyridine (5 mL:1 g starting material), to this solution was added *tert*-butyldiphenylsilyl chloride (1.1 equiv) and the reaction mixture stirred under an Ar atmosphere overnight. The reaction mixture was diluted with CH_2Cl_2 (40 mL:1 g starting material) and extracted with a saturated $NaHCO_3$ solution (2 × 40 mL:1 g starting material) and brine (40 mL:1 g starting material). The combined organics were then dried over anhydrous Na_2SO_4, filtered and the solvent removed in vacuo. The residue was purified as described below.

4.1.5.1. 5′-*O*-(*tert*-Butyldiphenylsilyl)uridine (12). The residue was purified by column chromatography on the basified silica gel, using 10% EtOH–CH_2Cl_2 for elution. Yield: 100% (white foam). Spectroscopic data correlated well to that previously reported by Sproat et al.[62] ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.38 (br s, 1H), 7.97 (d, J = 8.1 Hz, 1H), 7.65 (2 × d, each J = 6.1 Hz, 4H), 7.44–7.37(m, 6H), 5.93 (d, J = 2.1 Hz, 1H), 5.47 (br s, 1H), 5.37 (d, J = 8.1 Hz, 1H), 4.37–4.35 (m, 1H), 4.32–4.30 (m, 1H), 4.25–4.08 (m, 2H), 3.89 (d, J = 10.7 Hz, 1H), 3.38 (br d, J = 5.8 Hz, 1H), 1.08 (s, 9H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 164.1, 151.4, 140.4, 135.8, 135.6, 132.3, 130.3, 128.22, 128.19, 102.5, 90.5, 84.8, 75.7, 69.4, 62.8, 27.2, 19.5; HRMS (ESI) m/z calculated for $C_{25}H_{31}N_2O_6Si$ $(M+H)^+$ 483.19459, found 483.19407.

4.1.5.2. 5′-*O*-(*tert*-Butyldiphenylsilyl)-5-methyluridine (13). The residue was purified by the azeotropic distillation of pyridine, using toluene as the co-solvent. Yield: 100% (white foam). ^{1}H NMR (300 MHz, $CDCl_3$/MeOD): δ 8.53 (br d, 1H), 7.82 (s, 1H), 7.71–7.68 (m, 4H), 7.43–7.40 (m, 6H), 6.02 (br d, J = 5.7 Hz, 1H), 4.33–4.24 (m, 2H), 4.11–4.03 (m, 2H), 3.90 (dd, J = 2.3, 11.6 Hz, 1H), 1.49 (s, 3H), 1.12 (s, 9H); ^{13}C NMR (50 MHz, DMSO): δ 163.5, 150.6, 134.6, 132.6, 130.0, 127.9, 109.6, 87.3, 84.1, 72.9, 69.8, 64.2, 26.6, 18.6, 11.6; HRMS (ESI) m/z calculated for $C_{26}H_{33}N_2O_6Si$ $(M+H)^+$ 497.21024, found 497.20971.

4.1.5.3. 5′-*O*-(*tert*-Butyldiphenylsilyl)cytidine(14). The residue was purified by column chromatography using basified silica gel, with a gradient of EtOH–CH_2Cl_2 (10–20%) for elution. Yield: 100% (white foam).^{1}H NMR (300 MHz, d_6-DMSO): δ 7.66 (d, J = 7.4 Hz, 1H), 7.65–7.63 (m, 4H), 7.49–7.39 (m, 6H), 7.15 (br s, 2H), 5.83 (d, J = 3.4 Hz, 1H), 5.53 (d, J = 7.4 Hz, 1H), 5.43 (d, J = 5.1 Hz, 1H), 5.07 (d, J = 6.0 Hz, 1H), 4.11–4.08 (m, 1H), 3.95–3.90 (m, 3H), 3.79–3.74 (m, 1H), 1.02 (s, 9H); ^{13}C NMR (50 MHz, d_6-DMSO): δ 165.5, 155.1, 140.6, 135.0, 132.3, 130.0, 128.0, 93.7, 89.3, 82.9, 74.2, 68.9, 63.2, 26.7, 18.8; HRMS (ESI) m/z calculated for $C_{25}H_{32}N_3O_5Si$ $(M+H)^+$ 482.21057, found 482.20978.

4.1.5.4. 5′-*O*-(*tert*-Butyldiphenylsilyl)adenosine (15). The residue was purified by column chromatography, using basified silica gel and 10% EtOH–CH_2Cl_2 for elution. Yield: 73% (white foam). Proton NMR spectroscopic data correlated well to that previously reported by Beaton and co-workers.[63] ^{1}H NMR (300 MHz, $CDCl_3$/d_6-DMSO): δ 8.11 (s, 1H), 8.08 (s, 1H), 7.59 (2 × d, J = 7.0 Hz, 4H), 7.38–7.30 (m, 6H), 7.03 (br s, 2H), 5.95 (d, J = 4.9 Hz, 1H), 5.45 (d, J = 5.6 Hz, 1H), 5.07 (d, J = 5.4 Hz, 1H), 4.61–4.59 (m, 1H), 4.36–4.34 (m, 1H), 4.07–4.03 (m, 1H), 3.93 and 3.78 (2 × dd, J = 3.9, 11.3 Hz, 2H), 0.99 (s, 9H); ^{13}C NMR (50 MHz, $CDCl_3$/d_6-DMSO): δ 155.8, 152.3, 149.2, 138.5, 134.8, 132.4, 129.4, 127.4, 119.1, 87.5, 84.1, 73.5, 69.7, 63.5, 26.4, 18.7; HRMS (ESI) m/z calculated for $C_{26}H_{32}N_5O_4Si$ $(M+H)^+$ 506.22181, found 506.22098.

4.1.6. 2′,3′-*O*-Diacetyl-5′-*O*-(*tert*-butyldiphenylsilyl)uridine (16)

The silylated uridine **12** (4.19 g, 8.69 mmol) was stirred with distilled pyridine (1.41 mL, 17.4 mmol) until a solution had formed and was then cooled to 0 °C in an ice-water bath. To this cooled solution was then added drop-wise a mixture of distilled Ac_2O (2.50 mL, 26.1 mmol) and distilled pyridine (2.11 mL, 26.1 mmol). The reaction mixture was then warmed and stirred overnight under an Ar atmosphere. EtOAc (400 mL) was added to the reaction mixture and extracted with brine (3 × 400 mL), the combined aqueous layers were back-extracted with CH_2Cl_2 (3 × 400 mL) and the combined organic fractions were then extracted with saturated NH_4Cl that had been basified to pH ~ 10 using NH_3 (600 mL). The combined organics were dried over anhydrous $MgSO_4$, filtered and the solvent was removed in vacuo to give product **16** (4.39 g) as a cream-colored foam, yield: 89%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.39 (br s, 1H), 7.76–7.64 (m, 5H), 7.43–7.35 (m, 6H), 6.30 (d, J = 6.5 Hz, 1H), 5.53–5.51 (m, 1H), 5.47–5.40 (m, 2H), 4.18 (br s, 1H), 4.02 and 3.85 (2 × d, J = 11.8 Hz, 2H), 2.07 (s, 3H), 2.04 (s, 3H), 1.13 (s, 9H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 170.0, 169.8, 163.2, 150.8, 139.6, 135.9, 135.8, 132.8, 130.4, 129.8, 128.1, 127.8, 103.4, 85.4, 83.4, 73.3, 71.2, 63.7, 27.2, 20.9, 20.6, 19.5; HRMS (ESI) m/z calculated for $C_{29}H_{34}N_2O_8SiNa$ $(M+H)^+$ 589.19766, found 589.19699.

4.1.7. 3-*N*-Benzoyl-2′,3′-*O*-diacetyl-5′-*O*-(*tert*-butyldiphenylsilyl) uridine (17)

The protected uridine **16** (1.07 g, 1.88 mmol) was stirred with distilled pyridine (20 mL) and to this pale yellow solution was added *N*,*N*-diisopropylethylamine (1.64 mL, 9.41 mmol) with stirring. Distilled benzoyl chloride (1.10 mL, 9.41 mmol) was added, with the liberation of a white gas, and the reaction mixture was stirred under an Ar atmosphere for 2 h. The dark burgundy-colored reaction mixture was diluted with CH_2Cl_2 (100 mL) and washed with distilled H_2O (3 × 100 mL). The aqueous fractions were next extracted with CH_2Cl_2 (100 mL), and the combined organic layers dried over anhydrous Na_2SO_4 and the solvent was removed under reduced pressure. The residue was purified by column chromatography on silica gel and eluted with a gradient of EtOAc–hexane (10–50%). Product **17** (0.90 g) was obtained as a yellow foam, yield: 71%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.95 (d, J = 7.3 Hz, 2H), 7.86 (d, J = 8.3 Hz, 1H), 7.67–7.63 (m, 4H), 7.48–7.41 (m, 9H), 6.24 (d, J = 6.0 Hz, 1H), 5.55–5.48 (m, 3H), 4.20 (d, J = 2.1 Hz, 1H), 3.87 and 4.05 (2 × d, J = 11.9 Hz, 2H), 2.10 (s, 3H), 2.05 (s, 3H), 1.15 (s, 9H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 170.0, 168.6, 161.9, 149.7, 139.3, 135.9, 135.6, 135.3, 132.7, 131.5, 130.8, 130.6, 130.5, 130.3, 129.3, 128.4, 103.2, 85.9, 83.6, 73.5, 71.2, 63.7, 27.2, 20.8, 20.6, 19.5; HRMS (ESI) m/z calculated for $C_{36}H_{39}N_2O_9Si$ $(M+H)^+$ 671.24193, found 671.24173.

4.1.8. 3-Benzoyl-1-[(6a*R*,8*R*,9*R*,9a*S*)-9-hydroxy-2,2,4,4-tetraisopropyltetrahydro-6*H*-furo[3,2-*f*][1,3,5,2,4]trioxadisilocin-8-yl]pyrimidine-2,4(1*H*,3*H*)-dione (18)

1-[(6a*R*,8*R*,9*R*,9a*S*)-9-Hydroxy-2,2,4,4-tetraiso-propyltetrahydro-6*H*-furo[3,2-*f*][1,3,5,2,4]trioxadisilocin-8-yl]pyrimidine-2,4(1*H*,3*H*)-dione **10** (6.02 g, 12.5 mmol), Na_2CO_3 (10.10 g, 96.88 mmol) and tetrabutylammonium bromide (0.16 g, 0.48 mmol) were dissolved in a biphasic mixture of CH_2Cl_2 (240 mL) and distilled H_2O (480 mL). Benzoyl chloride (1.88 mL, 16.3 mmol) was added and the reaction mixture was vigorously stirred for 2 h. The reaction mixture was diluted with excess CH_2Cl_2 and the aqueous phase was further extracted with CH_2Cl_2 (3 × 480 mL). The combined organic fractions were dried over anhydrous Na_2SO_4, filtered and evaporated under reduced pressure. The residue was dissolved in 1,2-dichloroethane (120 mL) with gentle heating and the solution was allowed to stand for 66 h, to allow the *O*-/*N*-rearrangement to occur. The solvent was removed under reduced pressure and the residue was purified by column chromatography on silica gel, with gradient EtOAc–hexane (5–50%) used for elution. Compound **18** (7.26 g) was isolated as a white foam, yield: 99%. ^{1}H and ^{13}C NMR spectra obtained correlated well to those reported previously by Sekine.[64] ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.93 (d, J = 7.2 Hz, 2H), 7.81 (d, J = 8.2 Hz, 1H), 7.66 (t, J = 7.4 Hz, 1H), 7.50 (t, J = 7.7 Hz, 2H), 5.80 (d, J = 8.2 Hz, 1H), 5.76 (s, 1H), 4.37 (dd, J = 4.8, 8.6 Hz, 1H), 4.21–4.11 (m, 3H), 4.01 (dd, J = 2.6, 13.3 Hz, 1H), 2.04 (br s, 1H), 1.10–1.03 (m, 28H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 168.5, 162.1, 148.9, 139.6, 135.1, 131.4, 130.4, 129.1, 101.8, 90.8, 82.1, 75.2, 68.9, 60.2, 17.4, 17.3, 17.22, 17.19, 17.0, 16.9, 16.8, 16.7, 14.1, 13.3, 12.9, 12.5; HRMS (ESI) m/z calculated for $C_{28}H_{43}N_2O_8Si_2$ $(M+H)^+$ 591.25525, found 591.25494.

4.1.9. 1-[(6a*R*,8*R*,9a*R*)-2,2,4,4-Tetraisopropyl-9-oxotetrahydro-6*H*-furo[3,2-*f*][1,3,5,2,4]trioxadisilocin-8-yl]pyrimidine-2,4(1*H*,3*H*)-dione (19)

The oxidation complex was pre-formed by preparing 3 equiv. of the complex relative to the starting material **10**.[60] Initially, chromium(VI) trioxide (0.69 g, 6.8 mmol) was dissolved in freshly distilled CH_2Cl_2 (24 mL). To this solution was added distilled pyridine (1.10 mL, 13.5 mmol) and distilled Ac_2O (0.64 mL, 6.8 mmol). The mixture was stirred for a few minutes to allow for complete formation of the oxidation complex. A solution was then prepared of **10** (1.09 g, 2.25 mmol) in distilled CH_2Cl_2 (3 mL). The starting material solution was added drop-wise to the oxidation complex solution with stirring. The reaction mixture was allowed to stir under an Ar atmosphere for 2 h. After this time, the reaction mixture was poured drop-wise into stirring EtOAc (180 mL). The suspension was then pumped through a short silica gel column, using excess EtOAc to rinse the column. The filtrate was concentrated to dryness to yield a yellow-brown residue. This residue was then purified by column chromatography, with gradient EtOAc–hexane solutions (20–50%) for elution. Product **19** (0.87 g) was obtained pure as a white foam, yield: 80%. NMR spectroscopic data obtained correlates well to that reported by Matsuda and co-workers.[60] ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.80 (br s, 1H), 7.16 (d, J = 8.0 Hz, 1H), 5.76 (d, J = 8.0 Hz, 1H), 5.04 (d, J = 9.0 Hz, 1H), 5.00 (s, 1H), 4.14–4.13 (m, 2H), 3.97–3.91 (m, 1H), 1.14–1.03 (m, 28 H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 204.7, 163.0, 149.2, 143.7, 103.2, 85.5, 79.6, 71.8, 62.4, 17.4, 17.2, 16.9, 16.82, 16.79, 16.76, 16.67, 16.6, 13.4, 13.0, 12.5, 12.3; HRMS (ESI) m/z calculated for $C_{21}H_{37}N_2O_7Si_2$ $(M+H)^+$ 485.21338, found 485.21289.

4.1.10. 5′-*O*-(4,4′-Dimethoxytrityl)uridine (20)

Uridine (0.51 g, 2.1 mmol) was dissolved in pyridine (10 mL), and to this solution was then added 4,4′-dimethoxytrityl chloride (0.85 g, 2.5 mmol) and DMAP (0.0033 g, 0.025 mmol). The reaction mixture was stirred at 60 °C under an Ar atmosphere overnight and the solvent was removed under reduced pressure. The residue was purified by column chromatography on basified silica, using gradient MeOH–CH_2Cl_2 (0–10%) for elution. The cream foam isolated after chromatography was dissolved in MeOH (4.5 mL) and the solution was added drop-wise to vigorously stirring Et_2O (35 mL). A white precipitate was observed and the mixture was cooled to 0 °C in an ice-water bath to allow for complete crystallization. The white solid was filtered off through a sintered funnel and the mother liquor was evaporated and dried in vacuo. The mother liquor gave product **20** (0.95 g) as a cream-colored foam, yield: 83%. Spectroscopic data correlated well to that previously reported by Yang and co-workers.[65] ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.90 (d, J = 8.1 Hz, 1H), 7.22–7.39 (m, 9H), 6.83 (d, J = 8.7 Hz, 4H), 5.95 (d, J = 3.4 Hz, 1H), 5.34 (d, J = 8.1 Hz, 1H), 4.44 (t, J = 5.2 Hz, 1H), 4.34–4.32 (m, 1H), 4.20–4.18 (m, 1H), 3.78 (s, 6H), 3.49–3.45 (m, 2H), NH and OH peaks not observed; ^{13}C NMR (50 MHz, DMSO): δ 163.1, 158.1, 150.5, 144.6, 140.7, 140.5, 135.4, 135.2, 129.7, 128.9, 127.9, 127.7, 127.6, 126.7, 126.4, 113.2, 101.4, 88.9, 85.8, 82.4, 73.4, 69.6, 63.0, 55.0; HRMS (ESI) m/z calculated for $C_{30}H_{31}N_2O_8$ $(M+Na)^+$ 569.18944, found 569.18944.

4.1.11. 2′,3′-*O*-Diacetyl-5′-*O*-(4,4′-dimethoxytrityl)uridine (21)

The protected uridine **20** (2.00 g, 3.65 mmol) was dissolved in distilled pyridine (1.50 mL, 18.3 mmol), cooled to 0 °C in an ice-water bath and to the solution was added drop-wise a mixture of distilled Ac_2O (1.05 mL, 11.0 mmol) and distilled pyridine (0.90 mL, 11 mmol). The reaction mixture was then warmed and stirred under an Ar atmosphere overnight. The reaction mixture was diluted with EtOAc (200 mL) while stirring, and the organic fraction was washed with brine (3 × 200 mL) and the combined aqueous fractions were extracted with CH_2Cl_2 (3 × 200 mL). All of the organic fractions were combined and washed with saturated NH_4Cl that had been basified to pH ~ 10 with NH_3 (300 mL). The combined organics were dried over anhydrous $MgSO_4$, filtered and the solvent removed in vacuo to give product **21** (2.06 g) as a pale orange foam, yield: 90%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.34 (br s, 1H), 7.73 (d, J = 8.1 Hz, 1H), 7.29–7.40 (m, 9H), 6.88 (d, J = 8.1 Hz, 4H), 6.28 (d, J = 6.3 Hz, 1H), 5.65–5.62 (m, 1H), 5.60–5.58 (m, 1H), 5.36 (d, J = 8.1 Hz, 1H), 4.27–4.25 (m, 1H), 3.82 (s, 6H), 3.53–3.50 (m, 2H), 2.14 (s, 3H), 2.13 (s, 3H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 170.0, 169.8, 163.1, 159.0, 150.8, 144.0, 139.9, 134.9, 130.39, 130.34, 128.4, 128.3, 127.5, 113.6, 103.3, 87.8, 85.5, 82.2, 73.0, 71.5, 62.9, 55.4, 20.9, 20.7; HRMS (ESI) m/z calculated for $C_{34}H_{35}N_2O_{10}$ $(M+H)^+$ 631.22862, found 631.22851, $C_{34}H_{34}N_2O_{10}Na$ $(M+Na)^+$ 653.21057, found 653.21020.

4.1.12. 3-*N*-Benzoyl-2′,3′-*O*-diacetyl-5′-*O*-(4,4′-dimethoxytrityl)uridine (22)

The protected uridine **21** (0.39 g, 0.63 mmol) was stirred with distilled pyridine (8 mL). To this solution was then added *N*,*N*-diisopropylethylamine (0.55 mL, 3.15 mmol) and stirring continued for five min. To the solution was then added distilled benzoyl chloride (0.37 mL, 3.2 mmol) with the immediate liberation of a white-colored gas. The reaction mixture was stirred under an Ar atmosphere for 2 h, after which it was diluted with CH_2Cl_2 (40 mL) and extracted with distilled H_2O (3 × 40 mL), the combined aqueous layers were then extracted with CH_2Cl_2 (40 mL) and the combined organic layers were dried over anhydrous Na_2SO_4, filtered and evaporated. The residue was purified by column chromatography on silica gel, with gradient elution EtOAc–hexane (10–50%), to give product **22** (0.27 g) as a yellow foam, yield: 59%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.96 (d, J = 8.8 Hz, 2H), 7.84 (d, J = 8.2 Hz, 1H), 7.68–7.27 (m, 12H), 6.87 (d, J = 8.8 Hz, 4H), 6.21 (d, J = 6.2 Hz, 1H), 5.67–5.62 (m, 1H), 5.50–5.58 (m, 1H), 5.45 (d, J = 8.2 Hz, 1H), 4.25 (d, J = 2.6 Hz, 1H), 3.81 (s, 6H), 3.53–3.51

(m, 2H), 2.10 (s, 3H), 2.07 (s, 3H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 170.0, 169.9, 168.7, 162.0, 159.0, 149.7, 144.0, 139.7, 135.3, 135.1, 134.9, 131.6, 130.8, 130.4, 129.3, 128.4, 128.3, 127.6, 113.6, 103.1, 87.9, 86.1, 82.4, 73.4, 71.5, 62.8, 55.5, 20.8, 20.7; HRMS (ESI) *m/z* calculated for $C_{34}H_{35}N_2O_{10}$ $(M+H)^+$ 631.22862, found 631.22851, $C_{41}H_{38}N_2O_{11}Na$ $(M+Na)^+$ 757.23678, found 757.23653.

4.2. Bio-evaluation of nucleosides on cancer cell lines

Seven cell lines representing three different types of cancer (leukemia, colorectal and brain) were used for the bio-evaluation of the nucleoside derivatives. HL-60, K562 and Jurkat cells represent leukemic cell lines; HT-29 and Caco-2 cells originate from colorectal carcinomas, while U373 and Hs683 are glioblastoma cell lines. HL-60, K562, Jurkat, HT-29, Caco-2 and HS683 cell lines originated from the American Type Culture Collection (ATCC) Manassas, VA. The U373 cell line was purchased from the European Collection of Cell Culture (ECACC), Salisbury, UK.

4.2.1. Maintenance of cell lines

The HL-60, K562 and Jurkat leukemic cell lines were maintained in RPMI-1640 medium (Sigma–Aldrich, St. Louis, USA) supplemented with 10% (*v*/*v*) heat inactivated fetal bovine serum (FBS) obtained from Gibco-BRL. The colorectal cancer cell lines, HT-29 and Caco-2 as well as HS683 were maintained in Dulbecco's Modified Eagles Medium (DMEM) (Sigma–Aldrich, St. Louis, USA) supplemented with 5–10% (*v*/*v*) FBS. U373 cells were maintained in Earles's Modified Eagles medium supplemented with 10% FBS and 1 mM sodium pyruvate.

All cells were grown in 75 cm^3 tissue culture flasks at 37 °C in a humidified incubator with a 5% CO_2 atmosphere using standard aseptic culture techniques. The HL-60, K562 and Jurkat cell lines were grown in suspension and sub-cultured when the cells reached a density of 1.5×10^6/mL. Cell densities were reduced to 0.5×10^6/mL and the growth media replaced. The adherent cell lines, HT-29, Caco-2, U373 and Hs683 were sub-cultured when the cells were confluent using a Trypsin-versene solution and reseeded into the same culture flasks. Prior to cell viability studies, cells were harvested and their viability status confirmed by the trypan blue assay.

4.2.2. Assessment of cell viability using the MTT assay

The MTT assay was used to evaluate the effects of the nucleosides on cell survival. The assay used was essentially that of Mosman et al.[66] with the only major modification being the solubilization of the formazan crystals with 100 μL per well of DMSO. The colorectal and leukemic cell lines were subjected to an initial screening procedure by exposing them for 24 h to a concentration of 100 μM of the nucleoside derivatives. In this case, camptothecin, the topoisomerase I inhibitor and parent drug of irinotecan, was used as a positive control, since it is a known inducer of apoptosis and an inhibitor of cell proliferation of both leukemic and colorectal cancer cell lines.[67,68]

The evaluation of the test nucleosides on the glioma cell lines were carried out over a period of 72 h since they are known to respond slower to most potential anti-cancer agents. It was presumed that these compounds would show a significant effect and therefore the cells were exposed to a range of test concentrations (0.01–100 μM) in order to calculate the 50% growth inhibitory concentration (GI_{50}) value which is an indication of potency. Narciclasine, a plant growth regulator that has been previously demonstrated by Kiss and co-workers, to be proapoptotic to cancer cells at concentrations greater than 1 μM, was used as a positive control in the glioma cell lines.[56] In addition, the group observed that narciclasine displayed potent anti-proliferative activity, independent of the tumor type and independent of whether the cells were of human (GI_{50} range of 5–99 nM) or rodent origin (GI_{50} range of 28–35 nM).[57]

Using the same methodology to assess cell viability, freshly isolated human peripheral leucocytes (white blood cells) were also exposed to the test nucleosides and camptothecin for 24 h. This was done to obtain an indication of relative toxicity to normal non-cancerous cells. Permission to isolate white blood cells from human volunteers was obtained from the University of the Witwatersrand Human Ethics Committee clearance no: M070519.

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