Keto and Exomethylene Pyranonucleosides as Antitumor Agents

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Abstract: Nucleosides and their analogues take an important place in medicinal chemistry as the structural basis for the development of therapeutic agents. Recently, there has been a renewed interest in the synthesis of keto and exomethylene pyranonucleosides, due to their high cytotoxicity *in vitro* and powerful inhibitory action *in vivo*. Their mode of action probably involves their ability to act as acceptors in a Michael-addition mechanism, while it was revealed that 5-fluorouracil nucleosides represent novel prodrugs of 5-fluorouracil targeting thymidylate synthase. The present mini review summarizes the molecular design, chemical synthesis and biological activity of keto- and exomethylene pyranonucleoside analogues.

Keywords: Pyranonucleosides, ketonucleosides, exomethylene nucleosides, oxidation, Wittig reaction, cytotoxicity, antitumor activity.

1. INTRODUCTION

In recent years, numerous investigations have been directed towards the development of drugs for the treatment of cancer and viral infections. The vast majority of approved drugs are modified nucleosides, which play an important role in chemotherapy of cancer and viral diseases [1-4]. The fact that nucleosides and nucleoside analogues have achieved considerable attention for their antitumor properties, justifies the importance of pursuing research on these bioactive agents.

Recently, there has been a renewed interest in the synthesis of nucleosides containing pyranosyl rings, in order to develop potential antitumor [5, 6], antiviral [7-11], antioxidant [12] and antimicrobial [13] agents. Their biological activity is often modulated, by the systematic variation of the sugar ring and/or the nucleic base. Thus, introduction of reactive functionalities such as keto or/and exomethylene groups in the carbohydrate backbone of pyranose nucleosides has led to novel scaffolds endowed with interesting biological activities. These uncommon pyranonucleosides are key intermediates in synthetic and biosynthetic processes and a number of them exhibit interesting antitumor activity [14-16]. They have been shown to be highly cytotoxic *in vitro* [17, 18, 5] and potent inhibitors of L1210 murine leukemia *in vivo* [6, 19]. These agents are known to inhibit DNA, RNA, and protein synthesis [20] and to interact with sulfhydryl groups of cellular proteins and enzymes [21]. Furthermore, their mode of action probably involves their ability to act as acceptors in a Michael-addition fashion mechanism, while it was revealed that 5-FU nucleosides are likely to represent novel prodrugs of 5-FU, targeting thymidylate synthase [22, 23].

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For all these reasons, keto and exomethylene pyranonucleosides have been the subject of intense synthetic activity. However, to the best of our knowledge, none of the formerly published reviews have systematically addressed the synthesis of keto and exomethylene pyranonucleosides, although many aspects of the chemistry of these nucleoside analogues have been reported. The present mini review summarizes the molecular design, chemical synthesis and biological activity of keto- and exomethylene pyranonucleoside analogues as antitumor agents.

2. KETO-PYRANONUCLEOSIDES

2.1. 2′- and 4′-Keto-Pyranonucleosides

Over the past years, a large number of keto-pyranonucleoside analogues have been synthesized, giving rise to a new interesting chemistry. In particular, 2′- and 4′-ketopyranosyl-purines and -pyrimidines have proved to be versatile synthetic intermediates and provide an advantageous and often unique route to nucleosides of biological interest. In 1970's, 2′-keto-pyranonucleosides **2a** and **2b** were first synthesized starting from the already available purine nucleosides **1a** and **1b** (Fig. **1**) [24, 25]. Pfitzner-Moffatt oxidation of the 2′-hydroxyl group of compounds **1**, using DMSO-DCC [26], gave the 2′-keto intermediates, which were further subjected to acid-mediated removal of isopropylidene protecting group to deliver the desired (2′-keto-pyranosyl)theophylline **2a** and (2′-keto-pyranosyl)chloropurine **2b**.

The first reported biological activity in the ketonucleoside field concerned 7-(6-deoxy-*β*-L-lyxo-hexopyranosyl-2-ulose)theophylline (**2a**), which was found to inhibit the growth of KB human epidermoid carcinoma cells *in vitro* [17], whereas the parent nucleoside **1a** was inactive under the same conditions. It was also shown that chloropurine ketonucleoside **2b** was more active than the theophylline ketonucleoside **2a** [18].

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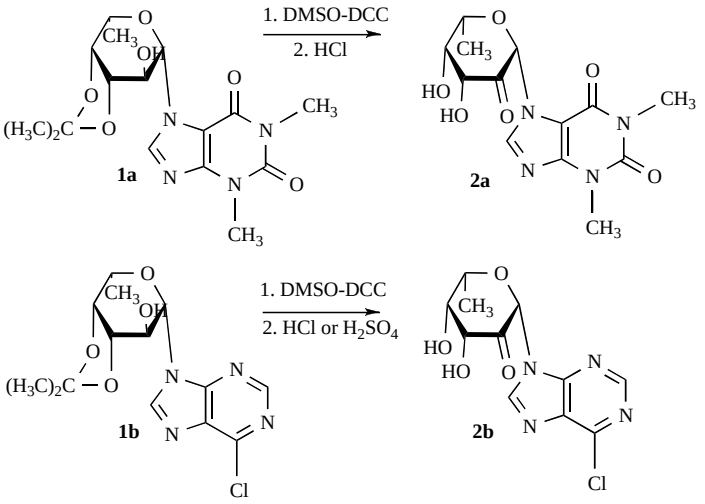


Fig. (1).

Access to 4′-ketonucleosides of theophylline **4a**, chloropurine **4b** and thymine **4c** was reported by Herscovici *et al*. (Fig. **2**) [27-29]. Their preparation utilized a similar strategy, namely, oxidation of the corresponding protected rhamno-pyranosylpurines and pyrimidines **3a-c** with the DMSO-DCC [26] reagent to afford the desired 4′-keto-lyxopyranonucleosides.

The inhibitory activity against KB cancerous cells of the 4′-ketonucleosides **4a-c** [18], confirmed the biological importance of the presence of a keto group in the sugar moiety, contrary to the parent nucleosides, which appeared to be inactive. The first *in vivo* study on the antitumor activity of ketonucleosides appeared in 1977 [19] and it became clear that the 1-(lyxo-hexopyranosyl-4-

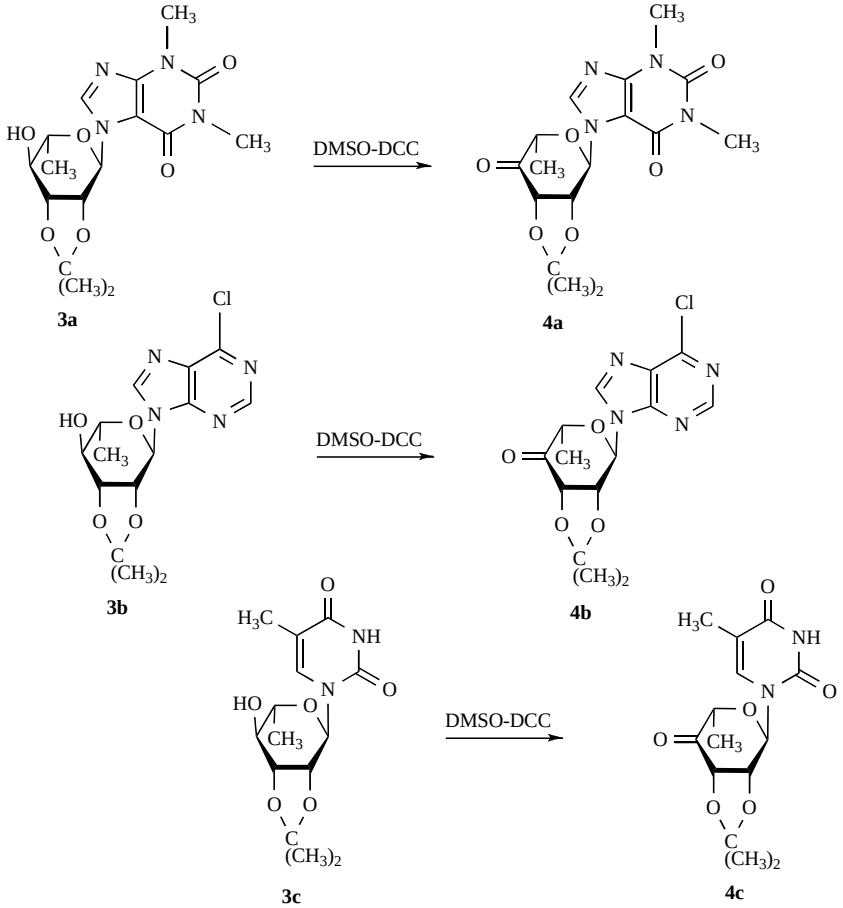


Fig. (2).

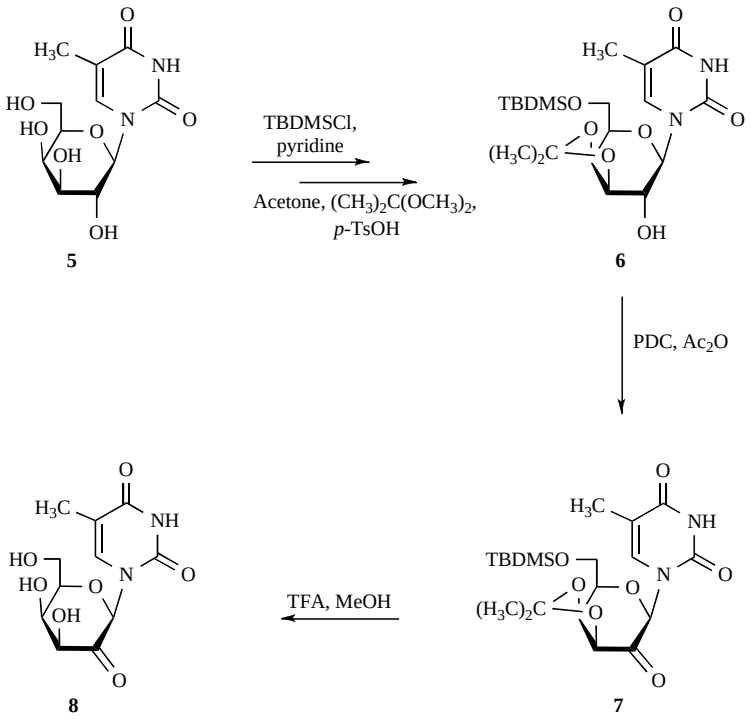


Fig. (3).

ulose)thymine **4c** was more active against L1210 leukemia in mice than the parent nucleoside **3c**, which was inactive.

Several years later, starting from the 1-(*β*-D-galacto-pyranosyl)thymine (**5**), the 2′-ketonucleoside, 1-(*β*-D-lyxo-hexopyranosyl-2-ulose)thymine (**8**) (Fig. **3**) was prepared [30]. Subjecting **5** to the sequence of selective protection of the primary hydroxyl group with a TBDMS group, specific acetalation, oxidation of **6** with PDC/Ac_2O gave **7** and deprotection of **7** afforded the 2′-ketonucleoside of thymine **8**. Compound **8** has been shown to be a potent anti-tumor agent, as it exhibited high cytotoxic and anti-proliferative action on tumor cells.

2.2. Fluorinated 2′- and 4′-Keto-Pyranonucleosides

The unique role of hydrogen or hydroxyl in nucleoside acids has prompted the investigation of biological properties of nucleosides having substituents, other than hydrogen or

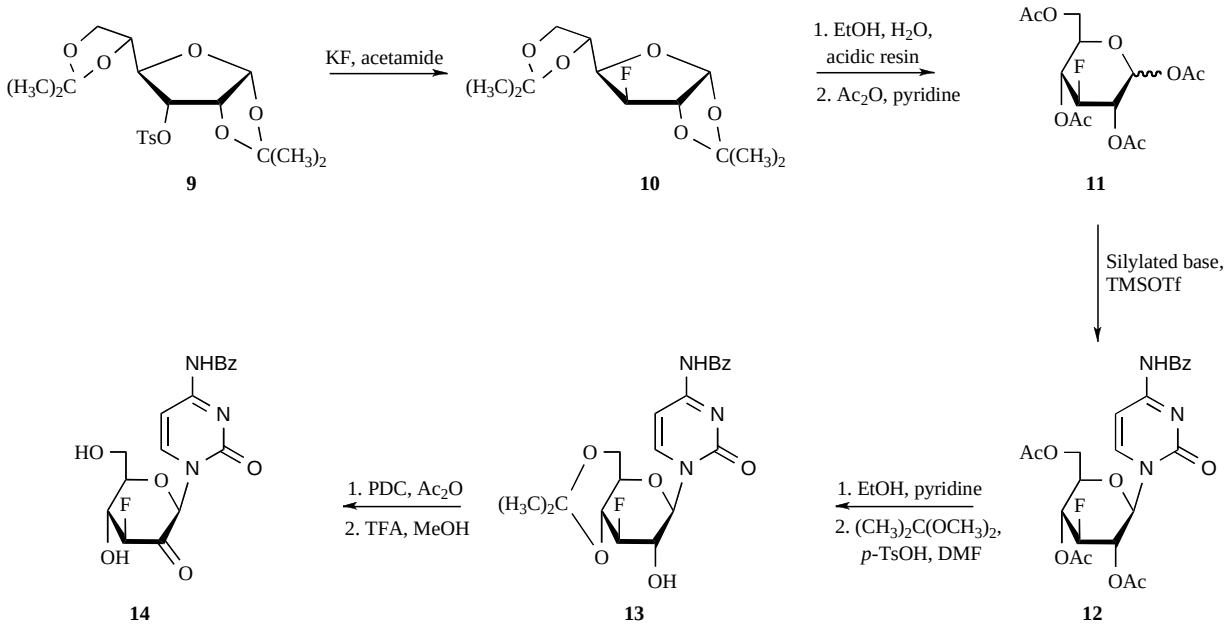


Fig. (4).

hydroxyl. Accordingly, it was of interest to study the biological properties of nucleosides containing fluorine atom(s) or fluorine-containing groups in the sugar or in the base moiety [31, 32]. Up to date various fluorinated nucleosides have been the subject of intense synthetic activity, due to the introduction of fluorine atom resulting in a substantial enhancement of the bioactivity and stability of the corresponding compounds. These outstanding results motivated organic chemists and pharmaceutical workers to synthesize various types of fluorinated ketonucleosides.

Very recently, an efficient synthesis of 3′-fluoro-2′-keto-glucopyranosyl nucleoside **14** (Fig. **4**) was described by Manta *et al*. [33]. In their synthesis, the 3′-fluorine atom was introduced *via* treatment of the tosylate **9** with KF/acetamide.

The resulting fluoro compound **10** was subjected to hydrolysis and acetylation, to afford the glucosyl donor **11** [34, 35], which was initially converted to the *β*-nucleoside of N^4-benzoyl cytosine **12** *via* Vorbruggen methodology [36] followed by selective deprotection and isopropylidenation to afford the intermediate alcohol **13**. Oxidation of compound **13** with PDC and Ac_2O, then deisopropylidenation with TFA, concluded the synthesis of the fluorinated 2′-ketonucleoside **14**. Biological assays demonstrated that ketonucleoside **14** is a promising antiadenocarcinoma and antirotavirus agent.

In view of the interesting bioactivities of ketonucleosides against cancer cells and several viruses, in 2009, the synthesis of 3′-fluorinated dideoxy-2′-keto-glucopyranosyl derivatives of N^4-benzoyl cytosine **18** and **19**, was recently reported (Fig. **5**) [11]. The synthesis of these compounds was accomplished *via* acetylation of the free hydroxyl group of the key-intermediate *β*-nucleoside **13**, deisopropylidenation with TFA and selective protection with a trityl group to give alcohol **15**. After phenoxythiocarbonylation of **15** under a commonly used condition, PhOC(S)Cl and an excess amount of DMAP [37-39], the resulting 4′-*O*-phenoxythiocarbonyl derivative **16** was subjected to deoxygenation with Bu_3SnH [39, 40] and deacetylation to afford the 4′-deoxynucleoside **17**. Oxidation of **17** with PDC and Ac_2O and subsequent removal of the trityl group from **18** with HCOOH [41] provided the target ketonucleoside **19**. The fluorinated 3′,4′-dideoxy-2′-ketonucleosides of N^4-benzoyl cytosine **18** and **19** showed antitumor activity against gastric cancer derived AGS cells and proved to be even more cytotoxic to all cancer cell lines tested than 5-FU.

Besides the aforementioned fluorinated dideoxy 2′-keto-pyranonucleosides, the synthesis of fluorinated dideoxy 4′-keto-pyranonucleosides **23** and **24** (Fig. **6**) was also carried out, starting from the suitably protected isopropylidene intermediate **13** [11]. Their synthetic strategy involved phenoxythiocarbonylation, followed by routine deoxygenation to afford the dideoxy nucleoside **20**. Treatment with aqueous TFA led to diol **21**, which upon selective protection of the primary 6′-hydroxyl group with a trityl group gave the partially protected derivative **22**.

After oxidation of the fluoro tritylated dideoxy precursor **22** with PDC/Ac_2O and detritylation with CH_2Cl_2/HCOOH [41] of the resulting 4′-ketonucleoside **23**, the target fluorinated dideoxy 4′-ketonucleoside **24** was obtained. Biological studies have confirmed that, although **23** and **24**

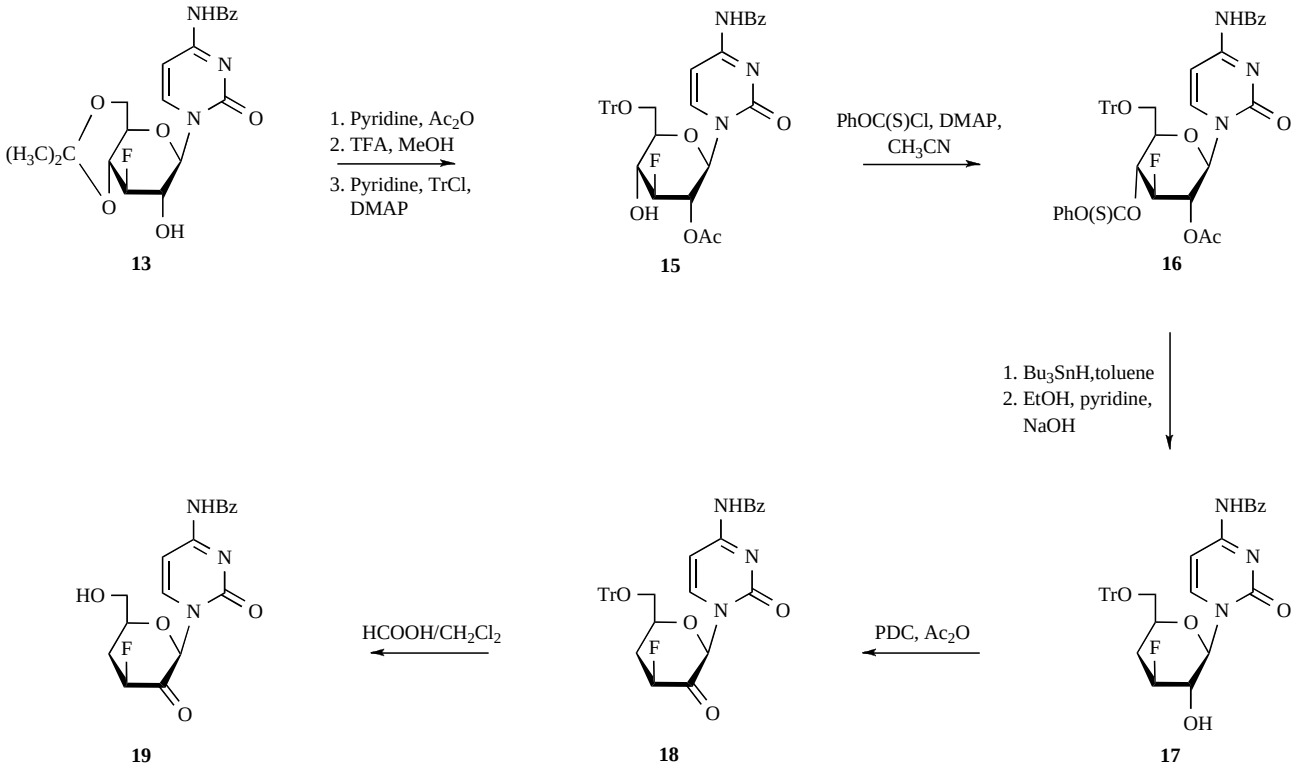


Fig. (5).

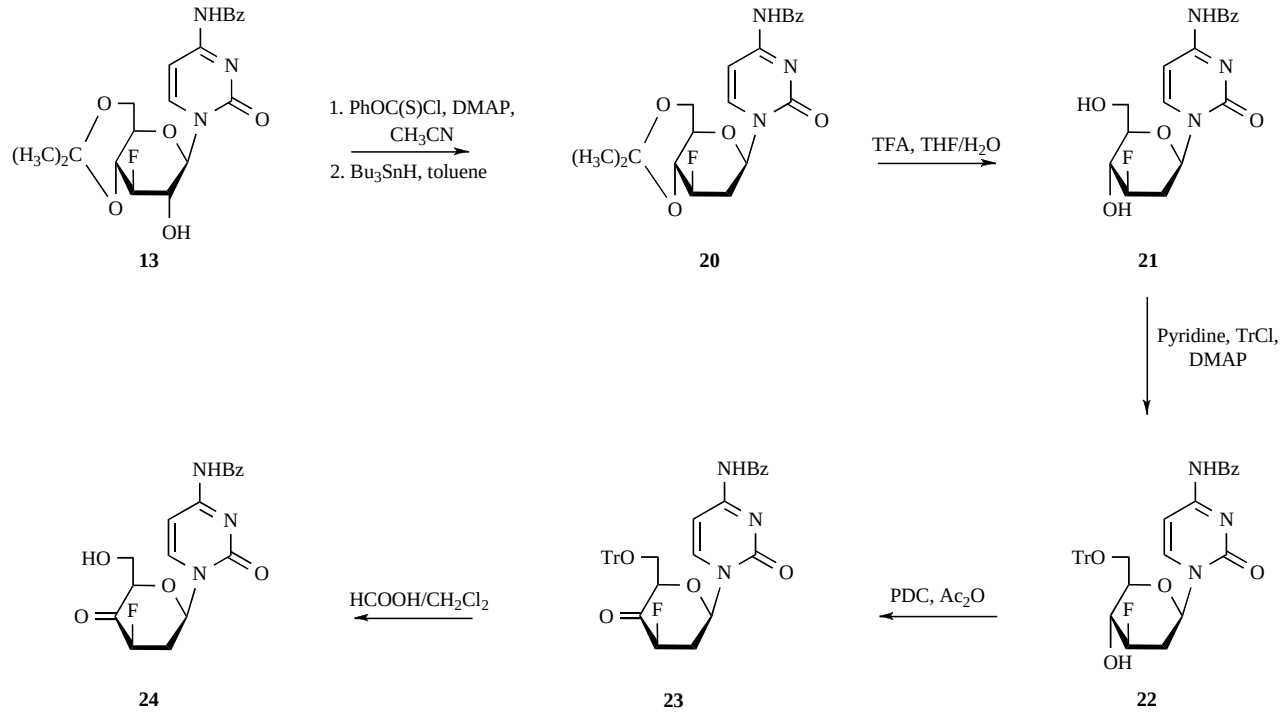


Fig. (6).

showed high antitumor activity, ketonucleoside **23**, bearing the 6′-*O*-protecting group, proved to be more cytotoxic, against all cancer cell lines tested, than 5-FU, implying that the presence of a primary hydroxyl might not be critical for biological activity. Flow cytometric BrdU-cell cycle analysis also revealed that the mechanism of antitumor activity of the synthesized compounds may be related to a delay in the G1/S and programmed cell death initiation. Moreover, further results indicated that compound **23** may have strong implication in clinical settings, especially for treatment of those tumors poorly responsive to the existing chemotherapy agents.

In retrospect, keto- and fluorinated ketonucleosides constitute new series of cytostatic drugs. The high antitumor activity of ketonucleosides and the cytotoxic effects of fluoro-ketonucleosides reveal the special influence of the keto group and fluorine atom on the biological properties of nucleosides. It was shown that 4′-ketonucleosides were more active than 2′-ketonucleosides, while 6′-*O*-protected ketonucleosides exhibited the most promising antitumor activity. Although the mechanism of action of these compounds is still unclear, the availability of ketonucleosides gave rise to interesting biological studies opening new horizons towards extensive investigations on the mode of action.

3. KETO-UNSATURATED PYRANONUCLEOSIDES

3.1. 2′- and 4′-Keto-Unsaturated Pyranonucleosides

During the last four decades it has been shown that unsaturated ketonucleosides have exceptional antineoplastic and immunosuppressive properties [14-16]. It appears that these nucleosides not only can be considered as bioisosters of natural nucleosides and nucleotides, but can play a major role in different domains like therapy, diagnosis, and biotechnology.

In order to investigate the possibility of increasing the biological activities of nucleosides containing keto group in the sugar moiety, Antonakis *et al.* designed and synthesized the first reported [42, 43] unsaturated ketonucleoside **25a** (Fig. **7**).

The synthesis of theophylline unsaturated 2′-ketonucleoside analogue **25a** was accomplished by acetylation of the known (2′-keto-pyranosyl)theophylline **2a**, followed by elimination of the 4′-acetoxyl group. In a similar manner, acetylation of (2′-keto-pyranosyl)chloropurine **2b** afforded 9-(*glycero*-hex-3-enopyranosyl-2-ulose)-6-chloropurine (**25b**), while the aforementioned method was proven unsuccessful when applied to the synthesis of the unsaturated 2′-ketonucleoside of theophylline **27** (Fig. **8**). An alternative approach involved the oxidation of a free hydroxyl group in partially acetylated nucleosides, leading to a *β*-elimination of the acetoxyl group [44, 45]. Thus, treatment of **26** with DMSO-DCC [26] gave 7-(dideoxy-*glycero*-hex-3-enopyranosyl-2-ulose)theophylline (**27**).

Although chloropurine ketonucleoside **25b** had a more pronounced inhibitory activity against KB cancerous cells than theophylline ketonucleoside **25a** [18], the latter was proved to impair DNA, RNA and protein synthesis, and strongly inhibited cell multiplication, while no cytotoxicity was apparent [20].

In 1979, unsaturated halogenoketonucleosides were synthesized by Herscovici *et al.* [46]. The unsaturated bromoketonucleoside **30** (Fig. **9**) was prepared by oxidation of the 3′,4′-anhydrohexosylpurine **28**, followed by the action of lithium halide on the ketoanhydro intermediate **29**. LiBr

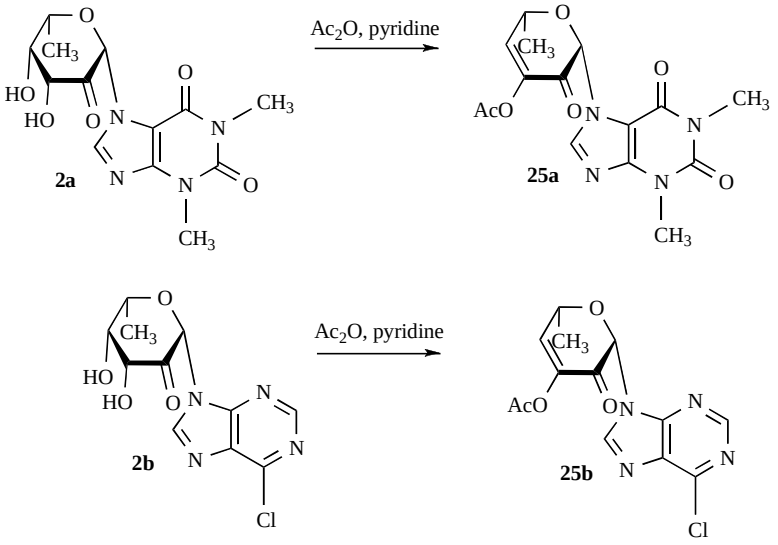


Fig. (7).

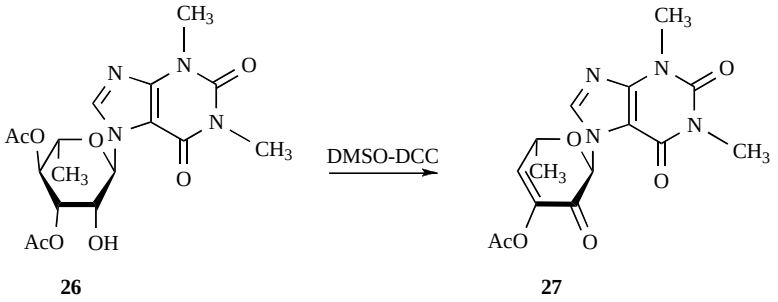


Fig. (8).

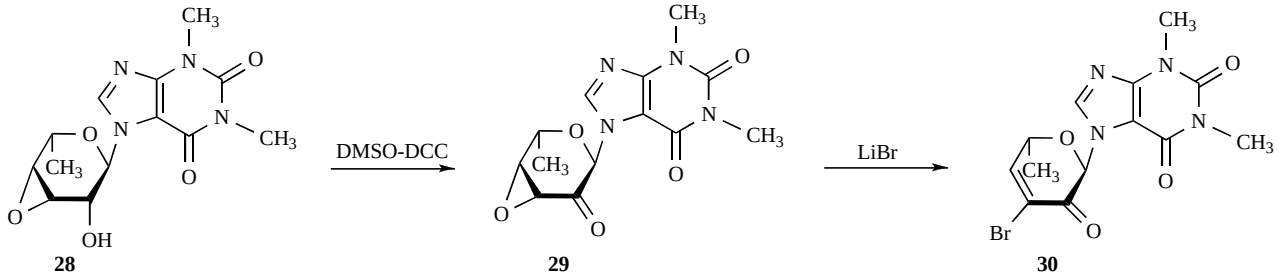


Fig. (9).

has been used to obtain 7-(3-bromo-*glycero*-hex-3-enopyranosyl-2-ulose)theophylline **30**.

The cytotoxicity of ketonucleoside **30** was explored comparatively with ketonucleoside **25a** in murine erythroleukemia cells [47], and it was found that unsaturated halogenoketonucleoside **30** was the most cytotoxic (IC_{50}: 0.21 μM). Although the mechanism of action of these compounds is still unknown, the addition of the electrophilic agent (Br-) increases the cytotoxic potency of the drug, in contrast to the presence of the *O*-acetyl group in the same position, which reduces this effect.

Based on the well-known fact that unsaturated 2′-ketonucleosides exhibit high bioactivity, the synthesis of unsaturated 4′-ketonucleosides **33** and **36** (Fig. **10**) was undertaken [6]. The approach involved the oxidation of the free hydroxyl group in partially benzoylated nucleoside, initiating the *β*-elimination of the benzoyloxy group. Thus, treatment of dibenzoate **31** with DMSO-DCC [26] gave 7-(6-*O*-triphenylmethyl-*glycero*-hex-2-enopyranosyl-4-ulose) theophylline **32**. Detritylation was performed with aqueous acetic acid, leading to the unsaturated ketonucleoside **33**.

The dibenzoylated nucleoside **34** constituted an important intermediate in the synthesis of the 7-(2,6-dideoxy-*glycero*-hex-2-enopyranosyl-4-ulose)theophylline (**36**), which was afforded after oxidation of 6′-deoxy nucleoside **35** with the DMSO-Ac_2O [48].

A thorough study [6] of the structure-activity relationship of the unsaturated ketonucleosides **25a**, **30**, **33**, **36** showed that all of the compounds examined exhibited significant activity against L1210 leukemia. Moreover, *in vitro*, these

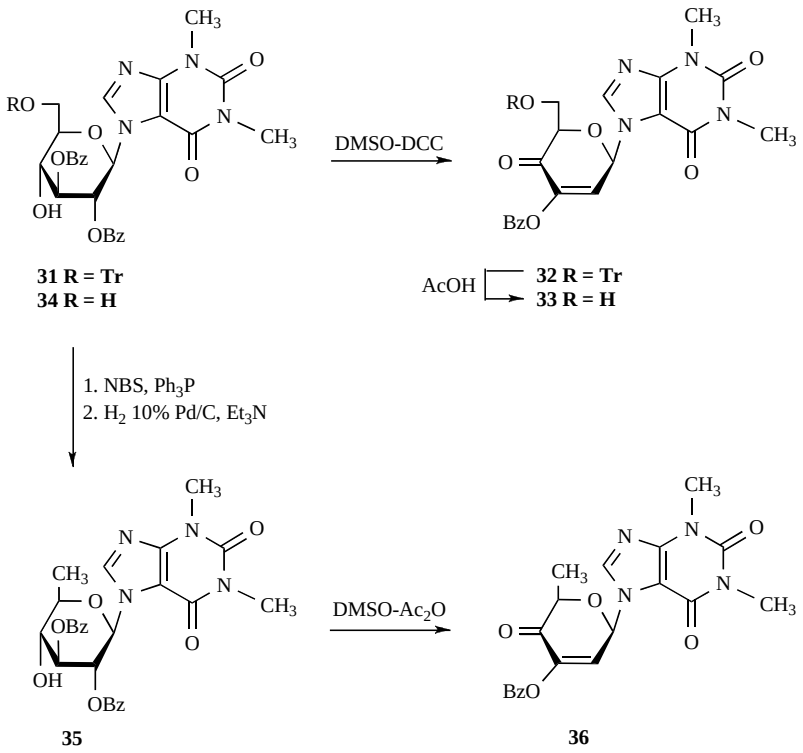


Fig. (10).

nucleosides have been shown to react as Michael acceptor with nucleophilic thiol groups of physiologically active molecules, e.g. glutathione, cysteine and lactate dehydrogenase [21]. *In vivo*, their reaction with plasma membrane surface thiols and soluble intracellular thiols has also been clearly demonstrated [21]. Furthermore, there exists a good correlation between the extent of *in vitro* plasma membrane alkylation and *in vivo* anticancer activity. Thus, the anticancer activity could be due to alkylation of thiol groups of key proteins involved in cell metabolism [21]. It is noteworthy that the absence of genotoxicity [49] makes some of these compounds particularly interesting.

Herscovici and co-workers also designed and synthesized the 7-(2,3,6-trideoxy-*glycero*-hex-2-enopyranosyl-4-ulose) theophylline **38** (Fig. **11**) [50], which constitutes an important intermediate in the synthesis of branched-chain nucleosides and has been obtained by reaction of the 2′,3′-anhydroketonucleoside **37** with NaI and CH_3COONa [51].

The disaccharide derivative **40** (Fig. **12**), an unsaturated ketonucleoside, which was reported by Halmos *et al*. [52], is an analogue of the biologically active compound **36** (Fig. **10**). The key intermediate for the synthesis of this unsaturated ketodisaccharide nucleoside was the partially protected, disaccharide nucleoside **39**. Treatment of **39** with DMSO-Ac_2O [48] afforded 7-[2′,3′-di-*O*-benzoyl-4′-*O*-(3′-*O*-benzoyl-2′,6′-dideoxy-*glycero*-hex-2′-enopyranosyl-4′-ulose)-6′-deoxy-glucopyranosyl]theophylline (**40**).

The significant *in vitro* and *in vivo* anticancer activity of theophylline nucleosides has intrigued medicinal chemists to focus their studies on unsaturated ketopyranosyl nucleosides, such as compounds **25a, 30, 33** and **36**.

In 1991, Ollapally's group reported the synthesis of 2′,3′-unsaturated 4′-keto-pyranosyl nucleosides of 5-FU **45** and **46** (Fig. **13**) [53].

In this approach, the protected nucleoside **42** was synthesized using the standard procedure, which involves coupling of the silylated base with peracetylated rhamnose, deacetylation using methanolic ammonia, conversion to 2′,3′-*O*-isopropylidene derivative **41** and then benzoylation in the usual manner. Finally, a sequential protection/

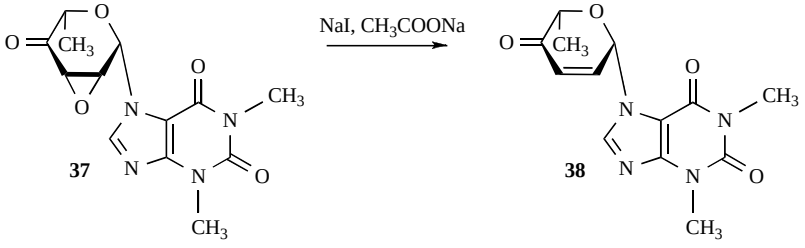
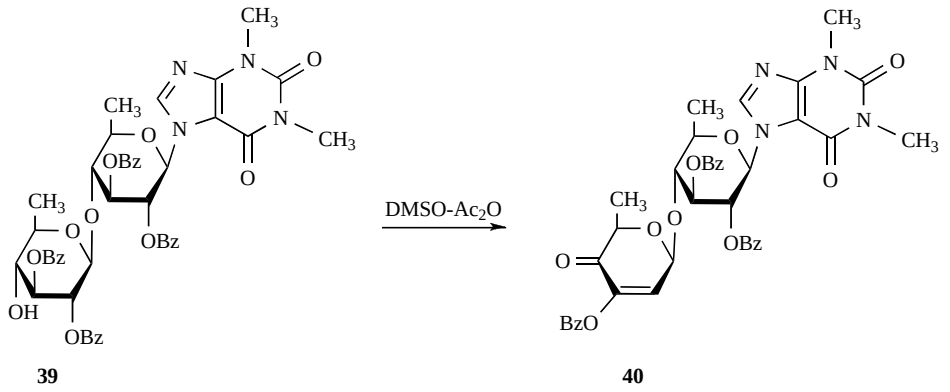


Fig. (11).



39 **40**

Fig. (12).

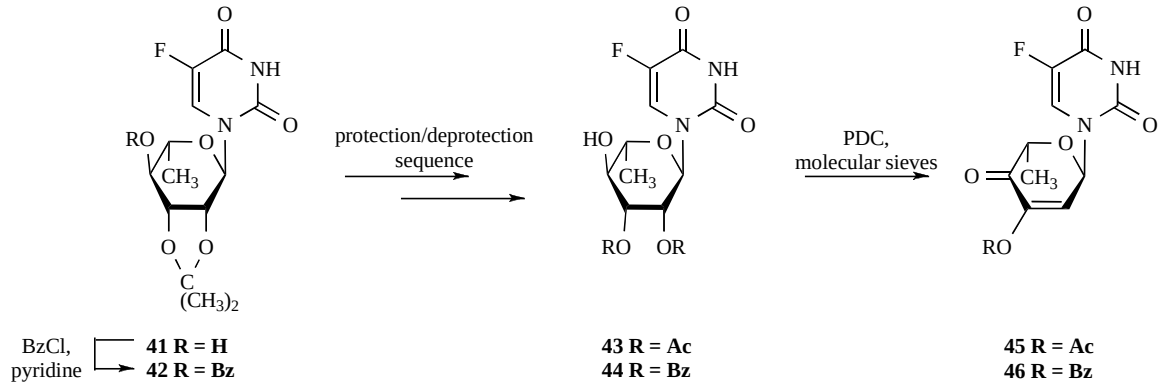


Fig. (13).

deprotection coherence on **42** allowed access to the corresponding alcohols **43** and **44**, whose oxidation using PDC/molecular sieves gave the target unsaturated 4′-ketonucleosides of 5-FU **45** and **46** [54]. The *in vitro* anticancer activity evaluation of the unsaturated 4′-ketonucleosides **45** and **46** showed antitumor action equivalent to that of 5-FU in four different cell lines [L1210, murine mammary carcinoma (FM3A), human B-lymphoblast (Raji) and human T-lymphoblast (Molt/4F)].

Encouraged by the significant anticancer properties as well as novel mode of action of unsaturated ketopyranosyl nucleosides, Sharma *et al*. also undertook the synthesis and bioevaluation of similar unsaturated 2′-ketonucleosides of pyrimidines (**49** and **51**) [55]. Tribenzoylnucleoside of 5-fluorocytosine **48** (Fig. **14**) was synthesized according to the earlier procedure described for 5-FU (Fig. **13**). Nucleoside **47a** was converted to the tribenzoylnucleoside **48** using a sequential protection/deprotection strategy, followed by oxidation, performed with PDC/molecular sieves to give the target unsaturated 2′-ketonucleoside of N^4-benzoyl-5-fluorocytosine **49**. Nucleoside dioxolane **47b** after protection and hydrolysis furnished the corresponding unsaturated alcohol **50**, which upon oxidation gave the target unsaturated 2′-ketonucleoside of 5-FU **51**.

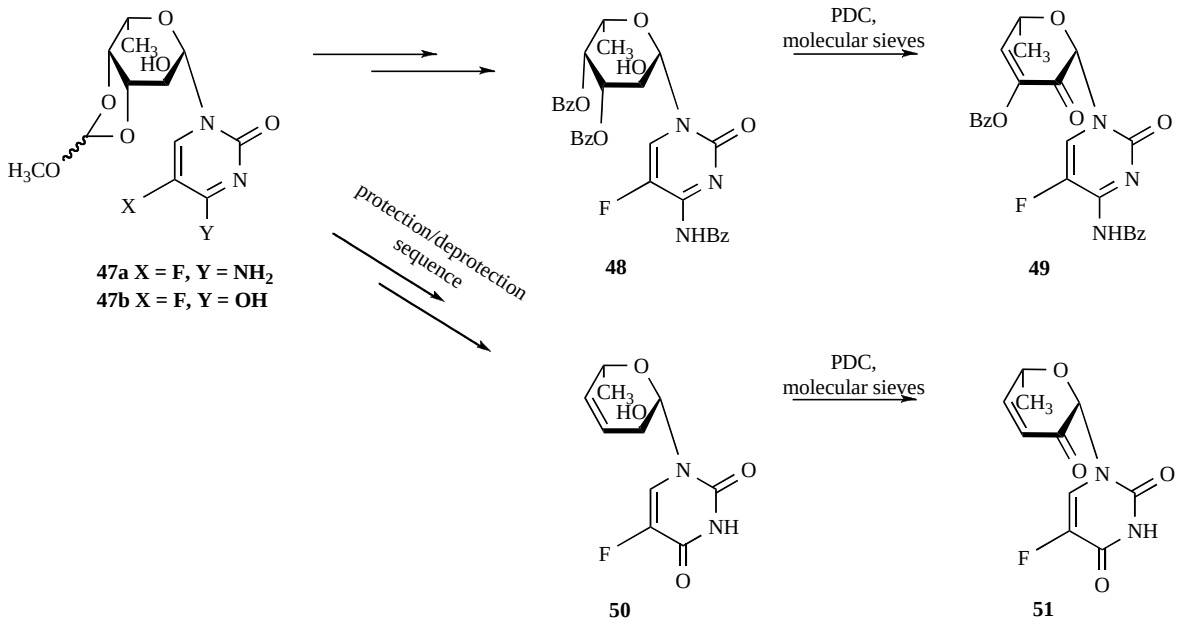
Apart from the above compounds, the synthesis of 2′,3′-unsaturated 4′-ketonucleosides **54a,b** and **56c,d** (Fig. **15**) was also reported [55]. The unsaturated ketonucleosides of uracil **54a** and 5-chlorouracil **54b** were prepared by benzoylation of unprotected nucleosides **52a,b**, followed by oxidation of the resulted di-*O*-benzoylnucleosides **53a,b**, with PDC/molecular sieves [54].

Key intermediates dioxolanes **55c** and **55d**, were readily prepared from alcohols **52c,d**, *via* a protection/deprotection sequence. Subsequent oxidation provided the desired 2′,3′-unsaturated 4′-ketonucleosides **56c** and **56d**.

In addition to the aforementioned unsaturated ketonucleosides, the preparation of 2′,3′-unsaturated 4′-ketonucleosides of 5-iodouracil **60a**, thymine **60b** and 5-trifluoromethyluracil **60c** (Fig. **16**) was also reported [55].

The unsaturated nucleosides **58a-c** were prepared by condensation of diacetylated rhamnal **57** with silylated bases. After deacetylation and subsequent oxidation, the desired 4′-keto-2′,3′-unsaturated nucleosides **60** were obtained. Although, unsaturated ketonucleosides **49**, **51**, **54a,b**, **56c,d** and **60** were all evaluated for their *in vitro* anticancer activities in different cancer cell lines, none of them showed significant anticancer activity.

Very recently, a novel synthetic strategy for the nucleoside **66** (Fig. **17**) was proposed by Agelis *et al*. [56]. Their synthesis started by transforming the acetylated mannopyranose **61** into 2′,3′-*O*-isopropylidene derivative **62**, which upon acetylation and subsequent deisopropylidenation gave the partially protected nucleoside **63**.



47a X = F, Y = NH_2
47b X = F, Y = OH

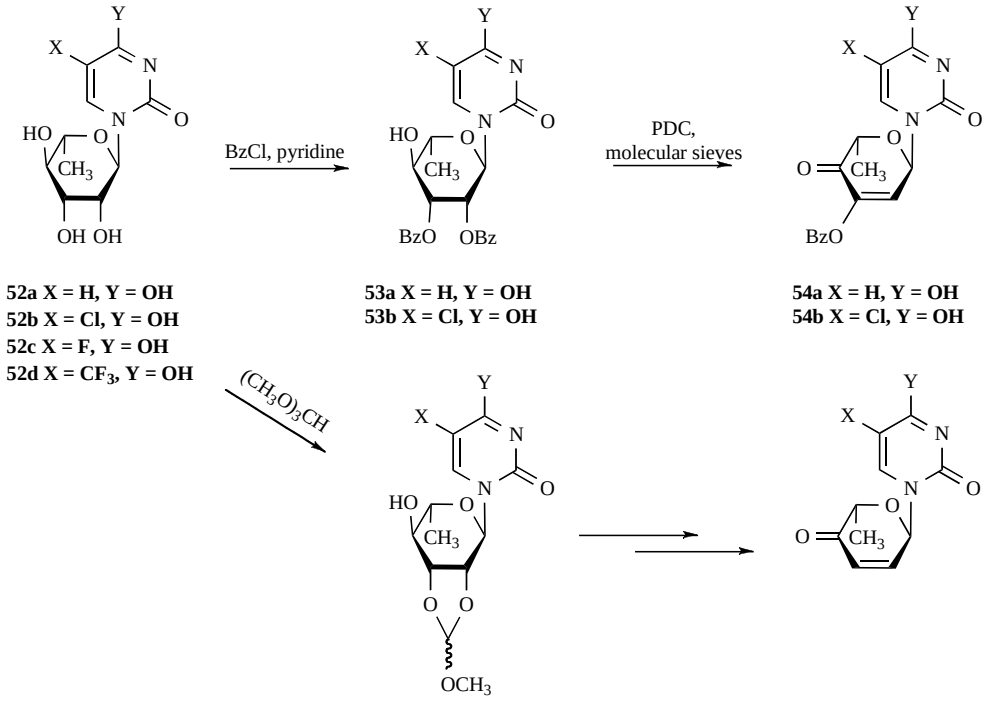
48

49

50

51

Fig. (14).



52a X = H, Y = OH
52b X = Cl, Y = OH
52c X = F, Y = OH
52d X = CF_3, Y = OH

53a X = H, Y = OH
53b X = Cl, Y = OH

54a X = H, Y = OH
54b X = Cl, Y = OH

55c X = F, Y = OH
55d X = CF_3, Y = OH

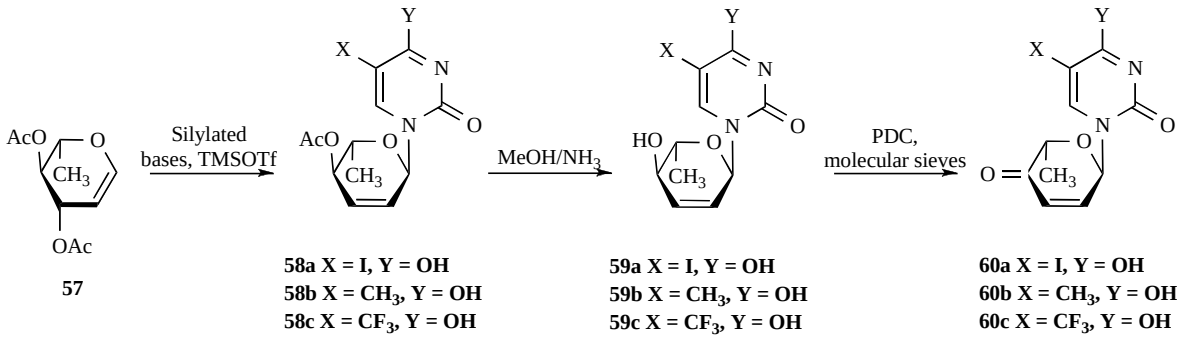
56c X = F, Y = OH
56d X = CF_3, Y = OH

Fig. (15).

Olefination of **63** with CH_3I/Ph_3P/imidazole [57-59], followed by deacetylation and selective tritylation of the primary hydroxyl group gave **64**. Oxidation of the free hydroxyl with PDC/Ac_2O and final detritylation of **65** with HCOOH/diethyl ether [60] afforded the 4′-keto-2′,3′-unsaturated derivative **66**.

In comparison to 5-FU, unsaturated 4′-ketonucleoside **66**, exhibited higher selectivity in all tested tumorgenic cell lines and higher cytotoxicity towards human epithelial colorectal adenocarcinoma (Caco-2) and breast cancer (MCF-7) cell lines. This selective activity of the tested compound is of great importance in its potential use as antitumor drug.

One year later, the synthesis and bioevaluation of unsaturated 4′-ketonucleosides **70** (Fig. **18**) was also reported [22]. The synthesis involved olefination of 1-(4-*O*-



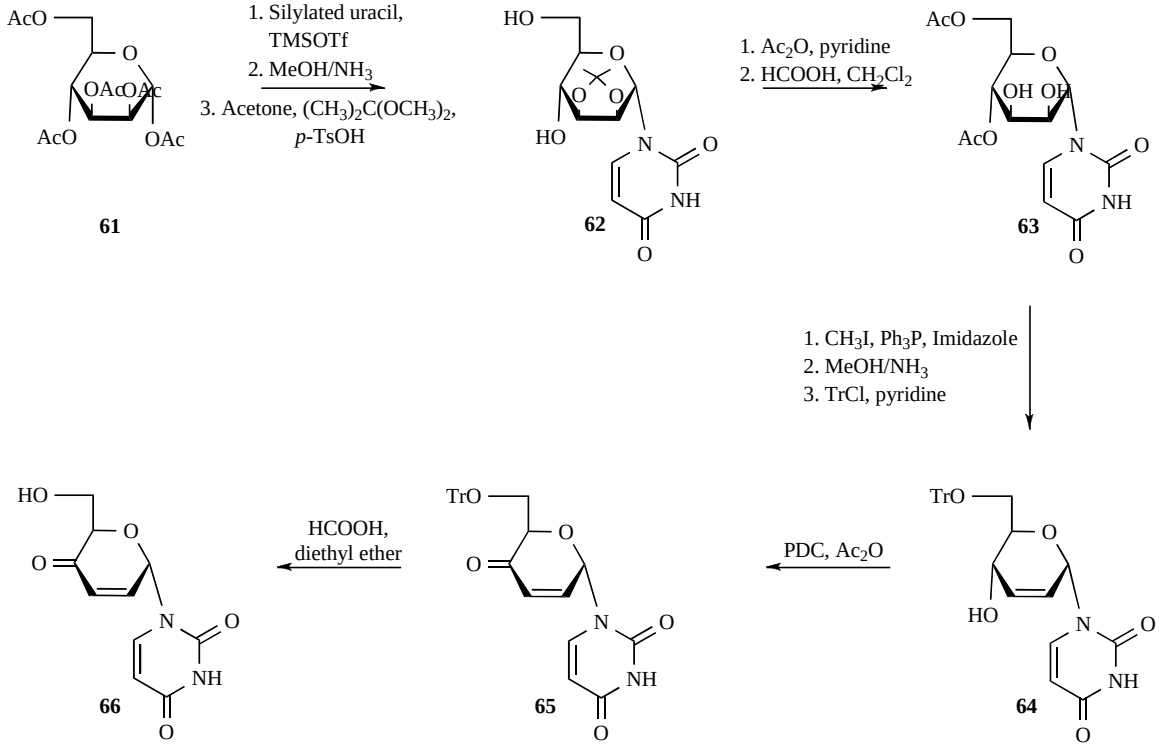
57

58a X = I, Y = OH
58b X = CH_3, Y = OH
58c X = CF_3, Y = OH

59a X = I, Y = OH
59b X = CH_3, Y = OH
59c X = CF_3, Y = OH

60a X = I, Y = OH
60b X = CH_3, Y = OH
60c X = CF_3, Y = OH

Fig. (16).

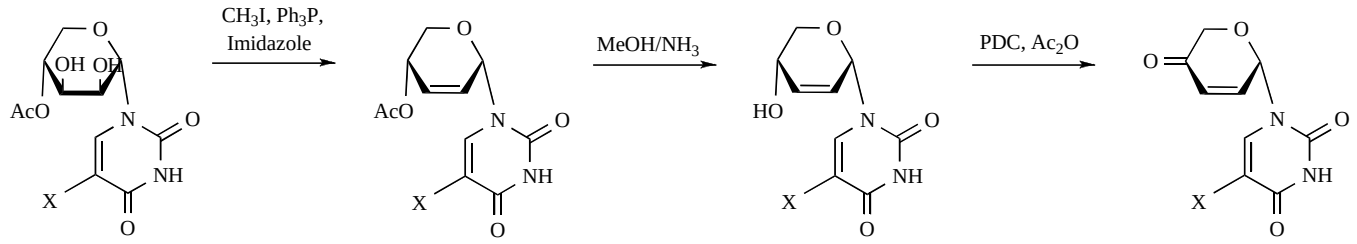


61 **62** **63** **64** **65** **66**

Fig. (17).

acetyl-α-D-lyxopyranosyl)thymine **67a** and 1-(4-*O*-acetyl-α-D-lyxopyranosyl)uracil **67b** with CH_3I/Ph_3P/imidazole [57-59], followed by deacetylation of unsaturated nucleosides **68a,b**, furnishing the unprotected derivatives **69a,b**.

Oxidation of the free hydroxyl group in 4′-position of the sugar moiety of **69** with PDC/Ac_2O led to the formation of the desired unsaturated 4′-ketonucleosides of thymine **70a** and uracil **70b**. Keto unsaturated nucleosides **70** were tested



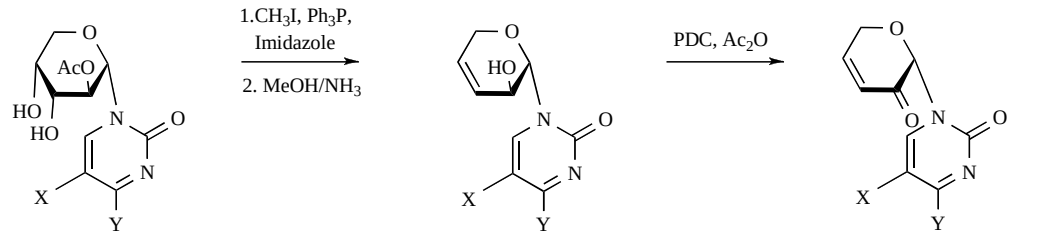
67a X = CH_3
67b X = H

68a X = CH_3
68b X = H

69a X = CH_3
69b X = H

70a X = CH_3
70b X = H

Fig. (18).



71a X = CH_3, Y = OH
71b X = H, Y = OH
71c X = F, Y = OH
71d X = H, Y = NHCOPh
71e X = CF_3, Y = OH

72a X = CH_3, Y = OH
72b X = H, Y = OH
72c X = F, Y = OH
72d X = H, Y = NHCOPh
72e X = CF_3, Y = OH

73a X = CH_3, Y = OH
73b X = H, Y = OH
73c X = F, Y = OH
73d X = H, Y = NHCOPh
73e X = CF_3, Y = OH

Fig. (19).

for their inhibitory effects on the proliferation of carcinoma cell lines. Thymine derivative **70a** showed 50%-inhibitory concentrations of low micromolar range against L1210, Molt4/C8, leukemic lymphoid (CEM) and MCF-7 cells (IC_{50}: 7 to 23 μM), while it was less cytostatic against Caco-2 cell cultures. The corresponding uracil derivative **70b** was cytostatic at 26-38 μM against L1210, Molt4/C8, CEM and MCF-7 cells. Alike **70a**, **70b** were also markedly less cytostatic against Caco-2 cells.

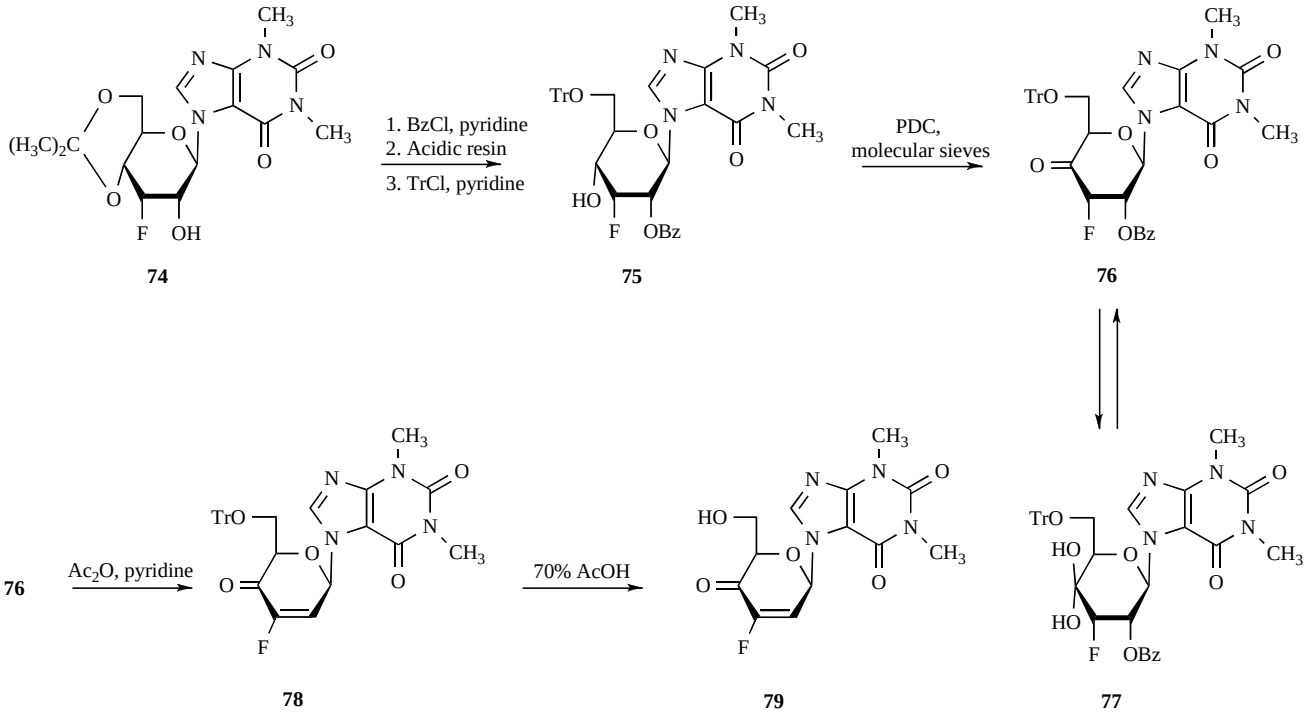
In 2011, Tzioumaki *et al.* designed and synthesized the "uncommon" unsaturated ketonucleosides **73a-e** (Fig. **19**) [23]. Starting with the olefination of the vicinal diol nucleosides **71a-e** with CH_3I/Ph_3P/imidazole [57-59], followed by deacetylation of the resulted derivatives with saturated methanolic ammonia or NaOH/EtOH/pyridine, alcohols **72a-e** were produced. After oxidation of the free hydroxyl group in the 2′-position of the sugar moiety with PDC/Ac_2O, the unsaturated alcohols were converted to the desired unsaturated 2′-ketonucleosides **73a-e**.

In general, the unsaturated 2′-ketonucleosides **73a-e** had a pronounced cytostatic activity. Particularly the 5-FU analogue **73c**, inhibited the proliferation of L1210, FM3A and human cervix carcinoma Hela cells at concentrations that were in the lower micromolar range (IC_{50}: 0.23 to 1.4 μM). Experimental evidence revealed that **73c** may act as a novel type of 5-FU releasing prodrug, and points to thymidylate synthase as target for its cytostatic action.

3.2. Fluoro-Keto Unsaturated Pyranonucleosides

3.2.1. 2′- and 4′-Keto-3′-Fluoro Unsaturated Pyranonucleosides

The significant *in vitro* and *in vivo* inhibitory activity of unsaturated ketonucleosides against various types of cancer



74 **75** **76** **78** **79** **77**

Fig. (20).

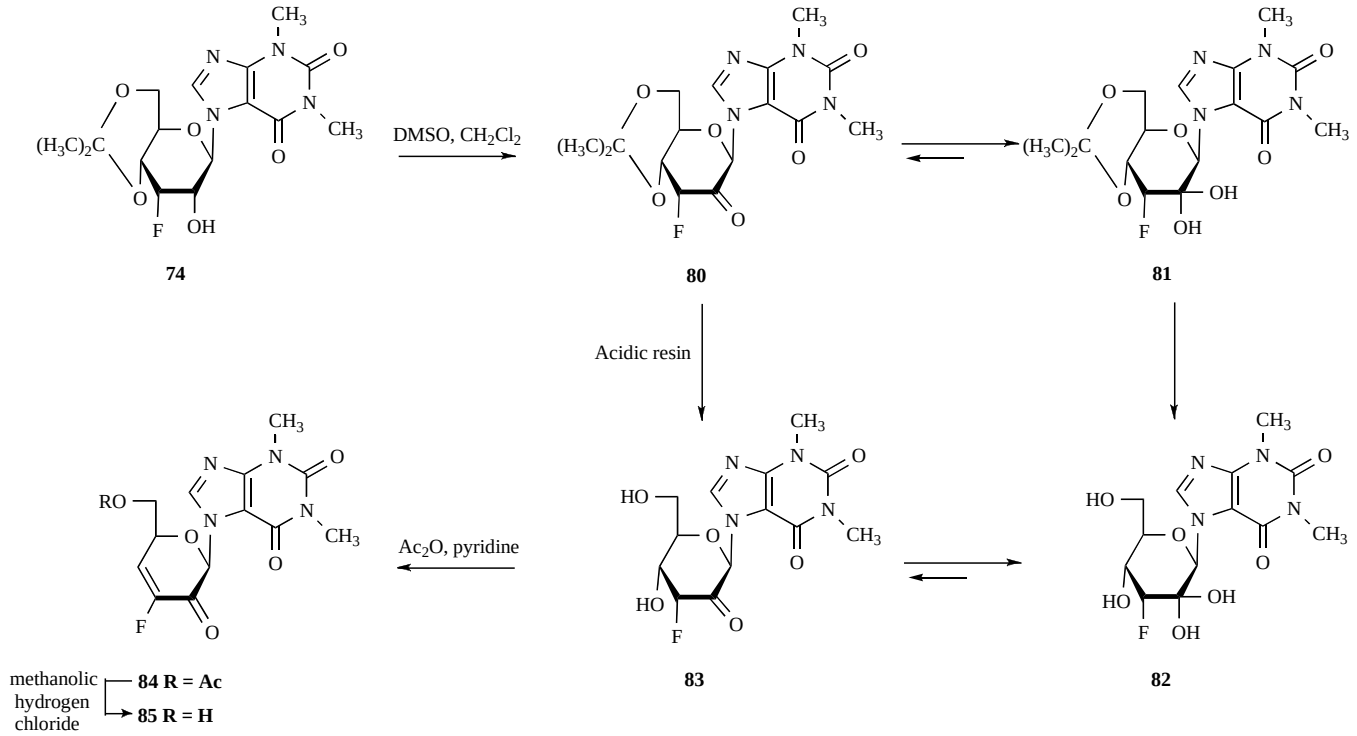


Fig. (21).

cells is well documented [47, 49]. In establishing structure-activity relationships, it was found that the introduction of a bromine atom at the double bond of **30** noticeably increased the toxicity but not the specificity towards cancer cells [61]. In order to minimize toxicity unsaturated ketonucleosides containing fluorine were designed and synthesized.

In 1992, the first synthesis of the unsaturated fluoroketo-nucleosides **78** and **79**, by direct oxidation of the parent fluoronucleoside **75**, was reported (Fig. **20**) [61].

Their synthetic strategy involved benzoylation of nucleoside **74,** followed by removal of the isopropylidene group and selective tritylation of the primary hydroxyl to furnish the partially protected nucleoside **75**. Oxidation of **75** gave ketone **76**, which due to the presence of an electron-withdrawing fluorine atom α to the carbonyl, was quickly transformed to the *gem*-diol **77**. To avoid hydration, the crude oxidation mixture was directly treated with Ac_2O in pyridine to afford **78** by a β-elimination mechanism, which upon detritylation gave **79**. Unsaturated 3′-fluoro-4′-ketonucleoside **79** had a much better antineoplastic activity and a lower immunosuppressive effect than its thymine analogue (its sugar residue differs to that of **79** as to the absence of the fluorine atom) towards splenic lymphocytes (steady state or stimulated by PHA) and to Raji and Human Burkitt's lymphoma (DAUDI) cells.

One year later, Antonakis' group developed another synthetic route to the synthesis of unsaturated 3′-fluoro-2′-ketonucleosides of theophylline **84** and **85**, from the intermediate **74** (Fig. **21**) [62]. Their synthesis involved oxidation of the alcohol **74** leading to the ketone **80**, which was quickly transformed into the hydrate **81**, due to the presence of the highly electronegative fluorine atom in the α position to the carbonyl group. In order to obtain better yields of ketone **80**, oxidation of **74** was performed with DMSO-$(COCl)_2$ [63]. Deacetonation of the crude mixture with acidic resin gave the diol **83**, which is in equilibrium with **82**; upon acetylation of the former followed by β-elimination, the unsaturated ketonucleoside **84** was obtained. Final deacetylation with methanolic hydrogen chloride led to the target analogue **85**. Compound **84**, possessing an *O*-acetyl group in the 6′-position, was found to be 2 to 3-fold more active towards human peripherical blood lymphocytes stimulated by PHA and to Raji and DAUDI cells, than ketonucleoside **85**, whose 6′-hydroxyl is unblocked.

In order to study any variation in biological activity, Ollapally's group accomplished the synthesis of the unsaturated 3′-fluoro-2′-ketonucleoside **89** starting from the fluorinated hexose **86** (Fig. **22**) [64, 65]. Coupling of peracetylated 3-deoxy-3-fluoro-α-D-glucopyranose **86** with silylated 5-FU gave nucleoside derivative **87**, which was easily converted into the alcohol **88** *via* a series of transformations of functional groups involved. Finally, oxidation of compound **88** with PDC provided the desired unsaturated fluoro-ketonucleoside **89**. The target compound showed moderate activity against human colon carcinoma (HT29) (IC_{50}: 5 μM) and KB cells (IC_{50}: 10 μM) *in vitro*.

In view of the high bioactivities of fluoro unsaturated ketonucleosides, Komiotis and co-workers designed and synthesized the 3′,4′-unsaturated-3′-fluoro-2′-ketonucleo-side of N^4-benzoyl cytosine **91** (Fig. **23**) [33]. Oxidation of **13** using PDC/molecular sieves [54], DMSO/Ac_2O [48], and DMSO/$(COCl)_2$ [63] gave invariably a mixture of the desired 2′-ketonucleoside, and its *gem*-diol in a 7:3 ratio.

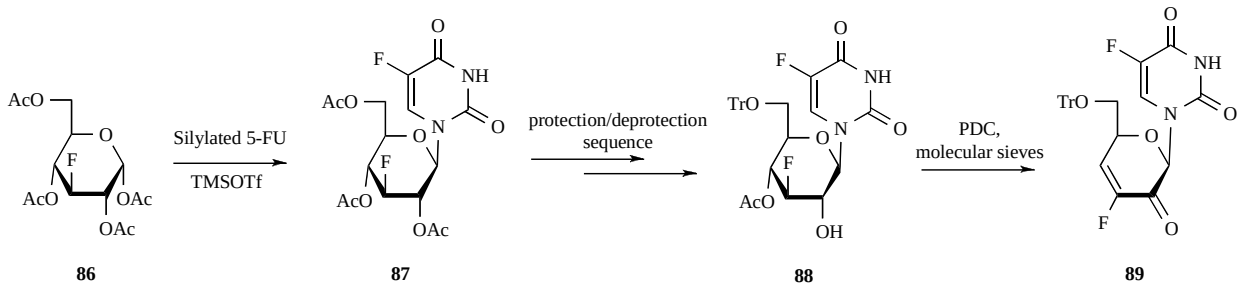


Fig. (22).

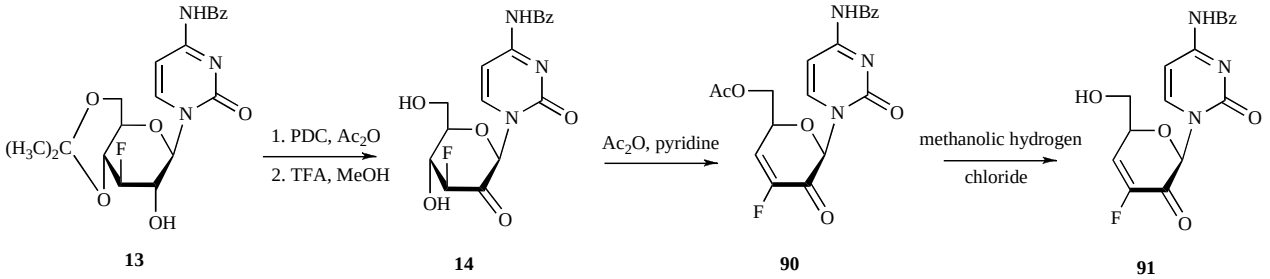


Fig. (23).

However, the target keto unsaturated compound **91** was obtained in a 66% overall yield when **13** was oxidized with PDC/Ac_2O, the crude reaction mixture deisopropylidenated, diol **14** was dehydrated and the resulting acetate **90** was deprotected. Biological assays demonstrated that nucleosides **90** and **91** are potential antiadenocarcinoma agents and have a promising potential in combating rotaviral infections.

It should be noted that, commencing from the fluorinated pyranose **11**, the unsaturated 3′-fluoro-4′-ketonucleosides of N^4-benzoyl cytosine **94a**, **95** and N^6-benzoyl adenine **94b**, (Fig. **24**) were also synthesized and their biological activity was evaluated [66]. The process started from the isopropylidene intermediates **13** and **92**. Compound **92** was obtained from pyranose **11**, as previously described for derivative **13**. 4′,6′-Isopropylidene alcohols **13** and **92** were first converted to the partially protected nucleosides **15** and **93** through simple transformations of functional groups.

PDC oxidation, followed by *in situ* elimination reaction of the *β*-acetoxyl group, provided the unsaturated 3′-fluoro-4′-keto-*β*-D-glucopyranosyl derivatives of N^4-benzoyl

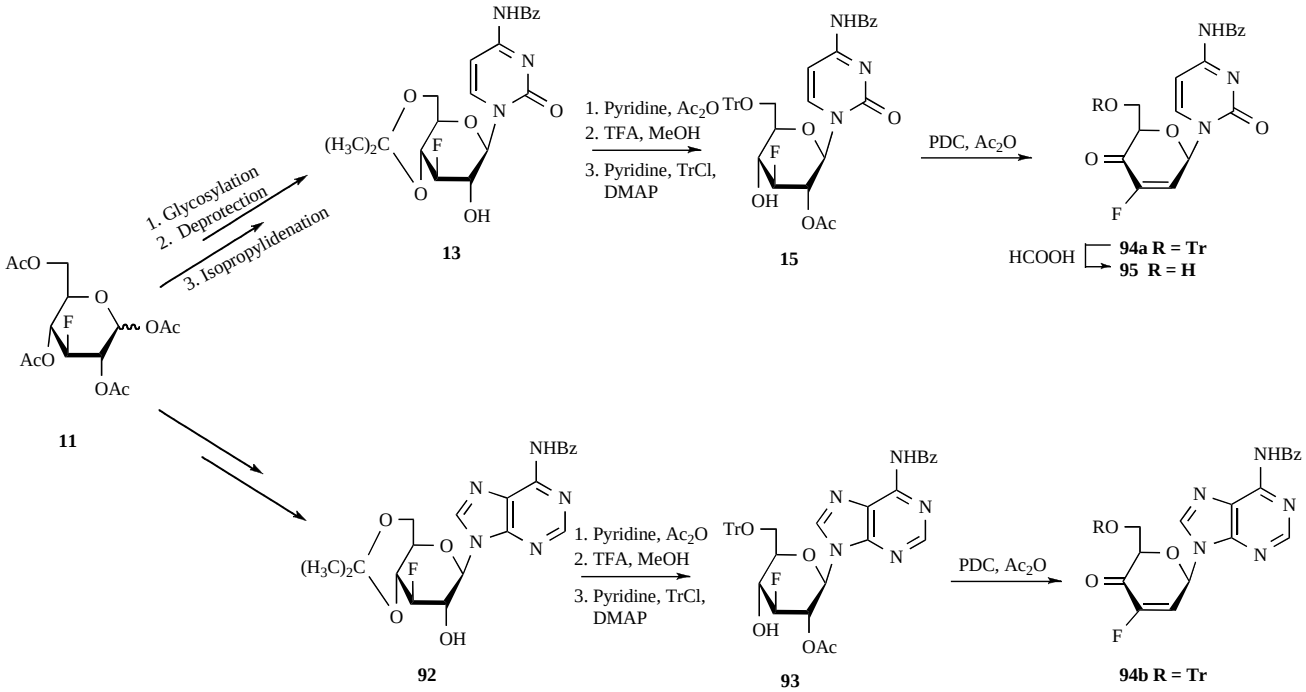
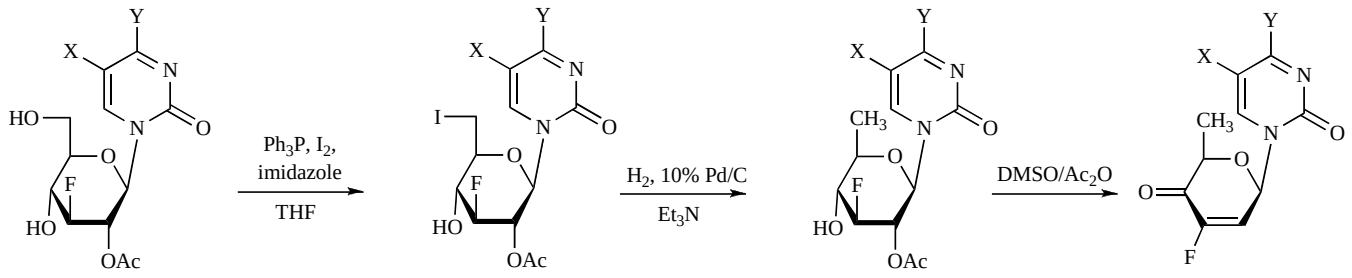


Fig. (24).



96a X = H, Y = OH
96b X = F, Y = OH
96c X = CH_3, Y = OH
96d X = H, Y = NHBz

97a X = H, Y = OH
97b X = F, Y = OH
97c X = CH_3, Y = OH
97d X = H, Y = NHBz

98a X = H, Y = OH
98b X = F, Y = OH
98c X = CH_3, Y = OH
98d X = H, Y = NHBz

99a X = H, Y = OH
99b X = F, Y = OH
99c X = CH_3, Y = OH
99d X = H, Y = NHBz

Fig. (25).

cytosine **94a** and N^6-benzoyl adenine **94b**. Finally, removal of the trityl group with HCOOH [41] gave only the cytosine analogue **95**. Compared to 5-FU, compounds **94a**, **94b** and **95** proved be more efficient as antitumor growth inhibitors, while cytosine analogue **94a** exhibited the best cytotoxic action against skin melanoma cells (CC_{50}: 3.3 μM).

Based on the fact that the 6′-protected fluoro unsaturated ketopyranonucleosides had the most promising anticancer and antiviral properties, the synthesis of a new class of unsaturated 3′-fluoro-4′-ketonucleoside analogues **99a-d** (Fig. **25**) bearing a methyl group in the 5′-position of the sugar moiety [67] was undertaken.

Replacement of the primary hydroxyl group of the partially acetylated nucleosides **96a-d** by an iodine atom using Ph_3P together with I_2 and imidazole [68], led to the desired iodo derivatives **97a-d**, which were hydrogenated in the presence of palladium-on-carbon to form the deoxy nucleosides **98a-d**. Finally, oxidation of the fluoro acetylated dideoxy precursors **98a-d** using the DMSO/Ac_2O [48] system afforded, after a *β*-elimination reaction, the desired unsaturated 2′,6′-dideoxy-3′-fluoro-*β*-D-glycero-hex-2′-enopyranosyl-4′-ulose derivatives of uracil (**99a**), 5-FU (**99b**), thymine (**99c**) and N^4-benzoyl cytosine (**99d**), respectively. The compounds markedly inhibited tumor cell proliferation of L1210, FM3A, Molt4/C8 and CEM cells (IC_{50}: 0.49 to16 μM).

In addition to the aforementioned unsaturated 4′-ketonucleosides, 3′-fluoro-2′-ketounsaturated nucleoside analogues, compounds **105a-d** (Fig. **26**) were also prepared and evaluated for their anti-proliferative activity against a panel of tumor cell lines [69]. Monoiodination of the primary hydroxyl group of key precursors **100a-d** followed by acetylation and subsequent catalytic hydrogenation afforded the deoxy nucleosides **101a-d**. Regioselectively 2′-*O*-deacylation of **101a-d** with $NH_2OH{\cdot}HCl$ and CH_3COONa [70] furnished only the partially acetylated analogue of 5-FU **102a**, which was further subjected to oxidation with DMSO/Ac_2O [48] to give the target nucleoside **105a**. In order to acquire the unsaturated carbonyl analogues of uracil (**105b**), thymine (**105c**) and N^4-benzoyl cytosine (**105d**), an alternatine plan was then devised. Thus, treatment of the acetylated fluoro dideoxy precursors **103b-d** with BzCl in pyridine followed by deacetylation with NaOH/EtOH/pyridine led to the partially protected derivatives **104b-d**. Finally, oxidation using the DMSO/Ac_2O system [48] afforded, the desired ketounsaturated derivatives **105b-d**. The 5-FU derivative **105a** showed pronounced cytostatic activity against the murine (L1210, FM3A) and human (HeLa) tumor cell lines (IC_{50}: 0.21 to 1.2 μM), while it was moderately inhibitory against CEM cell proliferation (IC_{50}: 12 μM). Analogues **105b-d** exhibited, moderate to poor inhibitory activity (IC_{50}: 7.8 to 219 μM) against tested tumor cell lines.

The inhibitory activity of unsaturated keto- and fluorinated ketonucleosides against a variety of tumor cells has been studied in some details and the results obtained ascertained that the presence of an *α,β*-unsaturated keto system in the sugar moiety enhances biological activity. Accumulated evidence also suggested that the correlation found between *in vivo* biological activity and reactions of keto unsaturated nucleosides with membrane thiols as Michael acceptors might be an important prerequisite for their biological activity. Moreover, 6′-deoxy and 6′-protected keto unsaturated analogues have emerged as more effective cytotoxic agents, indicating that the presence of a primary hydroxyl group might not be critical for biological action. It also became clear that the transposition of the keto group from C-4′ to C-2′ lowers the anticancer activity of this type of molecules, since the unsaturated 2′-ketopyranosyl derivatives proved to be less inhibitory than their 4′-keto congeners against a panel of tumor cell lines.

4. EXOMETHYLENE PYRANONUCLEOSIDES

Modified ribonucleosides containing an exocyclic methylene group in 2′-, 3′- or 4′-position exhibited interesting biological activities [71, 72]. Their adequate anticancer properties were at least partially attributed to the ability of these nucleosides to irreversibly inactivate ribonucleotide reductase after phosphorylation *via* specific nucleoside kinases [73, 74]. Experimental data also revealed that the exomethylene group of the sugar moiety is responsible for their inhibitory action [75]. In view of these factors and in order to discover new nucleoside derivatives with high anticancer activities, the introduction of an

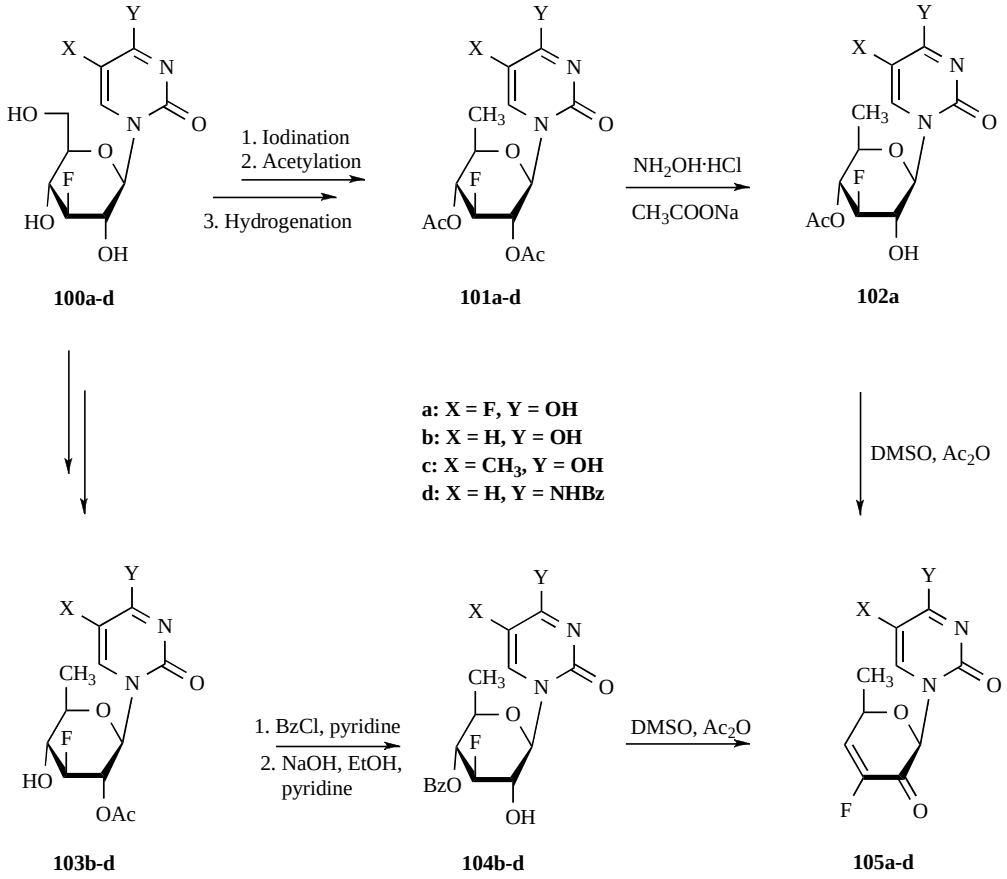


Fig. (26).

exomethylene group into pyranonucleosides has recently attracted considerable attention.

4.1. Exomethylene and Exomethylene Unsaturated Pyranonucleosides

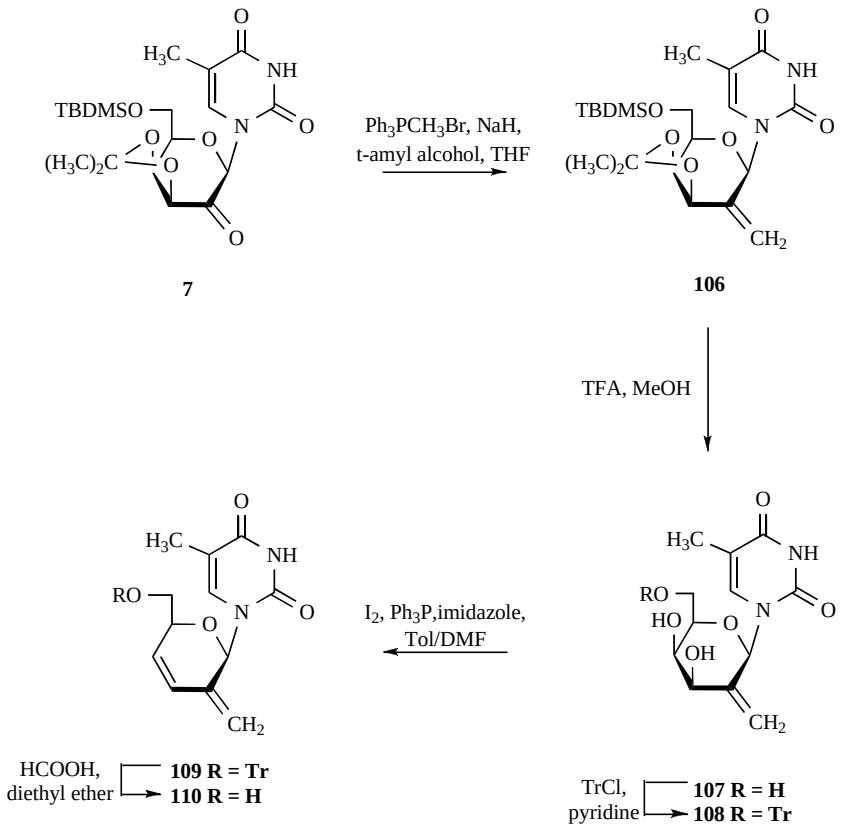
An efficient synthesis of 3′,4′-unsaturated 2′-exomethylene pyranonucleosides **109** and **110** (Fig. **27**) was first reported in 2007 [30]. Their synthesis started from the 2′-ketonucleoside of thymine **7**, which was subjected to the Wittig reaction with NaH and Ph_3PCH_3Br, in the presence of THF [72], to give the exomethylene nucleoside **106**. After deisopropylidenation of compound **106** in acidic conditions, the fully unprotected 2′-exomethylene derivative **107** was obtained and upon specific tritylation was converted to key intermediate **108**. Olefination according to Garegg-Samuelsson method, using I_2/Ph_3P/imidazole [76-79], led to the direct conversion of diol **108** to the unsaturated exomethylene **109**. Finally, detritylation afforded the desired unprotected nucleoside analogue **110**.

Biological assays demonstrated that compounds **109** and **110** did not show enhanced growth inhibition of Caco-2 cells, compared to the saturated exomethylene analogue **107**, which proved to be more cytotoxic and selective against the same cancer cells than 5-FU.

Using a similar strategy, Komiotis' group also carried out the synthesis of the 2′,3′-unsaturated 4′- exomethylene nucleoside analogues **116** and **117** (Fig. **28**) [56]. Tritylation of the previously described analogue **62** gave the partially protected nucleoside **111.** Oxidation of **111** led to the keto intermediate **112**, which upon Wittig olefination [72] gave the exomethylene nucleoside **113**. Full deprotection of uracil derivative **113** by treatment with TFA, led to the 4′-exomethylene nucleoside **114**. Two different routes were carried out for the completion of the synthetic strategy. In the first method, protection of the primary hydroxyl group of **114** led to the tritylated nucleoside **115**, which after olefination and direct deprotection gave the desired 2′,3′-unsaturated 4′- exomethylene **117**. Alternatively, Wittig reaction of the key intermediate **65**, led to the desired exomethylene nucleoside **116**, which after treatment with HCOOH furnished the unprotected analogue **117**.

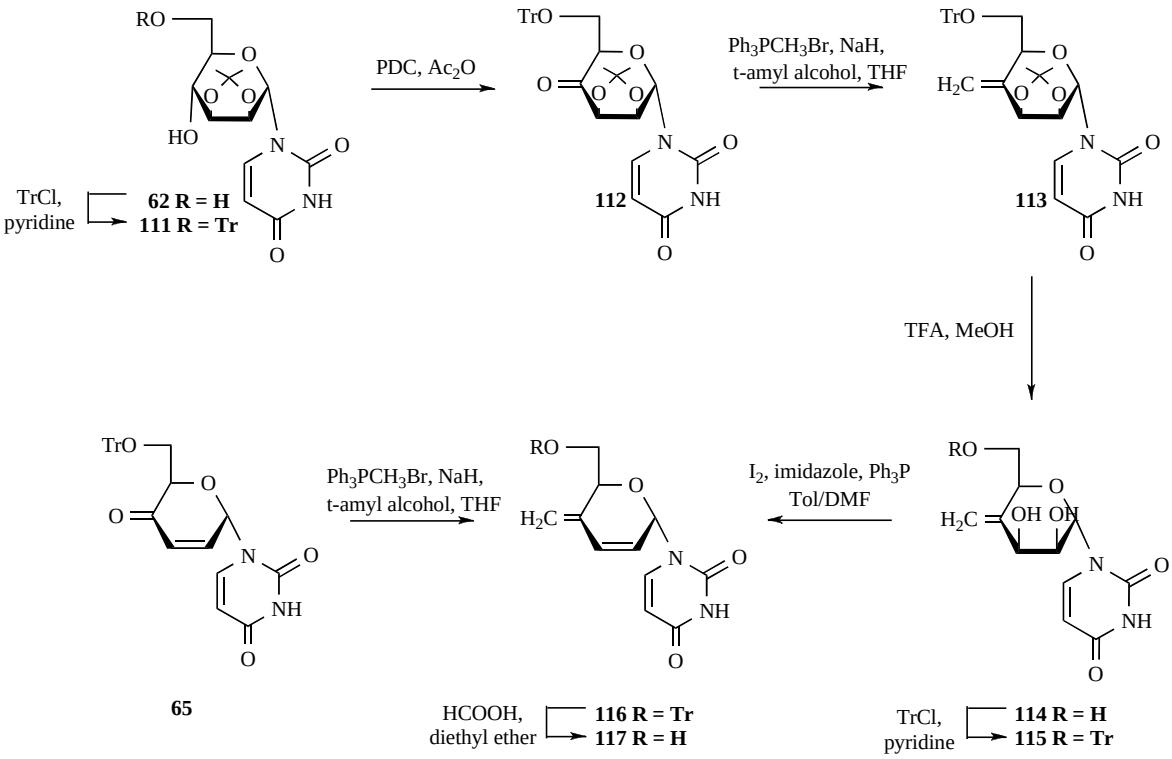
Compound **116** was found to be more selective than 5-FU towards MCF-7 and Caco-2 cells and as selective as 5-FU in skin melanoma cells. Compound **117** exhibited higher selectivity in all tested tumorgenic cell lines and higher cytotoxicity than 5-FU towards Caco-2 and MCF-7 cell lines. The selective activity of the tested compounds is of great importance in their potential use as antitumor drugs.

In 2010, the same group has also published the synthesis of keto-exomethylene-D-glucopyranosyl nucleoside analogues **124a-c** (Fig. **29**), where they combined both the pharmacologically active keto group and exomethylene moiety in the sugar part [80]. Wittig condensation of 3-keto glucoside **118** led to the formation of exomethylene sugar



7 **106**

Fig. (27).



65

Fig. (28).

119. Hydrolysis followed by acetylation furnished the precursor sugar **120**. Glucosyl donor **120** was condensed with silylated pyrimidines to afford the *β*-protected nucleosides **121a-c**. Removal of all acetoxyl groups gave the

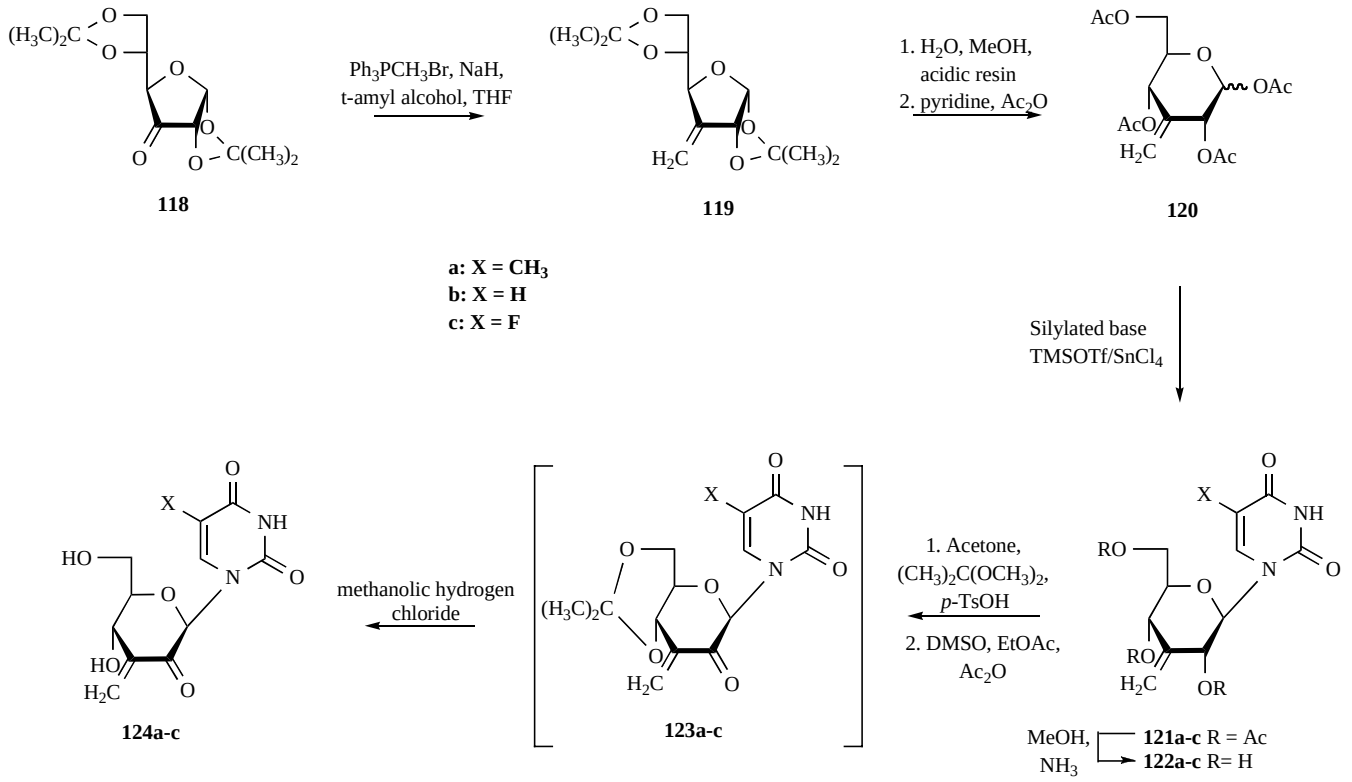


Fig. (29).

key intermediates exomethylene analogues **122a-c**. After acetalation and oxidation of the 2′-hydroxyl group, the labile derivatives **123a-c** were afforded and upon deisopropylidenation delivered the desired unprotected keto-exomethylene nucleosides **124a-c**.

The keto-exomethylene analogues **124a-c** were more cytostatic against the L1210, CEM and HeLa cells, than the corresponding saturated exomethylene derivatives **122a-c**. The 5-FU derivative **124c** was more cytostatic (IC_{50}: 0.56 to 9.4 μM) than the other compounds, which were compatible to the free parental 5-FU. It is noteworthy that, in contrast to the previously synthesized 2′- and 4′-exomethylene nucleosides **107** and **114**, no marked cytostatic activity was noticed for the 3′-exomethylene analogues **122a-c**. It appears that the transposition of the methylene moiety from C-2′ or C-4′ to C-3′ lowers the cytostatic activity of this type of molecules.

4.2. Exomethylene and Exomethylene Unsaturated Pentopyranonucleosides

As previously mentioned, studies on unsaturated pyranonucleoside analogues suggest that the presence of a primary hydroxyl does not seem to be essential for their biological activity. Taking into consideration these interesting results and in order to study any variation in biological activity, the unsaturated 4′-exomethylene hydroxymethyl-lacking pyranonucleoside analogues **128a,b** (Fig. **30**) were designed and synthesized [22]. Their synthesis was fulfilled through two different routes. The first synthetic strategy started from isopropylidene alcohols **125a,b**, which were derived from commercially available peracetylated lyxopyranose over three steps coherence. Oxidation of nucleosides **125a,b**, followed by Wittig reaction furnished the exomethylene derivatives **126a,b**. Deisopropylidenation gave the 2,3-vicinal diols **127a,b**, which upon olefination furnished the target compounds **128a,b**. The second route was performed by subjecting to the Wittig reaction the key intermediates **70a,b** to provide the desired deoxy 4′-exomethylene unsaturated nucleosides **128a,b**.

The target nucleosides **128a,b** and their key intermediates saturated exomethylene **127a,b** and keto unsaturated **70a,b** analogues, were tested for their inhibitory effects on the proliferation of L1210, Molt4/C8, CEM, MCF-7 and Caco-2 cell lines. Whereas the exomethylene **127a,b** and the unsaturated exomethylene lyxopyranonucleoside derivatives **128a,b** showed marginal, if any, significant cytostatic activity at 147 to >500 μM, the unsaturated ketolyxopyranonucleoside analogues **70a,b** were endowed with significantly more pronounced cytostatic activity.

Besides the aforementioned 4′-exomethylene 2′,3′-unsaturated lyxopyranonucleosides, the synthesis of 2′-exomethylene 3′,4′-unsaturated arabinopyranonucleoside analogues **133a-e** (Fig. **31**), also lacking the hydroxymethyl moiety, has been completed [23]. The synthesis was accomplished through two different synthetic routes.The first synthetic strategy started from isopropylidene intermediates **129a-d**, which were derived through a synthetic sequence, involving condensation of peracetylated arabinopyranose with pyrimidines, deacetylation and specific acetalation.

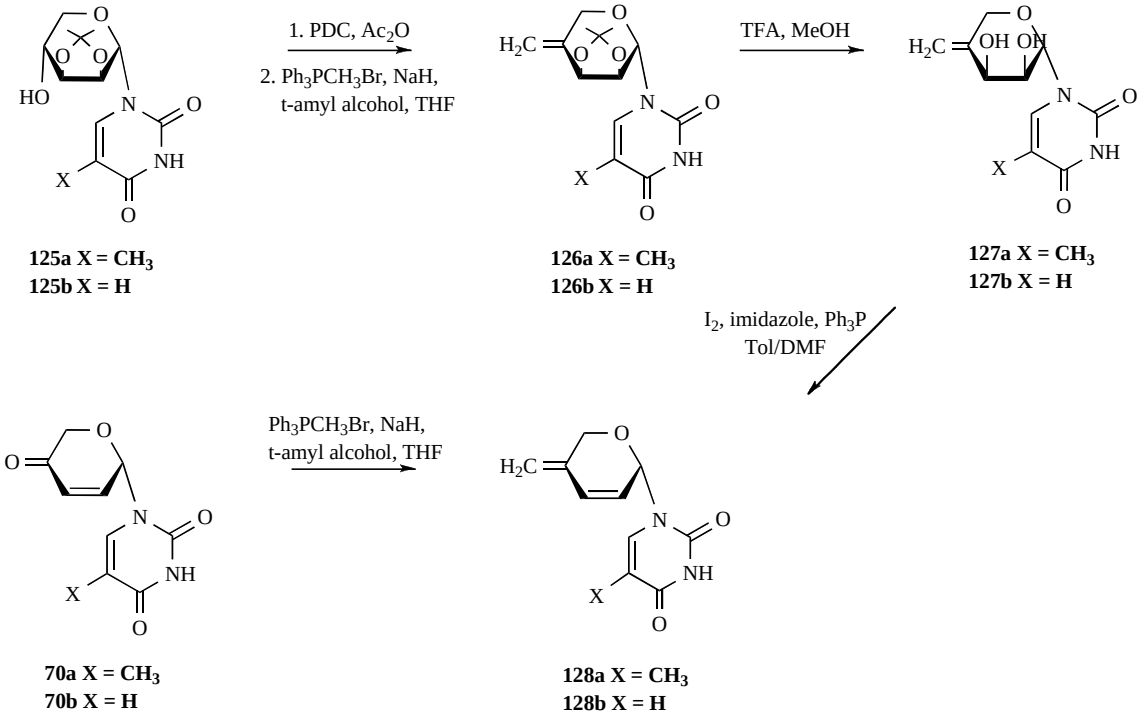


Fig. (30).

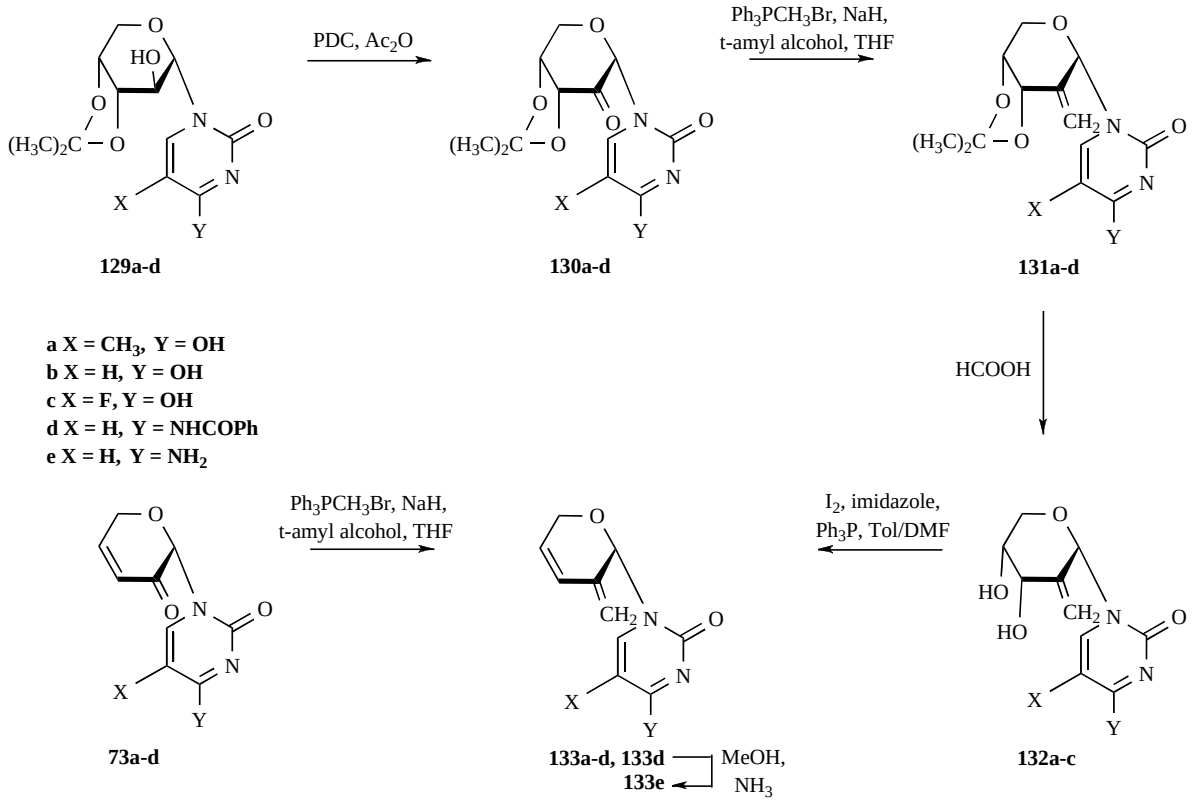


Fig. (31).

Access to exomethylene nucleosides **131a-d** was gained *via* oxidation of free hydroxyl group of key-intermediates **129a-d**, and Wittig reaction of the resulted 2′-ketonucleosides **130a-d**. Deisopropylidenation with HCOOH afforded the 2′-exomethylene nucleosides **132a-c**. It is noteworthy that deprotection of the isopropylidene derivative **131d** with various methods did not afford the desired analogue of N^4-benzoyl cytosine. Final olefination of **132a-c**

led to the target deoxy 3′,4′-unsaturated 2′-exomethylene nucleosides **133a-c**. Alternatively, Wittig condensation of the keto intermediates **73a-d**, afforded the exomethylene unsaturated derivatives **133a-d**, respectively. Deprotection of exomethylene **133d** with saturated methanolic ammonia gave the desired unsaturated 2′-exomethylene cytosine derivative **133e**.

The saturated exomethylene nucleoside analogue carrying the 5-FU base **132c** has been shown to be potential anti-tumor agent, as it exhibited anti-proliferative activity against L1210, FM3A and Hela cells in the lower micromolar range. Interestingly, the unsaturated exomethylene derivatives **133a-d** showed less cytostatic activity than the corresponding keto-arabinonucleoside analogues **73a-d.** Markedly, the 5-FU **133c** inhibited tumor cell proliferation of L1210, FM3A and Hela cells (IC_{50}: 0.23 to 1.4 μM). Experimental evidence suggested that **133c** – likewise its parent **73c** and **132c**- represents a novel prodrug of 5-FU and target thymidylate synthase to exert its cytostatic action.

Ultimately, the synthesis of several exomethylene and unsaturated exomethylene nucleosides, possessing significant antitumor activity, was explored. Interestingly, it appears that the transposition of the methylene moiety from C-2′or C-4′ to C-3′ lowers the cytotoxic activity of this type of molecules, since no marked cytostatic activity was noticed for the 3′-exomethylene analogues. Scientific data has repeatedly demonstrated that the presence of a primary hydroxyl and hydroxymethyl function did not seem to be a prerequisite for biological activity, as long as 6′-protected and hydroxymethyl lacking analogues were very effective cytotoxic agents. Antimetabolic experiments also revealed that thymidylate synthase is the principal target for the cytostatic activity of unsaturated keto and exomethylene 5-FU analogues, which may act as novel types of 5-FU prodrugs. The results enforced the importance of the presence of the keto-group together with the carbon-carbon double bond in the sugar moiety of the nucleosides, while it appeared that the unsaturated keto system might function as an acceptor in a Michael-addition type mechanism.

5. CONCLUSION

In this review, we have presented in a systematic way the recent advances in the synthesis of keto and exomethylene pyranonucleosides . Clearly, two main tactics have been employed for the synthesis of the aforementioned hexopyranosyl nucleosides. One strategy has focused on the direct oxidation of an isolated hydroxyl group in the sugar moiety of suitably protected nucleosides, initiating in some cases a simultaneous *β*-elimination reaction. The second strategy involved the Wittig condensation of suitably modified keto-derivatives. The bioactivity exhibited by these nucleosides has validated the rationale for molecular design and development of novel nucleoside drug candidates with new modes of action. In the next few years, some new and exciting syntheses of keto and exomethylene nucleosides are expected to surface, which could lead to the development of novel anti-cancer agents. To this end, the present review is thought to provide a useful background towards future developments.

ACKNOWLEDGEMENTS

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ABBREVIATIONS

5-FU	=	5-fluorouracil
DMSO	=	dimethyl sulfoxide
DCC	=	*N,N′*-dicyclohexylcarbodiimide
TBDMSCl	=	tert-butyldimethylsilyl chloride
PDC	=	pyridinium dichromate
Ac_2O	=	acetic anhydride
TFA	=	trifluoroacetic acid
AGS	=	human stomach adenocarcinoma cell line
PhOC(S)Cl	=	phenylchlorothionoformate
DMAP	=	4-dimethylaminopyridine
Bu_3SnH	=	tributyltin hydride
THF	=	tetrahydrofuran
NBS	=	*N*-bromosuccinimide
Et_3N	=	triethylamine
Ph_3P	=	triphenylphosphine
BzCl	=	benzoyl chloride
TMSOTf	=	trimethylsilyl trifluoromethanesulfonate
MeOH	=	methanol
p-TsOH	=	*p*-toluenesulfonic acid
TrCl	=	triphenylmethyl chloride
EtOH	=	ethanol
AcOH	=	acetic acid
Ph_3PCH_3Br	=	triphenylphosphonium bromide
Tol	=	toluene
DMF	=	dimethylformamide
EtOAc	=	ethyl acetate
BrdU	=	5-bromo-2′-deoxyuridine
PHA	=	phytohemagglutinin

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