Bioorganic & Medicinal Chemistry 22 (2014) 513–522

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

New carbocyclic nucleoside analogues with a bicyclo[2.2.1]heptane fragment as sugar moiety; Synthesis, X-ray crystallography and anticancer activity

Constantin I. Tănase [a,*], Constantin Drăghici [b], Miron Teodor Căproiu [b], Sergiu Shova [c], Christophe Mathe [d,1], Florea G. Cocu [a], Cristian Enache [e], Maria Maganu [b]

[a] National Institute for Chemical-Pharmaceutical Research and Development, 112 Vitan Av., 031299 Bucharest 3, Romania
[b] Organic Chemistry Center "C.D.Nenițescu", 202 B Splaiul Independentei, Bucharest 060023, Romania
[c] Institute of Macromolecular Chemistry "Petru Poni", Iasi, Romania
[d] Laboratoire de Chimie Organique Biomoléculaire de Synthèse, UMR 5625 CNRS-Université Montpellier II, Place Eugène Bataillon, 34095 Montpellier Cedex 5, France
[e] Central Laboratory for Phytosanitary Quarantine, 11, Voluntari Blvd., 077190 Voluntari, Romania

ARTICLE INFO

Article history:
Received 12 September 2013
Revised 23 October 2013
Accepted 31 October 2013
Available online 9 November 2013

Keywords:
Bicyclo[2.2.1]heptane nucleosides
X-ray crystallography
Anticancer
Chloropurine
Azide
Amine

ABSTRACT

An amine group was synthesized starting from an optically active bicyclo[2.2.1]heptane compound, which was then used to build the 5 atoms ring of a key 6-chloropurine intermediate. This was then reacted with ammonia and selected amines obtaining new adenine- and 6-substituted adenine conformationally constrained carbocyclic nucleoside analogues with a bicyclo[2.2.1]heptane skeleton in the sugar moiety. X-ray crystallography confirmed an *exo*-coupling of base to the ring and a L configuration of the nucleoside analogues. The compounds were tested for anticancer activity.

1. Introduction

For a long time, nucleosides and their analogues have been recognized as efficient antitumor[1] and antiviral[2] drugs. However, their side effects and resistance after prolonged use has led to numerous studies, mainly focused on the discovery of new analogues, more active and potentially less toxic, by changing both the base as well as the sugar moiety.[3] Remarkable metabolic stability of nucleoside analogues has been realized by replacing the endocyclic oxygen of the nucleoside sugar moiety with a methylene group to obtain carbanucleosides,[4] which are unaffected by phosphorylases and hydrolases that cleave the glycosidic bond.[5] Also, conformationally restricted nucleoside analogues are studied with great interest by fixing the sugar puckering in a rigid conformation that could have an optimal fitting to the enzymes involved in the metabolism of nucleosides or nucleotides.[6]

Following this trend, we have previously realized the synthesis of new compounds, in which we replaced the usual sugar moiety with an oxabicyclo[3.3.0]octane fragment from the skeleton of the Cloprostenol prostaglandin analogue. We have obtained new pyrimidine nucleoside analogues **I**, in racemic or optically active form.[7,8] These were tested for antitumor activity on standard Jurkat T lymphoblast and U937 cells. The more active analogue proved to be the one with 5-iodouracil as base (e.g., whilst uracil analogue has no major effect, 5-iodouracil analogue decreases by 64% uridine incorporation by U937 blasts at 30 μM concentration). We also have tried to introduce a bicyclo[2.2.1]heptane fragment as sugar moiety, by Mitsunobu reaction with pyrimidine bases, however instead of the desired products, mainly O-alkylated compounds were obtained.[9] Two of the compounds with thymine and 5-fluorouracil as base 'exhibited mainly a cytostatic activity in Jurkat lymphoblasts and on U937 monocytic blasts'.[9]

This motivated us to synthesize new carbocyclic nucleosides **II**, with a purine base (adenine and 6-substituted adenines) instead of a pyrimidine base and with the same bicyclo[2.2.1]heptane radical. The goal of our work was to find if these new structures could also have antitumor activity.

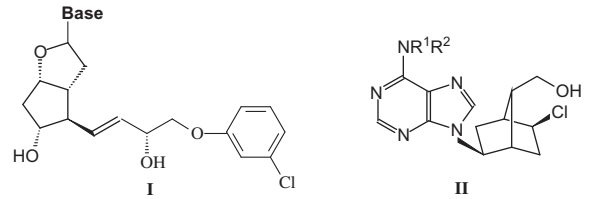
This bicyclo[2.2.1]heptane fragment will give a conformationally restricted nucleoside structure. Structure **II** mimics a L nucleo-

* Corresponding author. Tel.: +40 21 321 21 17; fax: +40 21 322 29 17.
E-mail address: cvtanase@gmail.com (C.I. Tănase).
[1] Fax: +33 4/670042029.

0968-0896/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmc.2013.10.056

side with 1,4 correlation between the base and the hydroxymethyl group on the upper part and 1,3 correlation on the lower part of the cyclopentane ring. On the other hand, a 1,4 correlation proved to be fruitful for antiviral activity of cyclohexene nucleosides,[10] and in compounds **II** there is a cyclohexane fragment constrained in a norbornane structure. It is also known that some N^6-substituted adenine nucleosides (studied mainly as adenosine A_3 receptor) are already in Phase II clinical trials for the treatment of colorectal cancer (IB-MECA[11a]) or are studied to be used in antitumor combination chemotherapy (Cl-IB-MECA[11b]).

We believe that the introduction of an adenine moiety substituted at the N^6-amino group could be useful for the antitumor activity of the new carbocyclic nucleosides **II**.



Base = U, IU, BrU, ClU, FU, T, C, 6-Aza-U

Therefore, we have worked to obtain new nucleoside analogues with adenine and N^6-substituted adenines as base linked *exo* to position 5 of a 2-chloro-(bicyclo[2.2.1]heptan-7-yl)methanol fragment used as sugar moiety. Our strategy, which we will describe below, is to build first the 6-chloro-purine ring on a bicyclo[2.2.1]heptane amine intermediate and finally to substitute the chlorine atom with selected amines.

2. Results and discussion

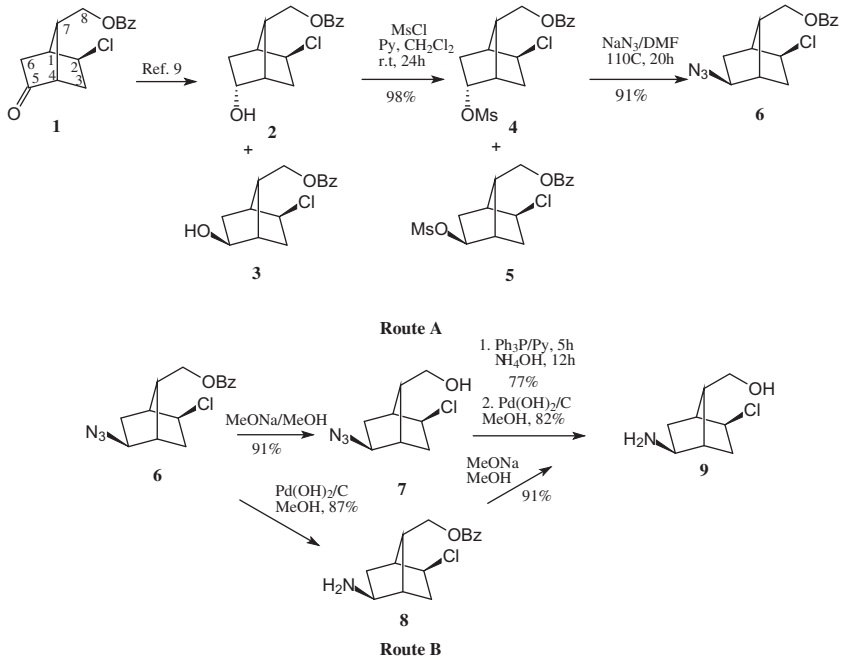
2.1. Chemistry

The synthesis of these nucleoside analogues started from an optically active intermediate **2**, which had been previously obtained by $NaBH_4$ reduction of the keto-intermediate **1**.[9] The synthesis strategy was to obtain an amine intermediate from **2**, and then to use it further to build the 5-atoms ring of the 6-chloropurine skeleton, and in the final stage, to substitute the chlorine atom with selected amines, with groups ranging from dimethyl to cycloalkyl (C-3, C-5, C-6), phenethyl, 1- and 3-methylpiperazine, morpholine and a diethylamino group spaced from 6-NH-group by a chain of two or three CH_2 groups.

Alcohol **2**, isolated pure by multiple column chromatography operations (CCO), was now mesylated to the easily crystallized compound **4** (Scheme 1). This enabled us to perform the mesylation of the crude reaction mixture of alcohols **2** and **3**, obtained in the sodium borohydride reduction of **1**, and to isolate the pure compound **4** by simple crystallization in 83% yield. The absolute configuration of the starting compound **4**, which will determine the configuration of the following compounds in the synthesis sequences, was obtained by X-ray crystallography, as is presented below. The isomer compound **5** was concentrated in mother liquor, and was isolated pure, as oil, through multiple CCO. The mesyl group was then substituted by a SN_2 reaction with NaN_3 to the azide compound **6**, with inversion of configuration. The reduction of the azide group to the amine group was performed by Staudinger reaction,[1] or by catalytic hydrogenation over Pd/C[13] or $Pd(OH)_2$/C[14] catalysts with benzoate–azide **6** (route **A**), respectively, with the deprotected compound **7** (route **B**), in 77–87% yield, to the corresponding amine **8**, respectively, **9**.

The benzoate group of azide **6** and, respectively, of amine **8** was removed by *trans*esterification (MeONa/MeOH), to the deprotected compound **7** and, respectively, **9**, in about 91% in both cases.

In order to build the 6-chloropurine skeleton of the key intermediate **12** (Scheme 2), amine **9** was first reacted with 4,6-dichloropyrimidine-5-amine,[15] and then with triethylorthoformate in the presence of concentrated HCl. The absolute configuration of intermediate **12**, which will give the configuration of all the nucleoside synthesized, was determined by X-ray crystallography and confirmed the entire stereocontrolled sequence of reactions from intermediate **1**–**12**. The chloropurine base is *exo*-linked to the bicyclo[2.2.1]heptane ring and is in a *cis* relation with the 7-hydroxymethyl group.



Scheme 1. Synthesis of the amine intermediate **9**.