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# Chemical Modifications on the Acyclic Moiety of 3-(2-Hydroxyethoxy)-1-Alkoxypropyl Nucleobases. 2.[1] Differentiation and Growth Inhibition in Rhabdomyosarcoma Cells after Exposure to a Novel 5-Fluorouracil Acyclonucleoside

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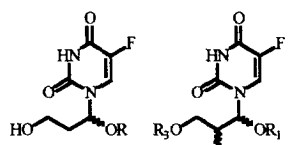
***Abstract:*** A series of new 5FU acyclonucleoside analogues has been synthesized and tested for their *in vitro* cytotoxicity versus HT-29 colon carcinoma. The only active compound is 1-{[3-(3-cloro-2-hydroxypropoxy)-1-methoxy]propoxy}-5-fluorouracil **14**, which is 8-fold less active than 5-fluorouracil. The rest of the newly prepared compounds showed no significant activity. We selected **14** as the drug in the treatment of an human embryonal cell line RD derived from rhabdomyosarcoma. Such treatment caused time-dependent growth inhibition. Interestingly, RD cells treated with **14** at a concentration of 90 μM for 6 days showed phenotypic differentiation, with increased expression of desmin, α-actinin and tropomyosin. We conclude that exposure of this human embryonal rhabdomyosarcoma cell line to a 90 μM concentration released the neoplastic cells from their blockade, allowing them to recover normal myogenic development. © 1997 Elsevier Science Ltd.

## INTRODUCTION

The area of seco-nucleosides (nucleoside analogues in which the "sugar" is linear instead of the normal cyclic) has been widely explored due to the successful development of the antiherpes drug, acyclovir.[2] Although progress has been made in the search for antitumour agents like **1**[3] and **2**,[4] the efforts are being continued and intensified.




**1** **2**

As part of a programme for the development of 5-fluorouracil derivatives,[5] we envisaged the formation of acyclonucleoside analogues *via* a tin (IV) chloride-mediated regiospecific opening of alkoxy-1,4-diheteroepanes.[1] Compound **3**[1,5] (see Table 1) proved to be 2-fold more active than 5-fluorouracil (5FU, $IC_{50}$ = 45 μM) against

HEp-2 cells in culture (human larynx tumour). On the other hand, **4** showed $IC_{50}$'s of 9.4, 3.94 and 5.79 μM against the CX-1 (human colon carcinoma), MX-1 (human mammary carcinoma) and the LX-1 (human lung carcinoma) tumour lines, respectively.[5] In view of the chemotherapeutic activities exhibited by the acyclonucleosides 1-{[3-(2-hydroxyethylhetero)-1-alkoxy]propyl}-5-fluorouracils, we initiated the present study to explore new acyclic nucleosides in which their 3-hydroxyethoxypropyl moiety is chemically modified in an attempt to search for more potent antitumour agents that can also be used as drugs with a differentiating action and to study its role in their biological activities.

Considerable evidence indicates that the malignant phenotype in cancers is not an irreversible state, but represents a disease of altered maturation. Recent years have seen the development of the concept of differentiation therapy based on the conversion of malignant cells to a more benign phenotype through induced differentiation using chemical substances.[6] Research has shown that rhabdomyosarcoma cells can be induced to a differentiated stage with no proliferative potential by antineoplastic drugs such as cytarabine[7] and the antibiotic actinomycin D.[8] Rhabdomyosarcomas are the most common malignant soft-tissue tumours in children comprising 5 % of all pediatric malignances.[9] The degree of rhabdomyosarcoma cell differentiation has been determined by the classic marker desmin;[10,11] more recently α-actinin and tropomyosin expression have also been used to determine the degree of cytoskeletal organization.[12]

## AIM

In this paper we report the results related with the synthesis of the acyclonucleosides **5-15** (Table 1), whose chemical modifications include the irreversible blocking of the hydroxy function (**5** and **6**), substitution of the hydroxy group by the chlorine atom (**7**) or the oxidation to a methoxycarbonyl group (**8**), in lipophilicity (**9-12,** in which the acyclic chain is linked to several nucleobases), the increase in hydrophilicity (**13**), and the inclusion of the chlorohydrin fragment in the two-carbon atom acyclic chain (**14**).The bis(5-fluorouracil-1-yl) compound **15** was also included in this study.

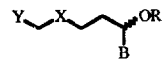
All the structures **5-15** can be seen as prodrugs of the nucleobases, with an acrolein moiety within them. After the expected chemical or enzymatic hydrolysis, two biologically active substances could thus be generated from the same drug as a consequence of drug activation. Acrolein itself inhibits the growth of Chinese hamster ovary cells ($IC_{50}$ = 50 μM).[13] To test behaviour of the products on cellular systems, cytotoxic activity against HT-29 colon carcinoma[14] was determined,[15] and the most active compound was used to study the modifications in proliferation and degree of differentiation in the human rhabdomyosarcoma cell line RD.

## CHEMISTRY

Compounds **3**,[1] **4**[1] were reported previously. As starting materials for the synthesis of **5, 6, 8-12, 14**, we used the acetals **16**[16]-**19**[17]-**20**[18] (Scheme 1), under our standard conditions.[1] These synthetic conditions were also used for the preparation of the adenine (**11** and **12**) and the uracil (**13**) derivatives, respectively. There are two facts that are worth pointing out:

(a) The condensation of olefin **19** with adenine in the presence of tin (IV) chloride, hexamethyldisilazane (HMDS) and chlorotrimethylsilane (CTS) in anhydrous acetonitrile at room temperature for 19 h proceeded regioselectively: the $N^7$- and $N^9$-acyclonucleosides **9** and **10** (see Scheme 1) were isolated in 31 % and 10 % yields, respectively ($N^7/N^9$ ratio ≈ 3:1).

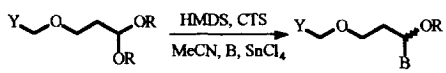
**Table 1.** Structures of acyclonucleosides **3-15**.



| Comp. | R | X | Y | Base |
|---|---|---|---|---|
| **3** | $Pr^i$ | O | $CH_2OH$ | 5FU |
| **4** | $Pr^i$ | S | $CH_2OH$ | 5FU |
| **5** | Me | O | $CH_2OTs$ | 5FU |
| **6** | Me | O | $CH_2OMe$ | 5FU |
| **7** | Me | O | $CH_2Cl$ | 5FU |
| **8** | Me | O | COOMe | 5FU |
| **9** | $Pr^i$ | O | $CH=CH_2$ | Ad-7-yl[a] |
| **10** | $Pr^i$ | O | $CH=CH_2$ | Ad-9-yl[a] |
| **11** | $Pr^i$ | O | $CH=CH_2$ | U |
| **12** | $Pr^i$ | O | $CH=CH_2$ | 5FU |
| **13** | $Pr^i$ | O | $CH(OH)CH_2OH$ | 5FU |
| **14** | Me | O | $CH(OH)CH_2Cl$ | 5FU |
| **15** | Me | O | CH(OMe)5FU | 5FU |

[a] Ad = Adenine

When tin (IV) chloride was replaced by trimethylsilyl trifluoromethanesulfonate, the reaction led to the formation of the $N^9$-acyclonucleoside **10** as the principal product (50 %) along with the formation of the $^7N$-acyclonucleoside **9** in 26 % yield ($N^7/N^9$ ratio ≈ 1:2).[19]




| Acetal | Y | R | B | Product | Yield (%) |
|---|---|---|---|---|---|
| **16** | $CH_2OTs$ | Me | 5FU | **5** | 68 |
| **17** | $CH_2OMe$ | Me | 5FU | **6** | 68 |
| **18** | COOMe | Me | 5FU | **8** | 95 |
| **19** | $CH=CH_2$ | $Pr^i$ | Ad-7-yl | **9** | 31[a] |
| **19** | $CH=CH_2$ | $Pr^i$ | U | **11** | 30 |
| **19** | $CH=CH_2$ | $Pr^i$ | 5FU | **12** | 72 |
| **20** | $CH(OH)CH_2Cl$ | Me | 5FU | **14** | 58 |

[a] Ad = Adenine. Compound **10** (Ad-9-yl derivative) was also obtained in 10 % yield.

**Scheme 1**

The structures of acyclic nucleosides **9** and **10** have been determined from their NMR spectra. The acyclic moieties were unequivocally characterized by $^1H$ and $^{13}C$ NMR spectra (see Experimental Part). As the $^1H$ NMR

spectra of **9** and **10** were very similar, the position of the acyclic substituent at the adenine base had to be deduced mainly from the $^{13}C$ NMR spectra (Table 2).

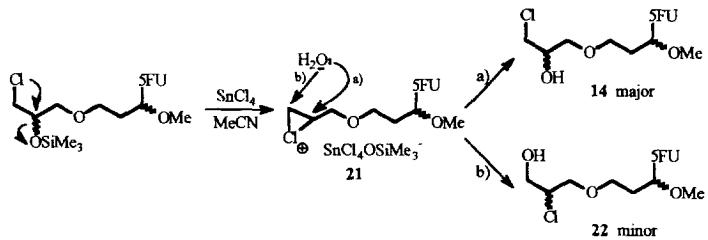
**Table 2.** $^{13}C$ NMR chemical shifts[a] (ppm) for the adenine[b] moiety in **9** (*N*-7) and **10** (*N*-9) for $CDCl_3$ solutions.

| | C-2 | C-4 | C-5 | C-6 | C-8 |
|---|---|---|---|---|---|
| **9** | 153.64 | 161.64 | 110.25 | 151.33 | 144.22 |
| **10** | 153.27 | 150.14 | 119.64 | 155.57 | 138.70 |

[a]Each reading was quoted to the nearest 0.05 ppm. [b]The locants correspond to the IUPAC enumeration of adenine.

The site of the adenine hemiaminal C-N bond was determined by comparison of the $^{13}C$ NMR spectra of the corresponding compounds with those of related adenine $N^7$- and $N^9$-glycosides and $N^7$- and $N^9$-methyl adenine derivatives.[20] We calculated the variation of $^{13}C$ NMR chemical shifts (**9** - **10**, $\Delta\delta$ in ppm) when the site of substitution for the 3-allyloxy-1-*iso*propoxy-1-propyl moiety changed from $N^7$ to $N^9$ in adenine. When the site of attachment of the acyclic moiety is changed from $N^7$ (**9**) to $N^9$ (**10**), one would expect a downfield shift of the C-4 signal approximately equivalent to an upfield shift on the C-5 resonance by analogy with the data obtained for adenine $N^7$- and $N^9$-glycosides and $N^7$- and $N^9$-methyl adenine derivatives.[20] In agreement with these data were the assignments for **9** which denoted a downfield shift of 11.5 ppm for the $C\text{-}4_{Ad}$ signal when compared with its position in **10** and an upfield shift of -9.39 ppm for the $C\text{-}5_{Ad}$ line. The $C\text{-}6_{Ad}$ resonance moved upfield -4.24 ppm because of a steric interaction between the acyclic moiety and the amino group and subsequent charge compression in the $H_2N$-C bond. The $C\text{-}8_{Ad}$ resonance position moved downfield 5.52 ppm while the $C\text{-}2_{Ad}$ resonance position was practically insensitive to the site of alkylation on the heterocyclic moiety ($N^7$ or $N^9$). The $^{13}C$-$^1H$ correlation spectra of **9** and **10** unambiguously allowed us to distinguish between $H\text{-}2_{Ad}$ (8.47 ppm for **9** and 8.45 ppm for **10**) and $H\text{-}8_{Ad}$ (8.00 ppm for **10** and 7.98 ppm for **9**). The assignment of the other signals was straightforward. The fact that it is not necessary to protect the amino group of the adenine and the possibility of synthesizing non-naturally-occurring $N^7$- and $N^9$- adenine acyclonucleoside analogues makes this new simplified preparation of particular value especially when these targets have to be prepared quickly;

(b) The reaction of **20** with 5-FU under standard conditions[1] led to acyclonucleoside **14**. In addition to **14**, a very minor close-moving spot was also separated by careful chromatography on silica gel ($CHCl_3$/MeOH: 100/3) whose $^1H$ (300 MHz) and $^{13}C$ NMR (75 MHz) data showed to be a mixture of diastereoisomers.

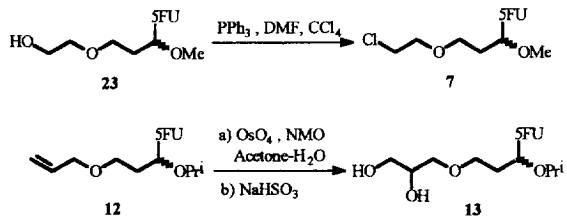



**Scheme 2**

Their mass spectrum showed the molecular ion at 310, corresponding to a 3:1 doublet due to the chlorine. All these data were consistent with the primary alcohol-bearing ayclonucleoside **22** formed through the chloronium ion **21** as depicted in Scheme 2. Cl is a very weak neighbouring group and can be shown to act in this way only

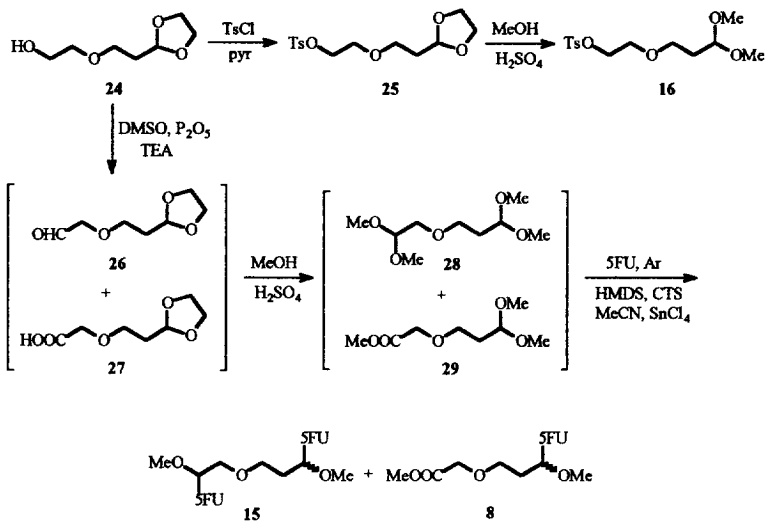
when the solvent does not interfere.[21] Compound **14** (as a mixture of diastereoisomers) turned out to be the only active compound against HT-29 cells (*vide infra*). All the attempts to separate both diastereoisomers were fruitless.

**7** and **13** (as a mixture of diastereoisomers) had to be prepared from the corresponding acyclonucleosides **23** and **12**, respectively, by using a mixture of $PPh_3$ and $CCl_4$ and DMF as a solvent, and by hydroxylation of the exocyclic double bond on treatment with osmium tetroxide and *N*-methylmorpholine *N*-oxide (NMO) in aqueous acetone, respectively (Scheme 3). On one hand, we previously reported[22] that 3-(2-hydroxyethoxy)propanal dimethyl acetal cyclized to 5-methoxy-1,4-dioxepane when treated with the $Ph_3P/CCl_4$ system, not yielding the expected 3-(2-chloroethoxy)propanal dimethyl acetal, which had to be the synthon for the one-pot preparation of **7**. On the other, 3-(2,3-dihydroxypropoxy)propanal dimethyl acetal, obtained by hydroxylation of 3-allyloxypropanal dimethyl acetal in 56 % yield, failed in its condensation with 5FU using $SnCl_4$, probably due to its inestability in the acidic medium.




**Scheme 3**

Acetal **19** was obtained by acid-catalyzed acetalization of 3-allyloxypropanal[17] with anhydrous *iso*propanol in 50% yield. Hydroxyacetal **24**[23] was the starting material that led to the tosyloxy acetal **25**, and to the acyclonucleosides **8** and **15** as depicted in Scheme 4.




**Scheme 4**

When **24** was subjected to dimethyl sulfoxide (DMSO) and $P_2O_5$-$Et_3N$ (TEA) conditions[24] in the hope of obtaining the aldehyde **26**, the chemical and spectroscopic behaviour of the reaction mixture showed us the presence of another substance, which proved to be carboxylic acid **27**, formed under the very mild conditions used.[25]

As we were unable to separate[26] both compounds using flash chromatography or distillation under diminished pressure, we decided to continue the synthetic route, hoping that the mono(5-fluorouracil-1-yl) derivative **8** and the bis(5-fluorouracil-1-yl) derivative **15** would be separated by flash chromatography, due to their different lipophilicities.

In fact, our assumptions proved to be correct and **8** and **15** were isolated by flash chromatography using a gradient elution ($CHCl_3$/MeOH: 100/2 → 100/5). The fact that on the one hand, two substances (**26** and **27**) were formed in the reaction, and on the other, the inability to separate them, might be overlooked because of its limited or null chemical synthetic value. But the failure in the oxidation of the primary alcohol of **24** to a formyl group using pyridinium chlorochromate,[27] pyridinium dichromate[28] or to a carboxylic acid group by potassium permanganate/18-crown-6,[29] or the Jones reagent[30] circumvented the above drawbacks and made the aforementioned procedure useful, especially when the two final products **8** and **15** were finally separated and isolated. Another approach such as the two possible Williamson syntheses, *i.e.*, from methyl hydroxyacetate and 2-(2-bromoethyl)-1,3-dioxolane, or methyl bromoacetate and 2-(2-hydroxyethyl)-1,3-dioxolane (unpublished results), also failed or gave exceedingly low yields preventing us from obtaining usable quantities of material to proceed further in the following steps of the convergent synthesis of **8**.

## BIOLOGICAL ACTIVITY

***In vitro Cytotoxicity versus HT-29.*** The cytotoxic activity of **3, 5-9, 11-15** was assessed with the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay as described.[15] The concentration of drug required to inhibit 50% of the cell growth ($IC_{50}$) after 72 h, of colon carcinoma HT-29 cells was calculated using an in-house programme. The only active compound turned out to be **14** ($IC_{50}$ = 70 μM),which is 8-fold less active than 5-fluorouracil (9 μM[31]), the rest of the series showing $IC_{50}$ values greater than 100 μM (insufficient activity up to this concentration to determine $IC_{50}$).

***In vitro differentiation and growth inhibition in the RD cell line.*** The rhabdomyosarcoma cell line (RD) was obtained from the American Type Culture Collection. Cell culture and preparation of cells for Fluorescence Activated Cell Sorting (FACScan[32]) were as described previously.[33] Four replicate culture flasks (75 $cm^2$) with 2 × $10^6$ cells each were exposed to a 90 μM solution of **14** together with controls. Cells were induced with **14**, both each day and separately, until the sixth day, in which the cells corresponding to the treatment of each day were collected from the first day to the sixth one. The number of collected cells was determined with a Neubauer chamber.

Figure 1 shows the plot of the cell number *versus* the incubation time (in days) for the RD control, 5-fluorouracil (5FU, 90 μM) and **14** (90 μM). 5FU showed a pronounced inhibition of the cell population. The inhibition was sustained until the third day (1.63 × $10^6$), from which a more accelerated decrease took place until the sixth day (5.1 × $10^5$). For **14** descending and ascending growing peaks were seen for the first 4 days with values within 6.87 × $10^5$ and 2.32 × $10^6$ cells. From the fourth to the fifth day there was an acceleration of the growth (5 × $10^6$) that was followed by a novel decrease until the day 6 (3.4 × $10^6$). There was a stastistically significant difference between the proliferation of RD cells growing in standard medium and RD cells growing in medium supplemented with **14** ($p < 0.001$). In short, tumour cells exposed to 5FU and to **14** showed a retarded growth rate during 6 days in comparison with the growth rate of control cells.

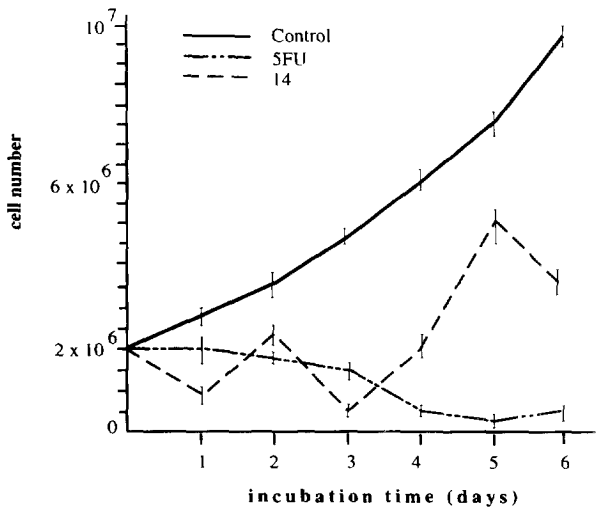



**Figure 1.** Effect of treatment with 5FU (90 μM) and **14** (90 μM) on the proliferation of RD cells.

Quantitative data on the changes in expression of desmin, a-actinin and tropomyosin in RD cell line in RD parental cell line (control), after 6 days of treatment with 5FU (90 μM), and after 6 days of treatment with **14** (90 μM) were provided by FACS analysis (Figure 2). 5FU led to clear differentiation signs in the RD cell line. This action has also been demonstrated in the colon carcinoma HT-29 cell line.[34] Nevertheless, 5FU showed a high cellular toxicity[35] and it was used in short treatments, after which cells were induced to differentiate by classic inductor agents.[35b] Such a differentiative action might imply an important morbility on healthy tissues. So, the use of **14** presents advantages over 5FU, *i.e.* a) for being less toxic than 5FU (Figure 1) and b) because gives rise to a significant increase in the differentiation markers in relation to 5FU (Figure 2). The inverse relation observed between proliferation and differentiation in tumour and normal cells suggests an alternative approach to cancer therapy that does not involve cell killing, but instead induces malignant cells to differentiate to benign forms with no proliferative potential. Further studies are in progress and will be reported in due course.

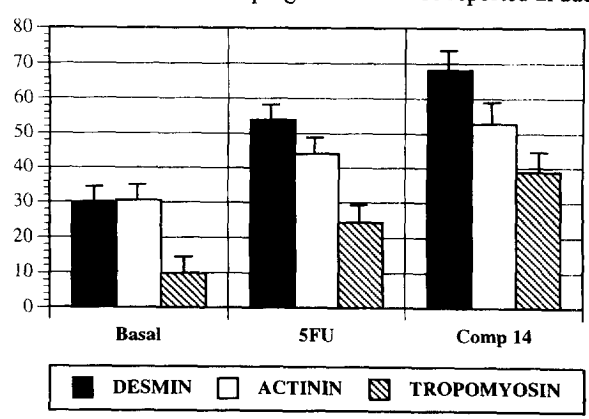



**Figure 2.** Analysis by fluorescence-activated cell sorting (FACScan). The results are expressed as the percentage of fluorescence corresponding to desmin, α-actinin and tropomyosin expression in RD parental cell line (basal) and RD cells treated with 5FU (90 μM) and **14** (90 μM).

## CONCLUSION

12 new acyclonucleoside-like compounds have been prepared. The new simplified synthesis of acyclonucleoside-like compounds with adenine moieties could be of particular value when the target compound has to be prepared quickly and without the need to protect the amino group. Such a reaction was regioselective yielding the corresponding $N^7$- and $N^9$-acyclonucleoside-like compounds in different proportions depending on the nature of the Lewis acid [tin (IV) chloride or trimethylsilyl trifluoromethylsulfonate]. The only active compound against HT-29 cells was **14** and so this was selected to induce differentiation in human embryonal cell line RD. Our results demonstrate the appearance of phenotypical markers of rhabdomyoblastic differentiation in the human rhabdomyosarcoma cell line RD after treatment with a 90 μM solution of **14**.

## EXPERIMENTAL

**General.** Melting points (mp) were taken in open capillaries on an Electrothermal melting point apparatus and are uncorrected. Infrared (IR) spectra were run on a Perkin-Elmer 782 spectrophotometer connected to a 3600 Data Station. Nuclear magnetic resonance (NMR) spectra were recorded on a 400.1 MHz $^1$H and 100.03 MHz $^{13}$C NMR Bruker ARX 400 or 300.13 MHz $^1$H and 75.78 MHz $^{13}$C NMR Bruker AMX-300 spectrometers, and chemical shifts (ppm) are reported relative to the solvent peak ($CHCl_3$ in $CDCl_3$ at 7.24 and 77.1 ppm). Signals are designated as follows: s, singlet; d, doublet; dd, doublet of doublet; ddd, double doublet of doublet; dddd, double double doublet of doublet; t, triplet; dt, double triplet; ddt, double double triplet; q, quadruplet; dq, double quadruplet; tq, triple quartet; quint, quintet; h, heptulet; m, multiplet. Coupling constants are expressed in hertz. The NMR, MS, and IR spectral data of all compounds were consistent with the assigned structures. The NMR spectra of **15-17** and **22** were obtained as two sets of frequencies due to the presence of the two diastereoisomers in the samples (ratio: 50/50). All final products had satisfactory (within ± 0.4%) C, H, and N analyses. The mass spectra (MS) were obtained using a VG-Platform II spectrometer (Fisons Instruments) at 70 eV, carrying out injection through a Carlo Erba GC 8000 chromatograph. Liquid secondary ion mass spectra (LSIMS) and high resolution mass spectra (HR) were carried out on a VG AutoSpec Q high resolution mass spectrometer (Fisons Instruments). Glc was performed on a Perkin-Elmer 8410 gas chromatograph equipped with a flame-ionisation detector and a steel column (2 × 0.125 in. i. d.) packed with 5 % CW 20 M. The $N_2$ flow rate was 30 mL/min, the injection port temperature was 250 °C, and the zone-detector temperature was 250 °C.

Analytical thin-layer chromatography (TLC) was performed using Merck Kieselgel 60F-254 plates as the solvent system. Chromatograms were visualized under a UV lamp (254 nm), by placing the air-dried plates in a tank of $I_2$ vapour, or charring with a 20 % sulfuric acid/methanol solution. Preparative separations were performed by flash chromatography on silica gel (Merck; 230-400 mesh) using mixtures of diethyl ether-hexane or $CHCl_3$-MeOH as eluents. Acetonitrile was dried by refluxing and distilling from calcium hydride; anhydrous methanol was prepared by the magnesium drying procedure, and ether was dried by storing over freshly prepared sodium wire. Dimethyformamide was distilled from phosphorus pentoxide and stored over molecular sieves (4 Å). *Iso*propanol was refluxed over calcium oxide, distilled and then allowed to stand over molecular sieves (5 Å). All reactions were carried out in dry glassware and under dry argon. Evaporations were carried out *in vacuo* with a rotary evaporator.

**CAUTION:** 5-fluorouracil, its derivatives and acrolein should be treated as highly toxic and handled accordingly.

**Statistical analyses.** The data derived from growth curves were subjected to analysis of variance with two independent factors. Student's *t* was used to analyze the differences between control and treated RD cells after FACS analysis. All data are means ± SEM of three separate experiments, and a *p* value less than or equal to 0.05 was considered significant.

**2-{2-[2-(*p*-toluenesulfonyloxy)ethoxy]ethyl}-1,3-dioxolane 25:** 2-[2-(2-hydroxyethoxy]ethyl}-1,3-dioxolane **24**[23] (5 g, 30.9 mmol) dissolved in anhydrous pyridine (10 mL) was cooled at 10° C under a flow of argon. *p*-toluenesulfonyl chloride (6.47 g, 33.9 mmol) was added slowly, taking care not to allow the temperature to reach 20 °C. After stirring for 3 h at rt, ice-water (50 mL) and concentrated HCl (20 mL) were added. After extraction with $CH_2Cl_2$ (3 × 30 mL), the organic layer was washed with brine (25 mL). The organic layer was separated, dried ($MgSO_4$), filtered and concentrated and the residue was purified by flash chromatography using a $CHCl_3$/MeOH (100/0.4) mixture yielding 3.47 g (35 %) of **25** as a thick oil. $R_f$ ($CHCl_3$/MeOH 100/1): 0.4. $^{13}C$ NMR (100.03 MHz, $CDCl_3$) δ 144.82 (C-$SO_{2Ts}$), 133.22 (C-$4_{Ts}$), 129.84 (C-$2_{Ts}$), 128.02 (C-$3_{Ts}$), 102.22 (C-1), 69.24 (C-5), 68.36 (C-6), 67.06 (C-3), 64.87 (dioxolane carbons), 34.19 (C-2) and 21.65 ($Me_{Ts}$). $^1H$ NMR (400.1 MHz, $CDCl_3$) δ 7.77 (d, $J_{2,3Ts}$ = 8.2, 2H, H-$2_{Ts}$), 7.31 (d, $J_{2,3Ts}$ = 8.2, 2H, H-$3_{Ts}$), 4.87 (t, $J_{1,2}$ = 4.9, 1H, H-1), 4.12 (t, $J_{6,5}$ = 4.8, 2H, H-6), 3.86 (m, 4H, dioxolane C$H_2$), 3.60 (t, $J_{5,6}$ = 4.8, 2H, H-5), 3.51 (t, $J_{3,2}$ = 6.5, 2H, H-3), 2.42 (s, 3H, $Me_{Ts}$) and 1.83 (q, *J* = 6.5 and 5.0, 2H, H-2). IR ($cm^{-1}$, neat): 2963, 2886 (s, aliphatic C-H groups), 1600 (w, aromatic ring multiple bond), 1452, 1358 (s, methoxy group), 1177, 1130, 1078 (s, C-O-C). MS (CI): *m/z* (%) 317 (20, $M^+$ + H), 101 (100, $C_5H_9O_2^+$), 73 (96, $C_3H_5O_2^+$). HR LSIMS calcd. for $C_{14}H_{19}O_6S$ ($M^+$ - H) 315.0902, found: 315.0899.

**3-[2-(*p*-toluenesulfonyloxy)ethoxy]propanal dimethyl acetal 16:** To **25** (2.16 g, 6.8 mmol) anhydrous MeOH (50 mL) containing $H_2SO_4$ (0.06 mL) were added. The solution was kept for 30 h at rt, basified (KOH/MeOH), filtered, concentrated and the residue was purified by flash chromatography using a diethyl ether/hexane (2/1) mixture yielding 1.94 g (89 %) ot **16** as a thick oil. $^{13}C$ NMR (100.03 MHz, $CDCl_3$) δ 144.88 (C-$SO_{2Ts}$), 133.15 (C-$4_{Ts}$), 129.88 (C-$2_{Ts}$), 128.05 (C-$3_{Ts}$), 102.12 (C-1), 69.25 (C-5), 68.37 (C-6), 67.35 (C-3), 53.22 (O*Me*), 33.01 (C-2) and 21.71 ($Me_{Ts}$). $^1H$ NMR (400.1 MHz, $CDCl_3$) δ 7.79 (dt, $J_{2,3Ts}$ = 8.2, $J_{2,MeTs}$ = 1.9, 2H, H-$2_{Ts}$), 7.33 (d, $J_{2,3Ts}$ = 8.2, 2H, H-$3_{Ts}$), 4.44 (t, $J_{1,2}$ = 5.8, 1H, H-1), 4.14 (q, $J_{6,5}$ = 4.8, 2H, H-6), 3.59 (q, $J_{5,6}$ = 4.8, 2H, H-5), 3.44 (t, $J_{3,2}$ = 6.4, 2H, H-3), 3.30 (s, 3H, *Me*O), 2.43 (s, 3H, $Me_{Ts}$) and 1.79 (q, *J* = 6.2, 2H, H-2). IR ($cm^{-1}$, neat): 2937, 2894 (s, aliphatic C-H groups), 1600 (s, aromatic ring multiple bond), 1451, 1361 (s, methoxy group), 1178, 1123, 1073 (s, C-O-C). MS (CI): *m/z* (%) 255 (52, $M^+$ - 2 × MeO + H), 71 (100). HR LSIMS calcd. for $C_{14}H_{21}O_6S$ ($M^+$ - H) 317.1059, found: 317.1053.

**3-allyloxypropanal di*iso*propyl acetal 19:** To 3-allyloxypropanal[17] (16 g, 0.13 mol) was added a solution of $H_2SO_4$ (1.6 mL) in anhydrous *iso*propanol (376 mL, 4.92 mol) to give a final 1 % concentration of the acid. The solution was kept for 16 h at rt, basified (KOH/$Pr^iOH$) and concentrated. The residue was dissolved in $CHCl_3$ (100 mL) and washed with $H_2O$ (2 × 20 mL). The organic layer was separated, dried ($MgSO_4$), filtered and concentrated and the residue distilled *in vacuo* (98-113 °C/ 16 torr) to give **19** (15 g, 50 %) as a colourless mobile liquid. $R_f$ (ether/hexane 1/1): 0.47. $^{13}C$ NMR (75.78 MHz, $CDCl_3$) δ 135.03 (C-6), 116.79 (C-7), 97.98 (C-1), 71.99 (C-5), 68.19 (*C*HMe$_2$), 66.62 (C-3), 35.99 (C-2), 23.48 and 22.61 (CH*Me*$_2$). $^1H$ NMR (300.13 MHz, $CDCl_3$) δ 5.89 (tq, $J_{6,7b}$ = 17.2, $J_{6,7a}$ = 10.3, $J_{6,5}$ = 5.6, 1H, H-6), 5.25 (dq, $J_{7b,6a}$ = 17.2, $J_{7b,7a}$ = 3.3, $J_{7b,5}$ = 1.7, 1H, H-$7_b$), 5.15 (dq, $J_{7a,6}$ = 10.3, $J_{7a,7b}$ = 3.3, $J_{7a,5}$ = 1.4, 1H, H-$7_a$), 4.73 (t, $J_{1,2}$ = 5.7, 1H, H-1), 3.93 (dt, $J_{5,7a}$ = 1.4, $J_{5,6}$ = 5.6,

2H, H-5), 3.84 (h, $J_{H,Me}$ = 6.2, 1H, C*H*Me$_2$), 3.48 (t, $J_{3,2}$ = 6.3, 2H, H-3), 1.85 (q, $J$ = 6.1, 2H, H-2), 1.18 (d, $J_{Me,H}$ = 6.2, 3H, CH*Me*$_2$) and 1.13 (d, $J_{Me,H}$ = 6.1, 3H, CH*Me*$_2$). IR (cm$^{-1}$, neat): 3084 (m, st =CH$_2$, 2974, 2935 (s, aliphatic C-H groups), 1381, 1369 (s, *iso*propoxy group), 1148 and 1109 (s, C-O-C). MS (CI): *m/z* (%) 157 (18, M$^+$ - OPr$^i$), 99 (24, M$^+$ - 2 × OPr$^i$ - H), 83 (100). HR LSIMS calcd. for $C_{12}H_{23}O_3$ (M$^+$ - H) 215.1647, found: 215.1653.

**(*RS*)-1-[3-(3-chloro-2-hydroxypropoxy)propanal dimethyl acetal 20**: Anhydrous MeOH (25 mL, 0.62 mmol) containing $H_2SO_4$ (0.13 mL) was added to 2-[2-(3-chloro-2-hydroxypropoxy)ethyl]-4-chloromethyl-1,3-dioxolane[18] (4.5 g, 17.37 mmol) to give a final 1 % concentration of the acid. The solution was kept for 6 d at rt, neutralized with anhydrous $NaHCO_3$ (0.315 g), filtered, concentrated and the residue was purified by flash chromatography using an ether/hexane (1/1) mixture, yielding 2.11 g (57 %) of **20** as a colourless liquid. $R_f$ (ether/hexane 1/1): 0.18. $^{13}$C NMR (75.78 MHz, $CDCl_3$) δ 102.23 (C-1), 71.53 (C-5), 70.21 (C-6), 67.52 (C-3), 53.04 (O*Me*), 45.91 (C-7) and 32.76 (C-2). $^1$H NMR (300.13 MHz, $CDCl_3$) δ 4.49 (t, $J_{1,2}$ = 5.7, 1H, H-1), 3.93 (quint, $J$ = 5.3, 1H, H-6), 3.60 (dd, $J_{gem}$ = 11.1, $J_{7,6}$ = 5.5, 2H, H-7), 3.53 (dd, $J_{gem}$ = 14.1, $J_{5,6}$ = 5.1, 2H, H-5), 3.52 (t, $J_{3,2}$ = 6.2, 2H, H-3), 3.30 (s, 3H, *Me*O), 2.67 (s, 1H, O*H*) and 1.86 (q, $J$ = 6.1, 2H, H-2). IR (cm$^{-1}$, neat): 3443 (m, st OH), 2940 (s, aliphatic C-H groups), 1388 (s, methoxy group), 1193 (s, C-O-C). MS (CI): *m/z* (%) 213 (13, M$^+$ + H), 181 (53, M$^+$ - OMe), 149 (100, M$^+$ - 2 × OMe - H). HR LSIMS calcd. for $C_8H_{18}O_4Cl$ (M$^+$ + 1) 213.0900, found: 213.0898.

**3-(2,2-dimethoxyethoxy)propanal dimethyl acetal 28 and methyl 2-(3,3-dimethoxypropoxy)acetate 29:** DMSO (4.41 mL, 61.7 mmol) and $P_2O_5$ (7.88 g, 55.5 mmol) were added successively with stirring to a cooled 0.2 M solution of 2-[2-[2-hydroxyethoxy)ethyl]-1,3-dioxolane **24**[22] in anhydrous $CH_2Cl_2$. The reaction was kept at rt for 30 min and, then tryethylamine (TEA, 15 mL, 108 mmol) was added with stirring and the cooling of the solution in a ice-water bath for 20 min. The mixture was left at rt for 30 minutes and then, was acidified with an aqueous solution of HCl (10 %). After washing with brine (2 × 40 mL) the organic layer was dried ($MgSO_4$), filtered and rotaevaporated off. After filtrating the solid which resulted from the addition of diethyl ether, the filtrate was concentrated to yield a liquid (3.89 g), whose analysis by glc showed the presence of a major peak (retention time, glc: 3.03 min, area: 92.9 %; programme: isotherm 150 °C/4 min, ramp 25 °C/min, isotherm 200 °C/1 min). Such a sample {identified as the mixture of 2-[2-(formylmethoxy)ethyl]-1,3-dioxolane **26** and 2[3-(carbomethoxy)ethyl]-1,3-dioxolane **27**} was used in the next step without further purification.

The above mixture (**26** + **27**, 3.6 g) was dissolved in anhydrous MeOH (75 mL, 1.86 mol) which contained concentrated $H_2SO_4$ (0.35 mL) to give a final concentration of the acid. The solution was kept for 5 d at rt, basified (NaOH/MeOH) and concentrated. The residue was dissolved in diethyl ether (50 mL) and washed with $H_2O$ (2 × 20 mL). The organic layer was separated, dried, filtered and concentrated, and the residue was purified by flash chromatography using an ether/hexane (2/3) mixture as eluant, yielding a mixture of **28** and **29** (**28/29** ratio: 63/37) as shown by the $^1$H NMR (1.25 g, retention time, glc: 3.49 min, area: 92.9 %; the programme was the same as above). $R_f$ (ether/hexane, 1/1): 0.38.

$^1$H NMR of **28**: (300.13 MHz, $CDCl_3$) δ 4.49 (t, $J_{1,2}$ = 5.8, 1H, H-1), 4.46 (t, $J_{6,5}$ = 5.2, 1H, H-7), 3.51 (t, $J_{3,2}$ = 6.5, 2H, H-3), 3.44 (d, $J_{5,6}$ = 5.2, 2H, H-5), 3.36 (s, 6H, *Me*O), 3.30 (s, 6H, *Me*O) and 1.86 (q, $J$ = 6.2, 2H, H-2).

$^1$H NMR of **29**: (300.13 MHz, $CDCl_3$) δ 4.52 (t, $J_{1,2}$ = 5.8, 1H, H-1), 4.04 (s, 2H, H-5), 3.72 (s, 3H,

*Me*O$_{ester}$), 3.56 (t, $J_{3,2}$ = 6.4, 2H, H-3), 3.31 (s, 6H, *Me*O$_{acetalic}$) and 1.89 (q, $J$ = 6.2, 2H, H-2).

**Synthesis of nucleobase acyclonucleosides 5-15. General procedure:** To a suspension of the acetal (1 mmol), the pyrimidine or the purine base (1-1.1 mmol), which contains trimethylchlorosilane (TMCS, 0.8 mmol) and hexamethyldisilazane (HMDS, 0.8 mmol) in dry acetonitrile (10 mL/mmol of acetal) was added a solution of anhydrous tin (IV) chloride (1.25 mmol) in dry acetonitrile (10 mL/mmol of the Lewis acid) dropwise with stirring under argon ar rt. After stirring, the reaction was neutralized by the addition of anhydrous $K_2CO_3$. The salts formed were filtered, the residue rotaevaporated off and extracted ($CHCl_3$). After filtration and concentration the residue was purified by flash chromatography using mixtures of $CHCl_3$/MeOH to give **5-6, 8-12, 14-15.** For an analytical sample the product was purified by gravity chromatography.

**(*RS*)-1-{1-methoxy-3-[2-(*p*-toluenesulfonyloxy)ethoxy]propyl}-5-fluorouracil 5:** Reaction of **16** (0.44 g, 1.38 mmol) with 5-fluorouracil (0.198 g, 1.52 mmol) for 6 h according to the general procedure yielded **5** (0.39 g, 68 %) as a syrup that, after standing gave rise to an amorphous white powder. Mp 138-140 °C. $R_f$ ($CHCl_3$/MeOH, 100/4): 0.29. $^{13}$C NMR (75.78 MHz, $CDCl_3$) δ 157.10 (d, $J_{4,F}$ = 26.4, C-4$_{5FU}$), 149.88 (s, C-2$_{5FU}$), 141.08 (d, $J_{5,F}$ = 238.4, C-5$_{5FU}$), 123.08 (d, $J_{6,F}$ = 33.1, C-6$_{5FU}$), 144.91 (C-SO$_2$), 132.89 (C-4$_{Ts}$), 129.90 (C-2$_{Ts}$), 127.94 (C-3$_{Ts}$), 85.34 (C-1), 69.15 (C-6), 68.53 (C-3), 66.37 (C-5), 56.74 (O*Me*), 34.85 (C-2) and 21.62 (*Me*$_{Ts}$). $^1$H NMR (300.13 MHz, $CDCl_3$) δ 9.85 (s, 1H, N*H*), 7.75 (d, $J_{2,3Ts}$ = 8.3, 2H, H-2$_{Ts}$), 7.32 (d, $J_{H,F}$ = 5.8, 1H, H$_{5F}$), 7.31 (d, $J_{3,2Ts}$ = 8.3, 2H, H-3$_{Ts}$), 5.67 (dt, $J_{1,2}$ = 6.4, $J_{1,F}$ = 1.9, 1H, H-1), 4.07 (t, $J_{6,5}$ = 4.8, 2H, H-6), 3.49 (m, 4H, H-3, H-5), 3.30 (s, 3H, *Me*O), 2.40 (s, 3H, *Me*$_{Ts}$) and 1.9 (m, 2H, H-2). IR (cm$^{-1}$, KBr): 3467 (m, NH), 3195, 3079 (s, aromatics), 2943 (s, aliphatic C-H groups), 1711, 1599 (s, aromatic ring multiple bond), 1468, 1355 (s, methoxy group), 1191, 1177, 1133 (s, C-O-C). MS (CI): *m/z* (%) 417 (13, M$^+$ + H), 131 (4, 5FU$^+$ + H), 71 (100). Anal. for $C_{17}H_{21}O_7N_2FS$: Calcd.: C, 49.03; H, 5.08; N, 6.73; S, 7.70. Found: C, 48.95; H, 5.20; N, 6.61; S, 7.50.

**(*RS*)-1-[1-methoxy-3-(2-methoxyethoxy)propyl]propyl]-5-fluorouracil 6:** Reaction of 3-(2-methoxyethoxy)propanal dimethylacetal **17**[16] (0.5 g, 2.81 mmol) with 5-fluorouracil (0.402 g, 3.09 mmol) for 14 h according to the general procedure yielded **6** (0.53 g, 68 %) as an amorphous white powder. Mp 116-119 °C. $R_f$ ($CHCl_3$/MeOH, 100/5): 0.38. $^{13}$C NMR (75.78 MHz, $CDCl_3$) δ 157.03 (d, $J_{4,F}$ = 27.2, C-4$_{5FU}$), 149.93 (s, C-2$_{5FU}$), 141.09 (d, $J_{5,F}$ = 238.5, C-5$_{5FU}$), 123.33 (d, $J_{6,F}$ = 33.2, C-6$_{5FU}$), 85.64 (C-1), 71.89 and 70.40 (C-5 and C-6), 66.28 (C-3), 59.08 (O*Me*$_{acetalic}$), 56.71 (O*Me*) and 34.94 (C-2). $^1$H NMR (300.13 MHz, $CDCl_3$) δ 9.5 (s, 1H, N*H*), 7.34 (d, $J_{H,F}$ = 5.8, 1H, H$_{5F}$), 5.72 (dt, $J_{1,2}$ = 6.4, $J_{1,F}$ = 1.9, 1H, H-1), 3.60-3.44 (m, 6H, H-3, H-5 and H-6), 3.33 (s, 3H, *Me*O), 3.33 (s, 3H, *Me*O) and 2.00 (m, 2H, H-2). IR (cm$^{-1}$, KBr): 3459 (m, NH), 3206, 3074 (s, aromatics), 2975, 2958 (s, aliphatic C-H groups), 1723, 1708 (s, aromatic ring multiple bond), 1116, 1088 (s, C-O-C). MS (CI): *m/z* (%) 277 (100, M$^+$ + H), 245 (6, M$^+$ - Me). Anal. for $C_{11}H_{17}O_5N_2F$: Calcd.: C, 47.82; H, 6.20; N, 10.14. Found: C, 47.82; H, 6.20; N, 10.14.

**(*RS*)-7-(3-allyloxy-1-*iso*propoxypropyl)adenine 9 and (*RS*)-9-(3-allyloxy-1-*iso*propoxypropyl)-adenine 10:** Reaction of 3-allyloxypropanal di*iso*propylacetal **19** (1 g, 4.62 mmol) with adenine (0.687 g, 5.08 mmol) for 19 h according to the general procedure yielded a first fraction which was identified as **10** (0.142 g, 10 %), an amorphous white powder. Mp 113-115 °C. $R_f$($CHCl_3$/MeOH, 10/1): 0.36. $^{13}$C NMR (100.03 MHz, $CDCl_3$) δ 155.57 (C-6$_{Ad}$), 153.27 (s, C-2$_{Ad}$), 150.14 (C-4$_{Ad}$), 138.70 (C-8$_{Ad}$), 119.64 (C-5$_{Ad}$), 134.62 (C-6), 117.19 (C-7), 80.24 (C-1), 72.13 (C-5), 70.98 (CHMe$_2$), 65.48 (C-3), 36.81 (C-2), 22.96 and 21.45 (CH*Me*$_2$). $^1$H NMR (400.1

MHz, $CDCl_3$) δ 8.37 (s, 1H, H-$2_{Ad}$), 7.98 (s, 1H, H-$8_{Ad}$), 6.01 (dd, $J_{1,2a}$ = 7.7, $J_{1,2b}$ = 5.5, 1H, H-1), 5.95 (s, 2H, N$H_2$), 5.83 (ddt, $J_{6,7b}$ = 17.1, $J_{6,7a}$ = 10.7, $J_{6,5a}$ = 5.7, 1H, H-6), 5.21 (dd, $J_{7b,5b}$ = 17.2, $J_{7b,7a}$ = 1.3, 1H, H-$7_b$), 5.13 (d, $J_{7a,6cis}$ = 10.4, 1H, H-$7_a$), 3.90 (m, 2H, H-5), 3.61 (h, $J$ = 6.1, 1H, C*H*$Me_2$), 3.56 (ddd, $J_{3,2b}$ = 9.3, $J_{3,2a}$ = 8.3, $J_{3a,3b}$ = 4.8, 1H, H-$3_b$), 3.40 (m, 1H, H-$3_a$), 2.35 (m, 1H, H-$2_b$), 2.21 (m, 1H, H-$2_a$), 1.22 (d, $J$ = 6.0, 3H, *CH*$Me_2$) and 0.98 (d, $J$ = 6.2, 3H, *CH*$Me_2$). IR ($cm^{-1}$, KBr): 3426 (m, $NH_2$), 3074 (s, aromatics), 1629 (m, st double bond), 1088 (s, C-O-C). MS (CI): *m/z* (%) 292 (100, $M^+$ + H). HR LSIMS calcd. for $C_{14}H_{22}N_5O_2$ ($M^+$ + H) 292.1774, found: 292.1775. Anal. for $C_{14}H_{21}O_2N_5.1/10H_2O$: Calcd.: C, 57.36; H, 7.29; N, 23.89. Found: C, 57.28; H, 7.69; N, 24.18.

The second fraction was identified as **9** (0.42 g, 31 %) after recrystallization (hexane/methanol). $R_f$ ($CHCl_3$/MeOH, 10/1): 0.26. Mp 155-158 °C. $^{13}C$ NMR (100.03 MHz, $CDCl_3$) δ 161.64 (C-$4_{Ad}$), 153.64 (s, C-$2_{Ad}$), 151.33 (C-$6_{Ad}$), 144.22 (C-$8_{Ad}$), 110.25 (C-$5_{Ad}$), 134.07 (C-6), 117.81 (C-7), 83.72 (C-1), 72.25 (C-5), 72.24 (*C*HMe$_2$), 64.76 (C-3), 37.73 (C-2), 22.60 and 21.22 (CH*Me*$_2$). $^1H$ NMR (400.1 MHz, $CDCl_3$) δ 8.47 (s, 1H, H-$2_{Ad}$), 8.00 (s, 1H, H-$8_{Ad}$), 6.25 (s, 2H, $NH_2$), 5.84 (tc, $J_{6,7b}$ = 17.2, $J_{6,7a}$ = 10.3, $J_{6,5}$ = 5.8, 1H, H-6), 5.75 (dd, $J_{1,2a}$ = 7.5, $J_{1,2b}$ = 6.4, 1H, H-1), 5.22 (dq, $J_{7b,6}$ = 17.2, $J_{7b,7a}$ = 3, 1H, H-$7_b$), 5.17 (ddd, $J_{7a,6}$ = 10.3, $J_{7a,7b}$ = 3, $J_{7b,5}$ = 1.6, 1H, H-$7_a$), 3.86 (dt, $J_{5,6}$ = 5.8, $J_{5,7}$ = 1.4, 2H, H-5), 3.80 (h, $J_{H,Me}$ = 6.2, 1H, C*H*$Me_2$), 3.46 (ddd, $J_{7b,7a}$ = 9.8, $J_{7b,6a}$ = 6.3, $J_{7b,6b}$ = 4.0, 1H, H-$3_b$), 2.88 (ddd, $J_{3a,3b}$ = 9.8, $J_{3a,2b}$ = 8.3, $J_{3a,2a}$ = 3.6, 1H, H-$3_a$), 2.33 (dddd, $J_{2b,2a}$ = 14.3, $J_{2b,3a}$ = 8.3, $J_{2b,1}$ = 6.4, $J_{2b,3b}$ = 4, 1H, H-$2_b$), 2.02 (1H, dddd, $J_{2a,2b}$ = 14.3, $J_{2a,1}$ =7.5, $J_{2a,3b}$ = 6.3, $J_{2a,3a}$ = 3.6, 1H, H-$2_a$), 1.26 (d, $J_{Me,H}$ = 6.0, 3H, CH*Me*$_2$) and 1.10 (d, $J_{Me,H}$ = 6.2, 3H, CH*Me*$_2$). IR ($cm^{-1}$, KBr) 3424 ($NH_2$), 3075 (s, aromatics), 1629 (m, st double bond), 1090 (s, C-O-C). MS (CI): *m/z* (%) 292 (100, $M^+$ + H). HR LSIMS calcd. for $C_{14}H_{22}N_5O_2$ ($M^+$ + H) 292.1774, found: 292.1771. Anal. for $C_{14}H_{21}O_2N_5.1/10H_2O$: Calcd.: C, 57.36; H, 7.29; N, 23.89. Found: C, 57.57; H, 7.42; N, 23.54.

The reaction of 3-allyloxypropanal di*iso*propylacetal **19** (2 g, 9.24 mmol) with adenine (1.37 g, 10.17 mmol) for 19 h yielded **10** (1.24 g, 50 %) and **9** (0.64 g, 26 %) when it was carried out according to the general procedure but using trimethylsilyl trifluoromethanesulfonate.

**(*RS*)-1-(3-allyloxy-1-*iso*propoxypropyl)uracil 11:** Reaction of 3-allyloxypropanal di*iso*propylacetal **19** (2.9 g, 13.4 mmol) with fluorouracil (1.5 g, 13.4 mmol) for 1 h according to the general procedure yielded **11** (1 g, 30 %), an amorphous white powder. Mp 118-122 °C. $R_f$($CHCl_3$/MeOH, 95/5): 0.37. $^{13}C$ NMR (75.78 MHz, $CDCl_3$) δ 163.55 (C-$4_U$), 151.00 (C-$2_U$), 139.65 (C-$6_U$), 102.76 (C-$5_U$), 134.56 (C-6), 117.11 (C-7), 81.09 (C-1), 72.02 (C-5), 70.91 (*C*HMe$_2$), 65.41 (C-3), 35.68 (C-2), 22.90 and 21.39 (CH*Me*$_2$). $^1H$ NMR (300.1 MHz , $CDCl_3$) δ 9.4 (s, 1H, N*H*), 7.37 (d, $J_{6,5U}$ = 8.1, 1H, H-$6_U$), 5.92 (t, $J_{1,2}$ = 6.8, 1H, H-1), 5.84 (tq, $J_{6,7b}$ = 17.3, $J_{6,7a}$ = 10.4, $J_{6,5}$ = 5.6, 2H, H-6), 5.75 (d, $J_{5,6U}$ = 8.0, 1H, H-$5_U$), 5.22 (dq, $J_{7b,6}$ = 17.3, $J_{7b,7a}$ = 1.6, $J_{7b,5}$ = 1.6, 1H, H-$7_b$), 5.13 (dq, $J_{7a,6}$ = 10.4, $J_{7a,7b}$ = 1.6, $J_{7a,5}$ = 1.3, 1H, H-$7_a$), 3.90 (m, 2H, H-5), 3.65 (h, $J_{H,Me}$ = 6.1, 1H, C*H*$Me_2$), 3.49 (m, 2H, H-3), 1.95 (m, 2H, H-2), 1.18 (d, $J_{Me,H}$ = 6.1, 3H, CH*Me*$_2$) and 1.09 (d, $J_{Me,H}$ = 6.2, 3H, CH*Me*$_2$). IR ($cm^{-1}$, KBr): 3198 (m, NH), 3066 (s, aromatics), 2977, 2934, 2877 (s, aliphatic C-H groups), 1693, 1680 (s, aromatic ring multiple bond), 1097 (s, C-O-C). MS (CI): *m/z* (%) 269 (35, $M^+$ + H), 157 (44, $M^+$ - U + H), 99 (100). Anal. for $C_{13}H_{20}O_4N_2$: Calcd.: C, 58.19; H, 7.51; N, 10.44. Found: C, 58.23; H, 7.55; N, 10.43.

**(*RS*)-1-(3-allyloxy-1-*iso*propoxypropyl)-5-fluorouracil 12:** Reaction of 3-allyloxypropanal di*iso*propylacetal **19** (1.1 g, 5.08 mmol) with 5-fluorouracil (0.727 g, 5.59 mmol) for 24 h according to the general procedure yielded **12** (1.05 g, 72 %) as an amorphous white powder. Mp 72-74 °C. $R_f$($CHCl_3$/MeOH, 100/3):

0.29. $^{13}$C NMR (75.78 MHz, $CDCl_3$) δ 156.95 (d, $J_{4,F}$ = 26.4, C-$4_{5FU}$), 149.49 (s, C-$2_{5FU}$), 140.88 (d, $J_{5,F}$ = 237.7, C-$5_{5FU}$), 123.70 (d, $J_{6,F}$ = 32.5, C-$6_{5FU}$), 134.47 (C-6), 117.23 (C-7), 81.09 (C-1), 72.07 (C-5), 71.19 (*C*HMe$_2$), 65.29 (C-3), 37.57 (C-2), 22.90 and 21.38 (CH*Me*$_2$). $^1$H NMR (300.1 MHz , $CDCl_3$) δ 9.4 (s, 1H, N*H*), 7.42 (d, $J_{H,F}$ = 5.9, 1H, H-$5_{5FU}$), 5.92 (ddd, $J_{1,2b}$ = 7.2, $J_{1,2a}$ = 5.7, $J_{1,F}$ = 1.9, 1H, H-1), 5.84 (tq, $J_{6,7b}$ = 17.3, $J_{6,7a}$ = 10.3, $J_{6,5}$ = 5.6, 1H, H-6), 5.22 (dq, $J_{7b,6}$ = 17.3, $J_{7b,5}$ = 1.6, $J_{7a,7b}$ = 1.6, 1H, H-$7_b$), 5.14 (dq, $J_{7a,6}$ = 10.3, $J_{7b,7a}$ = 1.6, $J_{7a,5}$ = 1.3, 1H, H-$7_a$), 3.90 (m, 2H, H-5), 3.67 (h, $J_{H,Me}$ = 6.1, 1H, C*H*Me$_2$), 3.5 (m, 2H, H-3), 1.92 (m, 2H, H-2), 1.19 (d, $J_{Me,H}$ = 6.1, 3H, CH*Me*$_2$) and 1.12 (d, $J_{Me,H}$ = 6.2, 3H, CH*Me*$_2$). IR (cm$^{-1}$, KBr): 3424 (m, NH), 3211 (m, NH), 3082 (s, aromatics), 2941 (s, aliphatic C-H groups), 1712, 1672 (s, aromatic ring multiple bond), 1387 (m, *iso*propoxy group), 1090 (s, C-O-C). MS (CI): *m/z* (%) 287 (23, M$^+$ + H), 157 (100, M - 5FU). Anal. for $C_{13}H_{19}O_4N_2F$: Calcd.: C, 54.54; H, 6.69; N, 9.78. Found: C, 54.94; H, 6.86; N, 10.09.

**1-{[3-(2-chloro-3-hydroxypropoxy)-1-methoxy]propyl}-5-fluorouracil 22 (mixture of diastereoisomers) and 1-{[3-(3-chloro-2-hydroxypropoxy)-1-methoxy]propyl}-5-fluorouracil 14 (mixture of diastereoisomers):** Reaction of **20** (2 g, 9.40 mmol) with fluorouracil (1.34 g, 10.34 mmol) for 24 h according to the general procedure yielded a crude which was purified by flash chromatography using a mixture of $CHCl_3$/MeOH (10/1): 0.38. The first fraction weighed 0.34 g (12 %) as a thick colourless oil and was identified as **22**. $R_f$($CHCl_3$ /MeOH, 100/5): $^{13}$C NMR (75.78 MHz, $CDCl_3$) δ 157.13 (d, $J_{4,F}$ = 26.4, C-$4_{5FU}$), 149.97 (s, C-$2_{5FU}$), 141.11 (d, $J_{5,F}$ = 238.3, C-$5_{5FU}$), 123.11 (d, $J_{6,F}$ = 33.2, C-$6_{5FU}$), 85.43, 85.40 (C-1), 71.85, 71.57 (C-5), 66.39, 66.37 (C-3), 56.72 (*Me*O), 50.74, 50.71 (C-6), 44.10, 44.08 (C-7). 34.99, 34.96 (C-2). $^1$H NMR (300.13 MHz, $CDCl_3$) δ 9.9, (s, 2H, N*H*), 7.34 (d, $J_{H,F}$ = 6.0, 2H, H-$5_{5FU}$), 5.73 and 5.71 (dt, $J$ = 6.4 and 1.8, 2H, H-1), 3.68 (ddd, $J$ = 11.5, 6.2 and 2.8, 2H), 3.63-3.42 (m, 6H), 3.32 (s, 6H, *Me*O), 3.28 (ddd, $J$ = 11.4 and 6.1, 2H), 3.07 (ddt, $J$ = 2.8, 2H), 2.74 (t, $J$ = 4.6, 2H, O*H*), 2.54 (dt, $J$ = 5.4 and 2.70, 2H), 1.97 (m, 4H, H-2). HR LSIMS calcd. for $C_{11}H_{17}O_5N_2FCl$ (M$^+$ + H): 311.0810, found: 311.0809.

The second fraction was identified as **14** (1.69 g, 58 %), a thick colourless oil. $R_f$($CHCl_3$/MeOH, 10/1): 0.36. $^{13}$C NMR (75.78 MHz, $CDCl_3$) δ 157.04 (d, $J_{4,F}$ = 26.5, C-$4_{5FU}$), 150.21 (s, C-$2_{5FU}$), 141.43 (d, $J_{5,F}$ = 239.6, C-$5_{5FU}$), 122.85 (d, $J_{6,F}$ = 32.9, C-$6_{5FU}$), 85.37 (C-1), 72.31, 71.34 (C-5), 70.27, 71.21 (C-6), 66.41, 66.38 (C-3), 56.85 (*Me*O), 45.65, 45.29 (C-7), 34.84 (C-2). $^1$H NMR (300.13 MHz , $CDCl_3$) δ 10.00, (d, $J$ = 4.8, 1H, N*H* of one diastereoisomer), 9.97 (d, $J$ = 4.8, 1H, N*H* of the other one), 7.34 (d, $J_{H,F}$ = 5.2, 2H, H-$5_{5FU}$), 5.78 (dt, $J_{1,2}$ = 6.5, $J_{1,F}$ = 1.8, 2H, H-1), 3.98 (ddd, $J$ = 11.6, 5.5 and 4.0, 2H, H-$6_b$), 3.90 (m, 2H, H-$6_a$), 3.39-3.67 (m, 12H, H-3, H-5 and H-7), 3.34 (s, 6H, *Me*O), 3.20 (s, 1H, O*H*), 2.07 (m, 2H, H-$2_b$), 1.93 (m, 2H, H-$2_a$). IR (cm$^{-1}$, neat): 3434 (m, NH), 3073 (s, aromatics), 2941 (s, aliphatic C-H groups), 1704 (s, aromatic ring multiple bond), 1242, 1126, 1080 (s, C-O-C). *m/z* (%) 313 (35, M$^+$ + 2), 311 (100, M$^+$), 281 (10, M$^+$ + 2 - OMe), 279 (28, M$^+$ - OMe), 131 (74, 5FU$^+$). Anal. for $C_{11}H_{16}O_5N_2FCl.H_2O.3/10CHCl_3$: Calcd.: C, 37.23; H, 5.06; N, 7.68. Found: C, 37.11; H, 4.73; N, 8.06.

**(*RS*)-1-[3-(2-chloroethoxy)-1-methoxy)-1-methoxypropyl]-5-fluorouracil 7:** Anhydrous $CCl_4$ (0.11 mL, 1.14 mmol) was added to anhydrous DMF (10 mL) that contained 1-[(2-hydroxyethoxy)-1-methoxypropyl]-5-fluorouracil **3**[1] (0.2 g, 0.76 mmol) and $Ph_3P$ (0.2 g, 0.76 mmol), and the solution stirred at 100 °C for 1 h. Afterwards the mixture was kept at 30 °C for 15 h further, concentrated to dryness and the crude obtained purified by flash chromatography ($CHCl_3$/MeOH, 100/1) to yield **7** (0.19 g, 89 %) as a thick colourless oil. $R_f$($CHCl_3$/ MeOH, 100/5): 0.36. $^{13}$C NMR (75.78 MHz, $CDCl_3$) δ 156.81 (d, $J_{4,F}$ = 27.2, C-$4_{5FU}$), 149.68 (s, C-$2_{5FU}$), 141.13 (d, $J_{5,F}$

= 238.8, C-$5_{5FU}$), 123.19 (d, $J_{6,F}$ = 33.0, C-$6_{5FU}$), 85.51 (C-1), 71.19 (C-5), 66.29 (C-3), 56.87 (*Me*O), 42.81 (C-6), 35.04 (C-2). $^1$H NMR ( 300.13 MHz, $CDCl_3$) δ 9.1 (s, 1H, N*H*), 7.35 (d, $J_{H,F}$ = 5.8, 1H, H-$5_{5FU}$), 5.74 (dt, $J_{1,2}$ = 6.4, $J_{1,F}$ = 1.9, 1H, H-1), 3.71-3.51 (m, 6H, H-3, H-5 and H-6), 3.35 (s, 3H, *Me*O) and 2.00 (m, 2H, H-2). IR ($cm^{-1}$, neat): 3203 (m, NH), 3078 (s, aromatics), 2960, 2927 (s, aliphatic C-H groups), 1665 (s, aromatic ring multiple bond), 1095, 1074 (s, C-O-C). MS (CI): *m/z* (%) 283 (11, $M^+$ + 2), 281 (31, $M^+$), 251 (5, $M^+$ - MeO - H), 249 (15, $M^+$ - MeO - H), 71 (100). Anal. for $C_{10}H_{14}O_4N_2FCl$: Calcd.: C, 42.79; H, 5.03; N, 9.98. Found: C, 42.97; H, 4.99; N, 9.70.

**1-[3-(2,3-dihydroxypropoxy)-1-*iso*propoxypropyl]-5-fluorouracil 13 (mixture of diastereoisomers):** A solution of 1-(3-allyloxy-1-*iso*propoxypropyl)-5-fluorouracil **12** (0.280 g, 0.98 mmol) in acetone (5.5 mL) and $H_2O$ (2 mL) was treated with a small crystal of osmium tetroxide. After being stirred at rt for 10 min, the solution was treated with *N*-methyl-morpholine *N*-oxide (0.230 g, 1.96 mmol) and stirred at rt for 68 h. Then, $NaHSO_3$ (0.215, 2.07 mmol) dissolved in water (5 mL) was added. The solvent was removed under reduced pressure and the residue chromatographed on silica gel, eluting with $CHCl_3$/MeOH (100/5) to give the title compound **13** as an oil (0.138 g, 44 %), which solidified with time. Mp 66-71 °C. $R_f$($CHCl_3$/MeOH, 10/1): 0.19. $^{13}$C NMR (75.78 MHz, $CDCl_3$) δ 157.38 (d, $J_{4,F}$ = 23.9, C-$4_{5FU}$), 150.26 (s, C-$2_{5FU}$), 141.32 (d, $J_{5,F}$ = 238.7, C-$5_{5FU}$), 123.32 (d, $J_{6,F}$ = 32.8, C-$6_{5FU}$), 81.62, 81.58 (C-1), 72.78, 72.17 (C-6), 71.21, 71.16 (C-5), 70.81 (*C*HMe$_2$), 66.36 (C-3), 63.73, 63.60 (C-7), 35.47, 35.37 (C-2). 22.92 (CH*Me*$_2$), 21.36, 21.33 (CH*Me*$_2$). $^1$H NMR (300 MHz, $CDCl_3$) δ 10.51, 10.44 (s, 2H, N*H* of the two diastereoisomers), 7.40 (d, $J_{H,F}$ = 5.7, 2H, H-$5_{5FU}$), 5.97 (ddd, $J_{1,2}$ = 7.7, $J_{1,F}$ = 1.9, 2H, H-1), 3.88-3.33 (m, 16H, H-3, H-5, H-6, H-7, C*H*Me$_2$), 3.10 (s, 2H, O*H*), 1.90 (m, 4H, H-2), 1.20 (d, $J_{Me,H}$ = 6.0, 6H, CH*Me*$_2$) and 1.13 (d, $J_{Me,H}$ = 6.2, 6H, CH*Me*$_2$). IR ($cm^{-1}$, neat): 3423 and 3206 (s, NH and OH), 3066 (s, aromatics), 2976, 2933 (s, aliphatic C-H groups), 1244, 1126, 1073 (s, C-O-C). *m/z* (%) 283 (49, $M^+$ + H), 261 (13, $M^+$ - OPr$^i$), 131 (47, $5FU^+$ + H), 99 (100). Anal. for $C_{13}H_{21}O_6N_2F.1/4H_2O$: Calcd.: C, 48.07; H, 6.67; N, 8.62. Found: C, 48.08; H, 6.77; N, 8.74.

**1-[1-methoxy-3-(methoxycarbonylmethoxy)propyl]-5-fluorouracil 8 and 3,8-bis(5-fluorouracil-1-yl)-2,5,9-trioxadecane 13 (mixture of diastereoisomers):** To a cooled suspension of the mixture **28** + **29** (0.66 g), 5-fluorouracil (0.907 g, 6.97 mmol) containing HMDS (1.32 mL, 6.34 mmol) and TMCS (0.8 mL, 6.34 mmol) in dry MeCN (19 mL), a solution of $SnCl_4$ (0.9 mL, 7.61 mmol) was added in $ClCH_2CH_2Cl$ (3 mL) dropwise with stirring and under argon. After 12 h of stirring at rt the reaction was quenched by the addition of an aqueous solution of $K_2CO_3$, the salts were filtered and the filtrate was rotaevaporated off and the residue purified by flash chromatography using a gradient elution ($CHCl_3$/MeOH, 100/2 → $CHCl_3$/MeOH, 100/5) to afford two compounds. The first fraction gave **8** (0.36 g, 95 %) as an off-white amorphous solid. Mp 93-98 °C. $R_f$($CHCl_3$/MeOH, 10/1): 0.56. $^{13}$C NMR (75.78 MHz, $CDCl_3$) δ 156.99 (d, $J_{4,F}$ = 26.6, C-$4_{5FU}$), 149.82 (s, C-$2_{5FU}$), 141.13 (d, $J_{5,F}$ = 238.7, C-$5_{5FU}$), 123.23 (d, $J_{6,F}$ = 33.1 C-$6_{5FU}$), 85.37 (C-1), 70.56 ($CO_{ester}$), 68.32 (C-5), 66.74 (C-3), 56.83 (*Me*$O_{ester}$), 51.94 (*Me*O), 34.99 (C-2). $^1$H NMR (300.13 MHz, $CDCl_3$) δ 9.4 (s, 1H, N*H*), 7.37 (d, $J_{H,F}$ = 5.8, 1H, H-$5_{5FU}$), 5.76 (dt, $J_{1,2}$ = 6.3, $J_{1,F}$ = 1.9, 1H, H-1), 4.03 (d, $J$ = 0.8, 2H, H-5), 3.72 (s, 3H, *Me*$O_{ester}$), 3.62 (m, 2H, H-3), 3.35 (s, 3H, *Me*O) 2.03 (q, $J$ = 6.3, 2H, H-2). IR ($cm^{-1}$, KBr): 3208 (m, NH), 3081 (s, aromatics), 2958 (s, aliphatic C-H groups), 1708 (s, C=O), 1136, 1081 (s, C-O-C). *m/z* (%) 291 (43, $M^+$ + H), 131 (45, $5FU^+$ + H), 71 (100). Anal. for $C_{11}H_{15}O_6N_2F$: Calcd.: C, 45.52; H, 5.21; N, 9.65. Found: C, 45.61; H, 5.21; N, 9.60.

Eluted secondly was **15** (0.3 g, 38 %) as an amorphous white solid. Mp 214-217 °C. $R_f$($CHCl_3$/MeOH,

10/1): 0.39. $^{13}C$ NMR (75.78 MHz, $CDCl_3$) δ (selected data) 157.50, 157.0 (C-$4_{5FU}$), 150.38, 150.23 (C-$2_{FU}$), 142.96, 139.79 (C-$5_{5FU}$), 123.75, 122.64 (C-$6_{5FU}$), 85.00 and 84.88 (C-1 and C-6), 70.61 (C-6), 66.89, 66.58 (C-3), 57.37, 56.83 (*Me*O), 35.03, 34.81 (C-2). $^1H$ NMR (300.13, $CDCl_3$) δ 10.4 (t, $J$ = 4.2, 2H, N*H*), 10.19 (d, $J$ = 4.7, 1H, N*H*), 10.06 (d, $J$ = 4.8, 1H, N*H*), 7.42 (t, $J_{H,F}$ = 5.6, $J_{H,F}$ = 5.4, 2H, H-$5_{5FU}$), 7.31 (t, $J_{H,F}$ = 6.3, $J_{H,F}$ = 5.8, 2H, H-$5_{5FU}$), 5.77-5.61 (m, 4H, H-1, H-6), 3.7-3.6 (m, 8H, H-3, H-5), 3.39 (s, 6H, *Me*O), 3.33 (s, 6H, *Me*O), 1.99-1.9 (m, 4H, H-2). IR ($cm^{-1}$, KBr): 3176 (m, NH), 3043 (s, aromatics), 2839 (s, aliphatic C-H groups), 1242, 1123, 1098 (s, C-O-C). *m/z* (%) 405 (14, $M^+$ + H), 373 (13, $M^+$ - OMe), 275 (50, $M^+$ - 5FU), 71 (100). Anal. for $C_{15}H_{18}O_7N_4F_2$: Calcd.: C, 44,56; H, 4.49; N, 13.86. Found: C, 44.41; H, 4.49; N, 13.68.

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