# Syntheses of novel modified acyclic purine and pyrimidine nucleosides as potential substrates of herpes simplex virus type-1 thymidine kinase for monitoring gene expression

**Michaela Grote, Steffi Noll, Bernhard Noll, Bernd Johannsen, and Werner Kraus**

**Abstract:** Suicide gene therapy with the herpes simplex virus type-1 thymidine kinase gene (HSV-1 tk) is considered to be a promising approach to the treatment of cancer. Making use of the lower specificity of the viral enzyme compared to human thymidine kinase, the therapy involves the administration of antiviral agents (e.g., ganciclovir) as prodrugs to induce enzymatic cell death in those cells that express the transferred gene. $^{18}$F-labelled derivatives have been described for monitoring location, duration, and magnitude of the viral kinase enzyme activity by positron emission tomography (PET). Since an optimal radiotracer has not been developed, novel substances were synthesized for monitoring gene expression. A group of 13 nucleoside analogues were synthesized, among them N$^1$-methyl-9-[(1,3-dihydroxy-2-propoxy)methyl]guanine (**5**) and N$^1$-methyl-9-[(4-hydroxy)-3-hydroxymethylbutyl]guanine (**7**) as methyl analogues of ganciclovir and penciclovir and their related fluoro compounds (**6**, **8**). Further novel derivatives include N$^6$-methyl-9-[(1,3-dihydroxy-2-propoxy)methyl]-, N$^6$-methyl-9-[(4-hydroxy)-3-hydroxymethylbutyl]adenine (**9**, **10**), as well as the uracil derivatives 5-hydroxy-1-[(1,3-dihydroxy-2-propoxy)methyl]uracil (**11**), 6-methyl-1-[(1,3-dihydroxy-2-propoxy)-methyl]uracil (**12**), and its 3-fluoro-derivative (**13**).

*Key words:* fluorinated nucleoside analogues, gene therapy, PET, thymidine kinase.

**Résumé :** La thérapie à l'aide de gènes suicidaires dérivés du gène kinase de la thymidine du virus de type-1 de l'herpès simplex (HSV-1 tk) est considérée comme une approche intéressante au traitement du cancer. En utilisant une spécificité plus faible de l'enzyme viral par comparaison à celle de la kinase de la thymidine humaine, la thérapie implique l'administration d'agents antiviraux (par exemple le ganciclovir) comme prodrogues pouvant induire la mort de cellules enzymatiques dans les cellules qui expriment le gène transféré. Des dérivés marqués au $^{18}$F ont été décrits pour la surveillance continue de la location, de la durée et du degré d'activité de l'enzyme kinase virale par tomographie à émission de positron (« PET »). Considérant qu'un marqueur isotopique optimal n'a pas encore été développé, de nouvelles substances ont été synthétisées pour la surveillance continue de l'expression génétique. On a synthétisé un groupe de 13 analogues de nucléosides, dont la N$^1$-méthyl-9-[(1,3-dihydroxy-2-propoxy)méthyl]guanine (**5**) et la N$^1$-méthyl-9-[(4-hydroxy)-3-hydroxyméthylbutyl]guanine (**7**) comme analogues méthylés du ganciclovir et du penciclovir et les composés fluorés apparentés (**6** et **8**). On a aussi synthétisé d'autres dérivés nouveaux, dont les N$^6$-méthyl-9-[(1,3-dihydroxy-2-propoxy)méthyl]- et N$^6$-9-[(4-hydroxy)-3-hydroxyméthylbutyl]adénine (**9** et **10**) ainsi que les dérivés uraciles 5-hydroxy-1-[(1,3-dihydroxy-2-propoxy)méthyl]uracile (**11**), 6-méthyl-1-[(1,3-dihydroxy-2-propoxy)méthyl]uracile (**12**) ainsi que son dérivé 3-fluoro (**13**).

*Mots clés :* analogues fluorés du nucléoside, thérapie génétique, « PET », kinase de la thymidine.

[Traduit par la Rédaction]

## Introduction

Recently, transfer of genes for therapy has become more of reality for the treatment of human malignancies (1). One of the promising approaches is suicide gene therapy with the herpes simplex virus type-1 thymidine kinase gene (HSV-1 tk) (2, 3). This approach, which is also referred to as virus-directed enzyme–prodrug therapy (VDEPT) (4) involves gene delivery into replicating tumor cells, and after sufficient expression of the enzyme herpes simplex virus type-1

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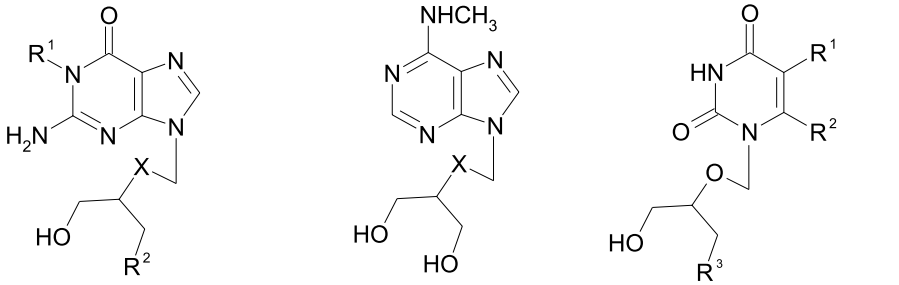
**Fig. 1.** Ganciclovir **1**, FHPG **2**, penciclovir **3**, FHBG **4**, and their related analogues **5**–**8**; acyclic derivatives of $N^6$-methyladenine **9**, **10** and the pyrimidines **11**–**13**.



**1.** $R^1$ = H, $R^2$ = OH, X = O
**2.** $R^1$ = H, $R^2$ = F*, X = O
**3.** $R^1$ = H, $R^2$ = OH, X = $CH_2$
**4.** $R^1$ = H, $R^2$ = F*, X = $CH_2$
**5.** $R^1$ = $CH_3$, $R^2$ = OH, X = O
**6.** $R^1$ = $CH_3$, $R^2$ = F*, X = O
**7.** $R^1$ = $CH_3$, $R^2$ = OH, X = $CH_2$
**8.** $R^1$ = $CH_3$, $R^2$ = F*, X = $CH_2$

**9.** X = O
**10.** X = $CH_2$

**11.** $R^1$ = OH, $R^2$ = H, $R^3$ = OH
**12.** $R^1$ = H, $R^2$ = $CH_3$, $R^3$ = OH
**13.** $R^1$ = H, $R^2$ = $CH_3$, $R^3$ = F*

F*: fluorine-18 or fluorine-19

thymidine kinase (HSV-1 TK), treatment with a prodrug, which is converted to a cell-toxic product by the viral enzyme.

Unlike the highly substrate-specific human TK, the viral enzyme (HSV-1 TK) accepts a diversity of pyrimidine- and purine-based nucleoside analogues, converting them into monophosphates. Subsequent phosphorylation by human (host) kinases leads to triphosphates that inhibit DNA polymerase and induce cell death. Inadequate site specific transfection and gene expression are some of the issues in gene therapy (5). Positron emission tomography (PET), a clinically valuable diagnostic modality for a noninvasive visualization of tissue functions in vivo, offers a possibility to monitor location and magnitude of gene expression, thereby helping to optimize protocols of gene and prodrug administration in humans. Fluorine-18, with a half-life of 110 min, is the most common isotope used for PET. Several nucleoside analogues labeled with $^{18}F$ for monitoring suicide gene therapy mediated by HSV-1 tk have been synthesized. They are usually derivatives of acyclonucleosides, which are selective anti-herpes agents, among them 9-[(3-[$^{18}F$]-fluoro-1-hydroxy-2-propoxy)methyl]guanine ([$^{18}F$]FHPG) **2**, derived from ganciclovir (GCV) **1**, and 9-[4-[$^{18}F$]-fluoro-3-hydroxymethylbutyl]guanine ([$^{18}F$]FHBG) **4**, derived from penciclovir **3** (6–11). Phosphorylation of these radioactive-labeled nucleoside analogues by virus-encoded thymidine kinase leads to metabolites trapped within the infected tumor cells, and the ensuing accumulation of radioactivity allows monitoring of the enzyme activity with PET (12).

In the search for improved PET radiotracers for gene therapy monitoring, we have synthesized and characterized a series of novel acyclic nucleosides.

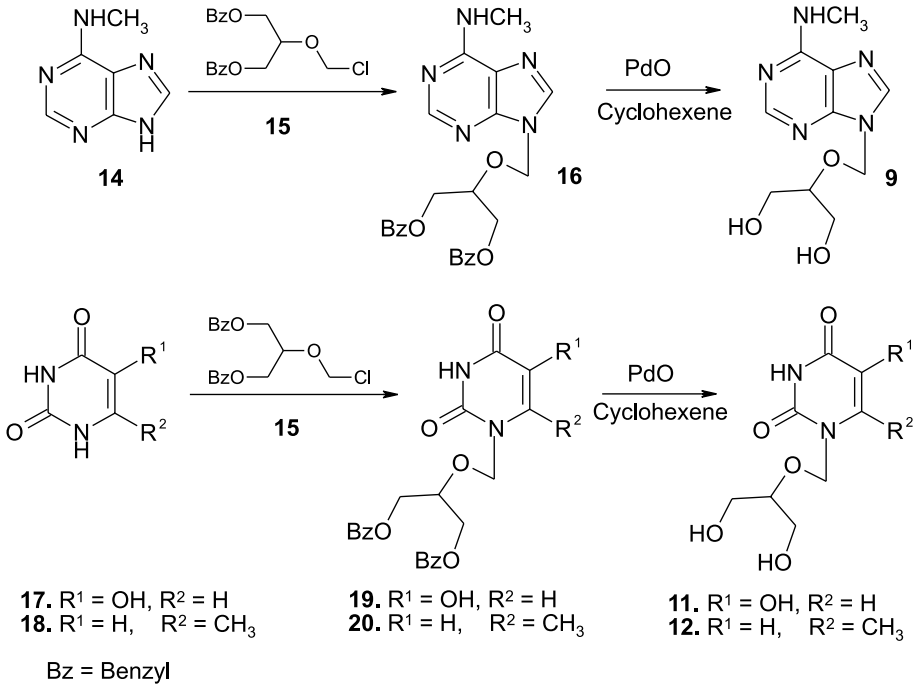
Here we report on the synthesis of acyclic analogues of guanine (**5**–**8**) and adenine (**9**, **10**), as well as the related pyrimidines (**11**–**13**) (Fig. 1).

Guanine-based nucleoside analogues offer several possibilities to be labeled with $^{18}F$, e.g., on the 8-position of the purine ring (13) or by replacement of a hydroxyl group in the side chain by fluorine (6, 10). Our strategy to replace fluorine for hydroxyl involves protection of one of the hydroxyl groups of the acyclic chain and, in the case of guanine derivatives, also the protection of the amino group in the 2-position, followed by introduction of a leaving group for the fluorination.

## Chemistry

Many methods for the preparation of acyclic nucleoside analogues have been described. In most cases they involve synthesis of a suitably protected side chain, followed by coupling to the nucleobase and subsequent deprotection. For example, Martin et al. (14) started from epichlorohydrin to obtain GCV via condensation of 2-*O*-(acetoxymethyl)-1,3-di-*O*-benzylglycerol with $N^2$-9-diacetylguanine. We used 1,3-dichloro-2-propanol, sodium hydride, and benzyl alcohol to produce 1,3-dibenzyloxy-2-propanol, which was added to 1,2-dichloroethane followed by paraformaldehyde and hydrogen chloride gas, to obtain 1,3-dibenzyloxy-2-chloromethoxypropane (**15**) (15, 16). $N^6$-Methyladenine **14** was coupled to **15** in dry dimethyl formamide with triethyl amine. Compound **14** may react with **15** either at the 7-position or the 9-position of the purine ring, but additional heating of the reaction mixture afforded only **16**. Treatment with palladium oxide in cyclohexene removed the benzyl protection groups, yielding $N^6$-methyl-9-[(1,3-dihydroxy-2-propoxy)methyl]adenine (**9**) (15, 16). The structure of **9** was assigned by its $^1H$ NMR spectra as the 9-isomer. It was found that the chemical shifts resulting from the $H_2C(1')$ (5.64 ppm) and HC(8) (8.23 ppm) were in good agreement with the almost similar compound 9-[(2-hydroxyethoxy)methyl]adenine (5.67 and 8.27 ppm) (17); also the NH shift of **9** amounted to 7.73 ppm, which was also analogous to 9-[(2-hydroxyethoxy)methyl]adenine (~7.3 ppm), taking into account that the downfield shift is caused by a methyl group. Because our substance showed a weak downfield shift, we synthesized the 9-substituted guanine derivative. In contrast, the corresponding signals of 7-[(2-hydroxyethoxy)methyl]adenine showed a strong downfield

**Scheme 1.** Synthesis of compounds **9**, **11**, and **12**.




shift ($H_2C(1')$ = 5.79 ppm, HC(8) = 8.60 ppm, $NH_2$ = 9.2 ppm resp. 10.0 ppm) (17).

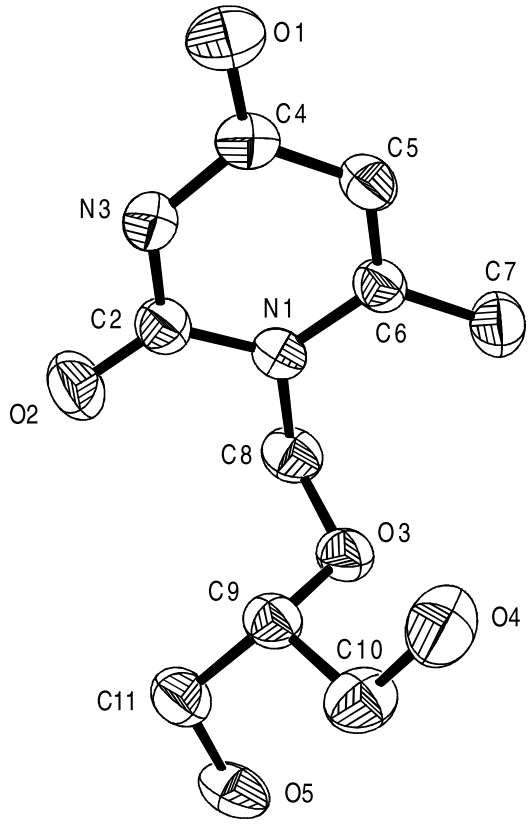
Nucleoside analogues can also be prepared by a coupling reaction of an activated base at the halogenated side chain (18). The pyrimidine derivatives were synthesized by direct condensation of the appropriate persilylated base with the chloromethyl ether **15** and tetra-*n*-butylammonium iodide as the catalyst to produce 5-hydroxy-1-[(1,3-dibenzyloxy-2-propoxy)methyl]uracil (**19**) and 6-methyl-1-[(1,3-dibenzyloxy-2-propoxy)methyl]uracil (**20**), respectively. Removal of the benzyl protection groups with palladium oxide resulted in 5-hydroxy-1-[(1,3-dihydroxy-2-propoxy)methyl]uracil (**11**) and 6-methyl-1-[(1,3-dihydroxy-2-propoxy)methyl]uracil) (**12**) (Scheme 1).

The molecular structure of **12** (Fig. 2) obtained by X-ray analysis proved that the acyclic chain has been coupled with the $N^1$-position of the pyrimidine ring.

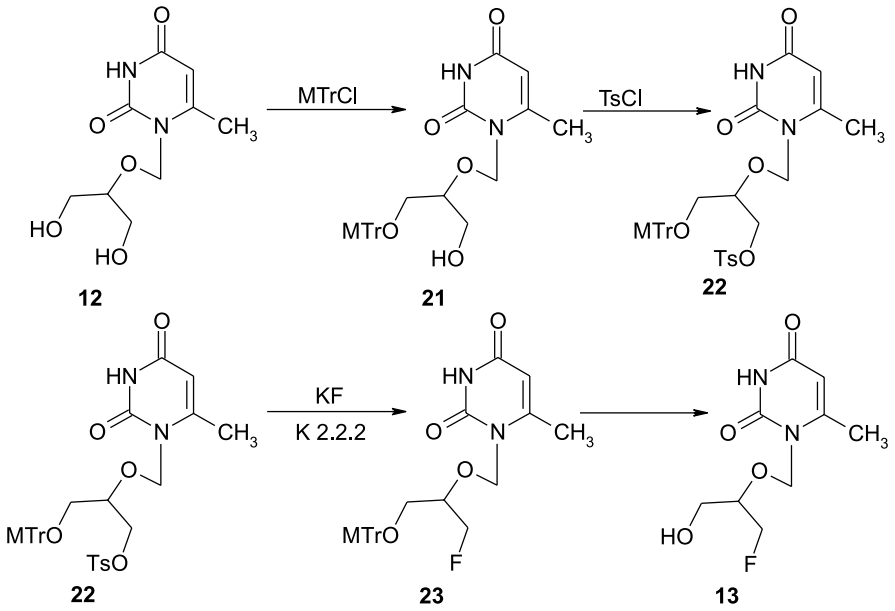
The precursor 6-methyl-1-[[1-(*p*-anisyldiphenylmethoxy)-3-(*p*-toluenesulfonyloxy)-2-propoxy]methyl]uracil (**22**) was obtained from **12** by treatment with *p*-anisylchloro-diphenylmethane in dimethyl formamide and with traces of dimethylamino pyridine to produce 6-methyl-1-[[1-(*p*-anisyldiphenylmethoxy)-3-hydroxy-2-propoxy]methyl]uracil (**21**) in 47% yield, followed by tosylation with tosyl chloride in anhydrous pyridine (63%). Fluorination with potassium fluoride and Kryptofix® 2.2.2 in acetonitrile at 120 °C, afforded 6-methyl-1-[[1-(*p*-anisyldiphenylmethoxy)-3-fluoro-2-propoxy]methyl]uracil (**23**) in a yield of 23%. Finally, the protection group was removed, to give 63% of the fluorinated compound 6-methyl-1-[(3-fluoro-1-hydroxy-2-propoxy)methyl]uracil (**13**) (Scheme 2).

The carba-analogue of GCV (18, 19), penciclovir **3** can be prepared by several procedures. There are three steps, including the synthesis of a suitable side chain, coupling to a purine derivative that is able to undergo highly regioselective alkylation at the N-9 position, and transformation of the resulting alkylated purine derivative into the target compound. Some of the reported alkylating agents that

**Fig. 2.** X-ray structure of **12**.



**Scheme 2.** Protection, tosylation, and subsequent fluorination of **12** to obtain **13**.




**Scheme 3.** Synthesis of $N^6$-methyl-9-[(4-hydroxy)-3-hydroxymethylbutyl]adenine (**10**).

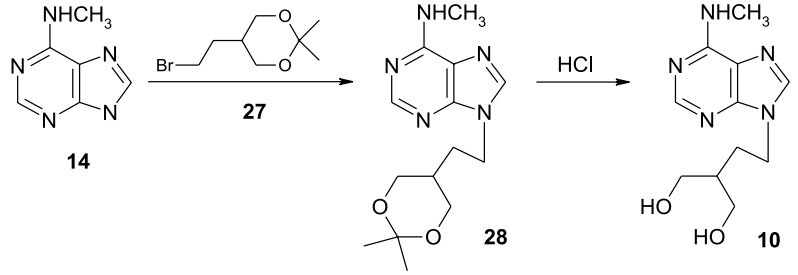
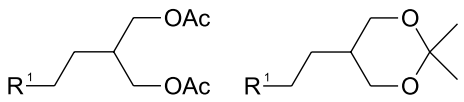



**Fig. 3.** Several alkylation agents used for the preparation of penciclovir.

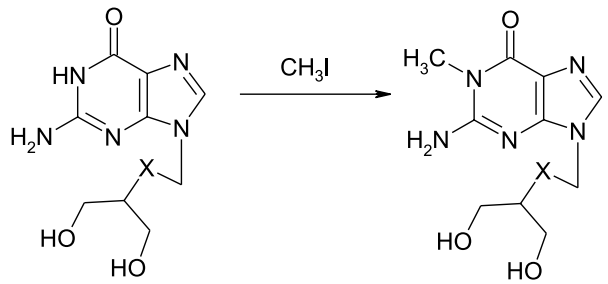



have been used are for instance, 4-acetoxy-3-acetoxymethyl-1-iodobutane (**24**) (20, 21), 4-acetoxy-3-(acetoxymethyl)butanol (**25**) (22), and 2,2-dimethyl-5-(2-*p*-toluenesulfonyloxyethyl)-1,3-dioxane (**26**) (23) (Fig. 3).

We used 5-(2-bromoethyl)-2,2-dimethyl-1,3-dioxane (**27**) to synthesize both penciclovir **3** (21, 24) and $N^6$-methyl-9-[(4-hydroxy)-3-hydroxymethylbutyl]adenine (**10**), using hydrochloric acid under refluxing conditions for deprotection (Scheme 3). The UV spectra of **10** and **9** were similar and suggested that it was the N-9 isomer.

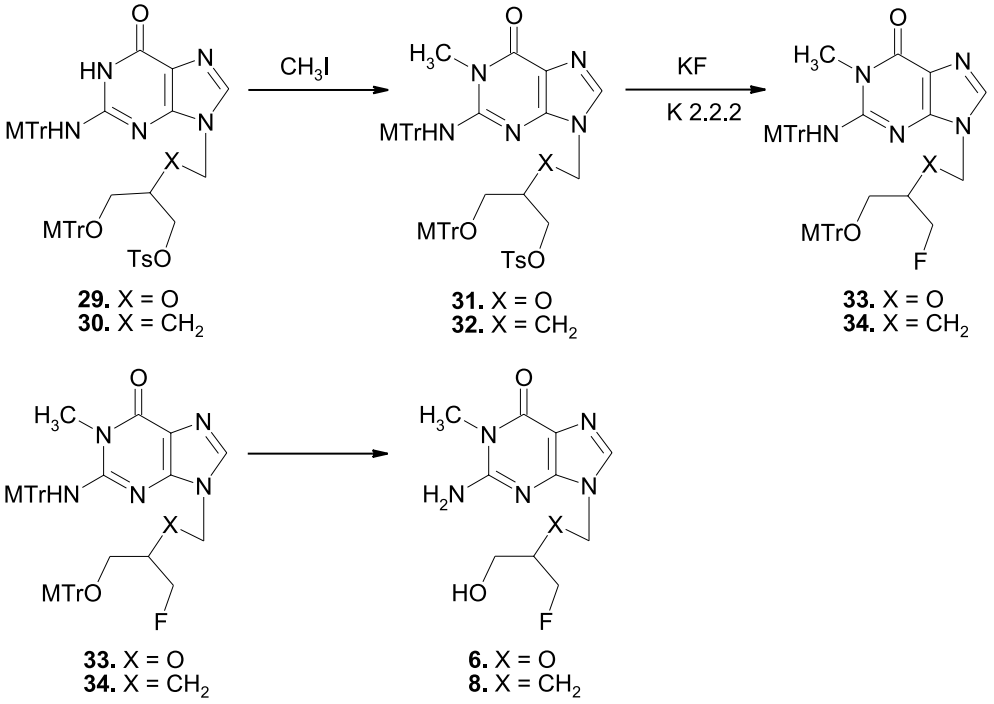
We decided to modify the substrate properties of GCV and penciclovir by introducing a methyl group and therefore changing the lipophilicity of the molecule (Scheme 4). As

**Scheme 4.** Methylation of ganciclovir and penciclovir.




described for acyclovir (25, 26), introduction of a methyl group resulted in the alteration of the antiviral activities. The desired products **5** and **7** were obtained from GCV **1** and penciclovir **3** by treatment with methyl iodide in dimethyl formamide followed by extensive preparative column chromatography.

**Scheme 5.** Methylation, followed by fluorination and splitting off of the protection groups to obtain **6** and **8**.




To obtain $N^1$-methylated guanine derivatives **6** and **8**, we started from $N^2$-(*p*-anisyldiphenylmethyl)-9-[[1-(*p*-anisyldiphenylmethoxy)-3-(*p*-toluenesulfonyloxy)-2-prop-oxy]methyl]-guanine (**29**) or $N^2$-(*p*-anisyldiphenylmethyl)-9-[(4-(*p*-toluenesulfonyloxy))-3-*p*-anisyldiphenylmethoxy-methyl-butyl]guanine (**30**), prepared by modified literature procedures (6, 27). After reaction with methyl iodide yielding 44% of **31** and 29% of **32**, fluorination followed. After fluorination, the removal of the methoxytrityl group was carried out. Details of both sysnthesis steps were described above (Scheme 5).

All synthesized substances were characterized by elemental analysis and $^1$H NMR spectroscopy, and derivatives containing fluorine were also determined by $^{19}$F NMR. The UV spectra of the compounds **3**, **5**, **7**, and **9**–**13** are listed in Table 1.

## Radiochemistry

To obtain [$^{18}$F]-labeled tracers the reaction conditions must consider the half-life of fluorine-18 (110 min). We synthesized the established [$^{18}$F]FHPG (**2**) and [$^{18}$F]FHBG (**4**) (6–11), as well as the new tracers [$^{18}$F]FMHPG (**6**) ($N^1$-methyl-9-[(3-[$^{18}$F]-fluoro-1-hydroxy-2-propoxy)methyl]guanine), [$^{18}$F]FMHBG (**8**) ($N^1$-methyl-9-(4-[$^{18}$F]-fluoro-3-hydroxymethyl-butyl)guanine), and [$^{18}$F]FMHPU (**13**) (6-methyl-1-[(3-[$^{18}$F]-fluoro-1-hydroxy-2-propoxy)methyl]-uracil. The tosylated and methoxytritylated precursors (**22**, **29**–**32**) were radiolabeled with a K[$^{18}$F]F/Kryptofix® 2.2.2 complex in acetonitrile, followed by splitting off the protection groups under acidic conditions. The labeled products were purified by HPLC. The synthesis time amounted to 90–110 min, the radiochemical purity was >98%, and an average specific activity of 19 GBq/μmol were determined.

**Table 1.** UV data for selected acyclic nucleosides.

| No. | max nm (ε) | |
|---|---|---|
| **1** | 258 (13 500) | (sh) 274 (9000) |
| **3** | 253 (11 400) | (sh) 274 (8100) |
| **5** | 254 (10 400) | (sh) 273 (7800) |
| **7** | 255 (7 900) | (sh) 272 (6200) |
| **9** | 265 (13 900) | |
| **10** | 266 (13 600) | |
| **11** | 278 (7 500) | |
| **12** | 262 (11 200) | |

## Experimental

### Instrumentation and reagents

$^1$H NMR spectra were obtained on a Varian Inova-400 (400 MHz) instrument with DMSO-$d_6$ as an internal standard. The chemical shifts are given as δ in ppm, the coupling constants as *J* in Hz. Fluorine $^{19}$F NMR spectra were obtained on a Varian Inova-400 (376 MHz) with $CFCl_3$ as the internal standard. Elemental analyses were carried out with a LECO CHNS 932 elemental analyzer. Melting points were determined on a BOËTIUS melting point apparatus and are uncorrected. UV spectra were recorded on a Specord UV–vis S10 instrument (Zeiss) in aqueous solution.

The X-ray data were collected at room temperature (293 K) on a SMART-CCD diffractometer (Siemens), using graphite-monochromatized Mo-$K_\alpha$ radiation (λ = 0.71073 Å). A summary of the crystallographic data is given in Table 2. The structure was solved by direct methods. All hydrogen atoms were located by a difference Fourier synthesis and refined freely. Empirical absorption corrections were

**Table 2.** Crystallographic data for X-ray diffraction studies of 6-methyl-1-[(1,3-dihydroxy-2-propoxy)methyl]guanine (**12**).

| | **12** |
|---|---|
| Formula | $C_9H_{14}N_2O_5 \cdot 2H_2O$ |
| fw | 230.22 |
| Crystal system | Triclinic |
| Space group | *P*-1 |
| *a* (Å) | 6.867(2) |
| *b* (Å) | 9.508(3) |
| *c* (Å) | 10.493(4) |
| α (°) | 101.593(7) |
| β (°) | 107.857(6) |
| γ (°) | 96.118(7) |
| *V* (Å$^3$) | 626.3(4) |
| *Z* | 2 |
| Temperature (K) | 293(2) |
| *d* (g/cm$^3$) | 1.217 |
| Absorption coefficient (mm$^{-1}$) | 0.100 |
| *F*(000) | 244 |
| Wavelength (Å) | 0.71073 |
| Crystal size (mm$^3$) | 1.5 × 0.6 × 0.4 |
| 2 θ Range (°) | 2.10–25.00 |
| Collection range | $-8 \le h \le 8$ |
| | $-7 \le k \le 11$ |
| | $-12 \le l \le 12$ |
| Reflections collected | 3100 |
| Independent reflections | 2186 ($R_{int}$ = 0.0344) |
| GoF | 0.984 |
| *R* ($I > 2\sigma(I)$) | |
| $R_1$ | 0.0433 |
| $wR_2$ | 0.1243 |
| *R* (all data) | |
| $R_1$ | 0.0530 |
| $wR_2$ | 0.1289 |

made using ψ scans. Most of the calculations were carried out in the SHELXTL system with some local modifications.

Position parameters and relevant bond lengths and angles are contained in Tables 3 and 4. Atomic positional and thermal parameters, full lists of bond lengths and angles, and $F_o/F_c$ values have been deposited as supplementary material.[2]

Chromatographic purification was performed using either Silica gel 60 (Merck) or RP-18 material (LiChroprep™). Furthermore, a MPLC (middle pressure liquid chromatography) system (Knauer) with two columns (silica gel: Eurosil Baselect, 25 mm × 200 mm, from Merck; RP-18 material, 30 × 480, from Kronlab) was used with pressures ranging from 2 to 5 bar (1 bar = 100 kPa). HPLC analysis was performed on a system using a PerkinElmer LC binary pump 250, UV detector (absorbance detector model 759A, Applied Biosystems) operating at 254 nm, and a radioactivity detector using a semipreparative C-18-column (Eurospher, Knauer, 8 mm × 250 mm) and an analytical C-18-column (Eurospher, Knauer, 5 mm × 250 mm).

**Table 3.** Position parameters of 6-methyl-1-[(1,3-dihydroxy-2-propoxy)methyl]guanine (**12**) and the standard deviations.

| | *x* | *y* | *z* | $U_{eq}$ |
|---|---|---|---|---|
| N(3) | 831(2) | 7617(1) | 3863(1) | 38(1) |
| N(2) | 3098(2) | 9173(2) | 5924(2) | 41(1) |
| O(7) | 572(2) | 5521(1) | 2088(1) | 45(1) |
| C(3) | 691(3) | 8793(2) | 3253(2) | 37(1) |
| O(8) | 2263(2) | 6765(1) | 5768(1) | 57(1) |
| C(2) | 2075(3) | 7782(2) | 5220(2) | 40(1) |
| O(2) | 1428(2) | 2490(1) | 1930(2) | 55(1) |
| C(1) | 2989(3) | 10398(2) | 5411(2) | 41(1) |
| C(6) | –307(3) | 6143(2) | 3067(2) | 45(1) |
| C(4) | 1717(3) | 10133(2) | 4002(2) | 42(1) |
| O(1) | 3957(2) | 11602(1) | 6152(1) | 60(1) |
| C(8) | 2567(3) | 5134(2) | 2650(2) | 41(1) |
| O(3) | 4180(3) | 6459(2) | 1357(2) | 64(1) |
| C(5) | –626(4) | 8514(2) | 1776(2) | 52(1) |
| C(9) | 2360(3) | 3713(2) | 3075(2) | 50(1) |
| C(10) | 3594(4) | 5069(2) | 1572(2) | 53(1) |
| OW2 | 4161(2) | 784(2) | 1390(1) | 51(1) |
| OW1 | 2722(2) | 8028(2) | –511(2) | 59(1) |

Solvents and reagents were purchased from the following commercial sources and used without further purification: Sigma, Fluka, Lancaster, Aldrich, and Merck.

Ganciclovir **1** was obtained by neutralization of the sodium salt (Cymeven™) with 0.1 mol L$^{-1}$ HCl and recrystallization from water; penciclovir **3** was synthesized according to a method of Harnden et al. (21). The precursors **29** and **30** were synthesized in modified procedures (10, 27).

## Syntheses of potential substrate and fluorinated reference compounds

### *N$^1$-Methyl-9-[(1,3-dihydroxy-2-propoxy)methyl]guanine (5)*

Ganciclovir (**1**, 214 mg, 0.838 mmol) and tetrabutylammonium hydroxide solution (1 mol L$^{-1}$, 0.88 mL, 0.88 mmol) were dissolved in 20 mL of dry DMF at room temperature. To this solution methyl iodide (120 µL, 1.93 mmol) in 1.2 mL DMF was slowly added and the mixture was stirred for an additional 45 min. The solvent was evaporated in vacuum and the crude product was purified by MPLC (RP-18, acetonitrile–water (1:9, v/v)) to afford **5** (136 mg, 61%), mp 198–200 °C. $^1$H NMR (DMSO-$d_6$) δ: 7.79 (s, 1H, 8-CH), 7.04 (s, 2H, -$NH_2$), 5.43 (s, 2H, -$H_2$C(1′)), 4.58 (t, 2H, *J* = 5.4 Hz, -OH), 3.53–3.50 (m, 1H, -CH-O-), 3.44–3.38 (m, 2H, -$CH_2$-), 3.32 (s, 3H, -$NCH_3$), 3.29–3.24 (m, 2H, -$CH_2$-). Anal. calcd. for $C_{10}H_{15}N_5O_4$: C 44.61, H 5.62, N 26.01; found: C 44.35, H 5.58, N 25.92.

### *N$^1$-Methyl-9-[(3-fluoro-1-hydroxy-2-propoxy)methyl]guanine (6) (FMHPG)*

N$^1$-Methyl-N$^2$-(*p*-anisyldiphenylmethyl)-9-[[1-(*p*-anisyldi-

[2] Supplementary data may be purchased from the Directory of Unpublished Data, Document Delivery, CISTI, National Research Council Canada, Ottawa, ON K1A 0S2, Canada (http://www.nrc.ca/cisti/irm/unpub_e.shtml for information on ordering electronically). CCDC 210605 contains the crystallographic data for this manuscript. These data can be obtained, free of charge, via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, U.K.; fax +44 1223 336033; or deposit@ccdc.cam.ac.uk).

**Table 4.** Bond lengths (Å) and angles for 6-methyl-1-[(1,3-dihydroxy-2-propoxy)-methyl]guanine (**12**).

| | |
|---|---|
| **Bond lengths (Å)** | |
| N(3)—C(2) | 1.384(2) |
| N(3)—C(3) | 1.394(2) |
| N(3)—C(6) | 1.473(2) |
| N(2)—C(2) | 1.371(2) |
| N(2)—C(1) | 1.378(2) |
| O(7)—C(6) | 1.408(2) |
| O(7)—C(8) | 1.435(2) |
| C(3)—C(4) | 1.343(2) |
| C(3)—C(5) | 1.488(3) |
| O(8)—C(2) | 1.219(2) |
| O(2)—C(9) | 1.420(2) |
| C(1)—O(1) | 1.233(2) |
| C(1)—C(4) | 1.422(3) |
| C(8)—C(10) | 1.500(3) |
| C(8)—C(9) | 1.511(3) |
| O(3)—C(10) | 1.422(2) |
| **Bond angles (°)** | |
| C(2)-N(3)-C(3) | 121.52(14) |
| C(2)-N(3)-C(6) | 117.73(14) |
| C(3)-N(3)-C(6) | 120.73(14) |
| C(2)-N(2)-C(1) | 126.25(16) |
| C(6)-O(7)-C(8) | 115.35(13) |
| C(4)-C(3)-N(3) | 119.82(15) |
| C(4)-C(3)-C(5) | 122.00(16) |
| N(3)-C(3)-C(5) | 118.17(15) |
| O(8)-C(2)-N(2) | 121.44(17) |
| O(8)-C(2)-N(3) | 122.80(16) |
| N(2)-C(2)-N(3) | 115.76(15) |
| O(1)-C(1)-N(2) | 120.47(16) |
| O(1)-C(1)-C(4) | 125.00(17) |
| N(2)-C(1)-C(4) | 114.53(15) |
| O(7)-C(6)-N(3) | 111.99(14) |
| C(3)-C(4)-C(1) | 122.05(16) |
| O(7)-C(8)-C(10) | 107.02(16) |
| O(7)-C(8)-C(9) | 111.31(14) |
| C(10)-C(8)-C(9) | 112.51(16) |
| O(2)-C(9)-C(8) | 112.75(16) |
| O(3)-C(10)-C(8) | 113.39(16) |

phenylmethyl)-3-fluoro-2-propoxy]-methyl]guanine (**33**, 50 mg, 0.061 mmol) was heated under reflux in 5 mL methanol and 5 mL acetic acid (80%) for 4.5 h. The solvent was evaporated and the residue purified by MPLC (RP-18, acetonitrile–water (1:9, v/v)) to give **6** (13 mg, 78%), mp 216–219 °C. $^1$H NMR (DMSO-$d_6$) δ: 7.81 (s, 1H, 8-CH), 7.08 (s, 2H, -$NH_2$), 5.43 (s, 2H, -$H_2$C(1′)), 4.89 (s, 1H, -OH), 4.54–4.27 (m, 2H, -$CH_2$-F), 3.85–3.77 (m, 1H, -CH-O-), 3.32 ($m_c$, 2H, -$CH_2$-), 3.30 (s, 3H, -$NCH_3$). $^{19}$F NMR (DMSO-$d_6$) δ: –231.0. Anal. calcd. for $C_{10}H_{14}N_5O_3F$: C 44.28, H 5.20, N 25.82; found: C 44.17, H 5.34, N 25.70.

### *$N^1$-Methyl-9-[(4-hydroxy)-3-hydroxymethylbutyl]guanine (7)*

Penciclovir (**3**, 231 mg, 0.841 mmol) and tetrabutylammonium hydroxide solution (1 mol $L^{-1}$, 0.88 mL, 0.88 mmol) were dissolved in 20 mL of dry DMF. A solution of methyl iodide (120 μL, 1.92 mmol) in 1.2 mL DMF was added under vigorous stirring at room temperature. After 2 h, the solvent was evaporated and the residue purified by MPLC (RP-18, acetonitrile–water (1:9, v/v)) to obtain **7** (48 mg, 21%), mp 221–227 °C. $^1$H NMR (DMSO-$d_6$) δ: 7.68 (s, 1H, 8-CH), 6.97 (s, 2H, -$NH_2$), 4.42 (t, 2H, $J$ = 5.2 Hz, -OH), 3.98 (t, 2H, $J$ = 7.4 Hz, -$H_2$C(1′)), 3.44–3.38 (m, 4H, -$CH_2$(OH)), 3.29 (s, 3H, N-$CH_3$), 1.69 ($m_c$, 2H, -$H_2$C(2′)), 1.45–1.40 (m, 1H, -CH(3′)). Anal. calcd. for $C_{11}H_{17}N_5O_3$: C 49.43, H 6.41, N 26.20; found: C 49.35, H 6.30, N 26.12.

### *$N^1$-Methyl-9-[(4-fluoro)-3-hydroxymethylbutyl]guanine (8) (FMHBG)*

$N^1$-Methyl-$N^2$-(*p*-anisyldiphenylmethyl)-9-[(4-fluoro)-3-*p*-anisyldiphenylmethoxy-methyl-butyl]guanine (**34**, 58 mg, 0.072 mmol) was heated under reflux with 5 mL methanol and 5 mL acetic acid (80%) for 4 h. The solvents were evaporated and the residue purified by MPLC (RP-18, acetonitrile–water (2:8, v/v)) to give **8** (17 mg, 88%), mp 135–138 °C. $^1$H NMR (DMSO-$d_6$) δ: 7.70 (s, 1H, 8-CH), 6.98 (s, 2H, -$NH_2$), 4.68 (s, 1H, -OH), 4.55–4.34 (m, 2H, -$CH_2$-F), 4.01 (t, 2H, $J$ = 7.2 Hz, -$H_2$C(1′)), 3.39 (d, 2H, $J$ = 6.4 Hz, -$CH_2$(OH)), 3.29 (s, 3H, $N^1CH_3$), 1.78–1.67 (m, 2H, -$H_2$C(2′)), 1.65–1.61 (m, 1H, -HC(3′)). $^{19}$F NMR (DMSO-$d_6$) δ: –227.4. Anal. calcd. for $C_{11}H_{16}N_5O_2F$: C 49.06, H 5.99, N 26.01; found: 49.01, H 5.78, N 25.88.

### *$N^6$-Methyl-9-[(1,3-dihydroxy-2-propoxy)methyl]adenine (9)*

Freshly activated palladium oxide (495 mg) and 10 mL cyclohexene were added to a solution of $N^6$-methyl-9-[(1,3-dibenzyloxy-2-propoxy)methyl]adenine (**16**, 1.0 g, 2.3 mmol) in 20 mL ethanol and refluxed for 30 h. After that, the reaction mixture was filtered and the solvent evaporated. The residue was washed first with chloroform, then with diethyl ether and dried. The crude product was redissolved in hot water, ethanol was added, and the substance was allowed to precipitate when cooling down to give **9** (365 mg, 63%), mp 160 °C. $^1$H NMR (DMSO-$d_6$) δ: 8.23 (s, 2H, 2-CH, 8-CH), 7.73 (s, 1H, -NH), 5.64 (s, 2H, -$H_2$C(1′)), 3.59 (t, 2H, $J$ = 5.5 Hz, -OH), 3.59 ($m_c$, 1H, -CH-O), 3.44–3.38 (m, 2H, -$CH_2$(OH)), 3.31–2.94 (m, 2H, -$CH_2$(OH)), 2.94 (s, 3H, $NCH_3$). Anal. calcd. for $C_{10}H_{15}N_5O_3$: C 47.43, H 5.97, N 27.65; found: C 47.37, H 5.86, N 27.52.

### *$N^6$-Methyl-9-[(4-hydroxy)-3-hydroxymethylbutyl]adenine (10)*

$N^6$-Methyl-9-[(2,2-dimethyl-1,3-dioxane-5-yl)ethyl]purine (**28**, 80 mg, 0.27 mmol) was dissolved in 1.5 mL methanol and 1.5 mL HCl (2 mol $L^{-1}$) and refluxed for 3 h. After cooling, the solvent was reduced and the precipitate filtered off. Crystallization from water gave **10** (23 mg, 34%), mp 134–136 °C. $^1$H NMR (DMSO-$d_6$) δ: 8.22 (s, 1H, 8-CH), 8.14 (s, 1H, 2-CH), 7.73 (s, 1H, -NH-), 4.44 (s, 2H, -OH), 4.20 (t, 2H, $J$ = 7.4 Hz, -$H_2$C(1′)), 3.39 ($m_c$, 4H, -$CH_2$(OH)), 2.94 (s, 3H, $NCH_3$), 1.78 ($m_c$, 2H, -$H_2$C(2′)), 1.44–1.40 (m, 1H, -HC(3′)). Anal. calcd. for $C_{11}H_{17}N_5O_2$: C 52.58, H 6.82, N 27.87; found: C 52.43, H 6.73, N 27.72.

### *5-Hydroxy-1-[(1,3-dihydroxy-2-propoxy)methyl]uracil (11)*

5-Hydroxy-1-[(1,3-dibenzyloxy-2-propoxy)methyl]uracil (**19**, 412 mg, 1.0 mmol) was refluxed with 6 mL of ethanol, 2.4 mL of cyclohexene, and freshly activated palladium ox-

ide (170 mg, 1.40 mmol) for 17 h. The catalyst was filtered off and washed with ethanol and water. The solutions were collected and the solvents were removed. The residue was purified by crystallization from ethanol to give **11** (111 mg, 48%), mp 147 to 148 °C. $^1$H NMR (DMSO-$d_6$) δ: 11.43 (s, 1H, -NH), 8.68 (s, 1H, 6-CH), 7.14 (s, 1H, -OH), 5.09 (s, 2H, -$H_2$C(1′)), 4.60 (s, 2H, -OH), 3.49 (m, 1H, -CH-), 3.45–3.29 (m, 4H, -$CH_2$(OH)). Anal. calcd. for $C_8H_{12}N_2O_6$: C 41.38, H 5.21, N 12.06; found: C 41.27, H 5.09, N 11.91.

### *6-Methyl-1-[(1,3-dihydroxy-2-propoxy)methyl]uracil (12)*

6-Methyl-1-[(1,3-dibenzyloxy-2-propoxy)methyl]uracil (**20**, 6.17 g, 15 mmol) was dissolved in 64 mL ethanol and treated with freshly activated palladium oxide (2.0 g, 16.3 mmol), 20 mL cyclohexene, and stirred for 18 h at room temperature. Afterwards the mixture was refluxed for 90 min and the catalyst was filtered. Precipitation of the product proceeded when cooling and recrystallization from ethanol gave 2.4 g (70%) of **12**, mp 147–150 °C. $^1$H NMR (DMSO-$d_6$) δ: 11.20 (s, 1H, -NH), 5.50 (s, 1H, 5-CH), 5.23 (s, 2H, -$H_2$C(1′)), 4.61 (t, 2H, $J$ = 5.6 Hz, -OH), 3.51–3.47 (m, 1H, -CH-O), 3.45–3.39 (m, 2H, -$CH_2$(OH)), 3.35–3.30 (m, 2H, -$CH_2$(OH)), 2.29 (s, 3H, -$CH_3$). Anal. calcd. for $C_9H_{14}N_2O_5$: C 46.95, H 6.13, N 12.17; found: 46.77, H 6.09, N 12.07.

### *6-Methyl-1-[(3-fluoro-1-hydroxy-2-propoxy)methyl]uracil (13) (FMHPU)*

6-Methyl-1-[[1-(*p*-anisyldiphenylmethoxy)-3-fluoro-2-propoxy]methyl]uracil (**23**, 55.0 mg, 0.109 mmol) was dissolved in 5 mL of ethanol and 5 mL of 80% acetic acid and refluxed for 1 h. The solvent was evaporated and the residue was dissolved in ethanol. Final purification was carried out with MPLC (RP-18, acetonitrile–water (1:4, v/v)) affording **13** (16.0 mg, 63%), mp 118–120 °C. $^1$H NMR (DMSO-$d_6$) δ: 11.24 (s, 1H, -NH), 5.52 (s, 1H, 5-CH), 5.34 (d, 1H, $J$ = 11.0 Hz, -$H_2$C(1′)), 5.30 (d, 1H, $J$ = 11.0 Hz, -$H_2$C(1′)), 4.88 (s, 1H, -OH), 4.58–4.29 (m, 2H, -$CH_2$-F), 3.78 ($m_c$, 1H, -CH-O-), 3.41 (d, 2H, $J$ = 5.6, -$CH_2$(OH)), 2.27 (s, 3H, 6-$CH_3$). $^{19}$F NMR (DMSO-$d_6$) δ: –232.0. Anal. calcd. for $C_9H_{13}N_2O_4F$: C 46.55, H 5.65, N 12.06; found: C 46.42, H 5.59, N 12.02.

### *$N^6$-Methyl-9-[(1,3-dibenzyloxy-2-propoxy)methyl]adenine (16)*

Triethylamine (3.8 mL) was added to $N^6$-methyladenine (**14**, 3.7 g, 24.8 mmol) dissolved in 20 mL of dry DMF under stirring. After addition of 1,3-dibenzyloxy-2-chloromethoxypropane (**15**, 8.0 g, 25.0 mmol) in 12.5 mL DMF, the solution was stirred for 1 h at room temperature and then heated for 3 h to 90 °C. The reaction mixture was cooled down and the precipitated triethylammonium chloride was removed. The solvent was evaporated and the residue purified by MPLC (RP-18, methanol–water (8:2, v/v)), yielding **16** (3.4 g, 31%), mp 91 °C. $^1$H NMR (DMSO-$d_6$) δ: 8.25 (s, 2H, 2-CH, 8-CH), 7.75 (s, 1H, -NH), 7.31–7.16 (m, 10H, -CH(arom)), 5.66 (s, 2H, -$H_2$C(1′)), 4.37 (s, 4H, -$CH_2$(Bz)), 4.09 (m, 1H, -CH-O), 3.45–3.39 (m, 4H, -$CH_2$-(OBz)), 2.95 (s, 3H, $NCH_3$). Anal. calcd. for $C_{24}H_{27}N_5O_3$: C 66.50, H 6.28, N 16.15; found: C 66.25, H 6.08, N 16.06.

### *5-Hydroxy-1-[(1,3-dibenzyloxy-2-propoxy)methyl]uracil (19)*

Isobarbituric acid (**17**, 384 mg, 3.0 mmol) was suspended in 17 mL of hexamethyldisilazane. Ammonium sulfate (225 mg, 1.70 mmol) was added and the mixture refluxed for 70 min. The solvent was evaporated and the residue suspended in 8 mL of dichloromethane, then tetra-*n*-butylammonium iodide (12.0 mg, 0.03 mmol) and 1,3-dibenzyloxy-2-chloro-methoxypropane (**15**, 1.44 g, 4.5 mmol) were added and the reaction mixture was refluxed for 90 min. After cooling, the solution was treated with 4 mL of methanol and 1 mL of water. The solvent was distilled off, the residue solved in $CH_2Cl_2$, washed with satd. NaCl solution and water. The organic layer was separated, dried, and the solvent was evaporated. The purification of the reaction product occurred by MPLC (silica gel, dichloromethane–methanol (25:1, v/v)) resulting in 853 mg (69%) of a yellowish viscous liquid of **19**. $^1$H NMR (DMSO-$d_6$) δ: 11.47 (s, 1H, -NH), 8.72 (s, 1H, 6-CH), 7.32–7.22 (m, 10H, -CH(arom)), 7.17 (s, 1H, -OH), 5.14 (s, 2H, -$H_2$C(1′)), 4.45 (s, 4H, -$CH_2$(Bz)), 3.96 (m, 1H, -CH-O), 3.51–3.42 (m, 4H, -$CH_2$-(OBz)). Anal. calcd. for $C_{22}H_{24}N_2O_6$: C 64.07, H 5.87, N 6.79; found: C 63.81, H 5.92, N 6.66.

### *6-Methyl-1-[(1,3-dibenzyloxy-2-propoxy)methyl]uracil (20)*

6-Methyluracil (**18**, 378 mg, 3.0 mmol) was refluxed with ammonium sulfate (150 mg, 1.14 mmol) and 11 mL of hexamethyldisilazane. The solvent was removed and the residue was dried. After dissolving the substance in $CH_2Cl_2$, it was treated with tetra-*n*-butylammonium iodide (12 mg, 0.03 mmol). 1,3-Dibenzyloxy-2-chloromethoxypropane (**15**, 1.44 g, 4.5 mmol) was added, followed by refluxing for 70 min. The reaction mixture was cooled, 1 mL of water and 4 mL of methanol were added and after stirring for 2 min, the solvent was removed. The residue was solved in $CH_2Cl_2$ and washed with satd. NaCl solution and water. The organic layer was separated, dried, and the solvent was removed. The reaction product was purified by column chromatography (silica gel, dichloromethane–methanol (25:1, v/v)) to afford **20** as yellow oil (426 mg, 35%). $^1$H NMR (DMSO-$d_6$) δ: 11.24 (s, 1H, -NH), 7.34–7.25 (m, 10H, -CH(arom)), 5.49 (s, 1H, 5-CH), 5.34 (s, 2H, -$H_2$C(1′)), 4.45 (s, 4H, -$CH_2$(Bz)), 3.95–3.93 (m, 1H, -CH-O), 3.49–3.43 (m, 4H, -$CH_2$-(OBz)), 2.25 (s, 3H, -$CH_3$). Anal. calcd. for $C_{23}H_{26}N_2O_5$: C 67.30, H 6.38, N 6.82; found: C 66.98, H 6.34, N 6.51.

### *6-Methyl-1-[[1-(*p*-anisyldiphenylmethoxy)-3-hydroxy-2-propoxy]methyl]uracil (21)*

6-Methyl-1-[(1,3-dihydroxy-2-propoxy)methyl]uracil (**12**, 460 mg, 2.00 mmol), *p*-anisylchloro-diphenylmethane (672 mg, 2.18 mmol), triethylethylamine (418 µL, 3.00 mmol), and DMAP (2.9 mg) in 7 mL DMF were stirred for 2 h at 50 °C. After removing the DMF, the residue was dissolved in ethyl acetate, washed with $NaHCO_3$ solution and water. The combined organic layers were dried, the solvent removed, and the crude product purified by chromatography (silica gel, dichloromethane–methanol (25:1, v/v)) to give **21** (468 mg, 47%), mp 83 °C. $^1$H NMR (DMSO-$d_6$) δ:11.31 (s, 1H, -NH), 7.36–6.86 (m, 14H, CH(arom)), 5.56 (s, 1H, 5-CH), 5.50 (d, 1H, $J$ = 12.3 Hz, -$H_2$C(1′)), 5.30 (d, 1H, $J$ = 12.3 Hz, -$H_2$C(1′)), 4.71 (t, 1H, $J$ = 5.5 Hz, -OH),

3.83 ($m_c$, 1H, -CH-O), 3.73 (s, 3H, -$OCH_3$), 3.36–3.30 (m, 2H, -$CH_2$(OH)), 3.00–2.92 (m, 2H, -$CH_2$-OMTr), 2.32 (s, 3H, -$CH_3$). Anal. calcd. for $C_{29}H_{30}N_2O_6$: C 69.31, H 6.02, N 5.57; found: C 69.55, H 6.13, N 5.30.

### *6-Methyl-1-[[1-(*p*-anisyldiphenylmethoxy)-3-(*p*-toluenesulfonyloxy)-2-propoxy]methyl]-uracil (22)*

**21** (508 mg, 1.01 mmol) and tosyl chloride (858 mg, 4.50 mmol) were suspended in 12 mL pyridine and left for 70 h at 30 °C. Subsequently, 2 mL of water were added and the reaction mixture was stirred for 2 h at 30 °C. The solvent was evaporated, the residue dissolved in ethyl acetate, and washed with water. The organic layer was dried, the solvent removed and the reaction mixture purified by chromatography (silica gel, dichloromethane–methanol (20:1, v/v)), yielding **22** (420 mg, 63%), mp 79 °C. $^1$H NMR (DMSO-$d_6$) δ: 11.30 (s, 1H, -NH), 7.68 (d, 2H, $J$ = 8.2 Hz, -CH, (m-Ts)), 7.41 (d, 2H, $J$ = 8.2 Hz, -CH, (o-Ts)), 7.31–6.85 (m, 14H, -CH(arom)), 5.52 (s, 1H, 5-CH), 5.33 (d, 1H, $J$ = 12.1 Hz, -$H_2$C(1′)), 5.21 (d, 1H, $J$ = 12.1 Hz, -$H_2$C(1′)), 4.10–4.04 (m, 1H, -CH-O), 4.02–3.95 (m, 2H, -$CH_2$-OTs), 3.00–2.87 (m, 2H, -$CH_2$-OMTr), 2.38 (s, 3H, -$CH_3$, (Ts)), 2.21 (s, 3H, 6-$CH_3$). Anal. calcd. for $C_{36}H_{36}N_2O_8S$: C 65.84, H 5.53, N 4.27, S 4.88; found: C 65.87, H 5.21, N 4.26, S 4.72.

### *6-Methyl-1-[[1-(*p*-anisyldiphenylmethoxy)-3-fluoro-2-propoxy]methyl]uracil (23)*

**22** (180 mg, 0.274 mmol), Kryptofix® 2.2.2 (1.00 g, 2.66 mmol), potassium carbonate (125 mg, 0.904 mmol), and potassium fluoride (1.00 g, 17.2 mmol) were suspended in 20 mL of dry acetonitrile and heated for 15 min to 120 °C. The mixture was cooled down, the precipitated substances were filtered off, and the solvent was evaporated. The residue was purified by column chromatography (RP-18, acetonitrile–water (2:1, v/v)) to afford **23** (42.9 mg, 31%), mp 80 °C. $^1$H NMR (DMSO-$d_6$) δ: 11.34 (s, 1H, -NH), 7.35–6.87 (m, 14H, -CH(arom)), 5.47 (d, 1H, $J$ = 11.5 Hz, -$H_2$C(1′)), 5.32 (d, 1H, $J$ = 11.5 Hz, -$H_2$C(1′)), 4.56–4.41 (m, 2H, -$CH_2$-F), 4.37–4.33 (m, 1H, -CH-O), 3.73 (s, 3H, -$OCH_3$), 3.07–2.97 (m, 2H, -$CH_2$-OMTr), 2.31 (s, 3H, 6-$CH_3$). $^{19}$F NMR (DMSO-$d_6$) δ: –229.5. Anal. calcd. for $C_{29}H_{29}N_2O_4F$: C 69.03, H 5.79, N 5.55; found: C 68.95, H 5.72, N 5.49.

### *$N^6$-Methyl-9-[(2,2-dimethyl-1,3-dioxane-5-yl)ethyl]purine (28)*

5-(2-Bromoethyl)-2,2-dimethyl-1,3-dioxane (**27**, 167 mg, 0.75 mmol) and potassium carbonate (138 mg, 1.00 mmol) were added to $N^6$-methyladenine (**14**, 113 mg, 0.75 mmol) suspended in 2 mL of dry DMF, and the mixture was stirred for 5 h at 20 °C. The mixture was then filtered to remove insoluble material. The combined filtrates were evaporated and the residue purified by MPLC (RP-18, water–acetonitrile (7:3, v/v)) yielding **28** (83 mg, 38%), mp 121–123 °C. $^1$H NMR (DMSO-$d_6$) δ: 8.23 (s, 1H, 2-CH), 8.15 (s, 1H, 8-CH), 7.69 (s, 1H, -NH), 4.15 (t, 2H, $J$ = 7.2 Hz, -$H_2$C(1′)), 3.76–3.72 (m, 2H, -$CH_2$-O-), 3.54–3.49 (m, 2H, -$CH_2$-O-), 2.93 (s, 3H, $NCH_3$), 1.77–1.73 (m, 2H, -$H_2$C(2′)), 1.55–1.54 (m, 1H, -HC(3′)), 1.30 (s, 3H, -$CH_3$), 1.23 (s, 3H, -$CH_3$). Anal. calcd. for $C_{14}H_{21}N_5O_2$: C 57.71, H 7.26, N 24.04; found: C 57.62, H 7.15, N 23.97.

### *$N^1$-Methyl-$N^2$-(*p*-anisyldiphenylmethyl)-9-[[1-(*p*-anisyldiphenylmethoxy)-3-(*p*-toluene-sulfonyloxy)-2-propoxy]methyl] guanine (31)*

$N^2$-(*p*-Anisyldiphenylmethyl)-9-[[1-(*p*-anisyldiphenylmethoxy)-3-(*p*-toluenesulfonyloxy)-2-prop-oxy]methyl]guanine (**29**, 200 mg, 0.210 mmol), and tetrabutylammonium hydroxide solution (1 mol $L^{-1}$, 0.22 mL, 0.22 mmol) were dissolved in 20 mL of dry DMF. A solution of methyl iodide (30 µL, 0.48 mmol) in 0.30 mL DMF was added under vigorous stirring at room temperature. After 45 min, the solvent was evaporated and the residue purified by MPLC (silica gel, dichloromethane–methanol (25:1, v/v)) to afford **31** (89.0 mg, 44%), mp 160–162 °C. $^1$H NMR (DMSO-$d_6$) δ: 7.81 (s, 1H, 8-CH), 7.67 (d, 2H, $J$ = 8.4 Hz, -CH, (m-Ts)), 7.49 (d, 2H, $J$ = 8.4 Hz, -CH, (o-Ts)), 7.32–6.66 (m, 29H, -NH, -CH(arom)), 5.08 (d, 1H, $J$ = 11.7 Hz, -$H_2$C(1′)), 4.85 (d, 1H, $J$ = 11.7 Hz, -$H_2$C(1′)), 3.75 (s, 3H, -$OCH_3$), 3.72 (s, 3H, -$NCH_3$), 3.54 (s, 3H, -$OCH_3$), 3.50–3.46 (m, 1H -CH-O-), 2.55–2.52 (m, 2H, -$CH_2$-OMTr), 2.43 (s, 3H, -$CH_3$, (Ts)), 2.37–2.34 (m, 2H, -$CH_2$-OTs). Anal. calcd. for $C_{57}H_{53}N_5O_8S$: C 70.72, H 5.52, N 7.23, S 3.31; found: C 70.28, H 5.42, N 7.15, S 3.19.

### *$N^1$-Methyl-$N^2$-(*p*-anisyldiphenylmethyl)-9-[(4-(*p*-toluene-sulfonyloxy))-3-*p*-anisyldiphenylmethoxy-methylbutyl]guanine (32)*

$N^2$-(*p*-Anisyldiphenylmethyl)-9-[(4-(*p*-toluenesulfonyloxy))-3-*p*-anisyldiphenylmethoxy-methyl-butyl]guanine (**30**, 300 mg, 0.315 mmol) and tetrabutylammonium hydroxide solution (1 mol $L^{-1}$, 0.33 mL, 0.33 mmol) were dissolved in 7.5 mL of dry DMF. A solution of methyl iodide (45 µL, 0.72 mmol) in 0.45 mL DMF was added under vigorous stirring at room temperature. After 75 min, the solvent was evaporated and the residue purified by MPLC (silica gel, dichloromethane–methanol (25:1, v/v)) to obtain **32** (134 mg, 44%), mp 117 °C. $^1$H NMR (DMSO-$d_6$) δ: 7.73 (d, 2H, $J$ = 8.0 Hz, -CH, (m-Ts)), 7.46 (d, 2H, $J$ = 8.0 Hz, -CH, (o-Ts)), 7.38 (s, 1H, 8-CH), 7.31–6.70 (m, 29H, $N^2$H, -CH(arom)), 3.73 (s, 3H, -$OCH_3$), 3.86–3.68 (m, 2H, -$CH_2$-OTs), 3.64 (s, 3H, $N^1CH_3$), 3.63 (s, 3H, -$OCH_3$), 3.29 ($m_c$, 2H, -$H_2$C(1′)), 2.72–2.51 (m, 2H, -$CH_2$-OMTr), 2.38 (s, 3H, -$CH_3$), 1.48 ($m_c$, 1H, -HC(3′)), 0.98–1.03 (m, 2H, -$H_2$C(2′)). Anal. calcd. for $C_{58}H_{55}N_5O_7S$: C 72.10, H 5.74, N 7.25, S 3.32; found: C 72.15, H 5.79, N 7.31, S 3.27.

### *$N^1$-Methyl-$N^2$-(*p*-anisyldiphenylmethyl)-9-[[1-(*p*-anisyl-diphenylmethyl)-3-fluoro-2-propoxy]methyl]guanine (33)*

**31** (200 mg, 0.207 mmol), potassium fluoride (182 mg, 3.13 mmol), potassium carbonate (125 mg, 0.904 mmol), and Kryptofix® 2.2.2 (500 mg, 1.32 mmol) in 20 mL acetonitrile were heated at 120 °C for 60 min. The mixture was cooled down, the precipitated substances were filtered off and the solvent was evaporated. The residue was purified by column chromatography (RP-18, acetonitrile–water (6:1, v/v)) to afford **33** (37 mg, 22%), mp 121–123 °C. $^1$H NMR (DMSO-$d_6$) δ: 7.83 (s, 1H, 8-CH), 7.35–6.65 (m, 29H, -NH, -CH(arom)), 5.07 (d, 1H, $J$ = 10.5 Hz, -$H_2$C(1′)), 4.95 (d, 1H, $J$ = 10.5 Hz, -$H_2$C(1′)), 4.60–3.80 (m, 2H, -$CH_2$-F), 3.74 (s, 3H, -$OCH_3$), 3.72 (s, 3H, -$NCH_3$), 3.72–3.46 (m, 1H, -CH-O-), 3.56 (s, 3H, -$OCH_3$), 2.61–2.52 (m, 2H, -$CH_2$-OMTr). $^{19}$F NMR (DMSO-$d_6$) δ: –229.5. Anal. calcd. for

**Table 5.** Syntheses times, HPLC conditions, and radiochemical yields of the radio-labelled compounds.

| Tracer | Synthesis time (after EOB) (min) | HPLC conditions (water–ethanol) | $R_t$ (min) | Radiochemical yield (d.c.) (%) |
|---|---|---|---|---|
| [$^{18}$F]FHPG (**2**) | 90 | 95:5 | 8.0 | 15 |
| [$^{18}$F]FHBG (**4**) | 95 | 92:8 | 10.0 | 10 |
| [$^{18}$F]FMHPG (**6**) | 85 | 90:10 | 7.0 | 12 |
| [$^{18}$F]FMHBG (**8**) | 95 | 92:8 | 16.0 | 19 |
| [$^{18}$F]FMHPU (**13**) | 85 | 95:5 | 12.0 | 10 |

**Note:** EOB is the end of bombardement; d.c. is decay corrected.

$C_{50}H_{46}N_5O_5F$: C 73.60, H 5.68, N 8.58; found: C 73.37, H 5.33, N 8.38.

***N*$^1$-Methyl-*N*$^2$-(p-*anisyldiphenylmethyl)-9-[(4-fluoro)-3-*p*-anisyldiphenylmethoxy-methylbutyl]guanine (34)**

**32** (190 mg, 0.197 mmol), potassium fluoride (333 mg, 5.73 mmol), potassium carbonate (125 mg, 0.904 mmol), and Kryptofix® 2.2.2 (500 mg, 1.32 mmol) were solved in 20 mL dry DMF and heated to 140 °C for 90 min. After cooling down, the precipitated substances were filtered off and washed well with DMF. The combined filtrates were evaporated and the residue purified by MPLC (RP-18, acetonitrile–water (9:1, v/v)) affording **34** (38.5 mg, 24%), mp 114–117 °C. $^1$H NMR (DMSO-$d_6$) δ: 7.47 (s, 1H, 8-CH), 7.34–6.72 (m, 29H, $N^2$H, -CH(arom)), 4.34–4.11 (m, 2H, -$CH_2$-F), 3.73 (s, 3H, -$OCH_3$), 3.65 (s, 3H, $N^1CH_3$), 3.64 (s, 3H, -$OCH_3$), 3.45 (t, 2H, $J$ = 7.1 Hz, -$H_2$C(1′)), 2.80–2.71 (m, 2H, -$CH_2$-OMTr), 1.60 ($m_c$, 1H, -HC(3′)), 1.22–1.07 (m, 2H, -$H_2$C(2′)). $^{19}$F NMR (DMSO-$d_6$) δ: –225.8. Anal. calcd. for $C_{51}H_{48}N_5O_4F$: C 75.26, H 5.94, N 8.60; found: C 75.07, H 5.83, N 8.57.

**General syntheses of the [$^{18}$F]-fluorinated compounds 2, 4, 6, 8, and 13**

A solution of 0.5 mL of Kryptofix® 2.2.2 (45 mM), 20 µL potassium carbonate (1 mol $L^{-1}$), and aqueous [$^{18}$F]fluoride solution (0.5–1.0 mL, 0.5–5.0 GBq) was azeotropically evaporated with acetonitrile (3 mL) at 100 °C; 2 to 3 mg of the appropriate precursors **22**, **29**–**32** solved in dry acetonitrile (300 mg) were added to the dry K[$^{18}$F]F/Kryptofix® 2.2.2 complex. The reaction mixture was heated at 160 °C for 20 min. After cooling down to room temperature, the mixture was passed through a LiChrolut Si (500 mg) cartridge. $^{18}$F-labelled compounds were eluted with 2 mL of dichloromethane–methanol (85:15). Methanolic hydrochloric acid (150 µL, 0.85 mol $L^{-1}$) was added to the eluate, the vial sealed and heated for 3 min at 120 °C, then the solvent was removed under a stream of nitrogen. A sodium hydroxide solution (400 µL, 0.2 mol $L^{-1}$) was added to the residue. The mixture was given to a LiChrolut RP-18 (200 mg) cartridge, the crude product eluted with 500 µL of a water–ethanol mixture (9:1) and subsequent purified by HPLC as described in Table 5 at a flow of 5 mL $min^{-1}$. The radiochemical purity and the identity of each probe with the reference standards was identified by analytical HPLC.

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