DOI: 10.1002/cmdc.201000244

A Novel Synthetic Route for the Anti-HIV Drug MC-1220 and its Analogues

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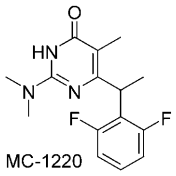
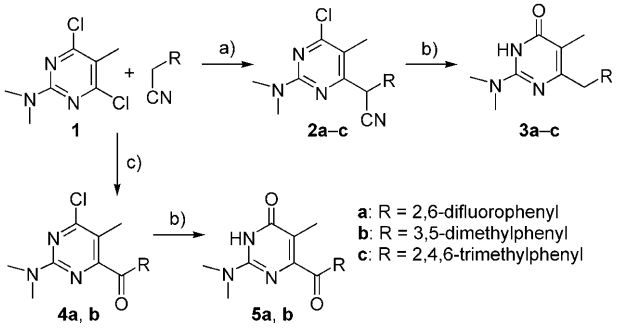
The human immunodeficiency virus type 1 (HIV-1) is the etiological agent of acquired immunodeficiency syndrome (AIDS). The viral reverse transcriptase (RT) of HIV-1 is one of the most promising targets for inhibition of HIV-1 replication; it can be inhibited by two classes of drugs that belong either to the nucleoside reverse transcriptase inhibitors (NRTIs) or to the non-nucleoside reverse transcriptase inhibitors (NNRTIs).[1–6]

The HIV pandemic continues to spread throughout the world, particularly affecting populations in developing countries. Efforts to limit this scourge need to be maximally implemented. Women now account for the majority of HIV-infected people worldwide, indicating the need for products that allow women the option of self-protection from HIV transmission without the knowledge of their sexual partners. The concepts of vaginal microbicides are a potentially promising strategy to prevent the spread of HIV. Microbicides are self-administered, prophylactic products designed to protect against sexually transmitted pathogens, including HIV-1. A special subgroup of NNRTIs, the dihydroalkoxybenzyloxopyrimidines (DABOs), have a proven capacity to irreversibly block HIV-1 replication.[7–10] This property correlates with their ability to bind HIV-1 RT with high affinity. One of the most important lead compounds of this series of tight-binding NNRTIs is MC-1220, which is used in racemic form.[11,12] The synthesis of MC-1220 and its analogues was previously published by Mai et al.[11] and Bartolini et al.[12] by condensation of the corresponding β-keto esters with guanidine sulfate derivatives. The β-keto esters, however, were prepared through multistep reactions.[11] Recently, Radi et al. reported the synthesis of arylmethyl-functionalized (*S*)-DABOs and related analogues from C6-protected formyl pyrimidinone.[13] Herein we describe a new and efficient synthetic route to racemic MC-1220 which can also be used for the preparation of its analogues.



The key step in our new synthetic route to MC-1220 and its analogues is an efficient procedure to introduce the arylmethyl moiety at C6 from a readily available pyrimidine precursor. As starting material we chose 4,6-dichloro-*N*,*N*,5-trimethylpyrimidin-2-amine (**1**)[14] due to the proper C2 substitution and the activated C6 position. Because compound **1** is not commercially available, it was necessary to start from diethyl methylmalonate and *N*,*N*-dimethylguanidine sulfate using sodium methoxide as a base to give 2-(dimethylamino)-5-methylpyrimidine-4,6-diol.[14] The diol was held at reflux in phosphorus oxychloride to give the starting compound **1**. Treatment of compound **1** with the sodium salt of the appropriate arylmethyl cyanides (2,6-difluorobenzyl cyanide, 3,5-dimethylbenzyl cyanide, and 2,4,6-trimethylbenzyl cyanide) afforded the corresponding 2-[6-chloro-2-(dimethylamino)-5-methylpyrimidin-4-yl]-2-(aryl)acetonitriles **2a**–**c** in high yields (86–98%). Nucleophilic substitution of compound **1** under the same conditions as mentioned above, followed by oxidation with a stream of oxygen bubbled through the reaction mixture for 3 h, afforded the 4-aryl-6-chloro-*N*,*N*-5-trimethylpyrimidin-2-amines **4a**,**b** in 52 and 99% yields, respectively. All five compounds were subsequently hydrolyzed, and in the case of **2a**–**c**, also decarboxylated with 7 M hydrochloric acid and acetic acid to furnish the corresponding 6-aryl-2-(dimethylamino)-5-methylpyrimidin-4(3*H*)-ones **3a**–**c** in 82–89% yield, and 6-aryl-2-(dimethylamino)-5-methylpyrimidin-4(3*H*)-ones **5a**,**b** in 80 and 94% yields, respectively, as new MC-1220 analogues (Scheme 1).



Scheme 1. Reagents and conditions: a) NaH, DMF, room temperature, 1 h; b) 7 M HCl, AcOH, reflux, 40 h; c) 1) NaH, DMF, room temperature, 2 h, 2) O_2, room temperature, 4 h.

Following the synthetic route shown in Scheme 2, we found it interesting to synthesize the MC-1220 analogues with a chloro substituent in the pyrimidine ring at C4. Reaction of **4a**,**b** with methylmagnesium bromide afforded the tertiary alcohols **6a**,**b**, which were dehydrated into the vinyl compounds **7a**,**b** in high yields using phosphorus pentoxide or phosphorus oxychloride, respectively. For a direct conversion of the carbonyl group in compound **4b** into a vinyl moiety, a Tebbe olefina-

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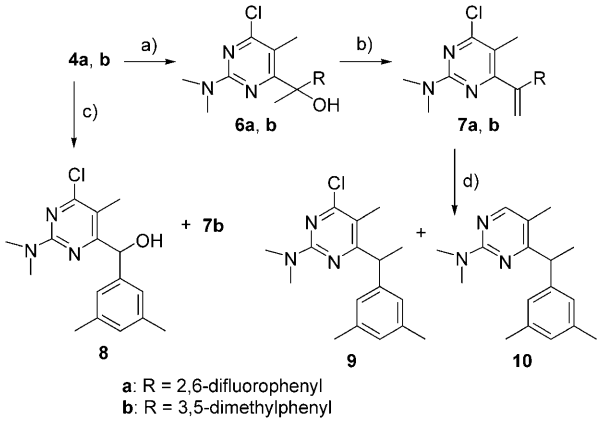
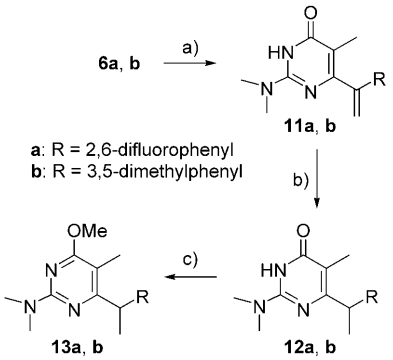
Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/cmdc.201000244: Full experimental details for the synthesis of compounds outlined in Schemes 1–3 and spectral data.



Scheme 2. Reagents and conditions: a) 1) MeMgBr, Et_2O, 20 °C, 14 h, 2) sat. NH_4Cl; b) P_2O_5, CH_2Cl_2, room temperature, 20 h, or $POCl_3$, CH_2Cl_2, reflux, 20 h; c) $TiCl_4$, Mg, THF, CH_2Cl_2, 0 °C, 20 min; d) 10% Pd/C, EtOH, 3.5 bar H_2, room temperature, 4 h.



Scheme 3. Reagents and conditions: a) 7 M HCl, AcOH, reflux, 42 h; b) 10% Pd/C, EtOH, 3.5 bar H_2, 5 h; c) MeI, NaH, DMF, room temperature, 2 h.

tion (methylenation)[15–19] was applied by using a titanium methylene complex. The desired product **7b** was isolated after column chromatography as the minor product in only 7% yield, while the major product was confirmed to be [6-chloro-2-(dimethylamino)-5-methylpyrimidin-4-yl]-(3,5-dimethylphenyl)methanol (**8**) in 27% yield.

Reduction of **7b** using 10% Pd/C and H_2 furnished 4-chloro-6-[1-(3,5-dimethylphenyl)ethyl]-*N*,*N*,5-trimethylpyrimidin-2-amine (**9**) in 5% yield and 4-[1-(3,5-dimethylphenyl)ethyl]-*N*,*N*,5-trimethylpyrimidin-2-amine (**10**) in 13% yield.

The synthetic route to MC-1220 is outlined in Scheme 3. Compounds **6a**,**b** were hydrolyzed under strong acidic conditions to afford 2-(dimethylamino)-6-(1-arylvinyl)-5-methylpyrimidin-4(3*H*)-ones **11a**,**b** in 70 and 89% yields, respectively. The products were reduced to MC-1220 (**12a**) and 2-(dimethylamino)-6-[1-(3,5-dimethylphenyl)-ethyl]-5-methylpyrimidin-4(3*H*)-one (**12b**). To study the structure–activity relationship of the pyrimidine ring, compounds **12a**,**b** were methylated by treatment of their sodium salts with methyl iodide to afford the corresponding methoxy derivatives **13a**,**b** in 86 and 91% yields, respectively.

The newly synthesized compounds were confirmed by comparison of NMR data.[11–14] The carbon signals C2, C4, C5, and C6 could not be observed in ^{13}C NMR spectra of compounds **3a–c**, **5a**,**b**, **11a**,**b**, and **12a**,**b**, especially when NMR spectra were processed using deuterated DMSO as a solvent. This can be explained by a rapid keto–enol tautomerism in the pyrimidinone ring. Only compound **12a** (MC-1220) was soluble in deuterated chloroform, and in this solvent carbon signals of C2, C4, C5, and C6 could be detected by ^{13}C NMR spectroscopy. However, elemental microanalysis, ^1H NMR spectroscopy, EIMS, and HRMS confirmed the proposed structures.

Table 1 lists the data for the inhibitory activities of the synthesized compounds against wild-type HIV-1 (strain HTLV-IIIB) and strains carrying the clinically relevant mutations that appear in the presence of NNRTIs: Y181C (strain N119), double mutant Y181C + K103N (strain A17), and the triple mutant K103R + V179D + P225H (EFV^R; efavirenz resistant strain) in MT-4 cells.

Table 1. Cytotoxicity and anti-HIV-1 activity of synthesized compounds **3a–c**, **5a**,**b**, **11a**,**b**, **12a** (MC-1220), **12b**, and **13a**,**b**.

Compd	CC_{50} [μM][a]	SI[b]	EC_{50} [μM][c]			
			wild-type	EFV^R	Y181C	K103N + Y181C
3a	> 100	> 1428	0.07	> 100	≥ 100	≥ 100
3b	> 100	> 200	0.5 ± 0.1	> 100	21 ± 6	> 100
3c	> 100	> 250	0.4 ± 0.1	> 100	> 100	> 100
5a	> 100	> 333	0.3 ± 0.1	> 100	94	> 100
5b	8 ± 0.1	–	> 8	> 8	> 8	> 8
11a	> 100	> 500	0.2 ± 0.05	> 100	44 ± 6	> 100
11b	> 100	> 40	2.5 ± 0.2	> 100	17 ± 2	≥ 100
12a	> 100	> 10000	0.01 ± 0.003	> 100	0.7 ± 0.2	90 ± 10
12b	44 ± 0.2	488	0.09 ± 0.01	> 44	3.5 ± 0.1	> 44
13a	46	–	> 46	> 46	> 46	> 46
13b	> 100	–	> 100	> 100	> 100	> 100

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

As is apparent from the results listed in Table 1, the majority of the novel compounds were non-cytotoxic toward MT-4 cells at doses as high as 100 μM (the highest concentration tested); however, compound **5b** was surprisingly cytotoxic relative to

all other synthesized compounds. Unfortunately, none of the newly synthesized compounds were more active than the lead compound MC-1220. In general, members of the 2,6-difluorophenyl series (**3a**, **5a**, **11a**, **12a**) were more active than both other series. When the arylalkyl group in compounds **3a**,**b** was replaced by an aroyl group in compounds **5a**,**b** or a vinyl group as in compound **11a**,**b**, the activity against HIV-1 was decreased. In the case of the methoxy compounds **13a**,**b**, the lack of a hydrogen at the N3 position in the pyrimidine ring may explain the complete loss of activity. Interestingly, the new compound **12b** was found to exhibit activity against the Y181C mutant in the micromolar range ($EC_{50} = 3.5$ μM). This compound could be a new lead for further screening efforts. Unfortunately, none of the other new compounds were found to exhibit activity against the Y181C mutant.

In summary, we present a new, rapid, and easy route for the synthesis of the lead compound MC-1220 (**12a**) and its analogue **12b** by reduction in only four steps to give total respective yields of 29 and 43%. The key step in this route involves reduction of the vinyl group in compounds **11a**,**b**. The intermediates from this route, which are readily available in large quantities, are also suitable for synthesizing new analogues of MC-1220 for broad medicinal screening campaigns. Although anti-HIV-1 activities were observed for the novel MC-1220 analogues, in all cases, they were lower than that of the lead compound MC-1220.

Acknowledgements

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).[22]

Keywords: antiviral agents · Grignard reaction · HIV · MC-1220 · NNRTIs · pyrimidines

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Received: June 9, 2010
Revised: July 12, 2010
Published online on August 16, 2010

