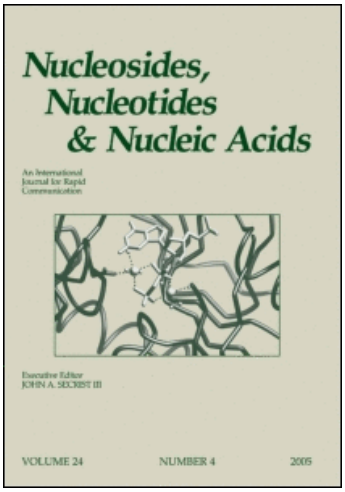

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Synthesis and Antiviral Evaluation of 3'-Substituted Thymidine Analogues Derived from 3'-Amino-3'-deoxythymidine

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To cite this Article Pannecouque, C. , Van Poppel, K. , Balzarini, J. , Claes, P. , De Clercq, E. and Herdewijn, P.(1995) 'Synthesis and Antiviral Evaluation of 3'-Substituted Thymidine Analogues Derived from 3'-Amino-3'-deoxythymidine', Nucleosides, Nucleotides and Nucleic Acids, 14: 3, 541 — 544
To link to this Article: DOI: 10.1080/15257779508012422
URL: http://dx.doi.org/10.1080/15257779508012422

NUCLEOSIDES & NUCLEOTIDES, 14(3-5), 541-544 (1995)

SYNTHESIS AND ANTIVIRAL EVALUATION OF 3'-SUBSTITUTED THYMIDINE ANALOGUES DERIVED FROM 3'-AMINO-3'-DEOXYTHYMIDINE

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Abstract: To assess the structure-activity relationship for antiviral activity, a series of 3'-deoxy-3'-*N*-functionalized thymidine analogues were synthesized. Several of these thymidine analogues show moderate *in vitro* activity against HIV-1 and HIV-2.

Introduction

In the scope of the development of antiviral and antitumoral nucleoside analogues, 3'-substituted nucleosides have attracted considerable interest[1]. Some of these compounds display high activity against human immunodeficiency virus type 1 (HIV-1): e.g. 3'-azido-3'-deoxythymidine (AZT)[2] and 3'-fluoro-3'-deoxythymidine (FLT) [3]. However, these drugs are not free of undesirable side effects. This has prompted various attempts at 3'-modifications of the sugar moiety[1].

In the past, different 1,2,3-triazole groups have been introduced at the 3-position of 2-deoxyribose in order to mimic the three-nitrogen system of AZT. However, no significant activity against HIV was found for these molecules[4,5]. Likewise 1,2,4-triazole derivatives have also been synthesized[6]. This report describes the synthesis and antiviral activity of a new series of 3'-substituted thymidine analogues.

Three of the synthesized compounds exhibited *in vitro* anti-HIV activity. Two of these compounds, namely 3'-(1,2,4-triazol-1-yl)carbimidoylamino-3'-deoxythymidine and 3'-deoxy-(3-amino-1-methyl-1,2,4-triazol-5-yl)aminothymidine, are original. The third compound, 3'-*N*-cyano-*O*-isophenylurea-3'-deoxythymidine[7] was already described, but its antiviral activity was not reported. As this compound was obtained as an intermediate in our synthesis scheme, it was also evaluated for its anti-HIV activity.

Chemistry

For the synthesis of all molecules 3'-azido-3'-deoxythymidine (AZT) **1a** served as the starting material. For the synthesis of compounds **2a**, **2b** and **3**, the primary 5' hydroxyl was tosylated, the tosyl group was replaced by an azido group and the obtained 3',5'-diazido-3',5'-dideoxythymidine **1c** was reduced with hydrogen in the presence of palladium on charcoal as catalyst according to the method described by Lin and Prusoff[8] affording **1d**.

Compound **1d** was reacted with 1.5 equivalents of *S,S*-dimethyl-*N*-cyanodithioimidocarbonate in ethanol at room temperature, yielding essentially 1-cyano-3-(3'-amino-3',5'-dideoxythymidin-5'-yl)-2-methylisothiourea **2a** and traces of the double substituted compound **2b**.

Using the method described for formation of *N*-cyanoguanidines[9], a 3',5'-cyclic nucleoside was obtained starting from **2a**. Therefore, in a first step the carbodiimide intermediate was formed by elimination of methyl mercaptan under the action of silver nitrate in a mixture of TEA and DMF (1:1). The newly formed compound **3** is a bicyclic nucleoside having an extra six-membered ring. The *N*-cyanoguanidine is not protonated at physiological *p*H because of the drop in pK_a of the guanidine function due to the presence of the electron-withdrawing cyano group.

The 1-cyano-3-(3'-deoxythymidin-3'-yl)-2-methylisothiourea derivative **5** was obtained by reaction of 3 equivalents of *S,S*-dimethyl-*N*-cyanodithioimidocarbonate with 3'-amino-3'-deoxythymidine **4** as described before[10].Compound **5** was used as the starting material for performing cyclo-addition reactions with different hydrazines.

The cyclisation with methylhydrazine was performed with an excess of hydrazine in ethanol. As two compounds were formed which were difficult to purify, the reaction was repeated using the 5'-monomethoxytritylated analogue of **5** and the reaction was stopped after one hour giving the isomers **8a** and **8b** in 94% yield. NMR analysis proved the compounds to be the topoisomers 3'-deoxy-3'-(3-amino-1-methyl-1,2,4-triazol-5-yl)aminothymidine **8a** as the predominant and 3'-deoxy-3'-(3-amino-2-methyl-1,2,4-triazol-5-yl)aminothymidine **8b** as the minor product. This reaction can be explained by a substitution of thiomethyl by hydrazine and an intramolecular cyclisation followed by aromatisation through rearrangement of the protons.

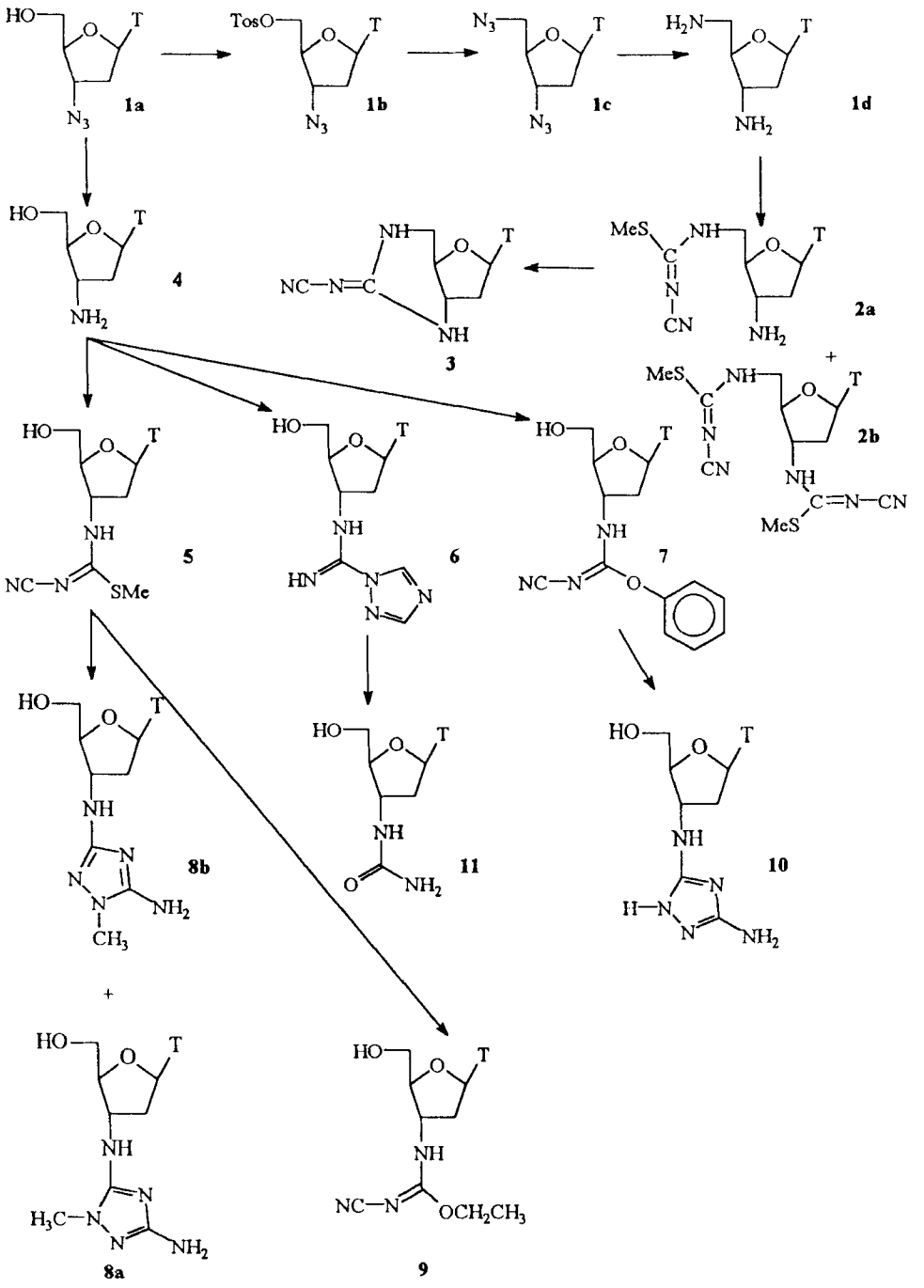
The same reaction was performed with hydrazine and 3'-*N*-cyano-*O*-isophenylurea-3'-deoxythymidine **7** as the reagents and yielded 89% of 3'-deoxy-3'-(3-amino-1,2,4-triazol-5-yl)aminothymidine **10**. The compound precipitated from the reaction mixture. This 3-amino-1,2,4-triazole nucleoside is extremely insoluble in all common solvents and NMR spectroscopy was performed in CF_3COOD.

When compound **5** was dissolved in ethanol and an excess of DBU was added, compound **9** was formed (24%).Compound **6**, 3'-(1,2,4-triazol-1-yl)carbimidoylamino-3'-deoxythymidine was obtained from the reaction of compound **4** with 1,1'-carbimidoyl-bis-(1,2,4-triazol)[11] in DMF (84%). Compound **6** was hydrolysed quantitatively in 80% aqeous acetic acid affording **11** and 1-*H*-1,2,4-triazole.

Biological activity

Compounds **3**, **5**, **6**, **7**, **8a**, **9**, **10** and **11** were evaluated for their inhibitory effect on the cytopathicity of herpes simplex virus type 1 (HSV-1, strain KOS), thymidine kinase deficient (TK^-) HSV-1 (strains B 2006 and VMW 1837), herpes simplex virus type 2 (HSV-2, strain G), vaccinia virus (VV), vesicular stomatitis virus(VSV) in human embryonic skin muscle (ESM) fibroblast cell cultures according to previously published procedures.[12] No activity was observed against these viruses at compound concentrations up to 400 μg/mL.

Compounds **3**, **5**, **6**, **7**, **8a**, **9**, **10** and **11** were also evaluated for their inhibitory effect on the cytopathicity of HIV-1 and HIV-2 in CEM cells. Compound **6** was inhibitory to HIV-1 at a 50% effective concentration (EC_{50}) of 16 μg /mL and its EC_{50} for HIV-2 was >20 μg /mL. This concentration also corresponded to the toxicity threshold of **6** [50% cytotoxic concentration(CC_{50}): 16μg /mL]. The compounds generated by hydrolysis of **6**, 1-H-1,2,4-triazole and compound **11**, were also evaluated. However, no anti-HIV activity



Anti-HIV-1 and HIV-2 activity of the 3'-substituted thymidine analogues in CEM cells

Compound	EC_{50} HIV-1 (III_B) (μg /mL)	EC_{50} HIV-2 (ROD) (μg /mL)	CC_{50} (μg /mL)
3	>100	>100	>100
5	>100	>100	>100
6	16 ± 5.7	>20	16 ± 0.5
7	25 ± 7	35 ± 7	>100
8a	55 ± 7	65 ± 7	>100
10	>100	>100	>100
11	>100	>100	>100
1-H, 1,2,4-triazole	>100	>100	>100

could be observed. Compound **6** might act as an alkylating agent. Compound **7** was inhibitory to HIV-1 and HIV-2 at an EC_{50} of 25 and 35 μg/mL, respectively. Compound **8a** showed inhibition of HIV-1 and HIV-2 at 55 ± 7 and 65 ± 7 μg/mL, respectively. Neither compound **7** nor compound **8a** proved cytotoxic at 100 μg/mL (see table).

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