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# Synthesis and antiviral activity of new dimeric inhibitors against HIV-1

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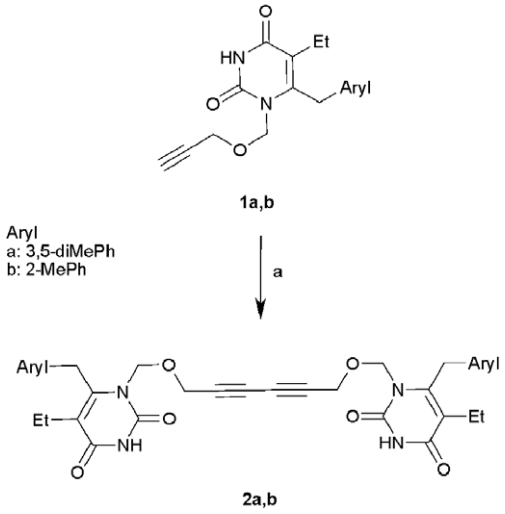
**Abstract**—This paper describes the synthesis and the antiviral activities of dimeric compounds derived from homo and asymmetric combinations of *N*-1 propynyloxymethyl analogues **1a**,**b** of MKC-442, an *N*-1 4-iodobenzyloxymethyl analogue of *TNK*-651 **5**, potent contraceptive norgestrel and AZT. They were obtained by Sonogashira reaction, 'click' chemistry or Pd-catalyzed oxidative coupling. The iodo precursor **5** turned out as a potent compound against wild type and mutated HIV-1 virus. All dimeric compounds showed lower activity against HIV-1 than MKC-442, except the asymmetric dimer of AZT and **1a** which showed an activity comparable to MKC-442.


## 1. Introduction

The spread of HIV-1 virus is still a serious problem. Currently there are a lot of possible therapeutical regimens available in hands of clinicians; however, only four, that is, nucleoside reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), protease inhibitors and fusion inhibitors are the best developed types of inhibitors so far.[1–6] This gives a total number of 21 drugs licensed for clinical use. The efforts to synthesize new inhibitors are undertaken both in academic and in industrial research centers all over the world. In our research group, we have been interested in different HEPT (1-[(2-hydroxyethoxy)methyl]-6-(phenylthio)thymine) derivatives as potential NNRTIs for a long time.[7–15] Recently, we have synthesized **1a** which is a very potent inhibitor of HIV-1 (Scheme 1).[13] This compound is among the first examples containing a terminal triple bond besides 4′-E-dC[5] targeting RT. In order to find new MKC-442




**Scheme 1.** Reagents and condition: (a) $PdCl_2(PPh_3)_2$, CuI, $Et_3N$, toluene, $O_2$, 74–90%.

*Keywords*: HIV-1; NNRTI; Synthesis; AZT; TNK-651; MKC-442; AZT; Sonogashira reaction; Click chemistry; NRTI; Nucleobase; Reverse transcriptase; Acetylene; D-norgestrel; Mutant; Virus; Drug-resistant; Efavirenz; Anti-viral activity; Uracil.
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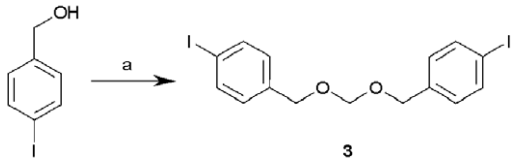
analogues with activity against HIV, it was therefore logic to perform arylation reactions on the terminal triple bond of **1a** and Sonogashira cross-coupling reaction. This idea was also based on the reports of using the alkyne moiety in related drug discovery.[16–22] When starting to make Sonogashira couplings on **1a**,**b**, we obtained the corresponding dimers **2a**,**b** through homo coupling of the acetylene bonds. These dimers were found active against HIV-1 and we realized that such dimeric molecules could be considered as a new type of double drugs against HIV-1 different from previous diacetylenes that have been reported in the literature to link nucleobases.[23–25] Therefore we decided to focus on the reactions on the acetylene bond in order to explore the potential of such double drugs. Our rationale is supported in relevant literature,[26,27] where small molecules can interfere with the HIV-1 RT dimerization process. We also decided to combine the commercially available NRTI-drug AZT with **1a** by a standard click chemistry.[28] The result is a hybrid between an NNRTI and an NRTI. Similar approaches have been reported recently.[29–31]

## 2. Chemistry

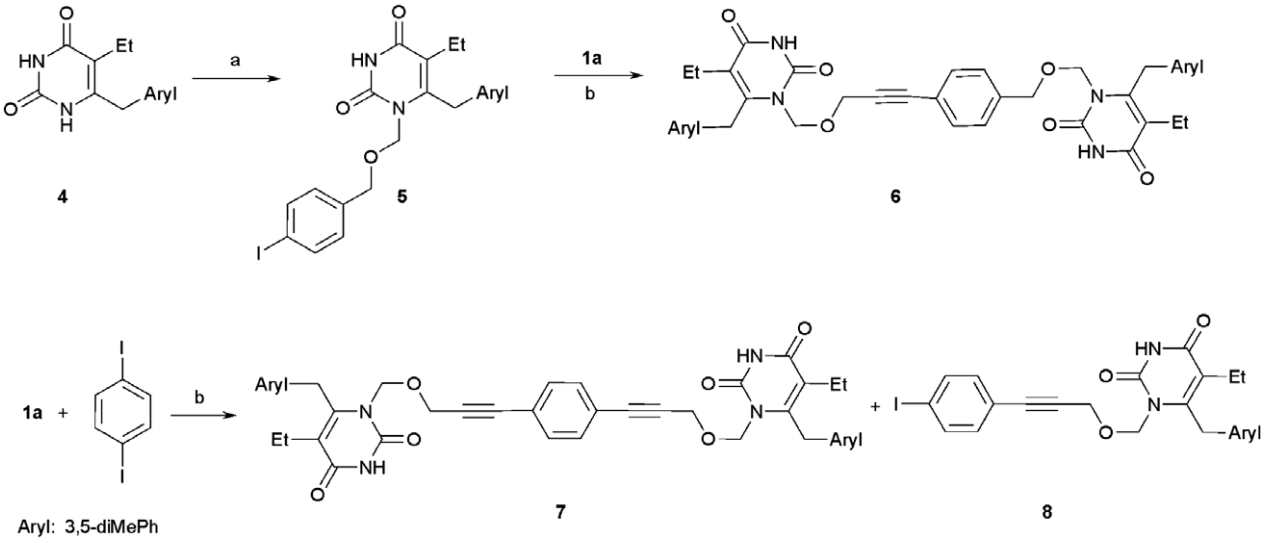
By changing the procedure of Sonogashira couplings on **1a**,**b**[13] into a procedure of oxidative coupling using the procedure of Ito et al.,[32] we obtained the corresponding diynes **2a**,**b** in 74–90% yield (Scheme 1). The compounds **2a**,**b** represent an idea of using a rigid linker which in turn can contribute with stacking properties upon binding of the drug to its target. In order to find the scope and limitation of this idea, we decided to replace one of the acetylene bonds with a para substituted benzene ring and also to further elongate the rigid and stacking linker by proper combinations of acetylene bonds and para substituted benzene rings. Therefore the iodine substituted analogue **5** of the extremely potent anti-HIV-1 compound TNK-651[33] was prepared by a Vorbrüggen reaction[34] between uracil **4**[8] and acetal **3** (Scheme 3). The latter compound was conveniently prepared according to a published method for preparation of formaldehyde acetals (Scheme 2).[13,35]



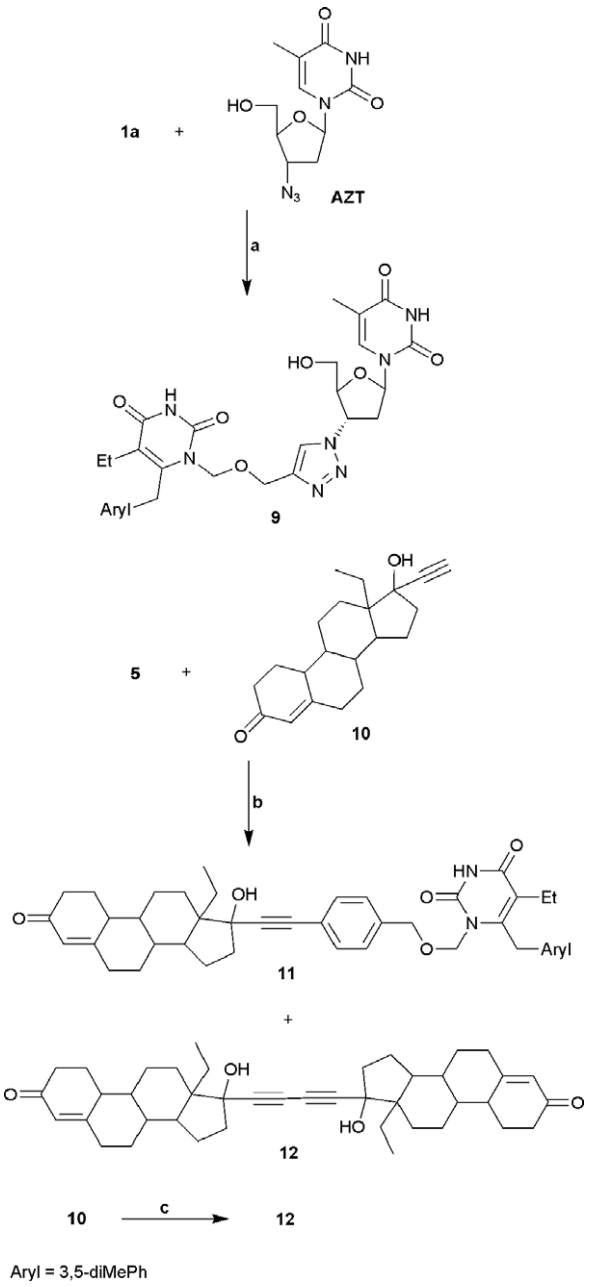

**Scheme 2.** Reagents and conditions: (a) KOH, $CH_2Br_2$, $NBu_4Br$, benzene, 85 °C, 71%.

A subsequent Sonogashira coupling of **1a** and **5**, under the exclusion of $O_2$, furnished **6** in 42% yield. On the other hand, it was easy to elongate the rigid linker by an additional acetylene bond by reaction of **1a** with diiodobenzene which gave the main product **7** together with a negligible amount of **8**.

It was also possible to use the acetylene bond of compound **1a** to synthesize asymmetric double drugs by taking advantage of click chemistry in a reaction with AZT (Scheme 4). The regioselectivity of triazole **9** was established by comparing the NMR spectral data with related compounds already published.[29,30] In order to lower the drug burden and to secure the administration of the HIV drug, the double drug idea[36] was further extended to the synthesis of potential double functional drugs by a Sonogashira coupling reaction between the aryl iodide **5** and the contraceptive**10** (−)-D-norgestrel[37] to obtain **11**, accompanied by a small amount of the homocoupled product **12**. The latter compound was also prepared independently by an oxidative coupling reaction (Scheme 4).




**Scheme 3.** Reagents and conditions: (a) MeCN, BSA, TMS triflate, **3**, −50 °C–rt, 81%; (b) $PdCl_2(PPh_3)_2$, CuI, $Et_3N$, 81% (**6**), 48% (**7**) and 21% (**8**).




**Scheme 4.** Reagents and conditions: (a) *t*-BuOH/$H_2O$, $CuSO_4 \cdot 5H_2O$, 1 M sodium ascorbate, 69%; (b) $PdCl_2(PPh_3)_2$, CuI, $Et_3N$, 85% (**11**); (c) $PdCl_2(PPh_3)_2$, CuI, $Et_3N$, toluene, $O_2$, 96%.

## 3. Antiviral activity

The HIV-1 strain HTLV-IIIB in MT-4 cells was used in our assay to investigate the anti-HIV-1 activity of MKC-442 analogues synthesized in the present study. The results are summarized in Table 1 together with those of MKC-442 and efavirenz. The monomer **1a** has been investigated before[13] and in the present series of compounds this is the most active compound against HIV-1 wild type. Among the new monomeric compounds, the TNK-651 analogue **5**, containing an iodine atom in the para position of the benzyl goup in the N-1 substituent, has an activity against HIV-1 wild type and the drug-resistant strains carrying Y181C and K103N + Y181C mutations, which is comparable to that found for compound **1a**. However, it is interesting to note that compound **5** showed a 20-fold higher activity than **1a** against the $EFV^R$ strain. The importance of iodine substitution has recently been reported in an investigation of three potent pyridinones where proper iodine substitution resulted in inhibition of drug-resistant variants, including Y181C and K103N RT.[38] It should be noticed that the activity of another iodo analogue **8** comprising an acetylene bond elongation of the N-1 substituent is considerably lower, which may be due to the rigid nature of the triple bond or to the length of the N-1 acyclic substituent. Also the dimer type compounds (**2a**,**b**, **6** and **7**) with combinations of acetylene bonds and benzene rings in the interlinking chain resulted in compounds with moderate activity against HIV-1. Compound **9**, representing an attempt to synthesize an asymmetric double drug by click chemistry in a reaction between **1a** and AZT, showed an activity against wild type HIV-1 and mutant strains which is comparable to those found for MKC-442. The low activity against the Y181C mutated strain makes it unlikely that the nucleoside part of **9** functions as a nucleoside reverse transcriptase inhibitor. In another attempt we intended to construct a bifunctional molecule with both contraceptive and anti-HIV-1 activity, the rationale being to reduce the drug burden for women by combining one of the HIV-1 drugs from the combination therapy with a contraceptive agent.

Compound **11** from coupling of **5** with commercially available **10** is an obvious possibility to test this idea. Unfortunately, the activity against HIV-1 dropped dramatically when compared with compound **5** and, therefore, it was not interesting to investigate compound **11** as a contraceptive agent.

## 4. Conclusion

In summary, we have described the synthesis of new dimeric series analogues of MKC-442, TNK-651 and AZT. However, the dimeric compounds only showed moderate activity against HIV. Instead, in this study we discovered the potent inhibitor **5**, with a promising activity against HIV-1 resistant mutants.

## 5. Experimental

### 5.1. Chemistry

NMR spectra were recorded on a Varian Gemini 2000 NMR spectrometer at 300 MHz for $^1H$ and 75 MHz for $^{13}C$ with TMS as internal standard. MALDI mass spectra were recorded on a 4.7 T Ultima (IonSpec, Irvine, CA) Fourier Transform Ion Cyclotron Resonance (FTICR) Mass Spectrometer. Melting points were determined on a Büchi melting point apparatus. Elemental analyses were performed at H.C. Ørsted Institute, University of Copenhagen. Silica gel (0.040–0.063 mm) used for column chromatography and analytical silica gel TLC plates 60 $F_{254}$ were purchased from Merck.

**Table 1.** Cytotoxicity and anti-HIV-1 activity of MKC-442 dimeric analogues **2a**–**b**, **6**, **11**, **12**, **7** and **9**, monomers **1a**, **1b**, **5**, and **8**, and the reference compounds MKC-442 and efavirenz (EFV)

| Compound | $CC_{50}$[b] (μM) | SI[c] | Wild type | $EC_{50}$[a] (μM) | | K103N + Y181C |
|---|---|---|---|---|---|---|
| | | | | $EFV^R$ | Y181C | |
| *Dimers* | | | | | | |
| **2a** | 9 | 18 | 0.5 | >9 | >9 | >9 |
| **2b** | 8 | 1 | >8 | >8 | >8 | >8 |
| **6** | 16 | 53 | 0.3 | >16 | 4 | >16 |
| **11** | >100 | >250 | 0.4 | >100 | 2.8 | >100 |
| **12** | 28 | 1 | >28 | >28 | >28 | >28 |
| **7** | 85 | 30 | 2.8 | >85 | >85 | >85 |
| **9** | >100 | >1250 | 0.08 | 26 | 8 | 27 |
| *Monomers* | | | | | | |
| **1a** | >100 | >33333 | 0.003 | 16 | 0.2 | 1.2 |
| **1b** | 33 | 82 | 0.4 | >33 | >33 | >33 |
| **5** | 19 | 2714 | 0.007 | 0.8 | 0.1 | 0.9 |
| **8** | 44 | 220 | 0.2 | >44 | 10 | >44 |
| MKC-442 | >100 | >3333 | 0.03 | 100 | 20 | >100 |
| EFV | 30 | 15000 | 0.002 | 3 | 0.008 | 0.3 |

[a] Compound dose required to reduce the viability of mock-infected cells by 50%, as determined by the MTT method.
[b] Compound dose required to achieve 50% protection of MT-4 cells from HIV-1-induced cytopathogenicity, as determined by the MTT method. The symbol (>) indicates that the $CC_{50}$ was not reached at the highest concentration tested.
[c] Selectivity index: Ratio $CC_{50}/EC_{50}$. $EC_{50}$ and $CC_{50}$ are expressed as the mean values of at least two separate experiments.

Solvents for chromatography were bought as HPLC grade or distilled prior to use. Reactions were in general carried out under argon atmosphere. $CH_3CN$ was dried over 3 Å molecular sieves. Compound **1a**,**b** was prepared according to earlier works.[8,13]

**5.1.1. 6-(3,5-Dimethylbenzyl)-1-[6-[6-(3,5-dimethylbenzyl)-5-ethyl-2,4-dioxo-1,2,3,4-tetrahydro-1-pyrimidinylmethoxy]-2,4-hexadiynyloxymethyl]-5-ethyl-1,2,3,4-tetrahydro-2,4-pyrimidinedione (2a).** A solution of **1a** (191 mg, 0.585 mmol), $PdCl_2$ $(PPh_3)_2$ (12 mg, 0.017 mmol), CuI (3.0 mg, 0.016 mmol), triethylamine (3.0 mL) in toluene (10 mL) was stirred at room temperature overnight under $O_2$ atmosphere. After evaporation of solvents, the residue was purified by column chromatography on silica gel with $CHCl_3$ to afford the compound **2a**: yellow foam. Yield: 140 mg (74%); $R_f$ 0.31 (3% MeOH/$CHCl_3$). $^1H$ NMR ($CDCl_3$) $\delta$ 1.07 (t, 6H, $J$ = 7.4 Hz, $CH_2CH_3$), 2.28 (s, 12H, $CH_3$), 2.48 (q, 4H, $J$ = 7.4 Hz, $CH_2CH_3$), 4.06 (s, 4H, $CH_2Ph$), 4.39 (s, 4H,$OCH_2C\equiv$), 5.28 (s, 4H, $NCH_2O$), 6.71 (s, 4H, aryl),6.90 (s, 2H, aryl), 9.89 (s, 2H, NH). $^{13}C$ NMR ($CDCl_3$) $\delta$ 13.9 ($CH_2CH_3$), 19.2 ($CH_2CH_3$), 21.3 ($CH_3$), 33.1 ($CH_2Ph$), 58.6 ($OCH_2C\equiv$), 69.4 ($CH_2C\equiv C–$), 73.6 ($NCH_2O$), 76.8 ($CH_2C\equiv C–$), 117.6 (C-5), 125.1, 129.0,134.9, 138.9 (aryl), 148.7 (C-2), 153.0 (C-6), 163.2 (C-4). HRMS (MALDI) $m/z$ Calcd for $C_{38}H_{42}N_4O_6Na^+$ ($MNa^+$) 673.2996, Found 673.2997.

**5.1.2. 5-Ethyl-1-[6-[5-ethyl-6-(2-methylbenzyl)-2,4-dioxo-1, 2,3,4-tetrahydro-1-pyrimidinylmethoxy]-2,4-hexadiynyloxymethyl]-6-(2-methylbenzyl)-1,2,3,4-tetrahydro-2,4-pyrimidinedione (2b).** It was prepared analogously to **2a**: yield 140 mg (90%); $R_f$ 0.29 (3% MeOH/$CHCl_3$). $^1H$ NMR ($CDCl_3$) $\delta$ 1.06 (t, 6H, $J$ = 7.4 Hz, $CH_2CH_3$), 2.37 (s, 6H, 2-$CH_3$), 2.43 (q, 4H, $J$ = 7.4 Hz, $CH_2CH_3$), 4.03 (s, 4H, $CH_2Ph$), 4.39 (s, 4H, $OCH_2C\equiv$), 5.18 (s,4H, $NCH_2O$), 6.86 (d, 2H, $J$ = 7.0 Hz, aryl), 7.11–7.24 (m, 6H, phenyl), 9.94 (s, 2H, NH). $^{13}C$ NMR ($CDCl_3$): $\delta$ 13.8 ($CH_2CH_3$), 19.1 ($CH_2CH_3$), 19.6 (2-$CH_3$), 30.6 ($CH_2Ph$), 58.6 ($OCH_2C\equiv$), 69.4 ($CH_2C\equiv C–$), 73.8 ($NCH_2O$), 76.8 ($CH_2C\equiv C–$), 117.9 (C-5), 125.7, 126.8, 127.3, 130.6, 133.3, 136.0 (aryl), 148.8 (C-2), 153.0 (C-6), 163.1 (C-4). HRMS (MALDI) $m/z$ Calcd for $C_{36}H_{38}N_4O_6Na^+$ ($MNa^+$) 645.2683 Found 645.2685.

**5.1.3. 1-Iodo-4-(4-iodobenzyloxymethoxymethyl)-benzene (3).** A mixture of KOH (1.33 g, 23.7 mmol), $Bu_4NBr$ (0.41 g, 1.27 mmol), $CH_2Br_2$ (2.01 g, 0.81 mL, 11.6 mmol) and 4-iodobenzylalcohol (5.50 g, 23.5 mmol) in benzene (8 mL) was heated at 85–87 °C (oil bath temperature) for 2 days. After cooling to room temperature, $H_2O$ (50 mL) was added and the mixture was extracted with $Et_2O$ (3 × 50 mL). The organic layer was dried with $MgSO_4$. After removing the volatiles, the residue was purified by silica gel column chromatography using $CH_2Cl_2$ as eluent to give compound **3** as a white crystalline solid. Yield: 4.02 g (71%); $R_f$ 0.91 (25% EtOAc/petroleum ether); mp 78–80 °C. $^1H$ NMR ($CDCl_3$): $\delta$ 4.56 (s, 4H, $PhCH_2O$), 4.80 (s, 2H, $OCH_2O$), 7.05 (d, 4H, $J$ = 8.3 Hz, aryl), 7.66 (d, 4H, $J$ = 8.3 Hz, aryl). $^{13}C$ NMR ($CDCl_3$): $\delta$ 68.9 ($PhCH_2O$), 93.2 (C–I), 94.1 ($OCH_2O$), 129.7, 137.4, 137.4 (aryl). HRMS (MALDI) $m/z$ Calcd for $C_{15}H_{14}I_2O_2Na^+$ ($MNa^+$) 502.8975 Found, 502.8966.

**5.1.4. 6-(3,5-Dimethylbenzyl)-5-ethyl-1-(4-iodobenzyl-oxymethyl)-1H-pyrimidine- 2,4-dione (5).** A suspension of uracil **4**[8] (1.08 g, 4.16 mmol) in dry MeCN (80 mL) was treated with $N,O$-bis-(trimethylsilyl)acetamidate (BSA) (1.86 g, 2.23 mL, 9.13 mmol) under a nitrogen atmosphere. When the mixture became clear, it was cooled to −50 °C, and TMS triflate (2.84 g, 2.32 mL, 12.8 mmol) was added dropwise, followed by the acetal **3** (3.30 g, 6.87 mmol). The mixture was kept at −50 °C for 15 min and then allowed to warm to room temperature.

Stirring was continued until uracil **4** was consumed as judged from TLC. A cold saturated solution of $NaHCO_3$ was added. The mixture was evaporated until dryness and further extracted with $Et_2O$ ($6 \times 50$ mL). The etheral fractions were combined, dried, and evaporated. The residue was purified by silica column chromatography (chloroform) and recrystallized from petroleum ether/ethyl acetate to deliver a pure compound. Yield: 1.69 g (81%); white solid; $R_f$ 0.43 (3% MeOH/$CHCl_3$); mp 175–177 °C. $^1$H NMR ($CDCl_3$): $\delta$ 1.06 (t, 3H, $J$ = 7.5 Hz, $CH_2CH_3$), 2.27 (s, 6H, $2 \times CH_3$), 2.46 (q, 2H, $J$ = 7.5 Hz, $CH_2CH_3$), 4.04 (s, 2H, $CH_2Ph$), 4.58 (s, 2H, $OCH_2$), 5.18 (s, 2H, $NCH_2O$), 6.64 (s, 2H,aryl), 6.88 (s, 1H, aryl), 7.06 (d, 2H, $J$ = 8.4 Hz, aryl), 7.66 (d, 2H, $J$ = 8.4 Hz, aryl), 9.41 (s, 1H, NH). $^{13}$C NMR ($CDCl_3$): $\delta$ 13.8 ($CH_2CH_3$), 19.2 ($CH_2CH_3$), 21.3 ($2 \times CH_3$), 33.3 ($CH_2Ph$), 71.0 ($OCH_2$), 72.7 ($NCH_2O$), 93.5 (C–I), 116.9 (C-5), 124.9, 129.0, 129.7, 134.7, 137.0, 137.5, 138.9 (aryl), 149.1 (C-2), 152.0 (C-6), 163.2 (C-4). HRMS (MALDI) $m/z$ calcd for $C_{23}H_{25}IN_2O_3Na^+$ ($MNa^+$) 527.0802 Found 527.0976. Anal. Calcd for $C_{23}H_{25}IN_2O_3 \cdot 0.25H_2O$: C, 54.29; H, 5.05; N, 5.50. Found: C, 54.09; H, 4.78; N, 5.40.

**5.1.5. 6-(3,5-Dimethylbenzyl)-1-[4-[3-[6-(3,5-dimethylbenzyl)-5-ethyl-2,4-dioxo-1,2,3,4-tetrahydro-1-pyrimidinylmethoxy]-1-propynyl]benzyloxymethyl]-5-ethyl-1,2,3,4-tetrahydro-2,4-pyrimidinedione (6).** A mixture of **1a** (163 mg, 0.5 mmol), **5** (252 mg, 0.5 mmol), $PdCl_2(PPh_3)_2$ (22 mg, 0.031 mmol), and CuI (12 mg, 0.063 mmol) was suspended in dry $Et_3N$ (30 mL) under argon. The mixture was stirred at room temperature for 24 h. The solvent was removed in vacuo and $CHCl_3$ (40 mL) was added. The organic phase was washed with $H_2O$ and dried over $Na_2SO_4$. After removing the volatiles, the residue was purified by silica gel column chromatography (chloroform). Yield: 148 mg (42%); white foam; $R_f$ 0.37 (3% MeOH/$CHCl_3$); mp 113–115 °C. $^1$H NMR ($CDCl_3$): $\delta$ 1.08 (t, 3H, $J$ = 7.4 Hz, $CH_2CH_3$), 1.18 (t, 3H, $J$ = 7.4 Hz, $CH_2CH_3$), 2.27 (s, 6H, $2 \times CH_3$), 2.28 (s,6H, $2 \times CH_3$), 2.42 (q, 2H, $J$ = 7.4 Hz, $CH_2CH_3$), 2.55 (q, 2H, $J$ = 7.4 Hz, $CH_2CH_3$), 4.09 (s, 2H, $CH_2Ph$), 4.16 (s, 2H, $CH_2Ph$), 4.54 (s, 2H, $OCH_2C\equiv$), 4.69 (s, 2H,$OCH_2$), 5.30 (br s, 4H, $NCH_2O$), 6.68 (s, 2H, aryl), 6.74 (s, 2H, aryl), 6.89 (s, 2H, aryl), 7.15 (d, 2H, $J$ = 8.6 Hz, aryl), 7.18 (d, 2H, $J$ = 8.6 Hz, aryl), 10.16 (s, 1H, NH), 10.56 (s, 1H, NH).$^{13}$C NMR ($CDCl_3$): $\delta$ 14.1 ($2 \times CH_2CH_3$), 19.0 ($CH_2CH_3$), 19.2 ($CH_2CH_3$), 21.3 ($4 \times CH_3$), 33.1 ($CH_2Ph$), 33.4 ($CH_2Ph$), 59.4 ($OCH_2C\equiv$), 73.1 ($NCH_2O$), 74.0 ($OCH_2$), 73.6 ($NCH_2O$), 85.2 ($CH_2C\equiv C$), 85.4 ($CH_2C\equiv C$), 116.4 (C-5), 116.7 (C-5), 121.4, 125.1, 126.9, 129.0, 131.0, 134.8, 134.9, 138.5, 138.9 (aryl), 149.1 (C-2), 149.8 (C-2), 151.1 (C-6), 151.9 (C-6), 164.4 (C-4), 164.9 (C-4). HRMS (MALDI) $m/z$ Calcd for $C_{42}H_{46}N_4O_6Na^+$ ($MNa^+$) 725.3310 Found, 725.3301.

**5.1.6. 6-(3,5-Dimethylbenzyl)-1-[3-[4-[3-[6-(3,5-dimethylbenzyl)-5-ethyl-2,4-dioxo-1,2,3,4-tetrahydro-1-pyrimidinylmethoxy]-1-propynyl]phenyl]-2-propynyloxymethyl]-5-ethyl-1,2,3,4-tetrahydro-2,4-pyrimidinedione (7).** A mixture of **1a** (196 mg, 0.60 mmol), diiobenzene (88 mg, 0.27 mmol), $PdCl_2(PPh_3)_2$ (52 mg, 0.074 mmol), CuI (28 mg, 0.147 mmol), and $Et_3N$ (10 mL) was stirred overnight under argon. After work up as described for compound **6**, the residue was subjected to silica gel column chromatography ($CHCl_3$) to give two fractions. The slow eluted fraction was the target compound **7**. Yield: 87 mg (48%); white solid; $R_f$ 0.15 (3% MeOH/$CHCl_3$); mp 180–182 °C. $^1$H NMR (DMSO-$d_6$): $\delta$ 0.84 (t, 6H, $J$ = 7.2 Hz, $CH_2CH_3$),2.18 (s, 12H, $4 \times CH_3$), 2.28 (q, 4H, $J$ = 7.2 Hz,$CH_2CH_3$),4.04 (s, 4H, $CH_2Ph$), 4.50 (s, 4H, $OCH_2C\equiv$), 5.15 (s, 4H, $NCH_2O$), 6.76 (s, 4H, aryl), 6.86 (s, 2H, aryl), 7.36 (s, 4H, aryl), 11.50 (s, 2H,NH). $^{13}$C NMR (DMSO-$d_6$): $\delta$ 13.4 ($CH_2CH_3$), 18.5($CH_2CH_3$), 20.7 ($2 \times CH_3$), 32.7($CH_2Ph$),56.6 ($OCH_2C\equiv$), 72.1 ($NCH_2O$), 85.0 ($OCH_2C\equiv C$), 87.5 ($OCH_2C\equiv C$), 115.5 (C-5), 121.9, 124.8, 128.3, 131.5, 135.7, 137.9 (aryl), 148.1 (C-2), 151.4 (C-6), 162.9 (C-4). HRMS (MALDI) $m/z$ Calcd for $C_{44}H_{46}N_4O_6Na^+$ ($MNa^+$) 749.3310 Found, 749.3314. Anal. Calcd for $C_{44}H_{46}N_4O_6 \cdot 2H_2O$: C, 69.65; H, 6.13; N, 7.34. Found: C, 69.27; H, 6.08; N, 7.34.

**5.1.7. 6-(3,5-Dimethylbenzyl)-5-ethyl-1-[3-(4-iodophenyl) prop-2-ynyloxymethyl]-1H-pyrimidine-2,4-dione (8).** Isolated as the fast eluted fraction. Yield: 30 mg (21%); brown foam; $R_f$ 0.36 (3% MeOH/$CHCl_3$). $^1$H NMR ($CDCl_3$): $\delta$ 1.01 (t, 3H, $J$ = 7.5 Hz, $CH_2CH_3$), 2.25 (s, 6H, $2 \times CH_3$), 2.44 (q, 2H, $J$ = 7.5 Hz, $CH_2CH_3$), 4.09 (s, 2H, $CH_2Ph$), 4.49 (s, 2H, $OCH_2C\equiv$), 5.25 (s, 2H, $OCH_2N$), 6.69 (s, 2H, aryl), 6.89 (s, 1H, aryl), 7.10 (d, 2H, $J$ = 9.0 Hz, aryl), 7.63 (d, 2H, $J$ = 9.0 Hz, aryl), 9.42 (s, 1H, NH). $^{13}$C NMR ($CDCl_3$): $\delta$ 13.7 ($CH_2CH_3$), 19.2 ($CH_2CH_3$), 21.2 ($2 \times CH_3$), 33.3 ($CH_2Ph$), 58.0 ($OCH_2C\equiv$),72.6 ($NCH_2O$), 85.6 ($OCH_2C\equiv$), 85.8 ($OCH_2C\equiv C$), 94.7 (C–I), 116.9 (C-5), 121.6, 125.0, 129.0, 133.3, 134.7, 137.5, 138.9 (aryl), 149.1 (C-2), 152.0 (C-6), 163.2 (C-4); HRMS (MALDI) $m/z$ calcd for $C_{25}H_{25}IN_2O_3Na^+$ ($MNa^+$), 551.0802 Found, 551.0791.

**5.1.8. 6-(3,5-Dimethylbenzyl)-5-ethyl-1-[1-[(2*S*,3*S*,5*R*)-2-hydroxymethyl-5-(5-methyl-2,4-dioxo-1,2,3,4-tetrahydro-1-pyrimidinyl)tetrahydro-3-furanyl]-1*H*-1,2,3-triazol-4-ylmethoxymethyl]-1,2,3,4-tetrahydro-2,4-pyrimidinedione (9).** AZT (133.5 mg, 0.50 mmol) and **1a** (163 mg, 0.50 mmol) were suspended in a 1:1 mixture of $H_2O$ and *tert*-butyl alcohol (3 mL). Sodium ascorbate (0.3 mmol, 300 μL of freshly prepared 1 M solution in $H_2O$) was added, followed by copper (II) sulfate pentahydrate (7.5 mg, 0.03 mmol, in 100 μL of $H_2O$). The heterogeneous mixture was stirred vigorously overnight. When TLC analysis indicated complete consumption of the reactants, the reaction mixture was diluted with $H_2O$ (50 mL), and the white precipitate was collected by filtration. After washing with cold $H_2O$ ($2 \times 25$ mL), it was dried under vacuum to afford 206 mg (69%) of pure product as a white powder; $R_f$ 0.10 (5% MeOH/$CHCl_3$); mp 132–134 °C. $^1$H NMR (DMSO-$d_6$): $\delta$ 0.90 (t, 3H, $J$ = 7.0 Hz, $CH_2CH_3$), 1.82 (s,3H, $CH_3$), 2.22 (s, 6H, $2 \times CH_3$), 2.28 (q, 2H,$J$ = 7.0 Hz, $CH_2CH_3$), 2.63–2.73 (m, 2H, H-2′), 3.60–3.64 (m, 2H, H-5′), 3.98 (s, 2H, $CH_2Ph$), 4.18–4.21 (m,1H, H-3′), 4.63 (s, 2H, $OCH_2$), 5.09 (s, 2H, $NCH_2O$), 5.29–5.35 (m, 2H, H-4′,OH), 6.42 (t, 1H, $J$ = 6 Hz, H-1′), 6.73 (s, 2H, aryl), 6.87 (s, 1H, aryl), 7.82 (s, 1H, C-6), 8.24 (s, 1H, CH, triazole), 11.36 (s,

1H, NH), 11.47 (s, 1H, NH). $^{13}$C NMR (DMSO-$d_6$): $\delta$ 12.2 ($CH_3$), 13.5 ($CH_2CH_3$), 18.5 ($CH_2CH_3$), 20.8 ($CH_3Ph$), 32.8 (C-2′), 37.1 ($CH_2Ph$), 59.1 (C-3′), 60.6 ($OCH_2$), 61.7 (C-5′), 72.0 ($NCH_2O$), 83.8 (C-4′), 84.4 (C-1′), 109.6 (C-5), 115.4 (C-5), 123.5, 124.9, 128.2, 135.8, 136.2, 138.0, 143.4, 148.2, 150.4, 151.5, 163.0, 163.6 (aryl, triazole, uracils). HRMS (MALDI) *m/z* Calcd for $C_{29}H_{35}N_7O_7Na^+$ ($MNa^+$) 616.2490 Found, 616.2460.

**5.1.9. 6-(3,5-Dimethylbenzyl)-5-ethyl-1-[4-(13-ethyl-17-hydroxy-3-oxo-2,3,6,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-17-ylethynyl)benzyloxymethyl]uracil (11).** Compound **5** (173 mg, 0.34 mmol), D-(−)-norgestrel **10** (107 mg, 0.34 mg), $PdCl_2(PPh_3)_2$ (22 mg, 0.031 mmol), and CuI (12 mg, 0.063 mmol) were stirred in $Et_3N$ (20 mL) at rt for 2 days. The mixture was evaporated to dryness, and $CHCl_3$ (15 mL) was added. The organic phase was washed with $H_2O$ (20 mL), dried and evaporated. The residue was purified by silica gel column chromatography (chloroform). A negligible amount of the homocoupled product **12** was also formed. Yield 200 mg (85%); yellow foam; $R_f$ 0.29 (3% MeOH/$CHCl_3$); mp 178−180 °C. $^1$H NMR ($CDCl_3$): $\delta$ 0.82–2.53 (m, 36H), 4.03 (s, 2H, $CH_2Ph$), 4.62 (s, 2H, $OCH_2$), 5.17 (s, 2H, $NCH_2O$), 5.83 (s, 1H, CH=), 6.63 (s, 2H, aryl), 6.88 (s, 1H, aryl), 7.25 (d, 2H, $J$ = 8.4 Hz, aryl), 7.37 (d, 2H, $J$ = 8.4 Hz, aryl), 8.95 (s, 1H, NH). $^{13}$C NMR ($CDCl_3$): $\delta$ 9.6, 13.7, 18.9, 19.0, 21.3, 22.5, 26.1, 26.2, 28.8, 30.7, 33.2, 35.5, 36.5, 39.4, 40.9, 42.4, 48.5, 49.0, 51.1 (alifatic), 71.2 ($OCH_2$), 72.7 ($NCH_2O$), 81.7, 85.7 (C-17, −C≡), 93.4 (−C≡*C*), 116.8 (C-5), 122.5, 124.6, 124.9, 127.6, 129.0, 131.6, 134.7, 137.5, 138.9 (aryl and −CH=), 149.1 (C-6), 151.8 (C-2), 163.0 (C-4), 166.6 (>C–), 199.9 (C=O). HRMS (MALDI) *m/z* Calcd for $C_{44}H_{52}N_2O_5Na^+$ ($MNa^+$) 711.3768 Found, 711.3742.

**5.1.10. 15-Ethyl-14-[4-(15-ethyl-14-hydroxy-5-oxotetracyclo[8.7.0.0$^{2,7}$.0$^{11,15}$]heptadec-6-en-14-yl)-1,3-butadiynyl]-14-hydroxytetracyclo.[8.7.0.0$^{2,7}$0$^{11,?}$]-heptadec-6-en-5-one (12).** D-(−)-norgestrel **10** (78 mg, 0.25 mmol), $PdCl_2(PPh_3)_2$ (6.2 mg, 0.0088 mmol), CuI (1.5 mg, 0.0081 mmol), $Et_3N$ (2 mL) and toluene (6 mL) were stirred under $O_2$ atmosphere overnight. The volatiles were removed in vacuo and the residue was further purified by silica gel column chromatography (chloroform). Yield: 75 mg (96%); yellowish solid; $R_f$ 0.30 (3% MeOH/$CHCl_3$); mp 215–217 °C. $^1$H NMR ($CDCl_3$): $\delta$ 0.86–2.51 (m, 50H), 5.83 (s, 2H, =CH). $^{13}$C NMR ($CDCl_3$): $\delta$ 9.5, 18.9, 22.5, 26.1, 26.5, 28.8, 30.6, 35.4, 36.5, 39.5, 40.9, 42.4, 48.7, 48.7, 51.1 (aliphatic), 70.3 (–C≡ *C*–*C*≡C–), 82.1, 83.6 (C-17, -*C*≡C–C≡*C*-), 124.6 (=CH), 166.4 (=C<), 199.9 (C=O). HRMS (MALDI) *m/z* Calcd for $C_{42}H_{54}O_4Na^+$ ($MNa^+$) 645.3914 Found, 645.3892. Anal. Calcd for $C_{42}H_{54}O_4 \cdot 3H_2O$: C, 74.46; H, 7.98. Found: C, 74.90; H, 8.13.

### 5.2. Antiviral assay procedures

Compounds were solubilized in DMSO at 100 mM and then diluted in culture medium.

**5.2.1. Virus and cells.** 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 $4.5 \times 10^6$ 50% cell culture infectious dose ($CCID_{50}$)/mL. The K103R + V179D + P225H mutant (EFV®) was derived from an IIIB strain passaged in MT-4 cells in the presence of efavirenz (up to 2 μM). 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). $EFV^R$, N119, and N117 stock solutions had titers of $4.0 \times 10^7$, $1.2 \times 10^8$, and $2.1 \times 10^7$ $CCID_{50}$/mL, respectively.

**5.2.2. 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 4 days of incubation. Virus titers were expressed as $CCID_{50}$/mL.

**5.2.3. Anti-HIV assays.** The activity of test compounds against multiplication of wild type HIV-1, $EFV^R$, N119, and N117 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 $1 \times 10^4$ 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.[39] Alternatively, p24 levels were determined by an immunoenzymatic kit (Abbott). 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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