Full Paper

# Synthesis and Anti-HIV-1 Activity of New MKC-442 Analogues with an Alkynyl-Substituted 6-Benzyl Group*

**Youssef L. Aly[1,2], Erik B. Pedersen[1], Paolo La Colla[3], and Roberta Loddo[3]**

[1] Nucleic Acid Center**, Department of Physics and Chemistry, University of Southern Denmark, Odense M, Denmark
[2] Chemistry Department, Faculty of Education Kafr El-Sheikh branch, Tanta University, Kafr El-Sheikh, Egypt (on leave)
[3] Dipartimento di Scienze e Tecnologie Biomediche, Sezione di Microbiologia e Virologia Generale e Biotecnologie Microbiche, Universita di Cagliari, Monserrato, Italy

Synthesis and antiviral activities are reported of a series of 6-(3-alkynyl benzyl)-substituted analogues of MKC-442 (6-benzyl-1-(ethoxymethyl)-5-isopropyluracil), a highly potent agent against HIV. The 3-alkynyl group is assumed to give a better stacking of the substituted benzyl group to reverse transcriptase (RT) and this was believed to improve antiviral activity against HIV-1. The bromo derivatives, 5-alkyl-6-(3-bromo-benzyl)-1-ethoxymethyl derivatives **7a, b** and 5-alkyl-6-(3-bromobenzyl)-1-allyloxymethyl derivatives **9a, b,** showed activity against HIV on the same level as their corresponding analogues **10a – d** with a 3-trimethylsilylalkynylbenzyl substituent and their desilylated analogues **11a – d.** However, they all showed activity against HIV-1 wild type in the range of more than 10fold lower than the one of MKC-442. Moderate activity against Y181C and Y181C + K103N mutated strains was also observed and, in some cases, they were marginally better than those found for MKC-442. A few amino-DABO and *S*-DABO analogues were also synthesized but they were found to be inactive against HIV.

**Keywords:** Human immunodeficiency virus (HIV-1) / MKC-442 / Non-nucleoside reverse transcriptase inhibitors (NNRTI) / Stacking / Sonogashira coupling

Received: October 4, 2006; accepted: February 6, 2007

DOI 10.1002/ardp.200600163

## Introduction

The reverse transcriptase (RT) of the human immunodeficiency virus type 1 (HIV-1) is a key enzyme, which plays an essential and multifunctional role in the replication cycle. This enzyme is responsible for the conversion of a single-stranded RNA genome of HIV-1 into a double-stranded DNA chain that subsequently is incorporated into the DNA of the infected host cell. Non-nucleoside inhibitors of HIV-1 reverse transcriptase (NNRTIs) [1] inhibit the enzyme by occupation of an induced allosteric binding site very close to the active site [2], they are highly specific as their binding site is a hydrophobic pocket located approximately 10 Å from the polymerase active site [3]. Several RT inhibitors have been developed and approved by FDA (US Food and Drugs Administration) and are currently in clinical use. In particular, the non-nucleoside RT inhibitors [4 – 6] are highly effective drugs with few side effects.

6-Benzyl-1-(ethoxymethyl)-5-isopropyluracil (MKC-442 or emivirine, Fig. 1) [7] showed high activity against HIV-1 and was chosen as a candidate for clinical trials with AIDS patients [8]. Unfortunately, it was reported that MKC-442 triggers the liver enzyme cytochrome P450,

**Correspondence:** Erik B. Pedersen, Nucleic Acid Center, Department of Physics and Chemistry, University of Southern Denmark, Campusvej 55, DK-5230 Odense M, Denmark.
**E-mail**: EBP@Chem.sdu.dk
**Fax**: +45 66158780

* The information in this document is provided as is and no guarantee or warranty is given that the information is fit for any particular purpose. The user thereof uses the information as its sole risk and liability.
** A research center funded by The Danish National Research Foundation for studies on nucleic acid chemical biology.

© 2007 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

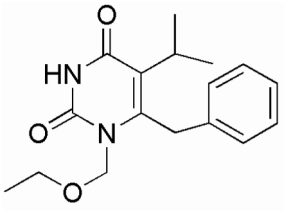


**Figure 1.** Chemical structure of MKC-442.

leading to drug interactions between MKC-442 and protease inhibitors [9].

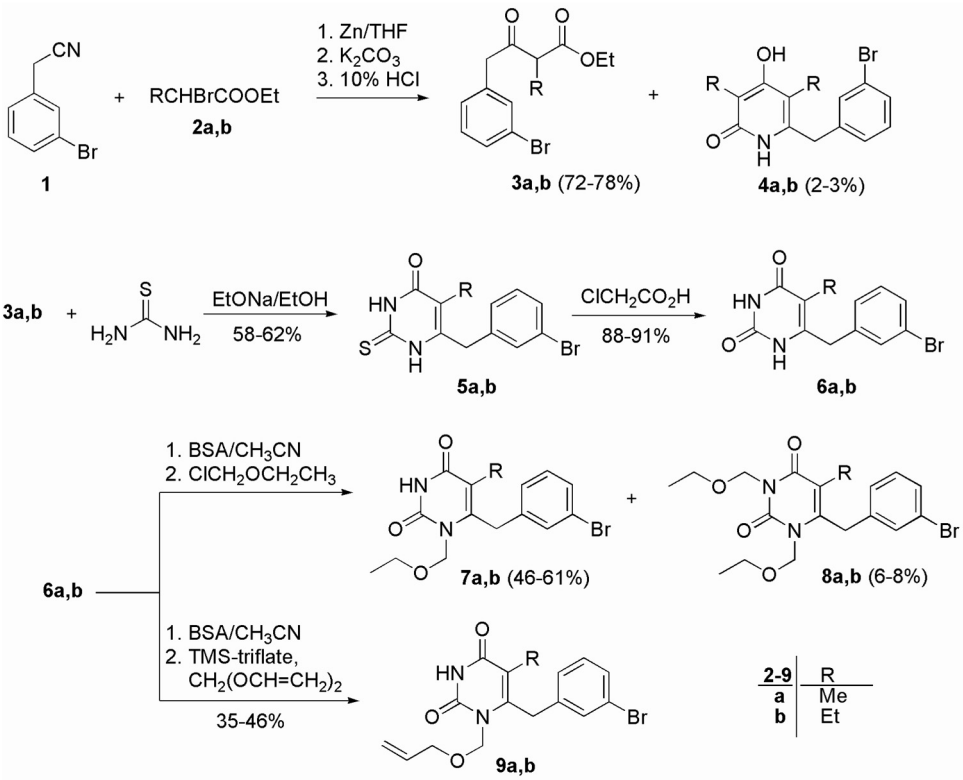
The inhibitor MKC-442 is held in place in the hydrophobic pocket by hydrogen bonds and hydrophobic bonds to the nearby amino acids. Although *m*-methyl groups on the benzyl substituent of MKC-442 can make favourable van-der-Waals contacts with Trp229 of the binding pocket, they are metabolically unfavourable, being open to electrophilic attack [3]. Instead, we considered *m*-ethynyl as an interesting possibility due to its better stacking properties. One could think improved stacking properties of the inhibitor to be important for inhibition of those mutated HIV that have a reduced number of aromatic rings in the binding pocket of RT, due to replacement of Tyr181, Tyr188 or Phe227 with non-aromatic amino acids.

As an attempt to optimize the MKC-442 lead structure [10–15], we have therefore introduced alkynyl groups at the *m*-position of the aromatic ring hoping to obtain better stacking MKC-442 analogues with improved activity against HIV.

## Results and discussion

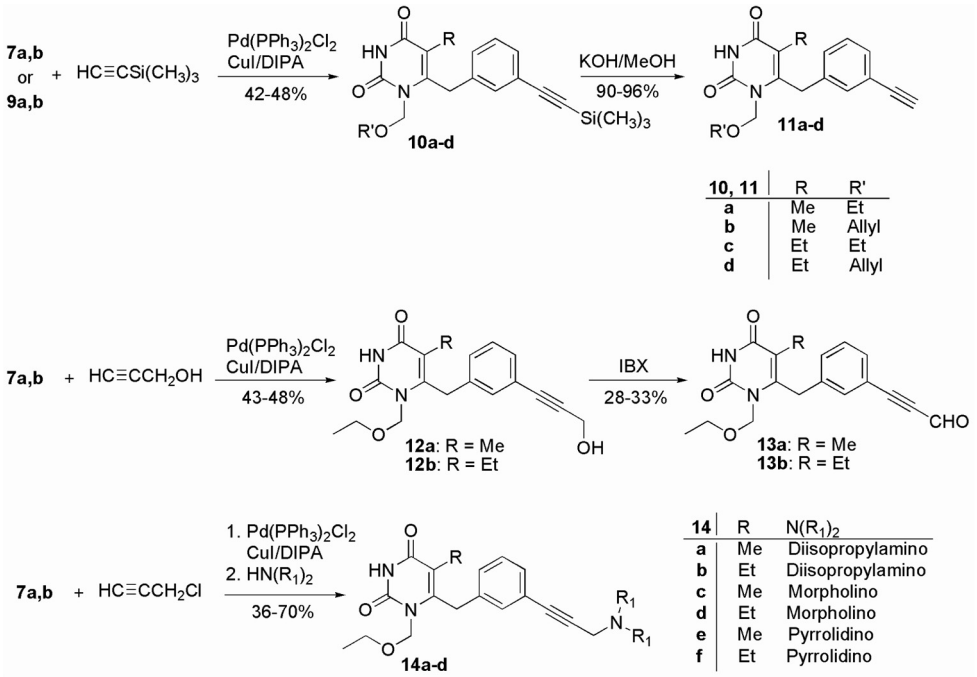
### Chemistry

For the synthesis of *m*-alkynyl MKC-442 analogous **11a–d**, the key intermediates ethyl 4-(*m*-bromophenyl)acetoacetates **3a, b** were synthesized in 72–78% yield by reaction of *m*-bromophenylacetonitrile **1** with a zinc organometallic reagent prepared from ethyl 2-bromopropionate or ethyl 2-bromobutyrate in anhydrous THF. In this reaction, the pyridinones **4a, b** were obtained as by-products in 2–3% yield. The acetoacetates **3a, b** were then used as starting materials for the synthesis of uracil rings in a ring-closure reaction with thiourea and sodium ethoxide in boiling ethanol. The so formed 6-(3-bromobenzyl)thiouracils **5a, b** were desulfurized with aqueous chloroacetic acid [16, 17] to give the corresponding uracil derivatives **6a, b** in an overall yield of 51–56% for the two steps from **3a, b**. Compounds **6a, b** were silylated using *N,O*-bis-(trimethylsilyl)acetamide (BSA) in acetonitrile




**Scheme 1.** Synthesis route of compounds **3–9**.

© 2007 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim 



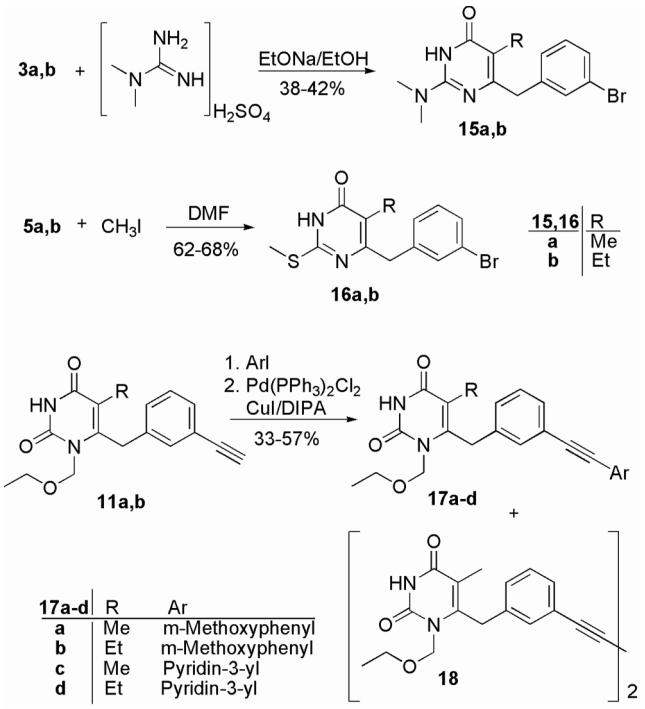

**Scheme 2.** Synthesis route of compounds **10**–**14**.

and subsequently N-1 alkylated using chloromethylethyl ether [15] to give the non-nucleoside analogues **7a, b** in 46–61% yield and the N-1, N-3 bis-alkylated by-products **8a, b** in 6–8% yield. The uracils **6a, b** were also silylated and reacted with bis-(allyloxy)methane in the presence of trimethylsilyl trifluoromethanesulfonate (TMS-triflate) as a Lewis-acid catalyst [18] to give the non-nucleoside analogues **9a, b** in 35–46% yield (Scheme 1).

Coupling of the bromo derivatives **7a, b** and **9a, b** with trimethylsilylacetylene (TMSA) in the presence of bis(triphenylphosphine)palladium(II)dichloride and CuI in diisopropylamine (DIPA) as solvent under nitrogen using the condition of Price and Tour [19] afforded the corresponding trimethylsilylethynyl derivatives **10a–d** in 42–48% yield. Desilylation of **10a–d** was achieved using potassium hydroxide and methanol to afford *m*-alkynylbenzyl analogues **11a–d** in 90–96% yield (Scheme 2). Coupling of compounds **7a, b** with propargyl alcohol using the above condition for the Sonogashira coupling afforded **12a, b** in 43–48% yield. Compounds **12a, b** were oxidized using *o*-iodoxybenzoic acid (IBX) in ethyl acetate to afford the aldehyde derivatives **13a, b** in 28–33% yield. These aldehydes were also considered interesting target compounds because a chemical reaction to form a thiohemiacetal could be anticipated to take place between the aldehyde group of the inhibitor and the mercapto group in cysteine of the Y181C RT mutation. This would be an alternative way to ensure strong binding of the inhibitor to this type of mutated RT. Also, we found it worthy to consider the effect of aminopropynyl substituents where additional chances were anticipated for an extra hydrogen bonding between their protonated forms and RT at physiological conditions. As the Sonogashira coupling can be done in a secondary amine as a solvent, it was considered obvious to form propargylamines *in situ* prior to the Sonogashira coupling. Reaction of **7a, b** with propargyl chloride in the presence of the secondary amines diisopropylamine, morpholine and pyrrolidine afforded the propargylamine products **14a–f** in 36–70% yield (Scheme 2).

For the synthesis of amino-DABOs **15a, b**, the β-ketoesters **3a, b** were reacted with 1,1-dimethylguanidine sulphate and EtONa in EtOH to afford **15a, b** in 38–42% yield. The reaction of thiouracil derivative **5a, b** with $CH_3I$ in DMF afforded the corresponding *S*-DABOs **16a, b** in 62–68% yield. Unfortunately, the Sonogashira coupling failed on both amino-DABOs **15a, b** and *S*-DABOs **16a, b**. Coupling of compounds **11a, b** with the aryl iodides 3-iodoanisole and 3-iodopyridine in the presence of bis(triphenylphosphine)palladium(II)dichloride and CuI in triethylamine as solvent under nitrogen afforded the coupling products **17a–d** in 33–57% yield and the homo-coupling product **18** as by-product in 3% yield (Scheme 3). The compounds **17a–d** were synthesized in order to further increase the staking ability of the benzylic part of the MKC-442 analogue and to explore

© 2007 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim 




**Scheme 3.** Synthesis route of compounds **15**–**18**.

how this could influence the activity against HIV-1, the risk being a too bulky substituent.

## Biological screening

The cytotoxicity and the anti-HIV activity of the synthesized alkynyl MKC-442 analogues are reported in Table 1. In general, the introduction of an alkynyl group in the benzylic part at the 3-position did not improve the activity against HIV-1 compared to MKC-442. In fact, compounds **10a–d**, **11a–d** and **12a, b** showed more than 10fold lower activity against the wild type HIV-1 than MKC-442. When compared with MKC-442, it was therefore surprising to find better activity for some of these compounds against the mutant Y181C than for MKC-442. When considering the bromo derivative **7b** as the reference compound, the activity against wild type HIV-1 was also lower in all cases when replacing the bromo substituent with substituents comprising acetylene. However, for the activity against the mutant Y181C, it was retained for the acetylene derivatives **10c, d**, **11c** and **13b**. Compound **7b** in turn was more potent against this mutant than MKC-442. An extra aminomethyl group or an aryl group on the ethynyl substituent was detrimental to the activity against mutated virus. Furthermore, the activity against wild type HIV-1 was also reduced. The lower activity of the compounds **17a–d** against wild type and the mutants indicates that the stacking principle is not a sufficient prerequisite for activity against the HIV mutants. The examples of amino-DABOs **15a, b** and *S*-DABOs **16a, b** with a 3-bromo substituent in the benzylic part were devoid of activity against HIV-1. One can conclude that the activity against HIV can not be increased by acetylene substitution in order to improve stacking properties of the benzylic part of the drug. It has previously been

**Table 1.** Antiviral activity of compounds **4–17** against HIV-1 in MT-4 cells[a].

| Compound N° | $CC_{50}$ (μM)[b] | $EC_{50}$ (μM)[c] | | | |
|---|---|---|---|---|---|
| | | Wild-type | $EFV^R$ | Y181C | K103N+-Y181C |
| **4a** | >100 | >100 | >100 | >100 | >100 |
| **4b** | >100 | >100 | >100 | >100 | >100 |
| **5a** | >100 | >100 | >100 | >100 | >100 |
| **5b** | >100 | 67 ± 12 | >100 | >100 | >100 |
| **6a** | >100 | >100 | >100 | >100 | >100 |
| **6b** | >100 | 41 ± 5 | >100 | >100 | >100 |
| **7a** | >100 | 0.5 ± 0.2 | >100 | 16 ± 1 | >100 |
| **7b** | >100 | 0.05 ± 0.01 | >100 | 5 ± 1 | 15 ± 5 |
| **9a** | >100 | 0.5 ± 0.02 | 88 ± 12 | 12 ± 1 | 90 ± 10 |
| **9b** | 58 ± 9 | 0.02 ± 0.05 | 17 ± 2 | 2.7 ± 0.3 | 12 ± 2 |
| **10a** | >100 | 4 ± 2 | >100 | 51 ± 5 | >100 |
| **10b** | 36 ± 5 | 1.3 ± 0.5 | >36 | >36 | >36 |
| **10c** | 41 ± 8 | 0.5 ± 0.05 | >41 | 15 ± 4 | >41 |
| **10d** | 30 ± 5 | 0.4 ± 0.05 | >30 | 9 ± 1 | >30 |
| **11a** | >100 | 2 ± 0.5 | >100 | 35 ± 4 | >100 |
| **11b** | >100 | 1.9 ± 0.1 | >100 | 50 ± 4 | >100 |
| **11c** | >100 | 0.3 ± 0.05 | >100 | 11 ± 0.5 | 42 ± 4 |
| **11d** | 26 ± 6 | 4 ± 0.05 | >26 | >26 | >26 |
| **12a** | >100 | 1.9 ± 0.01 | >100 | >100 | >100 |
| **12b** | >100 | 0.4 ± 0.05 | >100 | >100 | >100 |
| **13a** | 21 ± 7 | >21 | >21 | >21 | >21 |
| **13b** | >100 | 0.1 ± 0.01 | >100 | 9 ± 0.5 | 62 ± 7 |
| **14a** | >100 | 14 ± 3 | >100 | >100 | >100 |
| **14b** | 46 ± 2 | 0.6 ± 0.3 | >46 | >46 | >46 |
| **14c** | >100 | 56 ± 13 | >100 | >100 | >100 |
| **14c** | >100 | 56 ± 13 | >100 | >100 | >100 |
| **14e** | >100 | 75 ± 25 | >100 | >100 | >100 |
| **14f** | >100 | 6 ± 1 | >100 | >100 | >100 |
| **15b** | >100 | 9 ± 1 | >100 | 88 ± 5 | 85 ± 15 |
| **16a** | >100 | 6 ± 3 | >100 | 95 ± 5 | >100 |
| **16b** | >100 | 11 ± 1 | >100 | 90 ± 10 | >100 |
| **17a** | 32 ± 2 | 8 ± 0.2 | >32 | >32 | >32 |
| **17b** | 43 ± 5 | 7 ± 3 | >43 | >43 | >43 |
| **17c** | 65 ± 5 | 2 ± 0.5 | >65 | >65 | >65 |
| **17d** | 42 ± 1 | 10 ± 0.5 | >42 | >42 | >42 |
| MKC-442 | >100 | 0.03 ± 0.005 | 86 ± 14 | 24 ± 3 | >100 |

[a] Data represent mean values of at least two separate experiments.

[b] Compound dose required to reduce the viability of mock-infected cells by 50%, as determined by the MTT method.

[c] 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 $CC_{50}$ was not reached at the highest concentration tested. For description of assay see the Section 3 (Experimental).

© 2007 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim 

attempted to replace the benzylic part with a 1-naphthylmethyl substituent, but when using the same N-1 and 5-substituents as in the present investigation, this was also found a disadvantage when compared with the activity against MKC-442 [20 – 22].

*This work received funding from the European Community's Sixth Framework Programme under contract number LSHP-CT-2004-503162 (Selection and development of microbicides for mucosal use to prevent sexual HIV transmission/acquisition).*

## Experimental

### Chemistry

NMR spectra were recorded on a Varian Gemini 2000 NMR spectrometer (Varian, Palo Alto, CA, USA) at 300 MHz for $^1$H and 75 MHz for $^{13}$C with TMS as an internal standard. Chemical shifts are reported in parts per million (δ), and signals are expressed as s (singlet), d (doublet), t (triplet), q (quartet) or m (multiplet). MALDI spectra were recorded on an IonSpec Fourier Transform Ion Cyclotron Resonance Mass Spectrometer (IonSpec Corporation, Lake Forest, CA, USA). Melting points were determined on a Büchi melting point apparatus (Büchi Labortechnik, Flawil, Switzerland). Thin-layer chromatography (TLC) was performed on silica gel DC-alufolio 60 $F_{254}$ plates from Merck (Merck, Darmstadt, Germany). The silica gel (0.040 – 0.063 mm) used for column chromatography was purchased from Merck. Solvents used for column chromatography were distilled prior to use, while reagents were used as purchased.

#### *General procedure for preparation of compounds **3a, 3b** and **4a, 4b***

Zink dust (25 – 30 g) was activated by stirring with hydrochloric acid (4 M, 100 mL) for 5 min. The zinc dust was filtered and washed sequentially with $H_2O$ (100 mL), ethanol (100 mL) and anhydrous ether (100 mL). The zinc dust was dried by evaporation under reduced pressure at 80°C for 5 h, and kept *in vacuo* overnight. The active zinc was suspended in anhydrous THF (150 mL) and heated to reflux. A few drops of ethyl 2-bromopropionate **2a** or ethyl 2-bromobutyrate **2b** were added and the mixture was refluxed until a green colour appeared. *m*-Bromophenylacetonitrile (**1**, 66 mmol) was added in one portion and **2a** or **2b** (130 mmol) in 10 mL THF was added dropwise. After complete addition the mixture was refluxed for 5 h. After cooling to room temperature and dilution with THF (300 mL), the mixture was quenched by addition of saturated aqueous $K_2CO_3$ (100 mL). The mixture was stirred for 1 h and then the THF layer was decanted off, and the aqueous phase was washed with THF (3 × 100 mL). The combined THF fractions were stirred with 10% aqueous hydrochloric acid (90 mL) for 45 min, and the solution was evaporated under reduced pressure and dichloromethane (300 mL) was added. The organic phase was washed with saturated aqueous $NaHCO_3$ (2 × 100 mL), dried over anhydrous magnesium sulphate, and evaporated under reduced pressure. The residue was chromatographed on a silica gel column with petroleum ether (60 – 80°C)/ethyl acetate (v/v = 80 : 20) to give **3a, b** and **4a**, **b**.

#### *Ethyl 4-(3-bromophenyl)-2-methyl-3-oxo-butyrate **3a***

Yield 15.4 g (78%) as an oil. $^1$H-NMR ($CDCl_3$): δ (ppm) = 1.24 – 1.34 (m, 6H, 2 × $CH_3$), 3.59 (q, 1H, *J* = 7.2 Hz, CH), 3.80 (s, 2H, $ArCH_2$), 4.16 (q, 2H, *J* = 7.2 Hz, $CH_2O$), 7.11 – 7.21 (m, 4H, aryl). $^{13}$C-NMR ($CDCl_3$): δ (ppm) = 12.72 ($CH_3$), 14.01 ($CH_3$), 47.73 ($CH_2Ar$), 52.12 (CO*C*HCO), 61.50 (O*C*$H_2CH_3$), 122.55, 128.22, 130.07, 130.25, 132.55, 135.58 (aryl), 170.16 (C=O), 202.45 (C=O).

#### *Ethyl 4-(3-bromophenyl)-2-ethyl-3-oxo-butyrate **3b***

Yield 14.8 g (72%) as an oil. $^1$H-NMR ($CDCl_3$): δ (ppm) = 0.88 (t, 3H, *J* = 7.4 Hz, C*H*$_3$CH$_2$), 1.25 (t, 3H, *J* = 7.1 Hz, C*H*$_3$CH$_2$O), 1.89 (quint, 2H, *J* = 7.6 Hz, C*H*$_2$CH$_3$), 3.44 (t, 1H, *J* = 7.3 Hz, CH), 3.79 (s. 2H, ArC*H*$_2$), 4.15 (q, 2H, *J* = 7.1 Hz, $CH_2O$), 7.10 – 7.41 (m, 4H, $H_{arom}$). $^{13}$C-NMR ($CDCl_3$): δ (ppm) = 11.76 ($CH_3$), 14.05 ($CH_3$), 21.48 (*C*$H_2CH_3$), 48.15 ($CH_2Ar$), 59.84 (CO*C*HCO), 61.39 (O*C*$H_2CH_3$), 122.52, 128.26, 130.05, 130.24, 132.58, 135.46 (aryl), 169.40 (C=O), 201.85 (C=O). HRMS-MALDI: *m/z* = 335.0250 [M+Na$^+$] ($C_{14}H_{17}BrNaO_3$); requires 335.0253.

#### *6-(3-Bromobenzyl)-4-hydroxy-3,5-dimethylpyridin-2 (1H)-one **4a***

Yield 0.49 g (2%); m. p. 250 – 252°C. $^1$H-NMR (DMSO): δ (ppm) = 1.87, 1.88 (2 × s, 6H, 2 × $CH_3$), 3.39 (s, 1H, OH), 3.85 (s, 2H, $ArCH_2$), 7.19 – 7.43 (m, 4H, aryl), 11.26 (s, 1H, NH). $^{13}$C-NMR (DMSO): δ (ppm) = 8.93 ($CH_3$), 10.43 ($CH_3$), 34.80 ($CH_2Ar$), 104.77 (C-3), 105.17 (C-5), 121.64, 127.09, 129.18, 130.58, 130.72, 139.28 (aryl), 140.84 (C-6), 162.29 (C-4), 163.51 (C=O).

#### *6-(3-Bromobenzyl)-3,5-diethyl-4-hydroxypyridin-2 (1H)-one **4b***

Yield 0.7 g (3%); m. p. 225 – 227°C; $^1$H-NMR (DMSO): δ (ppm) = 0.82 – 0.91 (m, 6H, 2 × $CH_3$), 2.18 – 2.21 (m, 4H, 2 × $CH_2$), 3.88 (s, 2H, $ArCH_2$), 3.96 (br s, 1H, OH), 7.28 – 7.52 (m, 4H, aryl), 11.18 (br s, 1H, NH). $^{13}$C-NMR (DMSO): δ (ppm) = 13.11 ($CH_3$), 14.23 ($CH_3$), 16.21 (*C*$H_2CH_3$), 17.85 (*C*$H_2CH_3$), 34.92 ($CH_2Ar$), 105.93 (C-3), 106.55 (C-5), 121.51, 127.27, 129.23, 130.68, 138.32, 140.06, (aryl), 141.49 (C-6), 161.26 (C-4), 163.26 (C=O).

#### *General procedure for preparation of 5,6-substituted 2-thioxo-2,3-dihydro-1H-pyrimidin-4-one **5a, b***

Sodium (25.1 g, 1.1 mol) was dissolved in anhydrous ethanol (500 mL). Thiourea (58.23 g, 0.77 mol) was added and the mixture was heated to reflux. Compound **3a** or **3b** (51 mmol) was added dropwise, and the mixture was refluxed for 8 h. The solvent was evaporated *in vacuo* and the residue was dissolved in water (400 mL). The 2-thiouracil **5a** or **5b** was precipitated by neutralization with concentrated hydrochloric acid, and filtered off, washed with water and chromatographed on a silica gel column with petroleum ether (60 – 80°C)/ethyl acetate (v/v 1 : 1) to give **5a** and **5b**.

#### *6-(3-Bromobenzyl)-5-methyl-2-thioxo-2,3-dihydro-1H-pyrimidin-4-one **5a***

Yield 9.8 g (62%); m. p. 222 – 224°C. $^1$H-NMR ($CDCl_3$): δ (ppm) = 1.80 (s, 3H, $CH_3$), 3.86 (s, 2H, $CH_2Ar$), 7.28 – 7.50 (m, 4H, aryl), 12.26 (s, 1H, NH), 12.44 (s, 1H, NH). $^{13}$C-NMR ($CDCl_3$): δ (ppm) = 9.84 ($CH_3$), 34.33 ($CH_2Ar$), 111.53 (C-5), 121.75, 127.14, 129.66, 130.78, 131.06, 139.01 (aryl), 148.69 (C-6), 161.82 (C-4), 174.03 (C-

© 2007 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim 

2). HRMS-MALDI: $m/z$ = 310.9849 [M+H$^+$] ($C_{12}H_{11}BrN_2OS$); requires 310.9848.

### *6-(3-Bromobenzyl)-5-ethyl-2-thioxo-2,3-dihydro-1H-pyrimidin-4-one* ***5b***

Yield 9.6 g (58%); solid; m. p. 201–203°C. $^1$H-NMR ($CDCl_3$): δ (ppm) = 0.82 (t, 3H, $J$ = 7.2 Hz, C$H_3$CH$_2$), 2.25–2.32 (m, 2H, CH$_2$CH$_3$), 3.86 (s, 2H, CH$_2$Ar), 7.23–7.49 (m, 4H, aryl), 12.24 (s, 1H, NH), 12.44 (s, 1H, 1NH). $^{13}$C-NMR ($CDCl_3$): δ (ppm) = 12.84 (CH$_3$), 17.68 (CH$_2$CH$_3$), 33.96 (CH$_2$Ar), 117.32 (C-5), 121.71, 127.05, 129.61, 130.73, 130.97, 139.45 (aryl), 148.34 (C-6), 161.33 (C-4), 174.17 (C-2). HRMS-MALDI: $m/z$ = 324.9997 [M+H$^+$] ($C_{13}H_{13}BrN_2OS$); requires 325.0005.

### *General procedure for preparation of 5,6-substituted 1H-pyrimidine-2,4-diones* ***6a, b***

Compound **5a** or **5b** (0.1 mol) was suspended in aqueous chloroacetic acid (10%, 300 mL) and refluxed overnight. The mixture was cooled to room temperature, and the precipitated uracil was filtered off, washed with water, and dried *in vacuo* to give compounds **6a** and **6b**.

### *6-(3-Bromobenzyl)-5-methyl-1H-pyrimidin-2,4-dione* ***6a***

Yield 26.8 g (91%); m. p. 272–274°C. $^1$H-NMR ($CDCl_3$): δ (ppm) = 1.77 (s, 3H, CH$_3$), 3.77 (s, 2H, CH$_2$Ar), 7.28–7.51 (m, 4H, aryl), 10.79 (s, 1H, NH), 11.06 (s, 1H, 1NH). $^{13}$C-NMR ($CDCl_3$): δ (ppm) = 9.66 (CH$_3$), 34.79 (CH$_2$Ar), 105.36 (C-5), 121.74, 127.17, 129.59, 130.73, 130.95, 139.20 (aryl), 148.17 (C-6), 150.82 (C-2), 164.82 (C-4). HRMS-MALDI: $m/z$ = 316.9893 [M+Na$^+$] ($C_{12}H_{11}BrN_2NaO_2$); requires 316.9896.

### *6-(3-Bromobenzyl)-5-ethyl-1H-pyrimidin-2,4-dione* ***6b***

Yield 21.7 g (88%); m. p. 242–244°C. $^1$H-NMR ($CDCl_3$): δ (ppm) = 0.84 (t, 3H, $J$ = 7.2 Hz, C$H_3$CH$_2$), 2.25 (q, 2H, $J$ = 7.2 Hz, CH$_2$CH$_3$), 3.77 (s, 2H, CH$_2$Ar), 7.26–7.51 (m, 4H, aryl), 10.75 (s, 1H, NH), 11.05 (s, 1H, 1NH). $^{13}$C-NMR ($CDCl_3$): δ (ppm) = 13.48 (CH$_3$), 17.56 (CH$_2$CH$_3$), 34.41 (CH$_2$Ar), 111.55 (C-5), 121.71, 127.12, 129.56, 130.70, 130.95, 139.63 (aryl), 147.83 (C-6), 150.85 (C-2), 164.39 (C-4). HRMS-MALDI: $m/z$ = 309.0237 [M+H$^+$] ($C_{13}H_{13}BrN_2O_2$); requires 309.0233.

### *General procedure for the synthesis of* ***7a, b*** *and* ***8a, b***

N,*O-Bis*-(trimethylsilyl)acetamide (BSA, 6.2 mL, 2.5 mmol) was dissolved in anhydrous chloroform (30 mL) under $N_2$. Compound **6a** or **6b** (10 mmol) was added and after 10 min the mixture become a clear solution. Chloromethylethyl ether (1.5 mL, 15 mmol) was added and the reaction mixture was stirred overnight at room temperature under $N_2$, quenched with 25 mL ice cold saturated aqueous $NaHCO_3$, and extracted with dichloromethane (2 × 50 mL). The combined organic phases were dried over anhydrous magnesium sulphate, and evaporated under reduced pressure. The product was chromatographed on a column of silica gel with ethyl acetate/petroleum ether (60–80°C) (v/v = 1 : 1) to give compounds **7a, b** and **8a, b**.

### *6-(3-Brombenzyl)-1-ethoxymethyl-5-methyl-1H-pyrimidin-2,4-dione* ***7a***

Yield 1.6 g (46%); m. p. 102–104°C. $^1$H-NMR ($CDCl_3$): δ (ppm) = 1.16 (t, 3H, $J$ = 7.1 Hz, C$H_3$CH$_2$), 2.03 (s, 3H, CH$_3$), 3.59 (q, 2H, $J$ = 6.9 Hz, OC$H_2$CH$_3$), 4.14 (s, 2H, CH$_2$Ar), 5.14 (s, 2H, NCH$_2$O), 7.03–7.42 (m, 4H, aryl), 9.76 (s, 1H, NH). $^{13}$C-NMR ($CDCl_3$): δ (ppm) = 10.90 (CH$_3$), 14.95 (CH$_2$CH$_3$), 33.55 (CH$_2$Ar), 64.99 (OCH$_2$CH$_3$), 72.81 (NCH$_2$O), 111.16 (C-5), 123.34, 125.87, 130.30, 130.52, 130.70, 137.07 (aryl), 148.75 (C-6), 151.74 (C-2), 163.74 (C-4). HRMS-MALDI: $m/z$ = 375.0310 [M+Na$^+$] ($C_{15}H_{17}BrN_2NaO_3$); requires 375.0315.

### *6-(3-Bromobenzyl)-1-ethoxymethyl-5-ethyl-1H-pyrimidin-2,4-dione* ***7b***

Yield 2.2 g (61%); semisolid. $^1$H-NMR ($CDCl_3$): δ (ppm) = 1.04–1.20 (m, 6H, 2 × C$H_3$CH$_2$), 2.44 (q, 2H, $J$ = 7.3 Hz, CH$_2$CH$_3$), 3.60 (q, 2H, $J$ = 7.1 Hz, OC$H_2$CH$_3$), 4.14 (s, 2H, CH$_2$Ar), 5.11 (s, 2H, NCH$_2$O), 7.03–7.42 (m, 4H, aryl), 9.74 (s, 1H, NH). $^{13}$C-NMR ($CDCl_3$): δ (ppm) = 13.70 (CH$_3$), 14.97 (CH$_3$), 19.15 (CH$_2$CH$_3$), 32.96 (CH$_2$Ar), 65.05 (OCH$_2$), 72.75 (NCH$_2$O), 117.20 (C-5), 123.32, 125.84, 130.34, 130.51, 130.67, 137.61 (aryl), 148.13 (C-6), 151.88 (C-2), 163.28 (C-4). HRMS-MALDI: $m/z$ = 367.0642 [M+H$^+$] ($C_{16}H_{19}BrN_2O_3$); requires 367.0657.

### *6-(3-Bromobenzyl)-1,3-bis-(ethoxymethyl)-5-methyl-1H-pyrimidin-2,4-dione* ***8a***

Yield 0.24 g (6%); semisolid. $^1$H-NMR ($CDCl_3$): δ (ppm) = 1.15–1.25 (m, 6H, 2 × CH$_3$), 2.02 (s, 3H, CH$_3$ at C-5), 3.57–3.74 (m, 4H, 2 × C$H_2$CH$_3$), 4.14 (s, 2H, CH$_2$Ar), 5.16 (s, 2H, NCH$_2$O), 5.47 (s, 2H, NCH$_2$O), 7.18–7.42 (m, 4H, aryl). $^{13}$C-NMR ($CDCl_3$): δ (ppm) = 11.51 (CH$_3$ at C-5), 14.96 (CH$_2$CH$_3$), 15.15 (CH$_2$CH$_3$), 33.59 (CH$_2$Ar), 65.06, 65.90 (2 × OCH$_2$CH$_3$), 71.23 (N$^3$CH$_2$O), 73.58 (N$^1$CH$_2$O), 110.52 (C-5), 123.31, 125.89, 130.30, 130.49, 130.67, 137.14 (aryl), 147.33 (C-6), 152.41 (C-2), 163.02 (C-4). HRMS-MALDI: $m/z$ = 433.0721 [M+Na$^+$] ($C_{18}H_{23}BrN_2NaO_4$); requires 433.0733.

### *6-(3-Bromobenzyl)-1,3-bis-(ethoxymethyl)-5-ethyl-1H-pyrimidin-2,4-dione* ***8b***

Yield 0.33 g (8%); oil. $^1$H-NMR ($CDCl_3$): δ (ppm) = 1.06 (t, 3H, $J$ = 7.2 Hz, C$H_3$CH$_2$ at C-5), 1.15–1.28 (m, 6H, 2 × C$H_3$CH$_2$ at N$^1$and N$^3$), 2.47 (q, 2H, $J$ = 7.4 Hz, C$H_2$CH$_3$ at C-5), 3.57–3.74 (m, 4H, 2 × OC$H_2$CH$_3$), 4.13 (s, 2H, CH$_2$Ar), 5.13 (s, 2H, N$^1$CH$_2$O), 5.47 (s, 2H, N$^3$CH$_2$O), 7.04–7.42 (m, 4H, aryl). $^{13}$C-NMR ($CDCl_3$): δ (ppm) = 13.65, 14.98, 15.17 (3 × CH$_3$), 19.73 (CH$_2$CH$_3$ at C-5), 33.04 (CH$_2$Ar), 65.12, 65.95 (2 × OCH$_2$), 71.16, 73.53 (2 × NCH$_2$O), 116.46 (C-5), 123.30, 125.87, 130.35, 130.50, 130.66, 137.62 (aryl), 146.84 (C-6), 152.47 (C-2), 162.56 (C-4).

### *General procedure for the synthesis of compounds* ***9a, 9b***

Compound **6a** or **6b** (3 mmol) was dissolved in anhydrous acetonitrile (30 mL) under $N_2$ and BSA (2.6 mL, 10.5 mmol) was added. The reaction mixture became clear after 15 min and after cooling to −50°C trimethylsilyl trifluromethansulfonate (TMS triflate) (0.54 mL, 3 mmol) was added followed by dropwise addition of bis-(allyloxy)methane. The reaction mixture was stirred at room temperature for 30 h and quenched with a cold solution of saturated $NaHCO_3$ (5 mL). The solvent was evaporated under reduced pressure and the residue was extracted with diethyl ether (3 × 50 mL), dried over anhydrous magnesium sulphate, and evaporated under reduced pressure. The product was chromatographed on a silica gel with ethyl acetate/petroleum ether (60–80°C) (v/v = 1 : 1) to give **9a** and **9b**.

© 2007 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim 

### *1-Allyloxymethyl-6-(3-bromobenzyl)-5-methyl-1H-pyrimidin-2,4-dione* ***9a***

Yield 0.5 g (46%); oil. $^1$H-NMR ($CDCl_3$): δ (ppm) = 2.01 (s, 3H, $CH_3$), 4.11 (d, 2H, *J* = 6.3 Hz, O$CH_2$CH), 4.15 (s, 2H, $ArCH_2$), 5.17 (s, 2H, $NCH_2O$), 5.26 – 5.32 (m, 2H, $CH_2$=CH), 5.78 – 5.91 (m, 1H, $CH$=$CH_2$), 7.03 – 7.43 (m, 4H, aryl), 9.83 (s, 1H, NH). $^{13}$C-NMR ($CDCl_3$): δ (ppm) = 10.92 ($CH_3$), 33.59 ($CH_2Ar$), 70.47 (O$CH_2$CH), 72.57 ($NCH_2O$), 111.28 (C-5), 117.92 ($CH_2$=CH), 123.37, 125.86, 130.31, 130.56, 130.73, 136.98 (aryl), 133.36 ($CH$=$CH_2$), 148.57 (C-6), 151.77 (C-2), 163.70 (C-4). HRMS-MALDI: *m/z* = 387.0307 [M+Na$^+$] ($C_{16}H_{17}BrN_2NaO_3$); requires 387.0315.

### *1-Allyloxymethyl-6-(3-bromobenzyl)-5-ethyl-1H-pyrimidin-2,4-dione* ***9b***

Yield 0.4 g (35%); oil. $^1$H-NMR ($CDCl_3$): δ (ppm) = 1.06 (m, 3H, $CH_3$), 2.44 (q, 2H, *J* = 7.6 Hz, $CH_2CH_3$), 4.12 (d, 2H, *J* = 5.6 Hz, $OCH_2$), 4.14 (s, 2H, $CH_2Ph$), 5.13 (s, 2H, $NCH_2O$), 5.26 – 5.32 (m, 2H, $CH_2$=CH), 5.80 – 5.89 (m, 1H, $CH$=$CH_2$), 7.04 – 7.43 (m, 4H, aryl), 9.83 (s, 1H, NH). $^{13}$C- NMR ($CDCl_3$): δ (ppm) = 13.70 ($CH_3$), 19.13 ($CH_2CH_3$), 33.00 ($ArCH_2$), 70.53 ($OCH_2$), 72.51 ($NCH_2O$), 117.28 (C-5), 117.88 ($CH_2$ =CH), 123.33, 125.83, 130.34, 130.53, 130.69, 137.48 (aryl), 133.41 ($CH$=$CH_2$), 148.01 (C-6), 151.82 (C-2), 163.27 (C-4). HRMS-MALDI: *m/z* = 407.0456 [M+Na$^+$] ($C_{17}H_{19}BrN_2NaO_3$); requires 401.0471.

### *General procedure for the synthesis of compounds* ***10a – d***

Compounds **7a, b** or **9a, b** (14.24 mmol) were dissolved in diisopropylamine (DIPA, 50 mL) under $N_2$ and bis(triphenylphosphine)palladium(II)dichloride (0.3 g, 0.42 mmol), CuI (0.16 g, 0.85 mmol) were added. The reaction mixture was stirred for 15 min and trimethylsilylacetylene (TMSA; 2.21 mL, 15.66 mmol) was added. The reaction mixture was heated at 70°C for 15 h, cooled and filtered. The solvent was evaporated under reduced pressure and the residue was chromatographed on a silica gel with ethylacetate: petroleum ether (60 – 80°C) (v/v = 1 : 1) to give **10a – d**.

### *1-Ethoxymethyl-5-methyl -6-[3-(trimethylsilylethynyl)-benzyl]-1H-pyrimidin-2,4-dione* ***10a***

Yield 2.2 g (46%); brown foam. $^1$H-NMR ($CDCl_3$): δ (ppm) = 0.25 (s, 9H, $(CH_3)_3Si$), 1.17 (t, 3H, *J* = 7.2 Hz, $CH_3CH_2$), 2.01 (s, 3H, $CH_3$), 3.60 (q, 2H, *J* = 7.1 Hz, O$CH_2CH_3$), 4.13 (s, 2H, $CH_2Ar$), 5.13 (s, 2H, $NCH_2O$), 7.06 – 7.39 (m, 4H, aryl), 9.69 (s, 1H, NH). $^{13}$C-NMR ($CDCl_3$): δ (ppm) = 0.12 ($(CH_3)_3Si$), 10.91 ($CH_3$), 14.98 ($CH_2CH_3$), 33.65 ($CH_2Ar$), 64.99 (O$CH_2CH_3$), 72.80 ($NCH_2O$), 95.22, 104.17 (acetylene), 111.09 (C-5), 124.17, 127.49, 129.08, 130.41, 130.98, 134.89 (aryl), 149.06 (C-6), 151.82 (C-2), 163.80 (C-4). MS (EI): *m/z* = 371 [M+H$^+$] (22); 326 (100).

### *1-Allyloxymethyl-5-methyl-6-[3-(trimethylsilylethynyl)-benzyl]-1H-pyrimidin-2,4-dione* ***10b***

Yield 2.1 g (42%); oil. $^1$H-NMR ($CDCl_3$): δ (ppm) = 0.25 (s, 9H, $(CH_3)_3Si$), 2.01 (s, 3H, $CH_3$), 4.10 (d, 2H, *J* = 6.2 Hz, O$CH_2$CH), 4.13 (s, 2H, $ArCH_2$), 5.15 (s, 2H, $NCH_2O$), 5.20 – 5.32 (m, 2H, $CH_2$=CH), 5.79 – 5.92 (m, 1H, $CH$=$CH_2$), 7.06 – 7.39 (m, 4H, aryl), 9.71 (s, 1H, NH). $^{13}$C-NMR ($CDCl_3$): δ (ppm) = 0.13 ($(CH_3)_3Si$), 10.93 ($CH_3$), 33.70 ($CH_2Ar$), 70.48 (O$CH_2$CH), 72.58 ($NCH_2O$), 95.26, 104.15 (acetylene), 111.18 (C-5), 117.86 ($CH_2$=CH), 124.21, 127.49, 129.13, 130.41, 131.02, 134.81 (aryl), 133.44 ($CH$=$CH_2$), 148.95 (C-6) 151.81 (C-2), 163.77 (C-4). HRMS- MALDI: *m/z* = 405.1585 [M+Na$^+$] ($C_{21}H_{26}N_2NaO_3Si$); requires 405.1605.

### *1-Ethoxymethyl-5-ethyl-6-[3-(trimethylsilylethynyl)-benzyl]-1H-pyrimidin-2,4-dione* ***10c***

Yield 2.1 g (48%); brown foam. $^1$H-NMR ($CDCl_3$): δ (ppm) = 0.18 (s, 9H, $(CH_3)_3Si$), 0.96 – 1.21 (m, 6H, 2 × $CH_3CH_2$), 2.37 (q, 2H, *J* = 7.2 Hz, $CH_2CH_3$), 3.55 (q, 2H, *J* = 7.1 Hz, O$CH_2CH_3$), 4.05 (s, 2H, $CH_2Ar$), 5.02 (s, 2H, $NCH_2O$), 6.98 – 7.31 (m, 4H, aryl), 9.64 (s, 1H, NH). $^{13}$C-NMR ($CDCl_3$): δ (ppm) = 0.13 ($(CH_3)_3Si$), 13.69 ($CH_3$), 14.99 ($CH_3$), 19.14 ($CH_2CH_3$), 33.05 ($CH_2Ar$), 65.04 ($OCH_2$), 72.74 ($NCH_2O$), 95.19, 104.18 (acetylene), 117.09 (C-5), 124.14, 127.41, 129.05, 130.47, 130.95, 135.40 (aryl), 148.51 (C-6), 151.93 (C-2), 163.39 (C-4). HRMS-MALDI: *m/z* = 407.1761 [M+Na$^+$] ($C_{21}H_{28}N_2NaO_3Si$); requires 407.1747.

### *1-Allyloxymethyl-5-ethyl-6-[3-(trimethylsilylethynyl)benzyl]-1H-pyrimidin-2,4-dione* ***10d***

Yield 2.5 g (46%); foam. $^1$H-NMR ($CDCl_3$): δ (ppm) = 0.25 (s, 9H, $(CH_3)_3Si$), 1.06 (t, 3H, *J* = 7.6 Hz, $CH_3CH_2$), 2.45 (q, 2H, *J* = 7.6 Hz, $CH_2CH_3$), 4.10 (d, 2H, *J* = 6.1 Hz, O$CH_2$CH), 4.13 (s, 2H, $ArCH_2$), 5.12 (s, 2H, $NCH_2O$), 5.17 – 5.32 (m, 2H, $CH_2$=CH), 5.79 – 5.92 (m, 1H, $CH$=$CH_2$), 7.05 – 7.39 (m, 4H, aryl), 9.74 (s, 1H, NH). $^{13}$C-NMR ($CDCl_3$): δ (ppm) = 0.13 ($(CH_3)_3Si$), 13.70 ($CH_3$), 19.14 ($CH_2CH_3$), 33.11 ($CH_2Ar$), 70.55 (O$CH_2$CH), 72.53 ($NCH_2O$), 95.25, 104.15 (acetylene), 117.18 (C-5), 117.82 ($CH_2$=CH), 124.17, 127.42, 129.09, 130.48, 131.00, 135.30 (aryl), 133.49 ($CH$=$CH_2$), 148.40 (C-6) 151.92 (C-2), 163.36 (C-4). HRMS-MALDI: *m/z* = 419.1757 [M+Na$^+$] ($C_{22}H_{28}N_2NaO_3Si$); requires 419.1761.

### *General procedure for the deprotection of compounds* ***10a – d*** *and synthesis of compounds* ***11a – d***

Compounds **10a – d** (10 mmol) were dissolved in methanol (50 mL) and KOH (1 M, 10 mL) were added. The reaction mixture was stirred for 5 h. The solvent was evaporated under reduced pressure and the residue was extracted with diethyl ether to give **11a – d**.

### *1-Ethoxymethyl-6-(3-ethynylbenzyl)-5-methyl-1H-pyrimidin-2,4-dione* ***11a***

Yield 2.8 g (96%); brown foam. $^1$H-NMR ($CDCl_3$): δ (ppm) = 1.17 (t, 3H, *J* = 7.0 Hz, $CH_3CH_2$), 2.01 (s, 3H, $CH_3$), 3.11 (s, 1H, ethynyl), 3.62 (q, 2H, *J* = 7.1 Hz, O$CH_2CH_3$), 4.14 (s, 2H, $CH_2Ar$), 5.14 (s, 2H, $NCH_2O$), 7.10 – 7.41 (m, 4H, aryl), 9.83 (s, 1H, NH). $^{13}$C-NMR ($CDCl_3$): δ (ppm) = 10.89 ($CH_3$), 14.95 ($CH_3CH_2$), 33.62 ($CH_2Ar$), 64.95 (O$CH_2CH_3$), 72.77 ($NCH_2O$), 78.06, 82.92 (acetylene), 111.11 (C-5), 123.13, 127.78, 129.19, 130.75, 131.02, 135.10 (aryl), 148.92 (C-6), 151.82 (C-2), 163.77 (C-4). HRMS-MALDI: *m/z* = 321.1207 [M+Na$^+$] ($C_{17}H_{18}N_2NaO_3$); requires 321.1210.

### *1-Allyloxymethyl-6-(3-ethynylbenzyl)-5-methyl-1H-pyrimidin-2,4-dione* ***11b***

Yield 2.8 g (90%); oil. $^1$H-NMR ($CDCl_3$): δ (ppm) = 2.01 (s, 3H, $CH_3$), 3.11 (s, 1H, ethynyl), 4.10 (d, 2H, *J* = 5.7 Hz, O$CH_2$CH), 4.15 (s, 2H, $ArCH_2$), 5.16 (s, 2H, $NCH_2O$), 5.21 – 5.32 (m, 2H, $CH_2$=CH), 5.78 – 5.91 (m, 1H, $CH$=$CH_2$), 7.10 – 7.41 (m, 4H, aryl), 9.84 (s, 1H, NH). $^{13}$C-NMR ($CDCl_3$): δ (ppm) = 10.92 ($CH_3$), 33.68 ($CH_2Ar$), 70.45 (O$CH_2$CH), 72.55 ($NCH_2O$), 78.10, 82.89 (acetylene), 111.20 (C-5),

© 2007 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim 

117.85 ($CH_2$=CH), 123.17, 127.77, 129.23, 130.74, 131.07, 134.99 (aryl), 133.40 (*C*H=$CH_2$), 148.81 (C-6) 151.81 (C-2), 163.75 (C-4). HRMS-MALDI: *m/z* = 333.1209 [M+$Na^+$] ($C_{18}H_{18}N_2NaO_3$); requires 333.1210.

### *1-Ethoxymethyl-5-ethyl-6-(3-ethynylbenzyl)-1H-pyrimidin-2,4-dione* ***11c***

Yield 2.8 g (91%); brown foam. $^1$H-NMR ($CDCl_3$): δ (ppm) = 1.04–1.20 (m, 6H, 2 × C*H*$_3$CH$_2$), 2.45 (q, 2H, *J* = 7.1 Hz, C*H*$_2$CH$_3$), 3.11 (s, 1H, ethynyl), 3.60 (q, 2H, *J* = 7.0 Hz, OC*H*$_2$CH$_3$), 4.14 (s, 2H, $CH_2$Ar), 5.11 (s, 2H, $NCH_2O$), 7.10–7.41 (m, 4H, aryl), 9.87 (s, 1H, NH). $^{13}$C NMR ($CDCl_3$): δ (ppm) = 13.67, 14.94 (2 × $CH_3$), 19.11 (*C*H$_2$CH$_3$), 33.03 ($CH_2$Ar), 64.99 ($OCH_2$), 72.70 ($NCH_2O$), 78.03, 82.90 (acetylene), 117.11 (C-5), 123.08, 127.71, 129.14, 130.76, 130.98, 135.59 (aryl), 148.35 (C-6), 151.93 (C-2), 163.38 (C-4). HRMS-MALDI: *m/z* = 335.1365 [M+$Na^+$] ($C_{18}H_{20}N_2NaO_3$); requires 335.1366.

### *1-Allyloxymethyl-5-ethyl-6-(3-ethynylbenzyl)-1H-pyrimidin-2,4-dione* ***11d***

Yield 3.1 g (96%); foam. $^1$H-NMR ($CDCl_3$): δ (ppm) = 1.06 (t, 3H, *J* = 7.4 Hz, C*H*$_3$CH$_2$), 2.46 (q, 2H, *J* = 7.4 Hz, C*H*$_2$CH$_3$), 3.16 (s, 1H, ethynyl), 4.11 (d, 2H, *J* = 5.7 Hz, OC*H*$_2$CH), 4.16 (s, 2H, $ArCH_2$), 5.12 (s, 2H, $NCH_2O$), 5.19–5.32 (m, 2H, C*H*$_2$=CH), 5.79–5.92 (m, 1H, C*H*=$CH_2$), 7.12–7.41 (m, 4H, aryl), 10.60 (s, 1H, NH). $^{13}$C-NMR ($CDCl_3$): δ (ppm) = 13.49 ($CH_3$), 18.87 (*C*H$_2$CH$_3$), 32.88 ($CH_2$Ar), 70.22 (O*C*H$_2$CH), 72.25 ($NCH_2O$), 78.05, 82.71 (acetylene), 117.00 (C-5), 117.53 (*C*H$_2$=CH), 122.88, 127.53, 128.95, 130.57, 130.76, 135.39 (aryl), 133.30 (*C*H=$CH_2$), 148.02 (C-6), 151.95 (C-2), 163.46 (C-4). HRMS-MALDI: *m/z* = 347.1365 [M+$Na^+$] ($C_{19}H_{20}N_2NaO_3$); requires 347.1366.

### *General procedure for the synthesis of compounds* ***12a, b***

Compounds **7a, b** (14.24 mmol) were dissolved in diisopropylamine (DIPA, 50 mL) under $N_2$ and bis(triphenylphosphine)palladium(II)dichloride (0.3 g, 0.42 mmol), CuI (0.16 g, 0.85 mmol) were added. The reaction mixture was stirred for 15 min and propargyl alcohol (2.3 g, 42 mmol) was added. The reaction mixture was heated at 70°C for 36 h, cooled and filtered. The solvent was evaporated under reduced pressure and the residue was chromatographed on a silica gel with chloroform/methanol (v/v = 9 : 1) to give **12a, b**.

### *1-Ethoxymethyl-6-[3-(hydroxypropynyl)benzyl]-5-methyl-1H-pyrimidin-2,4-dione* ***12a***

Yield 2.0 g (43%); foam. $^1$H-NMR ($CDCl_3$): δ (ppm) = 1.16 (t, 3H, *J* = 7.1 Hz, C*H*$_3$CH$_2$), 1.99 (s, 3H, $CH_3$), 3.59 (q, 2H, *J* = 7.3 Hz, OC*H*$_2$CH$_3$), 3.77 (s, 1H, OH), 4.12 (s, 2H, $CH_2$Ar), 4.49 (s, 2H, C*H*$_2$OH), 5.12 (s, 2H, $NCH_2O$), 7.08–7.35 (m, 4H, aryl), 9.64 (s, 1H, NH). $^{13}$C-NMR ($CDCl_3$): δ (ppm) = 10.88 ($CH_3$), 14.95 (*C*H$_3$CH$_2$), 33.63 ($CH_2$Ar), 51.35 ($CH_2OH$), 64.96 (O*C*H$_2$CH$_3$), 72.78 ($NCH_2O$), 84.78, 88.29 (acetylene), 111.08 (C-5), 123.67, 127.59, 129.17, 130.15, 130.54, 135.00 (aryl), 149.09 (C-6), 151.79 (C-2), 163.79 (C-4). MS (EI): m/z = 328 [M+$H^+$] (15); 203 (100).

### *1-Ethoxymethyl-5-ethyl-6-[3-(hydroxypropynyl)benzyl]-1H-pyrimidin-2,4-dione* ***12b***

Yield 2.3 g (48%); foam. $^1$H-NMR ($CDCl_3$): δ (ppm) = 1.03–1.29 (m, 6H, 2 × C*H*$_3$CH$_2$), 2.43 (q, 2H, *J* = 7.4 Hz, C*H*$_2$CH$_3$), 3.59 (q, 2H, *J* = 7.1 Hz, OC*H*$_2$CH$_3$), 4.12 (s, 2H, $CH_2$Ar), 4.12 (s, 1H, OH), 4.49 (s, 2H, C*H*$_2$OH), 5.09 (s, 2H, $NCH_2O$), 7.09–7.35 (m, 4H, aryl), 8.18 (s, 1H, NH). $^{13}$C-NMR ($CDCl_3$): δ (ppm) = 13.68 ($CH_3$), 14.95 ($CH_3$), 19.12 (*C*H$_2$CH$_3$), 33.04 ($CH_2$Ar), 51.34 ($CH_2OH$), 65.09 (O*C*H$_2$CH$_3$), 72.72 ($NCH_2O$), 84.75, 88.29 (acetylene), 117.05 (C-5), 123.65, 127.52, 129.14, 130.18, 130.54, 135.50 (aryl), 148.52 (C-6), 151.84 (C-2), 163.30 (C-4). HRMS-MALDI: *m/z* = 365.1472 [M+$Na^+$] ($C_{19}H_{22}N_2NaO_4$); requires 365.1464.

### *General procedure for the synthesis of compounds* ***13a, b***

Compounds **12a, b** (2 mmol) were dissolved in ethylacetate (30 mL). Iodoxybenzoic acid (IBX) (1.68 g, 6 mmol) was added. The reaction mixture was heated at 80°C over night. The solvent was evaporated under reduced pressure, and the residue was chromatographed on a silica gel with ethyl acetate/petroleum ether (60–80°C) (v/v = 7 : 3) to give **13a, b**.

### *1-Ethoxymethyl-5-methyl-6-[3-(3-oxopropynyl)benzyl]-1H-pyrimidin-2,4-dione* ***13a***

Yield 0.24 g (33%); foam. $^1$H-NMR ($CDCl_3$): δ (ppm) = 1.16 (t, 3H, *J* = 7.0 Hz, C*H*$_3$CH$_2$), 2.01 (s, 3H, $CH_3$), 3.60 (q, 2H, *J* = 6.9 Hz, OC*H*$_2$CH$_3$), 4.18 (s, 2H, $CH_2$Ar), 5.14 (s, 2H, $NCH_2O$), 7.26–7.54 (m, 4H, aryl), 9.42 (s, 1H, CHO), 9.82 (s, 1H, NH). $^{13}$C-NMR ($CDCl_3$): δ (ppm) = 10.91 ($CH_3$), 14.92 (*C*H$_3$CH$_2$), 33.58 ($CH_2$Ar), 64.99 (O*C*H$_2$CH$_3$), 72.81 ($NCH_2O$), 88.60, 93.86 (acetylene), 111.30 (C-5), 120.49, 129.63, 130.28, 131.68, 132.09, 135.81 (aryl), 148.48 (C-6), 151.76 (C-2), 163.66 (C-4), 176.55 (CHO). MS (EI): m/z = 327 [M+$H^+$] (22); 59 (100) ($C_3H_7O$).

### *1-Ethoxymethyl-5-ethyl-6-[3-(3-oxopropynyl)benzyl]-1H-pyrimidin-2,4-dione* ***13b***

Yield 0.19 g (28%); foam. $^1$H-NMR ($CDCl_3$): δ (ppm) = 1.04–1.25 (m, 6H, 2 × C*H*$_3$CH$_2$), 2.47 (q, 2H, *J* = 7.5 Hz, C*H*$_2$CH$_3$), 3.59 (q, 2H, *J* = 6.5 Hz, OC*H*$_2$CH$_3$), 4.18 (s, 2H, $CH_2$Ar), 5.11 (s, 2H, $NCH_2O$), 7.27–7.54 (m, 4H, aryl), 9.42 (s, 1H, CHO), 9.72 (s, 1H, NH). $^{13}$C-NMR ($CDCl_3$): δ (ppm) = 13.69 ($CH_3$), 14.92 ($CH_3$), 19.15 (*C*H$_2$CH$_3$), 32.99 ($CH_2$Ar), 65.05 (O*C*H$_2$CH$_3$), 72.76 ($NCH_2O$), 88.59, 93.88 (acetylene), 117.28 (C-5), 120.45, 129.60, 130.25, 131,70, 132.10, 136.31 (aryl), 147.94 (C-6), 151.81 (C-2), 163.22 (C-4), 176.59 (CHO). HRMS-MALDI: *m/z* = 363.1465 [M+$Na^+$] ($C_{19}H_{20}N_2NaO_4$); requires 363.1472.

### *General procedure for the synthesis of compounds* ***14a–f***

Compounds **6a, b** (14.24 mmol) were dissolved in a secondary amine (50 mL) (diisopropylmine, morpholine or pyrrolidene) under $N_2$ and bis(triphenylphosphine)palladium(II)dichloride (0.3 g, 0.42 mmol), CuI (0.16 g, 0.85 mmol) were added. The reaction mixture was stirred for 15 min and cooled to 0°C. Propargyl chloride (2.3 g, 42 mmol) was added dropwise. The reaction mixture was heated at 70°C for 48 h. The solvent was evaporated under reduced pressure and the residue was washed with water and extracted with chloroform. The organic layer was dried over anhydrous magnesium sulphate, and evaporated under reduced pressure. The product was chromatographed on a silica gel with chloroform/methanol (v/v = 95 : 5) to give **14a–f**.

### *6-[3-(Diisopropylaminopropynyl)benzyl]-1-ethoxymethyl-5-methyl-1H-pyrimidin-2,4-dione* ***14a***

Yield 3.57 g (64%); oil. $^1$H-NMR ($CDCl_3$): δ (ppm) = 1.10–1.17 (m, 15H, 5 × $CH_3$), 2.01 (s, 3H, $CH_3$ at C-5), 2.95 (s, 2H, $NCH_2C$), 3.20–3.29 (m, 2H, 2 × CH[Pr$^i$]), 3.62 (q, *J* = 7.1 Hz 2H, OC*H*$_2$CH$_3$), 4.12 (s,

© 2007 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim 

2H, $CH_2Ar$), 5.13 (s, 2H, $NCH_2O$), 7.01 – 7.32 (m, 4H, aryl), 9.69 (s, 1H, NH). $^{13}$C-NMR ($CDCl_3$): δ (ppm) = 10.88 ($CH_3$ at C-5), 14.96 ($CH_3CH_2$), 20.56 ($4 \times CH_3[Pr^i]$), 33.64 ($CH_2Ar$), 34.76 ($CH[Pr^i]_2$), 48.49 ($CH_2N$), 64.91 ($OCH_2CH_3$), 72.74 ($NCH_2O$), 82.70, 90.07 (acetylene), 110.98 (C-5), 124.79, 126.61, 129.04, 130.05, 130.38, 134.83 (aryl), 149.06 (C-6), 151.75 (C-2), 163.67 (C-4). MS (EI): *m/z* = 397 [$M^+$] (24); 396 [M-H] (100).

### *6-[3-(Diisopropylaminopropynyl)benzyl]-1-ethoxymethyl-5-ethyl-1H-pyrimidin-2,4-dione* ***14b***

Yield 2.6 g (43%); oil. $^1$H-NMR ($CDCl_3$): δ (ppm) = 1.06 – 1.18 (m, 18H, $6 \times CH_3$), 2.44 (q, 2H, *J* = 7.5 Hz, $CH_2CH_3$), 3.20 – 3.29 (m, 2H, $2 \times CH[Pr^i]$), 3.62 (q, 2H, *J* = 7.0 Hz, $OCH_2CH_3$), 3.64 (s, 2H, $NCH_2C$), 4.11 (s, 2H, $CH_2Ar$), 5.10 (s, 2H, $NCH_2O$), 7.04 – 7.31 (m, 4H, aryl), 9.45 (s, 1H, NH). $^{13}$C-NMR ($CDCl_3$): δ (ppm) = 13.70 ($CH_3CH_2$), 14.99 ($CH_3CH_2$), 19.16 ($CH_2CH_3$), 20.59 ($4 \times CH_3[Pr^i]$), 33.09 ($CH_2Ar$), 34.79 ($CH[Pr^i]_2$), 48.51 ($CH_2N$), 64.99 ($OCH_2CH_3$), 72.72 ($NCH_2O$), 82.71, 90.13 (acetylene), 117.00 (C-5), 124.79, 126.60, 129.04, 130.11, 130.38, 135.33 (aryl), 148.56 (C-6), 151.80 (C-2), 163.17 (C-4). HRMS-MALDI: *m/z* = 424.2580 [M+H$^+$] ($C_{25}H_{35}N_3O_3$); requires 424.2595.

### *1-Ethoxymethyl-5-methyl-6-[3-(morpholinopropynyl)benzyl]-1H-pyrimidin-2,4-dione* ***14c***

Yield 2.02 g (36%); oil. $^1$H-NMR ($CDCl_3$): δ (ppm) = 1.20 (t, 3H, *J* = 7.0 Hz, $CH_3CH_2$), 2.00 (s, 3H, $CH_3$ at C-5), 2.66 (t, 4H, *J* = 4.6 Hz, morph), 3.51 (s, 2H, $NCH_2$), 3.59 – 3.62 (m, 4H, morph), 3.78 – 3.82 (m, 2H, $OCH_2CH_3$), 4.13 (s, 2H, $CH_2Ar$), 5.12 (s, 2H, $NCH_2O$), 7.06 – 7.36 (m, 4H, aryl), 9.54 (s, 1H, NH). $^{13}$C-NMR ($CDCl_3$): δ (ppm) = 10.89 ($CH_3$ at C-5), 14.97 ($CH_3CH_2$), 33.64 ($CH_2Ar$), 47.95 ($CH_2N$), 52.40 (morph), 64.94 ($OCH_2CH_3$), 66.78 (morph), 72.77 ($NCH_2O$), 84.83, 85.02 (acetylene), 111.01 (C-5), 124.00, 127.21, 129.14, 130.24, 130.71, 134.97 (aryl), 148.97 (C-6), 151.69 (C-2), 163.57 (C-4). HRMS-MALDI: *m/z* = 398.2067 [M+H$^+$] ($C_{22}H_{27}N_3O_4$); requires 398.2074.

### *1-Ethoxymethyl-5-ethyl-6-[3-(morpholinopropynyl)-benzyl]-1H-pyrimidin-2,4-dione* ***14d***

Yield 2.63 g (45%); oil. $^1$H-NMR ($CDCl_3$): δ (ppm) = 0.95 – 1.16 (m, 6H, $2 \times CH_3$), 2.37 (q, 2H, *J* = 7.4 Hz, $CH_2CH_3$), 2.54 (t, 4H, *J* = 4.7 Hz, morph), 3.42 (s, 2H, $NCH_2$), 3.56 – 3.61 (m, 4H, morph), 3.67 (q, 2H, *J* = 7.1 Hz, $OCH_2CH_3$), 4.03 (s, 2H, $CH_2Ar$), 5.00 (s, 2H, $NCH_2O$), 7.01 – 7.27 (m, 4H, aryl), 7.98 (s, 1H, NH). $^{13}$C-NMR ($CDCl_3$): δ (ppm) = 13.68 ($CH_3$), 14.13 ($CH_3$), 19.13 ($CH_2CH_3$), 33.04 ($CH_2Ar$), 47.95 ($CCH_2N$), 52.40 (morph), 64.96 ($OCH_2CH_3$), 66.76 (morph), 72.96 ($NCH_2O$), 84.82, 85.05 (acetylene), 116.97 (C-5), 123.95, 127.15, 129.09, 130.27, 130.67, 135.48 (aryl), 148.39 (C-6), 151.73 (C-2), 163.10 (C-4). HRMS-MALDI: *m/z* = 434.2056 [M+Na$^+$] ($C_{23}H_{29}N_3NaO_4$); requires 434.2050.

### *1-Ethoxymethyl-5-methyl-6-[3-pyrrolidinopropynyl)-benzyl]-1H-pyrimidin-2,4-dione* ***14e***

Yield 3.8 g (70%); oil. $^1$H-NMR ($CDCl_3$): δ (ppm) = 1.17 (t, 3H, *J* = 7.0 Hz, $CH_3CH_2$), 1.82 – 1.88 (m, 4H, pyrrolidino), 2.05 (s, 3H, $CH_3$ at C-5), 2.71 (s, 2H, $NCH_2$), 3.39 – 3.48 (m, 4H, pyrrolidino), 3.59 (q, 2H, *J* = 6.9 Hz, $OCH_2CH_3$), 4.16 (s, 2H, $CH_2Ar$), 5.13 (s, 2H, $NCH_2O$), 7.07 – 7.32 (m, 4H, aryl), 10.05 (s, 1H, NH). $^{13}$C-NMR ($CDCl_3$): δ (ppm) = 10.86 ($CH_3$ at C-5), 14.94 ($CH_3CH_2$), 23.72 (pyrrolidino), 33.62 ($CH_2Ar$), 43.63 ($CH_2N$), 52.57 (pyrrolidino), 64.87 ($OCH_2CH_3$), 72.70 ($NCH_2O$), 83.70, 86.27 (acetylene), 110.96 (C-5), 124.25, 126.96, 129.04, 130.24, 130.62, 134.93 (aryl), 148.89 (C-6), 151.82 (C-2), 163.78 (C-4). HRMS-MALDI: *m/z* = 382.2128 [M+H$^+$] ($C_{22}H_{27}N_3O_3$); requires 382.2125.

### *1-Ethoxymethyl-5-ethyl-6-[3-(pyrrolidinopropynyl)benzyl]-1H-pyrimidin-2,4-dione* ***14f***

Yield 3,76 g (67%); oil. $^1$H-NMR ($CDCl_3$): δ (ppm) = 0.95 – 1.10 (m, 6H, $2 \times CH_3CH_2$), 1.76 – 1.78 (m, 4H, pyrrolidino), 2.36 (q, 2H, *J* = 7.5 Hz, $CH_2CH_3$ at C-5), 2.66 (s, 2H, $NCH_2$), 3.48 – 3.58 (m, 6H, $OCH_2CH_3$, pyrrolidino), 4.03 (s, 2H, $CH_2Ar$), 5.02 (s, 2H, $NCH_2O$), 6.98 – 7.26 (m, 4H, aryl), 10.88 (s, 1H, NH). $^{13}$C-NMR ($CDCl_3$): δ (ppm) = 13.53 ($CH_3$), 14.80 ($CH_3$), 18.93 ($CH_2CH_3$), 23.61 (pyrrolidino), 33.88 ($CH_2Ar$), 43.23 ($CH_2N$), 52.11 (pyrrolidino), 64.69 ($OCH_2CH_3$), 72.45 ($NCH_2O$), 83.71, 85.93 (acetylene), 116.81 (C-5), 124.02, 126.74, 128.82, 130.16, 130.41, 135.38 (aryl), 148.05 (C-6), 152.05 (C-2), 163.60 (C-4).

### *General procedure for the synthesis of compounds* ***15a, b***

The β-keto esters **3a, b** (3.15 mmol) were dissolved in EtONa (Na, 0.14 g, 6.3 mmol in 50 mL anhydrous ethanol). *N*,*N*-Dimethylguanidine sulphate (1.17 g, 4.3 mmol) was added and the reaction mixture was refluxed for 36 h, cooled, filtered, and evaporated to the half volume *in vacuo*, poured into water and extracted with chloroform. The organic layer was washed with water ($2 \times 50$ mL), dried over anhydrous magnesium sulphate and evaporated *in vacuo*. The product was chromatographed on a silica gel with chloroform/methanol (v/v = 9 : 1) to give **15a, b**.

### *6-(3-Bromobenzyl)-2-dimethylamino-5-methyl-1H-pyrimidine-4-one* ***15a***

Yield 0.42 g (42%); m. p. 180 – 182°C. $^1$H-NMR ($CDCl_3$): δ (ppm) = 1.85 (s, 3H, $CH_3$), 2.98 (s, 6H, $2 \times NCH_3$), 3.74 (s, 2H, $CH_2Ar$), 7.23 – 7.47 (m, 4H, aryl), 10.83 (s, H, NH). $^{13}$C-NMR ($CDCl_3$): δ (ppm) = 10.02 ($CH_3$), 36.78 ($2 \times NCH_3$), 39.98 ($CH_2Ar$), 105.20 (C-5), 121.31, 127.70, 128.79, 130.21, 131.31, 141.47 (aryl), 152.72 (C-6), 161.15 (C-4), 164.54 (C-2). HRMS-MALDI: *m/z* = 322.0550 [M+H$^+$] ($C_{14}H_{16}BrN_3O$); requires 322.0550.

### *6-(3-Bromobenzyl)-2-dimethylamino-5-ethyl-1H-pyrimidine-4-one* ***15b***

Yield 0.40 g (38%); m. p. 153 – 155°C. $^1$H-NMR ($CDCl_3$): δ (ppm) = 0.99 (t, 3H, *J* = 7.1 Hz, $CH_3CH_2$), 2.43 (q, 2H, *J* = 7.3 Hz, $CH_2CH_3$), 3.11 (s, 6H, $2 \times NCH_3$), 3.77 (s, 2H, $CH_2Ar$), 7.09 – 7.47 (m, 4H, aryl), 8.47 (s, H, NH). $^{13}$C-NMR ($CDCl_3$): δ (ppm) = 13.78 ($CH_3CH_2$), 18.42 ($CH_2CH_3$), 37.42 ($2 \times NCH_3$), 40.35 ($CH_2Ar$), 112.69 (C-5), 122.14, 127.57, 129.13, 129.13, 132.00, 141.38 (aryl), 152.10 (C-6), 162.58 (C-4), 165.94 (C-2). HRMS-MALDI: *m/z* = 358.0514 [M+Na$^+$] ($C_{15}H_{18}BrN_3NaO$); requires 358.0525.

### *General procedure for the synthesis of compounds* ***16a, b***

Compounds **5a, b** (2 mmol) were dissolved in anhydrous DMF (30 mL) under $N_2$ and $CH_3I$ (0.56 g, 0.26 mL, 4 mmol) was added. The reaction mixture was stirred at room temperature for 48 h. The reaction mixture was poured on cold water and the solid formed was filtered off. The product was chromatographed on a silica gel with chloroform/methanol (v/v = 9 : 1) to give **16a, b**.

© 2007 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim 

### *6-(3-Bromobenzyl)-5-methyl-2-(methylthio)-1H-pyrimidine-4-one* ***16a***

Yield 0.40 g (62%); m. p. 174–176°C. $^1$H-NMR ($CDCl_3$): δ (ppm) = 2.08 (s, 3H, $CH_3$), 2.50 (s, 3H, $SCH_3$), 3.87 (s, 2H, $CH_2Ar$), 7.11–7.42 (m, 4H, aryl), 12.63, (s, 1H, NH). $^{13}$C-NMR ($CDCl_3$): δ (ppm) = 10.63 ($CH_3$), 13.26 ($SCH_3$), 40.52 ($CH_2Ar$), 116.29 (C-5), 122.36, 127.53, 129.56, 129.87, 132.04, 140.10 (aryl), 157.20 (C-6), 161.25 (C-4), 165.29 (C-2). HRMS-MALDI: *m/z* = 325.0004 [M+H$^+$] ($C_{13}H_{13}BrN_2OS$); requires 325.0005.

### *6-(3-Bromobenzyl)-5-ethyl-2-(methylthio)-1H-pyrimidine-4-one* ***16b***

Yield 0.46 g (68%); m. p. 174–176°C. $^1$H-NMR ($CDCl_3$): δ (ppm) = 0.83 (t, 3H, *J* = 7.5 Hz, $CH_3CH_2$), 2.32 (s, 3H, $SCH_3$), 2.37 (q, 2H, *J* = 7.5 Hz, $CH_2CH_3$), 3.77 (s, 2H, $CH_2Ar$), 7.13–7.44 (m, 4H, aryl), 12.47 (s, 1H, NH). $^{13}$C-NMR ($CDCl_3$): δ (ppm) = 12.56 ($CH_3CH_2$), 13.15 ($SCH_3$), 18.03 ($CH_2CH_3$), 39.43 ($CH_2Ar$), 116.05 (C-5), 121.31, 127.33, 129.01, 130.27, 131.73, 141.16 (aryl), 156.81 (C-6), 161.13 (C-4), 165.16 (C-2). HRMS-MALDI: *m/z* = 339.0166 [M+H$^+$] ($C_{14}H_{15}BrN_2OS$); requires 339.0161.

### *General procedure for the synthesis of compounds* ***17a–d*** *and* ***18***

Compounds **11a, b** (14.24 mmol) were dissolved in triethylamine (50 mL) under $N_2$ and bis(triphenylphosphine)palladium(II)-dichloride (0.3 g, 0.42 mmol), CuI (0.16 g, 0.85 mmol) and iodo derivatives (3-iodoanisole, 3-iodopyridine, 42 mmol) were added. The reaction mixture was stirred at room temperature for 15 h. The solvent was evaporated under reduced pressure and the residue was chromatographed on a silica gel with chloroform/methanol (v/v = 95 : 5) to give **17a-d** and **18**.

### *1-Ethoxymethyl-5-methyl-6-[3-(3-methoxyphenylethynyl)benzyl]-1H-pyrimidin-2,4-dione* ***17a***

Yield 2.12 g (37%); foam. $^1$H-NMR ($CDCl_3$): δ (ppm) = 1.18 (t, 3H, *J* = 7.1 Hz, $CH_3CH_2$), 2.03 (s, 3H, $CH_3$), 3.61 (q, 2H, *J* = 6.8 Hz, $OCH_2CH_3$), 3.82 (s, 3H, $OCH_3$), 4.16 (s, 2H, $CH_2Ar$), 5.16 (s, 2H, $NCH_2O$), 6.88–7.46 (m, 8H, aryl), 9.78 (s, 1H, NH). $^{13}$C-NMR ($CDCl_3$): δ (ppm) = 10.92 ($CH_3$ at C-5), 14.97 ($CH_3CH_2$), 33.69 ($CH_2Ar$), 55.25 ($OCH_3$), 64.97 ($OCH_2CH_3$), 72.79 ($NCH_2O$), 88.41, 90.10 (acetylene), 111.09 (C-5), 115.25, 116.18, 123.76, 124.18, 124.23, 127.31, 129.21, 129.37, 130.09, 130.57, 135.06, 149.07 (aryl), 151.82 (C-6), 159.28, 163.80 (2 × CO). HRMS-MALDI: *m/z* = 427.1624 [M+Na$^+$] ($C_{24}H_{24}N_2NaO_4$); requires 427.1628.

### *1-Ethoxymethyl-5-ethyl-6-[3-(3-methoxyphenylethynyl)benzyl]-1H-pyrimidin-2,4-dione* ***17b***

Yield 1.96 g (33%); foam. $^1$H-NMR ($CDCl_3$): δ (ppm) = 1.14–1.20 (m, 6H, 2 × $CH_3CH_2$), 2.44 (q, 2H, *J* = 7.4 Hz, $CH_2CH_3$), 3.59 (q, 2H, *J* = 7.2 Hz, $OCH_2CH_3$), 4.15 (s, 3H, $OCH_3$), 4.16 (s, 2H, $CH_2Ar$), 5.10 (s, 2H, $NCH_2O$), 7.13–7.71 (m, 8H, aryl), 9.52 (s, 1H, NH). $^{13}$C-NMR ($CDCl_3$): δ (ppm) = 13.71 ($CH_3$), 14.95 ($CH_3$), 19.16 ($CH_2CH_3$), 33.05 ($CH_2Ar$), 59.63 ($OCH_3$), 65.00 ($OCH_2CH_3$), 72.72 ($NCH_2O$), 74.44, 81.25 (acetylene), 117.12 (C-5), 122.63, 128.26, 128.33, 128.49, 129.31, 131.15, 131.32, 131.83, 131.95, 132.09, 135.86, 147.32 (aryl), 148.16 (C-6), 151.75 (C-2), 163.11 (C-4).

### *1-Ethoxymethyl-5-methyl-6-[3-(pyridin-3-ylethynyl)-benzyl]-1H-pyrimidin-2,4-dione* ***17c***

Yield 2.66 g (52%); foam. $^1$H-NMR ($CDCl_3$): δ (ppm) = 1.16–1.18 (t, 3H, *J* = 7.00 Hz, $CH_3CH_2$), 2.03 (s, 3H, $CH_3$), 3.62 (m, 2H, *J* = 6.9 Hz, $OCH_2CH_3$), 4.18 (s, 2H, $CH_2Ar$), 5.18 (s, 2H, $NCH_2O$), 7.14–7.48 (m, 4H, aryl), 7.82–8.81 (m, 4H, $H_{pyr}$), 10.63 (s, 1H, NH). $^{13}$C-NMR ($CDCl_3$): δ (ppm) = 10.81 ($CH_3$ at C-5), 14.87 ($CH_3CH_2$), 33.57 ($CH_2Ar$), 64.83 ($OCH_2CH_3$), 72.66 ($NCH_2O$), 85.54, 91.84 (acetylene), 111.05 (C-5), 122.97, 123.42, 127.76, 129.20, 130.06, 130.48, 135.22, 138.47, 148.43 ($C_{arom}$ and $C_{pyr}$), 148.75 (C-6), 151.93 (C-2), 163.97 (C-4). HRMS-MALDI: *m/z* = 398.1294 [M+Na$^+$] ($C_{24}H_{24}N_2NaO_4$); requires 398.1277.

### *1-Ethoxymethyl-5-ethyl-6-[3-(pyridin-3-ylethynyl)benzyl]-1H-pyrimidin-2,4-dione* ***17d***

Yield 3.2 g (57%); foam. $^1$H-NMR ($CDCl_3$): δ (ppm) = 1.04–1.19 (m, 6H, 2 × $CH_3CH_2$), 2.45 (q, 2H, *J* = 7.1 Hz, $CH_2CH_3$), 3.60 (q, 2H, *J* = 6.9 Hz, $OCH_2CH_3$), 4.15 (s, 2H, $CH_2Ar$), 5.12 (s, 2H, $NCH_2O$), 7.14–7.34 (m, 4H, $H_{arom}$), 7.64–8.02 (m, 4H, $H_{pyr}$), 10.27 (s, 1H, NH). $^{13}$C-NMR ($CDCl_3$): δ (ppm) = 13.56, 14.80 (2 × $CH_3CH_2$), 32.89 ($CH_2Ar$), 64.78 ($OCH_2CH_3$), 72.52 ($NCH_2O$), 74.28, 81.10 (acetylene), 116.97 (C-5), 122.40, 128.18, 128.34, 129.14, 131.01, 131.12, 131.69, 131.72, 131.78, 131.91, 135.80 ($C_{arom}$ and $C_{pyr}$), 147.95 (C-6), 151.83 (C-2), 163.27 (C-4).

### *1,4-Bis-[3-(3-Ethoxymethyl-5-methyl-2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-ylmethyl)phenyl]-butadiyne* ***18***

Yield 0.25 g (8%); brown foam. $^1$H-NMR ($CDCl_3$): δ (ppm) = 1.16 (t, 6H, *J* = 7.1 Hz, 2 × $CH_3CH_2$), 2.01 (s, 6H, 2 × $CH_3$), 3.60 (q, 4H, *J* = 7.1 Hz, 2 × $OCH_2CH_3$), 4.15 (s, 4H, 2 × $CH_2Ar$), 5.14 (s, 4H, 2 × $NCH_2O$), 7.13–7.54 (m, 8H, aryl), 9.95 (s, 2H, 2 × NH). $^{13}$C-NMR ($CDCl_3$): δ (ppm) = 10.89 (s, 2 × $CH_3$), 14.92 (s, 2 × $CH_3CH_2$), 33.61 (s, 2 × $CH_2Ar$), 64.93 (s, 2 × $OCH_2CH_3$), 72.76 (s, 2 × $NCH_2O$), 74.45, 81.23 (acetylene), 111.15 (s, 2 × C-5), 122.64, 129.31, 131.16, 131.29, 131.94, 135.34 (aryl), 148.74 (2 × C-6), 151.80 (2 × C-2), 163.75 (2 × C-4). HRMS- MALDI: *m/z* = 617.2344 [M+Na$^+$] ($C_{34}H_{34}N_4NaO_6$); requires 617.2371.

## 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 $4.5 \times 10^6$ 50% cell culture infectious dose ($CCID_{50}$)/mL. The K103R + V179D + P225H mutant ($EFV^R$) was derived from an IIIB strain passage 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 passage 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). K103R + V179D + P225H ($EFV^R$), Y181C, and

© 2007 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim 

K103N + Y181C 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.

### 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.

### Anti-HIV assays

The activity of test compounds against multiplication of wild type HIV-1, K103R + V179D + P225H ($EFV^R$), Y181C, and K103N + Y181C in acutely infected cells was based on inhibition of virus-induced cytopathicity 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 cytopathicity 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 [23]. 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.

## References

[1] E. De Clercq, *Chem. Biodivers* **2004**, *1*, 44 – 64.

[2] R. Esnouf, J. Ren, C. Ross, Y. Jones, *et al.*, *Nat. Struct. Biol.* **1995**, *2*, 303 – 308.

[3] A. L. Hopkins, J. Ren, R. M. Esnouf, B. E. Willcox, *et al.*, *J. Med. Chem*. **1996**, *39*, 1589 – 1600.

[4] J. M. J. Tronchet, M. Seman, *Curr. Top. Med. Chem*. **2003**, *3*, 1496 – 1511.

[5] C. M. Tarby, *Curr. Top. Med. Chem*. **2004**, *4*, 1045 – 1057.

[6] N. Sluis-Cremer, A. N. Temiz, I. Bahar, *Curr. HIV Res*. **2004**, *2*, 323 – 332.

[7] H. Tanaka, H. Takashima, M. Ubasawa, K. Sekiya, *et al.*, *J. Med. Chem*. **1995**, *38*, 2860 – 2865.

[8] M. Baba, H. Tanaka, T. Miyasaka, S. Yuasa, *et al.*, *Nucleosides Nucleotides*, **1995**, *14*, 575 – 583.

[9] G. M. Szczech, P. Furman, G. R. Painter, D. W. Barry, *et al.*, *Antimicrob. Agents Chemother.* **2000**, *44*, 123 – 130.

[10] O. S. Pedersen, E. B. Pedersen, *Synthesis* **2000**, *4*, 479 – 495.

[11] O. S. Pedersen, E. B. Pedersen, *Antivir Chem Chemother*. **1999**, *10*, 285 – 314.

[12] L. Petersen, E. B. Pedersen, C. Nielsen, *Synthesis* **2001**, *4*, 559 – 564.

[13] L. Petersen, T. H. Hansen, N. M. Khalifa, P. T. Jørgensen, *et al.*, *Monatsh. Chem.* **2002**, *133*, 1031 – 1043.

[14] M. Wamberg, E. B. Pedersen, N. R. El-Brollosy, C. Nielsen, *Bioorg. Med. Chem*. **2004**, *12*, 1141 – 1149.

[15] F. D. Therkelsen, A. L. Hansen, E. B. Pedersen, C. Nielsen, *Bioorg. Med. Chem*. **2003**, *1*, 2908 – 2918.

[16] K. Danel, E. Larsen, E. B. Pedersen, *Synthesis* **1995**, 934 – 936.

[17] T. B. Johnson, J. C. Ambelang, *J. Am. Soc*. **1938**, *60*, 2941 – 2944.

[18] H. Vorbrüggen, K. Krolikiewicz, B. Bennua, *Chem. Ber*. **1981**, *114*, 1234 – 1255.

[19] D. W. Price Jr., J. M. Tour, *Tetrahedron* **2003**, *59*, 3131 – 3156.

[20] K. Danel, C. Nielsen, E. B. Pedersen, *Acta Chem. Scand.* **1997**, *51*, 426 – 430.

[21] N. R. El-Brollosy, P. T. Jørgensen, B. Dahan, A. M. Boel, *et al.*, *J. Med. Chem.* **2002**, *45*, 5721 – 5726.

[22] G. Meng, F.-E. Chen, E. De Clercq, J. Balzarini, C. Pannecouque, *Chem Pharm. Bull.* **2003**, *51*, 779 – 789.

[23] R. Pauwels, J. Balzarini, M. Baba, R. Snoeck, *et al.*, *J. Virol. Methods* **1988**, *20*, 309 – 321.

© 2007 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim 