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RESEARCH ARTICLE

CHEMICAL SYNTHESIS, HYDROLYTIC STABILITY, AND BIOLOGICAL EVALUATION OF 4'-THIONUCLEOSIDES AND THEIR ANALOGS

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ABSTRACT

Synthesis of 4'-thionucleosides and their analogs (2'-Modified 4'-thionucleosides, 6-Azapyrimidine-2'-deoxy-4'-thionucleosides, 4'-Ethynyl-2'-deoxy-4'-thionucleosides, 5-Substituted 2'-Deoxy-2'-fluoro-4'-thionucleosides)has been discussed briefly in this review. Hydrolytic stability of purine and cytosine 2'-deoxy-4'-thionucleosidesand 2'-deoxy-4'-thiouridines and their 5-substituted analogs in aqueous acidic solutions was also elaborated. This study shows that cytosine and purine 2'-deoxy-4'-thionucleosides are more stable toward acidic hydrolysis than their unmodified counterparts. The reactivity ration ranges from 40 to 70 with purine thionucleosides and about 7 with cytosine thionucleosides. No other nucleosidic products or intermediates have been accumulating during the hydrolysis and the above mentioned thionucleosides. The N-glycosidic linkages of the 2'-deoxy-4'-thiouridines and their 5-substituted analogs are also more than one order of magnitude more stable toward acidic hydrolysis than are those of their native counterparts. A series of 4'thionucleosides has been evaluated for antitumor and antiviral activities.

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INTRODUCTION

Synthesis of promising molecules that enable the inhibition of viral enzymes has been optimized due the intensive medicinal chemistry work. This approach enabled chemists to develop selective molecules to treat viral infections (De Clercq, 2007; Ferir *et al.,* 2008; Lagoja and De Clercq, 2008). Those agents show excellent activity against target viruses' enzymes but cannot cover a wide range of enzymes. Therefore, it is advisable to evaluate the activities of promising molecules against an array of viruses to determine their spectra of activity which is not an easy task. Among those promising molecules are the nucleosides analogs. Those nucleosides have their oxygen atoms in the sugar moiety replaced by other atoms.An interesting family of those nucleoside analogs is the sugar and base modified nucleosides. They have recently received considerable attention and increasing interest especially thionucleosides in which one of the oxygen atoms has been replaced with sulfur. This thio-substitution may occur either in the sugar or in the base moiety. While base-modified thionucleosides (**1-3**,**Fig 1**) (Coleman, 1991, 1994;Webb and Matteucci, 1986)are usually used to construct oligonucleotides that may cross-link with their target nucleic acids or proteins. (Xu *et al.,* 1992; Meyer and Hanna, 1996; Saintome *et al.,* 1996).

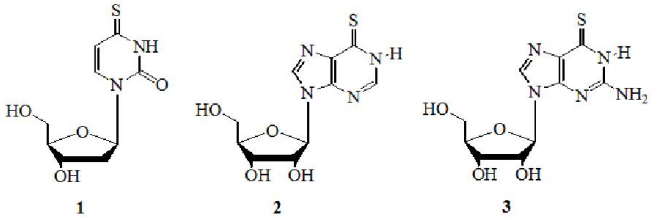

Figure 1. Examples of base-modified thionucleosides

Sugar-modified nucleosides (**4-6, Fig 2**) (Dyson *et al.,* 1991a; Secrist III *et al.,* 1991; Ichikawa *et al.,* 1999), in turn, have applications both as monomeric antiviral agents and as constituents of oligonucleotides (Inoue *et al.,* 2006; Matsugami *et al.,* 2008). For instance, the 4'-thio analogs (Basnak *et al.,* 1996; Hancox and Walker, 1996) of 2'-deoxynucleosides have been investigated as potential antiviral agents (Secrist *et al.,* 1991).It has been shown (Machida *et al.,* 1998)that 4'-deoxy-4'-thioguanosine and corresponding 2, 6-diaminopurine nucleoside exhibit marked anti-Human cytomegalovirus anti-proliferative activities, while others (Mirua *et al.,* 1996) and (Yoshimura *et al.,* 1997) have found that the anti-tumor effects of 2'-deoxynucleoside analogs are enhanced by the 4'-thiosubstitution.

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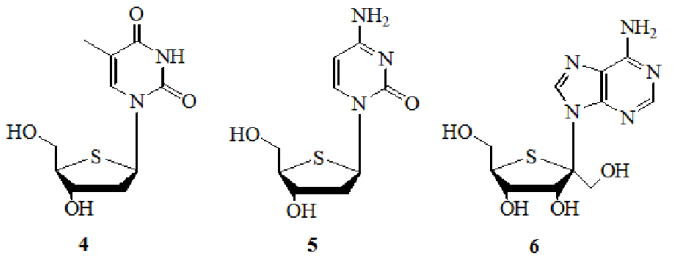


Figure 2. Examples of sugar-modified thionucleosides

For example, the exceptional reactivity of 3'-Azido-3'-deoxy-thymidine **8**(AZT) (Jung and Gardiner, 1991; Mitsuya *et al.,* 1985)as potential drug against infection by human immunodeficiency virus type 1 (HIV-1) has encouraged medicinal chemists to synthesize the thio analog**7**(**Fig 3**) with potentially higher activity and lowertoxicity (Jung and Gardiner, 1991; Mitsuya and Broder, 1986; De Clercq, 1994).Other 2'-deoxy-4'-thionucleosides such as 5-(2-bromovinyl)-2'-deoxy-4'-thiouridine (Dyson *et al.,* 1991a,c) **9** and 5-(2-thienyl)-6-aza-2'deoxy-4'-thiouridine (Basnak *et al.,* 1998)**10** (**Fig 3**) have shown a promise as anti-herpetic agents.

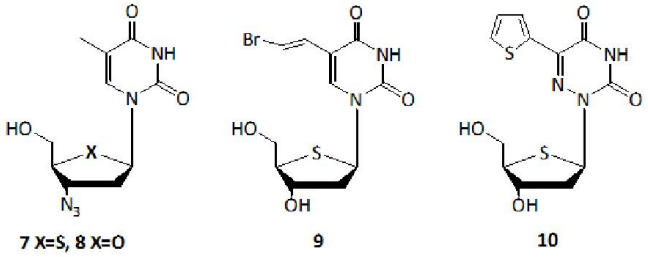


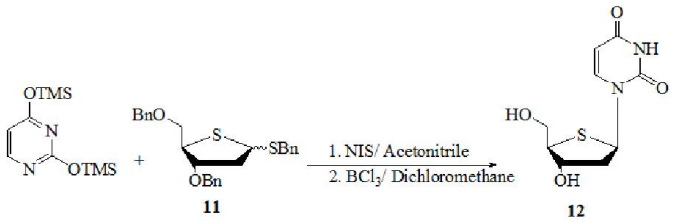
Figure 3. Examples of 4'- thionucleosides analogs

Due to the above mentioned biological applications of the 4'-thionucleosides, it appears important to also compare their intrinsic chemical properties to those of their native counter-parts. Although, there are published systematic reviews (Yokoyama, 2000; Gunaga *et al.,* 2004) for the thionucleosides, our current review covers not only their synthesis and biological evaluation but also gives a brief description of their hydrolytic stability (Elzagheid *et al.,* 1999; Otter *et al.,* 1998)which may to some extent influence the applicability of these compounds.

Chemical Synthesis

2'-Deoxy-4'-thionucleosides

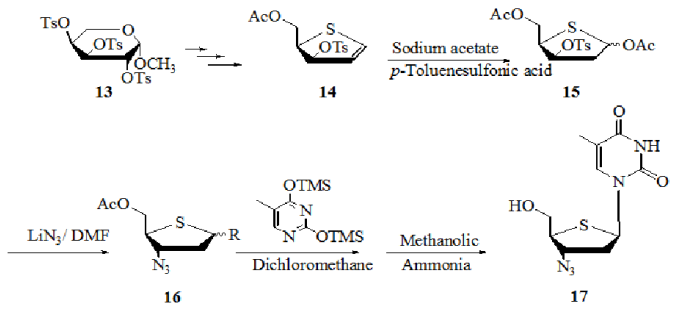
Synthesis of 2'-deoxy-4'-thionucleosides was reported earlierin 1975 (Bobek *et al.,* 1975).It was a multistep synthesis and based on coupling of the 4'-thiosugar with 5-fluorouracil. More successful syntheses were reported late (Dyson *et al.,* 1991a; Secrist III *et al.,* 1991; Bobek *et al.,* 1975) and those were based on coupling of a suitably protected 4'-thiosugar with a bistrimethylsilylated pyrimidine base.Despite difficulties in separation of the resulting anomeric mixture of products, the latter approach has remained widely used. A 7-step synthesis of the 2'-deoxy-4'-thionucleosideshas been described with 11 % overall yield (Dyson *et al.,* 1991b).This method has subsequently been optimized and used in multi-kilogram scale with an overall yield 50% without any chromatographic separation (Basnak *et al.,* 1996).Exploiting these possibilities, 2'-deoxy-4'-thiouridine **12** can be prepared by the condensation of the appropriate thio-sugar**11**(Dyson *et al.,* 1991c)with 2, 4-bis (trimethylsilyloxy) pyrimidine base promoted by *N*-iodosuccinimide(NIS) followed by one-step removal of all protecting groups by treatment with boron trichloride (BCl_3) (**Scheme 1**) (Otter *et al.,* 1998).



Scheme 1. Synthesis of 2'-deoxy-4'-thionucleosides

2'-Modified 4'-thionucleosides

3'-Deoxy-3'-azido-4'-thiothymidine (ThioAZT)**19** was synthesized from D-arabinose derivative **13** via the new thio-furanoid glycal **14** (Al-Masoudi *et al.,* 2003). Addition of sodiumacetate and *p*-toluene sulfonic acid to the thio-furanoid glycal**14**furnished the 2-deoxy-4-thiofuranose diacetate **15** as ananomeric mixture. Both elimination and substitution occurred when **15** was heated with lithiumazidein DMF to give the olefinic productas well as the desired azide**16**. Condensation of **16**with the silylated thymine, by applying the modified Vorbruggen method, afforded, after purification by chromatography, the acetylated nucleosideas an amorphous solid. Deacetylationwith methanolic ammonia gave, after chromatographic purification and precipitation, the thio AZT **17**(**Scheme 2**).



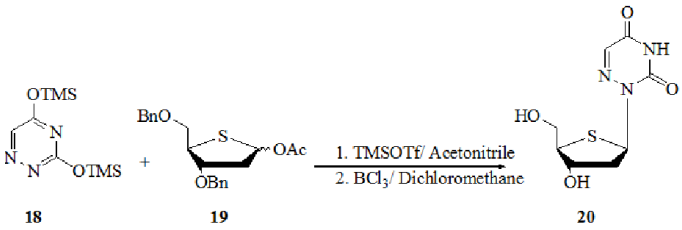
Scheme 2. Synthesis of 2'-modified 4'- thionucleosides

6-Azapyrimidine-2'-deoxy-4'-thionucleosides

The 4′-thionucleoside **20** was prepared by the reaction of the thiosuagr **19**(Dyson *et al.,* 1991b) with the bis-silylated azauracil base **18** in the presence of the Lewis acid trimethylsilyl triflate(TMSOTf). This Vorbrüggen methodology resulted in the best yields and anomeric ratio (90%, α: β 1:1) (Inguaggiato *et al.,* 1999). Thionucleosides **20** was obtained after deprotection with boron trichloride and separation of the α- and β-anomers (**Scheme 3**).

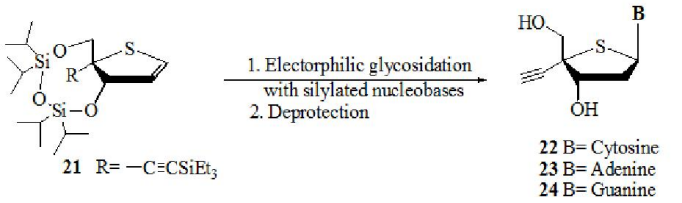
4'-Ethynyl-2'-deoxy-4'-thionucleosides

The synthesis of 4'-ethynyl-2'-deoxy-4'-thioribonucleosides **22, 23**, and **24** (Haraguchi, *et al.,* 2011)has been achieved by using an electrophilic glycosidation.



Scheme 3. 6-Azapyrimidine-2'-deoxy-4'-Thionucleosides

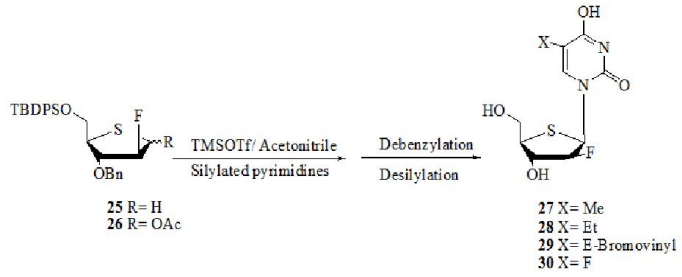
Here the 4-ethynyl-4-thiofuranoid glycal **21** served as a glycosyl donor. Glycosidation betweenthe thiosuagr **21** and the silylated nucleobases (N^4-acetylcytosine, N^6-benzoyladenine, and N^2-acetyl-O^6diphenylcarbamoylguanine)was carried out in the presence of *N*-iodosuccinimide(NIS) to form the desiredβ-anomers**22, 23**, and **24** (**Scheme 4**).



Scheme 4. 4'-Ethynyl-2'-deoxy-4'-Tionucleosides

5-Substituted 2'-Deoxy-2'-fluoro-4'-thionucleosides

Coupling ofselected pyrimidine bases with the 2-fluoro-4-thio-sugar **26** has led to the formation of 2'-fluoro-4'-thionucleosides **27-30** (Yoshimura *et al.,* 2000). Subsequent debenzylation, silica gel purification, and desilylation using ammonium fluoride gave the desired products in a very good yield (**Scheme 5**).

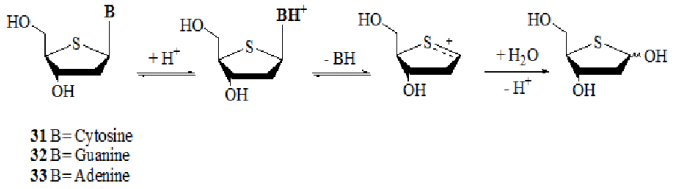


Scheme 5. 2'-Deoxy-2'-fluoro-4'-Thionucleosides

Hydrolytic Reactions

Hydrolysis of purine and cytosine 2'-deoxy-4'-thionucleosides

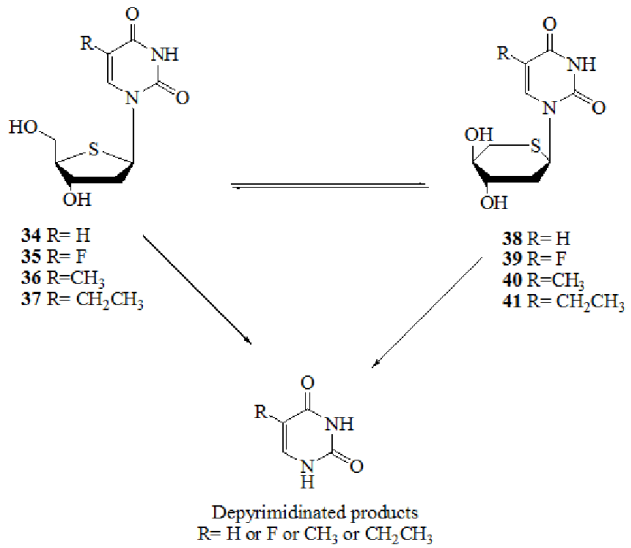
Hydrolysis of thionucleosides 31-33 in aqueous acid was followed by HPLC by analyzing the composition of the aliquots withdrawn at suitable intervals (Elzagheid *et al.,* 1999). The decomposition of the purine thionucleosides32-33 was accompanied by accumulation of a single chromophoric product, the free nucleoside base that was identified by spiking with an authentic sample. With the cytosine derivative, in contrast, deamination to 2'-deoxy-4'-thiouridine competed with the cleavage of *N*-glycosidic bond (release of cytosine). The deamination represents 15-20% of the total hydrolysis both in hydrochloric acid solutions (0.1M and 1.0M) and in formic and acetic acid buffers (pH 3 to 5). Both the hydrolysis and deamination reactions of **31-33** are acid-catalyzed at pH < 4 and pH-independent at pH > 4. This study shows that cytosine and purine 2'-deoxy-4'-thionucleosides are more stable toward acidic hydrolysis than their unmodified counterparts. The reactivity ration ranges from 40 to 70 with purine thionucleosides and about 7 with cytosine thionucleosides. It has been also observed that no other nucleosidic products or intermediates accumulate during the hydrolysis and degradation of these thionucleosides (**Scheme 6**).



Scheme 6. Hydrolysis of purine and cytosine 2'-deoxy-4'-thionucleosides

Hydrolysis of 2'-deoxy-4'-thiouridines and their 5-substituted analogs

Hydrolysis of the 2'-deoxy-4'-thiouridine and their 5-substiuted analogs **34-37** (Otter *et al.,* 1998)in hydrochloric acid solution (1 M), followed by RP-HPLC, releasedthe nucleobases and accompaniedwith the accumulation of the β-L-thiopyranoside isomers **38-41**. Isomerization reaction was reversible (**Scheme 7**).The *N*-glycosidic linkages of the 2'-deoxy-4'-thiouridines and their 5-substituted analogs are found to be more stable by one order of magnitude toward acidic hydrolysis than are those of their native counterparts.



Scheme 7.Hydrolysis of 2'-deoxy-4'- thionucleosidesand their 5 substituted analogs

Biological Evaluation

Antitumor activity

Activity evaluation of a novel 2'-deoxy-2'-fluoro-4′-thiocytidine **42** (4′-thioFAC) (**Fig 4**) against tumor (Miura

et al., 1998) has shown inhibition of the in vitro growth of various human cancer cell lines, in particular the growth of gastric and colorectal carcinomas cell lines. In contrast, the 1-(2-deoxy-2-fluoro-b-d-arabinofuranosyl) cytosine **43** (FAC) has shown little or no activity against the same solid cancer cell lines. It has also shown to have a remarkable antitumor effect against human tumors implanted into nude mice even when was administered orally. 4′-Thionucleoside **42** was less susceptible to deamination by cytidine deaminase than FAC and 2′-deoxy-2′, 2′-difluorocytidine (gemcitabine).This considered being a promising candidate for cancer chemotherapy. On the other hand, the 5-fluoro derivative **44** (5-F-4'-thioFAC) has shown a potent antitumor activity against both leukemia and solid tumors (Yoshimura *et al.,* 2000).

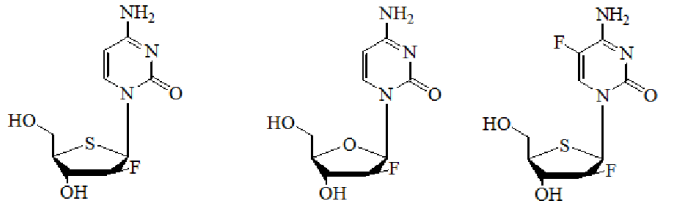


Figure 4. Chemical structures of 4'-thioFAC, and 5-F-4' thioFAC

Antiviral activity

It has also been shown that the 4'-ethynyl-2'-deoxy-4'-thioribonucleosides **22, 23,** and **24** (Haraguchi, *et al.,* 2011)(shown in **Scheme 4**), exhibit antiviral activity against herpes simplex virus and vaccinia virus without measurable cytotoxicity tothehostcells upto100μM.5-(2-thienyl)-6-aza-2'-deoxy-4'thiouridine **10** (Basnak *et al.,* 1998 and Inguaggiato *et al.,* 1999) has also shown to be an effective anti-herpesvirus agent (IC_{50} against HSV-1, 0.4 μM). On the other hand, 6-Aza-4'thiothymidine was moderately active against vaccinia virus, herpes simplex virus strains HSV-1(strain KOS) and HSV-2 (strain G) (Jasamai *et al*., 2008). A number of 5-substituted 4'-thionucleosides were also evaluated for their activity against vaccinia orthopoxvirus and cowpox viruses (Kern *et al.,* 2009 and Prichard *et al.,* 2009).The 5-iodo analog, 5-iodo-2'-deoxy-4'thiouridine (4'-thioIDU) was able to inhibit viral DNA synthesis atless than 1μM.Recombinant vaccinia virus that lacks a thymidine kinase was partially inhibited by this thionucleosides.

Conclusion

Nucleosides' sugar thiosubstitution, one of the oxygen atoms of the sugar moietyis replaced with sulfur, has remarkably enhanced both stability of the *N*-glycosidic linkage and biological activity of the 4'-thionucleosides.

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