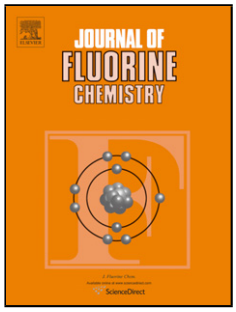

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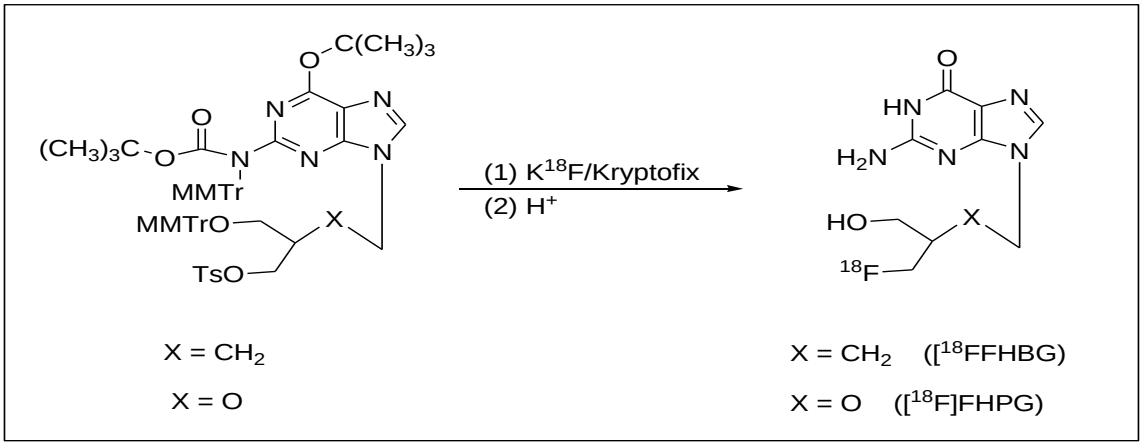
Graphical abstract



Highlights

- New O^6-carbamoyal protected precursors for [^{18}F]FHBG synthesized.
- Novel O^6-*tert*-butyl-N^2-monomethoxytrityl precursors for [^{18}F]FHBG and [^{18}F]FHPG synthesized.
- These precursors were fully characterized by NMR and MS.
- These precursors gave excellent radiochemical yields for [^{18}F]FHBG and [^{18}F]FHPG.

Abstract

A new, high radiochemical yield synthesis of [^{18}F]FHBG and [^{18}F]FHPG, the most popular imaging agents currently in use for monitoring gene therapy using positron emission tomography (PET), is reported in this work. Protection of sensitive sites in the precursors generally utilized for the preparation of [^{18}F]FHBG and [^{18}F]FHPG using the nucleophilic ^{18}F-fluorination reaction was found to be critical for good radiochemical yields, reliability and reproducibility of the synthetic process. As an initial approach, protection at O^6-oxygen in the guanine moiety of the currently used monomethoxytrityl-protected penciclovir tosylate derivative **9** with carbamoyl groups was carried out. Subsequently, full protection of both O^6-oxygen and N^2-nitrogen in the monomethoxytrityl-protected penciclovir and ganciclovir tosylate analogs **9** and **10** were achieved by their reaction with di-*tert*-butyl dicarbonate, which resulted in O^6-*tert*-butyl-N^2-Boc-monomethoxytrityl-protected penciclovir tosylate **18** and O^6-*tert*-butyl-N^2-Boc-monomethoxytrityl-protected ganciclovir tosylate **19**, respectively. The newly synthesized carbamoyl- and the Boc-protected precursors were first reacted with non-radioactive KF complexed with Kryptofix 222 to isolate the fluorinated products. Acid hydrolysis of the purified fluorinated intermediates provided the nucleosides FHBG and FHPG. Full characterization of the new

precursors as well as the products obtained by fluorination and hydrolysis reactions were carried out by one- and two-dimensional NMR spectroscopy and high resolution mass spectrometry. Single crystal X-ray crystallographic analysis of a model ganciclovir analog **22** confirmed the structural characterization of the new Boc-protected tosylate precursors **18** and **19** by NMR spectroscopy. The carbamoyl- and the Boc-protected precursors were further subjected to radiofluorination followed by acid hydrolysis reactions to furnish [^{18}F]FHBG and [^{18}F]FHPG reliably and reproducibly in excellent radiochemical yields (> 65%), much higher than those previously achieved.

Keywords: [^{18}F]FHBG; [^{18}F]FHPG; Penciclovir; Ganciclovir; ^{18}F-fluorination.

1. Introduction

Acyclic guanosine nucleosides 9-[4-hydroxy-3-(hydroxymethyl)butyl] guanine [Pencicolvir (**1**)] and 9-[(1,3-dihydroxy-2-propoxy)methyl]guanine [Ganciclovir (**2**)] (Figure 1) are potent antiviral drugs useful for the treatment of herpes simplex virus (HSV) [1, 2]. The antiviral activity of these drugs stems from the broad substrate specificity of the phosphorylating viral thymidine kinase (TK) enzyme as compared with the restrictive specificity of the mammalian TK enzyme. The established antiviral activity of guanine based nucleoside analogues has spurred renewed interest in the development of a new class of derivatives in order to further study the effects of structural modifications on their antiviral activity and toxicity [3]. These efforts led to the preparation of two N^9- fluorinated chain substituted guanine derivatives, namely 9-(4-fluoro-3-hydroxymethylbutyl)guanine (FHBG, **3**) and 9-[(3-fluoro-1-hydroxy-2-propoxy)methyl]guanine (FHPG, **4**) (Fig. 1).

The fluorinated analogs **3** and **4** displayed slightly lower antiviral activity [3] and altered cellular transport mechanism [4], but maintained good substrate activity for the HSV type -1 thymidine kinase (HSV-1 TK) enzyme [5,6]. These fluorinated substrates are specifically monophosphorylated by HSV-1 TK enzyme in cells that express the gene HSV-1-*tk* (this gene expresses the enzyme HSV-1 TK in high levels) [5,6]. The monophosphorylated nucleosides are subsequently converted to their corresponding di- and triphosphates by the endogenous cellular kinases. When these nucleoside triphosphates are incorporated into the host DNA, chain termination and cell death occurs. Thus, specific cells (e.g., malignant cells) can be selectively targeted and destroyed by delivering HSV-1-*tk* gene into them followed by treatment with these antiviral nucleosides. Similarly, this process of combination of a reporter gene (e.g., HSV-1-*tk*) and its complimentary reporter probe labeled with a positron emitter [e.g., [^{18}F]FHBG (**4**) or [^{18}F]FHPG (**6**), Figure 1; ^{18}F-isotope half-life = 109.74 min] forms the basis of *in vivo* imaging of endogenous gene expression in cancer and several other diseases by positron emission tomography (PET) [6]. As a prelude to mapping the thymidine kinase gene using PET, we previously developed a regiospecific fluorination (at carbon-8) method for purines with elemental fluorine [7] and extended that technique to the preparation of the corresponding 8-[^{18}F]fluoro-analogs as reporter probes [8]. Among all the PET reporter probes, [^{18}F]FHBG (**4**) [9-12], and to a lesser extent [^{18}F]FHPG (**6**) [13-15], have convincingly become the most useful purine based gene imaging agents for HSV1-tk enzyme and its mutated counterparts HSV1-sr39tk and HSV1-A167Ysr39tk [6,16]. Investigations have also demonstrated that [^{18}F]FHBG is more preferred over [^{18}F]FHPG for imaging gene

expression in tumors because of its higher accumulation in transduced cells [6,11]. The importance and usefulness of [^{18}F]FHBG as a PET imaging agent for monitoring gene expression in cancer gene therapy in humans is further underscored by its recent US Food and Drug Administration (FDA) approval as an investigational new drug (IND) [17].

The synthesis of [^{18}F]FHPG [18-23] and [^{18}F]FHBG [19,20,22-31] have been well described in the literature. However, many of these methods provide low radiochemical yields and/or suffer from reproducibility related issues. In the present investigation, we have developed a strategy using several new precursor compounds with specific design for the synthesis of both [^{18}F]FHBG and [^{18}F]FHPG. This approach has provided reliable and significantly higher radiochemical yields for [^{18}F]FHBG and [^{18}F]FHPG than all the previously reported methods. Described herein are the details on the synthesis of these novel precursors, their spectroscopic characterization as well as their reactions with [^{18}F]fluoride ion to yield pure [^{18}F]FHBG and [^{18}F]FHPG suitable for human utilization.

2. Results and Discussion

2.1. Probable causes for the low radiochemical yields of [^{18}F]FHBG and [^{18}F]FHPG in the previous synthetic approaches

The enticing property of [^{18}F]FHBG as a gene imaging agent in humans has elicited considerable interest in its synthesis. As the demand for [^{18}F]FHBG is steadily increasing, there is a compelling need for its production with high synthesis yields. To date, the various published radiosyntheses of [^{18}F]FHBG [19,20,22-31] and [^{18}F]FHPG [18-23] have all used tosylate precursors **9** and **10** (Scheme 1) in which the hydroxyl group in the side chain

and the exo-NH_2 group of the guanine moiety are protected by monomethoxytrityl (MMTr) groups.

After nucleophilic ^{18}F-fluorination of the tosylate precursors with [^{18}F]fluoride ion, the MMTr groups are deprotected by acid hydrolysis to provide [^{18}F]FHBG and [^{18}F]FHPG, which in all cases provide modest radiochemical yields. More importantly, the radiochemical yields obtained for these tracers frequently fluctuate and are erratic for unreported reasons [20,26]. Great efforts have been made by several research groups in the past fifteen years to improve the reliability and reproducibility of the radiosynthesis of [^{18}F]FHBG and [^{18}F]FHPG by changing the various reaction conditions, such as reaction solvent, temperature or purification procedure. For instance, conducting the reaction in acetonitrile [18,19,24], mixture of acetonitrile and DMF [26] or DMSO [23,30], or heating the reaction mixture at 90^{o} C [19] or higher [22,24,27,28,30], or heating by microwaves [26] has achieved varying levels of success with the radiochemical yields ranging between 10 and 34% (decay corrected). One-pot synthesis [19] (radiochemical yield:10-15%) or an optimized solid phase extraction method for purification [20,27] (radiochemical yield:15-30%) decreased the overall synthesis time to < 60 min [26]. but did not enhance the radiochemical yield. Similarly, the decay corrected radiochemical yields obtained for [^{18}F]FHBG with fully automated [25,28-31] modules ranged between 10 and 35% while utilization of a robotic module [23] provided a radiochemical yield of 31%.

The instability of the hemiaminal structure in the side chain of [^{18}F]FHPG under strong acidic deprotection condition (1M HCl) was suspected to be one of the reasons for the low

radiochemical yield. Accordingly, use of 1M acetic acid instead of 1M hydrochloric acid for the deprotection step was found to be beneficial, at least for the synthesis of [^{18}F]FHPG [22]. However, in the case of [^{18}F]FHBG which lacks the hemiaminal structure in the side chain, unlike [^{18}F]FHPG, little improvement in radiochemical yield was attained by using 1M acetic acid for the hydrolytic deprotection step. Use of alternative leaving groups such as mesylate, tresylate or brosylate in place of the tosylate in precursors **9** and **10** (Scheme 1) (Barrio et al, unpublished data from our laboratories) also provided radiochemical yields in the range of 8-35% for [^{18}F]FHBG, as previously realized with other methods. In spite of all these efforts from various laboratories, achievement of satisfactory reliability, reproducibility and good radiochemical yields for the synthesis of [^{18}F]FHBG has remained elusive. Only limited enhancement in the efficiency of the synthesis of [^{18}F]FHBG, over the original experimental conditions that provided radiochemical yields of 8-22% [24], has been demonstrated so far.

From the standpoint of the nucleophilic ^{18}F-fluorination method used for the preparation of the nucleosides [^{18}F]FHBG and [^{18}F]FHPG (Scheme 1), it is important to consider the active hydrogens present on N^1- and N^2-nitrogens in their precursors **9** and **10**, respectively. These two active hydrogens could potentially be sensitive for attack by a base (i.e., K_2CO_3) that is commonly present in the ^{18}F-fluorination reaction medium. For example, the lactam N^1-nitrogen in guanosine derivatives is known to be deprotonated under basic conditions [32]. Interestingly, it has been hypothesized that during the ^{18}F-fluorination of the precursors **9** and **10** (Scheme 1) a base-catalyzed abstraction of the N^1-hydrogen of the lactam, followed by an intramolecular cyclization with concomitant elimination of the tosylate took place and was the cause for the decrease in the radiochemical yield of

products **4** and **6** [22]. Since fluoride ion is a Lewis base and a strong hydrogen bond acceptor, the anhydrous reaction conditions generally required for the nucleophilic fluorination reactions unfortunately also enhances the basicity of the fluoride ion [33]. The precise role of [^{18}F]fluoride ion as a base and an abstractor of active hydrogens, however, is difficult to discern under the radiofluorination reaction conditions. We thus presumed the presence of the active hydrogens on N^1- and N^2-nitrogens in the tosylate precursors **9** and **10** as likely impediments for an efficient radiofluorination reaction. Accordingly, in this work we have developed and report synthetic schemes for the preparation of appropriately protected precursors devoid of active hydrogens for the preparation of [^{18}F]FHBG and [^{18}F]FHPG.

2.2. Optimization of the preparation of the monomethoxytrityl analogs **7** *and* **8**

The monomethoxytrityl (MMTr) is a very useful protecting group for amines and alcohols [34]. The MMTr group is quite stable under basic conditions typically used for nucleophilic fluorination reactions and is easily removed by dilute mineral acids. Accordingly, it has found use in the synthesis of [^{18}F]FHBG and [^{18}F]FHPG (Scheme 1) wherein a selective protection of one of the hydroxyl groups on the side chain on N^9-nitrogen along with the primary amino group at 2-position of the guanine moiety in penciclovir (**1**) and ganciclovir (**2**) has been achieved to provide the derivatives **7** and **8** (Scheme 1) [3,18,24]. The original literature procedure utilized 4-dimethylaminopyridine (DMAP) as catalyst in dimethylformamide and triethylamine as a base for the monomethoxytritylation reaction [3]. Subsequently, conditions for this reaction have been modified by Zheng and co-workers and a good discussion on the product formation has

been reported [21,27]. For slightly better yield, decrease of by-product formation and ease of product purification, we further modified the reaction conditions for this protection step adopting the method reported by Ogilvie *et al* [35]. In our syntheses, triethylamine was first added to a concentrated solution of the nucleosides **1** and **2** in DMSO followed by monomethoxytrityl chloride. Under this condition, the formation of side products was diminished and pure products **7** and **8** were easily obtained by flash column chromatography. The MMTr protected derivatives **7** and **8** were previously characterized by ^{1}H NMR spectroscopy [21, 27]. However, the presence of six phenyl rings in these products relatively confounds simple interpretation of ^{1}H and ^{13}C NMR signals. We have therefore extensively utilized two-dimensional COSY, HMQC and HMBC NMR techniques to alleviate this predicament to some extent and were able to assign the ^{13}C signals for the anisole part of the MMTr groups. For example, in the HMBC spectrum of the analog **7**, the N^2-H proton NMR signal (δ 7.56 ppm) correlated with the ^{13}C signals of the anisyl carbon C-1′ (136.87 ppm) and two phenyl C-1″ carbons (144.74 and 144.91 ppm) in the *N*-MMTr group on the guanine moiety (Fig. 2).

This pattern clearly distinguished the corresponding NMR signal arising from the *O*-MMTr group on the side chain. Further, the characteristic doublets due to H-3′/H-5′ protons in the anisole rings of both MMTr groups at δ 6.89 ppm (CH_3OPh-C-*O*) and δ 6.75 ppm (CH_3OPh-C-*N*) were shifted up-field from other aromatic protons (δ 7.07–7.33 ppm) due to the electron donating nature of the 4′-OCH_3 and exhibited correlations with the 4-methoxyphenyl C-1′ ^{13}C signals at 135.41 ppm and 136.87 ppm, respectively. The proton singlets for the CH_3O groups in both MMTr moieties at δ 3.73 ppm (*O*-MMTr), δ 3.64

ppm (*N*-MMTr) correlated with the C-4′ carbon NMR signals at 158.10 ppm (CH_3OPh-C-*O*), 157.62 ppm (CH_3OPh-C-*N*). The HMBC spectrum (Fig. 2) was also helpful in the assignment of ^{13}C signals in the guanine part of the molecule **7**. For instance, the C-5 carbon signal at 116.97 ppm showed correlation to the singlets of N^1-hydrogen (δ 10.48 ppm) and H-8 (δ 7.44 ppm), while C-4 carbon (149.46 ppm), as expected, correlated with only H-8. Surprisingly, H-8 displayed a relatively weak correlation with C-6 carbon (156.56 ppm), likely due to the four-bond separation between them. Curiously though the C-2 carbon (150.36 ppm) did not exhibit any correlation with N^1-H or N^2-H proton signals. The assignments of these NMR chemical shifts are all consistent with the data previously reported in the literature [36].

Standard tosylation reaction of the two monomethoxytrityl protected products **7** and **8** with *p*-toluenesulfonyl chloride in pyridine gave the precursors **9** and **10** in yields similar to a recently published work [37].

2.3. *O^6-Carbamoyl protected penciclovir anaogs*

A variety of protecting groups for the lactam moiety in guanine nucleosides (on N^1-nitrogen or O^6-oxygen) are known [34]. Examples among them are methoxymethyl (MOM) [38], 2,2,2-trichloro-*tert*-butyloxycarbonyl (TCBOC) [39] groups that protect the N^1- nitrogen (lactam tautomer) while 4-nitrophenylethyl (NPE)[40], diphenylcarbamoyl (DPC) [41,42] and dimethylcarbamoyl (DMC) [41,43] groups protect O^6-oxygen (lactim tautomer). We selected the carbamoyl groups DPC and DMC in an initial attempt to block the adverse effect, during the nucleophilic fluorination reaction, of N^1-H in the tosylate precursor **9**. Both these protecting groups are easily incorporated on the O^6-oxygen of

guanine nucleosides in excellent yields (> 80%) and also are efficiently deprotected by bases [41-43]. Thus, the penciclovir tosylate analog **9** reacted with ease with diphenyl- and dimethylcarbamoyl chloride to yield the corresponding carbamates **11** and **12** (Scheme 2).

The structures of the analogs **11** and **12** were confirmed by 1-D and 2-D NMR spectroscopic and mass spectrometric analyses. In the ^{1}H NMR spectrum, the down field lactam N^{1}-H signal of precursor **9** disappeared while the N^{2}-H signals of **11** and **12** remained around 7.8 ppm. In ^{13}C NMR spectrum, the carbonyl signal of the carbamoyl groups in **11** and **12** appeared at 150.32 and 151.76 ppm, respectively. In the HBMC spectrum of the compound **12**, correlation between the *N*-methyl proton singlets (δ 2.90 and 3.01 ppm) of the carbamoyl group and the ^{13}C resonance for the carbonyl group (151.76 ppm) was also observed.

*2.4. Nucleophilic fluorination of the carbamoyal precursors **11** and **12***

The O^{6}-diphenylcarbamoyal precursor **11** was subjected to nucleophilic fluorination with KF/Kryptofix in DMSO at 145^{o} C (Scheme 3).

After workup, the crude reaction mixture was separated by silica column flash chromatography to yield two distinct fractions. In fraction one, the fluorinated product **13** and the elimination product **14** (Scheme 3) were obtained as an inseparable mixture in 42% yield. Surprisingly, NMR spectroscopic and mass spectrometric analyses indicated the carbamoyl group was found to be cleaved off in all the products. The deprotection of the carbamoyl group is generally achieved with concentrated ammonia [41-43]. To our

knowledge, this is the first observation of the deprotection of the carbamoyl moiety by fluoride ion. The NMR spectroscopic analysis indicated the ratio of the fluorinated to the elimination product in the inseparable mixture was 8:2. In the electrospray ionization (ESI) mass spectrum, the molecular ion signal intensity of the elimination product **14** was about 30% of that of the desired fluorinated product **13**. The vinyl proton signal in the product **14** appeared as two broad singlets at 4.64 ppm and 5.24 ppm, each integrating for one proton. The ^{13}C NMR signal for the vinyl-CH_2 carbon appeared at 111.0 ppm. As expected, the presence of fluorine in the product **13** caused down field shifts for the proton (4.22 ppm, $^{1}J_{H,F}$ = 47.5 Hz) and ^{13}C (83.77 ppm, $^{1}J_{C,F}$ = 166.5 Hz) signals for the methylene chain carrying it compared with the corresponding signals in the tosylate precursor **11** or the hydroxyl derivative **7**. The ^{19}F NMR chemical shift for this product was observed at −225.4 ppm consistent for an aliphatic fluorinated compound.

The second fraction from the silica column chromatography was obtained in 22 % yield. The NMR spectroscopic analysis of this fraction showed that it was also a mixture of two products in a ratio of 3:1 and resisted all attempts at separation. Hence, the unseparated mixture was analyzed as such. The mass spectrometry data of this mixture revealed a molecular weight of exactly 20 Daltons less than that of the fluorinated product **13**, suggesting a loss of HF from it. The 2-D NMR spectroscopic analysis of the major component of this mixture suggested the possibility of a side chain CH_2-N^3-cyclized structure **15** for it (Scheme 3). Thus, in the HMBC spectrum clear correlations of the protons on the N^3-CH_2 group (δ 4.66 ppm) with C-2 (141.27 ppm) and C-4 (144.42 ppm) carbons were observed, strongly implying a cyclization of the side chain with the N^3-nitrogen. Further, the C-2, C-4 and C-5 NMR signals in the compound **15** were shifted up

field when compared with the corresponding carbons in the analogs **13** and **14** due to the well-known γ shielding effect [44] arising from the cyclic carbon chain attached to N^3-nitrogen. Accordingly, the C-2, C-4 and C-5 chemical shifts in the cyclic derivative **15** occur at 141.27, 144.42 and 114.05 ppm, respectively while the corresponding ^{13}C signals in the fluoro analog **13** appear at 150.93, 149.95 and 117.44, respectively. Support for the cyclic structure for compound **15** is also partly derived from the report of Holmes and Robins [45] who have previously synthesized an analogues cyclonucleoside from a 5′-tosylate derivative of guanosine. More recently, the formation of such a cyclonucleoside, as a side product, has been observed during the nucleophilic fluorination reaction of the tosylate **9** [22]. Since the formation of the cyclic product **15** from the precursor **11** would first require an availability of N^1-hydrogen [22], this side reaction must have happened after the cleavage of carbamoyl protecting group which would provide the necessary N^1-hydrogen. The cycloguanine derivatives in both these reports [22, 45] were shown to have an amidine tautomeric moiety wherein the exocylic N^2-nitrogen carrying a hydrogen and N^1 existing as a tertiary nitrogen. In contrast, the HMBC spectrum indicated the presence of an imidine tautomeric structure in **15** with N^1-nitrogen carrying the hydrogen instead of the N^2-nitrogen. Correspondingly, N^1-hydrogen (δ 6.50 ppm) displayed correlations with both C-5 (114.05 ppm) and C-6 (155.41 ppm) carbons in the HMBC spectrum.

In the ^{1}H NMR spectrum for the minor component in this mixture, chemical shifts presumed to be due to H-8, N^1-H of guanine moiety as well as signals resembling to those of the methylene protons in compound **15** were observed. It could be speculated that this product might have a side chain cyclized structure involving N^2-nitrogen instead of N^3-

nitrogen [45]. However, no further attempts were made to characterize this minor component.

The fluorination reaction of O^6-dimethylcarbamoyal precursor **12** (Scheme 4) provided a mixture of the fluorinated product **13** and the elimination product **14** in a total yield of 61%.

Similar to the diphenylcarbamoyal precursor (Scheme 3), the dimethylcarbamoyal group was also deprotected during the fluorination reaction. Interestingly, however, the product mixture was totally devoid of the cycloguanine derivative **15**. The lack of formation of the derivative **15** suggests a higher stability of the precursor **12** under the reaction conditions than that of the diphenylcarbamoyl analog **11** since the formation of the cyclic product would first require the presence of N^1-hydrogen [22], creation of which is possible only by the cleavage of carbamoyl protecting group.

Both the diphenylcarbamoyl precursor **11** and dimethylcarbamoyl precursor **12** were used in the radiosynthesis of [^{18}F]FHBG (**4**) (Scheme 5).

Decay corrected radiochemical yields of 38% and 43%, respectively were obtained for [^{18}F]FHBG with the precursors **11** and **12** with a total synthesis time of ~110 min. Relevant details are summarized in Table 1 below and full synthesis details are provided in the Experimental section.

These radiochemical yields are still more consistent and higher than those obtained with the commonly used precursor **9** [19,20,22-24]. The side products (i.e., **14** and **15**) formed during the fluorination reaction did not affect the final chemical purity of the product [^{18}F]FHBG. They were readily removed by semi-preparative HPLC purification after acid hydrolysis (see Experimental). Further discussion on the radiochemistry conditions is provided later in this section.

2.5. The need for fully protected tosylate precursors

Substitution of the hydrogen on the N^1-nitrogen with a methyl group in the tosylate precursors **9** and **10** was first strategized to suppress the formation of the cyclonucleoside **15** during the radiofluorination reactions and thereby improve the final radiochemical yields [22,46]. The results of the radiofluorination reactions of such N^1-blocked analogs of **9** and **10** were rather confounding. The ^{18}F-fluorination followed by acid hydrolysis of N^1-methyl substituted FHBG tosylate precursor surely provided a better radiochemical yield of 19% for N^1-methyl-[^{18}F]FHBG vs. 10% obtained for [^{18}F]FHBG with the tosylate derivative **9** (Scheme 1) attesting to the beneficial effect of N^1-nitrogen blockage. Unfortunately, analogous radiofluorination of the N^1-methyl substituted FHPG tosylate gave a lower radiochemical yield of 12% for N^1-methyl-[^{18}F]FHBG while the corresponding non-methylated precursor **10** provided a higher radiochemical yield of 15% for [^{18}F]FHPG. Thus, the effect of blockage of N^1-nitrogen with a methyl group in guanosine analogs seems rather unpredictable from the standpoint of improving the radiochemical yields. Besides, no viable reactions are known to de-methylate the N^1-

methyl-[^{18}F]FHBG or N^1-methyl-[^{18}F]FHPG to provide the desired radiolabeled nucleosides.

The nucleophilic fluorination reaction results with the O^6-carbamates (Schemes 3-5), in contrast to the N^1-methyl blocking studies [22,46] are quite encouraging. These results further indicate that protection of O^6-oxygen in the lactim tautomer is preferable to the protection of the N^1-nitrogen in the lactam tautomer of the tosylate derivatives **9** and **10**. The radiochemical yields for the ^{18}F-nucleosides obtained with the O^6-carbamate precursors **11** and **12** (Scheme 5) in this investigation are higher than those obtained with the free lactam tosylate precursors **9** and **10** (Scheme 1) [18,24]. The N^1-methyl group protection studies discussed above, however, clearly implies that N^1-hydrogen abstraction under the nucleophilic fluorination conditions [22,46] might not be the only reason for the poor radiochemical yields for preparation of [^{18}F]FHBG and [^{18}F]FHPG from the tosylates **9** and **10**. It is quite likely that the hydrogen on the exocyclic N^2-nitrogen of the guanine moiety in these tosylate precursors **9** and **10** may also be subjected to abstraction by base during the nucleophilic fluorination reaction in detriment to the radiochemical yields. We therefore envisioned that protection of the O^6-oxygen (lactim tautomer) as well as the N^2-nitrogen of the tosylate precursors **9** and **10** might be a prudent plan for more efficient and reproducible ^{18}F- labeling of these guanine derivatives.

The judicious approach of selectively protecting the N^2-nitrogen and only one of the hydroxyl groups in the side chain of penciclovir (**1**) and ganciclovir (**2**) with monomethoxytrityl groups [3] strategically permits the synthesis of the tosylate precursors **9** and **10** (Scheme 1) [18,24]. After careful considerations, we elected to maintain this core structure and embarked on to protect the O^6-oxygen (lactim tautomer) and the exocyclic

N^2-nitrogen in the tosylates **9** and **10** with appropriate groups. An alternative to the carbamoyl group was also considered because of its labile nature during the nucleophilic fluorination reaction condition (Schemes 3 and 4). A quite promising protecting group in this regard is *tert*-butyloxycarbonyl (Boc) which can be used to block both the exocyclic N^2-nitrogen and the O^6-oxygen in the lactim moiety [47,48]. An attractive feature of the Boc protection is the ease with which it can be deprotected by acid hydrolysis. Thus, we endeavored the preparation of new derivatives of tosylates **9** and **10** with Boc protection on the lactim moiety as well as on the N^2-nitrogen.

*2.6. Boc protection of O^6-oxygen and N^2-nitrogen in the tosylate precursors **9** and **10***

For the Boc protection, a solution of the tosylates **9** and **10** in dichloromethane were reacted, under reflux, with ~10 M excess of di-*tert*-butyl dicarbonate (Boc anhydride) in the presence of DMAP and triethylamine (Scheme 6) [47]. The reaction mixture underwent a quick color change from yellow to orange-red accompanied by a vigorous evolution of a gas, presumably carbon dioxide.

The bright red color of the reaction mixture soon faded back to yellow. The product was conveniently purified by flash column chromatography. Surprisingly, the expected N^2,^6O-di-Boc products **16** or **17** were not obtained after the column chromatographic purification. Instead, N^2-Boc-O^6-*tert*-butyl ethers **18** and **19** resulted in 52% and 72% yields, respectively, as carefully confirmed by spectroscopic analyses.

In the high resolution mass spectrum, the presence of both M+1 and M+23 (M+Na) ion signals corresponding to the products **18** and **19,** and not the di-Boc analogs **16** and **17**,

were observed. One- and two-dimensional (COSY, HMBC and HMQC) NMR spectroscopic analyses also indicated the products obtained in the Boc protection reaction were indeed N^2-Boc-O^6-*tert*-butyl ethers **18** and **19**. For example, in the 150 MHz ^{13}C NMR (600 MHz NMR spectrometer) spectrum of the derivative **18**, only a single carbonyl signal at 153.33 ppm was observed corresponding to the presence of only one Boc group in the molecule. The ^{13}C NMR carbonyl carbon signal in the Boc group usually appears in the region of 148 -158 ppm. The four ^{13}C NMR signals (apart from the carbonyl resonance at 153.33 ppm) of 151.92 ppm (C-4), 152.41ppm (C-2), 157.32 ppm (C-4′ of CH_3OPh-C-*N*) and 158.18 ppm (C-4′ of CH_3OPh-C-*O*) in this region were all assigned to other carbons in the compound **18** based on the HMBC spectrum. Only one ^{13}C carbonyl signal at ~153 ppm was observed even at a higher resolution and greater sensitive NMR frequency of 200 MHz for carbon-13 (800 MHz NMR spectrometer) indicating the presence of only one Boc group in the compound **18**. Overall, many correlations discerned between ^{1}H and ^{13}C NMR signals in the Boc protected analogs **18** and **19** mirrored those observed in the products **7-12**. Incidentally, the ^{1}H NMR signals for the protons in the N^2-MMTr group in compounds **18** and **19** were slightly broadened due to overlapping of closely spaced resonances suggesting the presence of rotamers due to the N^2-Boc group.

Reaction of Boc anhydride with alcohols to form *tert*-butyl ethers in the presence of Lewis acid catalysts is known [49]. In the reaction of the Boc anhydride with the tosylates **9** and **10** (Scheme 6), an acyl transfer from the Boc anhydride to O^6-oxygen [50] likely leads to the formation of the intermediates **16** and **17**. Spontaneous elimination of a molecule of CO_2 from these intermediates forms the O^6-*tert*-butyl ethers **18** and **19**. Conversely, the reaction could also proceed by first forming N^1-Boc derivative by an acyl

transfer from the O^6-Boc analog [50], which upon elimination of CO_2 followed by an internal transposition of *tert*-butyl group could lead to the O^6-*tert*-butyl ethers. In any event, analogous formation of O^6-*tert*-butyl ether has recently been well documented during the Boc protection reaction of closely related guanosine nucleoside analogs [51-54].

The tosylate derivatives **18** and **19**, after nucleophilic fluorination reaction, would be subjected to acid hydrolysis for deprotection of the monomethoxytrityl, Boc and *tert*-butyl groups. To test the ease with which these protecting groups could be hydrolyzed, a model guanine nucleoside containing all these groups was prepared (Scheme 7).

Monomethoxytritylation of ganciclovir (**2**) with six equivalents of MMTrCl gave the analog **20** in 76% yield which upon reaction with Boc anhydride provided the product **21**. The product **21** was fully characterized by NMR spectroscopy and mass spectrometry. As expected, the signals in the ^{1}H NMR spectrum for N^2-MMTr group were slightly broadened due to rotamers while sharp proton signals were observed for the other two MMTr groups on the oxygen in the side chain. The ^{13}C NMR spectrum of the derivative **21** showed the presence a single Boc carbonyl group. The high resolution mass spectrum was consistent with the structure **21**.

Deprotection of the Boc-protected derivative **21** with 1M HCl at 115^o C for 1 h gave ganciclovir (**2**) while the same hydrolysis for 3 min led to a partially deprotected product **22** containing a Boc group (Scheme 7). The structure of the Boc analog **22** was consistent with high resolution mass spectrometric and NMR spectroscopic data. Furthermore,

compound **22** was crystallized from methanol/water and analyzed by single crystal X-ray crystallography (Fig. 3).

As discussed earlier, the NMR spectroscopic and mass spectrometric data clearly showed the presence of only one Boc and one *tert*-butyl group in the tosylates **18** and **19**. The above X-ray structure (Fig. 3) now confirms the Boc group in the derivatives **18** and **19** must also be on the N^2-position and the *tert*-butyl group on the O^6-oxygen.

*2.7. Nucleophilic fluorination of fully protected tosylate precursors **18** and **19***

Nucleophilic fluorination reaction of the fully protected precursor **18** was carried out with potassium fluoride in the presence of Kryptofix 222 in DMSO at 145^o C (Scheme 8). The NMR spectroscopic analysis of the reaction mixture, after work up (see Experimental), indicated the N^2-Boc and O^6-*tert*-butyl ether moieties were stable under the fluorination reaction condition unlike the carbamoyl groups, as discussed earlier. Normal phase semi-preparative HPLC was used to cleanly separate the closely eluting fluorinated product **23** (53.9% yield) from the elimination side product **24** (29.6% yield). Interestingly, the HPLC analysis did not reveal the presence of the cycloguanine derivative **15**, attesting to the suitability of *tert*-butyl group for the protection of the lactam in the precursor **18** to prevent the formation of this undesired side product during nucleophilic fluorination reaction. The fluorinated product **23** and the elimination side product **24** were fully characterized by ^{1}H, ^{13}C and ^{19}F NMR spectroscopy and mass spectrometry. For decisive NMR signal assignments, HMBC and HMQC spectra were extensively utilized. As representative examples, Fig. 4 and 5 illustrate our efforts in this regard for the NMR analysis of the fluorinated analog **23**.

In the HMBC spectrum, many of the correlations previously observed for the monomethoxytrityl protected analog **7** (Fig. 2) in the aromatic region were also discerned for the fluorinated analog **23** (Fig. 4). In the guanine moiety in **23**, the H-8 singlet at δ 8.14 ppm correlated with C-4 (152.00 ppm) and C-5 (119.63 ppm) carbon signals. As expected, both C-2 and C-6 carbons having no two- or three-bond separated hydrogens showed no correlations in the HMBC spectrum. However, protons on the side chain on the N^9-nitrogen displayed rich HMBC correlations with carbons on the ring as well as on the aliphatic chain. For instance, the multiplet signal for H-10 at δ 3.99 ppm correlated with C-4 and C-8 (143.72 ppm) carbon resonances. The proton NMR signal for the methylene group (H-13) carrying the fluorine appeared as a doublet of doublets (J=47.4 and 4.6 Hz) at δ 4.55 ppm and also displayed two and three bond correlations with C-12 (37.16 ppm) and C-11 (27.71 ppm) and C-14 (61.73 ppm) carbons, respectively. Similarly, H-11 (δ 1.53 ppm) correlated with C-10 (41.21 ppm), C-12, C-13 (83.33 ppm) and C-14 carbons. The ^{1}H NMR multiplet signal for H-14 (δ 3.02–3.06 ppm) further manifested correlations with the resonances of carbons 11, 12 and 13. These HMBC correlations thus permitted definitive assignment of the chemical shifts for various carbons in the derivative **23**. The HMQC spectrum (Fig. 5) was also valuable for the assignment of ^{13}C NMR signals of carbons carrying a hydrogen.

For example, the ^{1}H NMR doublet of doublets at δ 4.55 ppm for H-13 showed correlation to a ^{13}C signal at 83.33 ppm which could undoubtedly be ascribed to the C-13 carbon in

the CH_2F group. The correlation of the H-10 proton signal at δ 3.99 ppm with the ^{13}C resonance of 41.21 ppm allowed the assignment of the C-10 carbon. Similarly, the correlation of the H-12 methine proton multiplet at δ 1.84 ppm with a ^{13}C signal at 37.16 ppm was surely attributed to the C-12 carbon on the side chain. The one bond correlation of the proton multiplet at δ 3.02-3.06 with the carbon signal of 61.73 ppm led to the assignment of C-14 carbon. In the guanine part of the molecule **23**, the lone H-8 proton at δ 8.14 ppm cleanly correlated with C-8 carbon resonance at 143.72 ppm establishing their one bond connectivity.

Overall, the HMBC and HMQC spectra complemented each other in the unambiguous assignment of the NMR chemical shifts in all the products analyzed.

As described in the Experimental section, acid hydrolysis (at 115^o C) of the HPLC purified fluorinated analog **23** for a short duration expectedly provided the N^2-Boc protected derivative **25** in 93.6% yield while conducting the reaction for a longer period led to full deprotection resulting in the formation of FHBG (**3**) in 75% yield (Scheme 9).

The crude fluorination reaction mixture containing the products **23** and **24** was also subjected to acid hydrolysis to mimic the conditions of ^{18}F-labeling process as described below. Semi-preparative HPLC purification of this reaction mixture provided pure FHBG (**3**) along with the deprotected elimination side product **26**.

The nucleophilic fluorination of the fully protected ganciclovir precursor **19** also provided only the fluorinated derivative **27** and the elimination product **28** (Scheme 10).

However, semi-preparative HPLC could not fully separate these two products. The HPLC purification provided a mixture containing the fluorinated product **27** (56.3% yield) along with the elimination product **28** as judged by ^{1}H NMR spectroscopy and mass spectrometry. The NMR and mass spectral characterization of the products **27** and **28** was performed only as a mixture.

The partial deprotection (see Experimental) of the mixture of derivatives **27** and **28** with 1M HCl at 115^{o} C for short duration followed by HPLC purification provided the N^{2}-Boc protected product **29** in 64.6% yield. The partially hydrolyzed product **29** could subsequently be fully deprotected to give pure FHPG (**5**) in 74.4% yield by further hydrolysis with 4M HOAc (Scheme 11).

*2.8. Preparation of [^{18}F]FHBG and [^{18}F]FHPG from the carbamoyl- (**11**, **12**) and Boc-protected (**18**, **19**) tosylate precursors*

The nucleophilic fluorination reaction of the carbamoyl- (**11** and **12**) and Boc-protected tosylate precursors (**18** and **19**) with [^{18}F]fluoride ion complexed with Kryptofix 222 followed by acid hydrolysis and semi-preparative HPLC purification provided chemically and radiochemically pure [^{18}F]FHBG (**4**) and [^{18}F]FHPG (**6**) (Schemes 5 and 12) in decicurie amounts.

The radiochemical yields obtained for these experiments are provided in Table 1. The radiofluorination reaction of the tosylate precursors **9** and **10** (Scheme 1) were generally

conducted in acetonitrile medium at 90-120^{o} C [18-21,24,27]. Alternatively, DMSO has also been used as a solvent for this ^{18}F-fluorination reaction at 100-135^{o} C [23,30]. Initially, we conducted a few selected radiofluorination reactions of the precursor **18** in acetonitrile medium in a sealed reaction vessel at 150^{o}-165^{o} C and obtained [^{18}F]FHBG in 55% radiochemical yield (Table 1). However, when the same reaction was conducted in DMSO at 150^{o} C, a significant improvement in the radiochemical yield of 68% was realized (Table 1). Thus, all further nucleophilic ^{18}F-fluorination reactions with the carbamoyl- and the Boc-protected precursors were conducted in DMSO medium. Protection of the lactam moiety in the tosylate **9** with diphenylcarbamoyl group (precursor **11**) was found to be beneficial and provided a radiochemical yield of ~38 % for [^{18}F]FHBG (Table 1), which is higher than those of all the previously reported methods [19,20,22-24,26,27]. Interestingly, the radiochemical yield was further improved to ~43 % when the dimethylcarbamoyl group (precursor **12**) was used to protect the lactam in the tosylate **9**. This strongly suggests the dimethylcarbamoyl group is more stable than the diphenylcarbamoyl moiety under the fluorination reaction condition. It is, however, quite gratifying that protection of both O^6-oxygen and N^2-nitrogen in the tosylates **9** and **10**, as realized with the derivatives **18** and **19**, had the most significant effect on the radiofluorination yields for [^{18}F]FHBG and [^{18}F]FHPG. The radiochemical yield obtained for [^{18}F]FHBG with the fully protected precursor **18** (68%) is substantially higher than that attained even with the derivative **12** (43%) (Table 1). Similarly, the 65% radiochemical yield accomplished for [^{18}F]FHPG with the precursor **19** (Table 1) is the highest achieved to date for this molecular imaging probe [18-23]. Thus, starting typically with 500-550 mCi of [^{18}F]fluoride ion, [^{18}F]FHBG or [^{18}F]FHPG can be reproducibly obtained in > 150 mCi yields (end of synthesis) and ready

for human administration in a total synthesis time of 110 min. In addition, an extremely attractive feature of the Boc- protected tosylate precursors **18** and **19** is their stability. No ill effects on the radiochemical yields were observed for these precursors stored for more than a year in a freezer at – 10^{o} C.

3. Conclusions

Several novel precursors for the high yield synthesis of the very valuable gene imaging PET imaging probes [^{18}F]FHBG (**4**) and [^{18}F]FHPG (**6**) have been developed. Previously utilized precursors **9** and **10** for the preparation of these tracers seem to suffer from undesired side reactions during the radiofluorination process, diminishing the radiochemical yields, as well as the reliability and reproducibility of the procedure. The new precursor strategies developed in this investigation is capable of mitigating these shortcomings. Protection of the O^6-oxygen in the precursor **9** with diphenyl- or dimethylcarbamoyl group (**11**, **12**) decidedly improved the radiochemical yield for [^{18}F]FHBG. More impressively, when the O^6-oxygen and the N^2-nitrogen in the precursors **9** and **10** were fully protected with *tert*-butyl and Boc groups, respectively (**18**, **19**) excellent radiochemical yields (> 60%) were reliably obtained for [^{18}F]FHBG and [^{18}F]FHPG, in decicurie amounts ready for human administration. The radiochemical yields achieved with these fully protected precursors are the highest reported to date.

4. Experimental

4.1. General

Penciclovir (**1**) was synthesized as previously reported [55] or purchased from Eurasia Trans Continental **(**Mumbai**,** India**).** Ganciclovir (**2**) was obtained by neutralization of an aqueous solution of Cytovene-IV (Roche Laboratories Inc, New Jersey, USA) with 1M HCl to yield a white powder which was filtered, washed with water and methanol and then dried over phosphorous pentoxide in a vacuum desiccator. All reagent chemicals and solvents were purchased from Aldrich. All solvents prior to use were distilled and dried under standard conditions. Flash column chromatography was carried out with 0.063-0.200 mm silica gel.

One and two dimensional [gradient selected heteronuclear multiple quantum coherence (HMQC) and gradient selected heteronuclear multiple bond coherence (HMBC)] ^{1}H and ^{13}C NMR spectra were acquired on Bruker Avance 300, DRX 500 or Avance 600 NMR spectrometer with a 5 mm broadband probe. The 800 MHz NMR spectra were recorded on a Bruker Avance III HD 800 spectrometer equipped with an inverse cryoprobe. The ^{19}F NMR was acquired on a Bruker Avance 300 MHz or 400 MHz spectrometer with a 5 mm QNP probe. The chemical shifts (δ) are expressed in parts per million (ppm) downfield from internal tetramethylsilane (TMS) for ^{1}H and ^{13}C and external fluorotrichloromethane (Freon 11) for ^{19}F NMR. Atmospheric pressure chemical ionization (APCI) and electrospray ionization (ESI) high resolution mass (HRMS) spectra were obtained on an Agilent LC TOF spectrometer at the analytical chemistry instrumentation facility of the University of California, Riverside. Single crystal X-ray crystallography was performed on a Bruker Smart 1000 CCD diffractometer.

4.2. Preparation of precursor compounds

*4.2.1. N^2-(p-Anisyldiphenylmethyl)-9-[3-(p-anisyldiphenylmethoxymethyl)-4-hydroxybutyl] guanine (**7**)*

To a suspension of penciclovir (**1**) **(**251 mg, 0.99 mmol) in DMSO (1.5 mL) was first added triethylamine (0.84 mL, 6 mmol) and then *p*-anisyldiphenylmethyl chloride (862 mg, 2.8 mmol). The reaction mixture was stirred at room temperature overnight. This resulted in a thick brown mixture to which brine (30 mL) was added. The solid that separated was collected on Celite 521 (Aldrich) by filtration. The solid was washed with water (30 mL) and then stirred with CH_2Cl_2 (30 mL). The CH_2Cl_2 solution was filtered and dried over anhydrous Na_2SO_4. Evaporation of the solvent at reduced pressure gave the crude product as a yellow foam (1.32 g). The crude product was purified by flash chromatography on silica gel (40 g), eluted with CH_2Cl_2/MeOH gradient (0-10% MeOH), to give the product **7** (453 mg, 56.8% yield) as a beige solid. ^{1}H NMR (600 MHz, DMSO-d_6): δ 1.20 (m, 2H, CH_2), 1.40 (m, 1H, CH), 2.71 (dd, J = 9.2 and 6.9 Hz, 1H, CH_2OMMTr), 2.80 (dd, J = 9.2 and 5.3 Hz, 1H, CH_2OMMTr), 3.14 and 3.29 (m, 2H, CH_2OH), 3.39 (m, 2H, *N*CH_2), 3.64 (s, 3H, OCH_3 of *N*-MMTr), 3.73 (s, 3H, OCH_3 of *O*-MMTr), 4.36 (t, J = 4.9 Hz, 1H, OH), 6.75 (d, J = 8.9 Hz, 2H, H-3′/H-5′ of CH_3OPh-C-*N*), 6.89 (d, J = 9.0 Hz, 2H, H-3′/H-5′ of CH_3OPh-C-*O*), 7.07-7.33 (m, 24H, Ar-H), 7.44 (s, 1H, H-8), 10.48 (s, 1H, N^1-H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 28.50 (CH_2), 35.30 (CH), 41.27 (*N*CH_2), 54.92 (OCH_3 of *N*-MMTr), 55.02 (OCH_3 of *O*-MMTr), 61.14 (CH_2OMMTr), 63.19 (CH_2OH), 69.60 (*N*-*t*C), 85.48 (*O*-*t*C), 112.77 (C3′/C5′ of CH_3OPh-C-*N*), 113.11 (C3′/C5′ of CH_3OPh-C-*O*), 116.97 (C5), 126.32, 126.73, 127.37, 127.42, 127.75, 127.89, 128.34, 128.43 (Ph-C), 129.78 (C2′/C6′ of CH_3OPh-C-*N*), 129.87 (C2′/C6′ of CH_3OPh-C-*O*),

135.41 (C1′ of CH_3OPh-C-*O*), 136.87 (C1′ of CH_3OPh-C-*N*), 137.41 (C8), 144.58 (C1″ of Ph-C-*O*), 144.74 and 144.91 (C1″ of Ph-C-*N*), 149.46 (C4), 150.36 (C2), 156.56 (C6), 157.62 (C4′ of CH_3OPh-C-*N*), 158.10 (C4′ of CH_3OPh-C-*O*); HRMS Calcd for $C_{50}H_{48}N_5O_5$ (M+H): 798.3655; Found: 798.3670.

*4.2.2. N^2-(p-Anisyldiphenylmethyl)-9-[(1-(p-anisyldiphenylmethoxy)-3-hydroxy-2-propoxy)methyl]guanine (**8**)*

Ganciclovir (**2**) **(**510 mg, 2 mmol) was dissolved in DMSO (3 mL) and triethylamine (1.67 mL, 12 mmol) was added to it followed by monomethoxytrityl chloride (1.85 g, 6 mmol). After stirring the reaction mixture at room temperature overnight brine (40 mL) was added to it. A solid that precipitated was collected on Celite 521 (Aldrich) by filtration. The filter cake was washed with water (30 mL) and stirred with CH_2Cl_2 (50 mL). The CH_2Cl_2 solution was filtered, dried over anhydrous Na_2SO_4 and evaporated at reduced pressure to give the crude product. The crude product was purified by flash chromatography on silica gel (80 g), eluted with CH_2Cl_2/MeOH gradient (0-5% MeOH), to give the product **8** as a beige solid (950 mg, 59.4% yield). ^{1}H NMR (600 MHz, DMSO-d_6): δ 2.59 (d, *J* = 9.1 Hz, 1H, CH_2OMMTr), 2.93 (dd, *J* = 9.1 and 6.6 Hz, 1H, CH_2OMMTr), 2.91 and 3.06 (m, 2H, CH_2OH), 3.41 (m, 1H, CH), 3.62 (s, 3H, OCH_3 of *N*-MMTr), 3.76 (s, 3H, OCH_3 of *O*-MMTr), 4.49 (t, *J* = 5.3 Hz, 1H, OH), 4.95 and 5.06 (d, *J* = 11.2 Hz, 2H, *N*CH_2O), 6.73 (d, *J* = 8.9 Hz, 2H, H-3′/H-5′ of CH_3OPh-C-*N*), 6.89 (d, *J* = 8.9 Hz, 2H, H-3′/H-5′ of CH_3OPh-C-*O*), 7.03-7.31 (m, 24H, Ar-H), 7.71 (s, 1H, N^2-H), 7.80 (s, 1H, H-8), 10.73 (s, 1H, N^1-H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 54.88 (OCH_3 of *N*-MMTr), 54.98 (OCH_3 of *O*-MMTr), 60.88 (CH_2OMMTr), 63.28 (CH_2OH), 69.65 (*t*C

of *N*-MMTr), 70.62 (*N*CH_2), 78.90 (CH), 85.04 (*t*C of *O*-MMTr), 112.79 (C3′/C5′ of CH_3OPh-C-*N*), 113.05 (C3′/C5′ of CH_3OPh-C-*O*), 116.68 (C5), 126.33, 126.61, 126.34, 127.47, 127.50, 127.65, 127.67, 127.87, 127.97, 128.39, 128.44 (Ph-C), 130.21 (C2′/C6′ of CH_3OPh-C-*N* and CH_3OPh-C-*O*), 135.20 (C1′ of CH_3OPh-C-*O*), 136.68 (C1′ of CH_3OPh-C-*N*), 137.88 (C8), 144.16 and 144.41 (C1″ of Ph-C-*O*), 144.78 and 144.83 (C1″ of Ph-C-*N*), 150.97 (C2), 156.70 (C6), 157.62 (C4′ of CH_3OPh-C-*N*), 158.08 (C4′ of CH_3OPh-C-*O*); HRMS Calcd for $C_{49}H_{46}N_5O_6$: 799.3370; Found: 800.3448 (M+H).

4.2.3. N^2-(p-Anisyldiphenylmethyl)-9-[3-(p-anisyldiphenylmethoxymethyl)-4-tosyloxybutyl]guanine ***(9)***

To a solution of the penciclovir derivative **7** (254 mg, 0.32 mmol) in pyridine (4 mL) at 0^o C, under argon, was added *p*-toluenesulfonyl chloride (182 mg, 0.96 mmol). The solution was stirred at 0^o C for 30 min and then at room temperature overnight. The dark yellow reaction mixture was quenched with ice-water and the solution was evaporated in a rotary evaporator. The residual pyridine from the reaction mixture was removed by co-evaporation with toluene under reduced pressure. The oily residue of the crude product was purified by flash chromatography on silica gel (40 g) with CH_2Cl_2/MeOH gradient (0-4% MeOH) eluent to give the product **9** (167 mg, 55.1% yield) as a white solid. ^{1}H NMR (600 MHz, DMSO-d_6): δ 1.15 (m, 2H, CH_2), 1.46 (m, 1H, CH), 2.38 (s, 3H, Ar-CH_3 of Ts), 2.64 (dd, *J* = 9.6 and 7.7 Hz, 1H, CH_2OMMTr), 2.74 (dd, *J* = 9.6 and 4.5 Hz, 1H, CH_2OMMTr), 3.29 (t, *J* = 7.1 Hz, 2H, *N*CH_2), 3.64 (s, 3H, OCH_3 of *N*-MMTr), 3.74 (s, 3H, OCH_3 of *O*-MMTr), 3.73 (m, 1H, CH_2OTs), 3.84 (dd, *J* = 9.7 and 5.2 Hz, 1H, CH_2OTs), 6.75 (d, *J* = 8.7 Hz, 2H, H-3′/H-5′ of CH_3OPh-C-*N*), 6.88 (d, *J* = 8.8 Hz, 2H, H-3′/H-5′ of CH_3OPh-C-

O), 7.02-7.38 (m, 24H, Ar-H), 7.45 (d, *J* = 8.4 Hz, 2H, H-3/H-5 of Ts), 7.72 (d, *J* = 8.4 Hz, 2H, H-2/H-6 of Ts), 7.44 (s, 1H, H-8), 7.60 (s, 1H, N^2-H), 10.45 (s, 1H, N^1-H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 21.11 (CH_3 of Ts), 27.15 (CH_2), 36.07 (CH), 39.92 (NCH_2), 54.92 (OCH_3 of *N*-MMTr), 55.04 (OCH_3 of *O*-MMTr), 61.59 (CH_2OMMTr), 69.56 (*N*-*t*C), 70.13 (CH_2OTs), 85.76 (*O*-*t*C), 112.78 (C3′/C5′ of CH_3OPh-C-*N*), 113.16 (C3′/C5′ of CH_3OPh-C-*O*), 117.02 (C5), 126.34, 126.87, 127.43 (Ph-C), 127.54 (C2/C6 of Ts), 127.78, 127.81, 128.37, 128.40 (Ph-C), 129.81 (C2′/C6′ of CH_3OPh-C-*O* and CH_3OPh-C-*N*), 130.14 (C3/C5 of Ts), 132.03 (C1 of Ts), 134.93 (C1′ of CH_3OPh-C-*O*), 136.93 (C1′ of CH_3OPh-C-*N*), 137.32 (C8), 144.08 and 144.23 (C1″ of Ph-C-*O*), 144.76 and 144.78 (C1″ of Ph-C-*N*), 144.98 (C4 of Ts), 149.41 (C4), 150.45 (C2), 156.57 (C6), 157.66 (C4′ of CH_3OPh-C-*N*), 158.21 (C4′ of CH_3OPh-C-*O*); HRMS: Calcd for $C_{57}H_{54}N_5O_7S$ (M+H): 952.3744; Found: 952.3532.

*4.2.4. N^2-(p-Anisyldiphenylmethyl)-9-[(1-(p-anisyldiphenylmethoxy)-3-tosyloxy-2-propoxy)methyl]guanine (**10**)*

The ganciclovir analog **6** (465 mg, 0.58 mmol) was dissolved in pyridine (20 mL) under argon and cooled to 0° C (ice bath) and *p*-toluenesulfonyl chloride (442 mg, 2.33 mmol) was added to it. After stirring the reaction mixture at 0° C for 30 min followed by at room temperature overnight, ice-water was added to quench the reaction. The solution was evaporated under reduced pressure and the residue was azeotroped with toluene in a rotary evaporator. The oily residue that resulted was purified by flash chromatography on silica gel (80 g) with CH_2Cl_2/MeOH gradient (0-4% MeOH) elution to provide the product **10** (410 mg, 74% yield) as a white foam. ^{1}H NMR (600 MHz, DMSO-d_6): δ 2.43 (m, 4H, CH_3

of Ts and CH_2OMMTr), 2.62 (dd, *J* = 10.1 and 6.2 Hz, 1H, CH_2OMMTr), 3.39 (dd, *J* = 10.3 and 2.4 Hz, 1H, CH_2OTs), 3.44 (dd, *J* = 10.3 and 6.4 Hz, 1H, CH_2OTs), 3.55 (s, 3H, OCH_3 of *N*-MMTr), 3.58 (m, 1H, CH), 3.75 (s, 3H, OCH_3 of *O*-MMTr), 4.80 and 5.02 (q, *J* = 11.6 Hz, 2H, *N*CH_2), 6.68 (d, *J* = 8.9 Hz, 2H, H-3′/H-5′ of CH_3OPh-C-*N*), 6.88 (d, *J* = 8.9 Hz, 2H, H-3′/H-5′ of CH_3OPh-C-*O*), 6.93-7.30 (m, 24H, Ar-H), 7.48 (d, *J* = 8.3 Hz, 2H, H-3/H-5 of Ts), 7.63 (d, *J* = 8.3 Hz, 2H, H-2/H-6 of Ts), 7.71 (s, 1H, N^2-H), 7.78 (s, 1H, H-8), 10.67 (s, 1H, N^1-H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 21.07 (CH_3 of Ts), 54.85 (OCH_3 of *N*-MMTr), 55.01 (OCH_3 of *O*-MMTr), 62.17 (CH_2OMMTr), 69.31 (CH_2OTs), 69.48 (*N*-*t*C), 70.38 (*N*CH_2), 74.95 (CH), 85.28 (*O*-*t*C), 112.77 (CH_3OPh-C-*N*), 113.13 (C3′/C5′ of CH_3OPh-C-*O*), 116.58 (C5), 127.73, 127.68, 127.44, 126.78, 126.75, 126.29, 126.25 (Ph-C), 127.75 (C2/C6 of Ts), 128.28, 128.24 (Ph-C), 129.70 (C3/C5 of Ts), 130.11 (C2′/C6′ of CH_3OPh-C-*O* and CH_3OPh-C-*N*), 131.66 (C1 of Ts), 134.59 (C1′ of CH_3OPh-C-*O*), 136.50 (C1′ of CH_3OPh-C-*N*), 137.30 (C8), 143.67 and 143.85 (C1″ of Ph-C-*O*), 144.39 and 144.65 (C1″ of Ph-C-*N*), 145.05 (C4 of Ts), 149.56 (C4), 150.92 (C2), 156.49 (C6), 157.57 (C4′ of CH_3OPh-C-*N*), 158.16 (C4′ of CH_3OPh-C-*O*); HRMS Calcd for $C_{56}H_{52}N_5O_8S$ (M+H): 954.3536; Found: 954.3534.

*4.2.5. N^2-(p-Anisyldiphenylmethyl)-O^6-diphenylcarbamoyl-9-[3-(p-anisyldiphenylmethoxymethyl)-4-tosyloxybutyl]guanine (**11**)*

To a solution of the tosylate derivative **9** (45 mg, 0.047 mmol) and diphenylcarbamoyl chloride (22 mg, 0.095 mmol) in pyridine (0.25 mL) was added *N*,*N*-diisopropylethylamine (52 μL, 0.30 mmol). The reaction mixture was stirred at room temperature for 3 h giving a dark red solution. Deionized water (100 μL) was added to quench the reaction. After

stirring at room temperature for 10 min, the reaction mixture was evaporated and the residue co-evaporated with toluene under vacuum. The resultant crude product was purified by chromatography on silica gel (20 g), eluted with CH_2Cl_2/EtOAc (97:3 and 95:5). Pure carbamoyl product **11** was obtained as a beige foam (36 mg, 66% yield). ^{1}H NMR (600 MHz, DMSO-d_6): δ 1.19 (m, 2H, CH_2), 1.50 (m, 1H, CH), 2.36 (s, 3H, CH_3 of Ts), 2.65 and 2.76 (m, 2H, CH_2OMMTr), 3.49 (m, 2H, *N*CH_2), 3.61 (s, 3H, OCH_3 of *N*-MMTr), 3.71 (s, 3H, OCH_3 of *O*-MMTr), 3.76 and 3.89 (m, 2H, CH_2OTs), 6.67 (d, *J* = 8.8 Hz, 2H, H-3′/H-5′ of CH_3OPh-C-*N*), 6.86 (d, *J* = 8.8 Hz, 2H, H-3′/H-5′ of CH_3OPh-C-*O*), 6.95 (m, 2H, Ar-H), 7.04 (m, 4H, Ar-H), 7.10 (d, *J* = 8.8 Hz, 2H, H-2′/H-6′ of CH_3OPh-C-*O*), 7.18 (d, *J* = 8.8 Hz, 2H, H-2′/H-6′ of CH_3OPh-C-*N*), 7.19-7.30 (m, 17H, Ar-H), 7.37-7.42 (m, 7H, Ar-H), 7.43 (d, *J* = 8.3 Hz, 2H, H-2/H-6 of Ts), 7.72 (d, *J* = 8.2 Hz, 2H, H-3/H-5 of Ts), 7.80 (s, 1H, H-8), 7.94 (broad s, 1H, N^2-H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 21.09 (CH_3 of Ts), 27.17 (CH_2), 36.16 (CH), 41.03 (*N*CH_2), 54.82 (OCH_3 of *N*-MMTr), 55.01 (OCH_3 of *O*-MMTr), 61.66 (CH_2OMMTr), 69.40 (*N*-*t*C), 70.13 (CH_2OTs), 85.80 (*O*-*t*C), 112.50 (C3′/C5′ of CH_3OPh-C-*N*), 113.18 (C3′/C5′ of CH_3OPh-C-*O*), 116.79 (C5), 127.20, 127.18, 126.86, 125.87 (Ph-C), 127.56 (C2′/C6′ of Ts), 129.28, 128.47, 128.43, 127.83, 127.80, 127.77, 127.74 (Ph-C), 129.77 (C2′/C6′ of CH_3OPh-C-*O*), 129.98 (C2′/C6′ of CH_3OPh-C-*N*), 130.14 (C3/C5 of Ts), 131.99 (C1 of Ts), 134.95 (C1′ of CH_3OPh-C-*O*), 137.26 (C1′ of CH_3OPh-C-*N*), 141.71 (C1″ of Ph-NCO), 142.43 (C8), 144.00 and 144.23 (C1″ of Ph-C-*O*), 144.99 (C4 of Ts), 145.15 and 145.23 (C1″ of Ph-C-*N*), 150.32 (OCON), 154.21 (C4), 154.69 (C2), 156.96 (C6), 157.35 (C4′ of CH_3OPh-C-*N*), 158.18 (C4′ of CH_3OPh-C-*O*); HRMS Calcd for $C_{70}H_{62}N_6O_8S$: 1146.4350; Found: 1147.4317 (M+H).

*4.2.6. N^2-(p-Anisyldiphenylmethyl)-O^6-dimethylcarbamoyl-9-[3-(p-anisyldiphenylmethoxymethyl)-4-tosyloxybutyl]guanine (**12**)*

The penciclovir tosylate analog **9** (53 mg, 0.056 mmol) was dissolved in a mixture of *N*,*N*-diisopropylethylamine (52 μL, 0.30 mmol) and pyridine (0.25 mL). Dimethylcarbamoyl chloride (15 μL, 0.16 mmol) was then added to that solution and the reaction mixture was stirred at room temperature for 3 h to give a red-brown solution. The reaction was quenched by addition of deionized water (100 μL) and the solution was concentrated in a rotary evaporator. The last traces of pyridine were removed by azeotropic distillation with toluene under vacuum. The residual crude product was purified by flash chromatography on silica gel (20 g) with an eluent mixture of CH_2Cl_2/EtOAc (9:1 and 8:2). Pure dimethylcarbamoyal derivative **12** was obtained as a colorless film (32.5 mg, 57% yield). ^{1}H NMR (600 MHz, DMSO-d_6): δ 1.18 (m, 2H, CH_2), 1.51 (m, 1H, CH), 2.38 (s, 3 H, CH_3 of Ts), 2.65 and 2.77 (m, 2H, CH_2OMMTr), 2.90 (s, 3H, *N*CH_3), 3.01 (s, 3H, *N*CH_3), 3.48 (t, *J* = 7.1 Hz, 2H, *N*CH_2), 3.62 (s, 3H, OCH_3 of *N*-MMTr), 3.73 (s, 3H, OCH_3 of *O*-MMTr), 3.76 and 3.89 (m, 2H, CH_2OTs), 6.67 (d, *J* = 8.9 Hz, 2H, H-3′/H-5′ of CH_3OPh-C-*N*), 6.87 (d, *J* = 9.0 Hz, 2H, H-3′/H-5′ of CH_3OPh-C-*O*), 6.95 (m, 2H, Ph-C-*N*), 7.04 (t, *J* = 7.6 Hz, 4H, 2 X H-3′/H-5′ of Ph-C-*N*), 7.09 (d, *J* = 9.0 Hz, 2H, H-4′/H-6′ of CH_3OPh-C-*O*), 7.17 (d, *J* = 8.9 Hz, 2H, H-2′/H-6′ of CH_3OPh-C-*N*), 7.26-7.31 (m, 10H, Ph), 7.29 (m, 4H, 2 X H-3′/H-5′ of Ph-C-*O*), 7.45 (d, *J* = 8.3 Hz, 2H, H-3/H-5 of Ts), 7.72 (d, *J* = 8.3 Hz, 2H, H-2/H-6 of Ts), 7.73 (s, 1H, H-8), 7.83 (broad s, 1H, N^2-H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 21.07 (CH_3 of Ts), 27.25 (CH_2), 36.16 (CH), 36.24 and 36.34 (2 X *N*CH_3), 40.95 (*N*CH_2), 54.82 (OCH_3 of *N*-MMTr), 55.02 (OCH_3 of *O*-MMTr), 61.65

(CH_2OMMTr), 69.34 (*N*-*t*C), 70.16 (CH_2OTs), 85.79 (*O*-*t*C), 112.48 (C3′/C5′ of CH_3OPh-C-*N*), 113.17 (C3′/C5′ of CH_3OPh-C-*O*), 117.07 (C5), 128.45, 128.40, 127.16, 127.14, 126.85, 125.83 (Ph-C), 127.54 (C2/C6 of Ts), 127.81, 127.78, 127.75, 127.73 (Ph-C), 129.77 (C2′/C6′ of CH_3OPh-C-*O*), 129.94 (C2′/C6′ of CH_3OPh-C-*N*), 130.14 (C3/C5 of Ts), 132.02 (C1 of Ts), 134.91 (C1′ of CH_3OPh-C-*O*), 137.34 (C1′ of CH_3OPh-C-*N*), 141.94 (C8), 144.02 and 144.19 (C1″ of Ph-C-*O*), 144.97 (C4 of Ts), 145.19 and 145.26 (C1″ of Ph-C-*N*), 151.76 (OCON), 154.03 (C4), 155.36 (C2), 156.89 (C6), 157.31 (C4′ of CH_3OPh-C-*N*), 158.18 (C4′ of CH_3OPh-C-*O*); HRMS Calcd for $C_{60}H_{58}N_6O_8S$: 1022.4037; Found: 1023.4109 (M+H).

*4.2.7. N^2-(p-Anisyldiphenylmethyl)-O^6-tert-butyl-N^2-tert-butyloxycarbonyl-9-[3-(p-anisyldiphenylmethoxymethyl)-4-tosyloxybutyl]guanine (**18**)*

To a stirring solution of the tosylate derivative **9** (308 mg, 0.32 mmol) and di-*tert*-butyl dicarbonate (890 μL, 3.88 mmol) in CH_2Cl_2 (2.5 mL) under argon was added triethylamine (180 μL, 1.3 mmol) in one portion. A solution of 4-(*N*,*N*-dimethylamino)pyridine (DMAP) (150 mg, 1.23 mmol) in dichloromethane (1 mL) was then added dropwise. The color of the reaction mixture quickly changed from light yellow to brownish-red and then back to yellow with evolution of gas bubbles presumably of carbon dioxide. The yellow solution was heated to reflux at 45° C under argon for 2 h. The reaction mixture was cooled to room temperature and subjected to flash column chromatography on silica gel (40 g). The flash column was eluted with CH_2Cl_2/EtOAc/Et_3N (95:5:0.1) to provide pure Boc-protected precursor **18** as a colorless oil (170 mg, 47.4% yield). Analytical HPLC analysis of this oily product indicated it to be >99 % pure (Fig. 6).

^{1}H NMR (800 MHz, DMSO-d_6): δ 0.96 (s, 9H, O^6-tC$_4$H$_9$), 1.42 (m, 2H, CH$_2$), 1.66 (s, 9H, Boc-tC$_4$H$_9$), 1.78 (m, 1H, CH), 2.34 (s, 3H, CH$_3$ of Ts), 2.92 and 3.02 (m, 2H, CH$_2$OMMTr), 3.57 (s, 3H, OCH$_3$ of *N*-MMTr), 3.70 (s, 3H, OCH$_3$ of *O*-MMTr), 3.80 (m, 2H, *N*CH$_2$), 4.11 (dd, *J* = 9.8 and 4.4 Hz, 1H, CH$_2$OTs), 4.16 (dd, *J* = 9.8 and 5.2 Hz, 1H, CH$_2$OTs), 6.54 (broad s, 2H, H-3′/H-5′ of CH$_3$OPh-C-*N*), 6.82 (d, *J* = 9.0 Hz, 2H, H-3′/H-5′ of CH$_3$OPh-C-*O*), 6.98-7.34 (m, 24H, Ar-H), 7.39 (d, *J* = 8.1 Hz, 2 H, H-3/H-5 of Ts), 7.74 (d, *J* = 8.4 Hz, 2H, H-2/H-6 of Ts), 8.05 (s, 1H, H-8); ^{13}C NMR (200 MHz, DMSO-d_6): δ 21.05 (CH$_3$ of Ts), 27.41 (O^6-tC$_4$H$_9$), 27.85 (CH$_2$), 28.04 (Boc-tC$_4$H$_9$), 35.85 (CH), 40.76 (*N*CH$_2$), 54.86 (OCH$_3$ of *N*-MMTr), 54.99 (OCH$_3$ of *O*-MMTr), 61.23 (CH$_2$OMMTr), 69.86 (CH$_2$OTs), 76.23 (*N*-*t*C), 79.37 (O^6-*t*C), 82.26 (Boc-*t*C), 85.75 (*O*-*t*C), 111.96 (C3′/C5′ of CH$_3$OPh-C-*N*), 113.12 (C3′/C5′ of CH$_3$OPh-C-*O*), 119.56 (C5), 126.12, 126.13, 126.72, 126.73, 126.83 (Ph-C), 127.53 (C2/C6 of Ts), 127.81 (Ph-C), 129.56 (C2′/C6′ of CH$_3$OPh-C-*N*), 129.84 (C2′/C6′ of CH$_3$OPh-C-*O*), 130.10 (C3/C5 of Ts), 130.95 (Ph), 132.01 (C1 of Ts), 134.93 (C1′ of CH$_3$OPh-C-*O*), 135.87 (C1′ of CH$_3$OPh-C-*N*), 143.60 (C8), 143.95 and 144.04 (C1″ of Ph-C-*N*), 144.11 and 144.21 (C1″ of Ph-C-*O*), 144.93 (C4 of Ts), 151.92 (C4), 152.41 (C2), 153.33 (*N*CO$_2$), 157.32 (C4′ of CH$_3$OPh-C-*N*), 158.18 (C4′ of CH$_3$OPh-C-*O*), 159.45 (C6); HRMS Calcd for C$_{66}$H$_{70}$N$_5$O$_9$S (M+H): 1108.4888; Found: 1108.4883.

*4.2.8. N^2-(p-Anisyldiphenylmethyl)-O^6-tert-butyl-N^2-tert-butyloxycarbonyl-9-[(1-(p-anisyldiphenylmethoxy)-3-tosyloxy-2-propoxy)methyl]guanine (**19**)*

The ganciclovir tosylate analog **10** (212 mg, 0.22 mmol) and Boc anhydride (625 μL, 2.67 mmol) were dissolved in CH_2Cl_2 (2.6 mL) and kept stirring under argon at room temperature. Triethylamine (123 μL, 0.89 mmol) was added to the solution in one lot followed by a dropwise addition of a solution of 4-(*N*,*N*-dimethylamino)pyridine (102 mg, 0.83 mmol) in CH_2Cl_2 (1 mL). A vigorous gas evolution of seemingly carbon dioxide ensued with the color of the reaction mixture changing from light yellow to brownish-orange and then back to yellow. The yellow reaction mixture was refluxed for 2 h to complete the reaction. The dichloromethane reaction mixture was and then applied onto a silica gel (40 g) column for flash chromatography purification. The column was eluted with CH_2Cl_2/EtOAc/Et_3N (95:5:0.1) to obtain the pure Boc-protected precursor **19** as a white foam (185 mg, 75% yield). ^{1}H NMR (600 MHz, DMSO-d_6): δ 0.93 (s, 9H, O^6-*t*C_4H_9), 1.67 (s, 9H, Boc-*t*C_4H_9), 2.37 (s, 3H, CH_3-of Ts), 2.94 (m, 2H, CH_2OMMTr), 3.37 (s, 3H, OCH_3 of *N*-MMTr), 3.38 (s, 3H, OCH_3 of *O*-MMTr), 3.78 (m, 1H, CH), 4.00 and 4.03 (q, *J* = 7.2 Hz, 1H, CH_2OTs), 5.49 and 5.55 (q, *J* = 11.4 Hz, 2H, *N*CH_2), 6.61 (broad s, 2H, H-3′/H-5′ of CH_3OPh-C-*N*), 6.65 (d, *J* = 8.8 Hz, 2H, H-3′/H-5′ of CH_3OPh-C-*O*), 7.08-7.34 (m, 24H, Ar-H), 7.39 (d, *J* = 8.1 Hz, 2H, H-3/H-5 of Ts), 7.68 (d, *J* = 8.1 Hz, 2H, H-2/H-6 of Ts), 8.37 (s, 1H, H-8); ^{13}C NMR (150 MHz, DMSO-d_6): δ 21.08 (CH_3 of Ts), 27.34 (*O*^{6}-*t*C_4H_9), 28.03 (Boc-*t*C_4H_9), 54.86 (OCH_3 of *N*-MMTr), 54.98 (OCH_3 of *O*-MMTr), 61.68 (CH_2OMMTr), 68.65 (CH_2OTs), 70.75 (*N*CH_2O), 74.69 (CH), 76.27 (*N*-*t*C), 79.46 (*O*^{6}-*t*C), 82.58 (Boc-*t*C), 85.75 (*O*-*t*C), 112.04 (C3′/C5′ of CH_3OPh-C-*N*), 113.13 (C3′/C5′ of CH_3OPh-C-*O*), 119.39 (C5), 126.18, 126.76, 126.85 (Ph-C), 127.54 (C2/C6 of Ts), 127.79 (Ph-C), 129.59 (C2′/C6′ of CH_3OPh-C-*N*), 129.88 (C2′/C6′ of CH_3OPh-C-*O*), 130.05 (C3/C5 of Ts), 130.98 (Ph-C), 131.73 (C1 of Ts), 134.49 (C1′ of CH_3OPh-C-*O*), 135.77

(C1′ of CH_3OPh-C-*N*), 143.75 and 143.81 (C1″ of Ph-C-*O*), 143.92 and 144.02 (C1″ of Ph-C-*N*), 144.02 (C8), 145.03 (C4 of Ts), 152.29 (C4), 153.22 (C2), 153.35 (*N*CO_2), 157.41 (C4′ of CH_3OPh-C-*N*), 158.22 (C4′ of CH_3OPh-C-*O*), 159.72 (C6); HRMS Calcd for $C_{65}H_{68}N_5O_{10}S$ (M+H): 1110.4686; Found: 1110.4671.

*4.2.9. N^2-(p-Anisyldiphenylmethyl)-9-[(1,3-bis-(p-anisyldiphenylmethoxy)-2-propoxy)methyl]guanine (**20**)*

Ganciclovir (**2**) (255 mg, 1.0 mmol) was dissolved in DMSO (3 mL) and triethylamine (1.67 mL, 12 mmol) and *p*-anisyldiphenylmethyl chloride (1.85g, 6.0 mmol) were added to it. The dark grey reaction mixture was stirred at room temperature for 12 h and then added with deionized water (20 mL). The solid that separated was collected on Celite 521 by filtration and washed with water (30 mL). The solid material was stirred with CH_2Cl_2 (100 mL) for 30 min and then filtered. The CH_2Cl_2 filtrate was dried over anhydrous Na_2SO_4 and evaporated to give the crude product as a yellow foam. The crude product was purified by flash chromatography on silica gel (40 g) and eluted with CH_2Cl_2/MeOH gradient (0-5% MeOH). The monomethoxytrityl protected product **20** obtained from the column was recrystallized from CH_2Cl_2 to provide a white solid (766 mg, 71.5% yield). ^{1}H NMR (500 MHz, DMSO-*d*6): δ 2.67 (m, 4H, 2 X CH_2OMMTr), 3.48 (s, 3H, OCH_3), 3.64 (m, 1H, CH), 3.75 (s, 6H, 2 X OCH_3), 4.94 (s, 2H, *N*CH_2O), 6.60 -7.30 (m, 43H, Ar-H and N^2H), 7.69 (s, 1H, H-8), 7.87 (s, 1H, N^1-H); ^{13}C NMR (125 MHz, DMSO-*d*6): δ 55.2 (OCH_3 of *N*-MMTr), 55.4 (OCH_3 of *O*-MMTr), 63.3 (CH_2OMMTr), 69.9 (*t*C of *N*-MMTr), 70.6 (*N*CH_2), 77.6 (CH), 85.5 (*t*C of *O*-MMTr), 113.1 (C3′/C5′ of CH_3OPh-C-*N*), 113.5 (2 X C3′/C5′ of CH_3OPh-C-*O*), 117.0 (C5), 126.6, 127.1, 127.8, 128.1, 128.2, 128.2, 128.6

(Ph-C), 130.1 (C2′/C6′ of CH_3OPh-C-*N*), 130.2 (C2′/C6′ of CH_3OPh-C-*O*), 135.3 (C1′ of CH_3OPh-C-*O*), 137.1 (C1′ of CH_3OPh-C-*N*), 138.2 (C8), 144.4 and 144.6 (C1″ of Ph-C-*O*) , 145.0 (C1″ of Ph-C-*N*), 150.1 (C4), 151.4 (C2), 157.0 (C6), 158.0 (C4′ of CH_3OPh-C-*N*), 158.5 (C4′ of CH_3OPh-C-*O*); HRMS Calcd for $C_{69}H_{62}N_5O_7$ (M+H): 1072.4636; Found: 1072.4649.

*4.2.10. N^2-(p-Anisyldiphenylmethyl)-O^6-tert-butyl-N^2tert-butyloxycarbonyl-9-[(1,3-bis-(p-anisyldiphenylmethoxy)-2-propoxy)methyl]guanine (**21**)*

To a solution of the monomethoxytrityl protected ganciclovir derivative **20** (214 mg, 0.2 mmol), Boc anhydride (550 μL, 2.67 mmol) and triethylamine (111 μL, 0.8 mmol) in CH_2Cl_2 (2 mL) was added dropwise a solution of 4-(*N*,*N*-dimethylamino)pyridine (92.7 mg, 0.78 mmol) in dichloromethane (0.5 mL) at room temperature. The bubbling beige reaction mixture was refluxed at 50° C for 2 h. The reaction mixture turned into a yellow solution after ~10 min of reflux. The reaction solution was cooled to room temperature and applied onto a silica gel (20 g) column for flash chromatography purification. The column was eluted with CH_2Cl_2/EtOAc gradient (0-5% EtOAc) and the pure Boc-protected product **21** was obtained as a colorless oil (130 mg, 52% yield). ^{1}H NMR (500 MHz, DMSO-d_6): δ 0.90 (s, 9H, *t*C_4H_9), 1.66 (s, 9H, *t*C_4H_9), 2.99, (m. 4H, 2 X CH_2OMMTr), 3.48 (s, 3H, OCH_3), 3.71 (s, 6H, 2 X OCH_3), 3.84 (m, 1H, CH), 5.59 (s, 2H, *N*CH_2O), 6.51 − 7.31 (m, 42H, Ar-H), 8.42 (s, 1H, H-8). ^{13}C NMR (125 MHz, DMSO-d_6,): δ 27.4 (O^6-*t*C_4H_9), 28.0 (Boc-*t*C_4H_9), 54.8 (OCH_3 of *N*-MMTr), 55.0 (OCH_3 of *O*-MMTr), 62.5 (CH_2OMMTr), 71.0 (*N*CH_2O), 76.3 (*N*-*t*C), 76.6 (CH), 79.3 (O^6-*t*C), 82.5 (Boc-*t*C), 85.5 (*O*-*t*C), 112.0 (C3′/C5′ of CH_3OPh-C-*N*), 113.1 (C3′/C5′ of CH_3OPh-C-*O*), 119.3 (C5), 126.1, 126.7, 126.8, 127.8, 127.9, 128.0 (Ph-C), 129.6 (C2′/C6′ of CH_3OPh-C-*N*), 129.9 (C2′/C6′ of

CH_3OPh-C-*O*), 130.9 (Ph), 134.7 (C1′ of CH_3OPh-C-*O*), 135.7 (C1′ of CH_3OPh-C-*N*), 143.98 − 144.04 (C1″ of Ph-C-*N*, C8, and C1″ of Ph-C-*O*), 152.4 (C4), 153.2 (C2), 153.3 (OCO), 157.4 (C4′ of CH_3OPh-C-*N*), 158.1 (C4′ of CH_3OPh-C-*O*), 159.7 (C6); HRMS Calcd for $C_{78}H_{78}N_5O_9$: 1227.5800; Found: 1228.5775 (M+H).

4.3. Fluorination – General procedure

Potassium fluoride (20 equiv.) and Kryptofix 222 (5 equiv.) were dissolved in water (0.1 mL) and CH_3CN (1 mL) in a 10 mL pear-shape flask. The solution was evaporated at ~40^o C under reduced pressure until a white solid was formed. The solid residue was further azeotropically dried with anhydrous CH_3CN (3 X 0.5 mL) to provide anhydrous KF/Kryptofix 222 complex. The tosylate precursor dissolved in anhydrous DMSO, as indicated below, was added to the fluoride ion complex and the reaction vessel was sealed with a glass stopper. The reaction mixture was heated at 145^o C for 30 min in an oil bath. After cooling the reaction mixture to room temperature, ice water (12 mL) was added to it. The precipitate that formed was collected on a pad of Celite 521 by filtration and washed with water (3 X 10 mL). The solid material was then stirred with CH_2Cl_2 (20 mL) for 30 min and filtered. The CH_2Cl_2 solution was dried over anhydrous Na_2SO_4 and filtered. Evaporation of the filtrate provided the crude fluorination reaction product mixture which was purified. Details on the fluorination reactions conducted are provided below.

*4.3.1. N^2-(p-Anisyldiphenylmethyl)-9-[3-(p-anisyldiphenylmethoxymethyl)-4-fluorobutyl]guanine (**13**), N^2-(p-Anisyldiphenylmethyl)-9-[3-(p-anisyldiphenylmethoxymethyl) but-3-ene]guanine (**14**) and 5-(p-*

*anisyldiphenylmethyl)amino-7-p-anisyldiphenylmethoxy-6,7,8,9-tetrahydro-2,4,5a,9a-tetraaza-benzo[cd]-azulen-3-one (**15**) (Scheme 3)*

Diphenylcarbamoyal penciclovir tosylate precursor **11** (28 mg, 0.024 mmol) in DMSO (0.7 mL); purified by flash column chromatography on silica gel (20 g) and eluted with a gradient constituting CH_2Cl_2/MeOH (98:2 to 95:5) to give two distinct fractions – an inseparable mixture of products of **13** and **14** (8.2 mg, 42% yield) and pure product **15** (4.2 mg, 22% yield) (Scheme 3). Product **13**: ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.25 (q, J = 7.3 Hz, 2H, CH_2), 1.57 (d, $^2J_{H,F}$ = 24.6 Hz, 1H, CH), 2.80 and 2.78 (2 X m, 2H, CH_2OMMTr), 3.42 (m, 2H, *N*CH_2), 3.66 (s, 3H, OCH_3 of *N*-MMTr), 3.75 (s, 3H, OCH_3 of *O*-MMTr), 4.21 (d, $^1J_{H,F}$ = 47.5 Hz, 2H, CH_2F), 6.77 (d, J = 8.8 Hz, 2H, H-3′/H-5′ of CH_3OPh-C-*N*), 6.91 (d, J = 8.8 Hz, 2H, H-3′/H-5′ of CH_3OPh-C-*O*), 7.05-7.35 (m, 24H, Ar-H), 7.47 (s, 1H, H-8), 7.61 (s, 1H, N^2-H), 10.52 (s, 1H, N^1-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 27.87 ($^3J_{C,F}$ = 4.8 Hz, CH_2); 37.70 ($^2J_{C,F}$ = 17.4 Hz, CH), 41.28 (*N*CH_2), 55.37 (OCH_3 of *N*-MMTr), 55.50 (OCH_3 of *O*-MMTr), 62.33 ($^3J_{C,F}$ = 6.6 Hz, CH_2OMMTr), 70.03 (*N*-*t*C), 83.77 ($^1J_{C,F}$ = 166.5 Hz, CH_2F), 86.06 (*O*-*t*C), 113.25 (C3′/C5′ of CH_3OPh-C-*N*), 113.67 (C3′/C5′ of CH_3OPh-C-*O*), 117.44 (C5), 126.86, 127.36, 127.93, 127.97, 128.32, 128.38, 128.84, 128.92 (Ph-C), 130.28 (C2′/C6′ of CH_3OPh-C-*N*), 130.36 (C2′/C6′ of CH_3OPh-C-*O*), 135.51 (C1′ of CH_3OPh-C-*O*), 136.12 (C1′ of CH_3OPh-C-*N*), 137.90 (C8), 144.83 (C1″ of Ph-C-*O*), 144.75 (C1″ of Ph-C-*N*), 149.95 (C4), 150.93 (C2), 157.02 (C6), 158.13 (C4′ of CH_3OPh-C-*N*), 158.66 (C4′ of CH_3OPh-C-*O*); ^{19}F NMR (282 MHz, DMSO-d_6): δ −226.4; MS Calcd for $C_{50}H_{46}FN_5O_4$: 799.35; Found: 822.33 (M+Na).

Product **14**: ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.84 (q, J = 7.3 Hz, 2H, CH_2), 3,21 (m, 2H, CH_2OMMTr), 3.48 (m, 2H, *N*CH_2), 3.66 (s, 3H, OCH_3 of *N*-MMTr), 3.75 (s, 3H,

OCH_3 of *O*-MMTr), 4.64 (m, 1H, vinyl-CH_2), 5.24 (m, 1H, vinyl-CH_2), 6.77 (d, *J* = 8.8 Hz, 2H, H-3′/H-5′ of CH_3OPh-C-*N*), 6.91 (d, *J* = 8.8 Hz, 2H, H-3′/H-5′ of CH_3OPh-C-*O*), 7.05-7.35 (m, 24H, Ar-H), 7.34 (s, 1H, H-8), 7.61 (s, 1H, N^2-H), 10.52 (s, 1H, N^1-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 32.45 (CH_2), 42. 44 (*N*CH_2), 55.37 (OCH_3 of *N*-MMTr), 55.41 (OCH_3 of *O*-MMTr), 65.62 (CH_2OMMTr), 66.56 (*N*-*t*C), 86.33 (*O*-*t*C), 111.17 (vinyl-CH_2), 113.25 (C3′/C5′ of CH_3OPh-C-*N*), 113.73 (C3′/C5′ of CH_3OPh-C-*O*), 117.47 (C5), 127.01, 126.37, 127.20, 127.68, 128.64, 128.44, 128.31, 129.05 (Ph-C), 130.43 (C2′/C6′ of CH_3OPh-C-*N*), 129.97 (C2′/C6′ of CH_3OPh-C-*O*), 135.56 (C1′ of CH_3OPh-C-*O*), 137.35 (C8), 137.90 (C1′ of CH_3OPh-C-*N*), 143.24 (vinyl-C), 144.73 (C1″ of Ph-C-*O*), 144.91 (C1″ of Ph-C-*N*), 149.88 (C4), 150.93 (C2), 157.75 (C6), 158.24 (C4′ of CH_3OPh-C-*N*), 158.46 (C4′ of CH_3OPh-C-*O*); MS Calcd for $C_{50}H_{45}N_5O_4$: 779.35; Found: 802.33 (M+Na).

Product **15**: ^{1}H NMR (600 MHz, DMSO-d_6): δ 1.61 and 2.38 (2 X m 2H, CH_2), 2.55 (m, 1H, CH), 7.49 (s, 1H, H-8), 3.05 (t, *J* = 9.2 Hz, 1H, CH_2OMMTr), 3.26 (dd, *J* = 9.2 and 6.4 Hz, 1H, CH_2OMMTr), 3.64 (s, 3H, OCH_3 of *N*-MMTr), 3.69 (s, 3H, OCH_3 of *O*-MMTr), 4.30 (m, 2H, N^9-CH_2), 7.33 (m, 6H, Ar-H), 4.52 (dd, *J* = 14.7 and 5.1 Hz, 1H, N^3-CH_2), 4.66 (dd, *J* = 14.7 and 3.2 Hz, 1H, N^3-CH_2), 6.50 (s, 1H, N^1H), 6.67 (d, *J* = 8.7 Hz, 2H, H-3′/H-5′ of CH_3OPh-C-*N*), 6.74 (d, *J* = 9.0 Hz, 2H, H-3′/H-5′ of CH_3OPh-C-*O*), 7.09 (d, *J* = 9.0 Hz, 2H, H-2′/H-6′ of CH_3OPh-C-*O*), 7.11-7.22 (m, 16H, Ar-H), 7.33 (m, 6H, Ar-H), 7.49 (s, 1H, H-8); ^{13}C NMR (75 MHz, DMSO-d_6): δ 29.74 (CH_2), 37.46 (CH), 43.62 (N^9-CH_2), 44.78 (N^3-CH_2), 55.39 (OCH_3 of *O*-MMTr), 55.46 (OCH_3 of *N*-MMTr), 66.50 (CH_2OMMTr), 70.12 (*N*-*t*C), 86.22 (*O*-*t*C), 113.52 (C3′/C5′ of CH_3OPh-C-*N*), 113.88 (C3′/C5′ of CH_3OPh-C-*O*), 114.05 (C5), 126.98, 127.18, 128.28, 128.36, 128.41,

128.43, 128.62, 128.65 (Ph-C), 129.97 (C4′/C6′ of CH_3OPh-C-*O*), 130.42 (C4′/C6′ of CH_3OPh-C-*N*), 135.49 (C1′ of CH_3OPh-C-*O*), 137.71 (C8), 138.10 (C1′ of CH_3OPh-C-*N*), 141.27 (C2), 144.42 (C4), 144.75 and 144.92 (C1″ of Ph-C-*O*), 146.97 and 147.39 (C1″ of Ph-C-*N*), 155.41 (C6), 158.22 (C4′ of CH_3OPh-C-*O*), 158.46 (C4′ of CH_3OPh-C-*N*); MS Calcd for $C_{50}H_{45}N_5O_4$:779.35; Found: 802.33 (M+Na).

*4.3.2. N^2-(p-Anisyldiphenylmethyl)-9-[3-(p-anisyldiphenylmethoxymethyl)-4-fluorobutyl]guanine (**13**) and N^2-(p-Anisyldiphenylmethyl)-9-[3-(p-anisyldiphenylmethoxymethyl) but-3-ene]guanine (**14**) (Scheme 4)*

Dimethylcarbamoyl tosylate precursor **12** (25.8 mg, 0.025 mmol) in DMSO (0.7 mL); purified by flash column chromatography on silica gel (20 g) and eluted with a gradient consisting of CH_2Cl_2/MeOH (98:2 to 95:5) to give an inseparable mixture of products **13** and **14** (12.3 mg, 61.2% yield) (Scheme 4). The NMR and mass spectral data for this mixture were identical to the information provided above.

*4.3.3. N^2-(p-Anisyldiphenylmethyl)-O^6-tert-butyl-N^2-tert-butyloxycarbonyl-9-[3-(p-anisyldiphenylmethoxymethyl)-4-fluorobutyl] guanine (**23**) and N^2-(p-Anisyldiphenylmethyl)-O^6-tert-butyl-N^2-tert-butyloxycarbonyl-9-[3-(p-anisyldiphenylmethoxymethyl) but-3-ene]guanine (**24**)(Scheme 8)*

Boc-protected penciclovir tosylate derivative **18** (40 mg, 0.035 mmol) in 1 mL of DMSO; purified by semi-preparative HPLC (column: Alltech Altima Silica, 10μ, 10 X 250 mm; eluent: CH_2Cl_2/CH_3CN 9:1; flow rate: 3 mL/min; detector: 254 nm UV). Pure fluorinated product **23** and elimination product **24** (Scheme 8) were isolated by this HPLC

method. Fluorinated product **23** (18.7 mg, 54.2% yield): R_t = 13.4 min; ^{1}H NMR (600 MHz, DMSO-d_6): δ 0.96 (s, 9H, O^6-tC$_4$H$_9$), 1.53 (m, 2H, CH$_2$), 1.66 (s, 9H, Boc-tC$_4$H$_9$), 1.84 (m, $^3J_{H,F}$ = 25.2 Hz, 1H, CH), 3.02 and 3.06 (m, 2H, CH$_2$OMMTr), 3.56 (s, 3H, OCH$_3$ of *N*-MMTr), 3.68 (s, 3H, OCH$_3$ of *O*-MMTr), 3.99 (m, 2H, *N*CH$_2$), 4.55 (dd, $^1J_{H,F}$ = 47.4 Hz, *J* = 4.6 Hz, 2H, CH$_2$F), 6.53 (broad s, 2H, H-3′/H-5′ of CH$_3$OPh-C-*N*), 6.85 (d, *J* = 8.9 Hz, 2H, H-3′/H-5′ of CH$_3$OPh-C-*O*), 7.04-7.40 (m, 24H, Ar-H), 8.14 (s, 1H, H-8); ^{13}C NMR (150 MHz, DMSO-d_6): δ 27.44 (O^6-tC$_4$H$_9$), 27.71 ($^3J_{C,F}$ = 4.3 Hz, CH$_2$), 28.08 (Boc-tC$_4$H$_9$), 37.16 ($^2J_{C,F}$ = 18.2 Hz, CH), 41.21 (*N*CH$_2$), 54.86 (OCH$_3$ of *N*-MMTr), 55.01 (OCH$_3$ of *O*-MMTr), 61.73 (CH$_2$OMMTr), 76.22 (*N*-*t*C), 79.40 (O^6-*t*C), 82.29 (Boc-*t*C), 83.33 ($^1J_{C,F}$ = 166.6 Hz, CH$_2$F), 85.73 (*O*-*t*C), 111.96 (C3′/C5′ of CH$_3$OPh-C-*N*), 113.21 (C3′/C5′ of CH$_3$OPh-C-*O*), 119.63 (C5), 126.18, 126.77, 126.88, 127.90 (Ph-C), 129.61 (C4′/C6′ of CH$_3$OPh-C-*N*), 129.93 (C4′/C6′ of CH$_3$OPh-C-*O*), 131.00 (Ph-C), 135.08 (C1′ of CH$_3$OPh-C-*O*), 135.92 (C1′ of CH$_3$OPh-C-*N*), 143.72 (C8), 143.97 and 144.01 (C1″ of Ph-C-*N*), 144.31 and 144.37 (C1″ of Ph-C-*O*), 152.00 (C4), 152.40 (C2), 153.35 (*N*CO$_2$), 157.31 (C4′ of CH$_3$OPh-C-*N*), 158.20 (C4′ of CH$_3$OPh-C-*O*), 159.48 (C6); ^{19}F NMR (376 MHz, DMSO-*d*6): δ −227.6; HRMS Calcd for C$_{59}$H$_{63}$FN$_5$O$_6$ (M+H): 956.4762; Found: 956.4759.

Elimination product **24** (11 mg, 29.6% yield): R_t = 11.6 min; ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.97 (s, 9H, O^6-tC$_4$H$_9$), 1.67 (s, 9H, Boc-tC$_4$H$_9$), 2.08 (t, *J* = 7.3 Hz, 2H, CH$_2$), 3.48 (broad s, 2H, CH$_2$OMMTr), 3.56 (s, 3H, OCH$_3$ of *N*-MMTr), 3.69 (s, 3H, OCH$_3$ of *O*-MMTr), 4.04 (t, *J* = 7.3 Hz, 2H, *N*CH$_2$), 4.18 (broad s, 1H, vinyl-H), 5.27 (broad s, 1H, vinyl-H), 6.52 (d, *J* = 8.0 Hz, 2H, H-3′/H-5′ of CH$_3$OPh-C-*N*), 6.86 (d, *J* = 8.7 Hz, 2H, H-3′/H-5′ of CH$_3$OPh-C-*O*), 6.99 − 7.11 (m, 8H, Ar-H), 7.22-7.43 (m, 16H, Ar-H), 8.05

(s, 1H, H-8); ^{13}C NMR (75 MHz, DMSO-d_6): δ 27.49 (O^6-tC$_4$H$_9$), 28.13 (Boc-tC$_4$H$_9$), 32.29 (CH$_2$), 41.97 (NCH$_2$), 54.91 (OCH$_3$ of *N*-MMTr), 55.01 (OCH$_3$ of *O*-MMTr), 65.39 (CH$_2$OMMTr), 76.29 (*N*-*t*C), 79.49 (O^6-*t*C), 82.35 (Boc-*t*C), 111.28 (vinyl-CH$_2$), 112.04 (C3′/C5′ of CH$_3$OPh-C-*N*), 113.32 (C3′/C5′ of CH$_3$OPh-C-*O*), 119.75 (C5), 126.29, 126.87, 127.03, 127.81, 128.03 (Ph-C), 129.60 (C4′/C6′ of CH$_3$OPh-C-*N*), 129.89 (C4′/C6′ of CH$_3$OPh-C-*O*), 130.99 (Ph-C), 135.22 (C1′ of CH$_3$OPh-C-*O*), 136.12 (C1′ of CH$_3$OPh-C-*N*), 142.46 (vinyl-C), 143.84 (C8), 144.07 and 144.49 (C1″ of Ph-C-*N*), 144.31 and 144.37 (C1″ of Ph-C-*O*), 152.00(C4), 152.40 (C2), 153.35 (NCO$_2$), 157.35 (C4′ of CH$_3$OPh-C-*N*), 158.29 (C4′ of CH$_3$OPh-C-*O*), 158.52 (C6); MS Calcd for C$_{59}$H$_{61}$N$_5$O$_6$: 935.47; Found: 958.20 (M+Na).

*4.3.4. N^2-(p-Anisyldiphenylmethyl)-O^6-tert-butyl-N^2-tert-butyloxycarbonyl-9-[(1-(p-anisyldiphenylmethoxy)-3-fluoro-2-propoxy)methyl]guanine (**27**) and N^2-(p-Anisyldiphenylmethyl)-O^6-tert-butyl-N^2-tert-butyloxycarbonyl-9-[(1-(p-anisyldiphenylmethoxy)-2-methylene-2-propoxy)methyl] guanine (**28**) (Scheme 10)*

Boc-protected ganciclovir tosylate precursor **19** (148 mg, 0.13 mmol) in DMSO (3 mL); purified on a silica gel (40 g) flash chromatography column and eluded with toluene/EtOAc (9:1 and 8:2) to give 105 mg of a mixture containing the fluorinated product **27** (75%) and the elimination side product **28** (25%) (Scheme 10). Fluorinated product **27**: ^{1}H NMR (600 MHz, DMSO-d_6): δ 0.94 (s, 9H, O^6-tC$_4$H$_9$), 1.66 (s, 9H, Boc-tC$_4$H$_9$), 3.01 (dq, J = 10.3 and 6.3 Hz, 1H, CH$_2$OMMTr), 3.05 (dq, J = 10.3 and 3.0 Hz, 1H, CH$_2$OMMTr), 3.56 (s, 3H, OCH$_3$ of *N*-MMTr), 3.71 (s, 3H, OCH$_3$ of *O*-MMTr), 3.81 (m, $^3J_{H,F}$ = 17.6 Hz, 1H, CH), 4.30 (ddq, $^1J_{H,F}$ = 47.7 Hz, J = 10.2 and 3.0 Hz, 1H, CH$_2$F), 4.42 (ddq, $^1J_{H,F}$ = 47.7 Hz, J =

10.2 and 6.2 Hz, 1H, CH_2F), 5.61 and 5.67 (q, *J* = 11.5 Hz, 2H, *N*CH_2), 6.56 (m, 2H, H-3′/H-5′ of CH_3OPh-C-*N*), 6.86 (d, *J* = 9.0 Hz, 2 H, H-3′/H-5′ of CH_3OPh-C-*O*), 7.04 -7.36 (m, 24H, Ar-H), 8.44 (s, 1H, H-8); ^{13}C NMR (150 MHz, DMSO-d_6): δ 27.35 (O^6-*t*C_4H_9), 27.99 (Boc-*t*C_4H_9), 71.05 (*N*CH_2), 54.82 (OCH_3 of *N*-MMTr), 54.96 (OCH_3 of *O*-MMTr), 61.79 ($^3J_{C,F}$ = 8.0 Hz, CH_2OMMTr), 76.19 ($^2J_{C,F}$ = 18.7 Hz, CH), 76.24 (*N*-*t*C), 79.36 (O^6-*t*C), 82.38 ($^1J_{C,F}$ = 169.5 Hz, CH_2F), 82.51 (Boc-*t*C), 85.69 (*O*-*t*C), 111.97 (CH_3OPh-C-*N*), 113.14 (C3′/C5′ of CH_3OPh-C-*O*), 119.37 (C5), 126.12, 126.73, 126.65, 127.75, 127.90, 129.61 (Ph-C), 129.93 (C4′/C6′ of CH_3OPh-C-*O* and CH_3OPh-C-*N*), 130.91 (Ph-C), 134.62 (C1′ of CH_3OPh-C-*O*), 135.79 (C1′ of CH_3OPh-C-*N*), 143.86 and 143.89 (C1″ of Ph-C-*O*), 143.44 and 143.96 (C1″ of Ph-C-*N*), 143.93 (C8), 152.33 (C4), 153.15 (C2), 153.30 (*N*CO_2), 157.32 (C4′ of CH_3OPh-C-*N*), 158.19 (C4′ of CH_3OPh-C-*O*), 159.64 (C6); ^{19}F NMR (282 MHz, DMSO-*d*6): δ −229.9; HRMS Calcd for $C_{58}H_{60}FN_5O_7Na$ (M+Na): 980.4374; Found: 980.4349.

Elimination product **28**: ^1H NMR (600 MHz, DMSO-d_6): δ 0.95 (s, 9H, O^6-*t*C_4H_9), 1.63 (s, 9H, Boc-*t*C_4H_9), 3.32 (m, 2H, CH_2OMMTr), 3.59 (s, 3H, OCH_3 of *N*-MMTr), 3.67 (s, 3H, OCH_3 of *O*-MMTr), 4.62 and 4.40 (d, *J* = 2.4 Hz, 2 X 1H, vinyl-CH_2), 5.81 (m, 2H, *N*CH_2), 6.63 (m, 2H, H-3′/H-5′ of CH_3OPh-C-*N*), 6.84 (d, *J* = 9.0 Hz, 2H, H-3′/H-5′ of CH_3OPh-C-*O*), 7.04-7.36 (m, 24H, Ar-H), 8.38 (s, 1H, H-8); ^{13}C NMR (150 MHz, DMSO-d_6): δ 27.35 (O^6-*t*C_4H_9), 27.95 (Boc-*t*C_4H_9), 54.85 (OCH_3 of *N*-MMTr), 54.91 (OCH_3 of *O*-MMTr), 62.90 (CH_2OMMTr), 71.05 (*N*CH_2), 76.24 (*N*-*t*C), 79.46 (O^6-*t*C), 82.54 (Boc-*t*C), 86.09 (*O*-*t*C), 111.97 (CH_3OPh-C-*N*), 113.14 (C3′/C5′ of CH_3OPh-C-*O*), 113.23 (vinyl-CH_2), 119.34 (C5), 126.12, 126.73, 126.90, 127.80, 127.85, 129.73, 129.65 (Ph-C), 130.91 (C4′/C6′ of CH_3OPh-C-*O* and CH_3OPh-C-*N*), 131.09 (Ph-C), 134.62 (C1′ of

CH_3OPh-C-*O*), 135.63 (C1′ of CH_3OPh-C-*N*), 143.44 and 143.96 (C1″ of Ph-C-*N*), 143.86 and 143.89 (C1″ of Ph-C-*O*), 143.93 (C8), 151.93 (C 4), 153.22 (C2), 153.30 (NCO_2), 156.60 (vinyl-C), 156.60 (C6), 157.39 (C4′ of CH_3OPh-C-*N*), 158.22 (C4′ of CH_3OPh-C-*O*); HRMS Calcd for $C_{58}H_{59}N_5O_7Na$ (M+Na): 960.4312; Found: 960.4405.

4.4. Acid hydrolysis and deprotection – General procedure

Two different conditions were employed for the acid hydrolysis of the protected derivatives. For a partial deprotection, the penciclovir as well as the ganciclovir substrates were dissolved in methanol (5 mL), treated with 1M HCl (1 mL) and heated at 115^o C for 3 min. The acidic mixtures were cooled to room temperature, neutralized with 1M NaOH to pH 7 and the solvents evaporated to dryness under reduced pressure. The crude products were then purified by flash chromatography on silica gel.

For full deprotection of penciclovir based derivatives, the protected compounds were dissolved in methanol (5 mL), treated with 1M HCl (1 mL) and heated at 115^o C for 1 h. The acidic mixtures were cooled to room temperature, neutralized with 1M NaOH to pH 7 and the solvents evaporated to dryness under reduced pressure. The residues were triturated with ether (3 mL) and centrifuged. After carefully pipetting off and discarding the ether layer, the solid residues were dissolved in hot deionized water (1 mL). The aqueous solutions were then purified by semi-preparative HPLC.

In the case of protected ganciclovir based products, the derivatives were dissolved in methanol (5 mL) and mixed with 4M HOAc (1 mL) and heated at 115^o C for 1 h for full deprotection. The solution was evaporated in a rotary evaporator and the last traces of

acetic acid were azeotropically distilled off with toluene. The residues were dissolved in hot water (1 mL) and purified by semi-preparative HPLC.

*4.4.1. N^2-tert-butyloxycarbonyl-9-[(1,3-di-hydroxy-2-propoxy)methyl]guanine (**22**) (Scheme 7)*

Fully protected ganciclovir derivative **21** (106 mg, 0.086 mmol) was partially deprotected and the product was purified on a silica gel (15 g) column with CH_2Cl_2/MeOH gradient (0-30% MeOH) to give the N^2-Boc protected analog **22** as a glassy solid (21.4 mg, 71% yield) (Scheme 7). ^{1}H NMR (600 MHz, DMSO-d_6): δ 1.48 (s, 9H, *t*C_4H_9), 2.28 and 3.42 (q, *J* = 5.2 and 5 Hz, 4H, 2 X CH_2OH), 3.84 (p, *J* = 5 Hz, 1H, CH), 5.52 (s, 2H, *N*CH_2O), 8.06 (s, 1H, H-8); ^{13}C NMR (150 MHz, DMSO-d_6,): δ 27.8 (*t*C_4H_9), 60.8 (2 X CH_2), 71.8 (*N*CH_2O), 80.3 (CH), 82.5 (Boc-*t*C), 119.4 (C5), 139.7 (C8), 147.9 (*N*COO), 149.3 (C4), 153.9 (C2), 155.2 (C6).

*4.4.2. N^2-tert-Butyloxycarbonyl-9-(4-fluoro-3-hydroxymethylbutyl)guanine (**25**) (Scheme 9)*

After partial deprotection of the fluoro penciclovir analog **23** (15.8 mg, 0.017 mmol), the crude product was purified on a silica gel (5 g) column with CH_2Cl_2/MeOH gradient (0-10% MeOH) to give the N^2-Boc protected product **25** (5.5 mg, 93.6% yield) (Scheme 9). ^{1}H NMR (500 MHz, DMF-d_7): δ 1.53 (s, 9H, *t*C_4H_9), 1.75 (m, $^3J_{H,F}$ = 23.7 Hz, 1H, CH), 1.91 (m, 2H, CH_2), 3.55 (t, *J* = 5.2 Hz, 2H, CH_2OH), 4.23 (t, *J* = 7.2 Hz, 2H, *N*CH_2), 4.50 and 4.57 (2 X dq, $^1J_{H,F}$ = 47.6 Hz, *J* = 9.1 and 5.1 Hz, 2H, CH_2F), 4.77 (t, *J* = 5.3 Hz, 1H, OH), 5.80 (s, 1H, N^2-H), 8.02 (s, 1H, H-8), 11.34 (broad s, 1H, N^1-H); ^{13}C NMR (125 MHz, DMF-d_7): δ 28.06 (*t*C_4H_9), 28.60 ($^3J_{C,F}$ = 4.9 Hz, CH_2), 40.11 ($^2J_{C,F}$ = 18.1 Hz, CH), 41.94

(*N*CH_2), 61.06 ($^3J_{C,F}$ = 6.0 Hz, CH_2OH), 83.43 (Boc-*t*C), 84.56 ($^1J_{C,F}$ = 164.4 Hz, CH_2F), 120.66 (C5), 139.95 (C8), 148.56 (*N*CO_2), 150.16 (C4), 155.12 (C2), 156.07 (C6); ^{19}F NMR (376 MHz, DMF-d_7): δ −229.1; HRMS Calcd for $C_{15}H_{23}FN_5O_4$ (M+H): 355.1734; Found: 355.1731.

*4.4.3. N^2-tert-butyloxycarbonyl-9-[(3-fluoro-1-hydroxy-2-propoxy)methyl]guanine (**29**) (Scheme 11)*

The inseparable product mixture of **27** and **28** (Scheme 10) (58 mg) was partially deprotected and the crude product was purified on a silica gel (20 g) column with CH_2Cl_2/MeOH gradient (0-10% MeOH) to give the N^2-Boc protected product **29** (17 mg, 64.6% yield) (Scheme 11). ^1H NMR (600 MHz, DMSO-d_6): δ 1.48 (s, 9H, Boc-*t*C_4H_9), 3.35 (m, 1H, CH_2OH), 3.40 (dq, *J* = 11.5 and 6.0 Hz, 1H, CH_2OH), 3.86 (m, $^3J_{H,F}$ = 21.5 Hz, 1H, CH), 4.36 (ddq, $^1J_{H,F}$ = 47.5 Hz, *J* = 10.1 and 5.6 Hz, 1H, CH_2F), 4.47 (ddq, $^1J_{H,F}$ = 47.5 Hz, *J* = 10.1 and 3.0 Hz, 1H, CH_2F), 4.83 (broad s, 1H, OH), 5.10 and 5.53 (q, *J* = 10.2 Hz, 2H, *N*CH_2), 8.07 (s, 1H, H-8), 11.37 (broad s, 2H, N^1-H and N^2-H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 27.77 (Boc-*t*C_4H_9), 59.30 ($^3J_{C,F}$ = 8.4 Hz, CH_2OH), 71.62 (*N*CH_2), 77.82 ($^2J_{C,F}$ = 17.6 Hz, CH), 82.67 ($^1J_{C,F}$ = 169.9 Hz, CH_2F), 82.56 (Boc-*t*C), 119.43 (C5), 139.66 (C8), 147.26 (C4), 147.97 (*N*CO_2), 153.87 (C2), 155.12 (C6); ^{19}F NMR (282 MHz, DMSO-*d*6): δ −231.4; HRMS Calcd for $C_{14}H_{21}FN_5O_5$ (M+H): 358.1527; Found: 358.1516.

*4.4.4. 9-(4-Fluoro-3-hydroxymethylbutyl)guanine (**3**, FHBG) (Scheme 9)*

The fluoro penciclovir analog **23** (16.7 mg, 0.017 mmol) was subjected to full deprotection condition with 1M HCl and the crude product was purified by semi-preparative HPLC (Alltech Econosil C18 column, 10μ, 10 X 500 mm; eluent: CH_3CN/H_2O 1:9; flow rate: 4.0 mL/min; detector: 254 nm UV), to give FHBG (**3**) with a retention time of 21.2 min (3.2 mg, 75% yield) (Scheme 9). ^{1}H NMR (600 MHz, DMSO-d_6): δ 1.74 (m, 2H, CH_2), 1.66 (m, $^3J_{H,F}$ = 23.6 Hz, 1H, CH), 3.38 (m, 2H, CH_2OH), 4.00 (t, J = 7.2 Hz, 2H, NCH_2), 4.42 and 4.46 (ddq, $^1J_{H,F}$ = 47.6 Hz, J = 9.1 and 5.0 Hz, 2H, CH_2F), 4.65 (t, J = 5.0 Hz, 1H, OH), 6.45 (broad s, 2H, NH_2), 7.69 (s, 1H, H-8), 10.60 (broad s, 1H, N^1-H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 27.62 ($^3J_{C,F}$ = 4.5 Hz, CH_2), 38.95 ($^2J_{C,F}$ = 19.2 Hz, CH), 40.60 (NCH_2), 59.81 ($^3J_{C,F}$ = 5.9 Hz, CH_2OH), 83.54 ($^1J_{C,F}$ = 165.1 Hz, CH_2F), 116.57 (C5), 137.24 (C8), 151.16 (C4), 153.68 (C2), 157.09 (C6); ^{19}F NMR (376 MHz, DMSO-*d*6): δ −227.5; HRMS Calcd for $C_{10}H_{15}FN_5O_2$ (M+H): 256.1209; Found: 256.1212.

*4.4.5. 9-[(3-Fluoro-1-hydroxy-2-propoxy)methyl]guanine (**4**, FHPG) (Scheme 11)*

The N^2-Boc protected ganciclovir derivative **29** (Scheme 11) (17 mg) was subjected to full deprotection condition with 4M HOAc and the crude product was purified by semi-preparative HPLC (Alltech Econosil C18 column, 10μ, 10 X 500 mm; eluent: CH_3CN/H_2O 5:95; flow rate: 4.0 mL/min; detector: 254 nm UV) to give FHPG (**4**) with a retention time of 23.0 min (9.1 mg, 74.4% yield) (Scheme 11). ^{1}H NMR (500 MHz, DMSO-d_6): δ 3.35 (m, 2H, CH_2OH), 3.80 (m, $^3J_{H,F}$ = 21.4 Hz, 1H, CH), 4.35 (ddq, $^1J_{H,F}$ = 47.7 Hz, J = 10.1 and 5.6 Hz, 1H, CH_2F), 4.46 (ddq, $^1J_{H,F}$ = 47.7 Hz, J = 10.1 and 3.0 Hz, 1H, CH_2F), 4.86 (broad s, 1H, OH), 5.31 and 5.54 (q, J = 11.1 Hz, 2H, NCH_2), 6.59 (broad s, 2H, NH_2), 7.79 (s, 1H, H-8), 10.85 (broad s, 1H, N^1-H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 59.30 ($^3J_{C,F}$ = 8.0 Hz, CH_2OH), 71.25 (NCH_2), 77.50 ($^2J_{C,F}$ = 17.8 Hz, CH), 82.73 ($^1J_{C,F}$ = 167.9 Hz,

CH_2F), 116.44 (C5), 137.53 (C8), 151.34 (C4), 154.19 (C2), 157.18 (C6); ^{19}F NMR (376 MHz, DMSO-d_6): δ −231.8; HRMS Calcd for $C_9H_{13}N_5O_3F$ (M+H): 258.1002; Found: 258.1006.

*4.4.6. 9-(3-Hydroxymethylbut-3-ene)guanine (**26**) (Scheme 9)*

The crude fluorination product mixture containing the derivatives **23** and **24** obtained from the Boc protected tosylate **18** (34 mg, 0.030 mmol) (Scheme 8) was hydrolyzed with HCl (1 M, 1 mL) at 115° C for 1 h. The reaction mixture was processed as described above and purified by semi-preparative HPLC (Alltech Econosil C18 column, 10μ, 10 X 500mm; eluent: CH_3CN/H_2O 1:9; flow rate: 4.0 mL/min; detector: 254 nm UV) to give FHBG (**3)** (R_t = 20.0 min; 1.6 mg, 22.6%) and the title product **26** (R_t = 19.0 min; 1.2 mg, 15.6% yield). Product **26**: ^1H NMR (600 MHz, DMSO-d_6): δ 2.44 (t, *J* = 7.2 Hz, 2H, CH_2), 3.67 (m, 2H, CH_2OH), 4.05 (t, *J* = 7.2 Hz, 2H, *N*CH_2), 4.69 (m, 1H, vinyl-H), 4.87 (t, *J* = 5.4 Hz, 1H, OH), 4.96 (q, *J* = 1.8 Hz, 1H, vinyl-H), 6.45 (broad s, 2H, *N*H_2), 7.64 (s, 1H, H-8), 10.59 (broad s, 1H, N^1-H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 32.71 (CH_2), 41.09 (*N*CH_2), 63.58 (CH_2OH), 110.21 (vinyl-CH_2), 116.45 (C5), 137.37 (C8), 146.19 (vinyl-C), 151.08 (C4), 153.59 (C2), 156.98 (C6); MS Calcd for $C_{10}H_{14}N_5O_2$: 234.11; Found: 235.07 (M+H) and 257.12 (M+Na).

*4.5. Single crystal X-ray crystallographic analysis of N^2-Boc-ganciclovir (**22**)*

N^2-*tert*-butyloxycarbonyl-9-[(1,3-di-hydroxy-2-propoxy)methyl]guanine (**22)** was crystallized from MeOH/H_2O. Crystal data and structure refinement – empirical formula: $C_{14}H_{21}N_5O_6$; molecular weight: 355.36; crystal system: triclinic; space group: P-1; unit cell dimensions: *a* = 6.7535(8) Å, *b* = 9.5174(11)Å, *c* = 12.9922(14)Å,

α = 87.5100 (11)°, β = 79.0030(10)°, γ = 87.3490 (11)°; volume: 818.36(16) Å^3, T = 100(2) K; Z = 2; crystal size: colorless prism of approximate size 0.2 x 0.1 x 0.10 mm^3; calculated density: 1.442 mg/m^3; μ(MoKα) = 0.114 mm^{-1}; reflections collected:7277; independent reflections: 3886 [R(int) = 0.0188]; absorption correction: semi-empirical from equivalents; structure refinement: Bruker Shelxtl software package (V6.12); refinement method: full-matrix least-squares on F^2; Final R indices [I>2sigma(I)] R1 = 0.0374, wR2 = 0.0887; R indices (all data) R1 = 0.0484, wR2 = 0.0946. Crystallographic data (excluding structure factors) for the compound **22** have been deposited with the Cambridge Crystallographic Data Center as supplementary publication no. CCDC-1560034. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EK, UK (fax: (+44)1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).

*4.6. Radiochemical synthesis of [^{18}F]FHBG (**4**) and [^{18}F]FHPG (**6**)*

*4.6.1. Nucleophilic [^{18}F]fluorination of N^2-(p-Anisyldiphenylmethyl)-O^6-diphenylcarbamoyl-9-[3-(p-anisyldiphenylmethoxymethyl)-4-tosyloxy-butyl]guanine (**11**) and N^2-(p-Anisyldiphenylmethyl)-O^6-dimethylcarbamoyl-9-[3-(p-anisyldiphenylmethoxymethyl)-4-tosyloxy-butyl]guanine (**12**) (Scheme 5) – preparation of 9-(4-[^{18}F]fluoro-3-hydroxymethylbutyl)guanine ([^{18}F]FHBG, **4**)*

No-carrier-added [^{18}F]fluoride ion was produced by 11 MeV proton bombardment of 98 % enriched [^{18}O]water (330 µL) in a silver target body using a Siemens RDS-112 cyclotron. A 60 min bombardment with 20 µA beam current typically produced 500-550 mCi of [^{18}F]fluoride ion. The aqueous [^{18}F]fluoride ion taken in a Pyrex glass reaction

vessel was treated with a solution of K_2CO_3 (0.7 mg) and Kryptofix 222 (7 mg) dissolved in water (0.04 mL) and acetonitrile (0.75 mL) mixture. The solution was evaporated at 115^0 C with a gentle stream of nitrogen gas (20-22 mL/min flow rate) bubbling into it. The residue was further dried by an azeotropic distillation with acetonitrile (3 X 0.5 mL). The diphenyl (**11**)- or dimethylcarbamoyl (**12**) precursors (2-3mg) dissolved in DMSO (0.7 mL) was added to the dried $K^{18}F$/Kryptofix 222 complex and reacted at 150^o C for 15 min. The reaction mixture was cooled to room temperature, diluted with 4 mL of deionized water and passed through a C-18 Sep-Pak (Waters; preconditioned with 5 mL of ethanol and then with 20 mL of deionized water). The Sep-Pak was eluted with deionized water (2 X 4 mL) and the eluents were rejected. The ^{18}F-intermediate product was subsequently eluted off the Sep-Pak with 2 mL of methanol and 1M HCl (0.4 mL) was added to it and heated to 95^o C for 10 min to hydrolyze the protecting groups during which time the reaction mixture got concentrated to a volume of about 0.7 mL due to the evaporation of the solvents. The reaction mixture was cooled to room temperature and partially neutralized with a solution of 1M NaOH (0.18 mL). The reaction mixture was diluted with 1.2 mL of a mixture of 7% ethanol and 93% 50 mM ammonium acetate in water and injected into a semi-preparative HPLC column (Phenomenex Aqua, 5µ, C-18 column; 25 X 1 cm; loop volume: 3 mL). The HPLC column was eluted with a mobile phase consisting of 7% ethanol and 93% 50 mM ammonium acetate in water at a flow rate of 5.0 mL/min. The effluent from the HPLC column passed through an alumina Sep-Pak (Waters) and was monitored with an UV detector (λ = 254 nm) and a gamma radioactive detector. Chemically and radiochemically pure [^{18}F]FHBG that eluted off with a retention time between 12 and 14 min was made isotonic with an addition of 0.4 mL of NaCl solution in

sterile water (180 mg/mL). The solution was then sterilized by passing through a Millipore sterilizing filter (0.22 μm) into a sterile multi-dose vial. The entire synthesis and the purification process took ~110 min. The radiochemical yield data are provided in Table 1.

*4.6.2. Preparation of 9-(4-[^{18}F]fluoro-3-hydroxymethylbutyl)guanine ([^{18}F]FHBG, **4**) using N^2-(p-Anisyldiphenylmethyl)-O^6-tert-butyl-N^2-tert-butyloxycarbonyl-9-[3-(p-anisyldiphenylmethoxymethyl)-4-tosyloxy-butyl]guanine (**18**) precursor (Scheme 12)*

Reaction of the Boc-protected tosylate precursor **18** (2-3 mg) with [^{18}F]fluoride ion in DMSO was exactly carried out as described above. Subsequent hydrolysis of the ^{18}F-intermediate and HPLC purification yielded chemically and radiochemically pure [^{18}F]FHBG (**4**). Table 1 summarizes the radiochemical yield data.

*4.6.3. Reaction of N^2-(p-Anisyldiphenylmethyl)-O^6-tert-butyl-N^2-tert-butyloxycarbonyl-9-[3-(p-anisyldiphenylmethoxymethyl)-4-tosyloxy-butyl] guanine (**18**) with [^{18}F]fluoride ion in acetonitrile (Scheme 12)- preparation of 9-(4-[^{18}F]fluoro-3-hydroxymethylbutyl)guanine (**4**)*

The [^{18}F]fluorination of the tosylate precursor **18** in acetonitrile was conducted slightly differently. To the dry [^{18}F]fluoride ion/Kryptofix 222 complex as prepared above, a solution of the precursor **18** (5 mg) in acetonitrile (1 mL) was added. The reaction vessel was hermetically sealed and heated at 150-165^{o} C for 15 min. The reaction mixture was cooled to 95^{o} C and treated with 1M HCl (0.4 mL) for 10 min at the same temperature. The acidic mixture was partially neutralized with 1 M NaOH (0.18 mL) after cooling to room temperature. The resultant solution was diluted with 1.2 mL of a mixture of 7% ethanol

and 93% 50 mM ammonium acetate in water and subjected to semi-preparative HPLC purification as described earlier. Relevant radiochemical yield data are furnished in Table 1.

*4.6.4. Preparation of 9-[(3-[^{18}F]fluoro-1-hydroxy-2-propoxy)methyl]guanine ([^{18}F]FHPG, **6**) (Scheme 12)*

Dry no-carrier-added [^{18}F]fluoride ion-Kryptofix 222 complex was produced as described previously and reacted with a solution of N^2-(*p*-Anisyldiphenylmethyl)-O^6-*tert*-butyl-N^2-*tert*-butyloxycarbonyl-9-[(1-(*p*-anisyldiphenylmethoxy)-3-tosyloxy-2-propoxy)methyl]guanine (**19**) (2-3 mg) in DMSO (0.7 mL) at 150^o C for 15 min. The reaction mixture, after dilution with water (4 mL), was processed with a C-18 Sep-Pak and the ^{18}F-intermediate was eluted off with 2 mL of acetonitrile. The acetonitrile solution was concentrated to ~ 0. 5 mL at 105^o C and hydrolyzed at that temperature with 4M acetic acid (0.4 mL) for 15 min. The acid reaction mixture was partially neutralized with 1M NaOH (1 mL) and diluted with 1.2 mL of a mixture of 7% ethanol and 93% 50 mM ammonium acetate in water. The crude product was purified by semi-preparative HPLC as detailed above. Chemically and radiochemically pure [^{18}F]FHPG product (**6**) eluted off the HPLC column with a retention time of 6-8 min. Pertinent radiochemical yield data are given in Table 1.

4.6.5. Chemical and radiochemical quality assurance

The chemical and radiochemical purities of [^{18}F]FHBG and [^{18}F]FHPG, as prepared above, were determined by an analytical HPLC method using a Phenomenex Luna C-18 column (25 cm X 0.46 cm, 5μ particle size). The column was eluted with 10% ethanol and

90% 50 mM ammonium acetate at a flow rate of 1.0 mL/min. The effluent from the HPLC column was passed through a UV detector (λ = 254 nm) followed by a gamma radioactivity detector. The chemical and radiochemical purities of both these tracers exceeded 99% as shown in a typical analytical HPLC chromatogram for [^{18}F]FHBG (Fig. 7).

Analytical HPLC also was used to determine the specific activities of [^{18}F]FHBG and [^{18}F]FHPG. A range of mass vs UV absorption at 254 nm wavelength for non-radiolabeled FHBG (**3**) and FHPG (**5**) were determined using the analytical HPLC method described above and the data set was used to construct calibration graphs. Using these calibration graphs, the specific activities of [^{18}F]FHBG and [^{18}F]FHPG were found to be 1-3 Ci/μmol, end of synthesis.

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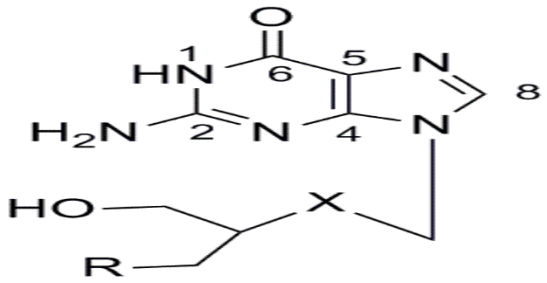
Figure captions



1, X = CH_2; R = OH (Penciclovir)

2, X = O; R = OH (Ganciclovir)

3, X = CH_2; R = F (FHBG)

4, X = CH_2; R = ^{18}F ([^{18}F]FHBG)

5, X= O; R = F (FHPG)

6; X = O; R = ^{18}F ([^{18}F]FHPG)

Fig. 1. Structures of acycloguanosine analogues.

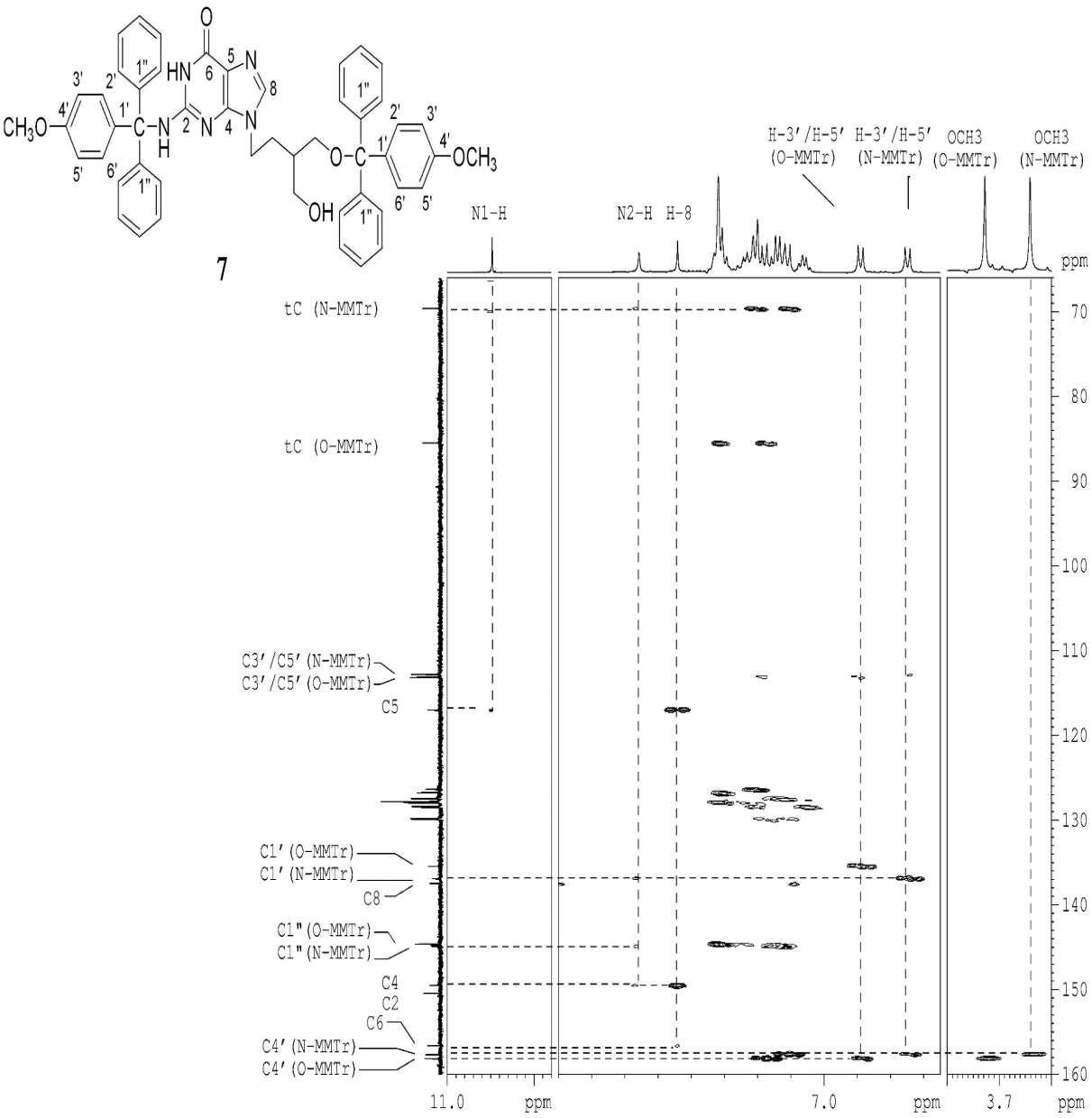


Fig. 2. HMBC spectrum (selected regions) of monomethoxytrityl protected derivative **7**.

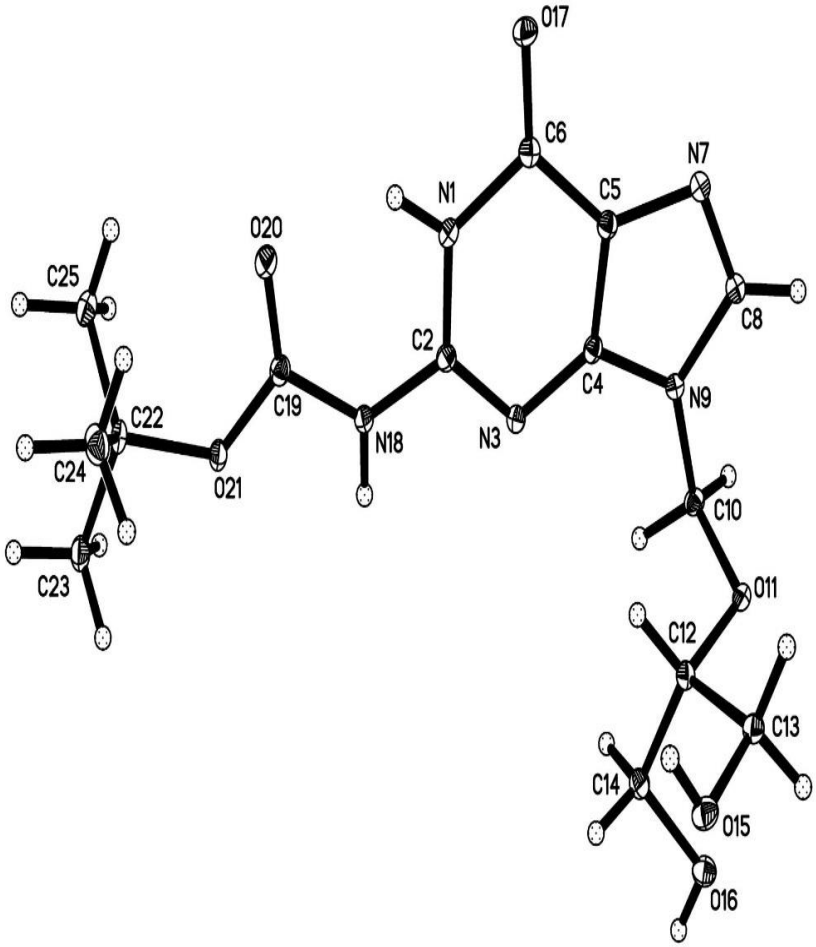


Fig.3. X-ray crystallographic structure of N^2-monomethoxytrityl protected ganciclovir derivative **22**.

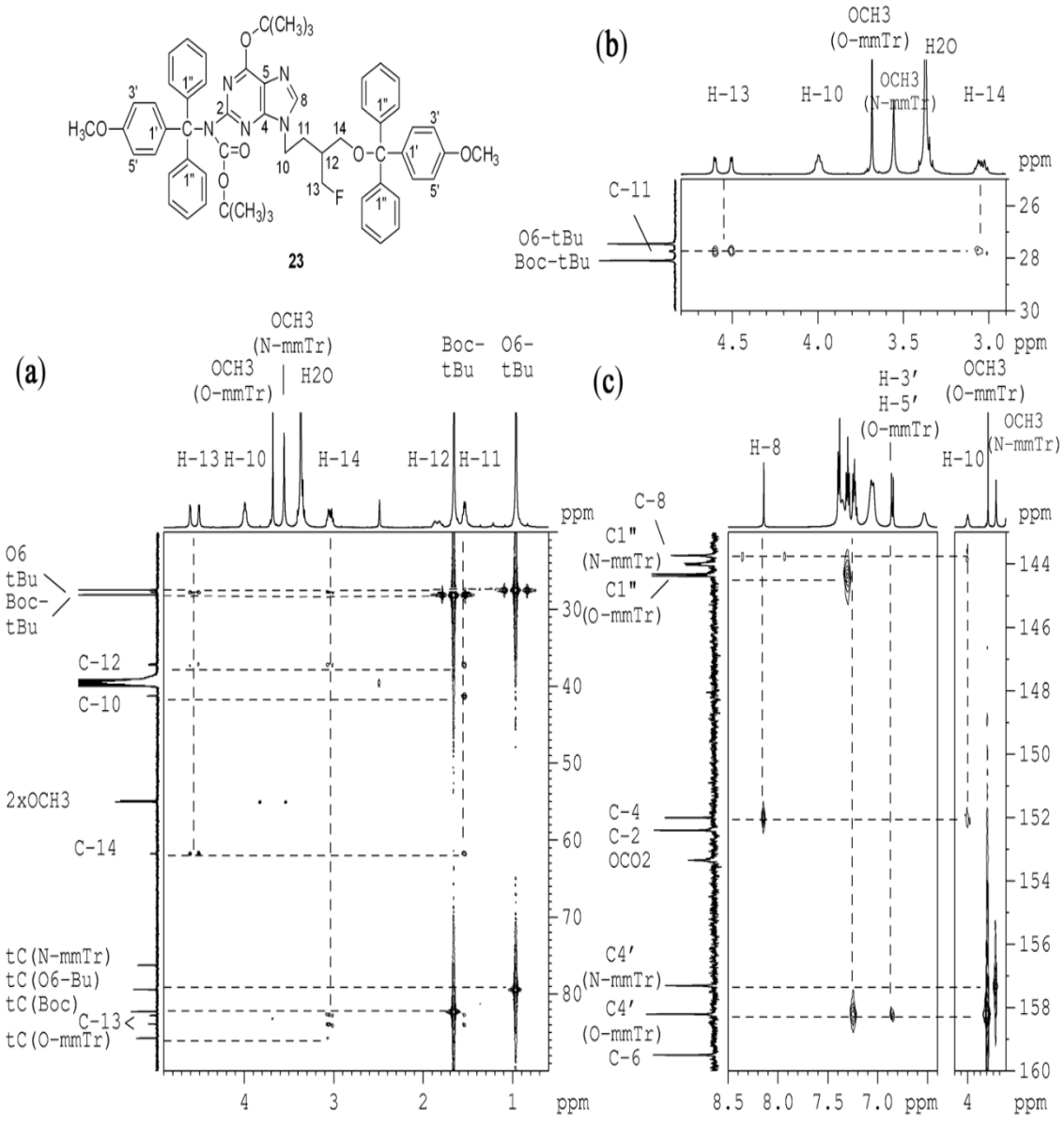


Fig. 4. Selected regions (**a**-**c**) of HMBC spectrum of the fully protected fluorinated product **23**.

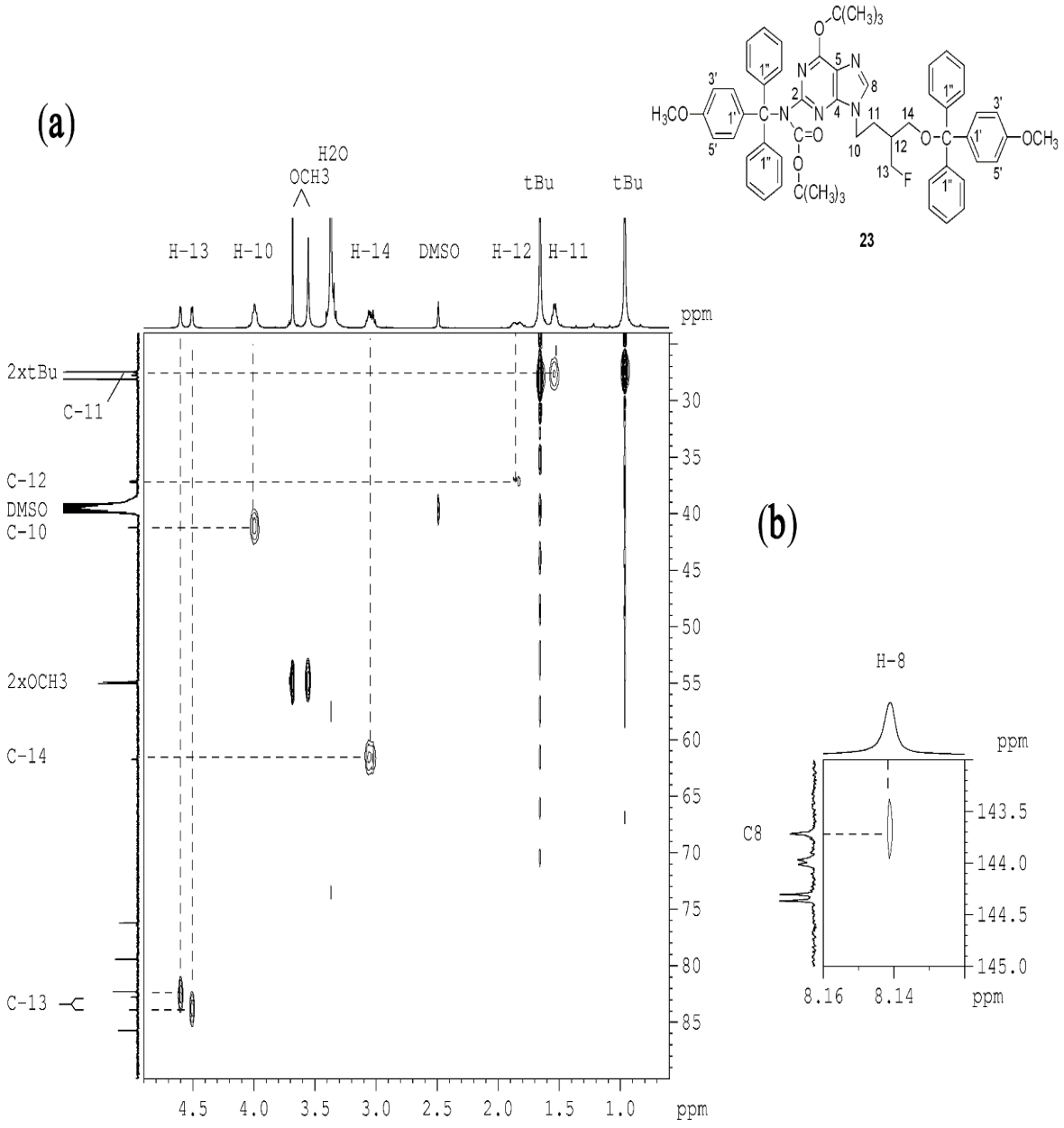


Fig. 5. Selected regions (**a**, **b**) of HMQC spectrum of the fully protected fluorinated product **23**.

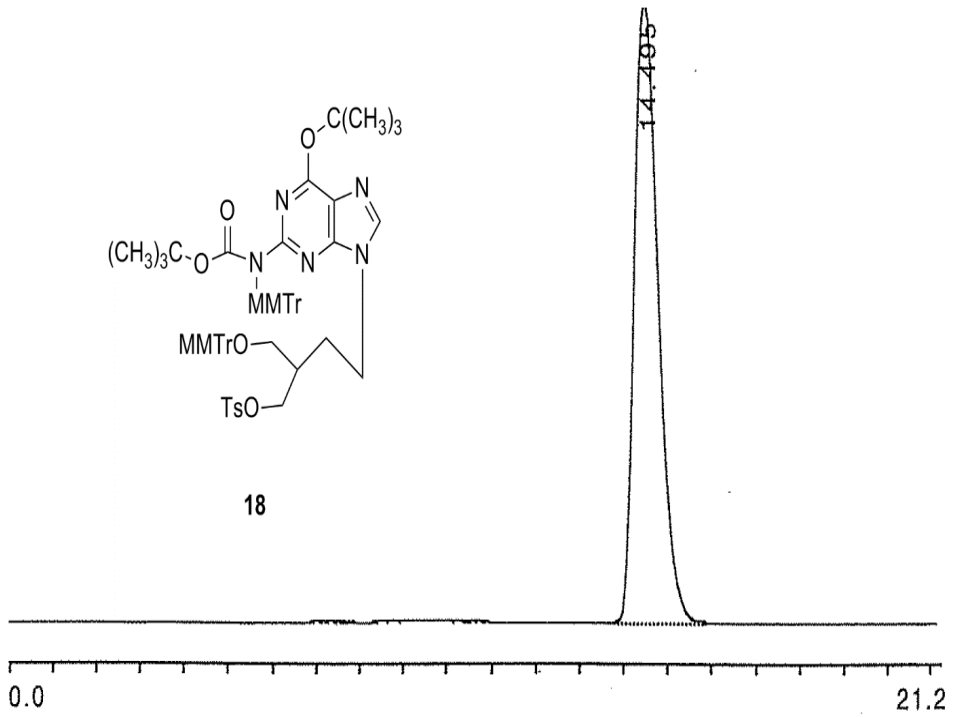


Fig. 6. The purity of the Boc-protected FHBG precursor **18** as determined by analytical HPLC (Grace Altima Silica, 5μ, 4.6 X 150 mm column; eluent: hexanes/THF (1:1); flow rate: 0.3 mL/min; 254 nm UV detection).

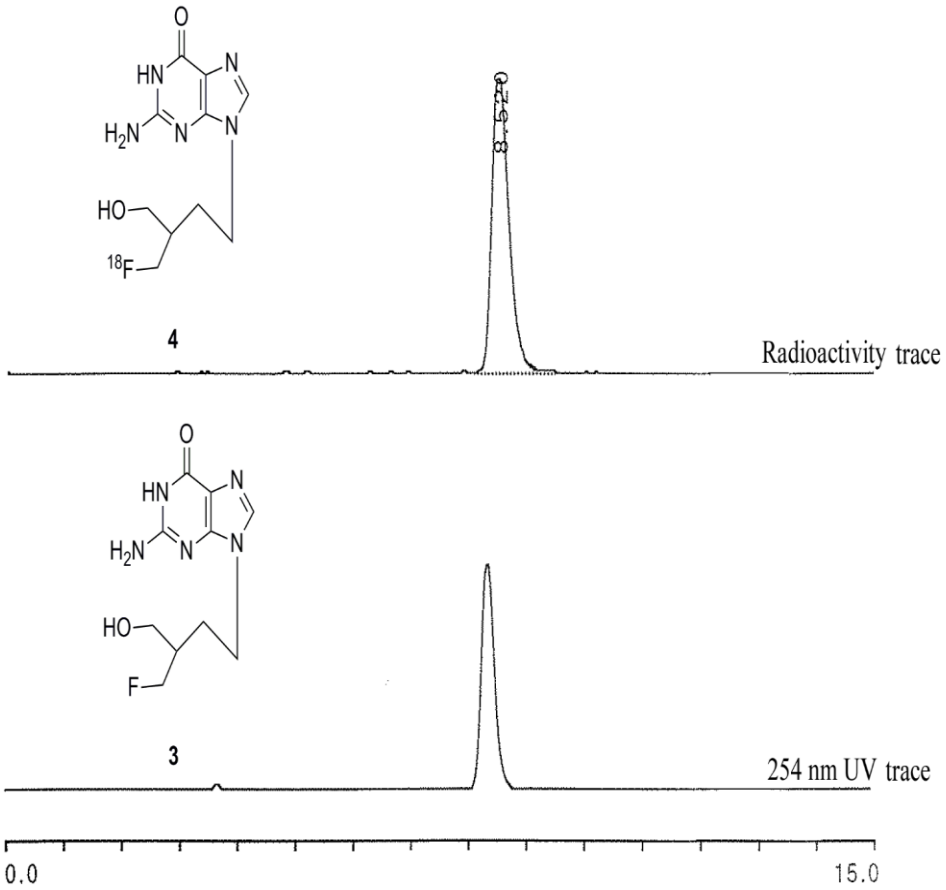
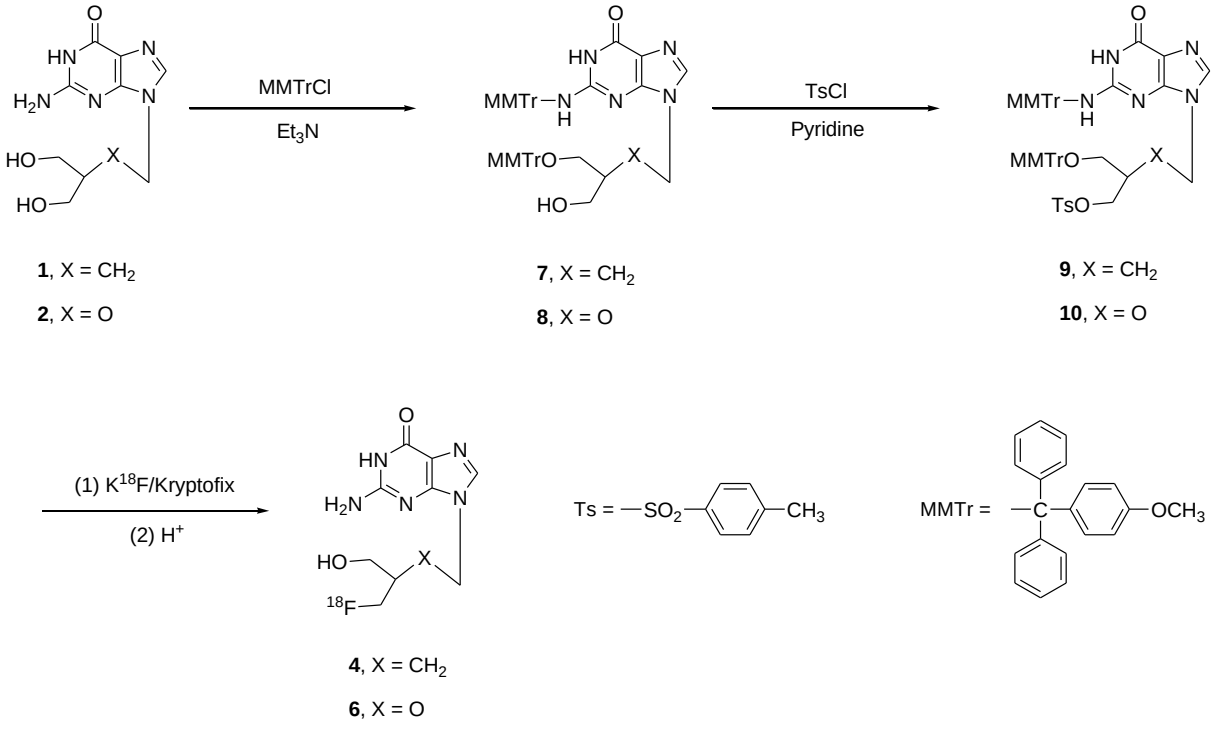


Fig. **7**. Analytical HPLC (Phenomenex Luna C18, 5μ, 4.6 X 250 mm column; eluent: EtOH/50 mM NH_4OAc (1:9); flow rate: 1.0 mL/min) diagram of [^{18}F]FHBG (top trace) co-injected with the FHBG standard (bottom trace; RT:8.5 min).

Scheme captions



1, X = CH_2

2, X = O

7, X = CH_2

8, X = O

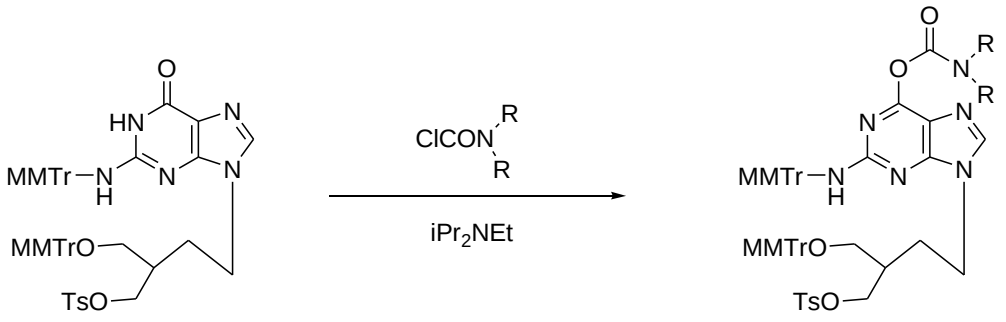
9, X = CH_2

10, X = O

4, X = CH_2

6, X = O

Scheme 1. The prevalent synthesis of [^{18}F]FHBG (**4**) and [^{18}F]FHPG (**6**).

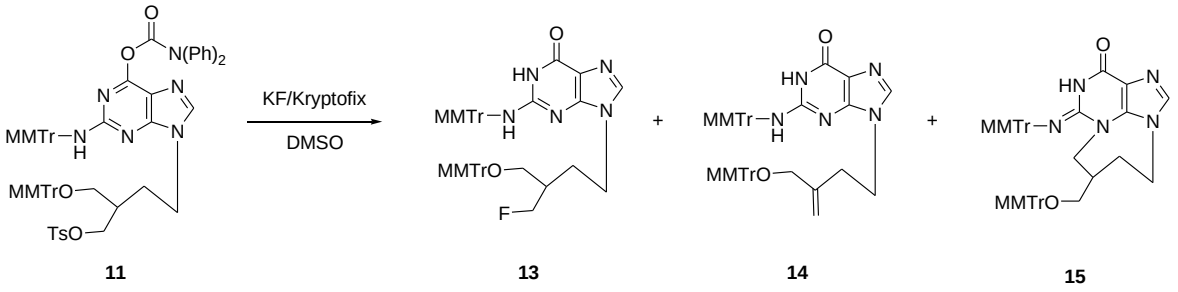


9

11, R = C_6H_5

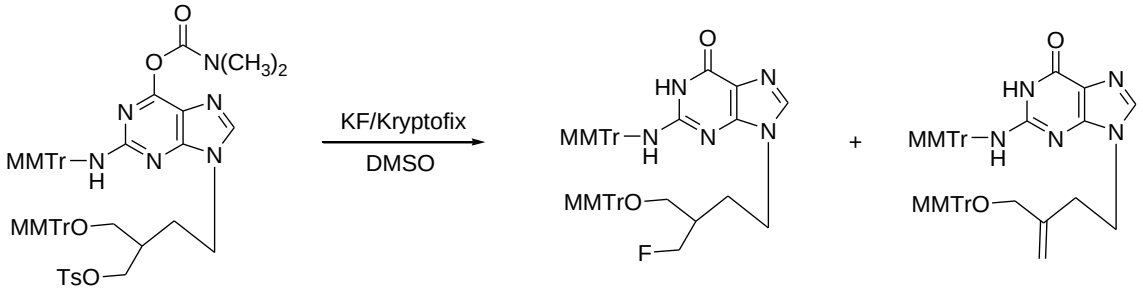
12, R = CH_3

Scheme 2. The synthesis of O^6-carbamoyl-protected penciclovir analogs.



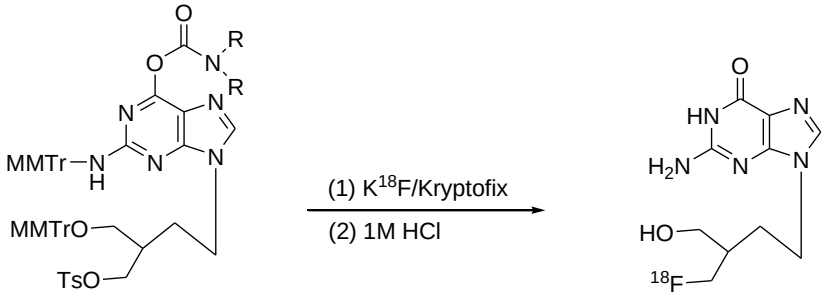
11 **13** **14** **15**

Scheme 3. Nucleophilic fluorination reaction of diphenylcarbamoyl-protected penciclovir derivative.



12 **13** **14**

Scheme 4. Reaction of dimethylcarbamoyl-protected penciclovir analog with the fluoride ion.

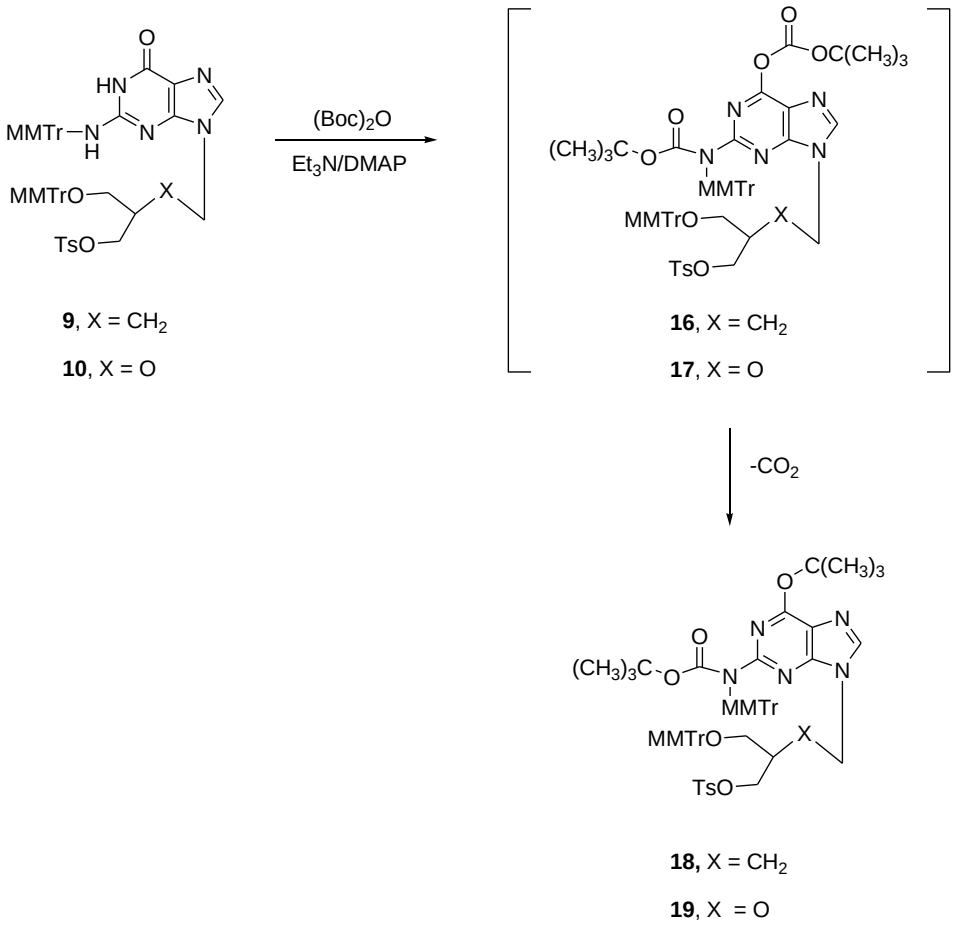


11, R = C_6H_5

12, R = CH_3

4

Scheme 5. Preparation of [^{18}F]FHBG from the carbamoyl-protected penciclovir derivatives.



9, X = CH_2

10, X = O

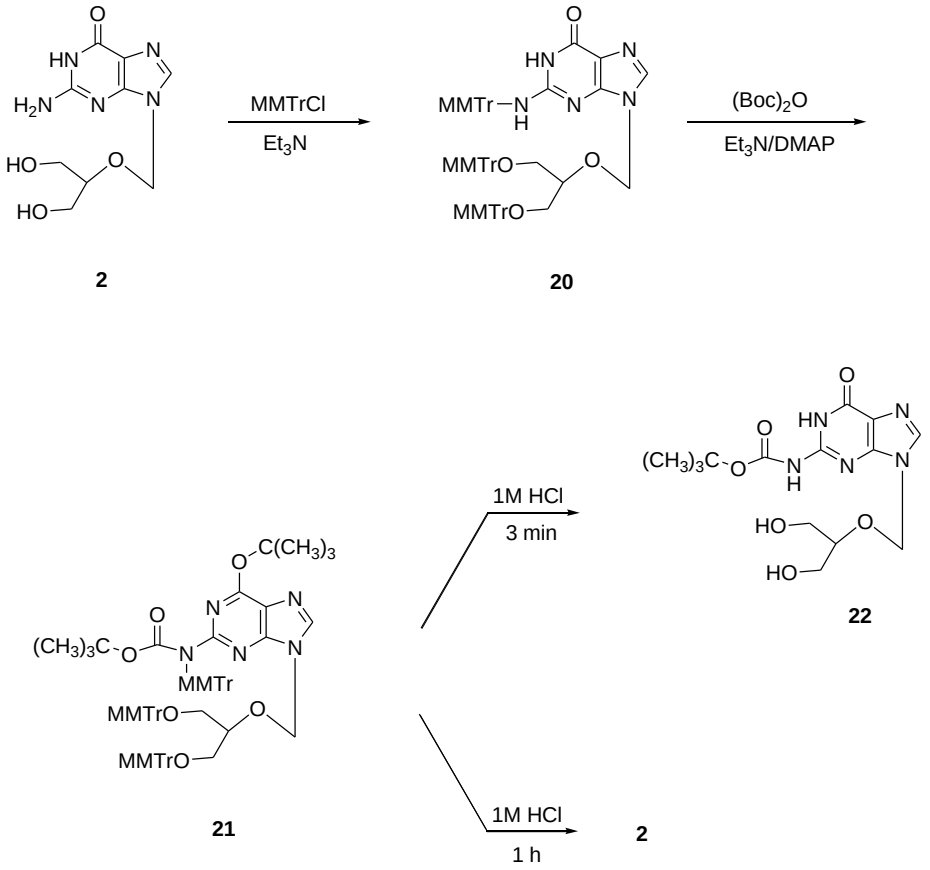
16, X = CH_2

17, X = O

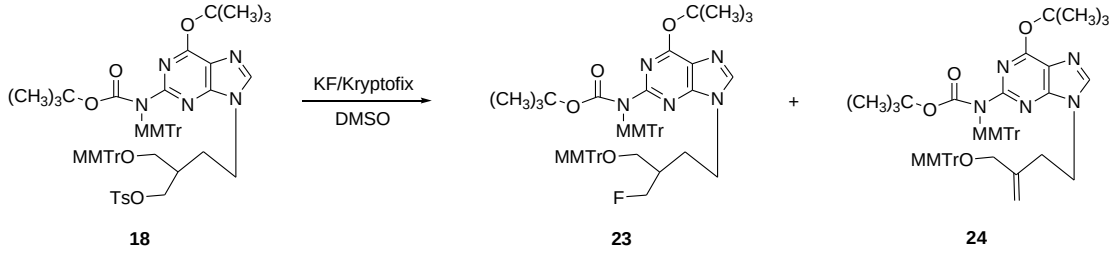
18, X = CH_2

19, X = O

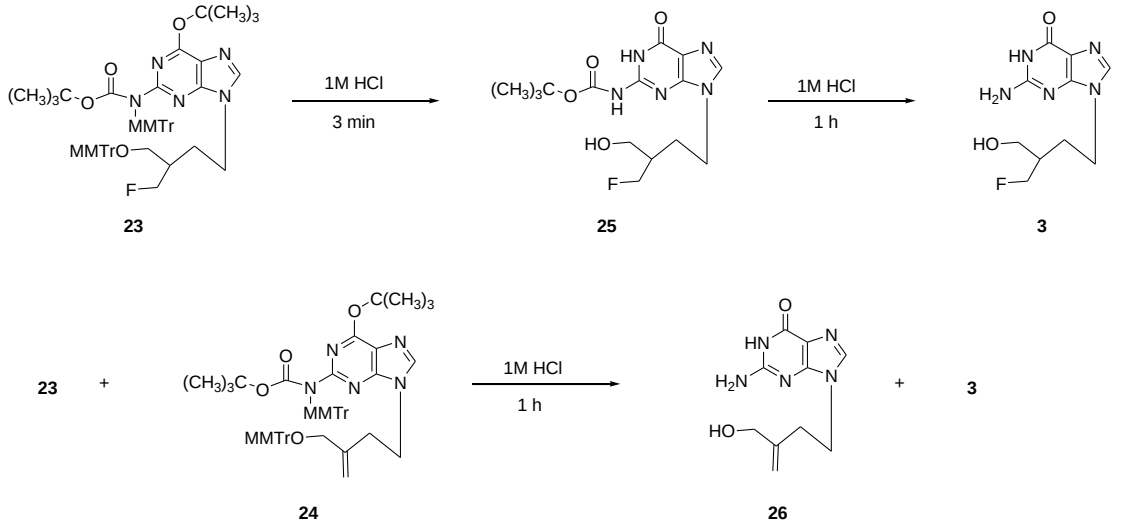
Scheme 6. Boc



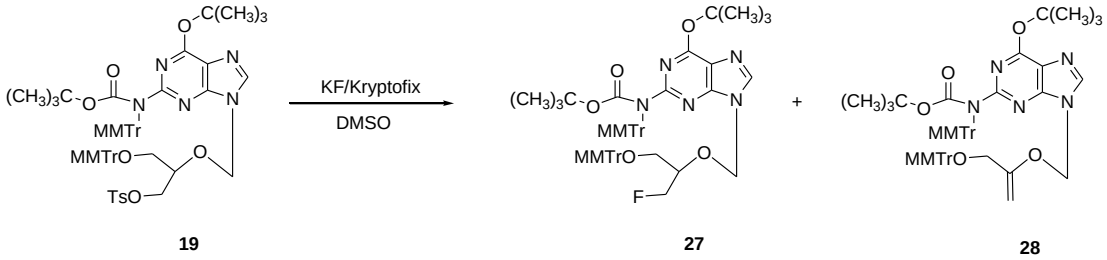
Scheme 7. Preparation of tri-monomethoxytrityl-Boc-protected ganciclovir **21** and its acid hydrolysis.



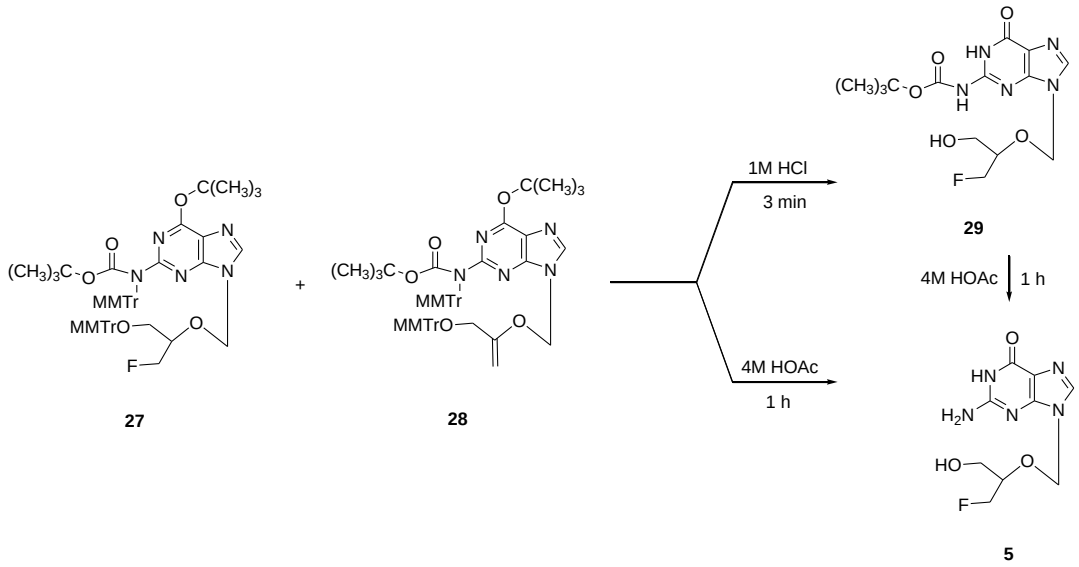
Scheme 8. Reaction of fully protected FHBG tosylate precursor **18** with fluoride ion.



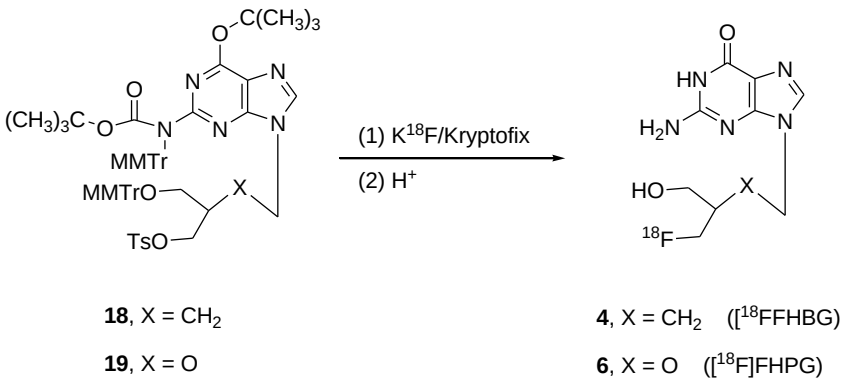
Scheme 9. Acid hydrolysis of pure protected FHBG analog **23** and a mixture of **23** and **24**.



Scheme 10. Nucleophilic substitution reaction of the protected FHPG tosylate precursor **19** with fluoride ion.



Scheme 11. Acid hydrolysis of a mixture of protected FHPG derivatives **27** and **28**.



18, X = CH_2

19, X = O

4, X = CH_2 ([^{18}FFHBG)

6, X = O ([^{18}F]FHPG)

Scheme 12. Preparation of [^{18}F]FHBG and [^{18}F]FHPG from their corresponding tosylate precursors **18** and **19**, respectively.

Table 1
Radiochemical yield data for [^{18}F]FHBG (**4**) and [^{18}F]FHPG (**6**)

Precursor yield[b]	Product	Radiochemical yield[a] (%)	End of synthesis (mCi)
11	[^{18}F]FHBG	37.9[c] (n=2)	95.0[c] (n=2)
12	[^{18}F]FHBG	42.7 ± 7.6 (n=3)	103.0 ± 17.6 (n=3)
18	[^{18}F]FHBG	68.2 ± 7.8 (n=28)	180.0 ± 25.9 (n=28)
18	[^{18}F]FHBG	55.2 ± 1.4[d] (n=3)	118.3 ± 13.3 (n=3)
19	[^{18}F]FHPG	65.0 ± 7.6 (n=4)	175.0 ± 34.0 (n=4)

[a]Values are decay corrected to end of cyclotron bombardment for the production of [^{18}F]fluoride ion and represent the mean ± standard deviation. The number of experiments used for the calculation of the data given in parentheses. [^{18}F]Fluorination reactions conducted in DMSO unless noted otherwise.
[b]Isolated yields for the number of experiments given in parentheses.
[c]Data for average of two experiments.
[d][^{18}F]Fluorination reaction conducted in acetonitrile.