Full Paper

Synthesis of Novel Uracil Non-Nucleoside Derivatives as Potential Reverse Transcriptase Inhibitors of HIV-1

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Novel emivirine and TNK-651 analogues **5a – d** were synthesized by reaction of chloromethyl ethyl ether and / or benzyl chloromethyl ether, respectively, with uracils having 5-ethyl and 6-(4-methylbenzyl) or 6-(3,4-dimethoxybenzyl) substituents. A series of new uracil non-nucleosides substituted at N-1 with cyclopropylmethyloxymethyl **9a – d**, 2-phenylethyloxymethyl **9e – h**, and 3-phenylprop-1-yloxymethyl **9i – l** were prepared on treatment of the corresponding uracils with the appropriate acetals **8a – c**. Some of the tested compounds showed good activity against HIV-1 wild type. Among them, 1-cyclopropylmethyloxymethyl-5-ethyl-6-(3,5-dimethylbenzyl)uracil **9c** and 5-ethyl-6-(3,5-dimethylbenzyl)-1-(2-phenylethyloxymethyl)uracil **9g** showed inhibitory potency equally to emivirine against HIV-1 wild type. Furthermore, compounds **9c** and **9g** showed marginal better activity against NNRTI resistant mutants than emivirine.

Keywords: Drug research / Emivirine analogues / HIV-1 / Non-nucleoside reverse transcriptase inhibitors / TNK-651 analogues

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Introduction

The reverse transcriptase (RT) enzyme is the pivot in the retroviral cycle of human immunodeficiency virus type-1 (HIV-1). It is responsible for the conversion of a single-stranded RNA viral genome into a double-stranded DNA required for the human cell's genetic machinery to replicate the virus [1, 2]. Two main classes of RT inhibitors, nucleoside reverse transcriptase inhibitors (NRTIs) such as zidovudine (AZT, Retrovir) [3], zalcitabine (ddC, Hivid) [4], didanosine (ddI, Videx) [5], and lamivudine (3TC, Epivir) [5], which act as chain terminators to stop the attachment of further nucleosides and so prevent an ongoing viral DNA synthesis, and non-nucleoside reverse transcriptase inhibitors (NNRTIs) which, in contrast to NRTIs, are highly specific as their binding site is a hydrophobic pocket located approximately 10 Å from the polymerase site. They bind allosterically forcing the RT-subunit into an inactive conformation [6, 7]. Much effort has been put into the synthesis and design of NNRTIs; more than 30 structurally different classes of NNRTIs have been reported [8, 9], One of the first NNRTIs was 1-[(2-hydroxyethoxy)methyl]-6-(phenylthio)thymine (HEPT) [10]. Although HEPT did not show very high activity against HIV-1, it was considered as an interesting lead compound for the synthesis of new analogues, among them the 6-benzyl-1-(ethoxymethyl)-5-isopropyluracil (Emivirine) [11], the corresponding 1-benzyloxymethyl analogues (TNK-651) [12] and 6-(3,5-dimethylbenzyl)-1-ethoxymethyl-5-isopropyluracil (GCA-186) [13], which all showed high activity against HIV-1. Emivirine was chosen

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Abbreviations: *N,O*-bis-(trimethylsilyl)acetamide (BSA); non-nucleoside reverse transcriptase inhibitors (NNRTIs); nucleoside reverse transcriptase inhibitors (NRTIs)

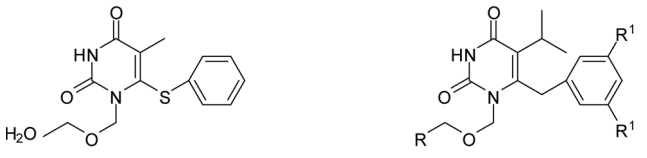
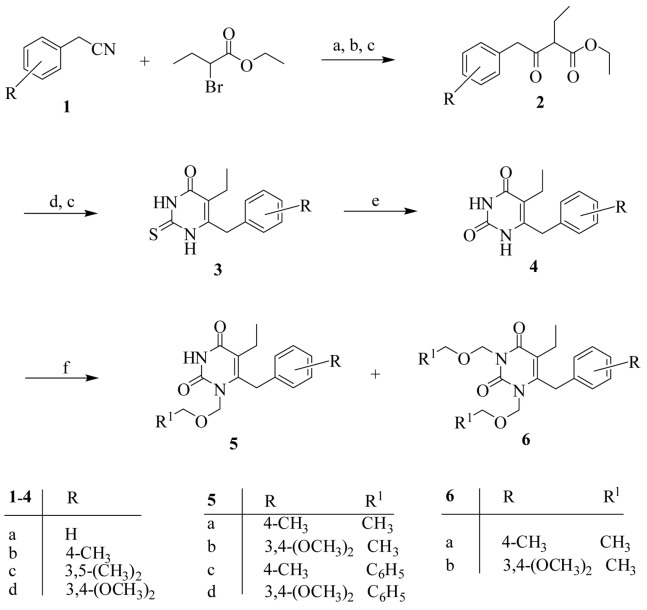


Figure 1. Structures of HEPT, Emivirine, TNK-651, GCA-186.

as a candidate for clinical trials with AIDS patients, however, the phase-III studies was halted when emivirine was found to activate a liver enzyme Cytochrome P450 which metabolizes protease inhibitors [14].

The mutations that appear in the presence of NNRTIs are the Y181C, Y181C + K103N, and the triple mutant K103R + V179D + P225H, which are critical for the binding of the NNRTIs to RT. GCA-186 differs structurally from Emivirine only in the introduction of two methyl substituents in the 3- and 5-position at the C-6 benzyl group. It showed higher antiviral activity against HIV-1 wild type and greater tolerance to the presence of the mutated HIV strains than Emivirine, due to binding of the two methyl substituents in two hydrophobic pockets in the NNRTI-binding site of RT as revealed by X-ray crystallography [13]. In an effort to improve the activity against HIV-1 wild type and NNRTI resistant mutants, and as a part of our interest in the chemistry of NNRTIs [15–25], the present study describes synthesis and antiviral evaluation of novel non-nucleosides analogues of emivirine and GCA-186 with a N-1 cyclopropylmethoxymethyl substituent. Also, a series of TNK-651 analogues substituted at N-1 with 2-phenylethyloxymethy and 3-phenylprop-1-yloxymethyl moieties has been investigated. The objective was to investigate if these substituents could lead to an improved antiviral activity against HIV-1. In addition, the synthesis of new HEPT analogues should also provide information regarding the ongoing investigation of the NNRTIs binding site.



1-4	R
a	H
b	4-CH_3
c	3,5-$(CH_3)_2$
d	3,4-$(OCH_3)_2$

5	R	R^1
a	4-CH_3	CH_3
b	3,4-$(OCH_3)_2$	CH_3
c	4-CH_3	C_6H_5
d	3,4-$(OCH_3)_2$	C_6H_5

6	R	R^1
a	4-CH_3	CH_3
b	3,4-$(OCH_3)_2$	CH_3

Reagents and Conditions: (a) Zn, THF; (b) K_2CO_3; (c) HCl, 91–93% (one-pot synthesis); (d) $(H_2N)_2CS$, NaOEt, 61–64%; (e) aq. $ClCH_2CO_2H$, 81–87%; (f) $R^1CH_2OCH_2Cl$, CsI, $CHCl_3$, 23–69%.

Scheme 1. Synthesis of compounds **2**-**6**.

Results and discussion

Chemistry

Phenylacetonitrile, 4-methylphenylacetonitrile, 3,5-dimethylphenylacetonotrile, and / or 3,4-dimethoxyphenylacetonitrile **1a–d** were reacted with the zinc organometallic reagent prepared from ethyl 2-bromobutyrate in anhydrous THF to afford the corresponding 4-aryl-2-ethyl-3-oxo esters **2a–d** in good yields. 5-Ethyl-6-substituted-2-thiouracils **3a–d** were synthesized by treating **2a–d** with thiourea in boiling ethanol in the presence of sodium ethoxide [26]. The NMR spectra of the crude compounds **2a–d** showed an impurity identified as another β-keto ester resulting from self-condensation of ethyl 2-bromobutyrate. On reaction with thiourea, this β-keto ester impurity also formed a pyrimidine compound as an impurity in the raw materials of **3a–d**. However, pure compounds **3a–d** were easily obtained by recrystallization from aqueous ethanol [27]. Desulfurization of **3a–d** was achieved by treatment with boiling aqueous chloroacetic acid to give the corresponding uracil derivatives **4a–d**. Silylation of 5-ethyl-6-(4-methylbenzyl)uracil **4b** and 5-ethyl-6-(3,4-dimethoxybenzyl)uracil **4d** with *N,O*-bis-(trimethylsilyl)acetamide (BSA) in anhydrous chloroform followed by treatment with chloromethyl ethyl ether and / or benzyl chloromethyl ether in the presence of cesium iodide gave the corresponding 1-ethyloxymethyl **5a**, **b** and 1-benzyloxymethyluracils **5c**, **d**, respectively. The 1,3-dialkylated uracils **6a**, **b** were also obtained in case of the reaction with chloromethyl ethyl ether (Scheme 1).

Bis-(cyclopropylmethyloxy)methane **8a**, bis-(2-phenylethyloxy)methane **8b** [23], and bis-(3-phenylprop-1-yloxy)-methane **8c** were prepared from the corresponding alcohols cyclopropylcarbinol **7a**, 2-phenylethanol **7b**, and 3-phenyl-1-propanol **7c**, respectively, in a reaction with dibromomethane using potassium hydroxide in anhydrous benzene in the presence of tetrabutylammonium bromide according to the method of Nazaretyan *et al.*

Table 1. Cytotoxicity and anti-HIV-1 activity of compounds **5a–d** and **9a–l**.

Compound	CC_{50} (μM)§ MT-4	EC_{50} (μM)#			
		wt_{IIIB}	N119 (Y181C)	A17 (K103N+Y181C)	EFV^R (K103R+V179D+P225H)
5a	>100	5.3 ± 0.3	>100	>100	>100
5b	>100	59±4.5	>100	>100	>100
5c	44 ± 0.5	1.1 ± 0.2	>44	>44	>44
5d	80 ± 6.5	12 ± 1	>80	>80	>80
9a	>100	1.1 ± 0.1	>100	>100	>100
9b	>100	37 ± 6	>100	>100	>100
9c	51 ± 3	0.03 ± 0.01	5.1 ± 0.8	>51	>51
9d	>100	>100	>100	>100	>100
9e	43 ± 2.5	0.4 ± 0.02	>43	>43	>43
9f	60	>60	>60	>60	>60
9g	41 ± 2	0.03 ± 0.01	5.6 ± 1.2	>41	=20
9h	73	>73	>73	>73	>73
9i	23 ± 0.5	0.2 ± 0.01	>23	>23	>23
9j	43	>43	>43	>43	>43
9k	43 ± 3	0.05 ± 0.01	8.8 ± 1.9	>43	>43
9l	38	>38	>38	>38	>38
Emivirine	>100	0.03 ± 0.005	>20	56	>100
EFV	38 ± 1.5	0.002 ± 0.0003	0.02 ± 0.005	0.1 ± 0.03	14 ± 2.5

§ Compound dose required to reduce the viability of mock-infected cells by 50%, as determined by the MTT method; # compound dose required to achieve 50% protection of MT-4 cells from HIV-1-induced cytopathogenicity, as determined by the MTT method.

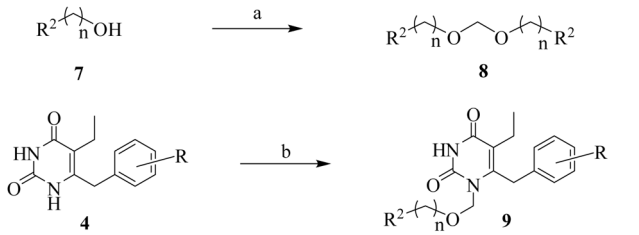
[28]. Compounds **4a–d** were silylated with BSA in anhydrous acetonitrile and reacted with the appropriate acetals **8a–c**, using trimethylsilyl trifluoromethanesulfonate (TMS triflate) as a Lewis acid catalyst [29], to give the corresponding N-1 cyclopropylmethyloxymethyl, 2-phenylethyloxymethyl and 3-phenylprop-1-yloxymethyl-uracils **9a–d**, **9e–h**, and **9i–l**, respectively (Scheme 2).

The newly synthesized compounds were confirmed by comparison of similar NMR data [15–20, 22, 24]. MS(EI), and HRMS are in full agreement with the proposed structures.

Biological screening

According to structure–activity relationships (SAR), studies of several crystal structures of the RT complex with inhibitors such as HEPT, GCA-186, and TNK-651 indicate that a methyl or isopropyl at C-5 and a benzyl or substituted benzyl, specially 3,5-dimethylbenzyl, at C-6 are the optimal substituents of the uracil ring with respect to HIV-inhibition [30]. The present study describes the synthesis of some novel uracil non-nucleosides having the optimal substituents at C-5 and C-6 with some new modifications at N-1 position of the uracil ring. Compounds **5a–d** and **9a–l** were tested for their biological activity against HIV-1 wild type and strains carrying the clinically relevant mutations N119 (Y181C), A17 (K103N + Y181C), and the triple mutant EFV^R (K103R + V179D + P225H) in MT-4 cells. Results were compared with the antiviral activity of emivirine, a well examined HEPT analogues, and efavirenz, the most active anti-HIV-1 drug used in therapy today. Results are listed in Table 1.

As seen from the results listed in Table 1, compounds **5c**, **9a**, **9c**, **9e**, **9g**, **9i**, and **9k** showed good activity against HIV-1 wild type with a wide range of EC_{50} values of 1.1 to 0.03 μM. The most active compounds were 1-cyclopropylmethyloxymethyl-5-ethyl-6-(3,5-dimethylbenzyl)uracil **9c** and 5-ethyl-6-(3,5-dimethylbenzyl)-1-(2-phenylethyloxymethyl)uracil **9g**, which showed inhibitory potency (EC_{50}) equally to emivirine against HIV-1 wild type. In general, the two methyl substituents at the 3- and 5-positions of the benzyl moiety improved the activity against HIV-1 wild type compared with those of unsubstituted benzyl non-nucleosides. Compounds having 1-cyclopropylmethyloxymethyl and 6-benzyl **9a** or 6-(3,5-dimethylbenzyl) **9c** showed activity against HIV-1 wild type with EC_{50} values of 1.1 and 0.03 μM, respectively. Compound **9c** was over 30-fold more potent than **9a**. Non-nucleosides bearing 2-phenylethyloxymethyl moiety at N-1, **9g**, showed inhibitory potency equal to those of emivirine, while **9e** was approximately 13 times less active than **9g**. Compounds having a 3-phenylprop-1-yloxymethyl moiety at N-1, **9i** and **9k**, showed good activity against HIV-1 wild type with EC_{50} values of 0.2 and 0.05 μM, respectively. Compounds **9c**, **9g**, and **9k** showed higher activity than emivirine against the mutant strain N119. Compounds **5c**, **9e**, **9g**, **9i**, **9j**, **9k**, and **9l** showed marginal higher activ-



7, 8	R^2	n
a		1
b		2
c		3

9	R	R^2	n
a	H		1
b	4-CH_3		1
c	3,5-$(CH_3)_2$		1
d	3,4-$(OCH_3)_2$		1
e	H	C_6H_5	2
f	4-CH_3	C_6H_5	2
g	3,5-$(CH_3)_2$	C_6H_5	2
h	3,4-$(OCH_3)_2$	C_6H_5	2
i	H	C_6H_5	3
j	4-CH_3	C_6H_5	3
k	3,5-$(CH_3)_2$	C_6H_5	3
l	3,4-$(OCH_3)_2$	C_6H_5	3

Reagents and Conditions: (a) CH_2Br_2, KOH, Bu_4NBr, benzene, 56–63%; (b) BSA, CH_3CN, **8**, TMS triflate, 44–67%.

Scheme 2. Synthesis of compounds **8** and **9**.

ity than emivirine against the mutant strains A17 and EFV^R.

In summary, we synthesized novel uracil non-nucleosides analogues of emivirine, GCA-186, and TNK-651 and investigated their antiviral activity against HIV-1. Compounds **9c**, **9g**, and **9k** showed higher activity than emivirine against the mutant strain N119. Compounds **5c**, **9e**, **9g**, **9i**, **9j**, **9k**, and **9l** showed marginal higher activity than emivirine against the mutant strains A17 and EFV^R.

Experimental

Chemistry

Melting points (uncorrected) were determined on a Gallenkamp melting point apparatus (Weiss-Gallenkamp, London, UK). NMR spectra were recorded on a Bruker AC 500 Ultra Shield NMR spectrometer (Bruker Bioscience, USA) at 500 MHz for ^1H and 125 MHz for ^{13}C with TMS as an internal standard. Electron impact mass spectra were recorded on Perkin Elmer Clarus 600 GC Mass Spectrometer (Perkin Elmer, Norwalk, CT, USA). MALDI spectra were recorded on a Fourier Transform (FT) Ion Cyclotron Resonance Mass Spectrometer (IonSpec Corporation, Lake Forest, CA, USA). The progress of reactions was monitored by TLC (DC-alufolio 60 F_{254}) from Merck (Darmstadt, Germany). For column chromatography, Merck silica gel (0.040-0.063 mm) was used.

*Ethyl 4-aryl-2-ethyl-3-oxobutyrates **2b** and **2d***

Zinc dust (14 g) was activated by stirring with 4 M HCl (30 mL) for 5 min. The zinc dust was filtered, washed sequentially with H_2O, EtOH, and dry Et_2O; then it was dried. The active zinc was suspended in dry THF (60 mL) and heated to reflux. Few drops of ethyl 2-bromobutyrate were added and the mixture was refluxed for 10 min. *p*-Methylphenylacetonitrile (3.94 g, 0.03 mol) and / or 3,4-dimethoxyphenylacetonitrile (5.32 g, 0.03 mol) was added in one portion and ethyl 2-bromobutyrate (0.06 mol) was added dropwise. After the addition was completed, the mixture was refluxed for 30 min, then it was diluted with THF (150 mL) and quenched by addition of sat. aq. K_2CO_3 (60 mL). The mixture was stirred for 1 h at room temperature. The THF layer was decanted and the residue was washed with THF (3 × 30 mL). The combined THF fractions were stirred with 10% aq. HCl (40 mL) for 30 min. The solution was concentrated under reduced pressure and diluted with CH_2Cl_2 (100 mL). The organic phase was washed with sat. aq. $NaHCO_3$ (2 × 60 mL), dried (Na_2SO_4), and evaporated under reduced pressure to give the product that was used for further synthesis without purification.

*Ethyl 2-ethyl-4-(4-methylphenyl)-3-oxobutyrate **2b***

Faint yellow oil; yield: 6.75 g (91%); ^1H-NMR ($CDCl_3$, 500 MHz) δ: 0.97 (t, *J* = 7.0 Hz, 3H, CH_3), 1.25 (t, *J* = 7.5 Hz, 3H, CH_3), 1.89–1.93 (m, 2H, CH_2), 2.37 (s, 3H, CH_3), 3.46 (t, *J* = 7.5 Hz, 1H, CH), 3.76 (s, 2H, CH_2), 4.12 (q, *J* = 7.0 Hz, 2H, CH_2), 7.08, 7.13 (2 × d, *J* = 8.0 Hz, 4H, H_{arom}); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 11.89 (CH_3), 13.52 (CH_3), 18.42 (CH_2), 21.00 (CH_3), 43.73 (CH_2), 60.72 (CH_2), 61.22 (CH), 127.76, 129.47, 130.12, 136.77 (C_{arom}), 169.64 (CO), 202.79 (CO).

*Ethyl 2-ethyl-4-(3,4-dimethoxyphenyl)-3-oxobutyrate **2d***

Faint yellow oil; yield: 8.18 g (93%); ^1H-NMR ($CDCl_3$, 500 MHz) δ: 0.82 (t, *J* = 7.0 Hz, 3H, CH_3), 1.24 (t, *J* = 7.5 Hz, 3H, CH_3), 1.81–1.83 (m, 2H, CH_2), 3.45 (t, *J* = 7.5 Hz, 1H, CH), 3.74 (s, 2H, CH_2), 3.85, 3.86 (2 × s, 6H, 2 × OCH_3), 4.12 (q, *J* = 7.0 Hz, 2H, CH_2), 6.70–6.82 (m, 3H, H_{arom}); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 13.90 (CH_3), 14.05 (CH_3), 18.40 (CH_2), 43.71 (CH_2), 55.86 (OCH_3), 55.88 (OCH_3), 60.69 (CH_2), 61.81 (CH), 111.45, 112.78, 121.87, 125.78, 148.31, 149.10 (C_{arom}), 169.69 (CO), 202.86 (CO).

*5-Ethyl-6-substituted-2-thiouracils **3b** and **3d***

Na (9.98 g, 0.434 mol) was dissolved in absolute EtOH (150 mL). Thiourea (22.84 g, 0.3 mol) was added and the mixture was heated to reflux. Compound **2b** and / or **2d** (0.02 mol) was added dropwise and the mixture was refluxed for 18 h. The solvent was evaporated to dryness under reduced pressure and the residue was redissolved in H_2O (150 mL). The product was precipitated by addition of conc. HCl (16 mL) and then glacial acetic acid till pH = 4. The precipitate was filtered off, washed with H_2O, dried, and crystallized from aq. EtOH.

*5-Ethyl-6-(4-methylbenzyl)-2-thiouracil **3b***

White solid; yield: 3.16 g (61%); m.p.: 203–205°C (dec.); ^1H-NMR (DMSO-d_6, 500 MHz) δ: 0.80 (t, *J* = 7.0 Hz, 3H, CH_3), 2.26 (s, 3H,

CH_3), 2.37 (q, J = 7.0 Hz, 2H, CH_2), 3.80 (s, 2H, CH_2), 7.06, 7.13 (2 × d, J = 8.2 Hz, 4H, H_{arom}), 12.29, 12.38 (2 × s, 2H, 2 × NH); ^{13}C-NMR (DMSO-d_6, 125 MHz) δ: 13.97 (CH_3), 18.17 (CH_2), 21.04 (CH_3), 34.63 (CH_2), 116.51 (C-5), 128.47, 129.66, 134.14, 136.29 (C_{arom}), 149.95 (C-6), 161.95 (C-4), 174.82 (C-2); EI MS *m/z* (%): 260 [M$^+$] (36).

5-Ethyl-6-(3,4-dimethoxybenzyl)-2-thiouracil ***3d***

White solid; yield: 3.94 g (64%); m.p.: 251–253°C (dec.); ^{1}H-NMR (DMSO-d_6, 500 MHz) δ: 0.85 (t, J = 7.0 Hz, 3H, CH_3), 2.29 (q, J = 7.0 Hz, 2H, CH_2), 3.72, 3.74 (2 × s, 6H, 2 × OCH_3), 3.76 (S, 2H, CH_2), 6.73–6.91 (m, 3H, H_{arom}), 12.17, 12.38 (2 × s, 2H, 2 × NH); ^{13}C-NMR (DMSO-d_6, 125 MHz) δ: 13.43 (CH_3), 18.22 (CH_2), 34.57 (CH_2), 56.05 (OCH_3), 56.07 (OCH_3), 116.52 (C-5), 112.63, 113.12, 120.53, 129.47, 148.26, 150.00 (C_{arom}), 149.25 (C-6), 161.95 (C-4), 174.69 (C-2); EI MS *m/z* (%): 306 [M$^+$] (14).

5-Ethyl-6-substituted-uracils ***4b*** *and* ***4d***

Compounds **3b**, **d** (0.01 mol) were suspended in 10% aq. $ClCH_2CO_2H$ (200 mL). The suspension was refluxed for overnight and filtered after cooling. The precipitate thus formed was washed with H_2O, cold EtOH, then Et_2O and dried.

5-Ethyl-6-(4-methylbenzyl)uracil ***4b***

White solid; yield: 1.98 g (81%); m.p.: 256–258°C (dec.); ^{1}H-NMR (DMSO-d_6, 500 MHz) δ: 0.81 (t, J = 7.0 Hz, 3H, CH_3), 2.22 (q, J = 7.0 Hz, 2H, CH_2), 2.26 (s, 3H, CH_3), 3.70 (s, 2H, CH_2), 7.12, 7.14 (2 × d, J = 8.0 Hz, 4H, H_{arom}), 10.71, 10.99 (2 × s, 2H, 2 × NH); ^{13}C-NMR (DMSO-d_6, 125 MHz) δ: 13.94 (CH_3), 18.05 (CH_2), 21.03 (CH_3), 35.10 (CH_2), 111.65 (C-5), 128.48, 129.60, 134.28, 136.20 (C_{arom}), 149.38 (C-6), 151.40 (C-2), 164.98 (C-4); EI MS *m/z* (%): 244 [M$^+$] (97).

5-Ethyl-6-(3,4-dimethoxybenzyl)uracil ***4d***

White solid; yield: 2.53 g (87%); m.p.: 211–213°C; ^{1}H -NMR (DMSO-d_6, 500 MHz) δ: 0.85 (t, J = 7.5 Hz, 3H, CH_3), 2.27 (q, J = 7.5 Hz, 2H, CH_2), 3.66 (s, 2H, CH_2), 3.72, 3.74 (2 × s, 6H, 2 × OCH_3), 6.75, 6.77 (2 × d, J = 8.0 Hz, 2H, H_{arom}), 6.93 (s, 1H, H_{arom}), 10.67, 10.97 (2 × s, 2H, 2 × NH); ^{13}C-NMR (DMSO-d_6, 125 MHz) δ: 14.03 (CH_3), 18.08 (CH_2), 35.05 (CH_2), 56.04 (OCH_3), 113.09 (C-5), 111.52, 112.61, 120.58, 129.63, 149.22, 149.47 (C_{arom}), 148.20 (C-6), 151.41 (C-2), 164.99 (C-4); EI MS *m/z* (%): 290 [M$^+$] (25).

Ethyloxymethyl and benzyloxymethyluracil derivatives ***5a–d*** *and* ***6a, b***

N,O-Bis-(trimethylsilyl)acetamide (BSA) (0.87 mL, 0.0035 mol) was added to a suspension of **4a–d** (0.001 mol) in anhydrous $CHCl_3$ (20 mL) and the mixture was stirred at room temperature under nitrogen. After a clear solution was obtained (10 min), chloromethyl ethyl ether and / or benzyl chloromethyl ether (0.015 mol) followed by CsI (0.26 g, 0.001 mol) were added. The reaction mixture was stirred at room temperature under nitrogen for 3–5 h. Saturated aq. $NaHCO_3$ (20 mL) was added and the mixture was extracted with CH_2Cl_2 (3 × 50 mL). The organic phase was collected, dried ($MgSO_4$), and evaporated under reduced pressure. The residue was chromatographed on silica gel column using $CHCl_3$ to give the corresponding *N*-1 alkylated **5a–d** and 1,3-bis-alkylated uracils **6a**, **b**.

5-Ethyl-1-ethyloxymethyl-6-(4-methylbenzyl)uracil ***5a***

White solid; yield: 115 mg (38%); m.p.: 137–138°C; ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 1.07 (t, J = 7.5 Hz, 3H, CH_3), 1.18 (t, J = 7.0 Hz, 3H, CH_3), 2.34 (s, 3H, CH_3), 2.47 (q, J = 7.5 Hz, 2H, CH_2), 3.58 (q, J = 7.0 Hz, 2H, CH_2), 4.13 (s, 2H, CH_2), 5.13 (s, 2H, CH_2), 7.01, 7.03 (2 × d, J = 8.0 Hz, 4H, H_{arom}), 9.68 (s, 1H, NH); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 13.75 (CH_3), 15.03 (CH_3), 19.16 (CH_2), 20.94 (CH_3), 33.04 (CH_2), 64.97 (CH_2), 72.71 (CH_2), 116.79 (C-5), 127.20, 129.87, 132.16, 136.98 (C_{arom}), 149.40 (C-6), 151.99 (C-2), 163.40 (C-4); MS (MALDI) *m/z* (%): 325 [M$^+$ + Na] (82). Anal. calcd. for $C_{17}H_{22}N_2NaO_3$: 325.1523. Found: 325.1514.

5-Ethyl-1-ethyloxymethyl-6-(3,4-dimethoxybenzyl)uracil ***5b***

White solid; yield: 143 mg (41%); m.p.: 89–91°C; ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 1.08 (t, J = 7.5 Hz, 3H, CH_3), 1.18 (t, J = 7.0 Hz, 3H, CH_3), 2.48 (q, J = 7.5 Hz, 2H, CH_2), 3.61 (q, J = 7.0 Hz, 2H, CH_2), 3.86, 3.87 (2 × s, 6H, 2 × OCH_3), 4.10 (s, 2H, CH_2), 5.14 (s, 2H, CH_2), 6.61, 6.80 (2 × d, J = 8.2 Hz, 2H, H_{arom}), 6.67 (s, 1H, H_{arom}), 9.78 (s, 1H, NH); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 13.81 (CH_3), 15.05 (CH_3), 19.15 (CH_2), 21.40 (CH_3), 32.97 (CH_2), 55.97 (OCH_3), 56.00 (OCH_3), 65.02 (CH_2), 72.68 (CH_2), 116.79 (C-5), 110.91, 111.87, 119.19, 127.56, 148.44, 149.68 (C_{arom}), 149.32 (C-6), 152.01 (C-2), 163.42 (C-4); MS (MALDI) *m/z* (%): 371 [M$^+$ + Na] (39). Anal. calcd. for $C_{18}H_{24}N_2NaO_5$: 371.1577. Found: 371.1577.

1-Benzyloxymethyl-5-ethyl-6-(4-methylbenzyl)uracil ***5c***

White solid; yield: 237 mg (65%); m.p.: 94–95°C; ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 1.07 (t, J = 7.5 Hz, 3H, CH_3), 2.36 (s, 3H, CH_3), 2.47 (q, J = 7.5 Hz, 2H, CH_2), 4.13 (s, 2H, CH_2), 4.68 (s, 2H, CH_2), 5.23 (s, 2H, CH_2), 6.97–7.41 (m, 9H, H_{arom}), 9.78 (s, 1H, NH); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 13.79 (CH_3), 19.16 (CH_2), 20.97 (CH_3), 33.08 (CH_2), 71.79 (CH_2), 72.83 (CH_2), 116.88 (C-5), 127.24, 127.82, 128.48, 129.99, 131.96, 132.05, 137.42, 141.08 (C_{arom}), 149.24 (C-6), 152.04 (C-2), 163.37 (C-4); MS (MALDI) *m/z* (%): 387 [M$^+$ + Na] (94). Anal. calcd. for $C_{22}H_{24}N_2NaO_3$: 387.1679. Found: 387.1668.

1-Benzyloxymethyl-5-ethyl-6-(3,4-dimethoxybenzyl)uracil ***5d***

White solid; yield: 284 mg (69%); m.p.: 131–133°C; ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 1.08 (t, J = 7.5 Hz, 3H, CH_3), 2.47 (q, J = 7.5 Hz, 2H, CH_2), 3.85, 3.87 (2 × s, 6H, 2 × OCH_3), 4.11 (s, 2H, CH_2), 4.68 (s, 2H, CH_2), 5.25 (s, 2H, CH_2), 6.57–7.38 (m, 8H, H_{arom}), 9.51 (s, 1H, NH); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 13.85 (CH_3), 19.15 (CH_2), 33.00 (CH_2), 55.97 (OCH_3), 56.00 (OCH_3), 71.86 (CH_2), 72.84 (CH_2), 116.85 (C-5), 110.86, 111.83, 119.18, 127.38, 127.78, 128.00, 128.48, 137.39, 148.46, 149.67 (C_{arom}), 149.16 (C-6), 151.93 (C-2), 163.19 (C-4); MS (MALDI) *m/z* (%): 433 [M$^+$ + Na] (96). Anal. calcd. for $C_{23}H_{26}N_2NaO_5$: 433.1734. Found: 433.1716.

1,3-Bis-(ethyloxymethyl)-5-ethyl-6-(4-methylbenzyl)uracil ***6a***

Colourless viscous oil, yield: 94 mg (26%); ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 1.07 (t, J = 7.0 Hz, 3H, CH_3), 1.19 (t, J = 7.0 Hz, 3H, CH_3), 1.24 (t, J = 7.0 Hz, 3H, CH_3), 2.34 (s, 3H, CH_3), 2.48 (q, J = 7.0 Hz, 2H, CH_2), 3.60 (q, J = 7.0 Hz, 2H, CH_2), 3.70 (q, J = 7.0 Hz, 2H, CH_2), 4.12 (s, 2H, CH_2), 5.14 (s, 2H, CH_2), 5.48 (s, 2H, CH_2), 7.01, 7.14 (2 × d, J = 8.0 Hz, 4H, H_{arom}); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 13.70 (CH_3), 15.03 (CH_3), 15.20 (CH_3), 19.74 (CH_2), 20.94 (CH_3), 33.11 (CH_2), 65.04 (CH_2), 65.90 (CH_2), 71.14 (CH_2), 73.49 (CH_2), 116.08 (C-5), 127.22, 129.86, 132.18, 136.97 (C_{arom}), 148.09 (C-6), 152.63 (C-2), 162.75 (C-4); EI MS *m/z* (%): 360 [M$^+$] (9).

1,3-Bis-(ethyloxymethyl)-5-ethyl-6-(3,4-dimethoxybenzyl)uracil ***6b***

Colourless viscous oil, yield: 93 mg (23%); ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 1.07 (t, *J* = 7.5 Hz, 3H, CH_3), 1.15–1.24 (m, 6H, 2 × CH_3), 2.48 (q, *J* = 7.5 Hz, 2H, CH_2), 3.48 (q, *J* = 7.0 Hz, 2H, CH_2), 3.68 (q, *J* = 7.0 Hz, 2H, CH_2), 3.84, 3.85 (s, 6H, 2 × OCH_3), 4.08 (s, 2H, CH_2), 5.15 (s, 2H, CH_2), 5.47 (s, 2H, CH_2), 6.59–6.81 (m, 3H, H_{arom}); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 13.75 (CH_3), 15.00 (CH_3), 15.05 (CH_3), 19.73 (CH_2), 33.03 (CH_2), 55.96 (OCH_3), 55.99 (OCH_3), 65.09 (CH_2), 65.88 (CH_2), 71.13 (CH_2), 73.45 (CH_2), 116.06 (C-5), 110.97, 111.83, 119.17, 127.57, 148.03, 148.43 (C_{arom}), 149.65 (C-6), 152.60 (C-2), 162.73 (C-4); EI MS *m/z* (%): 406 [M^+] (13).

Bis-(cyclopropylmethyloxy)methane ***8a*** *and bis-(3-phenylprop-1-yloxy)methane* ***8c***

Cyclopropylcarbinol (**7a**, 7.2 g, 0.1 mol) and / or 3-phenyl-1-propanol (**7c**, 13.6 g, 0.1 mol), dibromomethane (8.79 g, 0.0505 mol), and tetrabutylammonium bromide (1.74 g, 0.00535 mol) were added to potassium hydroxide (5.66 g, 0.101 mol) in anhydrous benzene (30 mL), and the suspension was heated under reflux for 5 h. After cooling, H_2O (50 mL) was added and the resulting solution was extracted with ether (3 × 50 mL). The ether phase was dried with anhydrous $MgSO_4$ and evaporated under reduced pressure to afford **8a** and **8c** as colourless oils. As determined from NMR, **8a** was contaminated with the starting material **7a** in a 5 : 2 ratio. The acetal **8a** was used for further synthesis without purification, while **8c** was purified on a silica gel column using petrol ether / $CHCl_3$ (1 : 1).

Bis-(cyclopropylmethyloxy)methane ***8a***

Colourless oil, yield: 4.4 g (56%); ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 0.55–0.58 (m, 8H, 4 × CH_2), 1.07–1.14 (m, 2H, 2 × CH), 3.46 (d, *J* = 7.0 Hz, 4H, 2 × CH_2), 4.83 (s, 2H, OCH_2O); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 2.65, 10.47 ($C_{cycloprpyl}$), 72.41 (CH_2), 94.64 (OCH_2O); EI MS *m/z* (%): 156 [M^+] (12).

Bis-(3-phenylprop-1-yloxy)methane ***8c***

Colourless oil, yield: 8.9 g (63%); ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 1.97–2.03 (m, 4H, 2 × CH_2), 2.77 (t, *J* = 7.5 Hz, 4H, 2 × CH_2), 3.64 (t, *J* = 6.5 Hz, 4H, 2 × CH_2), 4.78 (s, 2H, OCH_2O), 7.25–7.38 (m, 10H, H_{arom}); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 31.45 (CH_2), 32.51 (CH_2), 67.19 (CH_2), 95.48 (OCH_2O), 125.87, 128.40, 128.49, 141.94 (C_{arom}); EI MS *m/z* (%): 284 [M^+] (9).

1-Cyclopropylmethyloxymethyl-5-ethyluracils ***9a–d***

Compounds **4a–d** (1 mmol) were stirred in anhydrous CH_3CN (15 mL) under nitrogen and BSA (0.87 mL, 3.5 mol) was added. After a clear solution was obtained (10 min), the reaction mixture was cooled to −50°C and TMS triflate (0.18 mL, 1 mmol) was added followed by dropwise addition of bis-(cyclopropylmethyloxy)methane **8a** (312 mg, 2 mmol). The mixture was stirred at room temperature for 3–4 h. The reaction was quenched with sat. aq. $NaHCO_3$ solution (5 mL) and evaporated under reduced pressure. The residue was extracted with ether (3 × 50 mL), the combined organic fractions were dried ($MgSO_4$) and evaporated under reduced pressure. The residue was chromatographed on silica gel column with $CHCl_3$ to afford the corresponding nonnucleosides **9a–d**.

6-Benzyl-1-cyclopropylmethyloxymethyl-5-ethyluracil ***9a***

White solid; yield: 189 mg (60%); m.p.: 91–92°C; ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 0.21–0.22, 0.52–0.54 (2 × m, 4H, 2 × CH_2, $H_{cyclopropyl}$), 1.06 (t, *J* = 7.0 Hz, 3H, CH_3), 1.09–1.11 (m, 1H, CH, $H_{cyclopropyl}$), 2.48 (q, *J* = 7.0 Hz, 2H, CH_2), 3.40 (d, *J* = 7.0 Hz, 2H, CH_2), 4.20 (s, 2H, CH_2), 5.16 (s, 2H, CH_2), 7.13–7.36 (m, 5H, H_{arom}), 9.97 (s, 1H, NH); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 3.03, 13.74 ($C_{cyclopropyl}$), 10.47 (CH_3), 18.88 (CH_2), 33.41 (CH_2), 72.71 (CH_2), 74.09 (CH_2), 116.98 (C-5), 127.27, 127.34, 129.20, 135.33 (C_{arom}), 149.13 (C-6), 152.04 (C-2), 163.49 (C-4); MS (MALDI) *m/z* (%): 337 [M^+ + Na] (46). Anal. calcd. for $C_{18}H_{22}N_2NaO_3$: 337.1523. Found: 337.1534.

1-Cyclopropylmethyloxymethyl-5-ethyl-6-(4-methylbenzyl)uracil ***9b***

White solid; yield: 211 mg (64%); m.p.: 126–128°C; ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 0.12–0.24, 0.53–0.56 (2 × m, 4H, 2 × CH_2, $H_{cyclopropyl}$), 1.03–1.06 (m, 1H, CH, $H_{cyclopropyl}$), 1.07 (t, *J* = 7.5 Hz, 3H, CH_3), 2.35 (s, 3H, CH_3), 2.47 (q, *J* = 7.5 Hz, 2H, CH_2), 3.41 (d, *J* = 7.0 Hz, 2H, CH_2), 4.16 (s, 2H, CH_2), 5.16 (s, 2H, CH_2), 7.02, 7.15 (2 × d, *J* = 8.0 Hz, 4H, H_{arom}), 9.51 (s, 1H, NH); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 3.03, 13.76 ($C_{cyclopropyl}$), 10.47 (CH_3), 19.16 (CH_2), 20.95 (CH_3), 33.03 (CH_2), 72.70 (CH_2), 74.10 (CH_2), 116.80 (C-5), 127.22, 129.88, 132.13, 137.00 (C_{arom}), 149.44 (C-6), 151.90 (C-2), 163.31 (C-4); EI MS *m/z* (%): 328 [M^+] (5).

1-Cyclopropylmethyloxymethyl-5-ethyl-6-(3,5-dimethylbenzyl)uracil ***9c***

White solid; yield: 212 mg (62%); m.p.: 132–133°C; ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 0.23–0.25, 0.53–0.56 (2 × m, 4H, 2 × CH_2, $H_{cyclopropyl}$), 1.05–1.07 (m, 1H, CH, $H_{cyclopropyl}$), 1.08 (t, *J* = 7.5 Hz, 3H, CH_3), 2.30 (s, 6H, 2 × CH_3), 2.48 (q, *J* = 7.5 Hz, 2H, CH_2), 3.41 (d, *J* = 7.5 Hz, 2H, CH_2), 4.13 (s, 2H, CH_2), 5.17 (s, 2H, CH_2), 6.73 (s, 2H, H_{arom}), 6.92 (s, 1H, H_{arom}), 9.60 (s, 1H, NH); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 3.04, 13.75 ($C_{cyclopropyl}$), 10.49 (CH_3), 19.17 (CH_2), 21.27 (CH_3), 33.23 (CH_2), 72.74 (CH_2), 74.10 (CH_2), 116.82 (C-5), 125.05, 128.93, 135.04, 138.85 (C_{arom}), 149.46 (C-6), 151.46 (C-2), 163.41 (C-4); MS (MALDI) *m/z* (%): 365 [M^+ + Na] (48). Anal. calcd. for $C_{20}H_{26}N_2NaO_3$: 365.1836. Found: 365.1850.

1-Cyclopropylmethyloxymethyl-5-ethyl-6-(3,4-dimethoxybenzyl)uracil ***9d***

White solid; yield: 252 mg (67%); m.p.: 97–98°C; ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 0.22–0.24, 0.53–0.56 (2 × m, 4H, 2 × CH_2, $H_{cyclopropyl}$), 1.05–1.07 (m, 1H, CH, $H_{cyclopropyl}$), 1.09 (t, *J* = 7.5 Hz, 3H, CH_3), 2.48 (q, *J* = 7.5 Hz, 2H, CH_2), 3.41 (d, *J* = 7.0 Hz, 2H, CH_2), 3.87 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 4.14 (s, 2H, CH_2), 5.17 (s, 2H, CH_2), 6.61, 6.81 (2 × d, *J* = 8.0 Hz, 2H, H_{arom}), 6.68 (s, 1H, H_{arom}), 9.33 (s, 1H, NH); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 3.05, 13.83 ($C_{cyclopropyl}$), 10.48 (CH_3), 19.17 (CH_2), 32.96 (CH_2), 55.97 (OCH_3), 56.00 (OCH_3), 72.68 (CH_2), 74.17 (CH_2), 116.78 (C-5), 110.90, 111.82, 119.16, 127.48, 148.44, 149.40 (C_{arom}), 149.66 (C-6), 151.82 (C-2), 163.23 (C-4); MS (MALDI) *m/z* (%): 397 [M^+ + Na] (11). Anal. calcd. for $C_{20}H_{26}N_2NaO_5$: 397.1734. Found: 397.1728.

1-(2-Phenylethyloxymethyl)uracil derivatives ***9e–h***

Compounds **4a–d** (1 mmol) were stirred in anhydrous CH_3CN (15 mL) under nitrogen and BSA (0.87 mL, 3.5 mol) was added. After a clear solution was obtained (10 min), the reaction mixture was cooled to −50°C and TMS triflate (0.18 mL, 1 mmol) was added followed by dropwise addition of bis-(2-phenylethyloxy)-

methane **8b** (512 mg, 2 mmol). The reaction mixture was stirred at room temperature for 3–5 h and the mixture was worked up as described in preparation of compounds **9a–d** to give the non-nucleosides **9e–h**.

6-Benzyl-5-ethyl-1-(2-phenylethyloxymethyl)uracil ***9e***

White solid; yield: 214 mg (59%); m.p.: 96–97°C; ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 1.04 (t, *J* = 7.0 Hz, 3H, CH_3), 2.44 (q, *J* = 7.0 Hz, 2H, CH_2), 2.86 (t, *J* = 6.5 Hz, 2H, CH_2), 3.82 (t, *J* = 6.5 Hz, 2H, CH_2), 3.98 (s, 2H, CH_2), 5.11 (s, 2H, CH_2), 7.07–7.34 (m, 10H, H_{arom}), 9.43 (s, 1H, NH); ^{13}C-NMR ($CDCl_3$, 125 MHz) *d*: 13.71 (CH_3), 19.16 (CH_2), 33.20 (CH_2), 36.06 (CH_2), 70.07 (CH_2), 72.83 (CH_2), 126.37, 127.26, 127.30, 128.38, 128.88, 129.20, 135.30, 138.65 (C_{arom}), 149.08 (C-6), 151.89 (C-2), 163.19 (C-4); MS (MALDI) *m/z* (%): 387 [M^+ + Na] (94). Anal. calcd. for $C_{22}H_{24}N_2NaO_3$: 387.1679. Found: 387.1693.

5-Ethyl-6-(4-methylbenzyl)-1-(2-phenylethyloxymethyl)uracil ***9f***

Colourless viscous oil; yield: 198 mg (52%); ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 1.05 (t, *J* = 7.0 Hz, 3H, CH_3), 2.35 (s, 3H, CH_3), 2.42 (q, *J* = 7.0 Hz, 2H, CH_2), 2.86 (t, *J* = 6.5 Hz, 2H, CH_2), 3.81 (t, *J* = 6.5 Hz, 2H, CH_2), 3.94 (s, 2H, CH_2), 5.11 (s, 2H, CH_2), 6.95, 7.14 (2 × d, *J* = 8.0 Hz, 4H, H_{arom}), 7.21–7.32 (m, 5H, H_{arom}), 9.47 (s, 1H, NH); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 13.73 (CH_3), 19.15 (CH_2), 20.95 (CH_3), 32.82 (CH_2), 36.07 (CH_2), 70.03 (CH_2), 72.80 (CH_2), 116.74 (C-5), 126.44, 127.19, 128.37, 128.72, 129.86, 132.13, 136.96, 138, 65 (C_{arom}), 149.37 (C-6), 151.91 (C-2), 163.25 (C-4); EI MS *m/z* (%): 378 [M^+] (4).

5-Ethyl-6-(3,5-dimethylbenzyl)-1-(2-phenylethyloxymethyl)uracil ***9g***

White solid; yield: 201 mg (51%); m.p.: 123–125°C; ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 1.06 (t, *J* = 7.0 Hz, 3H, CH_3), 2.30 (s, 6H, 2 × CH_3), 2.43 (q, *J* = 7.0 Hz, 2H, CH_2), 2.87 (t, *J* = 6.5 Hz, 2H, CH_2), 3.83 (t, *J* = 6.5 Hz, 2H, CH_2), 3.92 (s, 2H, CH_2), 5.13 (s, 2H, CH_2), 6.66 (s, 2H, H_{arom}), 6.91 (s, 1H, H_{arom}), 7.21–7.33 (m, 5H, H_{arom}), 9.66 (s, 1H, NH); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 13.72 (CH_3), 19.16 (CH_2), 21.27 (CH_3), 33.05 (CH_2), 36.09 (CH_2), 70.09 (CH_2), 72.85 (CH_2), 116.75 (C-5), 125.02, 126.36, 128.38, 128.89, 135.06, 138.68, 138.84 (C_{arom}), 149.40 (C-6), 152.00 (C-2), 163.39 (C-4); MS (MALDI) *m/z* (%): 415 [M^+ + Na] (97). Anal. calcd. for $C_{24}H_{28}N_2NaO_3$: 415.1992. Found: 415.2001.

5-Ethyl-6-(3,4-dimethoxybenzyl)-1-(2-phenylethyloxymethyl)uracil ***9h***

White solid; yield: 198 mg (47%); m.p.: 109–111°C; ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 1.06 (t, *J* = 7.5 Hz, 3H, CH_3), 2.44 (q, *J* = 7.5 Hz, 2H, CH_2), 2.86 (t, *J* = 6.5 Hz, 2H, CH_2), 3.82 (t, *J* = 6.5 Hz, 2H, CH_2), 3.86 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 3.92 (s, 2H, CH_2), 5.12 (s, 2H, CH_2), 6.55, 6.79 (2 × d, *J* = 8.0 Hz, 2H, H_{arom}), 6.59 (s, 1H, H_{arom}), 7.20–7.31 (m, 5H, H_{arom}), 9.31 (s, 1H, NH); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 13.81 (CH_3), 19.15 (CH_2), 32.75 (CH_2), 36.08 (CH_2), 55.98 (OCH_3), 56.00 (OCH_3), 70.11 (CH_2), 72.76 (CH_2), 116.71 (C-5), 110.86, 111.82, 119.14, 126.37, 127.50, 128.37, 128.87, 138.66, 148.41, 149.29 (C_{arom}), 149.64 (C-6), 151.86 (C-2), 163.17 (C-4); MS (MALDI) *m/z* (%): 447 [M^+ + Na] (97). Anal. calcd. for $C_{24}H_{28}N_2NaO_5$: 447.1890. Found: 447.1904.

1-(3-Phenylprop-1-yloxymethyl)uracil derivatives ***9i–l***

Compounds **4a–d** (1 mmol) were stirred in anhydrous CH_3CN (15 mL) under nitrogen and BSA (0.87 mL, 3.5 mol) was added. After a clear solution was obtained (10 min), the reaction mixture was cooled to −50°C and TMS triflate (0.18 mL, 1 mmol) was added followed by dropwise addition of bis-(3-phenylprop-1-yloxy)methane **8c** (568 mg, 2 mmol). The reaction mixture was stirred at room temperature for 4–5 h and the mixture was worked up as described in preparation of compounds **9a–d** to give the non-nucleosides **9i–l**.

6-Benzyl-5-ethyl-1-(3-phenylprop-1-yloxymethyl)uracil ***9i***

Colourless viscous oil; yield: 185 mg (49%); ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 1.10 (t, *J* = 7.0 Hz, 3H, CH_3), 1.86 (m, 2H, CH_2), 2.50 (q, *J* = 7.5 Hz, 2H, CH_2), 2.67 (t, *J* = 6.5 Hz, 2H, CH_2), 3.58 (t, *J* = 6.0 Hz, 2H, CH_2), 4.20 (s, 2H, CH_2), 5.14 (s, 2H, CH_2), 7.14–7.39 (m, 10H, H_{arom}), 9.44 (s, 1H, NH); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 13.77 (CH_3), 19.21 (CH_2), 30.99 (CH_2), 32.16 (CH_2), 33.44 (CH_2), 68.62 (CH_2), 72.99 (CH_2), 116.99 (C-5), 125.93, 127.35, 128.38, 128.40, 129.26, 135.26, 141.46 (C_{arom}), 149.09 (C-6), 151.86 (C-2), 163.23 (C-4); MS (MALDI) *m/z* (%): 401 [M^+ + Na] (95). Anal. calcd. for $C_{23}H_{26}N_2NaO_3$: 401.1836. Found: 401.1845.

5-Ethyl-6-(4-methylbenzyl)-1-(3-phenylprop-1-yloxymethyl)uracil ***9j***

Colourless viscous oil; yield: 174 mg (44%); ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 1.11 (t, *J* = 7.5 Hz, 3H, CH_3), 1.88 (m, 2H, CH_2), 2.37 (s, 2H, CH_2), 2.51 (q, *J* = 7.5 Hz, 2H, CH_2), 2.68 (t, *J* = 6.5 Hz, 2H, CH_2), 3.59 (t, *J* = 6.0 Hz, 2H, CH_2), 4.16 (s, 2H, CH_2), 5.16 (s, 2H, CH_2), 7.03–7.32 (m, 9H, H_{arom}), 9.84 (s, 1H, NH); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 13.81 (CH_3), 19.20 (CH_2), 20.98 (CH_3), 31.03 (CH_2), 32.16 (CH_2), 33.05 (CH_2), 68.60 (CH_2), 72.97 (CH_2), 116.88 (C-5), 125.92, 127.25, 128.38, 128.41, 129.93, 132.14, 137.04, 141.50 (C_{arom}), 149.38 (C-6), 152.02 (C-2), 163.48 (C-4); EI MS *m/z* (%): 392 [M^+] (3).

5-Ethyl-6-(3,5-dimethylbenzyl)-1-(3-phenylprop-1-yloxymethyl)uracil ***9k***

White solid; yield: 209 mg (51%); m.p.: 110–112°C; ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 1.11 (t, *J* = 7.5 Hz, 3H, CH_3), 1.89 (m, 2H, CH_2), 2.32 (s, 6H, 2 × CH_3), 2.50 (q, *J* = 7.5 Hz, 2H, CH_2), 2.68 (t, *J* = 6.5 Hz, 2H, CH_2), 3.59 (t, *J* = 6.0 Hz, 2H, CH_2), 4.12 (s, 2H, CH_2), 5.16 (s, 2H, CH_2), 6.74 (s, 2H, H_{arom}), 6.93 (s, 1H, H_{arom}), 7.18–7.32 (m, 5H, H_{arom}), 9.54 (s, 1H, NH); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 13.77 (CH_3), 19.21 (CH_2), 21.28 (CH_3), 31.01 (CH_2), 32.16 (CH_2), 33.27 (CH_2), 68.59 (CH_2), 73.01 (CH_2), 116.86 (C-5), 125.05, 125.92, 128.38, 128.40, 128.98, 135.04, 138.90, 141.48 (C_{arom}), 149.38 (C-6), 151.95 (C-2), 163.37 (C-4); MS (MALDI) *m/z* (%): 429 [M^+ + Na] (96). Anal. calcd. for $C_{25}H_{30}N_2NaO_3$: 429.2150. Found: 429.2131.

5-Ethyl-6-(3,4-dimethoxybenzyl)-1-(3-phenylprop-1-yloxymethyl)uracil ***9l***

Colourless viscous oil; yield: 207 mg (47%); ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 1.11 (t, *J* = 7.5 Hz, 3H, CH_3), 1.88 (m, 2H, CH_2), 2.51 (q, *J* = 7.5 Hz, 2H, CH_2), 2.67 (t, *J* = 6.5 Hz, 2H, CH_2), 3.59 (t, *J* = 6.0 Hz, 2H, CH_2), 3.87 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 4.12 (s, 2H, CH_2), 5.16 (s, 2H, CH_2), 6.62, 6.82 (2 × d, *J* = 8.0 Hz, 2H, H_{arom}), 6.68 (s, 1H, H_{arom}), 7.16–7.30 (m, 5H, H_{arom}), 9.55 (s 1H, NH); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 13.86 (CH_3), 19.19 (CH_2), 30.99 (CH_2), 32.16 (CH_2), 32.98 (CH_2), 55.98 (OCH_3), 56.02 (OCH_3), 116.82 (C-5), 110.89,

111.87, 119.19, 125.93, 127.47, 128.38, 141.43, 148.47, 149.70 (C_{arom}), 149.32 (C-6), 151.89 (C-2), 163.32 (C-4); MS (MALDI) *m/z* (%): 461 [M^+ + Na] (93). Anal. calcd. for $C_{25}H_{30}N_2NaO_5$: 461.2047. Found: 461.2041.

Antiviral-assay procedures

Compounds were solubilized in DMSO at 100 mM and then diluted in culture medium. Cells and viruses: MT-4, C8166, and H9/IIIB cells were grown at 37°C in a 5% CO_2 atmosphere in RPMI 1640 medium supplemented with 10% fetal calf serum (FCS), 100 IU/mL penicillin G, and 100 μg/mL streptomycin. Cell cultures were checked periodically for the absence of mycoplasma contamination with a MycoTect Kit (Gibco). Human immunodeficiency viruses type 1 (HIV-1, IIIB strain) was obtained from supernatants of persistently infected H9/IIIB cells. The HIV-1 stock solutions had titers of 4.5×10^6 50% cell culture infectious dose ($CCID_{50}$)/mL. The Y181C mutant (NIH N119) was derived from an AZT-sensitive clinical isolate passaged initially in CEM and then in MT-4 cells in the presence of nevirapine (10 μM). The double mutant K103N + Y181C (NIH A17) was derived from the IIIB strain passaged in H9 cells in the presence of BI-RG 587 (1 μM). The triple mutant K103R + V179D + P225H (EFV^R) was derived from an IIIB strain passaged in MT-4 cells in the presence of efavirenz (up to 2 μM). N119, A17, and EFV^R stock solutions had titers of 1.2×10^8, 2.1×10^7, and 4.0×10^7 $CCID_{50}$/mL, respectively.

HIV titration: Titration of HIV was performed in C8166 cells by the standard limiting dilution method (dilution 1 : 2, four replica wells per dilution) in 96-well plates. The infectious-virus titer was determined by light microscope scoring of syncytia after four days of incubation. Virus titers were expressed as $CCID_{50}$/mL.

Anti-HIV assays

The activity of test compounds against multiplication of HIV-1 wild type III_B, N119, A17, and EFV^R in acutely infected cells, was based on inhibition of virus-induced cytopathogenicity in MT-4 cells. Briefly, an amount of 50 μL of culture medium containing 2×10^4 cells was added to each well of flat-bottom microtiter trays containing 50 μL of culture medium with or without various concentrations of test compounds. Then, an amount of 20 μL of HIV suspensions, containing the appropriate amount of $CCID_{50}$ to cause complete cytopathogenicity at day 4, was added. After incubation at 37°C, cell viability was determined by the 3-(4,5-dimethylthiazol-1-yl)-2,5-diphenyltetrazolium bromide (MTT) method [31]. The cytotoxicity of test compounds was evaluated in parallel with their antiviral activity and was based on the viability of mock-infected cells, as monitored by the MTT method.

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