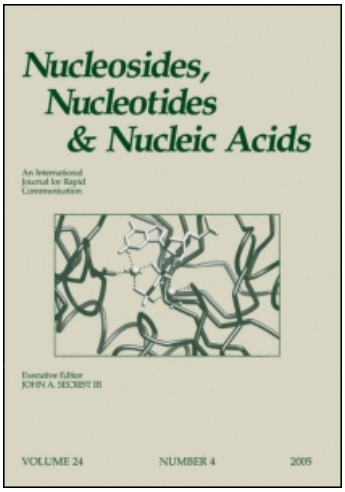

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SYNTHESIS OF 5-ALKENYLATED D4T ANALOGUES VIA THE Pd-CATALYZED CROSS-COUPLING REACTION

A. Ciurea,[1] C. Fossey,[1] S. Benzaria,[1] D. Gavriliu,[1] Z. Delbederi,[1] B. Lelong,[1] D. Ladurée,[1,*] A. M. Aubertin,[2] and A. Kirn[2]

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ABSTRACT

The target compounds 5-[N-(6-amino-hexyl)-acrylamide]-2′,3′-didehydro-2′,3′-dideoxy-uridine **(12)** and 5-{N-[5-(methoxycarbonyl)-pentyl]-acrylamide}-2′,3′-didehydro-2′,3′-dideoxy-uridine **(15)** were prepared by the palladium acetate-triphenylphosphine-catalyzed reaction of the 5′-O-acetyl-5-iodo-d4T analogue **(3)**. These compounds **12** and **15** can be used to prepare nucleotide probes carrying fluorescent labels and were nevertheless screened for their anti-HIV activity. The biological data demonstrated that none of them were active against HIV-1.

INTRODUCTION

In connection with our program on d4T analogues, we wanted to prepare chain-terminating fluorescence-tagged substrates for DNA polymerases[1,2]. Furthermore, among the derivatives of uridine, the more attractive analogues contain a reporter group at the C-5 position of the pyrimidine ring. This position is not involved in hydrogen bonding and is

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exposed into the major groove of DNA double-helix. This allows significant steric tolerance, and makes the C-5 position ideal for attachment of any reporter group with little effect on hybridization.

Pyrimidine nucleosides substituted at the C-5 position constitute a class of biologically significant molecules[3]. Especially, C-5 alkenyl and alkynyl substituents have shown various antiviral activities[4,5]. Thus, the introduction of unsaturated substituents at C-5 position of pyrimidine derivatives has become a considerable interest. In particular, the (*E*)-5-(2-bromovinyl)-2′-deoxyuridine (BVDU) is a highly potent and selective antiherpes agent, which inhibits herpes simplex virus type 1 (HSV-1)[6].

C-5 position of pyrimidine derivatives has become a considerable interest. In particular, the (*E*)-5-(2-bromovinyl)-2′-deoxyuridine (BVDU) is a highly potent and selective antiherpes agent, which inhibits herpes simplex virus type 1 (HSV-1)[6].

One critical feature of these substrates is the ''linker'' or group which covalently attaches the fluorescent moiety to the nucleotide substrate without interfering with enzymatic processing of the molecule. Although several linkers are known which meet these requirements[7], no general method is currently available for attaching fluorescent dyes or other reporters to all of the nucleotides found in DNA.

The choice of the attachment site of the tether on the C-5 position in the pyrimidine ring was made due to the low interference with base-pairing in DNA. Moreover, after 5'-phosphorylation, the C-5-modified triphosphate dideoxynucleosides are likely to be incorporated efficiently into DNA by polymerases. This suggested that there is an adequate steric clearance on the C-5 position in DNA duplex, and that such analogues did not significantly disrupt the normal base-pairing and helical conformation[8,9].

Nucleoside kinases play a pivotal role in the use of nucleosides for cancer and antiviral therapy. The binding of the kinases to nucleotides was weak when the extension arms contained four or fewer methylene groups. However when the tether increased to 10 Å by interposing additional methylene groups, 6 to 8, there was a substantial increase in the strength of enzyme binding[10].

Therefore, we now report the preparation of highly modified d4T analogues bearing a novel long chain 5-{N-[6-(trifluoroacetyl-amino)-hexyl]-, 5-[N-(6-amino-hexyl)-, 5-{N-[6-(*tert*-butoxycarbonyl)-hexyl]- and 5-{N-[5-(methoxycarbonyl)-pentyl]-acrylamide}.

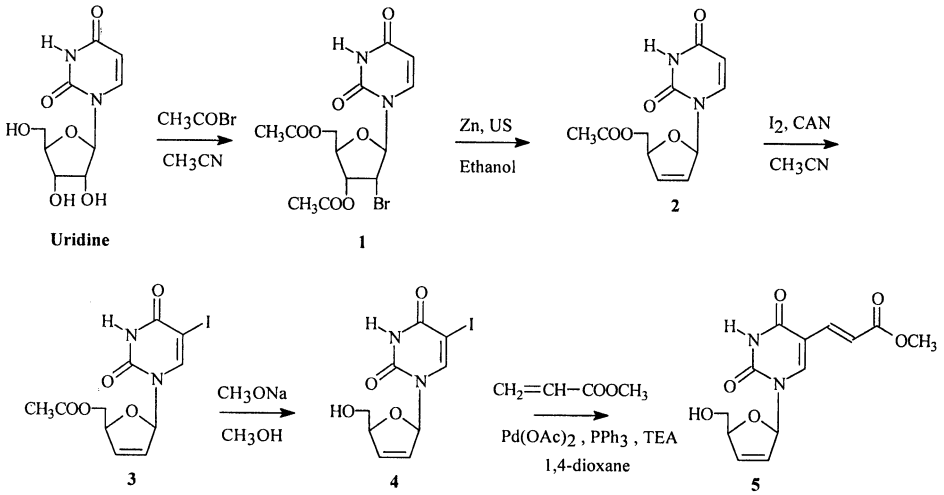
RESULTS AND DISCUSSION

Chemical

The synthetic method for the modified d4T analogues containing reactive functionalities tethered to the C-5 position involved the formation of

the key intermediate, the 5-iodo-2′,3′-didehydro-2′,3′-dideoxy-uridine **(4)**. Johsi *et al.* had previously reported that the parent 5-iodo-2′-deoxyuridine could readily be converted into the target 5-iodo-2′,3′-didehydro-2′,3′-dideoxy-uridine by a six-step procedure *via* the intermediate 2′-deoxy-3′-(phenylseleno) derivative in 25% overall yield[11]. However, the number of steps of this procedure and the 5-iodo-2′-deoxyuridine used as starting material had prompted us to investigate a more convenient and relatively efficient alternative procedure for the conversion of commercially available uridine which is relatively not expensive into the 5-iodo-unsaturated nucleoside **4**.

Also, we have developed an alternative four steps sequence starting from commercially available uridine leading to the versatile precursor **4** and our experiments were carried as follows. To introduce the olefinic bond between the C-2′ and C-3′ position of uridine, the uridine was first subjected to a bromoacetylation reaction using acetyl bromide in dry acetonitrile to provide the bromoacetate **(1)** in 95% yield (Scheme 1)[12]. The reductive β-elimination of the acetoxy-bromo nucleoside **(1)** proceeded by adding zinc dust in anhydrous ethanol to afford the olefinic nucleoside **(2)** in 44% yield after chromatography[13]. Halogenation of **2** to its 5-iodo counterpart **(3)** was further effected using elemental iodine and CAN at 60 °C in 64 yield[14]. This method has several advantages including readily available reagents and mild reaction conditions by comparison to a variety of methods which had been developed in the literature for electrophilic halogenation at the electron-rich C-5 position of uracil nucleosides. Kumar *et al.* had described a mild and efficient procedure for the C-5 iodination of



Scheme 1. Synthesis of 2′,3′-didehydro-2′3′-dideoxy-5-[2-(methoxycarbonyl)-vinyl]-uridine (**5**).

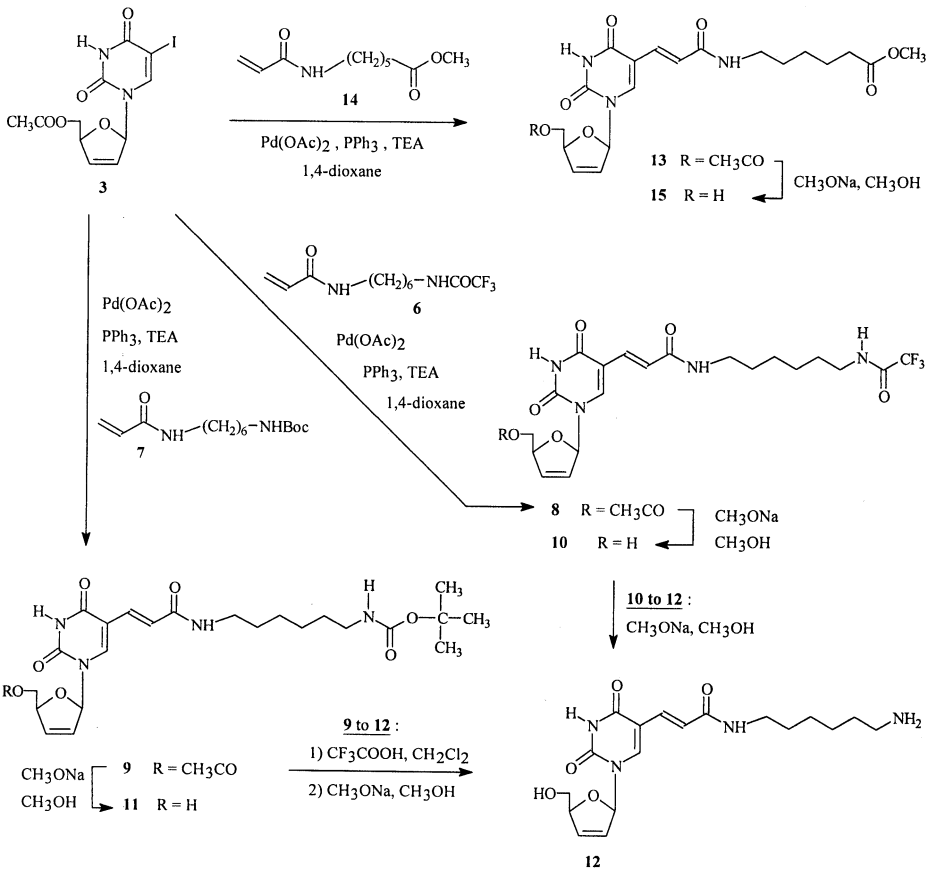
2′-deoxyuridine, uridine and arabinouridine using 2.5 mol equiv. of iodine monochloride in acetonitrile at 25 °C[15]. Recently, Lin *et al.* had originally reported the C-5 iodination of pyrimidine nucleosides with silver trifluoroacetate and iodine in dry dioxane[16]. For our part, this later method was too expensive and moreover the introduction of the 2′,3′ olefinic bond on the 5-iodouridine had failed and only the 5-iodouracil was isolated.

Finally, removal of the 5′-O-acetyl protecting group of **3** was performed using sodium methoxide in methanol at room temperature to give the expected 5-iodo-2′,3′-didehydro-2′,3′-dideoxy-nucleoside **(4)** in 91% yield after silica-gel column chromatography.

Using modified *Heck Reaction*, the C-5 modified target compounds **5, 8, 9** and **13** were then synthesized from a palladium catalyzed coupling of respectively methyl acrylate or conveniently protected acrylamides with the 5′-O-acetyl-5-iodo d4U intermediate **(3)** or **(4)** (the deblocked product of **3**)[17]. Thus, reaction of 5-iodo derivative **(4)** with methyl acrylate in anhydrous and deoxygenated dioxane in the presence of palladium(II)acetate, triphenylphosphine, and triethylamine afforded the 5-(carbomethoxyvinyl)-derivative **(5)** in 44% yield after silica-gel chromatography (Scheme 1).

In order to obtain the target compounds **8, 9** and **13**, we synthesized in a first step the two acrylamides **(6)** and **(7)** (Scheme 3)[18,19]. Reaction of 1,6-diaminohexane with acryloyl chloride (0.5 equiv.), followed by N protection of the terminal amino group by a trifluoroacetyl group gave **(6)** in 11% isolated yield. Condensation of *tert*-butoxycarbonyl-1,6-diaminohexane hydrochloride with acryloyl chloride afforded the N-Boc acrylamide **(7)** in 92%.

Condensation of 5′-O-acetyl-5-iodo-d4U **(3)** with the N-trifluoroacetyl acrylamide **(6)** (2 equiv. with regard to the iodo nucleoside) in anhydrous and deoxygenated dioxane in the presence of palladium(II)acetate, triphenylphosphine, and triethylamine gave as major product the expected C-5 substituted d4U **(8)** isolated in 38% yield after purification accomplished by chromatography (Scheme 2). Using N-Boc acrylamide **(7)**, we similarly obtained compound **9** in 32% yield. The *E* stereochemistry for the nucleosides **8** and **9** was indicated by the large vinylic coupling constants ($J = 15.5$ Hz and 15.3 Hz respectively for the olefin protons) measured at 400 MHz resolution. Selective 5′-O-deacylation of **8** and **9** with sodium methoxide in methanol respectively at 40 °C for 1 hour and at room temperature for 1 hour afforded respectively the target compounds comprising the 5-{N-[6-(trifluoroacetyl-amino)-hexyl]-acrylamide}-group (**10**) (13%) and the 5-{N-[6-(*tert*-butoxycarbonyl)-hexyl]-acrylamide}-group **(11)** (62%). The key intermediate **10** had its amino group deprotected with sodium methoxide in methanol to give the corresponding compound **12** in 54% yield isolated after silica-gel chromatography. At the end, removal of the N-Boc group of **9** with TFA and subsequent 5′-O-deacylation with sodium methoxide in methanol provided the target compound

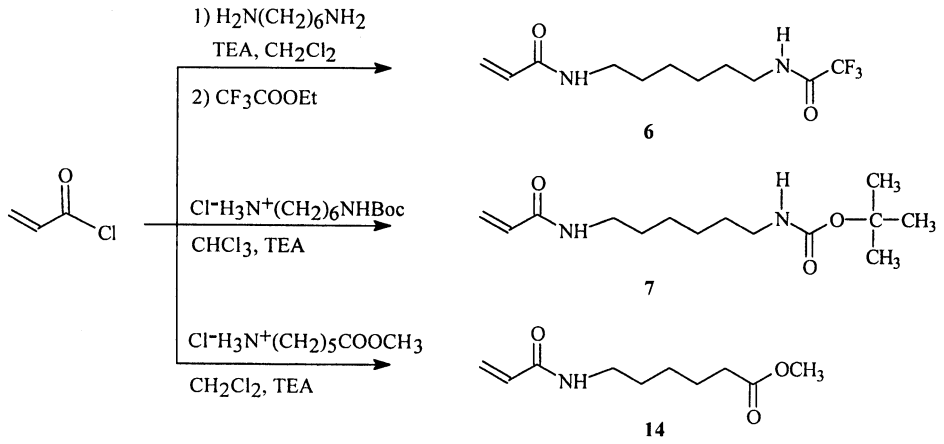


Scheme 2. Synthesis of 2′,3′-didehydro-2′3′-dideoxy-5-[N-(6-aminohexy)-acrylamide]-and 5-{N-[5-(methoxycarbonyl)-pentyl]acrylamide|-uridine (**12**) and (**15**).

5-[N-(6-amino-hexyl)-acrylamide]-d4U **(12)** in an overall yield of 41% after silica-gel chromatography.

In order to study the effect on activity/toxicity of the nature of the linkage, we extended this reaction to the preparation of the derivative **13** bearing a side chain including a terminal methyl ester group (Scheme 2). Likewise, condensation of 5-iodo derivative **3** with the methyl 6-(acryloyl-amino)hexanoate **(14)**[20] using $Pd(OAc)_2$ produced mainly the corresponding 5-{N-[5-(methoxycarbonyl)-pentyl]-acrylamide}-derivative **(13)** in 29% yield after purification by chromatography. Removal of the 5′-O-acetyl group was achieved using sodium methoxide in methanol as previously described for **10** (room temperature for 2 days) to give the target compound **15**.

The structure of the target compounds **10**, **11**, **12** and **15** was determined by analytical and spectroscopic data (see chemical procedures in experimental section).



Scheme 3. Synthesis of N-acrylol compounds (**6**), (**7**) and (**14**).

Biological

The newly synthesized 2′,3′-unsaturated-C-5-modified thymidine derivatives **10**, **11**, **12** and **15** were evaluated by comparison to AZT for inhibition of HIV-1 multiplication in cells of the lymphocytic lineage (CEM-SS and MT-4), and the results are summarized in Table 1. As shown in Table 1, the nucleosides **10**, **11**, **12** and **15** were unfortunately devoid of antiviral activity at non toxic concentration.

Table 1. Antiviral and Cytotoxicity Evaluation of β-D-d_4T Analogues Bearing Linker Arms **10**, **11**, **12** and **15**

	HIV-1_{LAI} in CEM-SS cells			HIV-1_{IIIB} in MT-4 cells		
Compd	IC50 (M)*	CC50 (M)†	SI‡	IC50 (M)	CC50 (M)	SI
AZT	$1.4\ 10^{-8}$			$9.8\ 10^{-9}$	$>10^{-6}$	>102
10	$5.6\ 10^{-4}$	$>10^{-4}$	>0.18	$>10^{-4}$	$>10^{-4}$	>1
11	$5\ 10^{-5}$	$>10^{-4}$	>2	$>10^{-4}$	$6.6\ 10^{-5}$	>0.66
12	$>10^{-4}$	$>10^{-4}$	>1	$>10^{-4}$	$>10^{-4}$	>1
15	$>10^{-4}$	$>10^{-4}$	>1	$>10^{-4}$	$>10^{-4}$	>1

*IC_{50} is the concentration required to inhibit HIV-1 multiplication by 50%.
†CC_{50} is the concentration of drug which causes 50% cytotoxicity to uninfected cells.
‡SI corresponds to the ratio CC_{50}/IC_{50}.
All data represent the mean values of three separate experiments ± SD.

EXPERIMENTAL SECTION

Chemical Procedures

Melting points were determined on a Kofler apparatus and are uncorrected. IR spectra were recorded on a Fourier transform Mattson spectrometer Genesis DTGS using WinFIRST^{TM} Macros and ApPro^{TM} and only noteworthy absorptions are listed. ^{1}H and ^{13}C NMR spectra were obtained on a JEOL Lambda 400 using TMS as an internal standard. NH and OH signals appeared as broad singlets exchangeable with D_2O (s = singlet, b = broad, d = doublet, t = triplet, q = quadruplet, m = multiplet). Thin layer chromatography (TLC) were performed on precoated silica gel 60 F_{254} sheets (0.2 mm layer, Macherey-Nagel), and compounds were detected by UV absorption at 254 nm. Column chromatography were effected by using Merck silica gel 60 (0.063−0.200 mm). All samples were kept in a drying oven at 50 °C over P_2O_5 for at least 24 hours prior to analysis. Reagent grade acetonitrile and dichloromethane were refluxed and distilled from phosphorus pentoxide. Anhydrous ethanol was prepared by using magnesium turnings. Anhydrous methanol p.a. was purchased from E. Merck. All coupling reactions were performed under an atmosphere of oxygen-free argon, and the solvent (or solution) was vigorously deoxygenated prior to addition of catalysts.

1-(3,5-di-O-acetyl-2-bromo-2-deoxy-β-D-ribofuranosyl)-uracil (1). Acetyl bromide (27.33 mL, 368.85 mmol, 6 equiv.) was added dropwise to a boiling suspension of uridine (15 g, 61.47 mmol) in anhydrous acetonitrile (250 mL) under an argon atmosphere. The reaction mixture was stirred at 80 °C for 30 min and then allowed to cool to room temperature. After evaporation to dryness under reduced pressure, the residue was dissolved in dichloromethane (400 mL) and the solution was washed with water (4 × 200 mL). The organic layer was separated, dried over magnesium sulphate and evaporated *in vacuo* to yield 23.77 g (95%) of **1** as white crystals: mp 70−74 °C; R_f 0.76 [EtOAc (100%)]; IR (KBr): 1749 (CO), 1696 (CO), 1459, 1379, 1229, 1044 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ (ppm) 2.06 (s, 3H, CH_3), 2.13 (s, 3H, CH_3), 4.22−4.33 (m, 3H, *H*-4′, *H*-5′), 4.99 (dd, J = 5.9 Hz, 7.3 Hz, 1H, *H*-2′), 5.23 (dd, J = 5.9 Hz, 3.6 Hz, 1H, *H*-3′), 5.76 (dd, J = 8.2 Hz, 2.1 Hz, 1H, *H*-5), 6.13 (d, J = 7.3 Hz, 1H, *H*-1′), 7.67 (d, J = 8.2 Hz, 1H, *H*-6), 11.54 (d, J = 2.1 Hz, 1H, *NH*); ^{13}C NMR (DMSO-d_6): δ (ppm) 20.5 (*C*H_3), 47.5 (*C*-2′), 62.8 (*C*-5′), 71.1 (*C*-3′), 79.6 (*C*-4′), 88.8 (*C*-1′), 102.9 (*C*-5), 140.0 (*C*-6), 150.5 (*C*-2), 162.8 (*C*-4), 169.3 (Me*C*OO), 170.1 (Me*C*OO).

1-(5-O-acetyl-2,3-dideoxy-β-D-*glycero*-pent-2-enofuranosyl)-uracil (2). The freshly activated zinc dust (2.49 g, 38.36 mmol, 3 equiv.) was added to a solution of **1** (5 g, 12.78 mmol) in anhydrous ethanol (100 mL) under an argon atmosphere. The heterogeneous reaction mixture was treated under

irradiation of 35 kHz ultrasound (150 W) for 30 min [the reaction was monitored by TLC in 100% ethyl acetate until no starting material remained], filtered on celite and the filtrate evaporated to dryness *in vacuo*. The oily residue was added with ethyl acetate (100 mL) and the precipitate (uracil) was collected by filtration. The filtrate was evaporated to dryness *in vacuo* and the residual yellow oil was chromatographed on a silica gel column (ethyl acetate). The fractions containing the desired product were pooled together, and the solvent was evaporated *in vacuo* to afford 1.42 g (44%) of **2** as white crystals: mp 80 °C; R_f 0.44 [EtOAc (100%)]; ^{1}H NMR (DMSO-d_6): δ (ppm) 2.00 (s, 3H, CH_3), 4.17 (d, J = 3.4 Hz, 2H, *H*-5′), 4.97 (s, 1H, *H*-4′), 5.66 (dd, J = 8.1 Hz, 2.1 Hz, 1H, *H*-5), 6.00 (d, J = 5.9 Hz, *H*-2′), 6.42 (d, J = 5.9 Hz, *H*-3′), 6.79 (s, 1H, *H*-1′), 7.44 (d, J = 8.1 Hz, 1H, *H*-6), 11.37 (d, J = 2.1 Hz, 1H, N*H*); ^{13}C NMR (DMSO-d_6): δ (ppm) 20.6 (*C*H_3), 64.5 (*C*-5′), 83.8 (*C*-4′), 89.4 (*C*-1′), 101.9 (*C*-5), 126.4 (*C*-2′), 133.9 (*C*-3′), 140.6 (*C*-6), 150.8 (*C*-2), 163.2 (*C*-4), 170.1 (Me*C*OO).

1-(5-*O*-acetyl-2,3-dideoxy-β-D-*glycero*-pent-2-enofuranosyl)-5-iodo-uracil (3). To a solution of dry 2 (0.62 g, 2.46 mmol) in anhydrous acetonitrile were added successively cerium ammonium nitrate (IV) (0.67 g, 1.23 mmol, 0.5 equiv.) and iodine (0.37 g, 1.47 mmol, 0.6 equiv.). The mixture was stirred at 60 °C for 2 h, then the solvent was evaporated to dryness *invacuo*. After addition of ethyl acetate (170 mL) to the residue, the solid product formed was removed by filtration. The filtrate was washed with a solution of 5% $NaHSO_3$ (2 × 100 mL) and water (2 × 100 mL). The organic layer was separated, dried over magnesium sulphate and evaporated *invacuo* to yield 0.6 g (64%) of **3** as a yellow product which is pure enough to be utilised in the further procedures. High purity sample for the analyses was obtained from purification by silica-gel column chromatography using ethyl acetate in dichloromethane (from 20% to 40%). Mp 167 °C; R_f 0.53 [CH_2Cl_2-EtOAc (50:50)]; IR (KBr): 1720 (CO), 1676 (CO), 1617, 1453, 1242, 1115, 1088, 1301, 787 cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ (ppm) 2.10 (s, 3H, CH_3), 4.13 (ddAB, J = 12.5 Hz, 2.3 Hz, 1H, *H*-5′), 4.30 (ddAB, J = 12.5 Hz, 3.1 Hz, 1H, *H*-5″), 5.01 (s, 1H, *H*-4′), 6.02 (d, J = 5.8 Hz, 1H, *H*-2′), 6.41 (d, J = 5.8 Hz, 1H, *H*-3′), 6.73 (s, 1H, *H*-1′), 7.73 (s, 1H, *H-6*), 11.80 (s, 1H, N*H*); ^{13}C-NMR (DMSO-d_6): δ (ppm) 21.0 (*C*H_3), 64.1 (*C*-5′), 69.7 (*C-5*), 83.9 (*C*-4′), 89.5 (*C*-1′), 126.4 (*C*-2′), 133.8 (*C*-3′), 144.3 (*C-6*), 150.2 (*C-2*), 160.2 (*C-4*), 169.9 (Me*C*OO).

1-(2,3-dideoxy-β-D-*glycero*-pent-2-enofuranosyl)-5-iodo-uracil (4). The protected nucleoside **3** (2.78 g, 7.35 mmol) was treated with a solution of sodium methoxide (0.397 g, 7.35 mmol, 1 equiv.) in anhydrous methanol (20 mL) at room temperature for 17 h. The reaction mixture was neutralized by addition of Amberlite IRN-77 (H^+) resin and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica-gel column chromatography using methanol in ethyl acetate (from 0% to

5%) as eluents to yield 2.25 g (91%) of **4** as yellow crystals: mp decomposed at 190 °C; R_f 0.51 [EtOAc (100%)]; ^{1}H-NMR (DMSO-d_6): δ (ppm) 3.60 (s, 2H, *H*-5′), 4.81 (s, 1H, *H*-4′), 5.12 (bs, 1H, *OH*), 5.91 (d, J = 5.9 Hz, 1H, *H*-2′), 6.39 (d, J = 5.9 Hz, 1H, *H*-3′), 6.76 (s, 1H, *H*-1′), 8.32 (s, 1H, *H-6*), 11.67 (s, 1H, N*H*).

The following is a general procedure for the coupling reactions of alkenyl amines with the 5-iodo nucleoside (**3**).

1-(2,3-dideoxy-β-D-*glycero*-pent-2-enofuranosyl)-5-[2-(methoxycarbonyl)-vinyl]-uracil (5). Palladium acetate (0.068 mmol, 15 mg, 0.05 equiv.), triphenylphosphine (0.137 mmol, 36 mg, 0.1 equiv.) and triethylamine (1.807 mmol, 0.25 mL, 1.32 equiv.) were combined in deoxygenated 1,4-dioxane (30 mL) and stirred at 60 °C under an argon atmosphere until a deep red coloration developed. Then **4** (1.37 mmol, 0.46 g) and methyl acrylate (2.738 mmol, 246 μL, 2 equiv.) were added, and the mixture was stirred at 80 °C for 7 h. The hot reaction mixture was filtered through celite, washed with hot 1,4-dioxane and the filtrate was evaporated under reduced pressure. The residue was purified by silica-gel column chromatography using ethyl acetate as eluent to yield 177 mg (44%) of **5**: R_f 0.45 [EtOAc (100%)]; ^{1}H-NMR (DMSO-d_6): δ (ppm) 3.65 (m, 5H, C*H*$_3$, *H*-5′), 4.83 (s, 1H, *H*-4′), 5.15 (bs, 1H, O*H*), 5.93 (d, J = 5.9 Hz, 1H, *H*-2′), 6.42 (d, J = 5.9 Hz, 1H, *H*-3′), 6.75 (dAB, J = 15.8 Hz, 1H, *CH*COOMe), 6.83 (s, 1H, *H*-1′), 7.26 (dAB, J = 15.8 Hz, 1H, C*H*=CHCOOMe), 8.38 (s, 1H, *H-6*), 11.68 (s, 1H, N*H*); ^{13}C-NMR (DMSO-d_6): δ (ppm) 51.3 (O*C*H$_3$), 61.9 (*C*-5′), 87.9 (*C*-4′), 89.6 (*C*-1′), 108.0 (*C-5*), 116.2 (*C*HCOOMe), 125.7 (*C*-2′), 135.4 (*C*-3′), 137.7 (H*C*=CHCOOMe), 144.6 (*C-6*), 149.6 (*C-2*), 161.8 (*C-4*), 167.0 (*C*OOMe).

N-acryloyl-N′-trifluoroacetyl-1,6-diaminohexane (6). A stirred solution of 1,6-diaminohexane (7 g, 60.3 mmol) in a mixture of dry dichloromethane (50 mL) and triethylamine (8.4 mL, 60.3 mmol, 1 equiv.) was cooled to 0 °C. Acryloyl chloride (2.4 mL, 30.1 mmol, 0.5 equiv.) was then added dropwise while the temperature was maintained at 0 °C. The reaction mixture was stirred at 0 °C for 20 min, then added dropwise with ethyl trifluoroacetate (18 mL, 150.8 mmol, 2.5 equiv.) and triethylamine (15.6 mL, 112.1 mmol, 1.86 equiv.) and allowed to stand at room temperature for an additional 3 days. The heterogeneous reaction mixture was filtered and the filtrate was evaporated to dryness *in vacuo*. The residue was triturated with ethyl acetate leaving an insoluble material which was collected by filtration. The organic filtrate was concentrated *in vacuo*, and the residue was purified by silica-gel column chromatography using ethyl acetate in cyclohexane (from 75% to 100%) as eluents to yield 1.84 g (11%) of **6**: mp 112 °C; R_f 0,60 [EtOAc (100%)]; ^{1}H-NMR (DMSO-d_6): δ (ppm) 1.25 (m, 4H, 2 × C*H*$_2$(CH$_2$)$_2$NHCO), 1.36–1.49 (m, 4H, 2 × C*H*$_2$CH$_2$NHCO),

3.09 (m, 2H, CH_2NHCOCH), 3.15 (t, J = 7 Hz, 2H, CH_2NHCOCF$_3$), 5.54 (dd, J = 10 Hz, 2 Hz, 1H, *Htrans*), 6.04 (ddAB, J = 17 Hz, 2 Hz, 1H, *Hcis*), 6.18 (qABX, J = 10 Hz, 17 Hz, 1H, =C*H*CONH), 8.05 (s, 1H, N*H*COCH), 9.40 (s, 1H, N*H*COCF$_3$); ^{13}C-NMR (DMSO-d$_6$): δ (ppm) 25.8 (*C*H$_2$(CH$_2$)$_2$NHCO), 26.0 (*C*H$_2$(CH$_2$)$_2$NHCO), 28.1 (*C*H$_2$CH$_2$NHCO), 28.9 (*C*H$_2$CH$_2$NHCO), 38.4 (*C*H$_2$NHCO), 39.4 (*C*H$_2$NHCO), 116.0 (q, J = 287 Hz, *C*F$_3$), 124.8 (=*C*H$_2$), 131.9 (=*C*HCONH), 156.1 (q, J = 36 Hz, *C*OCF$_3$), 164.4 (CH$_2$NH*C*OCH).

N-acryloyl-N'-(*tert*-butoxycarbonyl)-1,6-diaminohexane (7). To a solution of N-*tert*-butoxycarbonyl-1,6-diaminohexane hydrochloride (0.8 g, 3.16 mmol) in dry chloroform (50 mL) at 0 °C were added dropwise respectively triethylamine (0.88 mL, 6.32 mmol, 2 equiv.) and acryloyl chloride (0.27 mL, 306 mg, 1.07 equiv.). The reaction mixture was stirred at 0 °C for 20 min, allowed to warm to room temperature and stirred for an additional 1 h. The mixture was added with chloroform (130 mL) and washed with water (4 × 150 mL). The organic layer was separated, dried on anhydrous magnesium sulphate and evaporated under reduced pressure to yield 785 mg (92%) of **7** as white crystals: R_f 0.30 [EtOAc (100%)]; ^{1}H-NMR (DMSO-d$_6$): δ (ppm) 1.22 (m, 4H, 2 × C*H*$_2$(CH$_2$)$_2$NHCO), 1.35 (m, 13H, C(C*H*$_3$)$_3$, 2 × C*H*$_2$CH$_2$NHCO), 2.87 (m, 2H, C*H*$_2$NHBoc), 3.08 (m, 2H, C*H*$_2$NHCOCH), 5.54 (dd, J = 10 Hz, 2 Hz, 1H, *Htrans*), 6.04 (ddAB, J = 16.5 Hz, 2 Hz, 1H, *Hcis*), 6.18 (qABX, J = 10 Hz, 16.5 Hz, 1H, =C*H*), 6.77 (t, J = 5 Hz, 1H, N*H*Boc), 8.05 (t, J = 4 Hz, 1H, N*H*COCH); ^{13}C-NMR (DMSO-d$_6$): δ (ppm) 25.9 (*C*H$_2$(CH$_2$)$_2$NHCO), 26.1 (*C*H$_2$(CH$_2$)$_2$NHCO), 28.2 (C(*C*H$_3$)$_3$), 29.0 (*C*H$_2$CH$_2$NHCO), 29.4 (*C*H$_2$CH$_2$NHCO), 38.3 (*C*H$_2$NHCO), 39.8 (*C*H$_2$NHCO), 77.2 (*C*(CH$_3$)$_3$), 124.6 (=*C*H$_2$), 131.9 (=*C*H), 155.5 (*C*OOCMe$_3$), 164.3 (CH$_2$NHCOCH).

1-(5-*O*-acetyl-2,3-dideoxy-β-D-*glycero*-pent-2-enofuranosyl)-5-{N-[6-(trifluoroacetylamino)-hexyl]-acrylamide}-uracil (8). Compound **3** was converted to **8** by the same procedure as described for **5**, except that the reaction mixture was stirred at 80 °C for 14 h. The residue was purified by silica-gel column chromatography eluted with ethyl acetate in cyclohexane (from 50% to 100%) to yield 187 mg (38%) of **8**: R_f 0,26 [EtOAc (100%)]; ^{1}H-NMR (DMSO-d$_6$): δ (ppm) 1.25 [m, 4H, 2 × C*H*$_2$(CH$_2$)$_2$NH], 1.37–1.47 (m, 4H, 2 × C*H*$_2$CH$_2$NH), 1.97 (s, 3H, C*H*$_3$), 3.09 (m, 2H, C*H*$_2$NHCOCH), 3.15 (m, 2H, C*H*$_2$NHCOCF$_3$), 4.18 (dAB, J = 12.4 Hz, 1H, *H*-5′), 4.24 (ddAB, J = 12.4 Hz, 4.75 Hz, 1H, *H*-5″), 5.00 (s, 1H, *H*-4′), 6.02 (d, J = 6.22 Hz, 1H, *H*-2′), 6.43 (d, J = 6.22 Hz, 1H, *H*-3′), 6.81 (s, 1H, *H*-1′), 6.99 (dAB, J = 15.5 Hz, 1H, C*H*CONH), 7.13 (dAB, J = 15.5 Hz, 1H, C*H*=CHCONH), 7.75 (s, 1H, *H-6*), 8.07 (m, 1H, N*H*COCH), 9.39 (s, 1H, N*H*COCF$_3$), 11.65 (bs, 1H, *H*N-3); ^{13}C-NMR (DMSO-d$_6$): δ (ppm) 20.6 (*C*H$_3$COO), 25.9 (*C*H$_2$(CH$_2$)$_2$NH), 26.0 (*C*H$_2$(CH$_2$)$_2$NH), 28.1 (*C*H$_2$CH$_2$-

NH), 29.0 (CH_2CH_2NH), 38.5 (CH_2NH), 39.8 (CH_2NH), 64.7 (*C*-5′), 84.1 (*C*-4′), 89.9 (*C*-1′), 109.3 (*C*-5), 116.0 (q, J = 287 Hz, CF_3), 122.1 (CH=CHCONH), 126.1 (*C*-2′), 131.6 (CH=CHCONH), 134.0 (*C*-3′), 142.2 (*C*-6), 149.6 (*C*-2), 156.1 (q, J = 36 Hz, $COCF_3$), 161.8 (*C*-4), 165.3 (CHCONH), 170.1 (MeCOO).

1-(5-*O*-acetyl-2,3-dideoxy-β-D-*glycero*-pent-2-enofuranosyl)-5-{N-[6-(*tert*-butoxycarbonyl)-hexyl]-acrylamide}-uracil (9). Compound **3** was converted to **9** by the same procedure as described for **5**, except that the reaction mixture was stirred at 80 °C for 24 h. This product was purified by silica-gel chromatography using ethyl acetate in cyclohexane (from 50% to 100%) as eluents to yield 158 mg (32%) of **9**: R_f 0.18 [EtOAc (100%)]; ^{1}H-NMR (DMSO-d_6): δ (ppm) 1.22 (m, 4H, $2\times CH_2(CH_2)_2NH$), 1.35 (m, 13H, $C(CH_3)_3$, $2\times CH_2CH_2NH$), 1.97 (s, 3H, CH_3COO), 2.86 (m, 2H, CH_2NHBoc), 3.08 (m, 2H, $CH_2NHCOCH$), 4.18 (dAB, J = 12 Hz, 1H, *H*-5′), 4.24 (ddAB, J = 12 Hz, 2 Hz, 1H, *H*-5″), 5.00 (s, 1H, *H*-4′), 6.02 (d, J = 5.31 Hz, 1H, *H*-3′), 6.43 (d, J = 5.31 Hz, 1H, *H*-2′), 6.75 (t, J = 5.4 Hz, 1H, NHBoc), 6.80 (s, 1H, *H*-1′), 6.98 (dAB, J = 15.3 Hz, 1H, CHCONH), 7.12 (dAB, J = 15.3 Hz, 1H, CH=CHCONH), 7.75 (s, 1H, *H*-6), 8.05 (t, J = 5.2 Hz, 1H, NHCOCH), 11.63 (bs, 1H, *H*N-3); ^{13}C-NMR (DMSO-d_6): δ (ppm) 20.6 (CH_3COO), 26.0 ($CH_2(CH_2)_2NH$), 26.1 ($CH_2(CH_2)_2NH$), 28.2 ($C(CH_3)_3$), 29.1 (CH_2CH_2NH), 29.4 (CH_2CH_2NH), 38.5 (CH_2NH), 40.1 (CH_2NH), 64.7 (*C*-5′), 77.2 ($C(CH_3)_3$), 84.1 (*C*-4′), 89.9 (*C*-1′), 109.3 (*C*-5), 122.1 (CH=CHCONH), 126.1 (*C*-2′), 131.6 (CH=CHCONH), 134.0 (*C*-3′), 142.1 (*C*-6), 149.6 (*C*-2), 155.5 ($COOCMe_3$), 161.84(*C*-4), 165.2 (CHCONH), 170.1 (MeCOO).

1-(2,3-dideoxy-β-D-*glycero*-pent-2-enofuranosyl)-5-{N-[6-(trifluoroacetyl-amino)-hexyl]-acrylamide}-uracil (10). The 5-{N-[6-(trifluoroacetylamino)-hexyl]-acrylamide}-nucleoside (**8**) (77 mg, 0.149 mmol) was treated with a solution of sodium methoxide (9.6 mg, 0.179 mmol, 1.2 equiv.) in anhydrous methanol (5 mL). The reaction mixture was stirred at 40 °C for 1 h, neutralized by addition of Amberlite IRN-77 (H^+) resin and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica-gel column chromatography using methanol in ethyl acetate (from 0% to 5%) as eluents to yield 9 mg (13%) of **10**: R_f 0,24 [EtOAc-CH_3OH (95:5)]; ^{1}H-NMR (DMSO-d_6): δ (ppm) 1.24 (m, 4H, $2\times CH_2(CH_2)_2NH$), 1.38−1.47 (m, 4H, $2\times CH_2CH_2NH$), 3.08 (m, 2H, $CH_2NHCOCH$), 3.15 (m, 2H, $CH_2NHCOCF_3$), 3.64 (s, 2H, *H*-5′), 4.82 (s, 1H, *H*-4′), 5.10 (bs, 1H, O*H*), 5.93 (d, J = 5.6 Hz, 1H, *H*-2′), 6.41 (d, J = 5.6 Hz, 1H, *H*-3′), 6.83 (s, 1H, *H*-1′), 6.91 (dAB, J = 15.5 Hz, 1H, CH=CH), 6.98 (dAB, J = 15.5 Hz, 1H, CH=C*H*), 8.08 (m, 1H, NHCOCH), 8.21 (s, 1H, *H*-6), 9.42 (bs, 1H, $NHCOCF_3$), 10.88 (bs, 1H, *H*N-3); ^{13}C-NMR (DMSO-d_6): δ (ppm) 25.8 ($CH_2(CH_2)_2NH$), 26.0 ($CH_2(CH_2)_2NH$), 28.2 (CH_2CH_2NH), 29.2 (CH_2CH_2NH), 38.5 (CH_2NH),

39.8 (CH_2NH), 61.8 (*C*-5′), 87.7 (*C*-4′), 89.4 (*C*-1′), 108.8 (*C*-*5*), 116.0 (q, J = 287 Hz, CF_3), 121.5 (CH=*C*HCONH), 125.7 (*C*-2′), 131.6 (*C*H=CHCONH), 135.3 (*C*-3′), 142.9 (*C*-*6*), 149.7 (*C*-*2*), 156.1 (q, J = 36 Hz, $COCF_3$), 161.9 (*C*-*4*), 165.4 (CH*C*ONH). *Anal*. Calcd for $C_{20}H_{25}N_4O_6F_3$: C, 50.63; H, 5.31; N, 11.81; F, 12.01. Found: C, 50.61; H, 5.29; N, 11.77; F, 11.97.

1-(2,3-dideoxy-β-D-*glycero*-pent-2-enofuranosyl)-5-{N-[6-(*tert*-butoxy-carbonyl)-hexyl]-acrylamide}-uracil (11). Sodium methoxide (0.221 mmol, 11.9 mg, 1.2 equiv.) was added to a solution of the nucleoside **9** (0.185 mmol, 96 mg) in anhydrous methanol (20 mL) and the solution stirred at room temperature for 1 h. TLC indicated that deprotection was complete. The solution was carefully neutralized by addition of Amberlite IRN-77 (H^+) resin until moistened pH paper indicated pH ~ 6. The resin was filtered and then washed with methanol (2 × 20 mL). The combined filtrate was evaporated to dryness *in vacuo*. The residue was purified by silica-gel column chromatography using methanol in ethyl acetate (from 0% to 4%) as eluents to yield 54.8 mg (62%) of 11: R_f 0,25 [EtOAc-CH_3OH (95:5)]; ^{1}H-NMR (DMSO-d_6): δ (ppm) 1.22 (m, 4H, 2 × CH_2(CH_2)$_2$NH), 1.35 (m, 13H, C(CH_3)$_3$, 2 × CH_2CH_2NH), 2.86 (m, 2H, CH_2NHBoc), 3.08 (m, 2H, CH_2NHCOCH=CH), 3.63 (s, 2H, *H*-5′), 4.82 (s, 1H, *H*-4′), 5.17 (bs, 1H, O*H*), 5.93 (d, J = 5.6 Hz, 1H, *H*-2′), 6.42 (d, J = 5.6 Hz, 1H, *H*-3′), 6.77 (t, J = 5.4 Hz, 1H, N*H*Boc), 6.83 (s, 1H, *H*-1′), 6.90 (dAB, J = 15.5 Hz, 1H, C*H*=CH), 6.99 (dAB, J = 15.5 Hz, 1H, CH=C*H*), 8.06 (t, J = 5.2 Hz, 1H, N*H*COCH=CH), 8.21 (s, 1H, *H-6*), 11.60 (bs, 1H, *H*N-3); ^{13}C-NMR (DMSO-d_6): δ (ppm) 26.0 (CH_2(CH_2)$_2$NH), 26.2 (CH_2(CH_2)$_2$NH), 28.2(C(CH_3)$_3$),29.1(CH_2CH_2NH),29.4(CH_2CH_2NH),38.5(CH_2NH),40.140.1 40.1 (CH_2NH), 61.7 (*C*-5′), 77.1 (*C*(CH_3)$_3$), 87.6 (*C*-4′), 89.3 (*C*-1′), 108.8 (*C*-*5*), 121.3 (CH=*C*HCONH), 125.7 (*C*-2′), 131.4 (*C*H=CHCONH), 135.4 (*C*-3′), 142.8 (*C*-*6*), 149.8 (*C*-*2*), 155.4 (*C*OOCMe$_3$), 161.9 (*C*-*4*), 165.2 (CH*C*ONH). *Anal*. Calcd for $C_{23}H_{34}N_4O_7$: C, 57.73; H, 7.16; N, 11.71. Found: C, 57.71; H, 7.13; N, 11.69.

1-(2,3-dideoxy-β-D-*glycero*-pent-2-enofuranosyl)-5-[N-(6-amino-hexyl)-acryla-mide]-uracil (12). *Method 1:* To a solution of the 5-{N-[6-(trifluoroacetyl-amino)-hexyl]-acrylamide}-nucleoside (**10**) (0.235 mmol, 112 mg) in dry methanol was added sodium methoxide (0.282 mmol, 15.2 mg, 1.2 equiv.) and the reaction mixture was stirred at 60 °C for 24 h. TLC indicated that deprotection was complete. The solution was carefully neutralized by addition of Amberlite IRN-77 (H^+) resin until moistened pH paper indicated pH ~ 6. The mixture was filtered, and the resin was washed with methanol. The combined filtrate was evaporated to dryness *in vacuo*. The resulting powder was purified by silica-gel column chromatography eluted with ammonium hydroxide in methanol (from 0% to 2%) to yield 48 mg (54%) of **12**.

Method 2: To a solution of the 5-{N-[6-(*tert*-butoxycarbonyl)-hexyl]-acrylamide}-nucleoside (9) (104 mg, 0.200 mmol) in dry dichloromethane (1 mL) was added trifluoroacetic acid (0.68 g, 5.988 mmol, 30 equiv.). The reaction mixture was stirred at room temperature for 5.5 h, and then evaporated to dryness under reduced pressure. The resulting oil was dissolved in a small amount of dry methanol and the solution was added with sodium methoxide (21.5 mg, 0.399 mmol, 2 equiv.). The solution was stirred at room temperature for 1.5 h (TLC indicated that deprotection was complete) and then carefully neutralized by addition of Amberlite IRN-77 (H^+) resin until moistened pH paper indicated pH $\sim$ 6. The mixture was filtered, and the resin was washed with methanol. The combined filtrate was evaporated to dryness *in vacuo*. The residue was purified on silica-gel column chromatography eluted with ammonium hydroxide in methanol (from 0% to 2%) to yield 31 mg (41%) of **12**: R_f 0.25 [methanol-ammonium hydroxide (98:2)]; ^{1}H-NMR (DMSO-d_6): δ (ppm) 1.23 (m, 4H, 2 × C*H*$_2$(CH$_2$)$_2$NH), 1.32 (m, 2H, C*H*$_2$CH$_2$NH$_2$), 1.38 (m, 2H, C*H*$_2$CH$_2$NHCO), 2.51 (m, 2H, C*H*$_2$NH$_2$), 3.08 (m, 2H, C*H*$_2$NHCOCH), 3.63 (s, 2H, *H*-5′), 4.82 (s, 1H, *H*-4′), 5.10 (bs, 1H, O*H*), 5.93 (d, J = 5.6 Hz, 1H, *H*-2′), 6.41 (d, J = 5.6 Hz, 1H, *H*-3′), 6.83 (s, 1H, *H*-1′), 6.90 (dAB, J = 15.5 Hz, 1H, C*H*=CH), 6.98 (dAB, J = 15.5 Hz, 1H, CH=C*H*), 8.06 (t, J = 4.9 Hz, 1H, N*H*COCH), 8.20 (s, 1H, *H-6*), 10.88 (bs, 1H, *H*N-3); ^{13}C-NMR (DMSO-d_6): δ (ppm) 26.1 (*C*H$_2$(CH$_2$)$_2$NH), 26.4 (*C*H$_2$(CH$_2$)$_2$NH), 29.2 (*C*H$_2$CH$_2$NHCO), 32.6 (*C*H$_2$CH$_2$NH$_2$), 38.6 (*C*H$_2$NHCO), 41.3 (*C*H$_2$NH$_2$), 61.9 (*C*-5′), 87.7 (*C*-4′), 89.4 (*C*-1′), 108.9 (*C-5*), 121.5 (CH=*C*HCONH), 125.8 (*C*-2′), 131.7 (*C*H=CHCONH), 135.3 (*C*-3′), 142.9 (*C-6*), 149.9 (*C-2*), 162.1 (*C-4*), 165.3 (CH*C*ONH). *Anal*. Calcd for $C_{18}H_{26}N_4O_5$: C, 57.13; H, 6.92; N, 14.81. Found: C, 57.11; H, 6.88; N, 14.78.

1-(5-O-acetyl-2,3-dideoxy-β-D-*glycero*-pent-2-enofuranosyl)-5-{N-[5-(methoxy-carbonyl)-pentyl]-acrylamide}-uracil (13). Compound **3** (0.5 g, 1.32 mmol) was converted to **13** by the same procedure as described for **5**, except that the reaction mixture was stirred at 80 °C for 2 h. The residue was purified by silica-gel column chromatography eluted with methanol in dichloromethane (from 0% to 4%) to yield 172 mg (29%) of **13**: ^{1}H-NMR (DMSO-d_6): δ (ppm) 1.24 (m, 2H, C*H*$_2$(CH$_2$)$_2$COOMe), 1.39 (m, 2H, C*H*$_2$CH$_2$NHCO), 1.51 (m, 2H, C*H*$_2$CH$_2$COOMe), 1.97 (s, 3H, C*H*$_3$COO), 2.27 (t, J = 7 Hz, 2H, C*H*$_2$COOMe), 3.08 (m, 2H, C*H*$_2$NHCO), 3.56 (s, 3H, OC*H*$_3$), 4.18 (dAB, J = 12.6 Hz, 1H, *H*-5′), 4.24 (ddAB, J = 12.6 Hz, 4 Hz, 1H, *H*-5″), 5.00 (s, 1H, *H*-4′), 6.02 (d, J = 5.8 Hz, 1H, *H*-2′), 6.43 (d, J = 5.8 Hz, 1H, *H*-3′), 6.8 (s, 1H, *H*-1′), 6.98 (dAB, J = 13.8 Hz, 1H, C*H*CONH), 7.12 (dAB, J = 13.8 Hz, 1H, C*H*=CHCONH), 7.75 (s, 1H, *H-6*), 8.06 (m, 1H, CON*H*), 11.63 (s, 1H, *H*N-3).

Methyl 6-(acryloylamino)hexanoate (14).[20] Mp 50 °C; ^{1}H-NMR (DMSO-d_6): δ (ppm) 1.25 (m, 2H, C*H*$_2$(CH$_2$)$_2$COOMe), 1.40 (m, 2H, C*H*$_2$CH$_2$NHCO), 1.51 (m, 2H, C*H*$_2$CH$_2$COOMe), 2.28 (t, J = 7.3 Hz, 2H, C*H*$_2$COOMe), 3.09 (m, 2H, C*H*$_2$NHCO), 3.56 (s, 3H, OC*H*$_3$), 5.54 (dd, J = 10 Hz, 2 Hz, 1H, *Htrans*), 6.04 (ddAB, J = 17 Hz, 2 Hz, 1H, *Hcis*), 6.18 (qABX, J = 10 Hz, 17 Hz, 1H, =*CH*CONH), 8.03 (bs, 1H, *H*NCOCH); ^{13}C-NMR (DMSO-d_6): δ (ppm) 24.1 (*C*H$_2$CH$_2$COOMe), 25.9 (*C*H$_2$(CH$_2$)$_2$COOMe), 28.7 (*C*H$_2$CH$_2$NHCO), 33.2 (*C*H$_2$COOMe), 38.3 (*C*H$_2$NHCO), 51.1 (O*C*H$_3$), 124.7 (=*C*H$_2$), 131.9 (=*C*HCONH), 164.4 (*C*ONH), 173.3 (*C*OOCH$_3$).

1-(2,3-dideoxy-β-D-*glycero*-pent-2-enofuranosyl)-5-{N-[5-(methoxycarbonyl)-pentyl]-acrylamide}-uracil (15). Sodium methoxide (0.049 g, 0.91 mmol, 2.5 equiv.) was added to a solution of **13** (0.165 g, 0.36 mmol) in tetrahydrofuran (5 mL, previously eluted through a column of alumina). The solution was stirred at room temperature for 2 days and neutralized by addition of Dowex 50WX2 resin (in H^+ form). The resin was filtered and the filtrate was evaporated under reduced pressure. The crude material was purified by silica-gel column chromatography using methanol in dichloromethane (from 0% to 7%) as eluents to yield 50 mg (34%) of **15**: ^{1}H-NMR (DMSO-d_6): δ (ppm): 1.24 (m, 2H, C*H*$_2$(CH$_2$)$_2$COOMe), 1.38 (m, 2H, C*H*$_2$CH$_2$NHCO), 1.50 (m, 2H, C*H*$_2$CH$_2$COOMe), 2.27 (t, J = 7 Hz, 2H, C*H*$_2$COOMe), 3.07 (m, 2H, C*H*$_2$NHCO), 3.56 (s, 3H, OC*H*$_3$), 3.63 (s, 2H, *H*-5′), 4.82 (s, 1H, O*H*), 5.12 (s, 1H, *H*-4′), 5.94 (d, J = 5.8 Hz, 1H, *H*-2′), 6.42 (d, J = 5.8 Hz, 1H, *H*-3′), 6.83 (s, 1H, *H*-1′), 6.90 (dAB, J = 15.8 Hz, 1H, *H*C=CH), 6.98 (dAB, J = 15.8 Hz, 1H, HC=C*H*), 8.04 (m, 1H, N*H*CO), 8.20 (s, 1H, *H-6*), 11.57 (s, 1H, *H*N-3); ^{13}C-NMR (DMSO-d_6): δ (ppm) 24.1 (*C*H$_2$CH$_2$COOMe), 25.9 (*C*H$_2$(CH$_2$)$_2$COOMe), 28.8 (*C*H$_2$CH$_2$NHCO), 33.2 (*C*H$_2$COOMe), 38.4 (*C*H$_2$NHCO), 51.2 (O*C*H$_3$), 61.9 (*C*-5′), 87.7 (*C*-4′), 89.4 (*C*-1′), 108.8 (*C-5*), 121.5 (*C*HCONH), 125.7 (*C*-2′), 131.6 (*C*H=CHCONH), 135.3 (*C*-3′), 142.9 (*C-6*), 149.7 (*C-2*), 162.0 (*C-4*), 165.3 (*C*ONH), 173.3 (*C*OOMe). *Anal*. Calcd for C$_{19}$H$_{25}$N$_3$O$_7$: C, 56.01; H, 6.18; N, 10.31. Found: C, 55.98; H, 6.15; N, 10.29.

Antiviral Test Procedures

The cultures of CEM-SS and MT4 cells were maintained at 37 °C in a 5% CO_2 atmosphere in RPMI-1640 medium supplemented with 10% complement-depleted foetal bovine serum (FBS). The antiviral HIV-1 activity of a given compound in CEM-SS cells was measured by quantification of the reverse transcriptase activity (RT) associated with particles released from HIV-1$_{LAI}$-infected cells in the culture medium. CEM-SS cells were infected with 100 TCDI$_{50}$ (the virus stock was titrated under the same experimental

conditions); after 30 mn adsorption, free virus particles were washed out and cells were re-suspended in RPMI-1640 plus 10% foetal calf serum at a final concentration of 10^5 cells mL^{-1} in the presence of different concentrations of test compounds. After 5 days, virus production was measured by RT assay as already described[21]. The 50% inhibitory concentration (IC_{50}) was derived from the computer generated median effect plot of the dose-effect data[22]. The cytotoxicity of the drugs was evaluated in parallel by incubating uninfected cells in the presence of different concentrations of antiviral products. The cell viability was determined by a measure of mitochondrial dehydrogenase activity, enzymes reducing 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) into formazan (whose quantity was measured by the absorbance at 540 nm)[22]. The 50% cytotoxic concentration (CC_{50}) is the concentration of drug which reduces cell viability by 50% and was calculated with the program used in the determination of the IC_{50}. The assays using different cells and virus isolates were done according to previously published protocols; [21,24] virus production was quantified by the RT activity associated to virus particles released from the cells in the culture medium. Conditions in which the inhibitory properties were measured on HIV-1 reverse transcriptase *in vitro* has also been described[21]. *In vitro* RT inhibition was also described[21]. The CEM-SS cells were obtained from P. Nara through the AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH (Bethesda, Md., USA).

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