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SYNTHESIS OF ^{18}F LABELLED NUCLEOSIDE ANALOGUES

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SUMMARY

Several nucleoside analogues like 1-(β-D-glucopyranosyl)-5-fluorouracil **10**, 1-(β-D-galactopyranosyl)-5-fluorouracil **11** and 1-(2-deoxy-β-D-glucopyranosyl)-5-fluorouracil **12** have been synthesized. From the corresponding 1-(2',3',4',6'-tetra-O-acetyl-β-D-glycopyranosyl)-uracils **4**, **5** and **6**, the ^{18}F labelled compounds **16**, **17** and **18** have been prepared via the intermediates **13**, **14** and **15** in acetic acid using $[^{18}F]F_2$ and acidic deacetylating procedures. The ^{18}F labelled derivatives could be obtained, following preparative chromatography, in high purity and in yields of about $3 \cdot 10^8$ Bq - $5.7 \cdot 10^8$ Bq (18% - 34% related to the trapped radioactivity, not corrected for decay) for their *in-vitro* evaluation and for *in-vivo* studies with PET.

Key Words: Nucleoside synthesis, carbohydrates, 5-fluorouracil, $[^{18}F]F_2$, PET.

INTRODUCTION

In the last decade, chemotherapy with 5-fluorouracil has found extensive use for a variety of tumors especially in breast and gastrointestinal cancer [1]. The metabolism of fluorouracil has been studied extensively [2]. Its main actions are incorporation into the RNA, and irreversible inhibition of the enzyme thymidylate synthetase via a stable ternary complex, the *Friedkin* intermediate, by the false substrate 2'-deoxy-5-fluorouridine-5'-monophosphate [3 - 7]. In former studies with PET

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we have labelled 5-fluorouracil with ^{18}F [8] and we were able to show a relation between 5-fluorouracil accumulation in the malignant lesion and therapy response [1] which also may be monitored by ^{19}F-NMR spectroscopy [9]. However, 5-fluorouracil influx into the cells is much slower than the influx for other nucleobases [10]. In order to increase the transport into the cells and because of the low solubility of fluorouracil the synthesis of 1-(glucopyranosyl)-5-fluorouracil has been reported [11, 12]. Furthermore, 1-(2'-deoxy-glucopyranosyl)-thymine is known as a selective inhibitor of uridine phosphorylase [13]. In order to use glycopyranosyl nucleoside analogues as prodrugs for ADEPT (antibody directed enzyme prodrug therapy) and gene therapy with suicide enzymes we synthesized several glycopyranosyl pyrimidine nucleosides via a method introduced by *Vorbrüggen et al.* [14]. These derivatives were fluorinated (**10**, **11**, **12**, Fig. 1) and labelled with ^{18}F using $[^{18}F]F_2$ in a common direct fluorinating and labelling procedure, for the *in-vitro* examination of their biochemical pathways, and for the *in-vivo* measurement of their distribution, transport and action, with positron emission tomography (PET).

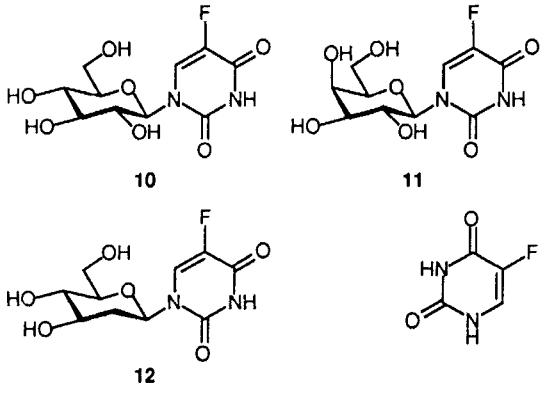
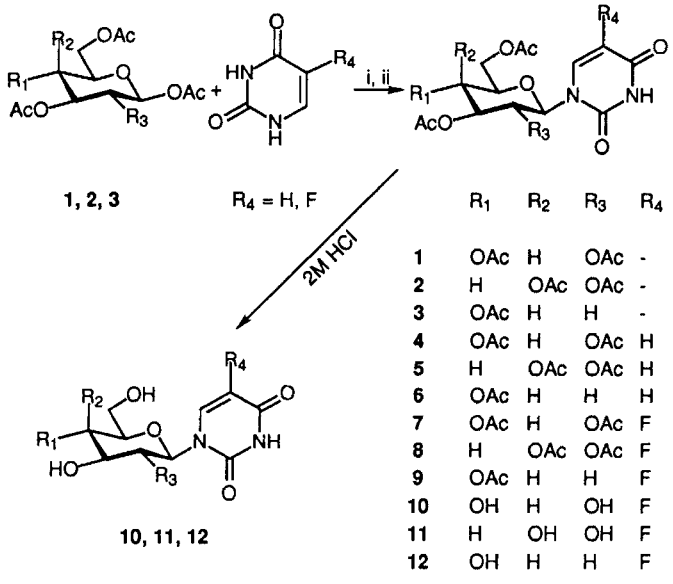


Figure 1: 1-Glycopyranosyl-5-fluorouracil nucleosides and the common chemotherapeutic agent 5-fluorouracil.

DISCUSSION

The preparation method of *Vorbrüggen et al.* using hexamethyldisilazane (HMDS), trimethylsilylchloride (Me_3SiCl) in presence of a catalyst, a peracetylated sugar and a nucleobase in acetonitrile solution was first applied for preparing native nucleosides. It was extended soon to the preparation of nucleoside analogues and has been used successfully in many syntheses since then. The

general problem for the synthesis of nucleosides is the α/β selectivity of the anomeric carbon of the carbohydrate residue. Since naturally occuring nucleosides have β-configuration, they are the desired synthetic products. Being also thermodynamically more stable, these may be selectively prepared at a moderate temperature, although some of the products prepared in this study contained an α-anomer contamination up to 5%.



	R_1	R_2	R_3	R_4
1	OAc	H	OAc	-
2	H	OAc	OAc	-
3	OAc	H	H	-
4	OAc	H	OAc	H
5	H	OAc	OAc	H
6	OAc	H	H	H
7	OAc	H	OAc	F
8	H	OAc	OAc	F
9	OAc	H	H	F
10	OH	H	OH	F
11	H	OH	OH	F
12	OH	H	H	F

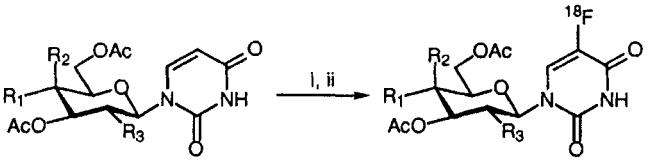
(i) 1 mmol of **1**, **2**, or **3**; pyrimidine1.1 - 1.2 mmol; HMDS 1.2 mmol; Me_3SiCl 1.25 mmol; CH_3CN 10 mL. 25 °C, 30 min.

(ii) $SnCl_4$ 0.16 mL. 45 °C, 24 h.

Figure 2: Synthesis of 1-glycopyranosyl-uracil nucleoside analogues.

We obtained and characterized the acetyl-protected uracil nucleoside derivatives **4**, **5**, **6** and their 5-fluoro analogues **7**, **8** and **9** as pointed out in Figure 2. The fluorinated reference compounds **10**, **11** and **12** were isolated as white solid materials following preparative chromatography, after deacetylation with diluted boiling hydrochloric acid. Labelling of **4**, **5** and **6** was carried out using $[^{18}F]F_2$ with 1% of carrier F_2 (0.5 mmol in total) in the target gas, according to our previous procedure [3]. Hydrolysis of intermediates **13**, **14** and **15** with HCl and workup by semi-preparative HPLC gave the labelled substrates **16**, **17** and **18** (Figure 3) in yields between 18% and 34% (related to the initially trapped radioactivity; not corrected for decay). The specific activity of the

isolated products was in the range of 19 to 22 GBq·mmol^{-1}. The samples were adjusted to a final specific activity of 15 GBq·mmol^{-1} (≈ 2.5 µmol·mCi^{-1}) at the time of calibration, for their *in-vivo* evaluation.



(i) [^{18}F]F_2, AcOH, 15 min; **13**, **14**, **15**.
(ii) 1 M HCl, 100 °C, 30 min; **16**, **17**, **18**.

	R_1	R_2	R_3
13	OAc	H	OAc
14	H	OAc	OAc
15	OAc	H	H
16	OH	H	OH
17	H	OH	OH
18	OH	H	H

Figure 3: Preparation of ^{18}F-labelled 1-glycopyranosyl-5-fluorouracil nucleoside analogues.

EXPERIMENTAL

Reagents and equipment. Chemicals and solvents were of analytical grade and used as delivered from Aldrich and Fluka. ^{1}H, ^{13}C and ^{19}F-NMR spectra were measured at the central department of spectroscopy of the German Cancer Research Center with a Bruker AC-250 spectrometer. Internal standards were TMS (^{1}H-NMR) and difluorotetrachloroethane (^{19}F-NMR; chemical shifts are relative to trifluoroacetic acid = 0 ppm). High resolution mass spectra were obtained from a Varian MAT 711 (HRMS). Electrospray mass spectra (ESI) were measured with a Finnigan TSQ 7000 triple-quadrupole system. The IR-data were obtained in KBr with a Perkin-Elmer 580B spectrometer. UV-Spectra were recorded using a L4500 diode array detector of Merck-Hitachi. Polygram Sil G/UV 254 plates of Machery and Nagel, Dueren, were used for TLC. Spots were developed with molybdatophosphoric acid (5% in ethanol) or sulfuric acid (10% in ethanol). Radio-TLC was analyzed by digital autoradiography using the multiwire proportional chamber LB 287 DAR of EG & G, Berthold. Flash column chromatography was performed on silica 60, Merck, Darmstadt, applying a 1.5 kg·cm^{-2} pressure of N_2.

Labelled precursor. $[^{18}F]F_2$ was prepared by the $^{20}Ne(d,\alpha)^{18}F$ nuclear process by isotopic exchange reaction with 1% F_2 as carrier (11 mL, approx. 0.5 mmol) in the target gas. An irradiation with a total charge of 20 μAh with 14 MeV deuterons delivered approximately $11 \cdot 10^9$ Bq $[^{18}F]F_2$ ($22 \cdot 10^9$ Bq·mmol^{-1}) which was subsequently bubbled at a maximum rate of 90 mL·min^{-1} through the solutions described below. An amount of $1.6 \cdot 10^9$ Bq of this radioactivity was typically trapped corresponding to ≈ 72 μmol F_2.

Standard procedure for the preparation of **4**, **5**, **6**, **7**, **8** *and* **9**. Pentaacetylated sugar **1**, **2** or **3** (1 mmol) and uracil (R_4 = H; 0.13 g, 1.16 mmol) or fluorouracil (R_4 = F; 0.15 g, 1.15 mmol) were dissolved in acetonitrile (10 mL). HMDS (0.25 mL, 1.2 mmol) and Me_3SiCl (0.16 mL, 1.25 mmol) were added. The mixture was stirred for 30 min at ambient temperature. Then $SnCl_4$ (0.16 mL) was added and the colorless solution was stirred continuously for 24 h at 45 °C. Ethyl acetate (EtOAc, 20 mL) and water (10 mL) were added after cooling. The layers were separated and the organic layer was dried with $MgSO_4$ and evaporated.

1-(2',3',4',6'-tetra-O-acetyl-β-D-glucopyranosyl)-Uracil **4**. It was obtained as colorless solid by flash column chromatography using hexane/EtOAc 2:1. Yield: 0.235 g, 53%). Data of **4**: TLC (EtOAc) R_f = 0.74; ^{1}H-NMR (250 MHz, $CDCl_3$): δ 2.00, 2.02, 2.06, 2.09 (s,s,s,s 12 H, C(=O)CH_3), 3.96 (ddd, 1 H, H-5', $^3J_{5',4'}$ = 10.2 Hz, $^3J_{5',6a'}$ = 4.9 Hz, $^3J_{5',6b'}$ = 2.1 Hz), 4.12 (dd, 1 H, H-6', $^2J_{6a',6b'}$ = 12,6 Hz), 4.28 (dd, 1 H, H-6'), 5.14 (dd, 1 H, H-4', $^3J_{4',3'}$ = 9.5 Hz), 5.18 (t, 1 H, H-3', $^3J_{3',2'}$ = 9.5 Hz), 5.40 (t, 1 H, H-2', $^3J_{2',1'}$ = 9.5 Hz), 5.83 (d, 1 H, H-5, $^3J_{5,6}$ = 8.2 Hz), 5.89 (d, 1 H, H-1'), 7.34 (d, 1 H, H-6), 9.24 (s, broad, 1 H, >NH); ^{13}C-NMR (62.89 MHz, $CDCl_3$): δ 20.27, 20.43, 20.47, 20.63 (C(=O)CH_3), 61.54 (C-6'), 67.73 (C-4'), 69.27 (C-2'), 72.58 (C-5'), 74.86 (C-3'), 80.24 (C-1'), 103.76 (C-5), 139.02 (C-6), 150.27 (C-2), 162.46 (C-4), 169.42, 169.49, 169.69, 170.43 (C(=O)CH_3); UV (MeOH): λ_{max} = 255.6 nm; IR (KBr) cm^{-1}: 770, 825, 930, 1035, 1230, 1370, 1450, 1690, 1750; HRMS: $C_{18}H_{22}O_{11}N_2$ 442.353.

1-(2',3',4',6'-tetra-O-acetyl-β-D-galactopyranosyl)-Uracil **5**. Compound **5** was isolated as a colorless solid by flash column chromatography with hexane/EtOAc 1: 1 Yield: 0.23 g, 52%. Data of **5**: TLC (EtOAc) R_f = 0.82; ^{1}H-NMR (250 MHz, $CDCl_3$): The spectrum could not be analyzed by simple inspection. Protons 6', 5', 4', 3', 2', 1' and 5 overlap and are strongly 2nd order, so that simulation and iterative fit to the measured spectrum should be done to assign shifts and couplings. This was not performed within this experiment. It is known from our previous work, that galactose

analogues gave strongly 2nd order spectra [15]. δ 2.00, 2.02, 2.05, 2.19 (s,s,s,s 12 H, C(=O)CH_3), 4.13 - 4.18 (m, 3 H), 5.19 - 5.30 (m, 2 H), 5.51 (d, 1 H, ^3J = 3.0 Hz), 5.84 (d,d, 2 H, H-1' and H-5, $^3J_{1',2'}$ = 8.6 Hz, $^3J_{5,6}$ = 8.1 Hz), 7.38 (d, 1H, H-6, $^3J_{5,6}$ = 8.1 Hz), 8.83 (s, broad, 1 H, >NH); ^{13}C-NMR (68.9 MHz, $CDCl_3$): δ 20.40, 20.44, 20.59, 20.61 (C(=O)CH_3), 61.17 (C-6'), 66.92 (C-4'), 67.09 (C-2'), 70.75 (C-3'), 73.73 (C-5'), 80.65 (C-1'), 103.68 (C-5), 139.38 (C-6), 150.19 (C-2), 162 (C-4), 169.65, 169.76, 169.78, 170.31 (C(=O)CH_3); UV (MeOH): λ_{max} = 256.3 nm; IR (KBr) cm^{-1}: 600, 825, 930, 960, 1060, 1090, 1130, 1230, 1370, 1460, 1700, 1750; HRMS: $C_{18}H_{22}O_{11}N_2$ 442.339.

1-(3',4',6'-tri-O-acetyl-2'-deoxy-β-D-glucopyranosyl)-Uracil **6**. Flash column chromatography with hexane/EtOAc 1:3 delivered **6** as a white foamy material. Yield: 0.3 g, 78%. Data of **6**: TLC (EtOAc/hexane 4:1) R_f = 0.30; ^{1}H-NMR (250 MHz, $CDCl_3$): Shifts could not be assigned simply by inspection, except of the pyrimidine protons (see compound **5**). We also suspect from the integrated spectrum, that a mixture of α/β anomers was obtained. δ 2.09, 2.14, 2.16 (s,s,s 9 H, C(=O)CH_3), 4.31 (m, 2 H), 4.56 (m, 1 H), 4.84 (s, 1 H), 5.21 (s, 1 H), 5.81 (d, 1 H, H-5, $^3J_{5,6}$ = 8.7 Hz), 6.08 (s, broad, 1 H, H-1'), 7.44 (d, 1 H, H-6) 8.86 (s, broad, 1 H, >NH); ^{13}C-NMR (68.9 MHz, $CDCl_3$): δ 20.71, 20.89, 20.98 (C(=O)CH_3), 30.44 (C-2'), 60.53 (C-6'), 65.60 (C-4'), 67.19 (C-3'), 74.82 (C-5'), 75.30 (C-1'), 103.02 (C-5), 139.66 (C-6), 149.70 (C-2), 162.77 (C-4), 169.13, 169.26, 170.51 (C(=O)CH_3); UV (MeOH): λ_{max} = 259.0 nm; IR (CH_2Cl_2) cm^{-1}: 1700, 1750, 2300, 3000; HRMS: $C_{16}H_{20}O_9N_2$ 384.319.

1-(2',3',4',6'-tetra-O-acetyl-β-D-glucopyranosyl)-5-Fluorouracil **7**. It was isolated as a white solid following flash column chromatography with hexane/EtOAc 3:2 Yield: 0.3 g, 65%. Data of **7**: TLC (EtOAc) R_f = 0.78; ^{1}H-NMR (250 MHz, $CDCl_3$): δ 2.01, 2.02, 2.06, 2.10 (s,s,s,s 12 H, C(=O)CH_3), 3.96 (ddd, 1 H, H-5', $^3J_{5',4}$ = 10.2 Hz, $^3J_{5',6a'}$ = 4.4 Hz, $^3J_{5',6b'}$ = 2.2 Hz), 4.13 (dd, 1 H, H-6', $^2J_{6a',6b'}$ = 12.7 Hz), 4.28 (dd, 1 H, H-6'), 5.11 (t, 1 H, H-3', $^3J_{3',4'} = {}^3J_{3',2'}$ = 9.5-Hz), 5.14 (dd, 1 H, H-4'), 5.40 (t, 1 H, H-2', $^3J_{2',1'}$ = 9.5 Hz), 5.86 (dd, 1 H, H-1', $^3J_{1,2}$ = 9.5 Hz, $^5J_{1',F}$ = 1.6 Hz), 7.42 (d, 1 H, H-6, $^3J_{6,F}$ = 5.5 Hz), 9.24 (s, broad, 1 H, >NH); ^{13}C-NMR (62.89 MHz, $CDCl_3$): δ 20.28, 20.45, 20.48, 20.65 (C(=O)CH_3), 61.47 (C-6'), 67.58 (C-4'), 69.39 (C-2'), 72.37 (C-5'), 74.94 (C-3'), 80.67 (C-1'), 123.15, 123.70 (C-6, $^2J_{C,F}$), 138.91, 142.74 (C-5, $J_{C,F}$), 148.86 (C-2), 155.96, 156.39 (C-4, $^2J_{C,F}$), 169.41, 169.58, 169.69, 170.45 (C(=O)CH_3); ^{19}F-NMR (235 MHz, $CDCl_3$): δ -86.76; UV (MeOH): λ_{max} = 262.0 nm; IR (KBr) cm^{-1}: 780, 890, 1030, 1050, 1090, 1130, 1220, 1360, 1430, 1670, 1740; HRMS: $C_{18}H_{21}O_{11}N_2F$ 460.351.

1-(2',3',4',6'-tetra-O-acetyl-β-D-galactopyranosyl)-5-Fluorouracil **8**. It was isolated as white solid following flash column chromatography with hexane/EtOAc 3:2. Yield: 0.345 g, 75%. Data of **8**: TLC (EtOAc) R_f = 0.83; ^{1}H-NMR (250 MHz, $CDCl_3$): Strongly 2nd order, assignments were not performed. δ 2.00, 2.02, 2.06, 2.21 (s,s,s,s 12 H, C(=O)CH_3), 4.14-4.18 (m, 3 H), 5.21-5.23 (m, 2 H), 5.51 (d, 1 H, J = 1.9 Hz), 5.82 (m, 1 H, H-1), 7.42 (d, 1 H, H-6, $^3J_{6,F}$ = 5.7 Hz), 8.86 (d, broad, 1 H, >NH, $^4J_{H,F}$ = 4.6 Hz); ^{13}C-NMR (68.9 MHz, $CDCl_3$): δ 20.39, 20.44, 20.62, 22.65 (C(=O)CH_3), 61.17 (C-6'), 66.79 (C-4'), 67.19 (C-2'), 70.55 (C-3'), 73.78 (C-5'), 81.04 (C-1'), 123.40, 123.94 (C-6, $^2J_{C,F}$), 138.91, 142.73 (C-5, $J_{C,F}$), 148.79 (C-2), 155.96, 156.39 (C-4, $^2J_{C,F}$), 169.63, 169.77, 169.88, 170.31 (C(=O)CH_3); ^{19}F-NMR (235 MHz, $CDCl_3$): δ -86.97; UV (MeOH): λ_{max} = 262.1 nm; IR (KBr) cm^{-1}: 420, 600, 790, 900, 950, 1060, 1090, 1140, 1230, 1370, 1750; HRMS: $C_{18}H_{21}O_{11}N_2F$ 460.201.

1-(3',4',6'-tri-O-acetyl-2'-deoxy-β-D-glucopyranosyl)-5-Fluorouracil **9**. It was obtained as colorless oil which crystallized slowly when standing in the cold. Flash column chromatography was done with hexane/EtOAc 1:1. Yield: 0.25 g, 62%. Data of **9**: TLC (EtOAc) R_f = 0.78; ^{1}H-NMR (250 MHz, $CDCl_3$): δ 1.79 (ddd, 1 H, H-2', $^2J_{2',2'}$ = 12.5 Hz, $^3J_{2',3'}$ = 11.3 Hz, $^3J_{2',1'}$ = 11.1 Hz), 2.04, 2.07, 2.10 (s,s,s 9 H, C(=O)CH_3), 2.48 (ddd, 1 H, H-2', $^3J_{2',3'}$ = 5.1 Hz, $^3J_{2',1'}$ = 2.3 Hz), 3.85 (ddd, 1 H, H-5', $^3J_{5',4'}$ = 9.8 Hz, $^3J_{5',6a'}$ = 5.0 Hz, $^3J_{5',6b'}$ = 2.2 Hz), 4.12 (dd, 1 H, H-6', $^2J_{6',6'}$ = 12.5 Hz), 4.30 (dd, 1 H, H-6'), 5.04 (dd, 1 H, H-4', $^3J_{4',3'}$ = 9.6 Hz), 5.18 (ddd, 1 H, H-3'), 5.86 (ddd, 1 H, H-1', $^5J_{1',F}$ = 1.8 Hz), 7.45 (d, 1 H, H-6, $^3J_{6,F}$ = 5.8 Hz), 8.89 (d, 1 H, >NH, $^4J_{H,F}$ = 4.4 Hz); ^{13}C-NMR (62.89 MHz, $CDCl_3$): δ 20.62, 20.72, 20.74 (C(=O)CH_3), 35.18 (C-2'), 61.92 (C-6'), 67.99 (C-4'), 70.07 (C-3'), 75.07 (C-5'), 79.36 (C-1'), 122.91, 123.45 (C-6, $^2J_{C,F}$), 138.90, 142.74 (C-5, $J_{C,F}$), 148.10 (C-2), 155.94, 156.37 (C-4, $^2J_{C,F}$), 169.71, 169.89, 170.54 (C(=O)CH_3); ^{19}F-NMR (235 MHz, $CDCl_3$): δ -87.54; UV (MeOH): λ_{max} = 261.5 nm; IR (CH_2Cl_2) cm^{-1}: 1730, 1790, 2340, 2900; HMRS: $C_{16}H_{19}O_9N_2F$ 402.317.

Standard hydrolysis procedure. Derivative **7**, **8** or **9** (0.15 mmol) was dissolved in 1 M HCl (2 mL) and heated to 120 °C for 30 min in a closed vessel. After cooling ion exchange resin (wet, 2 g, Biorad AG11 A8 50-100 mesh, OH^-) was added for neutralization and the mixture was stirred for 5 min. The resin was filtered off and the solution was evaporated to dryness.

1-(β-D-glucopyranosyl)-5-fluorouracil **10**. It was obtained following flash column chromatography with $CHCl_3$/MeOH 3:1 as a white solid. Yield: 0.042 g, 95%.

Data of **10**: TLC ($CHCl_3$/MeOH 3:1) R_f = 0.25; ^{1}H-NMR (250 MHz, DMSO-d6): δ 3.15-3.50 (m, 10 H, H-2' - H-6', -OH), 5.30 (d, 1 H, H-1', $^3J_{1,2}$ = 9.1 Hz), 8.02 (d, 1 H, H-6, $^3J_{6,F}$ = 7.1 Hz), 11.8 (s, broad, 1 H, >NH); ^{13}C-NMR (62.89 MHz, DMSO-d6): δ 60.81 (C-6'), 69.28 (C-4'), 70.63 (C-2'), 76.66 (C-5'), 79.79 (C-3'), 82.82 (C-1'), 125.38, 125.93 (C-6, $^2J_{C,F}$), 138.20, 141.88 (C-5, $J_{C,F}$), 149.82 (C-2), 157.08, 157.48 (C-4, $^2J_{C,F}$); ^{19}F-NMR (235 MHz, DMSO-d6): δ -91.90; UV (MeOH): λmax = 266.2 nm; ESI: $C_{10}H_{13}O_7N_2F$ 292.193.

1-(β-D-galactopyranosyl)-5-fluorouracil **11**. It was isolated as a foamy white solid following flash column chromatography with $CHCl_3$/MeOH 4:1. Yield 0.035 g, 80%. Data of **11**: TLC ($CHCl_3$/MeOH 4:1) R_f = 0.18; ^{1}H-NMR (250 MHz, DMSO-d6): δ 3.42-3.68 (m, 6 H, H-2' - H-6'), 4.36 - 5.22 (4 H, -OH), 5.27 (d, 1 H, H-1', $^3J_{1,2}$ = 9.0 Hz), 8.00 (d, 1 H, H-6, $^3J_{6,F}$ = 7.2 Hz), 11.75 (s, broad, 1 H, >NH); ^{13}C-NMR (62.89 MHz, DMSO-d6): δ 60.31 (C-6'), 68.38 (C-4'), 68.53 (C-2'), 73.41 (C-3'), 78.40 (C-5'), 83.26 (C-1'), 125.17, 125.71 (C-6, $^2J_{C,F}$), 138.16, 141.81 (C-5, $J_{C,F}$), 149.46 (C-2), 156.70, 157.11 (C-4, $^2J_{C,F}$); ^{19}F-NMR (235 MHz, $CDCl_3$): δ -91.99; UV (MeOH): λ_{max} = 266.4 nm; ESI: $C_{10}H_{13}O_7N_2F$ 292.204.

1-(2'-deoxy-β-D-glucopyranosyl)-5-fluorouracil **12**. It was obtained as a white material after flash column chromatography with $CHCl_3$/MeOH 4:1. Yield: 0.036 g, 86%. Data of **12**: TLC (EtOAc) R_f = 0.11; ^{1}H-NMR (250 MHz, D_2O): Strongly 2nd order, most assignments are tentative, δ 1.85 (dd, 1 H, H-2', $^2J_{2',2'}$ = 12.6 Hz, $^3J_{2',3'}$ = 6.3 Hz), 2.20 (dm, 1 H, H-2', $^3J_{2',1'}$ = 9.3 Hz), 3.42 (dd, 1 H, ^3J = 9.4 Hz), 3.57 (m, 1 H), 3.76 - 3.95 (m, 3 H), 4.71 (m, 1 H), 5.81 (dd, 1 H, H-1', J = 1.7 Hz), 8.03 (d, 1 H, H-6, $^3J_{6,F}$ = 6.5 Hz); ^{13}C-NMR (62.89 MHz, $CDCl_3$): δ 40.92 (C-2'), 65.05 (C-6'), 74.54 (C-4'), 74.83 (C-5'), 83.02 (C-3'), 84.47 (C-1'), 130.00, 130.54 (C-6), 143.43, 147.14 (C-5, $J_{C,F}$), 154.53 (C-2), 163.98, 164.39 (C-4, $^2J_{C,F}$); ^{19}F-NMR (235 MHz, $CDCl_3$): δ -90.71; UV (MeOH): λ_{max} = 266.8 nm; ESI: $C_{10}H_{13}O_6N_2F$ 276.194.

Standard procedure for the preparation of labelled derivatives **16**, **17** *and* **18**. The corresponding educt **4**, **5** or **6** (0.1 mmol) was dissolved in acetic acid (25 mL) and $[^{18}F]F_2$ was bubbled through the solution within 15 min. The solvent was evaporated under reduced pressure and the residue was dissolved using 2 mL of 1M HCl. Hydrolysis was stopped after 30 min of boiling. The solvent was evaporated again under reduced pressure until dryness. The crude product contained typically $1.6 \cdot 10^9$ Bq of radioactivity. It was dissolved in water (2 mL) and purified by semi-preparative HPLC. Products were collected at their corresponding retention volumes of V_R = 62 mL (**16**), V_R = 99.6 mL (**17**), and V_R = 117.6 mL (**18**). The total preparation time was 75 min.

Chromatography and analytical procedures for the labelled products. Pure **16**, **17**, or **18** was separated from the crude reaction mixture using a Knauer Eurosphere 100-C18 column, 250x19 mm (10 µm material). A Waters 590 solvent pump was used with a U6K injection valve with a 5 mL sample loop. The UV absorption of solutes was detected at 254 nm with Knauer fixed wavelength detector. Radioactivity was measured with a Beckman Model 170 flow-through radioactivity detector. A 20 mM Na_2HPO_4 elution buffer at pH = 7.8 was applied at a flow rate of 10 mL·min^{-1}. Compounds **16**, **17**, and **18** were collected and identified on analytical HPLC using the same solvent system at a flow rate of 1 mL·min^{-1} with a Eurospher 100-C18 column, 250x4.6 mm (5 µm material). Comparison of the UV spectra with the respective analytical standards using a photo diode array detector confirmed the chemical identity of the isolated labelled products. Quantitation of the radioactivity was possible using a Canberra FLOW-ONE A200 detection system (cell size 500 µL) or a Berthold LB 506 C-1 radioactivity detektor. On TLC the labelled material showed the same retention as their reference samples. Pure **16** (t_R = 6 min) was obtained with a radioactivity of $5.7 \cdot 10^8$ Bq, **17** (t_R = 8 min) was obtained with an amount of $3 \cdot 10^8$ Bq, **18** (t_R = 9.5 min) was isolated with $3.8 \cdot 10^8$ Bq. Around 18 - 34% of the initially trapped radioactivity were found in the isolated and pure products.

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