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Synthesis of nucleoside analogs and new Tat protein inhibitors*

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Abstract: Two studies, concerning the synthesis of original nucleoside analogs regarded as an application of heterochemistry on thiaazaheterocycle systems from thiaazabutadienes are discussed. The preparation of new *N*- and *C*-nucleosides is presented. In the second part, the discovery of aromatic polycyclic derivatives as inhibitors of Tat protein is exposed. The work presented takes into account the participation of African partners in further synthetic research programs carried out in collaboration with the laboratory of Nantes.

The HIV epidemic still requires considerable efforts to be contained, especially in Africa. Nucleoside analogs of reverse transcriptase (NARTIs) [1] occupied an essential position in the treatment of HIV infection and AIDS [2]. Even with the recent evolution of nonnucleoside analogs (NNRTIs) and the favored protease inhibitors, NARTIs still have a major role to play in combination therapy. There are different NARTIs currently available: Zidovudine (AZT, Retrovir), Didanosine (ddI, Videx), Zalcitabine (ddC, Hivid), Stavudine (d4T, Zerit), Lamivudine (3TC, Epivir), Adefovir (bis-POM PMEA), Abacavir (1592U89), FLT, and Carbovir. However, none of them are without problems of either viral resistance, tolerability, or ease of administration. Actually, the most popular two-drug combination cited as a first-line therapy was Zidovudine plus Lamivudine. Recent study of Stavudine with Didanosine suggest that this combinaition may also find favor in the clinic due to minimal dose required and lower cost.

NNRTIs are a structurally diverse group of compounds that are potent inhibitors at nanomolar concentration. They do not require intracellular metabolism to become active and lack the hematological toxicity associated with the NARTIs. NNRTIs such as Nevirapine (Viramune), TIPO, HEPT, E-BPU, MKC-442 and Delavirdine (Rescriptor, U-90152), Atevirdine (U-87201), α-APA (R89439), TSAO, and Efavirenz (Sustina), are promising partners for polytherapy. Actually, Neviparine, Delavirdine, and Efavirenz are prone to be used in combination with nucleoside analogs due to limiting development of resistance and despite relatively mild side effects such as skin rash, elevated transaminase levels, and gastrointestinal disturbances.

There are a few inhibitors on the market that target the HIV protease enzyme (pol protease), which stimulated the maturation. Protease inhibitors are mostly small peptide analogs: L'Indinavir

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(Crixivan), Ritonavir (Norvir), Saquinavir (Invirase), and Nelfinavir (Viracept). More recently, 141W94 (VX-478), a second-generation nonpeptidic inhibitor, has progressed through development with remarkable speed. Since all protease drugs have been approved under accelerated process, the long-term safety of these agents remains unknown.

The recommended treatment option for those initiating antiretroviral therapy is now recognized as triple combination therapy involving the use of protease inhibitors associated with NARTIs and NNRTs. However, many questions remain concerning their long-term efficacy. It is thought that in the future combinations of four or five drugs may emerge. Nucleotide analogs such as GS 840 (Adenovir dipivoxil), PMPA, and Zintevir seem to be characterized by their ability to inhibit viral replication for long periods. Recently, antisens technology, targeted at messenger RNA (mRNA) of conserved region of HIV genome, is regarded due to the advantage in avoiding the problem of resistance. The progress in the understanding of the viral events has aroused other promising targets to interact with the viral infection. Among the most advance research, we can cite: HIV integrase inhibitors, chemokine receptor inhibition, glycosylation inhibitors, ribozyme activators, and, more recently, immunotherapies. Another alternative concerning the inhibition of Tat or Rev proteins is actually under accelerated investigation.

We present here our investigation on two of the above topics:

- the synthesis of new *N*- and *C*-nucleoside analogs of reverse transcriptase and
- the synthesis of new active triphenylene inhibitors of Tat protein.

Our present purpose is mainly to illustrate the participation of African partners in the development of synthetic programs, in particular with the laboratories of organic synthesis of Abidjan (Ivory Coast) and Dakar (Sénégal).

SYNTHESIS OF NUCLEOSIDE ANALOGS

On the basis of the above considerations, original nucleoside analogs directed upon reverse transcriptase still aroused considerable interest [1]. In the hope of elucidating and/or finding better therapeutic agents, 3'-modified deoxynucleoside analogs seem to be one of the recommended targets [2]. They either act as enzyme inhibitors or as chain terminators of viral DNA-polymerization due to the lack or alteration of 3'-hydroxyl group. Modified *N*- or *C*-nucleosides tethered to original heterocycle at the anomeric sugar moiety, have also retained attention, and they have elicited a new class of heterocyle nucleoside analogs, which mostly behave like nonnucleoside RT substrates [3].

With respect to the previous studies carried out in our laboratory, *N*-thioacylamidine (1-thia-3-aza-1,3-butadienes) and thiocarbamate precursors [4] have been regarded as promising intermediates to produce five- and six-membered thiaazaheterocycles with some structural analogies with natural nucleobases. To illustrate our proposal, the synthesis of ribosides tethered to thiazolinone and thiazinone heterocycles, at 1'- and 3'-positions is presented (Fig. 1). In continuation of this program, the access to new

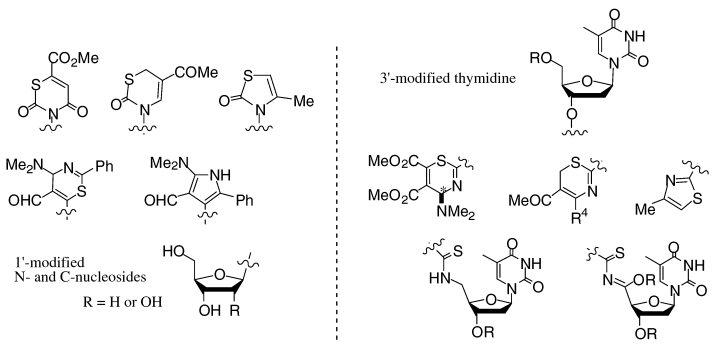
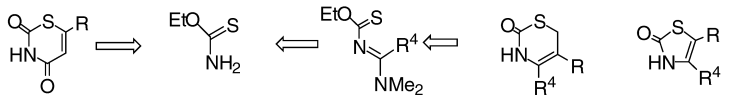


Fig. 1

C-nucleosides, aimed to present biological interest as antiviral or anticancer agents, has also been investigated.

1'-*N*-Nucleoside analogs

Access to the thiazaheterocycles was performed from thiocarbamate precursors or from dimethylamino-1-thia-3-azabut-1,3-dienes **A** resulting from the condensation of orthoamide acetals (Retrosynthesis 1).

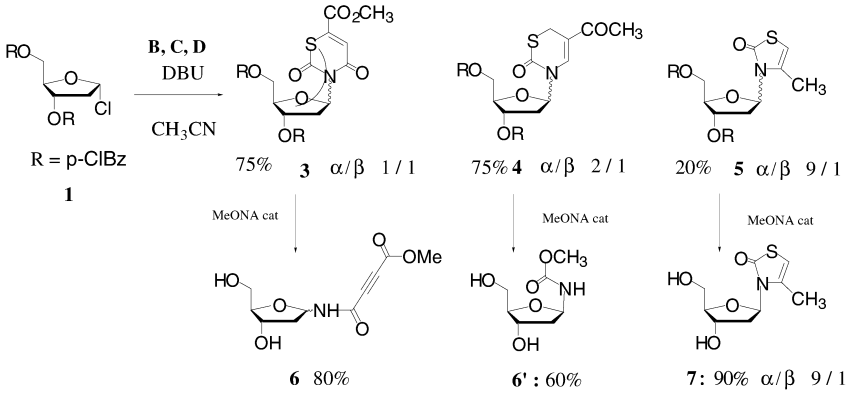


thiazindiones **B** thiocarbamates thiaazabutadiene **A** thiazinones **C** thiazolinone **D**

Retrosynthesis 1

6-Methoxycarbonyl-4*H*-1,3-thiazin-2,4dione **B** (R = CO_2Me) was obtained from ethylthiocarbame by [3 + 3] cycloaddition with dimethyl acetylene dicarboxylate (DMAD), followed by subsequent hydrolysis of the ethoxy group. [4 + 2] Cycloaddition from diene **A** (R^4 = H), with methylvinylketone (MVK) afforded the 5-acethyl-3,6-dihydro-2*H*-1,3-thiazin-2-one **C** (R = COMe, R^4 = H) after hydrolysis. Addition of sulphoxonium ylide on the 4-methyl thiaazaheterodiene **A** (R^4 = Me) furnished the thiazol-2-ine intermediate, following [4 + 1] heterocyclization and subsequent aromatization by a methyl iodide treatment. The formation of the targeted 4-methyl-2*H*-1,3-thiazolin-2-one **D** (R = H, R^4 = Me) requires the treatment of thiazoline precursor with phosphorus tribromide, as the latter remains stable under usual acidic hydrolysis.

Two strategies were designed for the synthesis of 1'-nucleoside analogs through inter- and intramolecular glycosylation process. In the first approach, which involves inter *N*-osidic coupling with thiazaheterocycle nucleobase analogs, 3,5-di-*O*-*p*-chlorobenzoyl-2-deoxy-ribofuranosyl [5] **1** (Scheme 1) and 5-*O*-*ter*butyldiphenylsilyl-2,3-*O*-isopropylidene-ribofuranosyl [6] **2** (Scheme 2), were chosen as glycosyl donors. This latter choice takes into account the stability of the targeted nucleoside analogs during the deprotection steps, which cannot be carried out under usual hydrogenolysis. *N*-osidic coupling of the thiaazaheterocycles discussed above was attempted in deoxyribofuranosyl series, giving a mixture of corresponding anomeric nucleosides **3**, **4**, and **5** in variable proportion (Scheme 1). The treatment of the thiazinones **3** and **4** under basic catalytic conditions, in order to deprotect the *p*-chlorobenzoyl group, induced a rearrangement of the heterocycle ring into the anomeric amidoacetylenic derivatives **6** and carbamate **6'** respectively. However, these derivatives can be regarded as new precursors for the synthesis of original interesting deoxynucleosides. The deprotection step of the thiazolinone compound **5** occured without alteration of the heterocycle, giving the targeted nucleoside analog **7**.



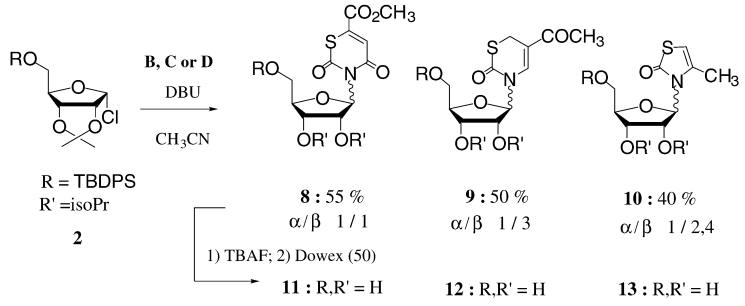
R = p-ClBz **1**

75% **3** α/β 1 / 1 75% **4** α/β 2 / 1 20% **5** α/β 9 / 1

6 80% **6' :** 60% **7:** 90% α/β 9 / 1

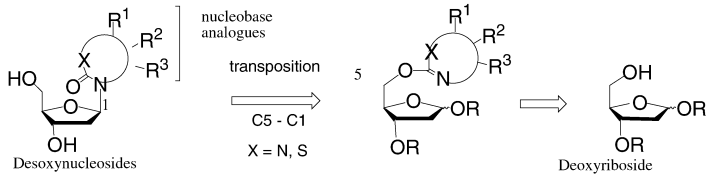
Scheme 1

The use of a similar glycosilation strategy, carried out from the 5-*O*-*ter*butyldiphenylsilyl-2,3-*O*-isopropylidene-ribofuranosyl **2**, afforded the nucleosides **11**, **12,** and **13** *via* the protected intermediates **8**, **9**, and **10**, respectively (Scheme 2).



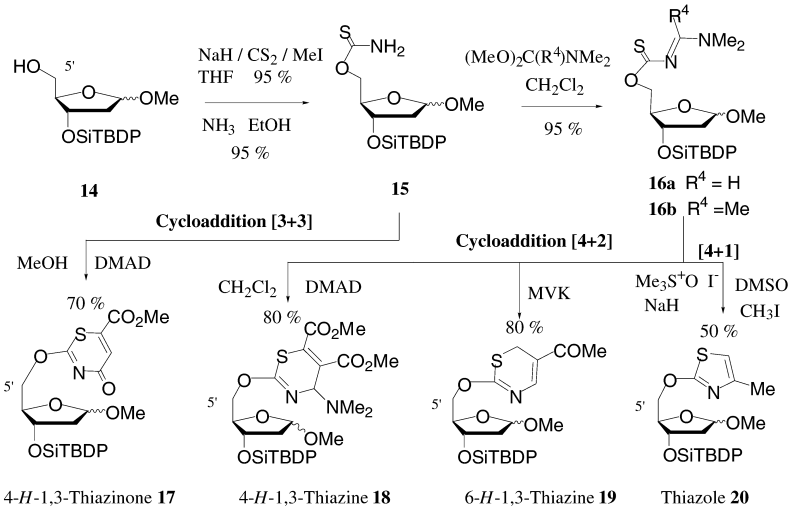
Scheme 2

Complementary studies through an intramolecular transposition process [7] were investigated in order to improve the synthesis of 2'-deoxynucleoside thiazolinone analogs. Following this hypothesis, the primary target appeared to be the elaboration of the thiazole at the 5'-position of a 3'-protected deoxyriboside precursor (Retrosynthesis 2).



Retrosynthesis 2

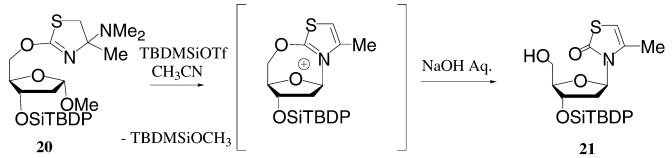
The procedure for the synthesis of *N*-thioacylamidine from ethoxythioamide discussed previously, was efficiently transposed to the primary hydroxyl of deoxyriboside **14** to give the corresponding thiaazaheterodienes **16** (Scheme 3). The formation of the thiaazaheterocycles at this position was performed



Scheme 3

by cycloaddition reactions from the heterodiene precursors **16** to lead to further isodeoxynucleosides **17**, **18**, **19**, and **20**, depending on the reagent partners.

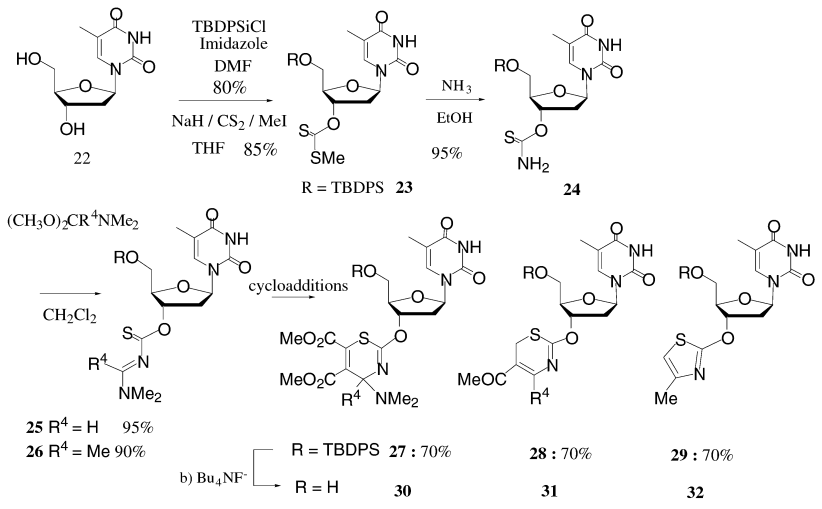
Preliminary results in the stereoselective transposition of heterocycle to the anomeric center of the deoxyriboside were observed with thiazole derivative **20**, which give stereoselectively the corresponding thiazolinone nucleoside analog **21** in moderate 20% yield (Scheme 4). Therefore, this result can be competitively compared to the yield obtained by interosidic pathway leading to an anomeric mixture of deoxynucleosides (<20%).



Scheme 4

3'-Nucleoside analogs

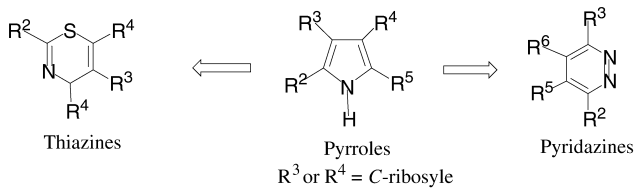
The synthesis of thiaazaheterocycles **27**, **28**, and **29** has been successfully carried out from the thymidine **22**, via *N*-thioacylamidine intermediates **25** or **26** (Scheme 5) [8].



Scheme 5

1'-*C*-Nucleoside analogs

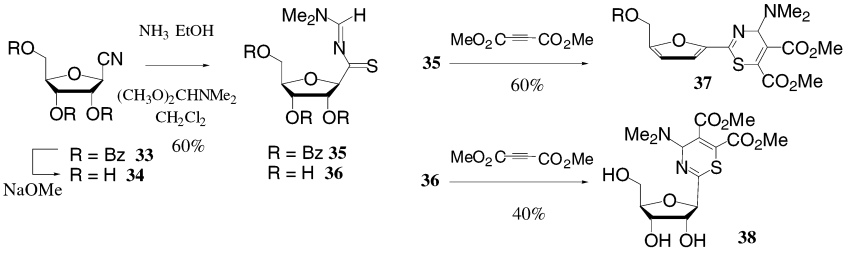
In the last part of our investigation on nucleoside analogs, we investigate the potentiality to acceed to new *C*-nucleosides bearing functionalized pyrroles (Retrosynthesis 3). These compounds are of interest in both antiviral and anticancer therapies.



Retrosynthesis 3

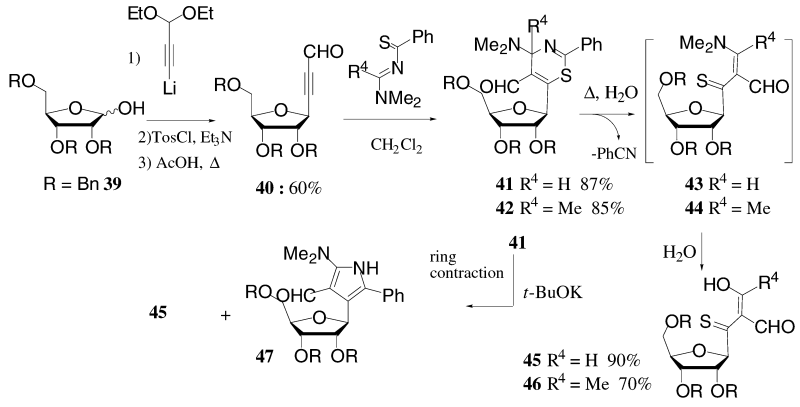
The synthesis makes use of the ability of thiazine or pyridazine derivatives to undergo ring contraction under basic or electrochemical conditions, emphazised by previous studies in laboratory [10,11]. The access to 1-*C*-nucleosidic thiazine heterocycles, resulting from [4 + 2] cycloaddition reaction of *N*-thioacylamidines with acetylenic dienophiles, was attempted starting from 1-*C*-thiaazabutadienes- **34** or from the 1-*C*-acetylenic-ribosyl precursor **39** (Schemes 6 and 7).

The introduction of the thiaazabutadiene systems at C-1 position of ribosyl ring was performed from β-1-cyanoribosyl intermediate **33** (Scheme 6). Therefore, Diels–Alder reactions with acetylenic dienophile attempted from dienic *C*-ribosyl **35** mainly afforded the furan derivative **37**, whereas, the deprotected diene **36** allowed to obtain the target thiazine *C*-nucleoside **38**, but in moderate yield.



Scheme 6

The second alternative from 1-*C*-acetylenic-ribosyl precursor **40** appears more efficient, as the thiazine nucleosides **41** and **42** are obtained in good yield by [4 + 2] cycloaddition with 4-dimethylamino-2-phenyl-1-thia-3-aza-butadiene (Scheme 7). The thiazines **41** and **42** can undergo a cycloreversion process under thermal conditions to give the corresponding thioamide vinylogues **43** and **44**, which are hydrolyzed into the thiadienes **45** and **46**, respectively. These types of derivatives open a new access route for original functionalized five- or six-membered ring azaheterocycles with strong biological potential. Therefore, the treatment of the thiazine **41** under controlled basic conditions at lower temperature afforded the expected pyrrole *C*-nucleoside **47** in 50% yield, by the extrusion of sulfur atom.



Scheme 7

SYNTHESIS OF NEW ACTIVE TAT PROTEIN INHIBITORS

The Erwann LORET [12] team designed a new family of organic molecules (TDS, triphenylene dimethyl succinimide) that should lead to a new therapy against AIDS (Fig. 2). They target specifically Tat, which is a viral protein that transactivates viral genes expression and is essential for HIV-1 repli-

cation. Tat is secreted by HIV-infected cells, and extracellular Tat are able to transactivate distant infected cells, induce immunodeficiency in noninfected T cells, and are directly involved in AIDS pathologies such as Kaposi's sarcoma lesions.

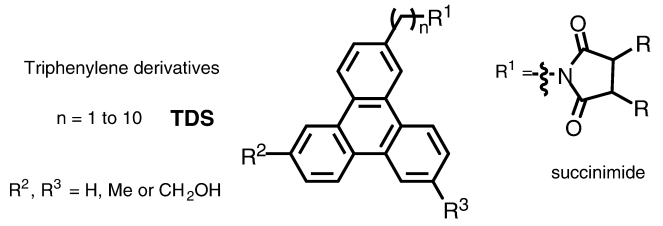


Fig. 2

We already synthesized about 10 derivatives of TDS [13] and verified the efficacy of these TDS molecules; Tat proteins from African, European, and U.S. HIV-1 isolates were chemically synthesized by Erwann LORET. They show with three different tests that these synthetic Tat proteins have the full Tat activity. TDS molecules bind directly to these different Tat variants, and the observed activity is not related to the inhibition of a cellular cofactor. There is no TDS binding with truncated Tat proteins or other proteins different from Tat. Virology tests show that TDS inhibits a Tat-induced activation of the HIV-LTR in transfected HeLa cells at a μM concentration. Moreover, (TDS1, triphenylene dihydroxymethyl succinimide n = 5) (the more interesting derivative) is able to inhibit Tat from European and North American HIV isolates, as well as Tat from highly virulent African HIV isolates. The HIV cytotoxicity on MT4 cells decreases significantly when the cells are preincubated with TDS1.

Our project is to optimize our TDS ligand, and we will prepare more than 100 derivatives to study the structure–activity relationship of Tat in collaboration with Erwann LORET and our virologist colleagues. Erwann LORET have identified six different Tat groups, but other groups may exist or could appear. They will synthesize these new Tat groups if they find new structural groups and go on with our structural studies on HIV-1 Tat variants using 2D-NMR, circular dichroism, and fluorescence. The data collected with these techniques should help to design new structures and keep up our ligands binding Tat, whatever the mutation and the structural changes of this viral protein.

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13. Patent: 236921 D17315 and international extension: WO 99/42,441 (1999).