Pergamon

Tetrahedron Letters 39 (1998) 3307–3310

TETRAHEDRON LETTERS

Solid Phase Synthesis of 2,6-Disubstituted-4(3*H*)-pyrimidinones Targeting HIV-1 Reverse Transcriptase

Emanuela Nizi, Maurizio Botta*, Federico Corelli*, Fabrizio Manetti, Flavia Messina, and Giovanni Maga[1]

Dipartimento Farmaco Chimico Tecnologico, Università di Siena, Banchi di Sotto 55-53100 Siena-Italy.

[1]Istituto di Genetica Biochimica ed Evoluzionistica del CNR, Via Abbiategrasso 207-27100 Pavia-Italy.

Received 29 January 1998; revised 23 February 1998; accepted 27 February 1998

Abstract: The solution and solid phase synthesis of 6-substituted 2-[(4-hydroxybutyl)amino]-4(3*H*)-pyrimidinones **1** as HIV-1 RT inhibitors is described.

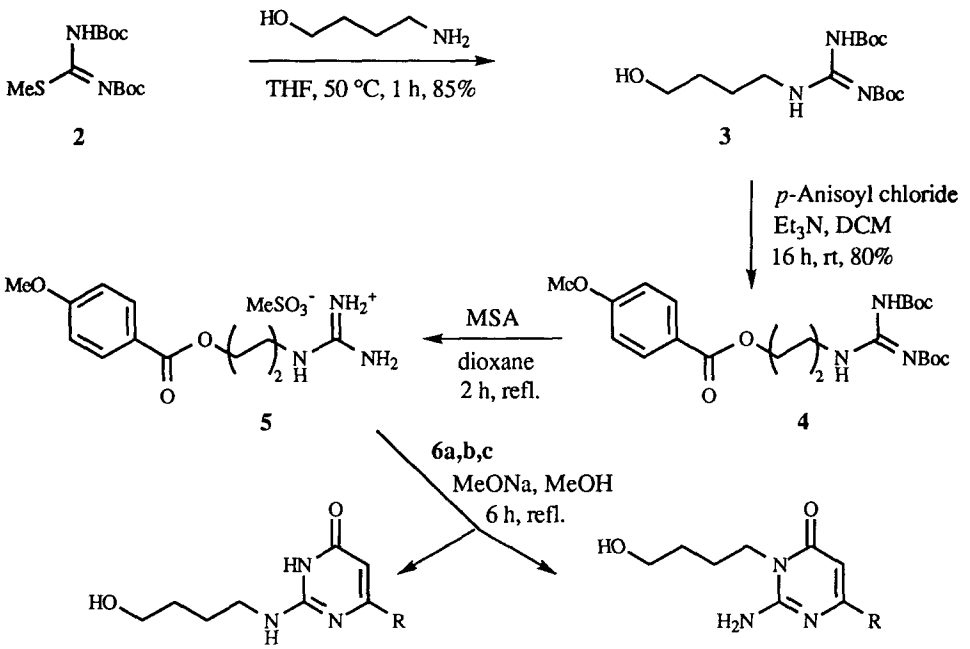
The synthesis and evaluation of libraries of compounds has become a powerful method for the identification and optimization of lead structures. Although interesting structures have been accessed by solution synthesis and lead compounds have been identified and optimized, solid phase synthesis (SPS) continues to be employed in the majority of the reported small-molecule library efforts;[1] hence, there is a growing need for developing new SPS methods for important classes of bioactive compounds.[2]

6-Substituted- and, more recently, 2,6-disubstituted-4(3*H*)-pyrimidinones have shown very good potential as antiviral agents[3] and in particular HIV-1 reverse transcriptase (RT) inhibitors.[4] Molecular modeling studies of our group demonstrated that compounds of general structure **1** (see Scheme 1) would interact effectively with the target enzyme.[5] As part of an ongoing research program on new anti-HIV-1 agents,[6] we desired to set up an SPS method for 2,6-disubstituted-4(3*H*)-pyrimidinones (**1**) which would allow for the maximization of the number and chemical diversity of these compounds. In general, 6-substituted isocytosine derivatives can be prepared following two different approaches, (i) by reaction of 2-methoxy- or 2-methylthiopyrimidinones with an excess of amide anions in refluxing tetraline, or (ii) by condensing guanidines with β-keto esters.[7] The first approach suffers from some drawbacks, as the experimental conditions required make problematic the use of aminoalcohols and sometimes cause the nucleophilic substitution to occur at the 4 position of the ring,[8] while the second method seems to be more appropriate in our case. Considering that a *N*-(4-hydroxybutyl)guanidine moiety would be a common feature of all library members, a convergent and versatile strategy calls for immobilizing this group first, and then subjecting it to condensation with different β-keto esters to prepare diverse sets of potential RT inhibitors. Since the presence of a free hydroxyl is not compatible with the conditions of the condensation reaction, we decided to employ *O*-immobilized *N*-4-(hydroxybutyl)guanidine, wherein the linker group, besides being a cleavable site of attachment for the molecule to a solid support, also serves as an oxygen protecting group. We report herein the preliminary results of our solution and solid phase syntheses of compounds **1**.

0040-4039/98/$19.00
PII: S0040-4039(98)0477-8

N^2,N^3-Bis(*tert*-butoxycarbonyl)-N^1-(4-hydroxybutyl)guanidine **3** (Scheme 1), obtained in 85% yield starting from N,N'-bis(*tert*-butoxycarbonyl)-*S*-methylisothiourea **2**[9] and 4-aminobutanol following a procedure already reported for similar compounds,[10] was *O*-protected using 4-methoxybenzoyl chloride to give the ester derivative **4** in 80% yield. The *O*/*N* selectivity in this acylation reaction is clearly due to the steric hindrance exerted by the bulky Boc groups. Removal of the *N*-protecting groups was best accomplished by heating **4** in dioxane in the presence of methanesulfonic acid (MSA, 1.2 mol equivalents), affording in quantitative yield the mesylate salt **5**. The latter was directly subjected to condensation with β-keto esters, namely ethyl 3-oxobutanoate (**6a**), ethyl 3-oxo-4-phenylbutanoate (**6b**)[4b], and dimethyl 3-oxoglutarate (**6c**), in refluxing methanol and in the presence of excess sodium methoxide. Under these conditions not only condensation to pyrimidinones, but also cleavage of 4-methoxybenzoate group occurred, leading to a mixture of fully deprotected 2,6-disubstituted-4(3*H*)-pyrimidinones **1a-c** along with 3,6-disubstituted isomers **7a-c**. Although the condensation reaction did not show a high degree of regioselectivity, the reaction mixtures were easily separated by column chromatography on silica gel to give the pure compounds, the ratio between pyrimidinones **1** and **7** being in every case > 2.6 (see Scheme 1 for yields).[11]

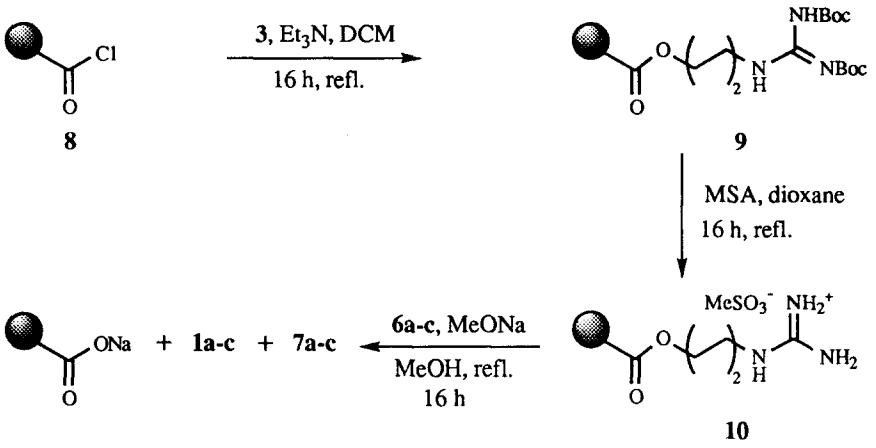
Scheme 1.



Having explored the performance of the above reaction sequence in solution, we went on to apply it on solid support. In order to reproduce as truly as possible the conditions used in solution, we chose as the solid support the modified[12] Merrifield resin **8** (Scheme 2). Thus, **8** was reacted with 3 mol equivalents of **3** in

refluxing dichloromethane (DCM) for 16 h, then **9** was collected on a glass frit and rinsed with the following sequence of wash solvents: DCM, MeOH, H_2O, MeOH, DCM, Et_2O. After drying in an N_2 atmosphere, the resin **9** was analyzed for residual Cl to determine the loading of **3** and this was found to be no more than 0.41 meq/g; therefore **9** was again exposed to excess **3** under the same conditions reported above to give the resin **9** showing a loading of 0.72 meq/g. In consideration of these results, all the following reactions were performed twice. The polymer-bound guanidine **9** was refluxed in dioxane for 16 h in the presence of MSA (1.5 mol equivalents) to provide the salt **10** which was in turn condensed with **6a** (10 mol equivalents of MeONa, MeOH, reflux, 16 h). Filtration of the reaction mixture afforded the resin (sodium salt form, which could be easily transformed into **8** by the action of $SOCl_2$) and a solution of **1a** and **7a** in 38 and 12% yield, respectively. Similarly, reaction of **10** with **6b** and **6c** gave **1b/7b** (45%/13%) and **1c/7c** (35%/traces). Yields correspond to purified products and they are calculated based on the acyl chloride functionality of the modified Merrifield resin. The structure and identity of the products were compared to those produced *via* solution synthesis and they exhibited satisfactory ^1H NMR and FAB-MS spectra. HPLC analysis of crude products cleaved from the resin showed purities ranging from 88-96%.

Scheme 2.



Compounds **1a-c** and **7a-c** were evaluated in enzyme assays against recombinant HIV-1 RTs from both wild type (wt) and clinically relevant mutant viruses resistant to TIBO/nevirapine (L100I, K103N and V106A), using nevirapine as reference drug. The ability of **1b**[13] to inhibit the recombinant enzymes, reported as K_i (μM) values, was found to be as follows: 410 (wt), 525 (L100I), 840 (K103N), and 75 (V106A). Although in general most of the enzymes are inhibited by **1b** at high concentration, the K_i value of 75 μM against V106A is not negligible and suggests that compounds like **1** can actually bind to RTs. Considering that this is the first example of isocytosine derivatives, and in particular *N*-(hydroxyalkyl)isocytosines, targeting HIV-1 RT,[14] compounds **1** may represent interesting leads for further structural optimization in the search of novel RT inhibitors.

In conclusion, the SPS method here reported allows the synthesis of the target compounds in an easy and profitable way, showing good potential for the preparation of combinatorial libraries of 2- and 3-[(4-hydroxybutyl)amino]-4(3*H*)-pyrimidinones bearing different substituents at the 5 and/or 6 position.

Acknowledgements. This investigation was supported by Ministero della Sanità, Istituto Superiore di Sanità, X Progetto AIDS 1997. One of us (M. B.) wishes to thank for a grant from the Merck Research Laboratories ("Academic Development Program in Chemistry").

References and Notes

1. Choong, I.C.; Ellman, J.A. In *Annu. Rep. Med. Chem.*; Bristol, J. A., Ed.; Academic Press: San Diego, **1996**; Vol. 31, p 309.
2. Patel, D.V. *Annual Reports in Combinatorial Chemistry and Molecular Diversity*; Moos, W.H., Pavia, M.R., Ellington, A.D., Kay, B.K., Eds.; ESCOM: Leiden, **1997**; Vol. 1, p 78.
3. Botta, M.; Saladino, R.; Gentile, G.; Summa, V.; Nicoletti, R.; Verri, A.; Focher, F.; Spadari, S. *Tetrahedron* **1994**, *50*, 3603.
4. (a) Artico, M. *Farmaco* **1996**, *51*, 305. (b) Massa, S.; Mai, A.; Artico, M.; Sbardella, G.; Tramontano, E.; Loi, A.G.; Scano, P.; La Colla, P. *Antiviral Chem. Chemother.* **1995**, *6*, 1.
5. Results to be published.
6. (a) Botta, M.; Saladino, R.; Delle Monache, G.; Gentile, G.; Nicoletti, R. *Heterocycles* **1996**, *43*, 1687 and references cited therein. (b) Botta, M.; Saladino, R.; Anzini, M.; Corelli, F. *Nucleosides Nucleotides* **1994**, *13*, 1769.
7. Botta, M.; Saladino, R.; Occhinero, F.; Nicoletti, R. *Trends in Organic Chemistry* **1995**, *5*, 57.
8. Botta, M.; Occhinero, F.; Saladino, R.; Crestini, C.; Nicoletti, R. *Tetrahedron Letters* **1997**, *38*, 8249.
9. Bergeron, R.J.; McManis, J.S. *J. Org. Chem.* **1987**, *52*, 1700.
10. Delle Monache, G.; Botta, B.; Delle Monache, F.; Espinal, R.; De Bonnevaux, S.C.; De Luca, C.; Botta, M.; Corelli, F.; Carmignani, M. *J. Med. Chem.* **1993**, *36*, 2956.
11. The structures of the new compounds were determined by FAB-MS and ^{1}H NMR spectroscopy. All the new compounds gave satisfactory (±0.4% of the theoretical values) elementary analyses, with the exception of **5** (not analyzed).
12. Aryes, J.T.; Mann, C.K. *Polymer Lett. (Part B)* **1965**, *3*, 505.
13. All the other compounds resulted to be less active than **1b**. Full description of their biological evaluation will be reported elsewhere.
14. Nizi, E. Doctorate Thesis, University of Siena, October 1997.