European Journal of Medicinal Chemistry 46 (2011) 993–1005

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

# Synthesis and biological evaluation of unsaturated keto and exomethylene D-arabinopyranonucleoside analogs: Novel 5-fluorouracil analogs that target thymidylate synthase

Niki Tzioumaki [a], Stella Manta [a], Evangelia Tsoukala [a], Vande Voorde Johan [b], Sandra Liekens [b], Dimitri Komiotis [a,**], Jan Balzarini [b,*]

[a] Department of Biochemistry and Biotechnology, Laboratory of Organic Chemistry, University of Thessaly, 26 Ploutonos Str., 41221 Larissa, Greece
[b] Rega Institute for Medical Research, Katholieke Universiteit Leuven, Minderbroedersstraat 10, 3000 Leuven, Belgium

ARTICLE INFO

*Article history:*
Received 30 June 2010
Received in revised form
6 October 2010
Accepted 6 January 2011
Available online 22 January 2011

*Keywords:*
Unsaturated nucleosides
Chemotherapy
5-Fluorouracil prodrugs
Ketonucleosides
Antiviral
Cytotoxicity

ABSTRACT

The synthesis of pyrimidine unsaturated keto and exomethylene arabinopyranonucleoside analogs as potential antitumor and antiviral agents is described. Commercially available 1,2,3,4-tetra-*O*-acetyl-D-arabinopyranose (**1**) was condensed with silylated thymine, uracil, 5-fluorouracil, $N^4$-benzoyl cytosine and 5-(trifluoromethyl)uracil, respectively, deacetylated and acetylated to afford 1-(3,4-*O*-isopropylidene-α-D-arabinopyranosyl)pyrimidine analogs **4**. Two different synthetic routes were investigated for the conversion of compounds **4** into the new 1-(2,3,4-trideoxy-2-methylene-α-pent-3-enopyranosyl)nucleoside derivatives of thymine (**10a**), uracil (**10b**), 5-fluorouracil (**10c**) and $N^4$-benzoyl cytosine (**10d**). Only the first approach could afford derivative **10d**. Debenzoylation of **10d** afforded 1-(2,3,4-trideoxy-2-methylene-α-pent-3-enopyranosyl)cytosine (**10f**). The first approach resulted also to the 2-keto-3,4-unsaturated analogs **9**. The new analogs did not show inhibition of DNA and RNA virus replication in cell culture. The 2′-ketonucleoside derivatives **9** were found to be more cytostatic than the corresponding 2′-exomethylene nucleosides **10**. The 5-fluorouracil unsaturated keto derivative **9c** and the exomethylene derivatives **10c** and **13c** showed antiproliferative activity in the lower micromolar range. Experimental evidence revealed that **9c**, **10c** and **13c** may act as novel types of 5-fluorouracil releasing prodrugs, and points to thymidylate synthase as target for their cytostatic action.



## 1. Introduction

Nucleosides and their analogs are considered as the cornerstone for the development of effective, selective and non-toxic antiviral and antitumor agents [1–6]. Extensive work has been conducted to search for new types of nucleosides with high biological activity, limited toxicity and less likely to develop drug resistance [7]. Over the past two decades, nucleoside/nucleotide chemistry has evolved to facilitate efficient routes to effective agents for the treatment of AIDS [3], herpes [2] and viral hepatitis [8].

Lately, nucleosides bearing a six-membered carbohydrate moiety have been evaluated for their potential antiviral [9–11], antioxidant [12] and antibiotic [13] properties and mimic of natural building blocks in nucleic acid synthesis [14,15]. Modifications in the sugar residue of the aforementioned nucleosides furnished enzymatically stable nucleic acid analogs [16], which can be considered as bioisosteres of natural nucleosides and nucleotides, and can play a major role in different domains like therapy, diagnosis and biotechnology. The unsaturated keto and exocyclic methylene nucleosides are a series of sugar modified pyranonucleosides, whose biological importance has been extensively emphasized. These agents were found to exhibit interesting antitumor and antiviral properties [17–22], while a number of them proved to be key intermediates in synthetic and biosynthetic processes. In addition, their activity is clearly independent of the anomeric configuration, the axial or equatorial position of the heterocyclic base, and the L or D configuration of the sugar [19,23].

As part of our program to search for new antitumor and antiviral agents, we have previously synthesized a series of unsaturated keto [24–28] as well as exomethylene pyranonucleoside analogs [29,30], which proved to be efficient as tumor cell growth inhibitors. Our

* Corresponding author. Katholieke Universiteit Leuven, Department of Microbiology and Immunology, Rega Institute for Medical Research, Minderbroedersstraat 10, 3000 Leuven, Belgium. Tel.: +32 16 337341; fax: +32 16 337340.
** Corresponding author. Tel.: +30 24 10 565285; fax: +30 24 10 565290.
*E-mail addresses:* dkom@bio.uth.gr (D. Komiotis), jan.balzarini@rega.kuleuven.be (J. Balzarini).

0223-5234/$ – see front matter © 2011 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmech.2011.01.005

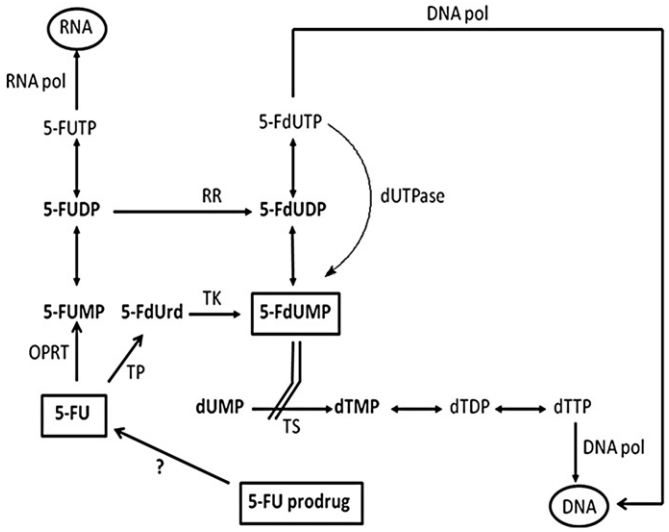
findings suggest that the presence of a primary hydroxyl and hydroxymethyl function does not seem to be critical for biological activity. This was clearly evident since early synthesized 6′-protected and hydroxymethyl-lacking analogs [31] were very effective cytotoxic agents.

Taking into consideration these interesting results, the aim of the present study is to synthesize a series of new hydroxymethyl-lacking pyrimidine nucleoside analogs in order to further investigate the structural features of the sugar and base moieties. Therefore, we report herein an efficient synthesis of 3′,4′-unsaturated 2′-keto and -exomethylene arabinonucleoside analogs, containing thymine, uracil, 5-fluorouracil, $N^4$-benzoyl cytosine, 5-(trifluoromethyl)uracil and cytosine as base moiety and the evaluation of their biological properties. Given the fact that for each series of compounds, the 5-fluorouracil nucleoside analogs were most cytostatic, thymidylate synthase (TS) could be considered as a potential target enzyme. TS converts 2′-deoxyuridylate (dUMP) to thymidylate (dTMP) and represents the only de novo pathway for the tumor cell to generate thymine nucleotide building blocks for DNA synthesis. It is well known that 5-fluorouracil (5-FU), after metabolic conversion by several enzymes of the pyrimidine nucleotide metabolism pathways is a potent inhibitor of TS as its 5-fluoro-2′-dUMP derivative. It can also be incorporated into DNA [through its 5-fluoro-2′-deoxyuridine-5′-triphosphate (FdUTP) derivative] and may also disrupt RNA synthesis [through its 5-fluoro-uridine-5′-triphosphate (FUTP) derivative] (Scheme 1) [32]. We could demonstrate that the unsaturated keto and exomethylene D-arabinopyranonucleoside analogs of 5-fluorouracil likely represent novel prodrugs of 5-FU and target (at least partially) TS to exert their cytostatic action.

## 2. Results and discussion

### 2.1. Synthesis

The starting materials, 1-(2,3,4-tri-*O*-acetyl-α-D-arabinopyranosyl)nucleosides of thymine (**2a**), uracil (**2b**), 5-fluorouracil (**2c**), $N^4$-benzoyl cytosine (**2d**) and 5-(trifluoromethyl)uracil (**2e**) were obtained by condensing commercially available 1,2,3,4-tetra-*O*-acetyl-D-arabinopyranose (**1**) with silylated thymine, uracil, 5-fluorouracil, $N^4$-benzoyl cytosine and 5-(trifluoromethyl)uracil, respectively, in the presence of trimethylsilyl trifluoromethanesulfonate ($Me_3SiOSO_2CF_3$) or $SnCl_4$, in refluxing 1,2-dichloroethane (1,2-$EtCl_2$) or acetonitrile (Scheme 2) [33]. Removal of all *O*-acetyl protecting groups of **2**(**a**–**c**, **e**) with saturated methanolic ammonia [34], gave compounds **3**(**a**–**c**, **e**), in excellent yields. Selective deprotection of **2d** using sodium hydroxide/ethanol/pyridine gave benzoylated derivative **3d**, in quantitative yield [35]. However, when compound **2d** was treated with methanolic ammonia the fully unprotected nucleoside was obtained. Specific acetylation of **3**(**a**–**e**) using 2,2-dimethoxypropane [34] in acetone led to the 3′,4′-*O*-isopropylidene derivatives **4**(**a**–**e**), respectively.




**Scheme 1.** Metabolic pathways to convert 5-FU to its active metabolites. OPRT: Orotate phosphoribosyltransferase, TK: thymidine kinase, TS: thymidylate synthase, TP: thymidine phosphorylase, RR: ribonucleotide reductase, dUTPase: 2′-deoxyuridine-5′-triphosphate hydrolase, RNA pol: RNA polymerase, DNA pol: DNA polymerase.

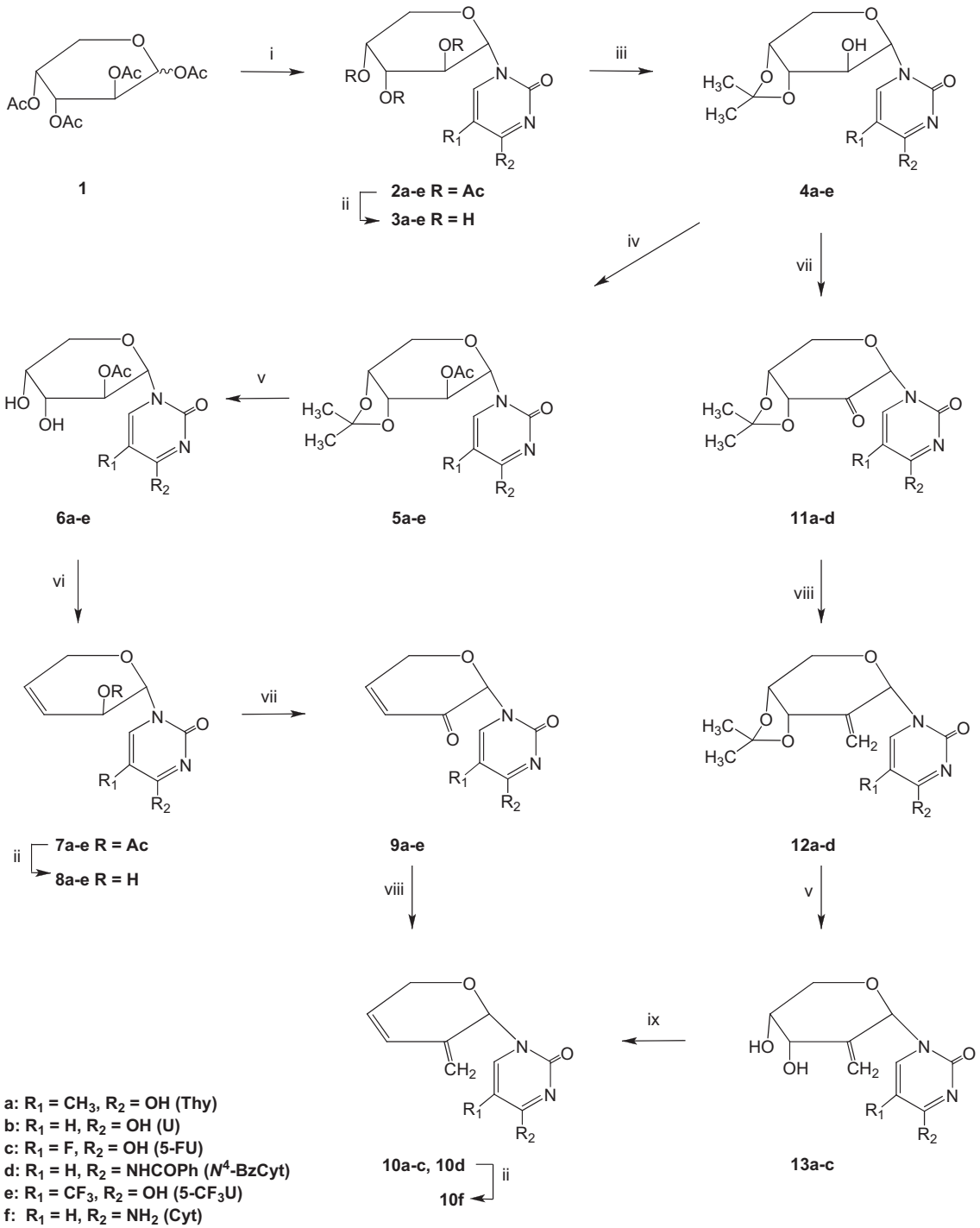
In order to elucidate the influence of keto and exomethylene moiety on the biological activity of the target molecules, two different synthetic routes were investigated for the conversion of compounds **4** into the desired olefinic derivatives **10**. Only the first approach resulted to keto unsaturated derivatives **9** and afforded cytosine nucleoside **10d**, while the second one led to saturated exomethylene analogs **13**. Therefore, in the first approach, acetylation of **4**(**a**–**e**) using acetic anhydride ($Ac_2O$) in pyridine followed by deisopropylidenation of the resulted derivatives **5**(**a**–**e**), in $CH_2Cl_2$/HCOOH, 1:1, led to the vicinal diol derivatives **6**(**a**–**e**), in high yields (79–95%). Olefination of **6**(**a**–**e**) with iodoform-$Ph_3P$-imidazole [36–38] afforded 1-(2-*O*-acetyl-3,4-dideoxy-α-D-*glycero*-pent-3-enopyranosyl)nucleosides of thymine (**7a**) in 65% yield, uracil (**7b**) in 66% yield, 5-fluorouracil (**7c**) in 56% yield, $N^4$-benzoyl cytosine (**7d**) in 61% yield and 5-(trifluoromethyl)uracil (**7e**) in 65% yield. Deacetylation of **7**(**a**–**c**, **e**) with saturated methanolic ammonia [34] and of **7d** with sodium hydroxide/ethanol/pyridine [35], gave **8**(**a**–**c**, **e**) and **8d**, respectively, which after oxidation of the free hydroxyl group in the 2′-position of the sugar moiety with pyridinium dichromate (PDC)/$Ac_2O$ [39], were converted to the desired unsaturated 2′-ketonucleosides **9**(**a**–**e**). Wittig condensation of the keto intermediates **9**(**a**–**d**), with NaH and methyl triphenylphosphonium bromide ($Ph_3PCH_3Br$) in the presence of *t*-amyl alcohol [40] in tetrahydrofuran (THF), afforded the exomethylene derivatives **10**(**a**–**d**), respectively. Debenzoylation of **10d** was performed with saturated methanolic ammonia to afford the desired unsaturated 2′-exomethylene cytosine derivative **10f**.

In the second approach, oxidation of the free hydroxyl group with PDC/$Ac_2O$ [39] led to the formation of the desired 2′-ketonucleosides **11**(**a**–**d**). Wittig reaction of the obtained ketonucleosides **11**(**a**–**d**) resulted in **12**(**a**–**d**), respectively. Deisopropylidenation of the resulted exomethylene compounds **12**(**a**–**c**) in $CH_2Cl_2$/HCOOH, 1:1, led to the vicinal diol nucleosides **13**(**a**–**c**), respectively. It is noteworthy that treatment of the isopropylidene derivative **12d** with a mixture of $CH_2Cl_2$ and HCOOH, 1:1, or 90% trifluoroacetic acid (TFA) in MeOH, or pyridinium p-toluenesulfonate (PPTS) in MeOH [41], or Amberlite IR 120 $H^+$ resin in MeOH [42], or 50% $CH_3COOH$ in $H_2O$ [34], unfortunately did not afford the desired 1-(2-deoxy-2-methylene-α-D-*erythro*-pentopyranosyl)-$N^4$-benzoyl cytosine, but it led instead to intractable materials. Olefination of **13**(**a**–**c**) with iodine-$Ph_3P$-imidazole [43–46] afforded 1-(2,3,4-trideoxy-2-methylene-α-pent-3-enopyranosyl)nucleosides of thymine (**10a**) in 57% yield, uracil (**10b**) in 53% yield, and 5-fluorouracil (**10c**) in 53% yield.

Compounds **9**(**a**–**e**), **10**(**a**–**d**, **f**) and **13**(**a**–**c**) were evaluated for their antiviral and cytostatic properties.

### 2.2. Biological activity

Whereas none of the compounds showed appreciable antiviral activity at subtoxic concentrations against a broad variety of DNA and RNA viruses (data not shown), there were striking differences in their cytostatic potential. In general, the unsaturated 2′-ketonucleosides **9** showed a more pronounced cytostatic activity than the 2′-exomethylene nucleosides **10** and **13** (Table 1). In all structural groups of compounds, the nucleosides carrying the 5-fluorouracil




**Scheme 2.** i) Silylated base, $Me_3SiOSO_2CF_3/SnCl_4$, dry 1,2-$EtCl_2/CH_3CN$; ii) a: ammonia/MeOH (B = Thy, U, 5-FU, 5-$CF_3$U), b: NaOH/pyridine/ethanol, 11 min (B = $N^4$-BzCyt); iii) acetone, 2,2-dimethoxypropane, *p*-toluenesulfonic acid; iv) $Ac_2O$, pyridine; v) $HCOOH/CH_2Cl_2$ (1:1); vi) dry Tol/DMF (4:1), iodoform-imidazole-$Ph_3P$, 120 °C, 90 min; vii) PDC, $Ac_2O$, dry $CH_2Cl_2$; viii) $Ph_3PCH_3Br$, NaH, *t*-amyl alcohol in THF, 0–25 °C; ix) dry Tol/DMF (4:1), iodine-imidazole-$Ph_3P$, 80 °C, 15 min.

base were far more cytostatic against the tumor cell lines than the corresponding thymine-, uracil-, cytosine-, $N^4$-benzoyl cytosine- and 5-(trifluoromethyl)uracil-carrying derivatives. With the exception of the human T-lymphocyte CEM cells that were inhibited by the 5-fluorouracil derivatives at higher micromolar concentrations, the murine leukemia L1210, the murine mammary carcinoma FM3A, and the human cervix carcinoma HeLa cells were inhibited in their proliferation by compounds **9c**, **10c** and **13c** at concentrations that were in the lower micromolar range. The reference compound 5-FU was only ~1.5- to 2.5-fold more cytostatic than compound **9c**. Interestingly, there was a strong correlation between the cytostatic activity of the individual compounds against the different tumor cell lines. The correlation coefficient ($R^2$) values for the $IC_{50}$ values were 0.942 between L1210 and FM3A (both murine cell lines, 2-day assay), 0.833 between CEM and HeLa (both human cell lines, 3-day assay), 0.976 between murine leukemia L1210 (2-day assay) and human lymphocyte CEM (3-day assay), 0.878 between murine L1210 (2-day assay) and human HeLa (3-day assay) and 0.845 between murine carcinoma FM3A (2-day assay) and human carcinoma HeLa (3-day assay) cells. Thus, irrespective of the time period of incubation,

**Table 1**
Cytostatic activity of test compounds.

| Compound | $IC_{50}$[a] (μM) | | | |
|---|---|---|---|---|
| | L1210 | FM3A | CEM | HeLa |
| **9a** | 34 ± 23 | 77 ± 32 | 104 ± 104 | 34 ± 37 |
| **9b** | 92 ± 5 | 121 ± 44 | 82 ± 14 | 42 ± 29 |
| **9c** | 1.3 ± 1.2 | 0.23 ± 0.07 | 17 ± 2 | 1.4 ± 0.6 |
| **9d** | 28 ± 20 | 62 ± 34 | 46 ± 41 | 72 ± 5 |
| **9e** | 23 ± 12 | 46 ± 2 | 39 ± 6 | 34 ± 3 |
| **10a** | 486 ± 60 | ≥ 900 | 504 ± 5 | 339 ± 46 |
| **10b** | 549 ± 83 | >1000 | 450 ± 39 | 760 ± 122 |
| **10c** | 8.6 ± 3.1 | 2.4 ± 0.1 | 85 ± 4 | 3.5 ± 0.7 |
| **10d** | 368 ± 68 | 379 ± 5 | 432 ± 0 | 296 ± 58 |
| **10f** | 1386 ± 594 | ≥1800 | 1550 ± 351 | 990 ± 117 |
| **13a** | 610 ± 251 | 775 ± 16 | ≥ 800 | ≥800 |
| **13b** | >800 | >800 | > 800 | ≥800 |
| **13c** | 21 ± 4 | 1.9 ± 0.4 | 97 ± 4 | 18 ± 4 |
| 5-FU | 0.56 ± 0.31 | 0.18 ± 0.02 | 14 ± 2 | 0.57 ± 0.23 |

[a] 50% Inhibitory concentration required to inhibit tumor cell proliferation by 50%. Data are the mean (±S.D.) of at least 2 to 3 independent experiments. The murine leukemia L1210 and mammary carcinoma FM3A cells were grown for 2 days, and the human lymphocyte CEM and cervix carcinoma HeLa cells were grown for 3 days before the cytostatic activity of the test compounds was determined by tumor cell counting.

species origin or tissue origin, the cytostatic data correlated very well.

Since compounds **9** and **10** lack a free hydroxyl group for potential phosphorylation, it was tempting to assume, that these molecules may act as prodrugs of the free base, and that the 2′-ketonucleoside derivative releases higher amounts of 5-FU, than the corresponding 2′-exomethylene nucleoside derivatives. When the compounds **9c**, **10c** and **13c** were solubilized in phosphate buffered saline (PBS), **13c** and **10c** were fully stable up to 3 days after bringing the compounds into solution. Compound **9c** was not stable and spontaneously and progressively released the free base 5-fluorouracil (data not shown). This may explain the higher cytostatic activity of **9c** versus **10c** and **13c**. When **9c**, **10c** and **13c** were exposed to phosphate buffer at pH 3.2 for 60 min, the compounds **10c** and **13c** were fully stable as attested by HPLC analysis, whereas the spontaneous release of 5-FU from **9c** was not higher than in neutral solution.

One may assume, that for those compounds that owe their inhibitory effect on cell growth to inhibition of TS, the inhibitory action would be more readily reversed by co-administration of thymidine (dThd) than by 2′-deoxyuridine (dUrd) (Scheme 1) [47]. Also, it has been demonstrated that compounds which inhibit the incorporation of (radiolabeled) dUrd into DNA and which do not inhibit the incorporation of (radiolabeled) dThd into DNA (or inhibit the latter to a significantly smaller extent than dUrd incorporation), selectively block TS [47]. To reveal whether the 5-fluorouracil derivatives **9c**, **10c** and **13c** behave mechanistically similar to the parent TS inhibitor 5-FU, we investigated whether their cytostatic activity can be reversed in the presence of uracil and the natural nucleosides such as dThd, dUrd and uridine. One murine (L1210) and one human (HeLa) cell line were chosen to perform the antimetabolic experiments since the cytostatic activity of the compounds against these cell lines were highly comparable. 5-FU, 5-fluoro-2′-deoxyuridine (5-FdUrd) and 5-fluorouridine (5-FUrd) were included as controls (Table 2). In L1210 cell cultures, addition of dThd (20 μM) markedly reversed the cytostatic activity of **9c**, **10c** and **13c** (25- − ∼40-fold) and also 5-FU (∼40-fold), 5-FUrd (∼100-fold) and 5-FdUrd (∼100,000-fold). In contrast, 20 μM dUrd only moderately reversed the cytostatic activity of the compounds (2- to 5-fold). These data indicate that the mechanism of cytostatic activity may be due to inhibition of TS. Indeed, addition of dThd circumvents the inhibition of TS (and thus, efficiently reverses the cytostatic activity of **9c**, **10c** and **13c**, 5-FU, 5-FUrd and 5-FdUrd), whereas dUrd does not efficiently reverse the cytostatic activity of TS inhibitors, because it needs to be converted to dTMP first by TS and for this reason TS has to remain active. Addition of uracil (500 μM) had no significant effect on the antiproliferative activity of **9c**, **10c** and **13c**, 5-FU, 5-FUrd and 5-FdUrd, whereas uridine (Urd) (500 μM) only reversed the cytostatic effect of 5-FUrd, but not of the other compounds. Obviously, uridine competes with 5-FUrd for its conversion (phosphorylation) by uridine kinase, which is not the case for **9c**, **10c** and **13c**, and 5-FU and 5-FdUrd.

We also examined whether **9c**, **10c**, **13c** and 5-FU differentially inhibit the incorporation of radiolabeled dThd and dUrd into DNA of L1210 cell cultures (Table 3). Whereas the compounds inhibited [$^3$H]dUrd incorporation into DNA, they did not significantly inhibit [$^3$H]dThd incorporation into DNA [$IC_{50}$ (50% inhibitory concentration): 349 to > 500 μM. Interestingly, a 24 h preincubation of the cells with **9c**, **10c** and **13c** made the inhibitory effect of the test compounds against [$^3$H]dUrd but not [$^3$H]dThd incorporation even more pronounced (Table 3). These data again point to TS as the main target for cytostatic action of **9c**, **10c** and **13c**, and 5-FU, they indicate that the compounds need some time for conversion to their active metabolite(s), and they are in full agreement with the reversal studies of the cytostatic activity in the presence of dThd and dUrd.

The activity of TS in intact tumor cells can also be directly monitored by measuring the tritium release by intact L1210 cell cultures that were exposed to [5-$^3$H]deoxyuridine ([5-$^3$H]dUrd) and

**Table 2**
Effect of natural nucleosides and nucleobases on the cytostatic activity of **9c**, **10c** and **13c** in murine L1210 and human HeLa cell cultures.

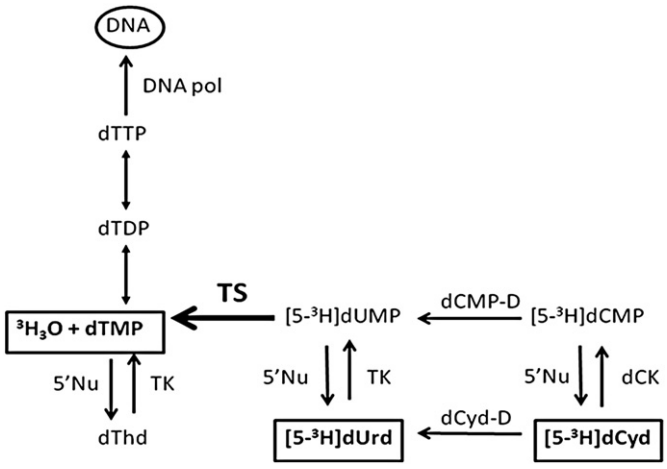
| | $IC_{50}$[a] (μM) | | | | |
|---|---|---|---|---|---|
| | As such | dThd (20 μM) | dUrd (20 μM) | Urd (500 μM) | Uracil (500 μM) |
| L1210 | | | | | |
| **9c** | 1.9 ± 0.4 | 75 ± 57 | 3.5 ± 0.2 | 2.0 ± 0.2 | 1.0 ± 0.3 |
| **10c** | 8.0 ± 0.2 | 182 ± 23 | 18 ± 3 | 5.7 ± 2.0 | 5.8 ± 0.9 |
| **13c** | 21 ± 4 | > 500 | 66 ± 0.0 | 18 ± 3 | 18 ± 3 |
| 5-FU | 0.56 ± 0.10 | 21 ± 1 | 1.3 ± 0.1 | 0.62 ± 0.02 | 0.47 ± 0.17 |
| 5-FUrd | 0.014 ± 0.000 | 1.2 ± 1.1 | 0.026 ± 0.001 | 0.72 ± 0.01 | 0.0075 ± 0.0003 |
| 5-FdUrd | 0.0011 ± 0.0005 | >100 | 0.0051 ± 0.0008 | 0.0023 ± 0.0006 | 0.00063 ± 0.00012 |
| HeLa | | | | | |
| **9c** | 0.97 ± 0.71 | 7.1 ± 2.2 | 0.62 ± 0.13 | 5.3 ± 2.2 | 8.0 ± 0.9 |
| **10c** | 3.5 ± 2.3 | 34 ± 1 | 1.4 ± 0.9 | 8.0 ± 1.4 | 18 ± 4 |
| **13c** | 18 ± 4 | 146 ± 3 | 12 ± 12 | 79 ± 51 | 58 ± 4 |
| 5-FU | 0.45 ± 0.07 | 1.5 ± 0.4 | 0.18 ± 0.06 | 1.1 ± 0.4 | 1.0 ± 0.8 |
| 5-FUrd | 0.014 ± 0.002 | 0.017 ± 0.000 | 0.022 ± 0.007 | 1.6 ± 0.4 | 0.016 ± 0.000 |
| 5-FdUrd | 0.0094 ± 0.0094 | 8.5 ± 4.7 | 0.021 ± 0.003 | 0.061 ± 0.069 | 0.11 ± 0.04 |

[a] 50% Inhibitory concentration required to inhibit tumor cell proliferation by 50%. Data are the mean (±S.D.) of at least 2 to 3 independent experiments.

**Table 3**
Inhibitory activity of compounds **9c**, **10c** and **13c** and 5-FU against incorporation of radiolabeled precursors of DNA and RNA synthesis in murine leukemia L1210 cells.

| Compound | $IC_{50}$[a] (μM) | | |
|---|---|---|---|
| | [*methyl*-$^3$H]dThd | [6-$^3$H]dUrd | [5-$^3$H]Urd |
| | Without preincubation | | |
| **9c** | 349 ± 82 | 3.1 ± 0.1 | 63 ± 31 |
| **10c** | >500 | 37 ± 9 | >100 |
| **13c** | >500 | 85 ± 22 | >100 |
| 5-FU | >500 | 0.97 ± 0.61 | 51 ± 33 |
| | Upon 24 h preincubation | | |
| **9c** | 294 ± 15 | 2.5 ± 0.6 | 16 ± 5 |
| **10c** | >500 | 7.9 ± 1.0 | 84 ± 6 |
| **13c** | >500 | 18 ± 2 | 282 ± 15 |
| 5-FU | >500 | 0.37 ± 0.05 | 4.9 ± 1.3 |

[a] 50% Inhibitory concentration, or compound concentration required to inhibit incorporation of radiolabel into DNA or RNA by 50%.




**Scheme 3.** *In situ* determination of TS activity by measurement of the tritium release from [5-$^3$H]dUrd or [5-$^3$H]dCyd added to the tumor cell cultures. dCK: 2′-Deoxycytidine kinase, dCyd-D: 2′-deoxycytidine deaminase, dCMP-D: 2′-deoxycytidylate deaminase, 5′Nu: 5′-nucleotidase, DNA pol: DNA polymerase, TK: thymidine kinase.

[5-$^3$H]deoxycytidine ([5-$^3$H]dCyd) (Scheme 3) [48]. Indeed, after [5-$^3$H]dUrd or [5-$^3$H]dCyd have been converted to [5-$^3$H]dUMP, the C-5 tritium atom on the pyrimidine base is released during the TS-catalyzed reductive methylation of the C-5 of the uracil ring (Scheme 3). The ability of **9c**, **10c** and **13c**, 5-FU and 5-FdUrd to inhibit tritium release from [5-$^3$H]dUrd and [5-$^3$H]dCyd was therefore evaluated in L1210 cell cultures at a variety of compound concentrations. 5-FdUrd proved to be a potent inhibitor of TS *in situ*. Its $IC_{50}$'s for tritium release from [5-$^3$H]dUrd and [5-$^3$H]dCyd ranked between 0.0004 and 0.0007 μM, irrespective of the time period of preincubation of the cells with 5-FdUrd (Table 4). The inhibitory activities of 5-FU and **9c**, **10c** and **13c** were much less pronounced than 5-FdUrd, especially after only 15 min preincubation of the drugs. However, a longer preincubation time of the cells (for 4 h and, even more, for 24 h) with 5-FU and the 5-fluorouracil derivatives before measuring TS activity in the intact cells revealed a much more pronounced inhibitory activity of the drugs against TS *in situ*. Taken all data together, our observations indicate that 5-FU and **9c**, **10c** and **13c** indeed need several metabolic conversion steps before reaching TS as the target enzyme for inhibition, and support the view that **9c**, **10c** and **13c** most likely act as prodrugs of 5-FU to exert their eventual cytostatic activity (Scheme 3).

The first predominant activation step for 5-FU to exert its cytostatic activity is catalyzed by orotic acid phosphoribosyltransferase (OPRT) converting it to 5-FUMP. This enzyme is selectively inhibited by K-oxonate [49]. Interestingly, when 5-FU and **9c** are combined with the K-oxonate inhibitor at 200, 100 and 50 μM in L1210 cell cultures, the cytostatic activity of both 5-FU and **13c** are markedly decreased (>90%). Instead, the cytostatic activity of 5-FdUrd (that does not need metabolic activation by OPRT) is unaffected by the addition of K-oxonate (data not shown). These findings are strongly suggestive for the obligatory release of 5-FU from **9c** to eventually display a pronounced cytostatic activity, and therefore point to **9c** acting as a prodrug of 5-FU. Finally, if **9c** would act as a direct inhibitor of TS, it would be expected that the 5-(trifluoromethyl) uracil derivative **9e** would also similarly act as a direct TS inhibitor and shows a similar cytostatic profile as **9c**. However, **9e** is equally and moderately cytostatic against all four cell lines ($IC_{50}$: 23–46 μM). This points to the release of 5-(trifluoromethyl)uracil, a base that is virtually inactive as a cytostatic agent.

The effect of the test compounds on TS was less pronounced in HeLa cell cultures than in L1210 cell cultures (esp. for **9c**, **10c** and **13c**, 5-FU and 5-FUrd) since co-administration of dThd with the compounds resulted in a much lesser degree of cytostatic reversal in HeLa than in L1210 cell cultures (Table 2). These observations are in agreement with our findings that addition of uracil and uridine had a more pronounced effect on the cytostatic activity of the compounds in HeLa cell than in L1210 cell cultures. Thus, the contribution of TS inhibition (and thus inhibition of DNA synthesis) in the eventual cytostatic activity of the compounds may be less pronounced, while the inhibitory effect of the compounds on uridine metabolism (and thus inhibition of RNA synthesis) may be more pronounced in HeLa cell cultures than in L1210 cell cultures. Indeed, addition of uracil or uridine markedly decreased the cytostatic activity of **13c** (5- to 10-fold) and 5-FdUrd (6- to 11-fold) in HeLa cells, a phenomenon not being observed in L1210 cells (Table 2).

Compounds **9c**, **10c** and **13c** have also been examined for their potential to act as substrate of purified recombinant human thymidine phosphorylase (TP) [50] and uridine phosphorylase type 1 (UP-1) [51] (which may release 5-FU from the prodrug). UP-1 represents an isoform of uridine phosphorylase (UP), that is more widely distributed and more abundantly expressed than UP-2 [51]. UP-1 plays an important role in the activation of 5-FU and its prodrug capecitabine. The prodrugs were found not to be

**Table 4**
Inhibitory activity of **9c**, **10c** and **13c**, 5-FU and 5-FdUrd against tritium release from [5-$^3$H]dUrd and [5-$^3$H]dCyd in L1210 cell cultures.

| Compound | [$^3$H]release ($IC_{50}$[a]) from | | | | | |
|---|---|---|---|---|---|---|
| | [5-$^3$H]dUrd | | | [5-$^3$H]dCyd | | |
| | Upon preincubation of L1210 cells with drug for following time period: | | | | | |
| | 15 min | 4 h | 24 h | 15 min | 4 h | 24 h |
| **9c** | 8.1 ± 2.8 | 0.83 ± 0.085 | 0.063 ± 0.003 | >10 | 0.95 ± 0.007 | 0.071 ± 0.001 |
| **10c** | >10 | 8.7 ± 1.9 | 0.43 ± 0.18 | >10 | 9.3 ± 1.1 | 2.2 ± 2.0 |
| **13c** | ≥10 | 3.6 ± 5.6 | 0.57 ± 0.43 | 8.2 ± 0.35 | 5.5 ± 4.7 | 5.4 ± 1.8 |
| 5-FU | 7.0 ± 1.7 | 0.22 ± 0.18 | 0.020 ± 0.013 | ≥10 | 0.60 ± 0.035 | 0.06 ± 0.016 |
| 5-FdUrd | 0.0006 ± 0.0001 | 0.0004 ± 0.0003 | 0.0004 ± 0.0 | 0.0006 ± 0.0001 | 0.0007 ± 0.0004 | 0.0007 ± 0.0001 |

[a] 50% Inhibitory concentration, or compound concentration required to inhibit tritium release from [5-$^3$H]dUrd or [5-$^3$H]dCyd by 50%.

substrates for these enzymes under conditions where dThd (for TP) and Urd (for UP) were very efficiently converted to thymine or uracil, respectively (data not shown). Moreover, the cytostatic activity of the 5-FU prodrugs was not decreased in the presence of benzyl acyclic uridine (BAU), a potent inhibitor of UP. Also, the cytostatic activity of **9c** (and 5-FU) in L1210 cell cultures was not influenced by superinfection of the tumor cell cultures by *Mycoplasma hyorhinis* that induce pronounced levels of TP [52]. In contrast, 5′-deoxy-5-fluorouridine (5′-dFUrd), a prodrug of 5-FU that obligatorily needs TP activity to release 5-FU, is poorly cytostatic against L1210 cells, but becomes highly cytostatic in the presence of the *Mycoplasma*-infected tumor cells (data not shown). Therefore, TP and UP-1 are clearly not the enzymes responsible for efficient release of 5-FU from the 5-FU analogs. Other enzymes should be investigated for their potential to recognize the 5-FU analogs as a substrate for hydrolysis. Additional studies on **9c**, **10c** and **13c** are currently ongoing to further reveal their (anti)-metabolic properties. It also remains to be determined whether the 5-FU analogues have a superior pharmacokinetic profile as compared to the free parent compound, and thus have a potential advantage over 5-FU in terms of clinical application.

## 3. Conclusion

In conclusion, the synthesis of unsaturated keto and exomethylene arabinopyranonucleoside analogues bearing thymine, uracil, 5-fluorouracil, $N^4$-benzoyl cytosine and 5-(trifluoromethyl) uracil was undertaken. The target unsaturated ketonucleoside **9** and their exomethylene **10** and **13** intermediates were found not to inhibit the replication of a broad variety of viruses. However, the 5-fluorouracil derivatives showed an interesting cytostatic potential in various cell culture assays. Antimetabolic experiments revealed that TS is the principal target for the cytostatic activity of **9c**, **10c** and **13c**, which may act as novel type of prodrugs of 5-FU.

## 4. Experimental

### 4.1. General procedure

Melting points were recorded in a Mel-Temp apparatus and are uncorrected. Thin-layer chromatography (TLC) was performed on Merck precoated $60F_{254}$ plates. Reactions were monitored by TLC on silica gel, with detection by UV light (254 nm) or by charring with sulfuric acid. Flash chromatography was performed using silica gel (240–400 mesh, Merck). $^1$H NMR spectra were recorded at room temperature with a Bruker 400 MHz spectrometer using chloroform-d ($CDCl_3$) and methanol-$d_4$ ($CD_3OD$). Chemical shifts are reported in parts per million ($\delta$) downfield from tetramethylsilane (TMS) as internal standard. Mass spectra were obtained with a Micromass Platform LC (ESI-MS). Optical rotations were measured using Autopol I polarimeter.

All reactions were carried out in dry solvents. $CH_2Cl_2$ and 1,2-$EtCl_2$ were distilled from phosphorous pentoxide and stored over 4E molecular sieves. Acetonitrile, toluene (Tol) and N,N-dimethylformamide (DMF) were distilled from calcium hydride and stored over 3E molecular sieves. THF was freshly distilled under nitrogen from sodium/benzophenone before use and pyridine stored over pellets of potassium hydroxide.

### 4.2. Synthesis of 1-(2,3,4-tri-O-acetyl-α-D-arabinopyranosyl) nucleosides 2(a–e)

#### 4.2.1. 1-(2,3,4-Tri-O-acetyl-α-D-arabinopyranosyl)thymine (2a)

A mixture of thymine (2.77 g, 21.99 mmol), hexamethyldisilazane (HMDS) (5.75 mL, 27.27 mmol) and saccharin (185.29 mg, 1.01 mmol) in dry 1,2-$EtCl_2$ (65 mL) was refluxed for 30 min under nitrogen. To this were added 1,2,3,4-tetra-*O*-acetyl-D-arabinopyranose (**1**) (5.0 g, 15.71 mmol) and $SnCl_4$ (2.6 mL, 21.99 mmol). The reaction mixture was refluxed for 3 h, neutralized with saturated sodium bicarbonate and then extracted with $CH_2Cl_2$. The organic extract was dried over anhydrous sodium sulfate, filtered and evaporated to dryness. The residue was purified by flash chromatography (EtOAc/hexane, 6:4) to give compound **2a** (4.53 g, 75%, $R_f = 0.38$) as a white solid, m.p. 122–124 °C. $[\alpha]_D^{22}$ −12.0 (c 0.500, $CHCl_3$); UV ($CHCl_3$): $\lambda_{max}$ 262 nm ($\epsilon$ 6557); $^1$H NMR ($CDCl_3$): $\delta$ 8.25 (br s, 1H, NH), 7.19 (s, 1H, H-6), 5.72 (d, 1H, $J_{1',2'} = 9.3$ Hz, H-1′), 5.40–5.33 (m, 2H, H-2′, H-4′), 5.20 (dd, 1H, $J_{2',3'} = 10.1$ Hz, $J_{3',4'} = 3.5$ Hz, H-3′), 4.10 (dd, 1H, $J_{4',5b'} = 3.9$ Hz, $J_{5a',5b'} = 13.5$ Hz, H-5b′), 3.87 (d, 1H, H-5a′), 2.22, 2.02, 2.01 (3s, 9H, 3OAc), 1.97 (s, 3H, 5-$CH_3$); ESI-MS ($m/z$) 385.34 [M + H$^+$]; Anal. Calcd for $C_{16}H_{20}N_2O_9$: C 50.00, H 5.25, N 7.29. Found: C 50.11, H 5.49, N 7.51.

#### 4.2.2. 1-(2,3,4-Tri-O-acetyl-α-D-arabinopyranosyl)uracil (2b)

A mixture of uracil (2.46 g, 21.99 mmol), HMDS (5.75 mL, 27.26 mmol) and saccharin (185.29 mg, 1.01 mmol) in dry $CH_3CN$ (68 mL) was refluxed for 30 min under nitrogen. To this were added 1,2,3,4-tetra-*O*-acetyl-D-arabinopyranose (**1**) (5.0 g, 15.71 mmol) and $SnCl_4$ (2.6 mL, 21.99 mmol). The reaction mixture was refluxed for 2 h, neutralized with saturated sodium bicarbonate and then extracted with $CH_2Cl_2$. The organic extract was dried over anhydrous sodium sulfate, filtered and evaporated to dryness. The residue was purified by flash chromatography (EtOAc/hexane, 7:3) to give compound **2b** (4.42 g, 76%, $R_f = 0.42$) as a white foam. $[\alpha]_D^{22}$ −24.0 (c 0.500, $CHCl_3$); UV ($CHCl_3$): $\lambda_{max}$ 256 nm ($\epsilon$ 7632); $^1$H NMR ($CDCl_3$): $\delta$ 8.29 (br s, 1H, NH), 7.40 (d, 1H, $J_{5,6} = 8.2$ Hz, H-6), 5.82 (dd, 1H, $J_{1',5} = 1.3$ Hz, H-5), 5.73 (d, 1H, $J_{1',2'} = 9.2$ Hz, H-1′), 5.40 (m, 1H, $J_{4',5b'} = 2.0$ Hz, H-4′), 5.33 (m, 1H, H-2′), 5.20 (dd, 1H, $J_{3',4'} = 3.4$ Hz, H-3′), 4.11 (dd, 1H, $J_{5a',5b'} = 13.5$ Hz, H-5b′), 3.86 (d, 1H, H-5a′), 2.20, 2.03, 2.02 (3s, 9H, 3OAc); ESI-MS ($m/z$) 371.33 [M + H$^+$]; Anal. Calcd for $C_{15}H_{18}N_2O_9$: C 48.65, H 4.90, N 7.56. Found: C 49.04, H 5.24, N 7.41.

#### 4.2.3. 1-(2,3,4-Tri-O-acetyl-α-D-arabinopyranosyl)5-fluorouracil (2c)

A mixture of 5-fluorouracil (2.54 g, 19.55 mmol), HMDS (5.75 mL, 27.26 mmol) and saccharin (164.73 mg, 0.90 mmol) in dry $CH_3CN$ (80 mL) was refluxed for 30 min under nitrogen. To this were added 1,2,3,4-tetra-*O*-acetyl-D-arabinopyranose (**1**) (4.8 g, 15.04 mmol) and $Me_3SiOSO_2CF_3$ (3.8 mL, 21.06 mmol). The reaction mixture was refluxed for 2 h, neutralized with saturated sodium bicarbonate and then extracted with $CH_2Cl_2$. The organic extract was dried over anhydrous sodium sulfate, filtered and evaporated to dryness. The residue was purified by flash chromatography (EtOAc/hexane, 6:4) to give compound **2c** (5.0 g, 85%, $R_f = 0.44$) as a white foam. $[\alpha]_D^{22}$ −24.0 (c 0.500, $CHCl_3$); UV ($CHCl_3$): $\lambda_{max}$ 263 nm ($\epsilon$ 7878); $^1$H NMR ($CDCl_3$): $\delta$ 8.99 (br s, 1H, NH), 7.48 (d, 1H, $J_{F5,6} = 5.6$ Hz, H-6), 5.75 (d, 1H, $J_{1',2'} = 8.5$ Hz, H-1′), 5.45–5.23 (m, 3H, H-2′, H-3′, H-4′), 4.13, 3.91 (q, AB-system, 2H, $J = 13.3$ Hz, H-5′), 2.24, 2.05, 2.04 (3s, 9H, 3OAc); ESI-MS ($m/z$) 389.32 [M + H$^+$]; Anal. Calcd for $C_{15}H_{17}FN_2O_9$: C 46.40, H 4.41, N 7.21. Found: C 46.14, H 4.64, N 7.49.

#### 4.2.4. 1-(2,3,4-Tri-O-acetyl-α-D-arabinopyranosyl)-$N^4$-benzoyl cytosine (2d)

A mixture of $N^4$-benzoyl cytosine (4.40 g, 20.42 mmol), HMDS (5.34 mL, 25.32 mmol) and saccharin (172.09 mg, 0.94 mmol) in dry $CH_3CN$ (80 mL) was refluxed for 30 min under nitrogen. To this were added 1,2,3,4-tetra-*O*-acetyl-D-arabinopyranose (**1**) (5.0 g, 15.71 mmol) and $Me_3SiOSO_2CF_3$ (4.0 mL, 21.99 mmol). The reaction mixture was refluxed for 1 h, neutralized with saturated sodium

bicarbonate and then extracted with $CH_2Cl_2$. The organic extract was dried over anhydrous sodium sulfate, filtered and evaporated to dryness. The residue was purified by flash chromatography (EtOAc/hexane, 7:3) to give compound **2d** (5.8 g, 78%, $R_f = 0.32$) as a white solid, m.p. 159–161 °C. $[\alpha]_D^{22}$ −22.0 (c 0.500, $CHCl_3$); UV ($CHCl_3$): $\lambda_{max}$ 262 nm ($\epsilon$ 17413); $^1$H NMR ($CDCl_3$): $\delta$ 7.62 (d, 1H, $J_{5,6}$ = 7.3 Hz, H-6), 7.90–7.50 (m, 6H, Bz and H-5), 6.01 (d, 1H, $J_{1',2'}$ = 8.9 Hz, H-1′), 5.43 (m, 1H, $J_{4',5b'}$ = 1.9 Hz, H-4′), 5.35 (m, 1H, H-2′), 5.24 (dd, 1H, $J_{2',3'}$ = 10.1 Hz, $J_{3',4'}$ = 3.4 Hz, H-3′), 4.15 (dd, 1H, $J_{5a',5b'}$ = 13.2 Hz, H-5b′), 3.90 (d, 1H, H-5a′), 2.22, 2.03, 2.00 (3s, 9H, 3OAc); ESI-MS (*m*/*z*) 474.44 [M + H$^+$]; Anal. Calcd for $C_{22}H_{23}N_3O_9$: C 55.81, H 4.90 N 8.88. Found: C 55.44, H 4.64, N 8.61.

#### 4.2.5. 1-(2,3,4-Tri-O-acetyl-α-D-arabinopyranosyl)5-(trifluoromethyl) uracil (**2e**)

A mixture of 5-(trifluoromethyl)uracil (0.99 g, 5.5 mmol), HMDS (1.45 mL, 6.82 mmol) and saccharin (46.3 mg, 0.25 mmol) in dry $CH_3CN$ (21 mL) was refluxed for 30 min under nitrogen. To this were added 1,2,3,4-tetra-*O*-acetyl-D-arabinopyranose (**1**) (1.6 g, 5.0 mmol) and $Me_3SiOSO_2CF_3$ (1.26 mL, 7.0 mmol). The reaction mixture was refluxed for 1 h, neutralized with saturated sodium bicarbonate and then extracted with $CH_2Cl_2$. The organic extract was dried over anhydrous sodium sulfate, filtered and evaporated to dryness. The residue was purified by flash chromatography (EtOAc/hexane, 4:6) to give compound **2e** (1.93 g, 88%, $R_f = 0.3$) as a white foam. $[\alpha]_D^{22}$ −12.0 (c 0.500, $CHCl_3$); UV ($CHCl_3$): $\lambda_{max}$ 260 nm ($\epsilon$ 6689); $^1$H NMR ($CDCl_3$): $\delta$ 8.32 (br s, 1H, NH), 7.86 (s, 1H, H-6), 5.73 (d, 1H, $J_{1',2'}$ = 8.9 Hz, H-1′), 5.43–5.21 (m, 3H, H-2′, H-3′, H-4′), 4.17–3.86 (m, 2H, H-5′), 2.19, 2.14, 2.10 (3s, 9H, 3OAc); ESI-MS (*m*/*z*) 439.31 [M + H$^+$]; Anal. Calcd for $C_{16}H_{17}F_3N_2O_9$: C 43.84, H 3.91, N 6.39. Found: C 43.92, H 3.98, N 6.42.

### 4.3. Synthesis of 1-(α-D-arabinopyranosyl)nucleosides **3**(**a**–**e**)

#### 4.3.1. 1-(α-D-Arabinopyranosyl)thymine (**3a**)

Compound **2a** (4.53 g, 11.78 mmol) was treated with ammonia/MeOH (saturated at 0 °C, 0.66 L). The solution was stirred overnight at room temperature and then was concentrated under reduced pressure to give compound **3a** (2.95 g, 97%, $R_f = 0.30$ in EtOAc/MeOH, 8:2) as a white solid, m.p. 249–251 °C. $[\alpha]_D^{22}$ −18.0 (c 0.570, MeOH); UV (MeOH): $\lambda_{max}$ 262 nm ($\epsilon$ 7201); ESI-MS (*m*/*z*) 259.25 [M + H$^+$]; Anal. Calcd for $C_{10}H_{14}N_2O_6$: C 46.51, H 5.46, N 10.85. Found: C 46.83, H 5.32, N 10.46.

#### 4.3.2. 1-(α-D-Arabinopyranosyl)uracil (**3b**), 5-fluorouracil (**3c**), 5-(trifluoromethyl)uracil (**3e**)

Uracil, 5-fluorouracil and 5-(trifluoromethyl)uracil derivatives **3b**, **3c** and **3e** were synthesized by similar procedure as described for **3a**.

**3b**:(2.86 g, 98%, $R_f = 0.39$ in EtOAc/MeOH, 8:2). $[\alpha]_D^{22}$ −12.0 (c 0.500, MeOH); UV (MeOH): $\lambda_{max}$ 258 nm ($\epsilon$ 6869); ESI-MS (*m*/*z*) 245.22 [M + H$^+$]; Anal. Calcd for $C_9H_{12}N_2O_6$: C 44.27, H 4.95, N 11.47. Found: C 44.56, H 5.12, N 11.78.

**3c**: (3.1 g, 92%, $R_f = 0.44$ in EtOAc/MeOH, 8:2), m.p. 257–259 °C. $[\alpha]_D^{22}$ −48.0 (c 0.500, MeOH); UV (MeOH): $\lambda_{max}$ 266 nm ($\epsilon$ 7158); ESI-MS (*m*/*z*) 263.17 [M + H$^+$]; Anal. Calcd for $C_9H_{11}FN_2O_6$: C 41.23, H 4.23, N 10.68. Found: C 41.62, H 4.09, N 10.71.

**3e**: (1.3 g, 97%, $R_f = 0.14$ in EtOAc). $[\alpha]_D^{22}$ −34.0 (c 0.605, MeOH); UV (MeOH): $\lambda_{max}$ 260 nm ($\epsilon$ 6156); ESI-MS (*m*/*z*) 313.21 [M + H$^+$]; Anal. Calcd for $C_{10}H_{11}F_3N_2O_6$: C 38.47, H 3.55, N 8.97. Found: C 38.57, H 3.71, N 8.86.

#### 4.3.3. 1-(α-D-Arabinopyranosyl)-$N^4$-benzoyl cytosine (**3d**)

$N^4$-Benzoyl cytosine derivative **2d** (5.8 g, 12.25 mmol) was dissolved in ethanol-pyridine (122.5 mL + 36.7 mL), 2M NaOH (12.2 mL) was added and the mixture stirred for 11 min at room temperature. Amberlite IR-120 (H$^+$) was added to neutralize the base. The suspension was filtered, the resin was washed with EtOH and pyridine (100 mL + 100 mL) and the filtrate was evaporated. The solid residue was triturated with diethyl ether (2 × 30 mL) and $CH_2Cl_2$ (2 × 30 mL) and filtered. **3d** was obtained (3.36 g, 79%, $R_f = 0.4$ in EtOAc/MeOH, 8:2) as a white foam, and it was used without further purification. $[\alpha]_D^{22}$ −12.0 (c 0.500, MeOH); UV (MeOH): $\lambda_{max}$ 261 nm ($\epsilon$ 15313); ESI-MS (*m*/*z*) 348.33 [M + H$^+$]; Anal. Calcd for $C_{16}H_{17}N_3O_6$: C 55.33, H 4.93, N 12.10. Found: C 55.56, H 5.02, N 12.47.

### 4.4. Synthesis of 1-(3,4-O-isopropylidene-α-D-arabinopyranosyl) nucleosides **4**(**a**–**e**)

#### 4.4.1. 1-(3,4-O-Isopropylidene-α-D-arabinopyranosyl)thymine (**4a**)

To a stirred suspension of **3a** (2.95 g, 11.43 mmol) in anhydrous acetone (197 mL) and 2,2-dimethoxypropane (10 mL) was added *p*-toluenesulfonic acid monohydrate (0.37 g, 1.94 mmol). After 6 h the resulting solution was neutralized with triethylamine so that pH did not exceed 7. The solution was concentrated and the residue was purified by flash chromatography (EtOAc) to give **4a** (2.86 g, 84%, $R_f = 0.39$) as a white foam. $[\alpha]_D^{22}$ −48.0 (c 0.500, MeOH); UV (MeOH): $\lambda_{max}$ 262 nm ($\epsilon$ 11760); $^1$H NMR ($CDCl_3$): $\delta$ 7.26 (s, 1H, H-6), 5.51 (d, 1H, $J_{1',2'}$ = 8.8 Hz, H-1′), 4.37–4.28 (m, 3H, H-3′, H-4′, H-5a′), 4.02 (dd, 1H, $J_{4',5b'}$ = 1.7 Hz, $J_{5a',5b'}$ = 13.9 Hz, H-5b′), 3.77 (dd, 1H, $J_{2',3'}$ = 8.5 Hz, H-2′), 1.89 (s, 3H, 5-$CH_3$), 1.62, 1.42 (2s, 6H, 2$CH_3$); ESI-MS (*m*/*z*) 299.29 [M + H$^+$]; Anal. Calcd for $C_{13}H_{18}N_2O_6$: C 52.34, H 6.08, N 9.39. Found: C 52.65, H 5.97, N 9.63.

#### 4.4.2. 1-(3,4-O-Isopropylidene-α-D-arabinopyranosyl)uracil (**4b**), 5-fluorouracil (**4c**), $N^4$-benzoyl cytosine (**4d**), 5-(trifluoromethyl) uracil (**4e**)

Uracil, 5-fluorouracil, $N^4$-benzoyl cytosine and 5-(trifluoromethyl)uracil derivatives **4b**, **4c**, **4d** and **4e** were synthesized by similar procedure as described for **4a**.

**4b**: (2.76 g, 83%, $R_f = 0.46$ in EtOAc). $[\alpha]_D^{22}$ 6.0 (c 0.485, MeOH); UV (MeOH): $\lambda_{max}$ 259 nm ($\epsilon$ 6836); $^1$H NMR ($CD_3OD$): $\delta$ 7.61 (d, 1H, $J_{5,6}$ = 8.1 Hz, H-6), 5.72 (d, 1H, H-5), 5.37 (d, 1H, $J_{1',2'}$ = 9.5 Hz, H-1′), 4.24–4.28 (m, 2H, H-4′, H-5a′), 4.16 (t, 1H, $J_{3',4'}$ = 6.0 Hz, H-3′), 3.99 (dd, 1H, $J_{4',5b'}$ = 2.3 Hz, $J_{5a',5b'}$ = 13.6 Hz, H-5b′), 3.76 (dd, 1H, $J_{2',3'}$ = 7.3 Hz, H-2′), 1.54, 1.36 (2s, 6H, 2$CH_3$); ESI-MS (*m*/*z*) 285.25 [M + H$^+$]; Anal. Calcd for $C_{12}H_{16}N_2O_6$: C 50.70, H 5.67, N 9.85. Found: C 50.85, H 5.80, N 9.98.

**4c**: (2.81 g, 79%, $R_f = 0.6$ in EtOAc), m.p. 183–185 °C. $[\alpha]_D^{22}$ −60.0 (c 0.500, MeOH); UV (MeOH): $\lambda_{max}$ 266 nm ($\epsilon$ 4650); $^1$H NMR ($CD_3OD$): $\delta$ 7.83 (d, 1H, $J_{F5,6}$ = 6.5 Hz, H-6), 5.38 (dd, 1H, $J_{1',2'}$ = 9.3 Hz, $J_{1',F5}$ = 1.5 Hz, H-1′), 4.30–4.16 (m, 3H, H-3′, H-4′, H-5a′), 4.01 (dd, 1H, $J_{4',5b'}$ = 2.5 Hz, $J_{5a',5b'}$ = 13.6 Hz, H-5b′), 3.76 (dd, 1H, $J_{2',3'}$ = 7.0 Hz, H-2′), 1.56, 1.37 (2s, 6H, 2$CH_3$); ESI-MS (*m*/*z*) 303.27 [M + H$^+$]; Anal. Calcd for $C_{12}H_{15}FN_2O_6$: C 47.68, H 5.00, N 9.27. Found: C 47.81, H 4.79, N 9.46.

**4d**: (3.15 g, 84%, $R_f = 0.58$ in EtOAc/MeOH, 9:1), m.p. 211–213 °C. $[\alpha]_D^{22}$ −22.0 (c 0.317, MeOH); UV (MeOH): $\lambda_{max}$ 262 nm ($\epsilon$ 8737); $^1$H NMR ($CD_3OD$): $\delta$ 8.12 (d, 1H, $J_{5,6}$ = 7.4 Hz, H-6), 7.99–7.52 (m, 6H, Bz and H-5), 5.62 (d, 1H, $J_{1',2'}$ = 8.9 Hz, H-1′), 4.34–4.22 (m, 3H, H-3′, H-4′, H-5a′), 4.08 (dd, 1H, $J_{4',5b'}$ = 2.4 Hz, $J_{5a',5b'}$ = 13.6 Hz, H-5b′), 3.86 (dd, 1H, $J_{2',3'}$ = 8.8 Hz, H-2′), 1.57, 1.38 (2s, 6H, 2$CH_3$); ESI-MS (*m*/*z*) 388.36 [M + H$^+$]; Anal. Calcd for $C_{19}H_{21}N_3O_6$: C 58.91, H 5.46, N 10.85. Found: C 58.75, H 5.70, N 10.78.

**4e**: (1.17 g, 78%, $R_f = 0.3$ in EtOAc/hexane, 8:2), m.p. 105–107 °C. $[\alpha]_D^{22}$ −36.0 (c 0.500, MeOH); UV (MeOH): $\lambda_{max}$ 259 nm ($\epsilon$ 8070); $^1$H NMR ($CD_3OD$): $\delta$ 9.79 (br s, 1H, NH), 7.96 (s, 1H, H-6), 5.56 (d, 1H, $J_{1',2'}$ = 7.5 Hz, H-1′), 4.36–4.28 (m, 3H, H-3′, H-4′, H-5a′), 4.04 (dd, 1H, $J_{4',5b'}$ = 2.5 Hz, $J_{5a',5b'}$ = 13.6 Hz, H-5b′), 3.80 (t, 1H, $J_{2',3'}$ = 6.5 Hz,

H-2′), 1.58, 1.40 (2s, 6H, $2CH_3$); ESI-MS (*m*/*z*) 353.25 [M + $H^+$]; Anal. Calcd for $C_{13}H_{15}F_3N_2O_6$: C 44.32, H 4.29, N 7.95. Found: C 44.51, H 4.31, N 7.98.

### 4.5. Synthesis of 1-(2-O-acetyl-3,4-O-isopropylidene-α-D-arabinopyranosyl)nucleosides **5**(**a**–**e**)

#### 4.5.1. 1-(2-O-Acetyl-3,4-O-isopropylidene-α-D-arabinopyranosyl) thymine (**5a**)

Compound **4a** (1.66 g, 5.56 mmol) was dissolved in a mixture of pyridine (4.6 mL) and $Ac_2O$ (2.5 mL). The reaction was carried out at room temperature for 2 h, then was quenched with MeOH at 0 °C and concentrated. The residue was purified by flash chromatography (EtOAc/hexane, 9:1) to give **5a** (1.78 g, 94%, $R_f$ = 0.50) as a white solid, m.p. 227–229 °C. $[\alpha]_D^{22}$ −52.0 (c 0.500, $CHCl_3$); UV ($CHCl_3$): $\lambda_{max}$ 262 nm ($\epsilon$ 8160); $^1$H NMR ($CDCl_3$): $\delta$ 8.40 (br s, 1H, NH), 7.23 (s, 1H, H-6), 5.55 (d, 1H, $J_{1',2'}$ = 9.3 Hz, H-1′), 5.12 (dd, 1H, $J_{2',3'}$ = 7.0 Hz, H-2′), 4.41–4.28 (m, 3H, H-3′, H-4′, H-5a′), 3.97 (dd, 1H, $J_{4',5b'}$ = 2.3 Hz, $J_{5a',5b'}$ = 13.8 Hz, H-5b′), 2.05 (s, 3H, OAc), 1.94 (s, 3H, 5-$CH_3$), 1.62, 1.40 (2s, 6H, $2CH_3$); ESI-MS (*m*/*z*) 341.34 [M + $H^+$]; Anal. Calcd for $C_{15}H_{20}N_2O_7$: C 52.94, H 5.92, N 8.23. Found: C 53.10, H 5.78, N 8.52.

#### 4.5.2. 1-(2-O-Acetyl-3,4-O-isopropylidene-α-D-arabinopyranosyl) uracil (**5b**), 5-fluorouracil (**5c**), 5-(trifluoromethyl)uracil (**5e**)

Uracil, 5-fluorouracil and 5-(trifluoromethyl)uracil derivatives **5b**, **5c** and **5e** were synthesized by similar procedure as described for **5a**.

**5b**: (1.53 g, 89%, $R_f$ = 0.67 in EtOAc), m.p. 232–234 °C. $[\alpha]_D^{22}$ −10.0 (c 0.205, $CHCl_3$); UV ($CHCl_3$): $\lambda_{max}$ 257 nm ($\epsilon$ 5838); $^1$H NMR ($CDCl_3$): $\delta$ 8.52 (br s, 1H, NH), 7.44 (d, 1H, $J_{5,6}$ = 8.2 Hz, H-6), 5.78 (d, 1H, H-5), 5.56 (d, 1H, $J_{1',2'}$ = 9.4 Hz, H-1′), 5.11 (dd, 1H, $J_{2',3'}$ = 6.8 Hz, H-2′), 4.40 (d, 1H, $J_{5a',5b'}$ = 13.7 Hz, H-5a′), 4.32 (m, 2H, H-3′, H-4′), 3.98 (dd, 1H, $J_{4',5b'}$ = 2.1 Hz, H-5b′), 2.06 (s, 3H, OAc), 1.60, 1.39 (2s, 6H, $2CH_3$); ESI-MS (*m*/*z*) 327.32 [M + $H^+$]; Anal. Calcd for $C_{14}H_{18}N_2O_7$: C 51.53, H 5.56, N 8.59. Found: C 51.79, H 5.38, N 8.82.

**5c**: (1.6 g, 93%, $R_f$ = 0.66 in EtOAc/hexane, 9:1), m.p. 210–212 °C. $[\alpha]_D^{22}$ −56.0 (c 0.505, $CHCl_3$); UV ($CHCl_3$): $\lambda_{max}$ 265 nm ($\epsilon$ 8074); $^1$H NMR ($CDCl_3$): $\delta$ 8.56 (br s, 1H, NH), 7.54 (d, 1H, $J_{F5,6}$ = 5.9 Hz, H-6), 5.55 (dd, 1H, $J_{1',2'}$ = 8.8 Hz, $J_{1',F5}$ = 1.4 Hz, H-1′), 5.03 (dd, 1H, $J_{2',3'}$ = 6.8 Hz, H-2′), 4.40–4.28 (m, 3H, H-3′, H-4′, H-5a′), 3.97 (dd, 1H, $J_{4',5b'}$ = 2.3 Hz, $J_{5a',5b'}$ = 13.8 Hz, H-5b′), 2.07 (s, 3H, OAc), 1.59, 1.39 (2s, 6H, $2CH_3$); ESI-MS (*m*/*z*) 345.31 [M + $H^+$]; Anal. Calcd for $C_{14}H_{17}FN_2O_7$: C 48.84, H 4.98, N 8.14. Found: C 48.70, H 5.09, N 8.45.

**5e**: (1.21 g, 93%, $R_f$ = 0.3 in EtOAc/hexane, 4:6). $[\alpha]_D^{22}$ −28.0 (c 0.389, $CHCl_3$); UV ($CHCl_3$): $\lambda_{max}$ 265 nm ($\epsilon$ 8163); $^1$H NMR ($CDCl_3$): $\delta$ 8.56 (br s, 1H, NH), 8.05 (s, 1H, H-6), 5.64 (d, 1H, $J_{1',2'}$ = 7.7 Hz, H-1′), 5.06 (m, 1H, $J_{2',3'}$ = 6.6 Hz, H-2′), 4.43–4.32 (m, 3H, H-3′, H-4′, H-5a′), 3.97 (dd, 1H, $J_{4',5b'}$ = 2.31 Hz, $J_{5a',5b'}$ = 13.6 Hz, H-5b′), 2.10 (s, 3H, OAc), 1.61, 1.42 (2s, 6H, $2CH_3$); ESI-MS (*m*/*z*) 395.29 [M + $H^+$]; Anal. Calcd for $C_{15}H_{17}F_3N_2O_7$: C 45.69, H 4.35, N 7.10. Found: C 45.81, H 4.49, N 7.41.

#### 4.5.3. 1-(2-O-Acetyl-3,4-O-isopropylidene-α-D-arabinopyranosyl)-$N^4$-benzoyl cytosine (**5d**)

Compound **4d** (1.57 g, 4.06 mmol) was dissolved in a mixture of pyridine (24.1 mL) and $Ac_2O$ (0.36 mL). The reaction was carried out at 4 °C overnight, then was quenched with MeOH at 0 °C and concentrated. It was purified by flash chromatography (EtOAc/hexane, 8:2) (1.45 g, 83%, $R_f$ = 0.53 in EtOAc/MeOH, 9.5:0.5) and **5d** was obtained as a white solid, m.p. 133–135 °C. $[\alpha]_D^{22}$ −42.0 (c 0.500, $CHCl_3$); UV ($CHCl_3$): $\lambda_{max}$ 261 nm ($\epsilon$ 18443); $^1$H NMR ($CDCl_3$): $\delta$ 7.65 (d, 1H, $J_{5,6}$ = 7.3 Hz, H-6), 7.98–7.54 (m, 6H, Bz and H-5), 5.83 (d, 1H, $J_{1',2'}$ = 8.7 Hz, H-1′), 5.15–5.12 (m, 1H, $J_{2',3'}$ = 7.4 Hz, H-2′), 4.43–4.34 (m, 3H, H-3′, H-4′, H-5a′), 4.04 (dd, 1H, $J_{4',5b'}$ = 1.3 Hz, $J_{5a',5b'}$ = 13.7 Hz, H-5b′), 2.07 (s, 3H, OAc), 1.63, 1.43 (2s, 6H, $2CH_3$); ESI-MS (*m*/*z*) 430.41 [M + $H^+$]; Anal. Calcd for $C_{21}H_{23}N_3O_7$: C 58.74, H 5.40, N 9.79. Found: C 58.89, H 5.38, N 9.85.

### 4.6. Synthesis of 1-(2-O-acetyl-α-D-arabinopyranosyl)nucleosides **6**(**a**–**e**)

#### 4.6.1. 1-(2-O-Acetyl-α-D-arabinopyranosyl)thymine (**6a**)

Compound **5a** (1.78 g, 5.23 mmol) was dissolved in a mixture of $CH_2Cl_2$ (17.8 mL) and formic acid (17.8 mL, 90%). The solution was stirred for 3 h at room temperature and then was concentrated under reduced pressure. The residue was purified by flash chromatography (EtOAc) to give **6a** (1.41 g, 90%, $R_f$ = 0.12) as a white solid, m.p. 232–234 °C. $[\alpha]_D^{22}$ −38.0 (c 0.500, MeOH); UV (MeOH): $\lambda_{max}$ 262 nm ($\epsilon$ 14420); $^1$H NMR ($CD_3OD$): $\delta$ 7.67 (br s, 1H, NH), 5.59 (d, 1H, $J_{1',2'}$ = 9.2 Hz, H-1′), 5.23 (t, 1H, $J_{2',3'}$ = 9.4 Hz, H-2′), 4.03, 3.82 (q, AB-system, 2H, $J$ = 12.6 Hz, H-5′), 3.97 (m, 1H, H-4′), 3.90 (dd, 1H, $J_{3',4'}$ = 3.0 Hz, H-3′), 2.02 (s, 3H, OAc), 1.90 (s, 3H, 5-$CH_3$); ESI-MS (*m*/*z*) 301.24 [M + $H^+$]; Anal. Calcd for $C_{12}H_{16}N_2O_7$: C 48.00, H 5.37, N 9.33. Found: C 47.72, H 5.77, N 9.64.

#### 4.6.2. 1-(2-O-Acetyl-α-D-arabinopyranosyl)uracil (**6b**), 5-fluorouracil (**6c**), $N^4$-benzoyl cytosine (**6d**), 5-(trifluoromethyl)uracil (**6e**)

Uracil, 5-fluorouracil, $N^4$-benzoyl cytosine and 5-(trifluoromethyl)uracil derivatives **6b**, **6c**, **6d** and **6e** were synthesized by similar procedure as described for **6a**.

**6b**: (1.13 g, 84%, $R_f$ = 0.14 in EtOAc), m.p. 115–117 °C. $[\alpha]_D^{22}$ −40.0 (c 0.500, MeOH); UV (MeOH): $\lambda_{max}$ 259 nm ($\epsilon$ 6471); $^1$H NMR ($CD_3OD$): $\delta$ 7.76 (d, 1H, $J_{5,6}$ = 8.1 Hz, H-6), 5.70 (d, 1H, H-5), 5.57 (d, 1H, $J_{1',2'}$ = 9.3 Hz, H-1′), 5.18 (t, 1H, $J_{2',3'}$ = 9.3 Hz, H-2′), 4.61 (dd, 1H, $J_{4',5b'}$ = 1.9 Hz, $J_{5a',5b'}$ = 12.7 Hz, H-5b′), 3.93 (m, 1H, H-4′), 3.86 (dd, 1H, $J_{3',4'}$ = 3.3 Hz, H-3′), 3.79 (d, 1H, H-5a′), 1.99 (s, 3H, OAc); ESI-MS (*m*/*z*) 287.25 [M + $H^+$]; Anal. Calcd for $C_{11}H_{14}N_2O_7$: C 46.16, H 4.93, N 9.79. Found: C 46.32, H 4.75, N 9.83.

**6c**: (1.26 g, 89%, $R_f$ = 0.28 in EtOAc). $[\alpha]_D^{22}$ −52.0 (c 0.505, MeOH); UV (MeOH): $\lambda_{max}$ 264 nm ($\epsilon$ 8416); $^1$H NMR ($CD_3OD$): $\delta$ 8.05 (d, 1H, $J_{F5,6}$ = 6.3 Hz, H-6), 5.60 (d, 1H, $J_{1',2'}$ = 8.8 Hz, H-1′), 5.15 (t, 1H, $J_{2',3'}$ = 8.8 Hz, H-2′), 4.04, 3.84 (q, AB-system, 2H, $J$ = 12.5 Hz, H-5′), 3.96 (m, 1H, H-4′), 3.91 (dd, 1H, $J_{3',4'}$ = 2.6 Hz, H-3′), 2.03 (s, 3H, OAc); ESI-MS (*m*/*z*) 305.21 [M + $H^+$]; Anal. Calcd for $C_{11}H_{13}FN_2O_7$: C 43.43, H 4.31, N 9.21. Found: C 43.51, H 4.66, N 9.43.

**6d**: (1.04 g, 79%, $R_f$ = 0.15 in EtOAc/MeOH, 9.5:0.5), m.p. 213–215 °C. $[\alpha]_D^{22}$ −50.0 (c 0.500, MeOH); UV (MeOH): $\lambda_{max}$ 263 nm ($\epsilon$ 11313); $^1$H NMR ($CD_3OD$): $\delta$ 8.25 (d, 1H, $J_{5,6}$ = 7.5 Hz, H-6), 7.98–7.51 (m, 6H, Bz and H-5), 5.80 (d, 1H, $J_{1',2'}$ = 9.2 Hz, H-1′), 5.21 (t, 1H, $J_{2',3'}$ = 9.2 Hz, H-2′), 4.06 (dd, 1H, $J_{4',5b'}$ = 1.8 Hz, $J_{5a',5b'}$ = 12.7 Hz, H-5b′), 3.97 (m, 1H, H-4′), 3.92 (dd, 1H, $J_{3',4'}$ = 3.3 Hz, H-3′), 3.86 (d, 1H, H-5a′), 1.96 (s, 3H, OAc); ESI-MS (*m*/*z*) 390.38 [M + $H^+$]; Anal. Calcd for $C_{18}H_{19}N_3O_7$: C 55.53, H 4.92, N 10.79. Found: C 55.32, H 4.75, N 10.83.

**6e**: (1.0 g, 95%, $R_f$ = 0.28 in EtOAc). $[\alpha]_D^{22}$ −28.0 (c 0.483, MeOH); UV (MeOH): $\lambda_{max}$ 260 nm ($\epsilon$ 7913); $^1$H NMR ($CD_3OD$): $\delta$ 8.37 (s, 1H, H-6), 5.57 (d, 1H, $J_{1',2'}$ = 9.3 Hz, H-1′), 5.06 (t, 1H, $J_{2',3'}$ = 9.4 Hz, H-2′), 3.99–3.75 (m, 4H, H-5′, H-4′, H-3′), 1.94 (s, 3H, OAc); ESI-MS (*m*/*z*) 355.26 [M + $H^+$]; Anal. Calcd for $C_{12}H_{13}F_3N_2O_7$: C 40.69, H 3.70, N 7.91. Found: C 40.82, H 3.78, N 7.99.

### 4.7. Synthesis of 1-(2-O-acetyl-3,4-dideoxy-α-D-glycero-pent-3-enopyranosyl)nucleosides **7**(**a**–**e**)

#### 4.7.1. 1-(2-O-Acetyl-3,4-dideoxy-α-D-glycero-pent-3-enopyranosyl)thymine (**7a**)

Imidazole (0.67 g, 9.88 mmol), $Ph_3P$ (5.18 g, 19.77 mmol) and iodoform (3.89 g, 9.88 mmol) were added to the suspension of **6a** (1.41 g, 4.71 mmol) in 40 mL of dry Tol/DMF (4:1). The reaction

mixture was heated (120 °C, oil bath) under nitrogen for 1.5 h, concentrated in vacuum and the residue diluted with EtOAc, washed with saturated sodium bicarbonate, sodium thiosulfate and water. The organic extract was dried over anhydrous sodium sulfate, filtered and evaporated to dryness. The residue was purified by flash chromatography (EtOAc/hexane, 6:4) yielded **7a** (0.81 g, 65%, $R_f = 0.68$ in EtOAc), as a colorless oil. $[\alpha]_D^{22}$ 4.0 (c 0.500, $CHCl_3$); UV ($CHCl_3$): $\lambda_{max}$ 264 nm ($\epsilon$ 8684); $^1$H NMR ($CDCl_3$): $\delta$ 8.44 (br s, 1H, NH), 7.19 (s, 1H, H-6), 6.00–5.97 (m, 1H, $J_{3',4'}$ = 10.3 Hz, H-3′), 5.79–5.75 (m, 2H, $J_{4',5b'}$ = 2.4 Hz, H-1′, H-4′), 5.51 (m, 1H, $J_{2',3'}$ = 7.9 Hz, H-2′), 4.48–4.37 (m, 2H, $J_{5a',5b'}$ = 17.0 Hz, H-5a′, H-5b′), 2.05 (s, 3H, OAc), 1.94 (s, 3H, 5-$CH_3$); ESI-MS (*m/z*) 267.26 [M + $H^+$]; Anal. Calcd for $C_{12}H_{14}N_2O_5$: C 54.13, H 5.30, N 10.52. Found: C 54.35, H 5.41, N 10.68.

#### 4.7.2. 1-(2-O-Acetyl-3,4-dideoxy-α-D-glycero-pent-3-enopyranosyl) uracil (**7b**), 5-fluorouracil (**7c**), $N^4$-benzoyl cytosine (**7d**), 5-(trifluoromethyl)uracil (**7e**)

Uracil, 5-fluorouracil, $N^4$-benzoyl cytosine and 5-(trifluoromethyl)uracil derivatives **7b**, **7c**, **7d** and **7e** were synthesized by similar procedure as described for **7a**.

**7b**: (0.66 g, 66%, $R_f = 0.68$ in EtOAc). $[\alpha]_D^{22}$ −4.0 (c 0.500, $CHCl_3$); UV ($CHCl_3$): $\lambda_{max}$ 258 nm ($\epsilon$ 7583); $^1$H NMR ($CDCl_3$): $\delta$ 8.41 (br s, 1H, NH), 7.38 (d, 1H, $J_{5,6}$ = 8.2 Hz, H-6), 5.99 (m, 1H, H-1′), 5.78 (m, 3H, H-2′, H-3′, H-4′), 5.50 (d, 1H, H-5), 4.48–4.37 (m, 2H, H-5a′, H-5b′), 2.06 (s, 3H, OAc); ESI-MS (*m/z*) 253.21 [M + $H^+$]; Anal. Calcd for $C_{11}H_{12}N_2O_5$: C 52.38, H 4.80, N 11.11. Found: C 52.51, H 5.02, N 11.48.

**7c**: (0.65 g, 56%, $R_f = 0.8$ in EtOAc). $[\alpha]_D^{22}$ −10.0 (c 0.460, $CHCl_3$); UV ($CHCl_3$): $\lambda_{max}$ 266 nm ($\epsilon$ 7726); $^1$H NMR ($CDCl_3$): $\delta$ 8.71 (br s, 1H, NH), 7.46 (d, 1H, $J_{F5,6}$ = 5.6 Hz, H-6), 6.02 (d, 1H, $J_{2',3'}$ = 9.9 Hz, H-3′), 5.77 (m, 2H, H-1′, H-4′), 5.47 (d, 1H, $J_{1',2'}$ = 6.8 Hz, H-2′), 4.51, 4.42 (q, AB-system, 2H, $J$ = 17.0 Hz, H-5′), 2.09 (s, 3H, OAc); ESI-MS (*m/z*) 271.22 [M + $H^+$]; Anal. Calcd for $C_{11}H_{11}FN_2O_5$: C 48.89, H 4.10, N 10.37. Found: C 48.72, H 4.43, N 10.44.

**7d**: (0.58 g, 61%, $R_f = 0.73$ in EtOAc/MeOH, 9.5:0.5). $[\alpha]_D^{22}$ −12.0 (c 0.300, $CHCl_3$); UV ($CHCl_3$): $\lambda_{max}$ 262 nm ($\epsilon$ 28770); $^1$H NMR ($CDCl_3$): $\delta$ 7.82 (d, 1H, $J_{5,6}$ = 7.4 Hz, H-6), 7.92–7.49 (m, 6H, Bz and H-5), 6.06–5.99 (m, 2H, H-1′, H-3′), 5.78 (dd, 1H, $J_{3',4'}$ = 10.1 Hz, $J_{4',5b'}$ = 1.8 Hz, H-4′), 5.55–5.51 (m, 1H, H-2′), 4.52–4.41 (m, 2H, $J_{5a',5b'}$ = 17.3 Hz, H-5a′, H-5b′), 2.05 (s, 3H, OAc); ESI-MS (*m/z*) 356.32 [M + $H^+$]; Anal. Calcd for $C_{18}H_{17}N_3O_5$: C 60.84, H 4.82, N 11.83. Found: C 60.51, H 4.42, N 11.48.

**7e**: (0.603 g, 65%, $R_f = 0.28$ in EtOAc/hexane, 3:7). $[\alpha]_D^{22}$ −16.0 (c 0.633, $CHCl_3$); UV ($CHCl_3$): $\lambda_{max}$ 258 nm ($\epsilon$ 8627); $^1$H NMR ($CDCl_3$): $\delta$ 8.25 (br s, 1H, NH), 7.79 (s, 1H, H-6), 5.98–5.92 (m, 1H, H-3′), 5.72–5.68 (m, 2H, H-1′, H-4′), 5.40–5.35 (m, 1H, H-2′), 4.47–4.31 (m, 2H, H-5′), 1.99 (s, 3H, OAc); ESI-MS (*m/z*) 321.24 [M + $H^+$]; Anal. Calcd for $C_{12}H_{11}F_3N_2O_5$: C 45.01, H 3.46, N 8.75. Found: C 45.17, H 3.52, N 8.84.

### 4.8. Synthesis of 1-(3,4-dideoxy-α-D-glycero-pent-3-enopyranosyl) nucleosides **8**(**a**–**e**)

#### 4.8.1. 1-(3,4-Dideoxy-α-D-glycero-pent-3-enopyranosyl)thymine (**8a**)

Compound **7a** (0.81 g, 3.06 mmol) was treated with ammonia/MeOH (saturated at 0 °C, 184 mL). The solution was stirred for 3 h at room temperature and then was concentrated under reduced pressure. Purification by flash chromatography (EtOAc) yielded **8a** (0.50 g, 73%, $R_f = 0.37$) as a white foam. $[\alpha]_D^{22}$ 22.0 (c 0.500, $CHCl_3$); UV ($CHCl_3$): $\lambda_{max}$ 260 nm ($\epsilon$ 7769); $^1$H NMR ($CD_3OD$): $\delta$ 7.76 (br s, 1H, NH), 7.53 (s, 1H, H-6), 5.92–5.81 (m, 2H, $J_{3',4'}$ = 10.3 Hz, $J_{4',5b'}$ = 3.0 Hz, H-3′, H-4′), 5.52 (d, 1H, $J_{1',2'}$ = 8.2 Hz, H-1′), 4.44–4.29 (m, 3H, $J_{5a',5b'}$ = 17.5 Hz, H-2′, H-5a′, H-5b′), 1.91 (s, 3H, 5-$CH_3$); ESI-MS: (*m/z*) 225.23 [M + $H^+$]; Anal. Calcd for $C_{10}H_{12}N_2O_4$: C 53.57, H 5.39, N 12.49. Found: C 53.42, H 5.47, N 12.58.

#### 4.8.2. 1-(3,4-Dideoxy-α-D-glycero-pent-3-enopyranosyl)uracil (**8b**), 5-fluorouracil (**8c**), 5-(trifluoromethyl)uracil (**8e**)

Uracil, 5-fluorouracil and 5-(trifluoromethyl)uracil derivatives **8b**, **8c** and **8e** were synthesized by similar procedure as described for **8a**.

**8b**: (0.40 g, 74%, $R_f = 0.23$ in EtOAc). $[\alpha]_D^{22}$ 4.0 (c 0.454, MeOH); UV (MeOH): $\lambda_{max}$ 260 nm ($\epsilon$ 3985); $^1$H NMR ($CDCl_3$): $\delta$ 7.66 (d, 1H, $J_{5,6}$ = 8.1 Hz, H-6), 5.89–5.87 (m, 1H, $J_{3',4'}$ = 10.2 Hz, H-3′), 5.81–5.79 (m, 1H, $J_{4',5b'}$ = 3.8 Hz, H-4′), 5.72 (d, 1H, H-5), 5.50 (d, 1H, $J_{1',2'}$ = 8.1 Hz, H-1′), 4.40–4.36 (m, 2H, H-2′, H-5a′), 4.32–4.28 (m, 1H, H-5b′); ESI-MS: (*m/z*) 211.17 [M + $H^+$]; Anal. Calcd for $C_9H_{10}N_2O_4$: C 51.43, H 4.80, N 13.33. Found: C 51.52, H 4.67, N 13.57.

**8c**: (0.40 g, 75%, $R_f = 0.55$ in EtOAc), m.p. 222–224 °C. $[\alpha]_D^{22}$ 18.0 (c 0.500, MeOH); UV (MeOH): $\lambda_{max}$ 269 nm ($\epsilon$ 6732); $^1$H NMR ($CDCl_3$): $\delta$ 7.93 (d, 1H, $J_{F5,6}$ = 6.5 Hz, H-6), 5.91 (dd, 1H, $J_{3',4'}$ = 10.9 Hz, $J_{4',5b'}$ = 1.9 Hz, H-4′), 5.81 (m, 1H, H-3′), 5.52 (d, 1H, $J_{1',2'}$ = 7.9 Hz, H-1′), 4.45–4.33 (m, 3H, H-2′, H-5a′, H-5b′); ESI-MS: (*m/z*) 229.16 [M + $H^+$]; Anal. Calcd for $C_9H_9FN_2O_4$: C 47.37, H 3.98, N 12.28. Found: C 47.59, H 4.08, N 12.57.

**8e**: (0.489 g, 95%, $R_f$ = 0.25 in EtOAc/hexane, 1:1), m.p. 119–121 °C. $[\alpha]_D^{22}$ −2.0 (c 0.500, $CHCl_3$); UV ($CHCl_3$): $\lambda_{max}$ 260 nm ($\epsilon$ 9787); $^1$H NMR ($CDCl_3$): $\delta$ 9.31 (br s, 1H, NH), 7.85 (s, 1H, H-6), 5.89 (m, 2H, H-4′, H-3′), 5.63 (d, 1H, $J_{1',2'}$ = 7.9 Hz, H-1′), 4.52–4.26 (m, 3H, H-2′, H-5a′, H-5b′); ESI-MS: (*m/z*) 279.20 [M + $H^+$]; Anal. Calcd for $C_{10}H_9F_3N_2O_4$: C 43.18, H 3.26, N 10.07. Found: C 43.51, H 3.34, N 10.26.

#### 4.8.3. 1-(3,4-Dideoxy-α-D-glycero-pent-3-enopyranosyl)-$N^4$-benzoyl cytosine (**8d**)

$N^4$-benzoyl cytosine derivative **8d** was synthesized from **7d** by similar procedure as described for **3d**. (0.33 g, 66%, $R_f$ = 0.36 in $CH_2Cl_2$/MeOH, 9.5:0.5), m.p. 108–110 °C. $[\alpha]_D^{22}$ −44.0 (c 0.500, MeOH); UV (MeOH): $\lambda_{max}$ 262 nm ($\epsilon$ 19310); $^1$H NMR ($CDCl_3$): $\delta$ 7.91 (d, 1H, $J_{5,6}$ = 7.6 Hz, H-6), 7.97–7.50 (m, 6H, Bz and H-5), 5.93–5.85 (m, 2H, H-3′, H-4′), 5.75 (d, 1H, $J_{1',2'}$ = 7.6 Hz, H-1′), 4.52–4.38 (m, 2H, H-5a′, H-5b′), 4.31–4.28 (m, 1H, H-2′); ESI-MS: (*m/z*) 314.28 [M + $H^+$]; Anal. Calcd for $C_{16}H_{15}N_3O_4$: C 61.34, H 4.83, N 13.41. Found: C 61.56, H 4.67, N 13.57.

### 4.9. Synthesis of 1-(3,4-dideoxy-α-pent-3-enopyranosyl-2-ulose) nucleosides **9**(**a**–**e**)

#### 4.9.1. 1-(3,4-Dideoxy-α-pent-3-enopyranosyl-2-ulose)thymine (**9a**)

A mixture of **8a** (0.50 g, 2.24 mmol), PDC (1.01 g, 2.69 mmol) and $Ac_2O$ (0.63 mL, 6.72 mmol) was stirred in dry $CH_2Cl_2$ (38 mL) for 3 h, under nitrogen at room temperature. Purification by flash chromatography (EtOAc/hexane, 8:2) yielded pure **9a** (0.40 g, 80%, $R_f = 0.53$ in EtOAc) as a white solid m.p. 186–188 °C. $[\alpha]_D^{22}$ 6.0 (c 0.500, $CHCl_3$); UV ($CHCl_3$): $\lambda_{max}$ 260 nm ($\epsilon$ 9409); $^1$H NMR ($CDCl_3$): $\delta$ 8.67 (br s, 1H, NH), 7.23–7.19 (m, 1H, $J_{3',4'}$ = 10.3 Hz, $J_{4',5b'}$ = 2.1 Hz, H-4′), 6.93 (s, 1H, H-6), 6.36–6.32 (m, 1H, H-3′), 6.18 (br s, 1H, H-1′), 4.78–4.63 (m, 2H, $J_{5a',5b'}$ = 18.9 Hz, H-5a′, H-5b′), 1.93 (s, 3H, 5-$CH_3$); $^{13}$C NMR ($CDCl_3$): $\delta$ 13.27, 63.12, 96.39, 112.30, 130.21, 139.01, 151.21, 154.32, 160.92, 198.39; ESI-MS (*m/z*) 223.18 [M + $H^+$]; Anal. Calcd for $C_{10}H_{10}N_2O_4$: C 54.05, H 4.54, N 12.61. Found: C 54.22, H 4.38, N 12.71.

#### 4.9.2. 1-(3,4-Dideoxy-α-pent-3-enopyranosyl-2-ulose)uracil (**9b**), 5-fluorouracil (**9c**), $N^4$-benzoyl cytosine (**9d**), 5-(trifluoromethyl) uracil (**9e**)

Uracil, 5-fluorouracil, $N^4$-benzoyl cytosine and 5-(trifluoromethyl)uracil derivatives **9b**, **9c**, **9d** and **9e** were synthesized by similar procedure as described for **9a**.

**9b**: (0.31 g, 78%, $R_f = 0.46$ in EtOAc). $[\alpha]_D^{22}$ −2.0 (c 0.500, $CHCl_3$); UV ($CHCl_3$): $\lambda_{max}$ 254 nm ($\epsilon$ 8902); $^1$H NMR ($CDCl_3$): $\delta$ 8.30 (br s, 1H, NH), 7.23–7.18 (m, 1H, $J_{3',4'} = 10.5$ Hz, $J_{4',5b'} = 2.3$ Hz, H-4′), 7.08 (d, 1H, $J_{5,6} = 8.1$ Hz H-6), 6.36–6.31 (m, 1H, H-3′), 6.16 (s, 1H, H-1′), 5.76 (d, 1H, H-5), 4.80–4.61 (m, 2H, H-5a′, H-5b′); $^{13}$C NMR ($CDCl_3$): $\delta$ 63.11, 93.56, 104.01, 127.93, 143.09, 148.10, 151.01, 161.99, 198.02; ESI-MS ($m/z$) 209.19 [M + H$^+$]; Anal. Calcd for $C_9H_8N_2O_4$: C 51.93, H 3.87, N 13.46. Found: C 52.24, H 3.68, N 13.61.

**9c**: (0.34 g, 84%, $R_f$ = 0.37 in EtOAc/hexane, 7:3), m.p. 182–184 °C. $[\alpha]_D^{22}$ −8.0 (c 0.385, $CHCl_3$); UV ($CHCl_3$): $\lambda_{max}$ 266 nm ($\epsilon$ 5501); $^1$H NMR ($CDCl_3$): $\delta$ 8.66 (br s, 1H, NH), 7.24–7.22 (m, 1H, $J_{3',4'} = 10.2$ Hz, $J_{4',5b'} = 1.4$ Hz, H-4′), 7.18 (d, 1H, $J_{F5,6} = 5.5$ Hz, H-6), 6.36 (d, 1H, H-3′), 6.20 (s, 1H, H-1′), 4.81–4.67 (m, 2H, $J_{5a',5b'} = 18.8$ Hz, H-5a′, H-5b′); $^{13}$C NMR ($CDCl_3$): $\delta$ 62.91, 96.11, 130.12, 130.97, 142.77, 151.52, 152.73, 163.77, 197.96; ESI-MS ($m/z$) 227.17 [M + H$^+$]; Anal. Calcd for $C_9H_7FN_2O_4$: C 47.80, H 3.12, N 12.39. Found: C 47.46, H 3.43, N 12.22.

**9d**: (0.21 g, 63%, $R_f = 0.68$ in $CH_2Cl_2$/MeOH, 9.5:0.5). $[\alpha]_D^{22}$ −12.0 (c 0.500, $CHCl_3$); UV ($CHCl_3$): $\lambda_{max}$ 262 nm ($\epsilon$ 24840); $^1$H NMR ($CDCl_3$): $\delta$ 7.62 (d, 1H, $J_{5,6} = 7.1$ Hz, H-6), 7.94–7.44 (m, 6H, Bz and H-5), 7.20 (d, 1H, $J_{3',4'} = 10.6$ Hz, H-4′), 6.37–6.34 (m, 2H, H-1′, H-3′), 4.81–4.66 (m, 2H, H-5a′, H-5b′); $^{13}$C NMR ($CDCl_3$): $\delta$ 61.72, 62.97, 96.97, 109.37, 128.36, 129.92, 130.18, 132.11, 132.27, 133.98, 151.71, 152.17, 174.16, 198.31; ESI-MS ($m/z$) 312.31 [M + H$^+$]; Anal. Calcd for $C_{16}H_{13}N_3O_4$: C 61.73, H 4.21, N 13.50. Found: C 61.44, H 4.28, N 13.71.

**9e**: (0.289 g, 58%, $R_f$ = 0.32 in $CH_2Cl_2$/MeOH, 9.8:0.2), m.p. 189–191 °C. $[\alpha]_D^{22}$ −4.0 (c 0.350, $CHCl_3$); UV ($CHCl_3$): $\lambda_{max}$ 260 nm ($\epsilon$ 9981); $^1$H NMR ($CDCl_3$): $\delta$ 8.58 (br s, 1H, NH), 7.57 (s, 1H, H-6), 7.24–7.19 (m, 1H, $J_{3',4'} = 10.2$ Hz, H-4′), 6.36 (d, 1H, H-3′), 6.19 (s, 1H, H-1′), 4.82–4.65 (m, 2H, $J_{5a',5b'} = 19.0$ Hz, H-5a′, H-5b′); $^{13}$C NMR ($CDCl_3$): $\delta$ 66.06, 104.5, 110.23, 126.63, 127.24, 142.41, 148.85, 149.0, 165.76, 192.67; ESI-MS ($m/z$) 277.18 [M + H$^+$]; Anal. Calcd for $C_{10}H_7F_3N_2O_4$: C 43.49, H 2.55, N 10.14. Found: C 43.62, H 2.71, N 10.33.

### *4.10. Synthesis of 1-(2,3,4-trideoxy-2-methylene-α-pent-3-enopyranosyl)nucleosides **10**(**a**–**d**, **f**)*

#### *4.10.1. 1-(2,3,4-Trideoxy-2-methylene-α-pent-3-enopyranosyl) thymine (**10a**)*

To a stirred suspension of $Ph_3PCH_3Br$ (2.11 g, 5.91 mmol) and *t*-amyl alcohol (0.71 mL, 6.45 mmol) in dry THF (18.5 mL) was added NaH (0.25 g, 60% in oil, 10.32 mmol) at 0 °C under nitrogen and the reaction mixture was stirred for 2 h at ambient temperature. To this yellow phosphorous ylide was added a solution of **9a** (0.40 g, 1.79 mmol) in dry THF (1.1 mL) dropwise, at 0 °C under nitrogen. After the mixture was stirred for 30 min at ambient temperature, the reaction mixture was quenched with saturated sodium bicarbonate and extracted with EtOAc. The organic layer was washed with water, dried with sodium sulfate and evaporated. The residue was purified by flash chromatography (EtOAc/hexane, 9:1) to give **10a** (0.24 g, 60%, $R_f = 0.64$), as a white foam. $[\alpha]_D^{22}$ 12.0 (c 0.500, $CHCl_3$); UV ($CHCl_3$): $\lambda_{max}$ 263 nm ($\epsilon$ 8478); $^1$H NMR ($CDCl_3$): $\delta$ 8.74 (br s, 1H, NH), 7.09 (s, 1H, H-6), 6.46 (s, 1H, H-1′), 6.42 (d, 1H, $J_{3',4'} = 10.0$ Hz, H-4′), 6.03 (d, 1H, H-3′), 5.17, 4.87 (br s, 2H, methylene), 4.51, 4.35 (q, AB-system, 2H, $J = 17.4$ Hz, H-5′) 1.94 (s, 3H, 5-$CH_3$); $^{13}$C NMR ($CDCl_3$): $\delta$ 13.24, 63.79, 92.36, 108.25, 112.23, 125.34, 138.92, 140.32, 154.11, 154.92, 161.33; ESI-MS ($m/z$) 221.21 [M + H$^+$]; Anal. Calcd for $C_{11}H_{12}N_2O_3$: C 59.99, H 5.49, N 12.72. Found: C 59.82, H 5.68, N 12.93.

#### *4.10.2. 1-(2,3,4-Trideoxy-2-methylene-α-pent-3-enopyranosyl) uracil (**10b**), 5-fluorouracil (**10c**), $N^4$-benzoyl cytosine (**10d**)*

Uracil, 5-fluorouracil and $N^4$-benzoyl cytosine **10b**, **10c** and **10d** were synthesized by similar procedure as described for **10a**.

**10b**: (0.19 g, 62%, $R_f = 0.62$ in EtOAc). $[\alpha]_D^{22}$ −4.0 (c 0.550, $CHCl_3$); UV ($CHCl_3$): $\lambda_{max}$ 258 nm ($\epsilon$ 7559); $^1$H NMR ($CDCl_3$): $\delta$ 8.43 (br s, 1H, NH), 7.17 (d, 1H, $J_{5,6} = 8.1$ Hz, H-6), 6.38 (s, 1H, H-1′), 6.33 (d, 1H, $J_{3',4'} = 10.1$ Hz, H-4′), 5.95 (d, 1H, H-3′), 5.66 (d, 1H, H-5), 5.11, 4.81 (br s, 2H, methylene), 4.41, 4.24 (q, AB-system, 2H, $J = 17.6$ Hz, H-5′); $^{13}$C NMR ($CDCl_3$): $\delta$ 65.57, 87.93, 103.27, 114.56, 125.36, 136.90, 142.07, 150.07, 151.92, 162.61; ESI-MS ($m/z$) 207.19 [M + H$^+$]; Anal. Calcd for $C_{10}H_{10}N_2O_3$: C 58.25, H 4.89, N 13.59. Found: C 58.03, H 5.08, N 13.89.

**10c**: (0.19 g, 56%, $R_f = 0.76$ in EtOAc/hexane, 9:1). $[\alpha]_D^{22}$ −4.0 (c 0.100, $CHCl_3$); UV ($CHCl_3$): $\lambda_{max}$ 266 nm ($\epsilon$ 4790); $^1$H NMR ($CDCl_3$): $\delta$ 8.76 (br s, 1H, NH), 5.91 (d, 1H, $J_{F5,6} = 5.9$ Hz, H-6), 6.43 (s, 1H, H-1′), 6.39 (m, 1H, H-4′), 6.03 (d, 1H, $J_{3',4'} = 9.4$ Hz, H-3′), 5.21, 4.91 (br s, 2H, methylene), 4.48, 4.32 (q, AB-system, 2H, $J = 17.7$ Hz, H-5′); $^{13}$C NMR ($CDCl_3$): $\delta$ 64.52, 93.72, 108.98, 123.36, 130.14, 141.99, 143.76, 152.76, 153.01, 162.02; ESI-MS ($m/z$) 225.20 [M + H$^+$]; Anal. Calcd for $C_{10}H_9FN_2O_3$: C 53.57, H 4.05, N 12.50. Found: C 53.88, H 4.28, N 12.71.

**10d**: (0.11 g, 53%, $R_f = 0.43$ in EtOAc/hexane, 9:1). $[\alpha]_D^{22}$ 96.0 (c 0.250, $CHCl_3$); UV ($CHCl_3$): $\lambda_{max}$ 266 nm ($\epsilon$ 3988); $^1$H NMR ($CD_3OD$): $\delta$ 7.92 (d, 1H, $J_{5,6} = 7.2$ Hz, H-6), 7.69–7.45 (m, 6H, Bz and H-5), 6.66 (s, 1H, H-1′), 6.43 (m, 1H, $J_{3',4'} = 10.2$ Hz, H-4′), 6.03 (d, 1H, H-3′), 5.17, 4.83 (br s, 2H, methylene), 4.50, 4.32 (q, AB-system, 2H, $J = 17.7$ Hz, H-5′); $^{13}$C NMR ($CD_3OD$): $\delta$ 61.27, 63.08, 92.17, 111.16, 113.22, 127.72, 129.39, 130.01, 130.99, 132.16, 134.23, 142.15, 152.11, 153.77, 173.48; ESI-MS ($m/z$) 310.33 [M + H$^+$]; Anal. Calcd for $C_{17}H_{15}N_3O_3$: C 66.01, H 4.89, N 13.58. Found: C 66.24, H 4.68, N 13.61.

#### *4.10.3. 1-(2,3,4-Trideoxy-2-methylene-α-pent-3-enopyranosyl) cytosine (**10f**)*

Cytosine derivative **10f** was synthesized from **10d** by the similar procedure as described for **3a**. It was purified by flash chromatography (EtOAc/MeOH, 9:1) (0.44 g, 61%, $R_f = 0.11$). $[\alpha]_D^{22}$ 2.0 (c 0.289, MeOH); UV (MeOH): $\lambda_{max}$ 277 nm ($\epsilon$ 1689); $^1$H NMR ($CD_3OD$): $\delta$ 7.53 (d, 1H, $J_{5,6}$ = 7.3 Hz, H-6), 7.29 (d, 1H, H-5), 6.44 (d, 1H, $J_{3',4'} = 10.3$ Hz, H-4′), 6.33 (s, 1H, H-1′), 6.06 (d, 1H, H-3′), 5.10, 4.67 (br s, 2H, methylene), 4.35, 4.18 (q, AB-system, 2H, $J = 17.7$ Hz, H-5′); $^{13}$C NMR ($CD_3OD$): $\delta$ 63.71, 63.99, 93.18, 110.31, 112.74, 128.10, 130.31, 140.15, 152.32, 153.96; ESI-MS ($m/z$) 206.22 [M + H$^+$]; Anal. Calcd for $C_{10}H_{11}N_3O_2$: C 58.53, H 5.40, N 20.48. Found: C 58.71, H 5.59, N 20.59.

### *4.11. Synthesis of 1-(3,4-O-isopropylidene-α-D-erythro-pentopyranosyl-2-ulose)nucleosides **11**(**a**–**d**)*

#### *4.11.1. 1-(3,4-O-Isopropylidene-α-D-erythro-pentopyranosyl-2-ulose)thymine (**11a**)*

A mixture of **4a** (1.20 g, 4.02 mmol), PDC (1.81 g, 4.82 mmol) and $Ac_2O$ (1.14 mL, 12.06 mmol) was stirred in 67 mL of dry $CH_2Cl_2$ for 1h under nitrogen at room temperature and was concentrated in vacuum. Purification by flash chromatography (EtOAc/hexane, 8:2) yielded pure **11a** (0.99 g, 83%, $R_f$ = 0.36 in EtOAc/hexane, 9:1) as a white foam. $[\alpha]_D^{22}$ −12.0 (c 0.500, $CHCl_3$); UV ($CHCl_3$): $\lambda_{max}$ 262 nm ($\epsilon$ 4948); $^1$H NMR ($CDCl_3$): $\delta$ 8.62 (br s, 1H, NH), 6.97 (s, 1H, H-6), 6.15 (s, 1H, H-1′), 4.73–4.67 (m, 2H, $J_{3',4'} = 5.5$ Hz, H-3′, H-4′), 4.49 (d, 1H, $J_{5a',5b'} = 13.6$ Hz, H-5a′), 4.25 (dd, 1H, $J_{4',5b'} = 1.7$ Hz, H-5b′), 1.94 (s, 3H, 5-$CH_3$), 1.53, 1.45 (2s, 6H, 2$CH_3$); ESI-MS ($m/z$) 297.26 [M + H$^+$]; Anal. Calcd for $C_{13}H_{16}N_2O_6$: C 52.70, H 5.44, N 9.46. Found: C 52.92, H 5.78, N 9.61.

#### *4.11.2. 1-(3,4-O-Isopropylidene-α-D-erythro-pentopyranosyl-2-ulose)uracil (**11b**), 5-fluorouracil (**11c**), $N^4$-benzoyl cytosine (**11d**)*

Uracil, 5-fluorouracil and $N^4$-benzoyl cytosine derivatives **11b**, **11c** and **11d** were synthesized by similar procedure as described for **11a**.

**11b**: (0.99 g, 79%, $R_f = 0.42$ in EtOAc). $[\alpha]_D^{22}$ −4.0 (c 0.298, $CHCl_3$); UV ($CHCl_3$): $\lambda_{max}$ 257 nm ($\epsilon$ 8850); $^1$H NMR ($CDCl_3$): $\delta$ 9.02 (br s, 1H, NH), 7.15 (d, 1H, $J_{5,6} = 8.1$ Hz, H-6), 6.16 (s, 1H, H-1′), 5.79 (d, 1H, H-5), 4.74 (d, 1H, $J_{3',4'} = 5.6$ Hz, H-3′), 4.67 (d, 1H, H-4′), 4.49 (d, 1H, $J_{5a',5b'} = 13.7$ Hz, H-5a′), 4.26 (dd, 1H, $J_{4',5b'} = 1.0$ Hz, H-5b′), 1.49, 1.44 (2s, 6H, $2CH_3$); ESI-MS ($m/z$) 283.22 [M + H$^+$]; Anal. Calcd for $C_{12}H_{14}N_2O_6$: C 51.06, H 5.00, N 9.93. Found: C 51.38, H 5.11, N 9.68.

**11c**: (1.12 g, 85%, $R_f$ = 0.36 in EtOAc/hexane, 9:1), m.p. 146–148 °C. $[\alpha]_D^{22}$ 16.0 (c 0.500, $CHCl_3$); UV ($CHCl_3$): $\lambda_{max}$ 265 nm ($\epsilon$ 2354); $^1$H NMR ($CDCl_3$): $\delta$ 8.47 (s, 1H, H-6), 6.11 (s, 1H, H-1′), 4.73–4.69 (m, 2H, $J_{3',4'}$ = 5.6 Hz, H-3′, H-4′), 4.52 (d, 1H, $J_{5a',5b'} = 13.7$ Hz, H-5a′), 4.26 (dd, 1H, $J_{4',5b'} = 1.2$ Hz, H-5b′), 1.53 and 1.46 (2s, 6H, $2CH_3$); ESI-MS ($m/z$) 301.25 [M + H$^+$]; Anal. Calcd for $C_{12}H_{13}FN_2O_6$: C 48.00, H 4.36, N 9.33. Found: C 48.38, H 4.08, N 9.12.

**11d**: (1.3 g, 83%, $R_f = 0.43$ in EtOAc/MeOH, 9.5:0.5). $[\alpha]_D^{22}$ −36.0 (c 0.250, $CHCl_3$); UV ($CHCl_3$): $\lambda_{max}$ 261 nm ($\epsilon$ 17661); $^1$H NMR ($CDCl_3$): $\delta$ 7.97 (d, 1H, $J_{5,6} = 7.3$ Hz, H-6), 7.72–7.50 (m, 6H, Bz and H-5), 6.40 (s, 1H, H-1′), 4.88 (d, 1H, $J_{3',4'} = 4.7$ Hz, H-3′), 4.70 (d, 1H, H-4′), 4.51, 4.38 (q, AB-system, 2H, $J = 13.4$ Hz, H-5′), 1.47, 1.43 (2s, 6H, $2CH_3$); ESI-MS ($m/z$) 386.39 [M + H$^+$]; Anal. Calcd for $C_{19}H_{19}N_3O_6$: C 59.22, H 4.97, N 10.90. Found: C 59.38, H 5.05, N 10.88.

### 4.12. Synthesis of 1-(2-deoxy-3,4-O-isopropylidene-2-methylene-α-D-erythro-pentopyranosyl)nucleosides **12(a–d)**

#### 4.12.1. 1-(2-Deoxy-3,4-O-isopropylidene-2-methylene-α-D-erythro-pentopyranosyl)thymine (**12a**)

To a stirred suspension of $Ph_3PCH_3Br$ (3.94 g, 11.02 mmol) and *t*-amyl alcohol (1.32 mL, 12.02 mmol) in dry THF (34 mL) was added NaH (0.46 g, 60% in oil, 19.23 mmol) at 0 °C under nitrogen and the reaction mixture was stirred for 2 h at ambient temperature. To this yellow phosphorous ylide was added a solution of **11a** (0.99 g, 3.34 mmol) in dry THF (5 mL) dropwise, at 0 °C under nitrogen. After the mixture was stirred for 30 min at ambient temperature, the reaction mixture was quenched with saturated sodium bicarbonate and extracted with EtOAc. The organic layer was washed with water, dried with sodium sulfate and evaporated. The residue was purified by flash chromatography (EtOAc/hexane, 1:1) to give **12a** (0.57 g, 58%, $R_f = 0.3$), as a white solid, m.p. 110–112 °C. $[\alpha]_D^{22}$ −20.0 (c 0.500, $CHCl_3$); UV ($CHCl_3$): $\lambda_{max}$ 264 nm ($\epsilon$ 9276); $^1$H NMR ($CDCl_3$): $\delta$ 8.27 (br s, 1H, NH), 7.81 (s, 1H, H-6), 6.47 (s, 1H, H-1′), 5.66, 5.50 (br s, 2H, methylene), 4.86 (d, 1H, $J_{3',4'} = 7.4$ Hz, H-3′), 4.35 (dd, 1H, $J_{4',5b'} = 1.7$ Hz, H-4′), 3.98 (d, 1H, $J_{5a',5b'} = 13.5$ Hz, H-5a′), 3.58 (dd, 1H, H-5b′), 1.92 (s, 3H, 5-$CH_3$), 1.61, 1.44 (2s, 6H, $2CH_3$); ESI-MS ($m/z$) 295.32 [M + H$^+$]; Anal. Calcd for $C_{14}H_{18}N_2O_5$: C 57.13, H 6.16, N 9.52. Found: C 57.40, H 6.42, N 9.43.

#### 4.12.2. 1-(2-Deoxy-3,4-O-isopropylidene-2-methylene-α-D-erythro-pentopyranosyl)uracil (**12b**), 5-fluorouracil (**12c**), $N^4$-benzoyl cytosine (**12d**)

Uracil, 5-fluorouracil and $N^4$-benzoyl cytosine derivatives **12b**, **12c** and **12d** were synthesized by similar procedure as described for **12a**.

**12b**: (0.64 g, 65%, $R_f$ = 0.6 in EtOAc), m.p. 213–215 °C. $[\alpha]_D^{22}$ −28.0 (c 0.600, $CHCl_3$); UV ($CHCl_3$): $\lambda_{max}$ 259 nm ($\epsilon$ 5157); $^1$H NMR ($CDCl_3$): $\delta$ 8.51 (br s, 1H, NH), 7.92 (d, 1H, $J_{5,6} = 8.2$ Hz, H-6), 6.46 (s, 1H, H-1′), 5.72 (d, 1H, H-5), 5.67, 5.49 (br s, 2H, methylene), 4.85 (d, 1H, $J_{3',4'} = 7.3$ Hz, H-3′), 4.35 (dd, 1H, $J_{4',5b'} = 2.3$ Hz, H-4′), 3.97 (d, 1H, $J_{5a',5b'} = 13.4$ Hz, H-5a′), 3.58 (dd, 1H, H-5b′), 1.56, 1.42 (2s, 6H, $2CH_3$); ESI-MS ($m/z$) 281.29 [M + H$^+$]; Anal. Calcd for $C_{13}H_{16}N_2O_5$: C 55.71, H 5.75, N 9.99. Found: C 56.02, H 5.93, N 9.85.

**12c**: (0.65 g, 58%, $R_f = 0.71$ in EtOAc/hexane, 9:1). $[\alpha]_D^{22}$ −20.0 (c 0.500, $CHCl_3$); UV ($CHCl_3$): $\lambda_{max}$ 265 nm ($\epsilon$ 8347); $^1$H NMR ($CDCl_3$): $\delta$ 8.81 (br s, 1H, NH), 8.18 (d, 1H, $J_{F5,6} = 6.5$ Hz, H-6), 6.49 (s, 1H, H-1′), 5.71, 5.58 (br s, 2H, methylene), 4.87 (d, 1H, $J_{3',4'} = 7.4$ Hz, H-3′), 4.37 (dd, 1H, $J_{4',5b'} = 1.9$ Hz, H-4′), 3.98 (d, 1H, $J_{5a',5b'} = 13.4$ Hz, H-5a′), 3.56 (dd, 1H, H-5b′), 1.64, 1.45 (2s, 6H, $2CH_3$); ESI-MS ($m/z$) 299.25 [M + H$^+$]; Anal. Calcd for $C_{13}H_{15}FN_2O_5$: C 52.35, H 5.07, N 9.39. Found: C 52.11, H 5.21, N 9.55.

**12d**: (0.63 g, 49%, $R_f$ = 0.42 in EtOAc). $[\alpha]_D^{22}$ −34.0 (c 0.500, $CHCl_3$); UV ($CHCl_3$): $\lambda_{max}$ 261 nm ($\epsilon$ 21256); $^1$H NMR ($CDCl_3$): $\delta$ 7.92 (d, 1H, $J_{5,6} = 7.9$ Hz, H-6), 7.91–7.63 (m, 6H, Bz and H-5), 6.67 (s, 1H, H-1′), 5.64, 5.58 (br s, 2H, methylene), 4.85 (d, 1H, $J_{3',4'} = 7.2$ Hz, H-3′), 4.37 (dd, 1H, $J_{4',5b'} = 2.3$ Hz, H-4′), 4.03 (d, 1H, $J_{5a',5b'} = 13.4$ Hz, H-5a′), 3.66 (dd, 1H, H-5b′), 1.59, 1.43 (2s, 6H, $2CH_3$); ESI-MS ($m/z$) 384.41 [M + H$^+$]; Anal. Calcd for $C_{20}H_{21}N_3O_5$: C 62.65, H 5.52, N 10.96. Found: C 62.72, H 5.63, N 10.89.

### 4.13. Synthesis of 1-(2-deoxy-2-methylene-α-D-erythro-pentopyranosyl)nucleosides **13(a–c)**

#### 4.13.1. 1-(2-Deoxy-2-methylene-α-D-erythro-pentopyranosyl) thymine (**13a**)

Compound **12a** (0.57 g, 1.94 mmol) was dissolved in a mixture of $CH_2Cl_2$ (6.9 mL) and formic acid (6.9 mL, 90%). The solution was stirred for 3h at room temperature and then was concentrated under reduced pressure. The residue was purified by flash chromatography (EtOAc/hexane, 9:1) to give **13a** (0.43 g, 87%, $R_f = 0.14$) as a white foam. $[\alpha]_D^{22}$ −2.0 (c 0.500, MeOH); UV (MeOH): $\lambda_{max}$ 262 nm ($\epsilon$ 8524); $^1$H NMR ($CDCl_3$): $\delta$ 7.69 (s, 1H, H-6), 6.16 (s, 1H, H-1′), 5.47, 4.83 (br s, 2H, methylene), 4.41 (m, 1H, H-4′), 4.06–4.04 (m, 2H, $J_{5a',5b'} = 12.5$ Hz, H-5a′, H-5b′), 3.92 (d, 1H, $J_{2',3'} = 5.1$ Hz, H-3′), 1.91 (s, 3H, 5-$CH_3$); $^{13}$C NMR ($CDCl_3$): $\delta$ 13.23, 65.78, 67.82, 76.38, 83.16, 112.11, 113.42, 139.43, 153.12, 154.01, 161.94; ESI-MS ($m/z$) 255.26 [M + H$^+$]; Anal. Calcd for $C_{11}H_{14}N_2O_5$: C 51.97, H 5.55, N 11.02. Found: C 52.23, H 5.76, N 10.88.

#### 4.13.2. 1-(2-Deoxy-2-methylene-α-D-erythro-pentopyranosyl) uracil (**13b**), 5-fluorouracil (**13c**)

Uracil and 5-fluorouracil derivatives **13b** and **13c** were synthesized by similar procedure as described for **13a**.

**13b**: (0.46 g, 85%, $R_f$ = 0.15 in EtOAc/hexane, 9:1). $[\alpha]_D^{22}$ −8.0 (c 0.300, MeOH); UV (MeOH): $\lambda_{max}$ 259 nm ($\epsilon$ 8241); $^1$H NMR ($CDCl_3$): $\delta$ 7.75 (d, 1H, $J_{5,6}$ = 8.1 Hz, H-6), 6.14 (s, 1H, H-1′), 5.73 (d, 1H, H-5), 5.43, 4.81 (br s, 2H, methylene), 4.38 (m, 1H, H-4′), 4.02 (dd, 1H, $J_{4',5b'}$ = 2.3 Hz, $J_{5a',5b'}$ = 12.6 Hz, H-5b′), 3.91 (d, 1H, H-5a′), 3.88 (br s, 1H, H-3′); $^{13}$C NMR ($CDCl_3$): $\delta$ 62.85, 70.29, 75.37, 86.22, 103.59, 117.29, 139.98, 151.27, 152.02, 162.90; ESI-MS ($m/z$) 241.22 [M + H$^+$]; Anal. Calcd for $C_{10}H_{12}N_2O_5$: C 50.00, H 5.04, N 11.66. Found: C 50.36, H 5.22, N 11.43.

**13c**: (0.5 g, 89%, $R_f$ = 0.14 in EtOAc/hexane, 9:1), m.p. 182–184 °C. $[\alpha]_D^{22}$ −8.0 (c 0.500, MeOH); UV (MeOH): $\lambda_{max}$ 265 nm ($\epsilon$ 1407); $^1$H NMR ($CDCl_3$): $\delta$ 8.04 (d, 1H, $J_{F5,6} = 6.7$ Hz, H-6), 6.16 (s, 1H, H-1′), 5.49, 4.92 (br s, 2H, methylene), 4.41 (m, 1H, H-4′), 4.05 (dd, 1H, $J_{4',5b'} = 2.0$ Hz, $J_{5a',5b'} = 12.6$ Hz, H-5b′), 3.95 (d, 1H, H-5a′), 3.91 (br s, 1H, H-3′); $^{13}$C NMR ($CDCl_3$): $\delta$ 66.03, 67.98, 75.89, 84.02, 113.58, 132.93, 141.39, 153.12, 153.97, 160.07; ESI-MS ($m/z$) 259.22 [M + H$^+$]; Anal. Calcd for $C_{10}H_{11}FN_2O_5$: C 46.52, H 4.29, N 10.85. Found: C 46.43, H 4.20, N 10.78.

### 4.14. Synthesis of 1-(2,3,4-trideoxy-2-methylene-α-pent-3-enopyranosyl)nucleosides **10(a–c)**

#### 4.14.1. 1-(2,3,4-Trideoxy-2-methylene-α-pent-3-enopyranosyl) thymine (**10a**)

$Ph_3P$ (1.33 g, 5.07 mmol), iodine (0.64 g, 2.53 mmol), and imidazole (0.17 g, 2.53 mmol) were added to the suspension of **13a** (0.43 g, 1.69 mmol) in 47 mL of dry Tol/DMF (4:1). The reaction mixture was heated (80 °C, oil bath) under nitrogen for 15 min and concentrated in vacuum. The residue was purified by flash chromatography (EtOAc/hexane, 9:1) yielded **10a** (0.21 g, 57%), as a white foam.

*4.14.2. 1-(2,3,4-Trideoxy-2-methylene-α-pent-3-enopyranosyl) uracil (**10b**), 5-fluorouracil (**10c**)*

Uracil and 5-fluorouracil derivatives **10b** and **10c** were synthesized by similar procedure as described for **10a**.

**10b**: (0.21 g, 53%).

**10c**: (0.23 g, 53%).

*4.15. Methods for measurement of biological activity*

*4.15.1. Cytostatic assays*

Murine leukemia L1210, murine mammary carcinoma FM3A, human T-lymphocyte CEM and human cervix carcinoma (HeLa) cells were suspended at 300,000–500,000 cells/mL of culture medium, and 100 μL of a cell suspension was added to 100 μL of an appropriate dilution of the test compounds in wells of 96-well microtiter plates. After incubation at 37 °C for two (L1210, FM3A) or three (CEM, HeLa) days, the cell number was determined using a Coulter counter. The $IC_{50}$ was defined as the compound concentration required to inhibit cell proliferation by 50%.

The cytostatic activity ($IC_{50}$) of the 5-FU derivatives and 5-FU, 5-Furd and 5-FdUrd was also examined in the presence of 20 μM dThd, 20 μM dUrd, 500 μM Urd and 500 μM uracil. The appropriate fixed concentration of the natural nucleosides/nucleobase were added to the serial concentrations of **9c**, **10c** and **13c** and the fluorinated control compounds before exposure to the tumor cells (either L1210 or HeLa).

*4.15.2. Antimetabolic assays*

Compounds **9c**, **10c** and **13c** and 5-FU were evaluated for their capacity to inhibit the incorporation of [$CH_3$-$^3$H]dThd and [6-$^3$H]dUrd into DNA, or [5-$^3$H]Urd into RNA. The assays were carried out in 96-well microtiter plates. To each well were added $10^5$ L1210 cells, 0.25 μCi of [*methyl*-$^3$H]dThd, 1 μCi of [6-$^3$H]dUrd or 0.25 μCi [5-$^3$H]Urd (all derived from Amersham, U.K.), and a given amount of the test compound was added to each well. The cells were allowed to proliferate for 20 h at 37 °C in a humidified, $CO_2$-controlled atmosphere. At the end of this incubation period, the contents of the wells (200 μL) were transferred onto 25-mm glass fiber filters (type A/E, Gelman Instrument Company, Ann Arbor, Mich.), mounted on a Millipore 3025 sampling manifold apparatus. The filters were washed twice with cold NaCl/Pi (phosphate buffered saline), twice with cold 10% trichloroacetic acid, twice with cold 5% trichloroacetic acid, once with cold ethanol, and once with cold ether and assayed for radioactivity in the presence of a scintillant (Optiphase 'Hisafe' 2, Perking Elmer, Waltham, MA).

The activity of TS in intact L1210 cells was measured by evaluation of tritium release from [5-$^3$H]dUMP (formed in the cells from [5-$^3$H]dUrd or [5-$^3$H]dCyd) in the reaction catalyzed by TS. Cell cultures (0.5 mL DMEM culture medium) were prepared containing $\sim 5 \times 10^6$ L1210 cells and appropriate amounts of the test compounds (**9c**, **10c** and **13c**, 5-FU and 5-FdUrd). After 15 min, 4 or 24 h preincubation at 37 °C, 1 μCi of [5-$^3$H]dUrd (radiospecificity: 15.9 Ci/mmol) (Moravek Biochemicals, Brea, CA) or 1 μCi of [5-$^3$H]dCyd (radiospecificity: 22 Ci/mmol) (Moravek Biochemicals) was added to the cell cultures. After 30 min incubation, 100 μL of the cell suspensions were withdrawn and added to a cold suspension of 500 μL activated charcoal (VWR, Haasrode, Belgium) (100 mg/mL in TCA 5%). After 10 min, the suspension was centrifuged at 13,000 rpm for 10 min, after which the radioactivity in 400 μL supernatant was counted in a liquid scintillator using OptiPhase HiSafe (Perkin Elmer, Waldham, MA).

*4.15.3. Thymidine and uridine phosphorylase assays*

The conversion of dThd to thymine, Urd to uracil and potentially **9c**, **10c** and **13c** to 5-FU by human recombinant TP [47] and uridine phosphorylase type 1 [48] (kindly provided by Dr. T.P. Roosild, Las Vegas, Nevada, USA) was measured by high pressure liquid chromatography (HPLC) analysis. To determine the conversion activity, a high amount of recombinant enzyme was incubated with 100 μM of dThd or **9c**, **10c** and **13c** in TP-buffer (10 mM Tris.HCl, pH 7.6, 1 mM EDTA, 2 mM $KH_2PO_4$/$K_2HPO_4$ and 150 mM NaCl) or UP buffer (same as TP-buffer, but 300 mM NaCl instead of 150 mM). At 20, 40 and 60 min, 100 μL aliquots of the reaction mixtures were withdrawn and heated at 95 °C for 3 min to inactivate the enzyme. dThd and Urd were separated from thymine and uracil and **9c**, **10c** and **13c** were separated from 5-FU on a reverse-phase RP-8 column (Merck, Darmstadt, Germany) and quantified by high pressure liquid chromatography (HPLC) analysis (Alliance 2690, Waters, Milford, MA). The separation was performed by a linear gradient from 98% buffer B (50 mM $NaH_2PO_4$ and 5 mM heptane sulfonic acid, pH 3.2), to 20% buffer B + 80% acetonitrile (8 min 98% buffer B + 2% acetonitrile; 5 min linear gradient of 98% buffer B + 2% acetonitrile to 20% buffer B + 80% acetonitrile; 10 min 20% buffer B + 80% acetonitrile, followed by equilibration at 98% buffer B + 2% acetonitrile). Retention times for thymine and dThd were respectively 5.1 and 10.8 min, for uracil and Urd respectively 2.1 and 2.4 min and for **9c**, **10c**, **13c** and 5-FU respectively, 8.4, 11.1, 6.8 and 2.4 min. UV/VIS-based detection was performed at 267 nm.

*4.15.4. Antiviral assays*

The antiviral assays [except anti-human immunodeficiency virus (HIV) assays] were based on inhibition of virus-induced cytopathicity in HEL [herpes simplex virus type 1 (HSV-1), HSV-2 (G), vaccinia virus and vesicular stomatitis virus], Vero (parainfluenza-3, reovirus-1, Sindbis, Coxsackie B4, and Punta Toro virus), or HeLa (vesicular stomatitis virus, Coxsackie virus B4, and respiratory syncytial virus) cell cultures. Confluent cell cultures in microtiter 96-well plates were inoculated with 100 cell culture inhibitory dose-50 ($CCID_{50}$) of virus (1 $CCID_{50}$ being the virus dose to infect 50% of the cell cultures). After a 1 h virus adsorption period, residual virus was removed, and the cell cultures were incubated in the presence of varying concentrations (200, 40, 8, … μM) of the test compounds. Viral cytopathicity was recorded as soon as it reached completion in the control virus-infected cell cultures that were not treated with the test compounds. The methodology of the anti-HIV assays was as follows: human CEM ($\sim 3 \times 10^5$ cells/cm$^3$) cells were infected with 100 $CCID_{50}$ of HIV-1($III_B$) or HIV-2(ROD)/mL and seeded in 200 μL wells of a microtiter plate containing appropriate dilutions of the test compounds. After 4 days of incubation at 37 °C, HIV-induced CEM giant cell formation was examined microscopically.

## Acknowledgments

This work was supported in part by the Postgraduate Programmes "Biotechnology-Quality assessment in Nutrition and the Enviroment", "Application of Molecular Biology-Molecular Genetics-Molecular Markers", Department of Biochemistry and Biotechnology, University of Thessaly. Financial support was also provided by the "Geconcerteerde Onderzoeksacties" (GOA no. 10/014) of the K.U.Leuven and the FWO (no. G-486-08). The authors are grateful to Dr. Angeliki Panagiotopoulou, technical scientist of the NMR Laboratory of NCSR "Demokritos", for obtaining the NMR spectra, and to Mrs. Lizette van Berckelaer, Leentje Persoons, Frieda De Meyer, Vickie Broeckx and Leen Ingels for dedicated technical help.

## References

[1] A. Holý, Antiviral Res. 71 (2006) 248–253.
[2] R.C. Brandy, D.I. Bernstein, Antiviral Res. 61 (2004) 73–81.

[3] D.C. Meadows, J. Gervay-Hague, Chem. Med. Chem. 1 (2006) 16–29.
[4] C. Mathé, G. Gosselin, Antiviral Res. 71 (2006) 276–281.
[5] R.F. Schinazi, B.I. Hernandez-Santiago, S.J. Hurwitz, Antiviral Res. 71 (2006) 322–334.
[6] D. Komiotis, S. Manta, E. Tsoukala, N. Tzioumaki, Curr. Med. Chem. Anti-Infect. Agents 7 (2008) 219–244.
[7] W.D. Meng, F.L. Qing, Curr. Top. Med. Chem. 6 (2006) 1499–1528.
[8] G.V. Papatheodoridis, E. Dimou, V. Papadimitropoulos, Am. J. Gastroenterol. 97 (2002) 1618–1628.
[9] I. Verheggen, A. Van Aerschot, L. Van Meervelt, J. Rozenski, L. Wiebe, R. Snoeck, G. Andrei, J. Balzarini, P. Claes, E. De Clercq, P. Herdewijn, J. Med. Chem. 38 (1995) 826–835.
[10] T. Ostrowski, B. Wroblowski, R. Busson, J. Rozenski, E. De Clercq, M.S. Bennet, J.N. Champness, W.C. Summers, M.R. Sanderson, P. Herdewijn, J. Med. Chem. 41 (1998) 4343–4353.
[11] Y. Maurinsh, J. Schraml, H. De Winter, N. Blaton, O. Peeters, E. Lescrinier, J. Rozenski, A. Van Aerschot, E. De Clercq, R. Busson, P. Herdewijn, J. Org. Chem. 62 (1997) 2861–2871.
[12] C. Spanou, S. Manta, D. Komiotis, A. Dervishi, D. Kouretas, Int. J. Mol. Sci. 8 (2007) 695–704.
[13] A. Haouz, V. Vanheusden, H. Munier-Lechman, M. Froeyen, P. Herdewijn, S. Van Galenbergh, M. Delarue, J. Biol. Chem. 278 (2003) 4963–4971.
[14] K. Vastmans, S. Pochet, A. Peys, L. Kerremans, A. Van Aerschot, C. Hendrix, P. Marliere, P. Herdewijn, Biochemistry 39 (2000) 12757–12765.
[15] K. Vastmans, M. Froeyen, L. Kerremans, S. Pochet, P. Herdewijn, Nucleic Acids Res. 29 (2001) 3154–3163.
[16] P. Herdewijn, E. De Clercq, Bioorg. Med. Chem. Lett. 11 (2001) 1591–1597.
[17] M.J. Egron, F. Leclercq, K. Antonakis, I. Bennani-Baiti, C. Frayssinet, Carbohydr. Res. 248 (1993) 143–150.
[18] M. Alaoui, M.J. Egron, M. Bessodes, K. Antonakis, I. Chouroulinkov, Eur. J. Med. Chem. 22 (1987) 305–310.
[19] F. Leclercq, K. Antonakis, Carbohydr. Res. 263 (1994) 309–313.
[20] G.S. Bisacchi, S.T. Chao, C. Bachard, J.P. Daris, S. Innaimo, G.A. Jacobs, O. Kocy, P. Lapointe, A. Martel, Z. Merchant, W.A. Slusarchyk, J.E. Sundeen, M.G. Young, R. Colonno, R. Zahler, Bioorg. Med. Chem. Lett. 7 (1997) 127–132.
[21] S.J. Yoo, H.O. Kim, Y. Lim, J. Kim, L.S. Jeong, Bioorg. Med. Chem. 10 (2002) 215–226.
[22] P. Gunaga, M. Baba, L.S. Jeong, J. Org. Chem. 69 (2004) 3208–3211.
[23] F. Leclercq, K. Antonakis, Carbohydr. Res. 181 (1988) 217–222.
[24] S. Manta, G. Agelis, T. Botić, A. Cencič, D. Komiotis, Bioorg. Med. Chem. 15 (2007) 980–987.
[25] S. Manta, G. Agelis, T. Botić, A. Cencič, D. Komiotis, Eur. J. Med. Chem. 43 (2008) 420–428.
[26] S. Manta, E. Tsoukala, N. Tzioumaki, A. Goropevšek, R.T. Pamulapati, A. Cencič, J. Balzarini, D. Komiotis, Eur. J. Med. Chem. 44 (2009) 2696–2704.
[27] S. Manta, E. Tsoukala, N. Tzioumaki, C. Kiritsis, J. Balzarini, D. Komiotis, Bioorg. Chem. 38 (2010) 48–55.
[28] S. Manta, N. Tzioumaki, E. Tsoukala, A. Panagiotopoulou, M. Pelecanou, J. Balzarini, D. Komiotis, Eur. J. Med. Chem. 44 (2009) 4764–4771.
[29] G. Agelis, N. Tzioumaki, T. Botić, A. Cencič, D. Komiotis, Bioorg. Med. Chem. 15 (2007) 5448–5456.
[30] G. Agelis, N. Tzioumaki, T. Tselios, T. Botić, A. Cencič, D. Komiotis, Eur. J. Med. Chem. 43 (2008) 1366–1375.
[31] N. Tzioumaki, E. Tsoukala, S. Manta, G. Agelis, J. Balzarini, D. Komiotis, Arch. Pharm. 342 (2009) 353–360.
[32] D.B. Longley, D.P. Harkin, P.G. Johnston, Nat. Rev. Canc. 3 (2003) 330–338.
[33] E. Tsoukala, G. Agelis, J. Dolinsek, T. Botić, A. Cencič, D. Komiotis, Bioorg. Med. Chem. 15 (2007) 3241–3247.
[34] V. Vanheusden, R. Busson, P. Herdewijn, S. Van Calenbergh, J. Org. Chem. 69 (2004) 4446–4453.
[35] J. Milecki, A. Földesi, A. Fischer, R.W. Adamiak, J. Chattopadhyaya, J. Labelled Compd. Rad. 44 (2001) 763–783.
[36] M. Bessodes, E. Abushanab, P.R. Panzica, J. Chem. Soc. Chem. Commun. (1981) 26–28.
[37] B.K. Chun, G.Y. Song, C.K. Chu, J. Org. Chem. 66 (2001) 4852–4858.
[38] M. Bessodes, D. Komiotis, K. Antonakis, Tetrahedron Lett. 27 (1986) 579–580.
[39] J. Herscovici, K. Antonakis, J. Chem. Soc. Chem. Commun. 12 (1980) 561–564.
[40] L.S. Jeong, S.J. Yoo, Bioorg. Med. Chem. Lett. 8 (1998) 847–852.
[41] R. Van Rijsbergen, M.J.O. Anteunis, A. De Bruyn, J. Carbohydr. Chem. 2 (1983) 395–404.
[42] M.J. Kim, L.S. Jeong, J.H. Kim, J.H. Shin, S.Y. Chung, S.K. Lee, M.W. Chun, Nucleosides, Nucleotides Nucleic Acids 23 (2004) 715–724.
[43] P.J. Garegg, B. Samuelsson, Synthesis (1979) 469–470.
[44] Z. Liu, B. Classon, B. Samuelsson, J. Org. Chem. 55 (1990) 4273–4275.
[45] P.J. Garegg, B. Samuelsson, Synthesis (1979) 813–814.
[46] A.F. Luzzio, E.M. Menes, J. Org. Chem. 59 (1994) 7267–7272.
[47] E. De Clercq, J. Balzarini, P.F. Torrence, M.P. Mertes, C.L. Schmidt, D. Shugar, P.J. Barr, A.S. Jones, G. Verhelst, R.T. Walker, Mol. Pharmacol. 19 (1981) 321–330.
[48] J. Balzarini, E. De Clercq, Biochim. Biophys. Acta 785 (1984) 36–45.
[49] Y. Yamada, H. Yasui, A. Goto, T. Arai, T. Ura, T. Hamaguchi, K. Muro, Y. Shimada, K. Shirao, Int. J. Clin. Oncol. 8 (2003) 374–380.
[50] A. Bronckaers, L. Aguado, A. Negri, M.J. Camarasa, J. Balzarini, M.J. Pérez-Pérez, F. Gago, S. Liekens, Biochem. Pharmacol. 78 (2009) 231–240.
[51] T.P. Roosild, S. Castronovo, M. Fabbiani, G. Pizzorno, BMC Struct. Biol. 9 (2009) 14.
[52] S. Liekens, A. Bronckaers, J. Balzarini, Lancet Oncol. 10 (2009) 628–635.