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SYNTHESIS OF 5-METHYLAMINO-2'-DEOXYURIDINE DERIVATIVES

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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 synthetase¹² (TS) and thymidine kinase¹³. 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 DNA²².

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 NaBH₃CN 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 thymidine²³ 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 product of partial oxidation, namely, 3',5'-O-(tetrawith the isopropildisilyloxane-1,3-diyl)-2'-deoxy-5-hydroxymethyluridine (23% yield).

5-METHYLAMINO-2'-DEOXYURIDINE DERIVATIVES

Reductive amination of aldehyde **3** was initially carried out following the procedure described by Mattson and coworkers²⁷, which required titanium (IV) isopropoxide [Ti(O-Prⁱ)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-Prⁱ)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-Prⁱ)₄. In any case, we did not observe such differences in the yields and rates between the reactions performed with or without Ti(O-Prⁱ)₄ to justify the use of the catalyst. Similarly, the direct addition of NaBH₃CN 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-5formyluridine (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 NaBH₃CN.

	Yield of 4 (%)			Yield of 5 (%)		
Amine	CH ₂ Cl ₂	THF	DMF	CH ₂ Cl 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

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

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 CH₂Cl₂ as a solvent. Furthermore, we have found that, in this case, addition of Ti(O-Prⁱ)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 ¹³C-NMR spectra were recorded in CDCl₃ on a Bruker WM 500 spectrometer. Residual proton and carbon signals of the solvent (CDCl₃ $\delta = 7.24$ and 77.5, respectively) were used as internal references. NMR signals were assigned to the pertinent nuclei through two-dimensional ¹H-¹H and ¹H-¹³C 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 spectro-photometer. $[\alpha]_D^{25^\circ}$ 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 CH₂Cl₂ (8 mL) were added dropwise, in 20 min under stirring. After 4 h, the reaction was quenched with CH₃OH and the solvent evaporated *invacuo*. The residue, dissolved in CHCl₃ (300 mL), was washed with a 1M aqueous sodium bicarbonate solution $(3 \times 0.5 \text{ L})$. The organic phase was dried over anhydrous sodium sulphate, filtered and evaporated *invacuo*. After repeated additions and evaporations of toluene $(3 \times 1 \text{ L})$ *in vacuo*, 49.9 g of **2** were obtained and identified by comparison of spectral data with those reported in the literature²⁸.

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

K₂S₂O₈ (10.8 g, 40 mmol) and CuSO₄ × 5H₂O (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 H₂O (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 CH₃OH in CHCl₃ (0 to 2%). Fractions eluted with 2% CH₃OH in CHCl₃ gave pure **3** (6.2 g, 62% yield). ¹H 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(CH₃)₂]. ¹³C 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<u>C</u>H(CH₃)₂]; 17.7-17.2 [4CH(<u>C</u>H₃)₂]. MS ESI (+) = 499. UV (CHCl₃) $\lambda_{max} = 290$ nm (ε = 14000). [α]_D^{25°} (CHCl₃) = -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 NaBH₃CN (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 H₂O (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 (CHCl₃/CH₃OH 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 CH₃OH in CHCl₃. Fractions eluted with 4% CH₃OH in CHCl₃, gave **5a** (0.045 g, 4%) while fractions eluted with 12% CH₃OH in CHCl₃, gave **4a** (0.98 g, 88%). **4a**: ¹H 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, CH₂NH(CH₂)₃CH₃]; 2.60 [2H, m, CH₂NHCH₂(CH₂)₂CH₃]; 2.45 (1H, m,

H-2'a); 2.35 (1H, m, H-2'b); 1.50 (2H, m, NHCH₂CH₂CH₂CH₃); 1.35 (2H, m, NH(CH₂)₂CH₂CH₃); 1.10-0.90 [28H, 4CH(CH₃)₂]; 0.85 [3H, t, CH₂NH (CH₂)₃CH₃]; ¹³C 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 [CH₂NH(CH₂)₃CH₃]; 46.7 [CH₂NH<u>C</u>H₂(CH₂)₂CH₃]; 40.0 (C-2'); 32.0 (CH₂NHCH₂<u>C</u>H₂CH₂CH₂CH₃); 20.7 $[CH_2NH(CH_2)_2CH_2CH_3];$ 17.77-17.1 $[4CH(CH_3)_2];$ 14.2 $[CH_2NH(CH_2)_3];$ <u>CH</u>₃]; 13.6-12.7 [4<u>C</u>H(CH₃)₂]. MS ESI(+) = 556. UV (CHCl₃) $\lambda_{\text{max}} = 268 \text{ nm}$ $(\epsilon = 22000)$. $[\alpha]_{D}^{25}(CH_{3}OH) = -4.63$; **5a**: ¹H 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, NHCH₂(CH₂)₂CH₃]; 2.33 (1H, m, H-2'a); 2.10 (1H, m, H-2'b); 1.62 (2H, m, NHCH₂CH₂CH₂CH₃); 1.41 [2H, m, NH(CH₂)₂CH₂CH₃]; 1.00 [3H, t, NH(CH₂)₃C<u>H₃]</u>; 1.12-0.80 [28H, 4CH(CH₃)₂]. ¹³C 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 [NHCH₂(CH₂)₂CH₃]; 42.1 (C-2'); 31.9 (NHCH₂CH₂CH₂ CH₃); 22.3 [NH(CH₂)₂CH₂CH₃]; 18.1-17.1 [8C, 4CH(CH₃)₂]; 14.0 [NH(CH₂)₃ <u>CH</u>₃]; 13.4-12.5 [4C, 4<u>C</u>H(CH₃)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 (CHCl₃/CH₃OH 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 CH₃OH in CHCl₃. Fractions eluted with 10% of CH₃OH gave **5b** (0.12 g, 10%) while fractions eluted with 12% of CH₃OH gave 4b (1.0 g, 88%). **4b:** ¹H 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, CH₂NHCH₂(CH₂)₃CH₂OH]; 3.5 [2H, dd, CH₂NHCH₂(CH₂)₃CH₂OH] 2.60 [2H, m, CH₂NHCH₂(CH₂)₃CH₂OH]; 2.45 (1H, m, H-2'a); 2.25 (1H, m, H-2'b); 1.55 (4H, m, CH₂NHCH₂C<u>H₂CH₂CH₂CH₂OH)</u>; 1.40 [2H, m, CH₂NHCH₂CH₂CH₂CH₂CH₂OH]; 1.10-0.80 [28H, 4CH(CH₃)₂]; ¹³C 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 $[CH_2NHCH_2(CH_2)_3CH_2OH];$ 60.7 (C-5'); 48.8 [<u>CH</u>₂NHCH₂(CH₂)₃CH₂OH]; 46.5 [CH₂NH<u>C</u>H₂(CH₂)₃CH₂OH]; 39.7 (C-2'); $[CH_2NHCH_2(CH_2)_2CH_2CH_2OH];$ 29.0 $[CH_2NHCH_2CH_2(CH_2)_2]$ 32.2 CH₂OH]; 23.2 (CH₂NHCH₂CH₂CH₂CH₂CH₂OH); 17.4-13.4 [4CH(<u>C</u>H₃)₂]; 13.4-12.4 [4CH(CH₃)₂]; MS ESI(+) = 586; UV (CHCl₃) = λ_{max} = 268 nm $(\epsilon = 28000); \ [\alpha]_D^{25^{\circ}}(CH_3OH) = -8.27; \ 5b: \ ^1H \ NMR \ \delta \ 11.0 \ (1H, s, NH-3);$ 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, NHCH₂(CH₂)₃CH₂OH]; 3.40 [2H, m,

NHC<u>H</u>₂(CH₂)₃CH₂OH]; 2.30 (1H, m, H-2'a); 2.10 (1H, m, H-2'b); 1.55 (4H, m, NHCH₂C<u>H</u>₂CH₂CH₂CH₂CH₂OH); 1.40 (2H, m, NHCH₂CH₂<u>H</u>₂CH₂CH₂CH₂OH); 1.03-0.80 [28H, 4CH(CH₃)₂]; ¹³C 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 NHCH₂(CH₂)₃<u>C</u>H₂OH]; 44.5 [NH<u>C</u>H₂(CH₂)₃CH₂OH]; 39.6 (C-2'); 33.2 [NHCH₂(CH₂)₂<u>C</u>H₂CH₂OH]; 29.0 [NHCH₂<u>C</u>H₂(CH₂)₂CH₂OH]; 23.5 (NHCH₂CH₂<u>C</u>H₂CH₂CH₂OH); 17.4-13.4 [4CH(<u>C</u>H₃)₂]; 13.4-12.4 [4<u>C</u>H (CH₃)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 (CHCl₃/CH₃OH 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 CH₃OH in CHCl₃. Fractions eluted with 15% CH₃OH in CHCl₃ gave 5c (0.18 g, 16%) while fractions eluted with 30% CH₃OH in CHCl₃, gave 4c (0.87 g, 76%). 4c: ¹H NMR δ 7.61 (1H, s, H-6); 6.02 (1H, m, H-1'); 4.45 (1H, m, H-3'); 4.08 [4H, m, CH₂NHCH(CH₂OH)₂]; 3.65 (3H, m, H-4', H-5'a and H-5'b); 3.58 [2H, m, CH₂NHCH(CH₂OH)₂]; 2.75 [1H, m, CH₂NHCH (CH₂OH)₂]; 2.55 (1H, m, H-2'a); 2.35 (1H, m, H-2'b); 1.10-0.90 [28H, 4CH(CH₃)₂]; ¹³C 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 [CH₂NHCH(CH₂OH)₂]; 62.0 [CH₂NHCH(CH₂OH)₂]; 62.4 (C-5'); 45.0 [CH₂NHCH(CH₂OH)₂]; 39.7 (C-2'); 17.3-6.9 [4CH(\underline{CH}_3)₂]; 13.3-12.3 [4 \underline{C} H(CH₃)₂]; MS ESI(+) = 574; UV $(CHCl_3) \lambda_{max} = 266 \text{ nm} (\epsilon = 11000); [\alpha]_D^{25^{\circ}}(CH_3OH) = -6.80; 5c: {}^{1}H \text{ NMR }\delta$ 11.0 (1H, s, NH-3); 10.2 (1H, m, NH-7); 8.85 (1H, s, CHO); 8.84 [1H, d, NHCH(CH₂OH)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(CH₂OH)₂]; 3.55 [1H, m, NHCH (CH₂OH)₂] 2.25 (1H, m, H-2'a); 2.05 (1H, m, H-2'b); 1.03-0.80 [28H, 4CH(CH₃)₂]; ¹³C 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(CH₂OH)₂]; 48.7 [NHCH(CH₂OH)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 (CHCl₃/CH₃OH 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 CH₃OH in CHCl₃. Fractions eluted with 15% CH₃OH in CHCl₃, gave 5d (0.24 g, 20%) while fractions eluted with 35% of CH₃OH gave 4d (0.89 g, 78%). **4d:** ¹H 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, $CH_2NHCH_2CH(OH)CH_2OH$; 2.76 [2H, m, $CH_2NHCH_2CH(OH)$ -CH₂OH]; 2.45 (1H, m, H-2'a); 2.28 (1H, m, H-2'b); 1.10-0.90 [28H, 4CH(CH₃)₂]; ¹³C 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 [CH₂NHCH₂CH(OH)CH₂OH]; 65.2 [CH₂NHCH₂CH(OH)CH₂OH]; 61.2 (C-5'); 51.0 [CH₂NHCH₂CH(OH)-CH₂OH]; 46.3 [CH₂NHCH₂CH(OH)CH₂OH]; 40.0 (C-2'); 17.3-6.9 $[4CH(CH_3)_2]; 13.3-12.3 [4CH(CH_3)_2]; MS ESI(+) = 574; UV (CHCl_3)$ $\lambda_{\text{max}} = 266 \text{ nm} \ (\epsilon = 24000); \ [\alpha]_{\text{D}}^{25^{\circ}}(\text{CH}_{3}\text{OH}) = -3.72; \ \text{5d:} \ ^{1}\text{H} \ \text{NMR} \ \delta \ 11.0$ (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, NHCH₂C<u>H</u>(OH)CH₂OH]; 4.03 (1H, dd, H-5'b); 3.90 (1H, dd, H-5'a); 3.85 (1H, m, H-4'); 3.72 [2H, m, NHCH₂CH(OH)CH₂OH]; 3.60 [2H, m, NHCH₂CH(OH)CH₂OH]; 2.35 (1H, m, H-2'a); 2.20 (1H, m, H-2'b); 1.03-0.80 [28H, 4CH(CH₃)₂]; ¹³C 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 [NHCH₂CH(OH)CH₂OH]; 67.2 [NHCH₂CH(OH)CH₂OH]; 62.1 (C-5'); 48.3 [NHCH₂CH(OH)CH₂OH]; 41.5 (C-2'); 17.3-6.9 [4CH(CH₃)₂]; 13.3-12.3 $[4CH(CH_3)_2];$ 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 (CHCl₃/CH₃OH 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 CH₃OH in CHCl₃. Fractions eluted with 30% CH₃OH in CHCl₃, gave **4e** (1.17 g, 99%). **4e:** ¹H 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 CH₂NHC₆H₄CH₃); 3.70 (1H, m, H-4'); 2.45 (1H, m, H-2'a); 2.20 (3H, s, CH₃ tolyl); 2.15 (1H, m, H-2'b); 1.08-0.90 [28H, 4CH(CH₃)₂]; ¹³C 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 (<u>C</u>H₂NHC₆H₄CH₃); 39.6 (C-2'); 20.7 (CH₃ tolyl); 17.3 – 16.9 [4CH(<u>C</u>H₃)₂]; 13.3-12.3 [4<u>C</u>H(CH₃)₂]; MS ESI(+) = 590; UV (CHCl₃) $\lambda_{max} = 254$ nm ($\epsilon = 19000$; $[\alpha]_{25^{\circ}}^{25^{\circ}}$ (CH₃OH) = -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 (CHCl₃/CH₃OH 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 $(200 \text{ g}, 150 \times 2.5 \text{ cm})$, eluted with increasing amounts (from 0 to 35%) of CH₃OH in CHCl₃. Fractions eluted with 35% CH₃OH in CHCl₃, gave 4f (1.07 g, 91%). 4f: ¹H 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, CH₂NHC₆H₄NH₂); 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(CH₃)₂]; ¹³C 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 (<u>CH₂NHC₆H₄NH₂);</u> 40.0 (C-2'); 17.7-17.1 [4CH(<u>CH₃)₂];</u> 13.7-12.7 $[4\underline{C}H(CH_3)_2]$; MS ESI(+) = 591; UV (CHCl₃) $\lambda_{max} = 258 \text{ nm}$ ($\varepsilon = 25000$); $[\alpha]_{D}^{25^{\circ}}(CH_{3}OH) = -14.8.$

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