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# Synthesis of *N*-1-(Indanyloxymethyl) and *N*-1-(4-Hydroxybut-2-enyloxymethyl) Analogues of the HIV Drugs Emivirine and GCA-186

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**Summary.** A series of Emivirine and GCA-186 analogues substituted at N-1 with indan-1-yloxymethyl (**6a–6c**) and indan-2-yloxymethyl (**6d–6f**) were synthesized by reaction of the corresponding bis(indanyloxy)methans with uracils having 5-ethyl or 5-isopropyl and 6-benzyl or 6-(3,5-dimethylbenzyl) substituents. A route to the corresponding *N*-1 substituted 4-hydroxybut-2-enyloxymethyl analogue was also devised. All newly synthesized compounds showed potent activity against wild-type HIV-1, the most active compound being 5-ethyl-1-(indan-1-yloxymethyl)-6-(3,5-dimethylbenzyl)uracil (**6b**), which was 50-fold more active than Emivirine.

**Keywords.** Bioorganic chemistry; Drug research; HIV; Non-nucleoside reverse transcriptase inhibitors; Emivirine analogues.

## Introduction

Effective treatment regimens for the human immunodeficiency virus (HIV-1) infection have included both HIV protease and reverse transcriptase inhibitors (RTIs). The non-nucleoside reverse transcriptase inhibitors (NNRTIs) in contrast to nucleoside reverse transcriptase inhibitors (NRTIs) such as *AZT* [1], *ddC* [2], *ddI* [3], 3*TC* [3], are highly specific as their binding site is a hydrophobic pocket located approximately 10 Å from the polymerase active site [4]. NNRTIs

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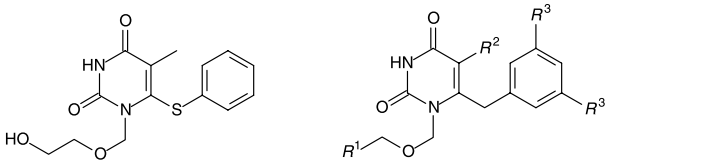


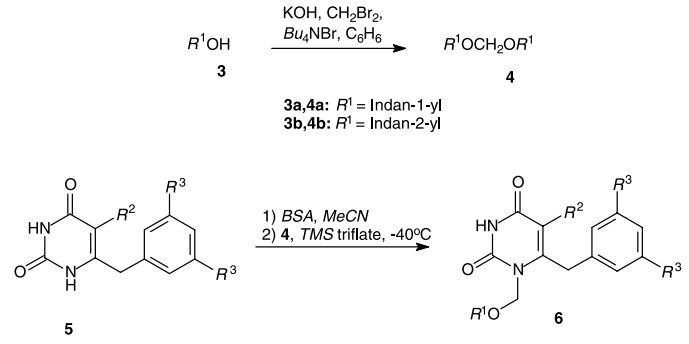

**Fig. 1.** Chemical structure of *HEPT* (**1**), MKC-442, TNK-651, and GCA-186

consist of many classes of compounds [5] and among them the most potent anti-HIV agents are found. One of the first NNRTIs was 1-[(2-hydroxyethoxy)methyl]-6-(phenylthio)thymine (*HEPT*, **1**, Fig. 1) [6, 7]. Although *HEPT* did not show very high activity against HIV-1, it was considered an interesting lead compound for the synthesis of new analogues, among them 6-benzyl-1-(ethoxymethyl)-5-isopropyluracil (Emivirine, formerly MKC-442, **2a**, Fig. 1) [8], the corresponding 1-benzyloxymethyl analogue (TNK-651, **2b**, Fig. 1) [4], and the 6-(3,5-dimethylbenzyl) analogue (GCA-186, **2c**, Fig. 1) [9] which all showed high activity against HIV-1.

Emivirine was chosen as a drug candidate for clinical trials [10] by Triangle Pharmaceuticals, but phase III was abandoned when comparative studies showed Emivirine to be less potent than other HIV inhibitors [11]. It was stated that Emivirine triggers the liver enzyme Cytochrome P 450, which metabolizes protease inhibitors [12]. According to structure activity relationship (SAR), studies of several crystal structures of the reverse transcriptase (RT) complex with inhibitors, such as *HEPT* [13], MKC-442 [4], TNK-651 [4], and GCA-186 [9], indicate that ethyl or isopropyl group at C-5, and benzyl or (3,5-dimethylbenzyl) group at C-6 are the optimal substituents of the uracil ring with respect to HIV-1 inhibition. The only site where new modifications may be tried is the N-1 position of the uracil [14]. The substituents at N-1 may have larger size and length, and even bulky N-1 substituents may be accommodated because of the flexibility of the Pro 236 loop region [9, 14]. Recently, we have synthesized a series of novel active Emivirine analogues [15–17]. The present work reports a series of novel Emivirine and GCA-186 analogues modified at N-1 position with larger bulky substituents.

## Results and Discussions

Bis(indanyloxy)methanes (**4a**, **4b**) [17, 18] were synthesized according to the method of *Nazaretyan et al.* [19] by refluxing the corresponding indanols **3**, dibromoethane, potassium hydroxide, and tetrabutylammonium bromide in anhydrous benzene (Scheme 1). 5,6-Disubstituted uracils **5a**–**5c** were prepared according to the procedure described by *Danel et al.* [20] from the corresponding 2-alkyl-4-aryl-3-oxo esters which in turn were prepared by reaction of phenylacetonitrile or (3,5-dimethylphenyl)acetonitrile with the appropriate $\alpha$-bromo esters in *THF* in the presence of activated zinc. The so-formed 3-oxo esters were condensed with thiourea to give the corresponding 2-thiouracils which were desulfurized with aqueous chloroacetic acid to furnish the required uracils **5a**–**5c**. The uracil derivatives **5a**–**5c** were silylated with *N,O*-bis-(trimethylsilyl)acetamide (*BSA*) in




| 5,6 | $R^1$ | $R^2$ | $R^3$ |
|---|---|---|---|
| a |  | $(CH_3)_2CH$ | H |
| b | | $CH_3CH_2$ | $CH_3$ |
| c | | $(CH_3)_2CH$ | $CH_3$ |
| d |  | $(CH_3)_2CH$ | H |
| e | | $CH_3CH_2$ | $CH_3$ |
| f | | $(CH_3)_2CH$ | $CH_3$ |

**Scheme 1**

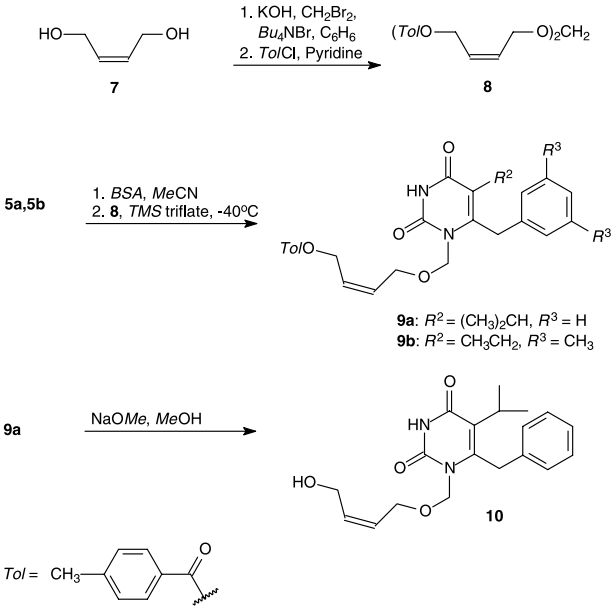
acetonitrile and alkylated at the N-1 position with bis(indan-1-yloxy)methane (**4a**) and bis(indan-2-yloxy)methane (**4b**) in the presence of trimethylsilyl trifluoromethanesulfonate (*TMS* triflate) to afford the corresponding Emivirine and GCA-186 analogues **6a**–**6f** in 51–71% yields (Scheme 1).

Compounds **6a**–**6c** are also regarded as TNK-651 analogues with an ethylene link between $CH_2$ group of Ph$CH_2$O and C-2 of the benzene ring, whereas **6d**–**6f** are TNK-651 analogues with an extra methylene group.

Another type of acetal for derivatizing the uracils was synthesized by reaction of *cis*-but-2-enediol (**7**) with dibromomethane in the presence of potassium hydroxide and tetrabutylammonium bromide in boiling dry benzene. Treatment of the product with *p*-toluoyl chloride in pyridine gave bis(4-*p*-toluoyloxy-*cis*-but-2-en-1-yloxy)methane (**8**) in 22% overall yield (Scheme 2). The uracils **5a** and **5b** were silylated with *BSA* in acetonitrile and treated with **8** in the presence of *TMS* triflate to afford the corresponding *N*-1-(4-*p*-toluoyloxy-*cis*-but-2-en-1-yloxymethyl) derivatives **9a** and **9b** in 71% and 68% yields (Scheme 2). Compounds **9a** and **9b** are interesting new Emivirine analogues with a large N-1 substituent. The N-3 regioisomer was neither isolated nor observed.

We found it of importance to study the activity when a bulky group, such as toluoyl moiety, is located distantly from the N-1 position of the uracil. Also we found it of interest to prepare an example of the corresponding detoluoylated analogue. Removal of toluoyl group from **9a** was achieved by its treatment with sodium methoxide in anhydrous methanol to furnish the corresponding 1-(4-hydroxy-*cis*-but-2-en-1-yloxymethyl) derivative in 66% yield (Scheme 2). N-1 substitution was proved by the N*O*E enhancement in the benzyl protons at C-6 when N-1 $CH_2$ was irradiated.

The newly synthesized Emivirine and GCA-186 analogues **6a**–**6f**, **9a**, **9b**, and **10** were tested against wild-type HIV-1 strain IIIB and against the resistant strain N119 which contain the mutation Y181C in MT-4 cells. As shown in Table 1, all the tested compounds are active against HIV-1 (wild-type). Compounds **6b**, **6c**, **6e**,




**Scheme 2**

**Table 1.** Inhibitory and cytotoxic concentrations against HIV-1 in MT-4 cells

| Compd | HIV-1 III (wild-type) | | | N119 (Y181C) $EC_{50}/\mu M$[a] |
|---|---|---|---|---|
| | $EC_{50}/\mu M$[a] | $CC_{50}/\mu M$[b] | $SI$[c] | |
| **6a** | 0.2 | 24 | 120 | –[d] |
| **6b** | 0.0004 | 23 | 57500 | 1.0 |
| **6c** | 0.003 | 34 | 11333 | 1.8 |
| **6d** | 0.05 | 26 | 520 | –[d] |
| **6e** | 0.001 | 31 | 31000 | 7.2 |
| **6f** | 0.002 | 28 | 14000 | –[e] |
| **9a** | 0.02 | 36 | 1800 | –[e] |
| **9b** | 0.003 | 34 | 11333 | –[e] |
| **10** | 0.03 | >100 | >3300 | –[e] |
| MKC-442 | 0.02 | >100 | >5000 | 44 |

[a] Inhibitory concentration of compounds achieving 50% inhibition of HIV multiplication in MT-4 infected cells; [b] cytotoxic concentration of compound required to reduce the viability of normal uninfected MT-4 cells by 50%; [c] selectivity index: ratio $CC_{50}/EC_{50}$, the symbol (>) indicates that $CC_{50}$ was not reached at the highest concentration test; [d] not tested; [e] inactive at subtoxic concentration

**6f**, and **9b** exhibited higher activity than MKC-442. The most active compound is **6b** which possessed the highest inhibition activity ($EC_{50} = 0.4\,nM$). It showed 50 fold higher activity against HIV-1 than MKC-442 and $SI$ was 11-fold higher than the one observed for MKC-442. Compound **6e** was over 20-fold more potent than MKC-442, whereas compounds **6c**, **6f**, and **9b** were *ca*. 10-fold more active than MKC-442. Three compounds **6b**, **6c**, and **6e** showed higher activity than MKC-442 against the mutant strain N119. The activities for the compounds **6b**

and **6c** were close to the one found for Efavirenz (0.3 $\mu M$) using the same strain and assay for the testing [16].

## Experimental

NMR spectra were recorded on a Varian Gemini 2000 NMR spectrometer at 300 MHz for $^1$H and 75 MHz for $^{13}$C with *TMS* as an internal standard. Chemical shifts are reported in ppm ($\delta$), and signals are expressed as s (singlet), d (doublet), t (triplet), q (quartet), or m (multiplet). MALDI mass spectra were recorded on an IonSpec Fourier Transform Ion Cyclotron Resonance Mass Spectrometer. Melting points were determined on a Büchi melting point apparatus. Elemental analyses were performed at H. C. Ørsted Institute, University of Copenhagen; the found values agreed favourably with the calculated ones. The progress of reactions was monitored by TLC (DC-alufolio 60 $F_{254}$) from Merck. For column chromatography Merck silica gel (0.040–0.063 mm) was used.

*General Procedure for Preparation of 1-(Indanyloxymethyl)uracils **6a–6f***

5,6-Disubstituted uracil (**5a**–**5c**, 1.0 mmol) was stirred in 15 cm$^3$ dry $CH_3CN$ under nitrogen and 0.87 cm$^3$ of *N,O*-bis(trimethylsilyl)acetamide (*BSA*, 3.5 mol) were added. After a clear solution was obtained, the mixture was cooled to −50°C and 0.18 cm$^3$ *TMS* triflate (1.0 mmol) were added followed by the dropwise addition of 0.56 g of bis(indan-1-yloxy)methane (**4a**) or bis(indan-2-yloxy)methane (**4b**) (2.0 mmol). The reaction mixture was stirred at room temperature for 5–8 h, and the mixture was quenched by addition of 5 cm$^3$ ice cold sat. aq. $NaHCO_3$ solution, and evaporated under reduced pressure. The residue was extracted with 3 × 50 cm$^3$ $Et_2O$ and the combined organic fractions were dried ($MgSO_4$), and evaporated under reduced pressure. The products were purified by silica gel column chromatography (20% $Et_2O$ in petroleum ether (60–80°C)) to afford **6a**–**6f**.

*6-Benzyl-1-(indan-1-yloxymethyl)-5-isopropyluracil* (**6a**, $C_{24}H_{26}N_2O_3$)

White foam; yield 0.22 g (56%); $^1$H NMR ($CDCl_3$, 300 MHz): $\delta = 1.29$ (d, $J = 6.9$ Hz, 6H, 2 × $CH_3$), 2.01–2.07 (m, 1H, 2′-H), 2.35–2.42 (m, 1H, 2′-H), 2.76–2.91 (m, 2H, 3′-H, CH), 3.01–3.09 (m, 1H, 3′-H), 4.18 (s, 2H, $CH_2$), 5.14–5.20 (m, 3H, 1′-H, $CH_2$), 7.04–7.35 (m, 9H$_{arom}$), 8.82 (s, 1NH) ppm; $^{13}$C NMR ($CDCl_3$, 75 MHz): $\delta = 20.46$ ($CH_3$), 28.35 (CH), 30.15 ($CH_2$), 32.63 ($CH_2$), 33.48 ($CH_2$), 71.85 (CH), 82.32 ($CH_2$), 119.85 (C-5), 124.91, 124.96, 126.56, 127.22, 127.27, 128.70, 129.18, 135.31, 141.96, 143.90 ($C_{arom}$), 148.66 (C-6), 151.72 (C-2), 162.20 (C-4) ppm; HRMS (MALDI, peak matching): $m/z = [M + Na]^+$ calcd 413.1839, found 413.1836.

*5-Ethyl-1-(indan-1-yloxymethyl)-6-(3,5-dimethylbenzyl)uracil* (**6b**, $C_{25}H_{28}N_2O_3$)

White foam; yield 0.23 g (57%); $^1$H NMR ($CDCl_3$, 300 MHz): $\delta = 1.06$ (t, $J = 7.4$ Hz, 3H, $CH_3$), 1.98–2.05 (m, 1H, 2′-H), 2.27 (s, 6H, 2 × $CH_3$), 2.33–2.42 (m, 1H, 2′-H), 2.45 (q, $J = 7.4$ Hz, 2H, $CH_2$), 2.75–2.83 (m, 1H, 3′-H), 3.02–3.09 (m, 1H, 3′-H), 4.07 (s, 2H, $CH_2$), 5.12–5.21 (m, 3H, 1′-H, $CH_2$), 6.65 (s, 2H$_{arom}$), 6.88 (s, 1H$_{arom}$), 7.20–7.36 (m, 4H$_{arom}$), 8.91 (s, 1NH) ppm; $^{13}$C NMR ($CDCl_3$, 75 MHz): $\delta = 13.85$ ($CH_3$), 19.22 ($CH_2$), 21.29 ($CH_3$), 30.16 ($CH_2$), 32.80 ($CH_2$), 33.24 ($CH_2$), 71.73 (CH), 82.28 ($CH_2$), 116.86 (C-5), 124.91, 124.96, 125.01, 126.54, 128.70, 128.96, 134.88, 138.85, 141.99, 143.88 ($C_{arom}$), 149.58 (C-6), 151.73 (C-2), 163.13 (C-4) ppm; MS (MALDI): $m/z = 427$ ($M + Na^+$).

*1-(Indan-1-yloxymethyl)-5-isopropyl-6-(3,5-dimethylbenzyl)uracil* (**6c**, $C_{26}H_{30}N_2O_3$)

White foam; yield 0.215 g (51%); $^1$H NMR ($CDCl_3$, 300 MHz): $\delta = 1.29$ (d, $J = 6.9$ Hz, 6H, 2 × $CH_3$), 2.01–2.08 (m, 1H, 2′-H), 2.27 (s, 6H, 2 × $CH_3$), 2.31–2.39 (m, 1H, 2′-H), 2.75–2.91 (m, 2H, CH, 3′-H), 3.02–3.08 (m, 1H, 3′-H), 4.10 (s, 2H, $CH_2$), 5.14–5.16 (m, 3H, 1′-H, $CH_2$), 6.65 (s, 2H, H$_{arom}$), 6.89 (s, 1H, H$_{arom}$), 7.22–7.36 (m, 4H, H$_{arom}$), 8.84 (s, 1NH) ppm; $^{13}$C NMR ($CDCl_3$, 75 MHz): $\delta = 20.49$ ($CH_3$), 21.26 ($CH_3$), 28.34 (CH), 30.13 ($CH_2$), 32.78 ($CH_2$), 33.29 ($CH_2$), 71.84 (CH), 82.23 ($CH_2$), 119.68 (C-5), 124.90, 124.93, 124.99, 126.51, 128.66, 128.82, 134.99, 138.77, 141.99, 143.88

($C_{arom}$), 148.92 (C-6), 151.76 (C-2), 162.22 (C-4) ppm; HRMS (MALDI, peak matching): $m/z =$ $[M + Na]^+$ calcd 441.2165, found 441.2149.

*6-Benzyl-1-(indan-2-yloxymethyl)-5-isopropyluracil* (**6d**, $C_{24}H_{26}N_2O_3$)
White solid; yield 0.265 g (68%); mp 135–136°C; $^1$H NMR ($CDCl_3$, 300 MHz): $\delta = 1.28$ (d, $J = 7.0$ Hz, 6H, $2 \times CH_3$), 2.84 (hept, $J = 7.0$ Hz, 1H, CH), 2.91, 3.14 (2m, 4H, $2 \times CH_2$), 4.16 (s, 2H, $CH_2$), 4.57 (quint, $J = 3.4$ Hz, 1H, 2′-H), 5.21 (s, 2H, $CH_2$), 7.08–7.35 (m, $9H_{arom}$), 9.72 (s, 1 NH) ppm; $^{13}$C NMR ($CDCl_3$, 75 MHz): $\delta = 20.39$ ($CH_3$), 28.29 (CH), 33.45 ($CH_2$), 39.49 ($CH_2$), 71.57 (CH), 78.91 ($CH_2$), 119.85 (C-5), 124.61, 126.59, 127.20, 129.12, 135.33, 140.47 ($C_{arom}$), 148.51 (C-6), 152.05 (C-2), 162.53 (C-4) ppm; MS (MALDI): $m/z = 413$ ($M + Na^+$).

*5-Ethyl-6-(3,5-dimethylbenzyl)-1-(indan-2-y-oxymethyl)uracil* (**6e**, $C_{25}H_{28}N_2O_3$)
White solid; yield 0.288 g (71%); mp 132–133°C; $^1$H NMR ($CDCl_3$, 300 MHz): $\delta = 1.05$ (t, $J = 7.4$ Hz, 3H, $CH_3$), 2.27 (s, 6H, $2 \times CH_3$), 2.47 (q, $J = 7.4$ Hz, 2H, $CH_2$), 2.92, 3.15 (2dd, $J = 3.7$, 16.3 Hz, $J = 6.4$, 16.3 Hz, 4H, $2 \times CH_2$), 4.06 (s, 2H, $CH_2$), 4.57 (quint, $J = 3.4$ Hz, 1H, 2′-H), 5.19 (s, 2H, $CH_2$), 6.68 (s, $2H_{arom}$), 6.88 (s, $1H_{arom}$), 7.15–7.21 (m, 4H, $H_{arom}$), 9.78 (s, 1NH) ppm; $^{13}$C NMR ($CDCl_3$, 75 MHz): $\delta = 13.79$ ($CH_3$), 19.14 ($CH_2$), 21.24 ($CH_3$), 33.18 ($CH_2$), 39.51 ($CH_2$), 71.45 (CH), 78.89 ($CH_2$), 116.90 (C-5), 124.61, 124.95, 126.60, 128.89, 134.91, 138.80, 140.49 ($C_{arom}$), 149.43 (C-6), 152.06 (C-2), 163.54 (C-4) ppm; MS (MALDI): $m/z = 427$ ($M + Na^+$).

*1-(Indan-2-yloxymethyl)-5-isopropyl-6-(3,5-dimethylbenzyl)uracil* (**6f**, $C_{26}H_{30}N_2O_3$)
White foam; yield 0.268 g (64%); $^1$H NMR ($CDCl_3$, 300 MHz): $\delta = 1.29$ (d, $J = 7.0$ Hz, 6H, $2 \times CH_3$), 2.28 (s, 6H, $2 \times CH_3$), 2.85 (hept, $J = 7.0$ Hz, 1H, CH), 2.92, 3.15 (2dd, $J = 3.9$, 16.5 Hz, $J = 6.4$, 16.5 Hz, 4H, $2 \times CH_2$), 4.08 (s, 2H, $CH_2$), 4.58 (quint, $J = 3.4$ Hz, 1H, 2′-H), 5.20 (s, 2H, $CH_2$), 6.68 (s, $2H_{arom}$), 6.89 (s, $1H_{arom}$), 7.13–7.25 (m, $4H_{arom}$), 9.40 (s, 1NH) ppm; $^{13}$C NMR ($CDCl_3$, 75 MHz): $\delta = 20.42$ ($CH_3$), 21.24 ($CH_3$), 28.32 (CH), 33.28 ($CH_2$), 39.52 ($CH_2$), 71.59 (CH), 78.87 ($CH_2$), 119.70 (C-5), 124.63, 124.96, 126.61, 128.79, 135.02, 138.75, 140.51 ($C_{arom}$), 148.81 (C-6), 152.01 (C-2), 162.49 (C-4) ppm; MS (MALDI): $m/z = 441$ ($M + Na^+$).

*Bis(4-p-toluoyloxy-cis-2-buten-1-yloxy)methane* (**8**, $C_{25}H_{28}O_6$)
A mixture of 5.66 g KOH (0.101 mol), 8.88 g of *cis*-2-butene-1,4-diol (**7**, 0.10 mol), 3.54 $cm^3$ of dibromomethane (0.0505 mol), and 1.74 g of tetrabutylammonium bromide (5.35 mmol) was heated under reflux in 50 $cm^3$ of anhydrous benzene for 4 h. The reaction mixture was left to cool and 100 $cm^3$ $H_2O$ were added. The mixture was extracted with $3 \times 100$ $cm^3$ $Et_2O$, and the combined $Et_2O$ extracts were dried ($MgSO_4$), and evaporated under reduced pressure to give 2.75 g (29%) of bis(4-hydroxy-*cis*-2-buten-1-yloxy)methane, which was stirred in 20 $cm^3$ of anhydrous pyridine at 0°C, when 4.67 g of *p*-toluoyl chloride (0.0302 mol) were added slowly. The reaction mixture was stirred at 0°C for 2 h, and left at room temperature for overnight. The solvent was removed under reduced pressure and the residue was extracted with $3 \times 100$ $cm^3$ $Et_2O$. The $Et_2O$ fractions were collected, dried ($MgSO_4$), and evaporated under reduced pressure to give compound **8** as a colourless oil in 22% (4.7 g) overall yield, which was used in the next step without further purification.

*5,6-Disubstituted 1-(4-p-Toluoyloxy-cis-2-buten-1-yloxymethyl)uracils **9a** and **9b***
5,6-Disubstituted uracils **5a** and **5b** (1.0 mmol) were stirred in 15 $cm^3$ dry $CH_3CN$ under $N_2$, and 0.87 $cm^3$ *BSA* (3.5 mmol) were added. After a clear solution was obtained (10 min), the mixture was cooled to −50°C and 0.18 $cm^3$ *TMS* triflate (1.0 mmol) was added followed by addition of 0.85 g **8** (2.0 mmol). The reaction mixture was stirred under $N_2$ at room temperature for 6 h. Cold aq. $NaHCO_3$ solution (5 $cm^3$) was added, and the solvent was evaporated under reduced pressure. The residue was extracted with $3 \times 50$ $cm^3$ $Et_2O$. The $Et_2O$ extracts were dried ($MgSO_4$), evaporated under reduced pressure, and the residue was purified by chromatography on a silica gel column (20% $Et_2O$ in petroleum ether (60–80°C)) to afford **9a** and **9b**.

*6-Benzyl-5-isopropyl-1-(4-p-toluoyloxy-cis-2-buten-1-yloxymethyl)uracil*
(**9a**, $C_{27}H_{30}N_2NaO_5$)
Obtained as an oil; yield 0.328 g (71%); $^1$H NMR ($CDCl_3$, 300 MHz): $\delta = 1.28$ (d, $J = 7.0$ Hz, 6H, 2×$CH_3$), 2.40 (s, 3H, $CH_3$), 2.82 (hept, $J = 7.0$ Hz, 1H, CH), 4.29 (s, 2H, $CH_2$), 4.31 (d, $J = 6.3$ Hz, 2H, $CH_2$), 4.85 (d, $J = 6.0$ Hz, 2H, $CH_2$), 5.16 (s, 2H, $CH_2$), 5.70–5.87 (m, 2H, 2×CH), 7.10–7.36 (m, 7$H_{arom}$), 7.90 (d, $J = 8.2$ Hz, 2$H_{arom}$), 9.13 (brs, 1NH) ppm; $^{13}$C NMR ($CDCl_3$, 75 MHz): $\delta = 20.36$ ($CH_3$), 21.61 ($CH_3$), 28.34 (CH), 33.53 ($CH_2$), 60.47 ($CH_2$), 65.27 ($CH_2$), 72.83 ($CH_2$), 119.85 (C-5), 127.23, 127.61, 129.04, 129.20, 129.56, 129.61, 135.25, 143.64 ($C_{arom}$, CH), 148.34 (C-6), 151.83 (C-2), 162.36 (C-4), 166.30 (CO) ppm; HRMS (MALDI, peak matching): $m/z =$ $[M + Na]^+$ calcd 485.2033, found 485.2047.

*5-Ethyl-6-(3,5-dimethylbenzyl)-1-(4-p-toluoyloxy-cis-2-buten-1-yl-oxymethyl)uracil*
(**9b**, $C_{28}H_{32}N_2O_5$)
White solid; yield 0.322 g (68%); mp 132–134°C; $^1$H NMR ($CDCl_3$, 300 MHz): $\delta = 1.05$ (t, $J = 7.3$ Hz, 3H, $CH_3$), 2.28 (s, 6H, 2×$CH_3$), 2.39 (s, 3H, $CH_3$), 2.44 (q, $J = 7.3$ Hz, 2H, $CH_2$), 4.08 (s, 2H, $CH_2$), 4.30 (d, $J = 5.6$ Hz, 2H, $CH_2$), 4.86 (d, $J = 5.7$ Hz, 2H, $CH_2$), 5.16 (s, 2H, $CH_2$), 5.74–5.85 (m, 2H, 2×CH), 6.71 (s, 2$H_{arom}$), 6.88 (s, 1$H_{arom}$), 7.20 (d, $J = 8.0$ Hz, 2$H_{arom}$), 7.90 (d, $J = 8.0$ Hz, 2$H_{arom}$), 9.90 (brs, 1NH) ppm; $^{13}$C NMR ($CDCl_3$, 75 MHz): $\delta = 13.68$ ($CH_3$), 19.10 ($CH_2$), 21.20 ($CH_3$), 21.55 ($CH_3$), 33.23 ($CH_2$), 60.44 ($CH_2$), 65.25 ($CH_2$), 72.65 ($CH_2$), 116.89 (C-5), 124.94, 127.22, 128.90, 129.56, 129.60, 134.82, 143.55 ($C_{arom}$), 127.53 (CH), 138.80 (CH), 149.15 (C-6), 152.04 (C-2), 163.51 (C-4), 166.23 (CO) ppm; MS (EI): $m/z = 476$ ($M^+$).

*6-Benzyl-1-(4-hydroxy-but-2-en-1-yloxymethyl)-5-isopropyluracil* (**10**, $C_{19}H_{24}N_2O_4$)
Compound **9a** (0.462 g, 1.0 mmol) was dissolved in a solution prepared from 0.026 g Na (1.1 mmol) and 15 $cm^3$ anhydrous $CH_3OH$. The mixture was stirred at room temperature for 2 h. The solvent was evaporated under reduced pressure and the residue was purified by chromatography on a silica gel column with $CHCl_3$ to afford compound **10** as a colourless oil in 66% (0.227 g) yield. $^1$H NMR ($CDCl_3$, 300 MHz): $\delta = 1.28$ (d, $J = 6.8$ Hz, 6H, 2×$CH_3$), 2.86 (hept, $J = 6.8$ Hz, 1H, CH), 3.17 (brs, 1OH), 4.16–4.26 (m, 6H, 3×$CH_2$), 5.15 (s, 2H, $CH_2$), 5.50–5.58 (m, 1H, CH), 5.80–5.89 (m, 1H, CH), 7.10–7.37 (m, 5$H_{arom}$), 9.94 (s, 1NH) ppm; $^{13}$C NMR ($CDCl_3$, 75 MHz): $\delta = 20.35$ ($CH_3$), 28.30 (CH), 33.48 ($CH_2$), 57.91 ($CH_2$), 64.98 ($CH_2$), 72.21 ($CH_2$), 120.30 (C-5), 127.17 (CH), 127.22, 129.19, 135.20 ($C_{arom}$), 133.22 (CH), 148.40 (C-6), 152.60 (C-2), 162.40 (C-4) ppm; HRMS (MALDI, peak matching): $m/z = [M + Na]^+$ calcd 367.1630, found 367.1623.

*Viruses and Cells*
The inhibitory activity against HIV-1 infection was evaluated using MT-4 cells [21] as target cells and the HIV-1 strain HTLV-IIIB [22] and the NNRTI resistant strain N119 [23] as infectious virus. The virus was propagated in H9 [21] cells at 37°C, 5% $CO_2$ using RPMI 1640 with 10% heat-inactivated fetal calf serum (FCS) and antibiotics (growth medium). Culture supernatant was filtered (0.45 nm), aliquoted, and stored at −80°C until use. Both HIV-1 strains were obtained from the NIH AIDS Research and Reference Program.

*Inhibition of HIV-1 Replication*
Compounds were examined for possible antiviral activity against both strains of HIV-1 using MT-4 cells as target cells. MT-4 cells were incubated with virus (0.005 MOI) and growth medium containing the test dilutions of compounds for six days in parallel with virus-infected and uninfected control cultures without compound added. Expression of HIV in the cultures was indirectly quantified using the MTT assay [24]. Compounds mediating less than 30% reduction of HIV expression were considered without biological activity. Compounds were tested in parallel for cytotoxic effect in uninfected MT-4 culture containing the test dilutions of compound as described above. A 30% inhibition of cell growth relative to control cultures was considered significant. The 50% inhibitory concentration ($EC_{50}$)

and 50% cytotoxic concentration ($CC_{50}$) were determined by interpolation from the plots of percent inhibition *versus* concentration of compound.

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