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Use of *N*-Methylpiperazine for the Preparation of Piperazine-Based Unsymmetrical Bis-Ureas as Anti-HIV Agents

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Substituted piperazines are common motifs in a large number of compounds with biological activity.[1–7] Furthermore, *N*-urea and *N*,*N*′-bis-urea piperazine derivatives have interesting antiviral properties as well as attributes for other therapeutic indications.[8–15]

Unsymmetrical substituted piperazine derivatives require monoprotected piperazines, which can be tedious to prepare and expensive. Furthermore, their use calls for an additional deprotection step. Herein we propose *N*-methylpiperazine for the preparation of unsymmetrical derivatives containing at least one *N*-urea moiety as a substitute of the piperazine core. The methyl group acts as protecting group of one of the nitrogen atoms of the piperazine and is concomitantly removed during the formation of a synthetic intermediate.

In one of our ongoing research programs devoted to the development of novel proton acceptor immonium-type coupling reagents, we address the preparation of aminium chlorides, shown in Figure 1.[16,17] Although the reaction of *N*,*N*-dimethylmorpholine-4-carboxamide and *N*,*N*-dimethylthiomorpholine-4-carboxamide with bistrichloromethyl carbonate (BTC) or phosgene rendered the corresponding oxygen- and sulfur-containing aminium salts,[17] this result was not achieved if *N*,*N*-4-trimethylpiperazine-1-carboxamide was used. Thus, reaction of this compound with BTC followed by the addition of potassium benzotriazolate (KOBt) or potassium 7-azabenzotriazolate (KOAt) (Figure 1, left), a pure white solid was obtained. This product showed a ^{1}H NMR spectrum similar to that expected (aromatic region from the OBt/OAt and protons from the piperazine cycle and dimethylamino group), but with a distinct molecular weight, as determined by MS, and with extra

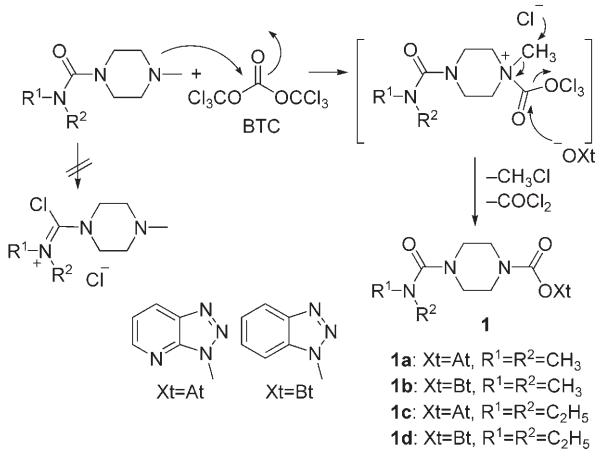

Figure 1. Left side: failed reaction to obtain aminium chlorides; right side: preparation of piperidine-containing active carbamates.

^{13}C NMR signals (2 carbonyls) and one missing CH_3 (N-CH_3). X-ray crystallography (Figure 2) confirmed that the product was active carbamate piperazine **1**.

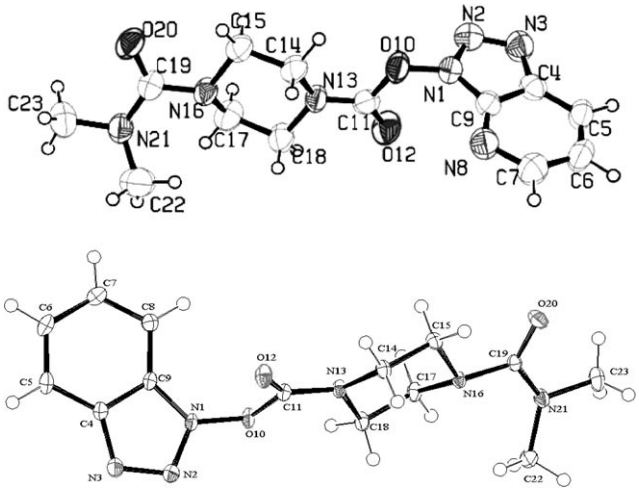

Figure 2. X-ray crystal structures of **1a** (top) and **1b** (bottom).

The mechanism of the reaction (Figure 1, right) can be interpreted by a nucleophilic attack at the carbonyl group of BTC by NCH_3 and finally, entrance of the OXt moiety and nucleophilic attack at CH_3 by Cl^- to release the final compound.

Following this strategy, key compounds **1a**–**d** were easily prepared with excellent yields and purities. Furthermore, compounds **1a**–**d** were totally stable at 4 °C for several months and highly stable at room temperature. Finally, compounds

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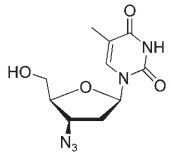
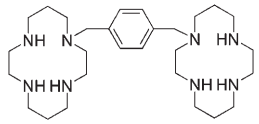
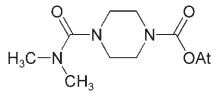
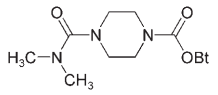
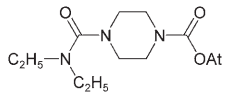
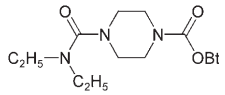
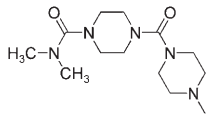
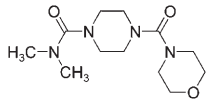
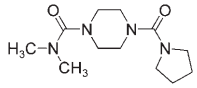
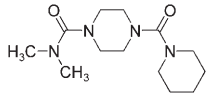
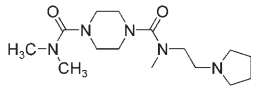
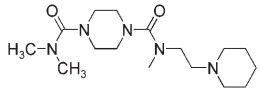
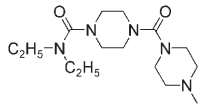
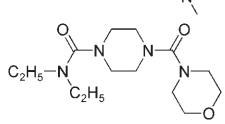
1a–d reacted smoothly with amines to render piperazine-containing ureas (**2**–**13**, see Table 1 for structures) with excellent yields (85–92% overall yield) and purities (>95%, without a single chromatography purification). Moreover, reaction of either BTC or phosgene with other amino-containing ureas such as 1-(3-(dimethylamino)propyl-1,3,3-trimethylurea or 1,1,3-trimethyl-3-(2-(pyrrolidin-1-yl)ethyl)urea rendered complex mixtures.

Anti-HIV activity and cytotoxicity measurements with MT-4 cells were based on the viability of lymphoid MT-4 cells infected or not with HIV-1 at a multiplicity of infection (moi) of 0.003 and exposure to various concentrations of the test compound. After five days of infection, the number of viable cells was quantified by a tetrazolium-based colorimetric method (MTT method), as described.[18,19]

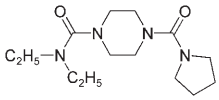
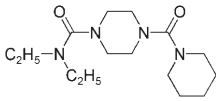
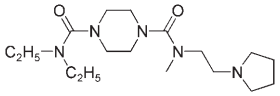
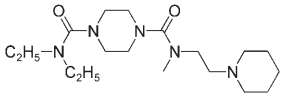
Table 1. Structures and biological activities of the acyl carbamate and bis-urea piperazine derivatives.[a]

Compd	Structure	NL4-3 wt EC_{50} [$\mu g\,mL^{-1}$][b]	No virus CC_{50} [$\mu g\,mL^{-1}$][c]
AZT[d]		0.0007	>1
AMD3100[d]		0.001	>1
1a		>23	23
1b		>39.3	39.3
1c		>125	>125
1d		>58.4	58.4
2		>125	>125
3		>125	>125
4		>125	>125
5		>125	>125
6		72.0	125
7		9.6	125
8		>125	>125
9		>125	>125

Whereas active carbamate derivatives **1** showed cytotoxicity, none of the bis-urea piperazine derivatives (**2**–**13**) did. More interestingly, compounds **6** and **7**, and **12** and **13**, which respectively contain either a pyrrolidine or a piperidine moiety at the end of one of the urea chains, exhibited anti-HIV activity in the absence of drug-induced cytotoxicity at the concentrations tested. In this regard, it is important to note that other compounds, such as **2** and **8**, derived from the reaction of the active carbamate urea **1** with *N*-methylpiperazine and which also contain a tertiary amine, did not show any activity. This finding could be explained in terms of either the higher hydrophobicity or higher flexibility of compounds **6**, **7**, **12**, and **13** versus **2** and **8**. The importance of hydrophobicity is reflected by the observation that piperidine derivatives (compounds **7** and **13**) were more active than pyrrolidines **6** and **12**. Finally, methyl (**6** and **7**) and ethyl (**12** and **13**) derivatives showed similar activity.

In conclusion, we describe a straightforward and efficient strategy to prepare unsymmetrical substituted piperazine derivatives containing at least one *N*-urea moiety as a substituent of the piperazine core, and we exemplify this strategy by the preparation of unsymmetrical *N*,*N*′-bis-urea piperazines. The

Table 1. (Continued)

Compd	Structure	NL4-3 wt EC_{50} [$\mu g\,mL^{-1}$][b]	No virus CC_{50} [$\mu g\,mL^{-1}$][c]
10		> 125	> 125
11		> 125	> 125
12		36.4	> 125
13		11.2	> 125

[a] Values are the average of two measurements; the highest concentration tested was 1 $\mu g\,mL^{-1}$ for control compounds, AZT and AMD3100, whereas the remaining compounds were tested at 125 $\mu g\,mL^{-1}$. [b] EC_{50}: concentration required to inhibit 50% of the HIV-induced cytopathic effect, as evaluated by the MTT method in MT-4 cells. [c] CC_{50}: concentration required to induce 50% death of uninfected MT-4 cells, as evaluated by the MTT method. [d] AMD3100 and AZT are well characterized anti-HIV compounds that were used only as controls in the anti-HIV assay.

methyl group acts as protecting group of one of the nitrogen atoms of the piperazine core and is concomitantly removed once the first urea has been formed during the reaction with phosgene or related reagents required for the preparation of an active carbamate intermediate. This intermediate, after reaction with the corresponding amine, renders the desired bis-urea. Derivatives that contain a pyrrolidine or a piperidine moiety at the end of one of the urea chains are not cytotoxic and exhibit anti-HIV activity. This synthetic strategy is currently being used to prepare new libraries of unsymmetrical urea piperazine derivatives containing sulfonamide or aryl moieties at the second nitrogen atom of the piperazine in order to increase biological activity.

Experimental Section

General procedure for the preparation of urea derivatives:[20] *N*,*N*-Dialkylcarbamoyl chloride (0.6 mol) was added dropwise to a stirring mixture of secondary amine (0.5 mol) and triethylamine (TEA, 0.5 mol) in CH_2Cl_2 (400 mL) at 0 °C. When the addition was completed, the mixture was stirred for 3–4 h at room temperature. The reaction mixture was made alkaline with 10% NaOH, the organic layer was collected, and the aqueous layer was washed with 100 mL CH_2Cl_2. The combined CH_2Cl_2 solutions were washed with H_2O (2×100 mL) and a saturated solution of NaCl (2×100 mL). Finally, the organic solvent was dried over anhydrous $MgSO_4$, filtered, and the solvent was removed under reduced pressure. The oily residue obtained was purified by vacuum distillation. All compounds were characterized by IR, ^1H and ^{13}C NMR.

General procedure for the preparation of active carbamates: A solution of urea (10 mmol) in CH_2Cl_2 (10 mL) was added dropwise to a solution of BTC (0.989 g, 3.3 mmol) in CH_2Cl_2 (5 mL) at 0 °C under nitrogen atmosphere. After being stirred for 4–5 h, the solution was evaporated under vacuum, and the residue was dissolved in dry acetonitrile (20 mL), followed by addition of KPF_6 (1.8 g, 10 mmol) and KOAt (1.66 g, 10 mmol). The reaction mixture was stirred at room temperature under nitrogen atmosphere overnight, then filtered and washed with acetonitrile (10 mL). The filtrate and the wash were removed to give a sticky foamy product. While the dimethyl derivatives solidified under vacuum, the diethyl derivatives were obtained as oils. Alternatively, the reaction was carried out with phosgene (10 mL of 20% phosgene in toluene) at −20 °C with a similar yield. All compounds were characterized by ^1H and ^{13}C NMR and elemental analysis; the structures of **1a** and **1b** were furthermore confirmed by X-ray crystallography.

General procedure for the preparation of bis-urea derivatives: The OXt ester (1 mmol) was added to a solution of amine (1 mmol) and TEA (1 mmol) in CH_2Cl_2 (10 mL) at room temperature [in the case of morpholine, pyrrole, and piperidine, these compounds themselves were used as a base instead of TEA (2 mmol)]. The reaction mixture was stirred for 3 h (IR showed complete reaction by the disappearance of the peak at 1778 cm^{-1}). After completion of the reaction, CH_2Cl_2 (20 mL) was added and then washed with sat. Na_2CO_3 (2×10 mL), sat. NaCl (2×10 mL), dried ($MgSO_4$), and filtered. The solvent was removed under vacuum to give the product in pure form, as shown by NMR spectroscopy. All compounds were characterized by ^1H and ^{13}C NMR and elemental analysis.

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