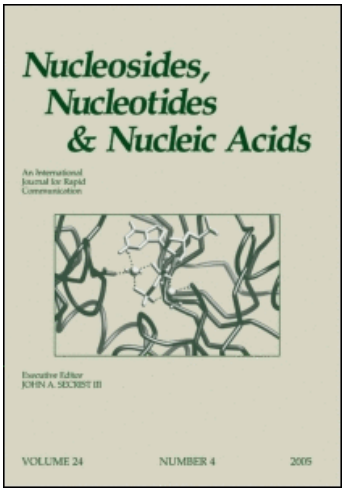

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SYNTHESIS OF 5-METHYLAMINO-2′-DEOXYURIDINE DERIVATIVES

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ABSTRACT

Reductive amination of 3′,5′-O-(tetraisopropyldisilyloxane-1,3-diyl)-2′-deoxy-5-formyluridine with several aliphatic and aromatic amines, in various solvents, is described. In the case of aliphatic amines, the expected C-5 substituted methylamino pyrimidine nucleosides are formed along with by-products deriving from opening of the pyrimidine ring. Relative amounts of the by-products depend upon the polarity of the solvent employed.

INTRODUCTION

Pyrimidine nucleoside analogues, variously modified at C-5 of the base moiety, have great potential as drugs for treatment of viral diseases and cancer[1–9]. 5-(2-Chloroethyl)- and (2-fluoroethyl)-2′-deoxyuridine, for an example, interfere with herpes simplex virus type 1 (HSV 1) and varicella zoster virus (VZV) replication[10,11] whereas a number of 5-methylamino derivatives have been found to be potent inhibitors of thymidylate synthetase[12] (TS) and thymidine kinase[13]. Incorporation of such compounds may also cause favourable variations of physico-chemical and/or biological properties in

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synthetic oligonucleotides[14–18], consequently improving their performance as antisense or antigene agents[19–21]. Particularly, substitution of 5-(propyn-1-yl)-2′-deoxyuridine for thymidine leads to DNA fragments characterized by an increased stability of both double and triple helix structures, when compared with their natural counterparts[19,21]. A further possibility to exploit C-5 position of pyrimidines to obtain new compounds with selected biological properties is offered by the conjugation with intercalating or metal complexing molecules capable of cleaving abasic sites in DNA[22].

It follows from the above that there is a great deal of interest in new C-5 modified pyrimidine nucleosides, thus justifying the considerable efforts so far fulfilled, aimed at developing new synthetic routes towards compounds of this type.

In this paper, we report the synthesis and structural characterization of a number of C-5 substituted methylamino pyrimidine nucleosides, prepared by reductive amination of 5-formyl-3′,5′-O-(tetraisopropylsilyloxy)-2′-deoxyuridine with a set of primary amines in various experimental conditions. This reaction is useful to obtain new derivatives which may turn out to be of biological interest on their own or as intermediates for the construction of other C-5 conjugated derivatives. A competitive opening of the pyrimidine ring, promoted by $NaBH_3CN$ in polar solvents, is also described.

RESULTS AND DISCUSSION

The first step of this work was to select a practical synthetic procedure to secure a large amount of the synthone, namely 3′,5′-O-(tetraisopropyldisiloxane-1,3-diyl)-5-formyl-2′-deoxyuridine (**3**, fdU scheme). Among the several routes reported in the literature, photochemical oxidation of thymidine[23] was ruled out, due to the disadvantage of not being suitable for a scale up of the process. Oxidation with MnO_2[24] was also judged not convenient, since it requires a preliminary preparation of 5-hydroxymethyl-2′-deoxyurine, followed by a further synthetic step. Finally, oxidation of thymidine by peroxodisulphate ion, as reported by Itahara and coworkers[25,26] was adopted with minor modifications, which led to formation of fdU in good yields. A nucleoside protected at the sugar moiety was used since, in some cases, the product of the following reductive amination was to be used for further modification. For this purpose, the tetraisopropyldisilyloxane group appeared to be the most suitable. Oxidation of the 5-methyl group of the protected nucleoside **2** with sodium peroxodisulfate in phosphate buffer at pH 7.0 gave 3′,5′-O-(tetraisopropyldisilyloxane-1,3-diyl)-2′-deoxy-5-formyluridine **3** (62% yield) along with the product of partial oxidation, namely, 3′,5′-O-(tetraisopropildisilyloxane-1,3-diyl)-2′-deoxy-5-hydroxymethyluridine (23% yield).

Reductive amination of aldehyde **3** was initially carried out following the procedure described by Mattson and coworkers[27], which required titanium (IV) isopropoxide [$Ti(O\text{-}Pr^i)_4$] as a mild Lewis acid catalyst. According to this methodology, however, the solvent and the reducing agent must be added only after 1 h, to allow an intermediate titanium/aldehyde/amine adduct to form. Since in our cases mostly solid amines (b–f, scheme) were to be used, these were dissolved in the solvent along with aldehyde **3** and $Ti(O\text{-}Pr^i)_4$. After 1 h, sodium cyanoborohydride was added. We have evaluated the influence of the catalyst performing the reactions in the absence of $Ti(O\text{-}Pr^i)_4$. In any case, we did not observe such differences in the yields and rates between the reactions performed with or without $Ti(O\text{-}Pr^i)_4$ to justify the use of the catalyst. Similarly, the direct addition of $NaBH_3CN$ to the initial solution did not seem to significantly affect the outcome of the reaction and, therefore, the one pot procedure described in the experimental section was adopted.

The above reaction, performed on amines a–d, afforded, besides the expected product of reductive amination, a by-product derived from opening of the pyrimidine ring (**5a–f**). The amount of by-product was dependent upon the polarity of the starting amine as well as that of the solvent employed, as summarized in the Table. Particularly, it could be observed that, for a given amine, yields of the target product (**4a–d**) decreased by increasing the polarity of the solvent. On the other hand, solvent being equal, the higher the number of hydroxyl groups on the amine, the lower the yields of **4a–d**. Consequently, when 3′,5′-O-(tetraisopropyldisilyloxane-1,3-diyl)-2′-deoxy-5-formyluridine (**3**) was reacted with 2-amino-1,3-propanediol (c) or R-(+)-1-amino-2,3-propanediol (d) in anydrous DMF, only products deriving from opening of the pyrimidine ring (**5c** and **5d**, respectively) could be detected. Interestingly, no by-products were observed in the reductive amination with the two aromatic amines, *p*-toluidine (e) and 1,4-diaminobenzene (f), in any solvent. It is also noteworthy that the opening of the pyrimidine ring, most likely involving a nucleophilic attack to C-6, did not occur in the absence of $NaBH_3CN$.

Table. Reaction Yields of Compound **4** and **5** for Amines a–f in Several Solvents

	Yield of **4** (%)			Yield of **5** (%)		
Amine	CH_2Cl_2	THF	DMF	CH_2Cl_2	THF	DMF
$NH_2(CH_2)_3CH_3$ (a)	88	70	50	< 5	10	35
$NH_2(CH_2)_5OH$ (b)	85	60	8	10	35	42
$NH_2CH(CH_2OH)_2$ (c)	76	45	0	16	41	70
$NH_2CH_2CH(OH)CH_2OH$ (d)	78	49	0	20	40	82
$NH_2C_6H_4NH_2$ (e)	99	86	90	0	0	0
$NH_2C_6H_4CH_3$ (f)	91	72	85	0	0	0

In conclusion, we have described a reductive amination of 3′,5′-O-(tetra isopropyldisilyloxane-1,3-diyl)-2′-deoxy-5-formyluridine, as a convenient synthetic approach to obtain C-5 substituted methylamino pyrimidine nucleosides. Particularly, we have shown that, when an aliphatic amine is employed, the target molecule is always accompanied by a side product, derived from opening of pyrimidine ring. By-product formation can be minimized by the usage of dry CH_2Cl_2 as a solvent. Furthermore, we have found that, in this case, addition of $Ti(O\text{-}Pr^i)_4$, a catalyst commonly used according to Mattson's procedure, does not improve either reaction rate or yields of the target product. The obtained products, particularly those containing an aromatic moiety, will be tested for their potential activities against TS.

EXPERIMENTAL

General Methods

^1H- and ^{13}C-NMR spectra were recorded in $CDCl_3$ on a Bruker WM 500 spectrometer. Residual proton and carbon signals of the solvent ($CDCl_3$ $\delta = 7.24$ and 77.5, respectively) were used as internal references. NMR signals were assigned to the pertinent nuclei through two-dimensional ^1H-^1H and ^1H-^{13}C COSY experiments. TLC was performed on silica gel plates 20×20 cm, 0.25 mm (MERCK). Mass spectra were registered by a Finnigan MAT instrument. UV spectra were recorded on a Jasco V-530 spectrophotometer. $[\alpha]_D^{25^\circ}$ values were measured by a Perkin-Elmer 243 B polarimeter. General reagents and solvents were purchased from Sigma-Aldrich-Fluka.

3′,5′-O-(Tetraisopropyldisiloxane-1,3-diyl)-2′-deoxythymidine (**2**)

10 g (40 mmol) of 2′-deoxythymidine (**1**) and 12.5 g of imidazole (182 mmol) were co-evaporated in anhydrous DMF and then dissolved in 60 ml of the same solvent. To the ice-cooled solution, 12 mL of 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (44 mmol) in dry CH_2Cl_2 (8 mL) were added dropwise, in 20 min under stirring. After 4 h, the reaction was quenched with CH_3OH and the solvent evaporated *in vacuo*. The residue, dissolved in $CHCl_3$ (300 mL), was washed with a 1M aqueous sodium bicarbonate solution (3×0.5 L). The organic phase was dried over anhydrous sodium sulphate, filtered and evaporated *in vacuo*. After repeated additions and evaporations of toluene (3×1 L) *in vacuo*, 49.9 g of **2** were obtained and identified by comparison of spectral data with those reported in the literature[28].

3′,5′-O-(Tetraisopropyldisiloxane-1,3-diyl)-5-formyl-2′-deoxyuridine (**3**)

Compound 2 (10.0 g, 20 mmol) was dissolved in CH_3CN (125 mL) and, then, 7.4 g (70 mmol) of 2,6-lutidine and 25 mL of an aqueous solution of

$K_2S_2O_8$ (10.8 g, 40 mmol) and $CuSO_4 \times 5H_2O$ (2.0 g, 8 mmol) were added. The mixture was kept at 65 °C for 4.5 h under stirring, cooled and filtered and the resulting solution dried *in vacuo*. The crude material was dissolved in ethyl acetate (0.5 L), washed with H_2O (3×1 L) and then with an aqueous solution of EDTA (2×1 L). The organic phase was dried over anhydrous sodium sulphate, filtered and evaporated *in vacuo*. The product was purified on a silica gel column (800 g 300×4 cm) eluted with increasing concentrations of CH_3OH in $CHCl_3$ (0 to 2%). Fractions eluted with 2% CH_3OH in $CHCl_3$ gave pure **3** (6.2 g, 62% yield). ^{1}H NMR δ 9.97 (1H, s, CHO); 8.5 (1H, s, H-6); 6.0 (1H, dd, H-1′); 4.43 (1H, m, H-3′); 4.1 (2H, m, H-5′a and H-5′b); 3,82 (1H, m, H-4′) 2.55 (1H, m, H-2′a); 2.30 (1H, m, H-2′b); 1.15-0.9 [28H, $4CH(CH_3)_2$]. ^{13}C NMR δ 187.5 (CHO); 163.0 (C-4); 151.2 (C-2); 149.8 (C-6); 112.5 (C-5); 89.8 (C-4′); 89.3 (C-1′); 70.22 (C-3′); 62.5 (C-5′); 42.5 (C-2′); 13.7-12.8 [$4\underline{C}H(CH_3)_2$]; 17.7-17.2 [$4CH(\underline{C}H_3)_2$]. MS ESI (+) = 499. UV ($CHCl_3$) $\lambda_{max} = 290$ nm ($\varepsilon = 14000$). $[\alpha]_D^{25°}$ ($CHCl_3$) = −48.6.

General Procedure for Reductive Amination

Compound **3** (1 g, 2.0 mmol), dried by addition and evaporation of dry CH_2Cl_2 *in vacuo* (×3), was dissolved in the same solvent (150 mL) and 40 mmol of amine (a–f) and 0.5 g of $NaBH_3CN$ (1.02 mmol) were added to the solution, under stirring. After 24 h, silica gel TLC in *n*-hexane/ethyl acetate 1:1 (v/v) showed the complete consumption of **3**. The mixture was washed with H_2O (3×0.5 L), dried over anhydrous sodium sulphate, filtered and evaporated *in vacuo*, thus giving a solid residue which was chromatographed as specified below. The same conditions of reaction, analytical TLC and purification were applied when reductive amination of product **3** was performed in dry DMF or dry THF. The yields of the reactions are summarised in the Table.

3′,5′-O-(Tetraisopropyldisiloxane-1,3-diyl)-5-[(N-butyl)aminomethyl]-2′-deoxyuridine (**4a**)

The crude product from reductive amination of **3** with amine **a** was analysed by TLC ($CHCl_3/CH_3OH$ 85:15 v/v), which showed the presence of two spots at R_f 0.5 (**4a**) and R_f 0.75 (**5a**), and chromatographed on a silica gel column (200 g, 150×2.5 cm), eluted with increasing amounts (from 0 to 15%) of CH_3OH in $CHCl_3$. Fractions eluted with 4% CH_3OH in $CHCl_3$, gave **5a** (0.045 g, 4%) while fractions eluted with 12% CH_3OH in $CHCl_3$, gave **4a** (0.98 g, 88%). **4a**: ^{1}H NMR δ 7.55 (1H, s, H-6); 6.05 (1H, m, H-1′); 4.50 (1H, m, H-3′); 4.07 (2H, m, H-5′a and H-5′b); 3.75 (1H, m, H-4′); 3.50 [2H, m, $C\underline{H}_2NH(CH_2)_3CH_3$]; 2.60 [2H, m, $CH_2NHC\underline{H}_2(CH_2)_2CH_3$]; 2.45 (1H, m,

H-2′a); 2.35 (1H, m, H-2′b); 1.50 (2H, m, $NHCH_2C\underline{H}_2CH_2CH_3$); 1.35 (2H, m, $NH(CH_2)_2C\underline{H}_2CH_3$); 1.10-0.90 [28H, $4CH(CH_3)_2$]; 0.85 [3H, t, $CH_2NH(CH_2)_3C\underline{H}_3$]; ^{13}C NMR δ 163.6 (C-4); 150.2 (C-2); 137.5 (C-6); 112.3 (C-5); 85.3 (C-4′); 84.3 (C-1′); 68.9 (C-3′); 61.1 (C-5′); 49.1 [$\underline{C}H_2NH(CH_2)_3CH_3$]; 46.7 [$CH_2NH\underline{C}H_2(CH_2)_2CH_3$]; 40.0 (C-2′); 32.0 ($CH_2NHCH_2\underline{C}H_2CH_2CH_3$); 20.7 [$CH_2NH(CH_2)_2\underline{C}H_2CH_3$]; 17.77-17.1 [$4CH(\underline{C}H_3)_2$]; 14.2 [$CH_2NH(CH_2)_3\underline{C}H_3$]; 13.6-12.7 [$4\underline{C}H(CH_3)_2$]. MS ESI(+) = 556. UV ($CHCl_3$) λ_{max} = 268 nm (ε = 22000). $[\alpha]_D^{25°}$ (CH_3OH) = −4.63; **5a:** ^{1}H NMR δ 11.0 (1H, s, NH-3); 9,96 (1H, m, NH-1); 8.96 (1H, s, CHO); 8.84 (1H, d, NH-7); 7.72 (1H, d, H-6); 5.82 (1H, m, H-1′), 4.02 (1H, dd, H-5′a); 3.80 (2H, m, H-5′b and H-4′); 4.44 [2H, m, $NHC\underline{H}_2(CH_2)_2CH_3$]; 2.33 (1H, m, H-2′a); 2.10 (1H, m, H-2′b); 1.62 (2H, m, $NHCH_2C\underline{H}_2CH_2CH_3$); 1.41 [2H, m, $NH(CH_2)_2C\underline{H}_2CH_3$]; 1.00 [3H, t, $NH(CH_2)_3C\underline{H}_3$]; 1.12-0.80 [28H, $4CH(CH_3)_2$]. ^{13}C NMR δ 183.9 (CHO); 159.7 (C-4); 146.3 (C-2); 144.5 (C-6); 122.4 (C-5); 85.3 (C-4′); 75.7 (C-1′); 68.9 (C-3′); 61.1 (C-5′); 50.1 [$NH\underline{C}H_2(CH_2)_2CH_3$]; 42.1 (C-2′); 31.9 ($NHCH_2\underline{C}H_2CH_2CH_3$); 22.3 [$NH(CH_2)_2\underline{C}H_2CH_3$]; 18.1-17.1 [8C, $4CH(\underline{C}H_3)_2$]; 14.0 [$NH(CH_2)_3\underline{C}H_3$]; 13.4-12.5 [4C, $4\underline{C}H(CH_3)_2$]; MS ESI (+) = 572.

3′,5′-O-(Tetraisopropyldisiloxane-1,3-diyl)-5-[N-(5-hydroxypentyl)aminomethyl]-2′-deoxyuridine (**4b**)

The crude product from reductive amination of **3** with amine b was analysed by TLC ($CHCl_3/CH_3OH$ 8:2 v/v), which showed the presence of two spots at R_f 0.4 (**4b**) and R_f 0.6 (**5b**), and chromatographed on a silica gel column (200 g, 150 × 2.5 cm), eluted with increasing amounts (from 0 to 25%) of CH_3OH in $CHCl_3$. Fractions eluted with 10% of CH_3OH gave **5b** (0.12 g, 10%) while fractions eluted with 12% of CH_3OH gave **4b** (1.0 g, 88%). **4b:** ^{1}H NMR δ 7.55 (1H, s, H-6); 6.02 (1H, m, H-1′); 4.45 (1H, m, H-3′); 4.00 (2H, m, H-5′a and H-5′b); 3.75 (3H, m, H-4′); 3.62 [2H, t, $CH_2NHCH_2(CH_2)_3C\underline{H}_2OH$]; 3.5 [2H, dd, $C\underline{H}_2NHCH_2(CH_2)_3CH_2OH$] 2.60 [2H, m, $CH_2NHC\underline{H}_2(CH_2)_3CH_2OH$]; 2.45 (1H, m, H-2′a); 2.25 (1H, m, H-2′b); 1.55 (4H, m, $CH_2NHCH_2C\underline{H}_2CH_2C\underline{H}_2CH_2OH$); 1.40 [2H, m, $CH_2NHCH_2CH_2C\underline{H}_2CH_2CH_2OH$]; 1.10-0.80 [28H, $4CH(CH_3)_2$]; ^{13}C NMR δ 163.6 (C-4); 149.8 (C-2); 137.4 (C-6); 111.6 (C-5); 85.0 (C-4′); 84.0 (C-1′); 68.4 (C-3′); 62.5 [$CH_2NHCH_2(CH_2)_3\underline{C}H_2OH$]; 60.7 (C-5′); 48.8 [$\underline{C}H_2NHCH_2(CH_2)_3CH_2OH$]; 46.5 [$CH_2NH\underline{C}H_2(CH_2)_3CH_2OH$]; 39.7 (C-2′); 32.2 [$CH_2NHCH_2(CH_2)_2\underline{C}H_2CH_2OH$]; 29.0 [$CH_2NHCH_2\underline{C}H_2(CH_2)_2CH_2OH$]; 23.2 ($CH_2NHCH_2CH_2\underline{C}H_2CH_2CH_2OH$); 17.4-13.4 [$4CH(\underline{C}H_3)_2$]; 13.4-12.4 [$4\underline{C}H(CH_3)_2$]; MS ESI(+) = 586; UV ($CHCl_3$) = λ_{max} = 268 nm (ε = 28000); $[\alpha]_D^{25°}$ (CH_3OH) = −8.27; **5b:** ^{1}H NMR δ 11.0 (1H, s, NH-3); 10.0 (1H, m, NH-7); 8.98 (1H, s, CHO); 8.84 (1H, d, NH-1); 7.30 (1H, d, H-6); 5.80 (1H, m, H-1′), 4.48 (1H, m, H-3′); 4.02 (2H, m, H-5′a and H-5′b); 3.80 (1H, m, H-4′); 3.65 [2H, t, $NHCH_2(CH_2)_3C\underline{H}_2OH$]; 3.40 [2H, m,

NHC$\underline{H}_2$$(CH_2)_3CH_2OH$]; 2.30 (1H, m, H-2′a); 2.10 (1H, m, H-2′b); 1.55 (4H, m, $NHCH_2C\underline{H}_2CH_2C\underline{H}_2CH_2OH$); 1.40 (2H, m, $NHCH_2CH_2\underline{H}_2CH_2CH_2OH$); 1.03-0.80 [28H, $4CH(CH_3)_2$]; ^{13}C NMR δ 189.3 (CHO); 162.1(C-4); 149.9 (C-2); 145.8 (C-6); 125.6 (C-5); 84.5 (C-4′); 73.6 (C-1′); 68.9 (C-3′); 61.0 [C-5′ and $NHCH_2(CH_2)_3\underline{C}H_2OH$]; 44.5 [$NH\underline{C}H_2(CH_2)_3CH_2OH$]; 39.6 (C-2′); 33.2 [$NHCH_2(CH_2)_2\underline{C}H_2CH_2OH$]; 29.0 [$NHCH_2\underline{C}H_2(CH_2)_2CH_2OH$]; 23.5 ($NHCH_2CH_2\underline{C}H_2CH_2CH_2OH$); 17.4-13.4 [$4CH(\underline{C}H_3)_2$]; 13.4-12.4 [$4\underline{C}H(CH_3)_2$]; MS ESI(+) = 602.

3′,5′-O-(Tetraisopropyldisiloxane-1,3-diyl)-5-[N-(1,3-dihydroxy-1-propyl)-aminomethyl]-2′-deoxyuridine (**4c**)

The crude product from reductive amination of **3** with amine c was analysed by TLC ($CHCl_3/CH_3OH$ 75:25 v/v), which showed the presence of two spots at R_f 0.45 (**4c**) and R_f 0.70 (**5c**), and chromatographed on a silica gel column (200 g, 150 × 2.5 cm), eluted with increasing amounts (from 0 to 30%) of CH_3OH in $CHCl_3$. Fractions eluted with 15% CH_3OH in $CHCl_3$ gave **5c** (0.18 g, 16%) while fractions eluted with 30% CH_3OH in $CHCl_3$, gave **4c** (0.87 g, 76%). **4c:** ^{1}H NMR δ 7.61 (1H, s, H-6); 6.02 (1H, m, H-1′); 4.45 (1H, m, H-3′); 4.08 [4H, m, $CH_2NHCH(C\underline{H}_2OH)_2$]; 3.65 (3H, m, H-4′, H-5′a and H-5′b); 3.58 [2H, m, $C\underline{H}_2NHCH(CH_2OH)_2$]; 2.75 [1H, m, $CH_2NHC\underline{H}$ $(CH_2OH)_2$]; 2.55 (1H, m, H-2′a); 2.35 (1H, m, H-2′b); 1.10-0.90 [28H, $4CH(CH_3)_2$]; ^{13}C NMR δ 164.3 (C-4); 150.1(C-2); 137.5 (C-6); 111.6 (C-5); 85.0 (C-4′); 84.1 (C-1′); 68.0 (C-3′); 61.4 [$CH_2NH\underline{C}H(CH_2OH)_2$]; 62.0 [$CH_2NHCH(\underline{C}H_2OH)_2$]; 62.4 (C-5′); 45.0 [$\underline{C}H_2NHCH(CH_2OH)_2$]; 39.7 (C-2′); 17.3-6.9 [$4CH(\underline{C}H_3)_2$]; 13.3-12.3 [$4\underline{C}H(CH_3)_2$]; MS ESI(+) = 574; UV ($CHCl_3$) λ_{max} = 266 nm (ε = 11000); $[\alpha]_D^{25°}$ (CH_3OH) = − 6.80; **5c:** ^{1}H NMR δ 11.0 (1H, s, NH-3); 10.2 (1H, m, NH-7); 8.85 (1H, s, CHO); 8.84 [1H, d, $N\underline{H}CH(CH_2OH)_2$]; 7.50 (1H, d, H-6); 5.78 (1H, m, H-1′), 4.48 (1H, m, H-3′); 4.02 (2H, m, H-5′b and H-4′); 3.75 (1H, m, H-5′a); 3.90 [4H, m, $NHCH(C\underline{H}_2OH)_2$]; 3.55 [1H, m, $NHC\underline{H}$ $(CH_2OH)_2$] 2.25 (1H, m, H-2′a); 2.05 (1H, m, H-2′b); 1.03-0.80 [28H, $4CH(CH_3)_2$]; ^{13}C NMR δ 184.6 (CHO); 160.5 (C-4); 145.9 (C-2); 143.8 (C-6); 122.6 (C-5); 84.7 (C-4′); 76.3 (C-1′); 68.9 (C-3′); 61.0 (C-5′); 60.6 [$NHCH(\underline{C}H_2OH)_2$]; 48.7 [$NH\underline{C}H(CH_2OH)_2$]; 43.1 (C-2′); 13.9 − 12.6 [$4CH(CH_3)_2$]; MS ESI(+) = 590.

3′,5′-O-(Tetraisopropyldisiloxane-1,3-diyl)-5-[N-(2R,3-dihydroxy-1-propyl)-aminomethyl]-2′-deoxyuridine (**4d**)

The crude product from reductive amination of **3** with amine d was analysed by TLC ($CHCl_3/CH_3OH$ 75:25 v/v), which showed the presence of two spots at R_f 0.55 (**4d**) and R_f 0.75 (**5d**), and chromatographed on a silica

gel column (200 g, 150 × 2.5 cm), eluted with increasing amounts (from 0 to 35%) of CH_3OH in $CHCl_3$. Fractions eluted with 15% CH_3OH in $CHCl_3$, gave **5d** (0.24 g, 20%) while fractions eluted with 35% of CH_3OH gave **4d** (0.89 g, 78%). **4d:** ^{1}H NMR δ 7.58 (1H, s, H-6); 6.08 (1H, dd, H-1′); 4.46 (1H, m, H-3′); 4.05 (2H, m, H-5′a and H-5′b); 3.75 (1H, m, H-4′); 3.71-3.40 [5H, m, C$\underline{H}_2$NHCH$_2$C$\underline{H}$(OH)C$\underline{H}_2$OH]; 2.76 [2H, m, CH$_2$NHC$\underline{H}_2$CH(OH)-CH$_2$OH]; 2.45 (1H, m, H-2′a); 2.28 (1H, m, H-2′b); 1.10-0.90 [28H, 4CH(CH$_3$)$_2$]; ^{13}C NMR δ 164.9 (C-4); 150.7 (C-2); 139.2 (C-6); 109.8 (C-5); 85.5 (C-4′); 84.6 (C-1′); 69.6 (C-3′); 69.0 [CH$_2$NHCH$_2$C$\underline{H}$(OH)CH$_2$OH]; 65.2 [CH$_2$NHCH$_2$CH(OH)C$\underline{H}_2$OH]; 61.2 (C-5′); 51.0 [CH$_2$NHC$\underline{H}_2$CH(OH)-CH$_2$OH]; 46.3 [C$\underline{H}_2$NHCH$_2$CH(OH)CH$_2$OH]; 40.0 (C-2′); 17.3-6.9 [4CH(C$\underline{H}_3$)$_2$]; 13.3-12.3 [4C$\underline{H}$(CH$_3$)$_2$]; MS ESI(+) = 574; UV ($CHCl_3$) λ_{max} = 266 nm (ε = 24000); $[\alpha]_D^{25^\circ}$ (CH_3OH) = −3.72; **5d:** ^{1}H NMR δ 11.0 (1H, s, NH-3); 10.2 (1H, m, NH-7); 9.0 (1H, s, CHO); 8.84 (1H, d, NH-1); 7.78 (1H, d, H-6); 5.75 (1H, m, H-1′); 4.60 (1H, m, H-3′); 4.48 [1H, m, NHCH$_2$C$\underline{H}$(OH)CH$_2$OH]; 4.03 (1H, dd, H-5′b); 3.90 (1H, dd, H-5′a); 3.85 (1H, m, H-4′); 3.72 [2H, m, NHCH$_2$CH(OH)C$\underline{H}_2$OH]; 3.60 [2H, m, NHC$\underline{H}_2$CH(OH)CH$_2$OH]; 2.35 (1H, m, H-2′a); 2.20 (1H, m, H-2′b); 1.03-0.80 [28H, 4CH(CH$_3$)$_2$]; ^{13}C NMR δ 185.8 (CHO); 163.4 (C-4); 147.6 (C-2); 145.8 (C-6); 123.4 (C-5); 84.9 (C-4′); 75.3 (C-1′); 69.8 (C-3′); 69.3 [NHCH$_2$C$\underline{H}$(OH)CH$_2$OH]; 67.2 [NHCH$_2$CH(OH)C$\underline{H}_2$OH]; 62.1 (C-5′); 48.3 [NHC$\underline{H}_2$CH(OH)CH$_2$OH]; 41.5 (C-2′); 17.3-6.9 [4CH(C$\underline{H}_3$)$_2$]; 13.3-12.3 [4C$\underline{H}$(CH$_3$)$_2$]; MS ESI(+) = 590.

3′,5′-O-(Tetraisopropyldisiloxane-1,3-diyl)-5-[N-(4-methylphenyl)aminomethyl]-2′-deoxyuridine (**4e**)

The crude product from reductive amination of **3** with amine e was analysed by TLC ($CHCl_3$/CH_3OH 7:3 v/v), which showed the presence of a single spot at R_f 0.65 (**4e**), and chromatographed on a silica gel column (200 g, 150 × 2.5 cm), eluted with increasing amounts (from 0 to 35%) of CH_3OH in $CHCl_3$. Fractions eluted with 30% CH_3OH in $CHCl_3$, gave **4e** (1.17 g, 99%). **4e:** ^{1}H NMR δ 7.48 (1H, s, H-6); 6.98 (2H, d, H-2 tolyl and H-6 tolyl); 6.50 (2H, d, H-3 tolyl and H-5 tolyl); 6.02 (1H, dd, H-1′); 4.45 (1H, dd, H-3′); 4.03 (4H, m, H-5′a, H-5′b and C$\underline{H}_2$NHC$_6$H$_4$CH$_3$); 3.70 (1H, m, H-4′); 2.45 (1H, m, H-2′a); 2.20 (3H, s, CH_3 tolyl); 2.15 (1H, m, H-2′b); 1.08-0.90 [28H, 4CH(CH$_3$)$_2$]; ^{13}C NMR δ 163.0 (C-4); 149.6 (C-2); 145.0 (C-1 tolyl); 136.7 (C-6); 129.6 (C-2 tolyl and C-6 tolyl); 127.3 (C-4 tolyl); 113.5 (C-3 tolyl and C-5 tolyl); 111.5 (C-5); 84.8 (C-4′); 83.7 (C-1′); 68.7 (C-3′); 60.9 (C-5′); 41.5 (C$\underline{H}_2$NHC$_6$H$_4$CH$_3$); 39.6 (C-2′); 20.7 (CH_3 tolyl); 17.3 − 16.9 [4CH(C$\underline{H}_3$)$_2$]; 13.3-12.3 [4C$\underline{H}$(CH$_3$)$_2$]; MS ESI(+) = 590; UV ($CHCl_3$) λ_{max} = 254 nm (ε = 19000); $[\alpha]_D^{25^\circ}$ (CH_3OH) = −9.12.

3′,5′-O-(Tetraisopropyldisiloxane-1,3-diyl)-5-[N-(**4**-aminophenyl)aminomethyl]-2′-deoxyuridine (**4f**)

The crude product from reductive amination of **3** with amine f was analysed by TLC ($CHCl_3/CH_3OH$ 7:3 v/v), which showed the presence of a single spot at R_f 0.45 (**4f**), and chromatographed on a silica gel column (200 g, 150 × 2.5 cm), eluted with increasing amounts (from 0 to 35%) of CH_3OH in $CHCl_3$. Fractions eluted with 35% CH_3OH in $CHCl_3$, gave **4f** (1.07 g, 91%). **4f:** ^{1}H NMR δ 7.44 (1H, s, H-6); 6.55 (2H, d, H-2 aminophenyl and H-6 aminophenyl); 6.45 (2H, d, H-3 aminophenyl and H-5 aminophenyl); 6.00 (1H, dd, H-1′); 4.45 (1H, dd, H-3′); 3.98 (4H, m, H-5′a, H-5′b and, $C\underline{H}_2NHC_6H_4NH_2$); 3.72 (1H, m, H-4′); 2.42 (1H, m, H-2′a); 2.13 (1H, m, H-2′b); 1.08-0.90 [28H, $4CH(CH_3)_2$]; ^{13}C NMR δ 163.7 (C-4); 150.3 (C-2); 140.5 (C-1 aminophenyl); 137.1 (C-6); 117.0 (C-2 aminophenyl and C-6 aminophenyl); 138.6 (C-4 aminophenyl); 115.7 (C-3 aminophenyl and C-5 aminophenyl); 112.1 (C-5); 85.2 (C-4′); 84.1 (C-1′); 69.0 (C-3′); 61.2 (C-5′); 42.8 ($\underline{C}H_2NHC_6H_4NH_2$); 40.0 (C-2′); 17.7-17.1 [$4CH(\underline{C}H_3)_2$]; 13.7-12.7 [$4\underline{C}H(CH_3)_2$]; MS ESI(+) = 591; UV ($CHCl_3$) λ_{max} = 258 nm (ε = 25000); $[\alpha]_D^{25^\circ}$ (CH_3OH) = −14.8.

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