EurJOC
European Journal of Organic Chemistry

A Journal of
ChemPubSoc Europe

Accepted Article

Title: Fluorine at the C5 position of 2'-deoxyuridine enhances repair of an O4-methyl adduct by O6-Alkylguanine DNA Alkyltransferases.

Authors: Lauralicia Sacre and Christopher James Wilds

To be cited as: *Eur. J. Org. Chem.* 10.1002/ejoc.201700466

Link to VoR: http://dx.doi.org/10.1002/ejoc.201700466

Supported by
ACES Asian Chemical Editorial Society

WILEY-VCH

Fluorine at the C5 position of 2'-deoxyuridine enhances repair of a O^4-methyl adduct by O^6-Alkylguanine DNA Alkyltransferases.

Lauralicia Sacre and Christopher J. Wilds*[a]

Abstract: Alkylation damage at the O^6- and O^4-atoms of 2'-deoxyguanosine (dG) and thymidine (T), respectively, can be removed by O^6-alkylguanine-DNA alkyltransferases (AGTs). Previous studies have shown that human AGT (hAGT) repairs small adducts poorly at the O^4-atom of T, in comparison to the *E. coli* variants (OGT and Ada-C). The C5 methyl group of the thymine nucleobase is suspected to contribute to hAGT repair proficiency possibly due to steric effects in the protein active site. In the present study, repair of oligonucleotides containing a 5-fluoro-O^4-methyl-2'-deoxyuridine (**dFU-Me**) insert by hAGT, *E.coli* AGT variants (OGT and Ada-C) and a chimeric hAGT/OGT protein was evaluated. All AGT variants, particularly hAGT and the hAGT/OGT chimera, demonstrated improved proficiency at removing the O^4-methyl group from substrates containing **dFU-Me**, relative to the thymidine and 2'-deoxyuridine counterparts.

Introduction

DNA damage can result from various agents and events from within and external to the cell.[1,2] For example, alkylating agents such as *N*-methyl-*N*'-nitrosourea have been shown to introduce alkyl lesions at the O^6 and O^4 atoms of 2'-deoxyguanosine (dG) and thymidine (T) respectively.[1,3] Such modifications to DNA are disruptive to the cell, a feature that has been exploited by drugs used to treat of cancer, which are designed to introduce alkyl lesions. Examples of chemotherapeutic drugs whose primary mode of action is the introduction of DNA damage includes Temozolamide and BCNU (1,3-bis-(2-chloroethyl)-1-nitrosourea).[4]

The detrimental effects of O^6-methyl-dG (O^6MedG) and O^4-methyl-T (O^4MeT) to hinder DNA polymerase activity and cause transitional events (G:C to A:T) have been well documented.[4–6] The mutagenic character arises mainly from the persistence of these lesions in addition to how frequently they occur.[7,8] O^6MedG incidences occur predominantly compared to O^4MedT in both *in vitro* and *in vivo* experiments where DNA is subjected to alkylating agents such as *N*-methyl-*N*'-nitrosourea and methyl methanesulfonate.[9–11] O^4MeT, however exhibits a higher level of cytotoxicity due to its reduced susceptibility to repair by the cellular repair machinery.[9,12] The detrimental effect of O^4MeT arises from a non-wobble base pair with the incorrect dG nucleotide leading to a possible mutagenic outcome if left unrepaired.[6]

O^6-alkylguanine DNA alkyltransferase proteins (AGTs) are able to locate and remove the aforementioned alkylated lesions via a direct repair mechanism.[13] The alkylated nucleotide is flipped out of the DNA duplex into the active site of the protein, where an activated cysteine residue (Cys145 in the human variant) irreversibly transfers the alkyl group from the DNA.[3,4] The alkylated protein product is then degraded rapidly by the ubiquitin pathway.[14] AGT proteins are found throughout life, and crystal structures of different AGT variants depict a similar overall structure, despite high or low homology.[13]

The range of substrates that can be acted upon and repair rates varies amongst AGTs. For example, Ada-C and OGT from *E. coli* have been shown to repair small adducts such as methyl groups at either the O^6-atom of dG or O^4-atom of T, with the latter repaired more efficiently.[12,15,16] Human AGT (hAGT) is proficient at repairing a vast range of adducts at the O^6-position of dG including mono-adducts, intrastrand, and interstrand cross-links.[12,15,17–19] Moreover, O^6-benzylguanine is also a very efficient pseudo-substrate for hAGT.[20–22] While Ada-C and OGT have been shown to be efficient at repairing O^4MeT in DNA, hAGT displays poor activity despite its ability to recognize and bind to DNA containing this adduct.[4,12,15] The evasion of the mutagenic and cytotoxic O^4MeT lesion by MMR and hAGT to undergo repair,[4,23] coupled with no available crystal structure of hAGT with a O^4MeT containing DNA substrate, warrants further investigation of this lesion.

In previous work from our laboratory, the influence of the methyl group at the C5-atom of T towards repair of various O^4-alkylated adducts by hAGT was evaluated.[3] It was proposed that a potential steric clash between the C5-methyl of T and Arg135 of hAGT could hinder repair. To address this, hAGT repair of DNA containing O^4MedU (**dU-Me**, Figure 1) was investigated and it was concluded that steric factors of the C5-methyl group may contribute, at least in part, as dU substrates were processed more efficiently relative to their dT counterparts by hAGT.[3,24] It was observed that an AGT chimera (hOGT), consisting of the hAGT protein with replaced residues (139-159) from the OGT active site, demonstrated a twenty fold repair enhancement of **dU-Me** compared to hAGT.[3]

Given the differences observed for AGT-mediated repair of O^4-alkyl groups in T versus dU, we have initiated studies to explore the influence of other groups at the C5-position. Fluorine and hydrogen have a similar van der Waals radius (1.47 versus 1.20 Å) with the former having a higher electronegativity allowing for the straightforward evaluation of the influence of an electronegative group at this position.[25,26] Replacement of hydrogen with fluorine has been exploited for applications in drug discovery. Fluorine has shown to increase lipophilicity and fat solubility of small molecules.[25–27] 5-fluorouracil and

[a] *Lauralicia Sacre and Christopher J. Wilds**
Department of Chemistry and Biochemistry and Centre for Structural and Functional Genomics, Concordia University, 7141 Sherbrooke St. West, Montréal, Québec (Canada) H4B 1R6
E-mail: chris.wilds@concordia.ca

Supporting information for this article is given via a link at the end of the document.((Please delete this text if not appropriate))

flunitrazepam are examples of potent fluorine containing drugs.[27-29] In chemically modified oligonucleotides, the introduction of fluorine has been shown to confer interesting properties to the nucleic acid scaffold. For example, replacing the 2'-hydroxyl group of RNA with fluorine has been shown to influence siRNA recognition by RISC, an important feature in strand selectivity and gene silencing.[29] In the present study, the influence of having fluorine at the C5-position with oligonucleotides containing 5-fluoro-O^4-methyl-2'-deoxyuridine (**dFU-Me**) on duplex stablity, structure and proficiency of AGT-mediated repair relative to T and dU is described.

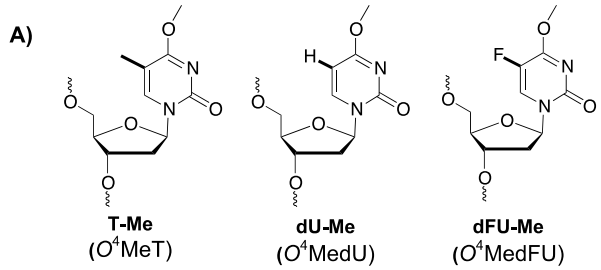
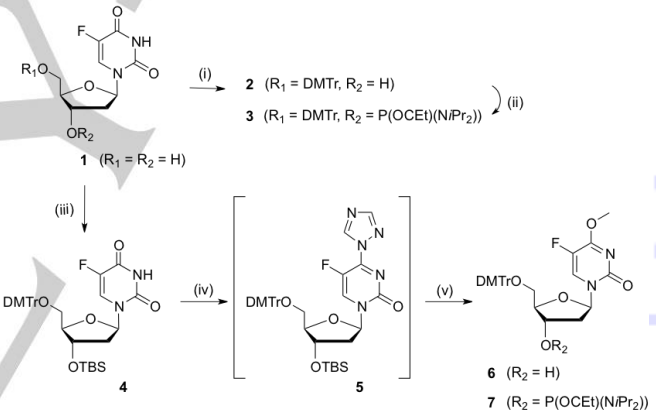


Figure 1. A) Structures of the modified O^4-alkyl pyrimidines, and B) DNA sequence where **X** corresponds to the adduct.

Results and Discussion

Synthesis of nucleosides and oligonucleotides

The structure of O^4-Me-dFU and the sequence of the 14-mer DNA used for biophysical and repair studies are shown in **Figure 1**. The corresponding 14-mer T and dU control sequences were also prepared**.** The oligonucleotides containing the modified nucleobases were prepared by a combination of solution and automated solid-phase synthesis. The synthetic pathway is shown in **Scheme 1** and it begins with the commercially available 5-fluoro-2'deoxyuridine (**dFU**), in which the 5'-hydroxyl was protected with a 4,4'-dimethoxytrityl group which was introduced by the portionwise addition of 4,4'-dimethoxytrityl chloride in pyridine. This was followed by silylation of the 3'-hydroxyl with *tert*-butyldimethylsilyl chloride in dichloromethane.[30,31] A convertible nucleoside was prepared by the addition of a 1,2,4-triazole group at the *C*4 position of the nucleoside. This reaction was optimized since it did not reach completion and when following the procedure commonly used for the T and dU series undesired by-product formation such as the detritylated nucleoside was observed.[3,30] This may be due to the presence of the C5-fluorine, which could contribute to increased reactivity at the *C*4 position. The formation of convertible intermediate **5** was accomplished by the slow addition of 1,2,4-triazole, triethylamine and phosphoryl chloride in three portions at 30 min intervals with stirring at 0°C to a solution containing compound **4**. After four hours, the reaction was complete and the convertible nucleoside was then converted to compound **6** by using sodium methoxide in methanol. Despite the increased reactivity of the convertible nucleoside, it allows the incorporation of different adducts at the C4 position. The incorporation of the O^4-methyl adduct was followed by the removal of the 3'-*O*-*tert*-butyldimethylsilyl group by a fluoride treatment using TBAF at room temperature for 30 min to yield compound **6** (74% over 3 steps). In a similar fashion, removal of the 3'-*O*-*tert*-butyldimethylsilyl protecting group from compound **1** to yield **2** was performed. Finally, compounds **2** and **6** were phosphitylated by the addition of diisopropylethylamine in THF and immediately followed by N,N-diisopropylaminocyanoethylphosphonamidic chloride to generate phosphoramidites **3** and **7**, respectively, in good yields, using previously published procedures.[3] Purification was achieved by short flash column chromatography. The ^{31}P NMR spectra (**Supporting Figures S4** and **S11**) revealed two sharp signals in the 147-149 ppm region characteristic of phosphoramidites.[3]



Scheme 1. Reagents and conditions: (i) DMTr-Cl, pyridine, DMAP, 16 h, 21 °C; (ii) *N,N*-diisopropylaminocyanoethylphosphonamidic chloride, DIPEA, THF, 30 min; (iii) 1. DMTr-Cl, pyridine, DMAP, 16 h, 21 °C. 2. TBS-Cl, Imidazole, DCM, 16 h, 21 °C; (iv) 1,2,4-Triazole, triethylamine, $POCl_3$, MeCN, 4 h, 0 °C; (v) 1. MeOH, NaOMe, 4h, 21 °C. 2. TBAF (1M in THF), 30 min; (vi) *N,N*-diisopropylaminocyanoethylphosphonamidic chloride, DIPEA, THF, 30 min.

Solid-phase synthesis of the oligonucleotide was conducted using "fast-deprotecting" commercially available 3'-*O*-phosphoramidites due to the labile nature of the **dFU-Me** adduct.[18] Phenoxyacetic anhydride was employed as the capping reagent to avoid transamidation.[32] Total deprotection and cleavage of the oligomers from solid support was accomplished with an anhydrous solution of potassium carbonate in methanol (0.05 M) for four hours at 22°C with gentle rocking.[18,30] Excess base was neutralized with an equimolar amount of acetic acid and the solvent removed in a speed-vacuum concentrator. The oligonucleotide containing **dFU-Me** was purified by SAX-HPLC. Characterization by ESI-MS of DNA containing the **dFU-Me** confirms the presence of the

modification after the deprotection process and it was in agreement with the expected mass (**Supporting Figure S13**). Enzymatic digestion of modified oligonucleotides using snake venom phosphodiesterase and calf intestinal phosphatase enzymes also confirmed nucleoside composition consistent with expected ratios (**Supporting Figure S14**).

UV thermal denaturation and CD spectroscopy

In order to evaluate the effect of the fluorine atom at the *C*5 position and the methyl adduct at the O^4-atom on the stability of the DNA duplex (formed by hybridizing the modified oligonucleotide with its corresponding complementary sequence containing 2'-deoxyadenosine as the base pairing partner of the O^4-methylated nucleobase), UV thermal denaturation studies were conducted. The results are summarized in **Figure 2**. The introduction of the methyl group at the O^4 position resulted in a decrease in thermal stability by approximately 11 °C relative to the unmodified controls. This is similar to the decrease in T_m observed for the **T** and **dU** counterparts suggesting that the decrease in duplex stability resulting from the O^4-Me lesions is independent of the identity of the nucleobase for the series studied (**T**, **dU** or **dFU**). The overall decrease in thermal stability may be attributed to the disruption of the H-bonding between the O^4-alkylated **dFU** and its base-pair partner 2'-deoxyadenosine and less optimal stacking with the adjacent bases similar to previous observations with the oligonucleotides containing **dU**.[3] Coincidentally, the duplexes containing **dU** and **dFU** displayed the same T_m value of 58 °C, as well as their respective modifications (**dFU-Me** and **dU-Me**), implying that the presence of the fluorine at the *C*5 position had minimal influence on the DNA duplex stability.

The CD analysis of the profiles from **dFU**, **dFU-Me**, and the control **T** display spectroscopic signatures consistent with the B-form DNA family, exhibiting a maximum positive signal at 280 nm, a cross-over near 256 nm, and a negative signal at 245 nm. The CD signatures of the duplex containing **dFU-Me** was similar to the unmodified control DNA duplex profile, which suggested that the O^4-methyl group did not cause any major effect on the overall DNA duplex structure, as previously reported for the dU series.[3]

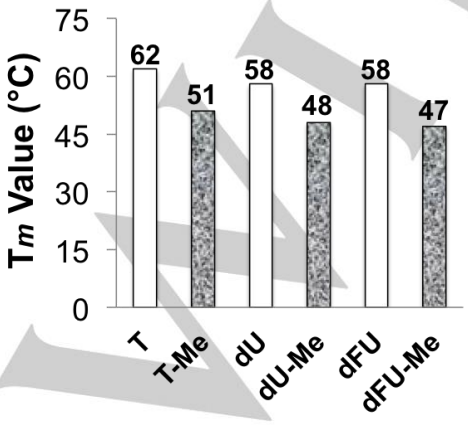


Figure 2. T_m values (°C) of duplexes containing **T, T-Me, dU, dU-Me, dFU, dFU-Me**. Colorless and grey bars represent the unmodified controls and O^4-methylated adducts, respectively. Hyperchromicity change (A_{260}) *versus* temperature (°C) profiles are shown in **Supporting Figure S16**.

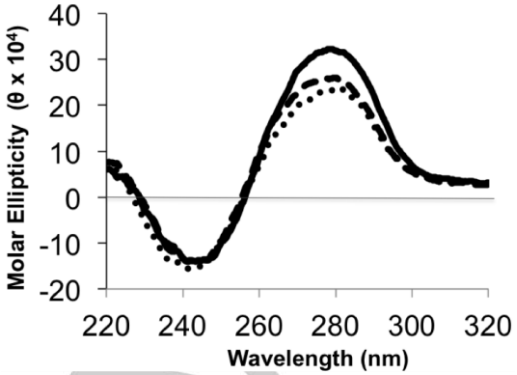


Figure 3. Circular dichroism spectra of DNA duplexes containing **dFU•dA (——), dFU-Me•dA (—)** and unmodified control **T•dA DNA (•••)**

Repair Assays

The repair of **dFU-Me** in a DNA duplex was studied with three recombinant AGT proteins (hAGT, Ada-C and OGT), and the chimera AGT protein (hOGT). Preliminary repair assays were performed as described previously by our group, where denaturing PAGE is used to monitor the production of a product generated from BclI cleavage which occurs at a specific site engineered into the DNA duplex.[3] The **dFU-Me** oligonucleotide is 5'-radiolabeled and if AGT repair occurs, BclI can cleave the duplex into smaller fragments including a radiolabeled 5-mer detected by PAGE.[3] If the O^4-alkyl group persists, the intact radiolabeled 14-mer is observed.

The total repair assay (performed overnight at 37 °C) revealed that **dFU-Me** was efficiently repaired by all four AGTs evaluated at five-molar equivalences to the DNA (reaching approximately 80 % repair). These results are consistent with the trends observed previously for the total repair assays with the O^4-methyl-dU versus O^4-methyl-T containing oligonucleotides.[3,18]

Interestingly, time course assays revealed that **dFU-Me** was repaired faster compared to their **dU-Me** and **T-Me** counterparts. Time course repair studies were performed with the four AGTs variants at room temperature. Both *E.coli* variants (OGT and Ada-C) took less than 15s to achieve full repair of **dFU-Me**. This result is in agreement with the total repair observed towards **dU-Me** versus **T-Me** occurring in less than 15s by OGT and Ada-C.[3]

Remarkably, repair of **dFU-Me** by hAGT only required approximately 90 s for the reaction to achieve completion. The repair observed of **dFU-Me** by hAGT occurred significantly faster relative to the **dU-Me** and **T-Me** analogues. It had previously been shown that **dU-Me** undergoes 25% repair by hAGT in 2.5 min, whereas similar repair levels were only obtained for **T-Me** after 30 min. **dU-Me** hAGT-mediated repair was approximately

12-fold faster compared to **T-Me** in terms of time to reach 25% substrate repair.[3] **dFU-Me** 25% repair by hAGT occurred in less than 15 s, suggesting that the fluorinated analogue is a better substrate than **dU-Me** and therefore even better than **T-Me**. Complete repair of **dFU-Me** by the hOGT chimera occurred in less than 1.5 min, displaying a similar repair profile to hAGT. Moreover, repair by the hOGT chimera on **dFU-Me** was similar compared to **dU-Me**. When compared with the results of our previous studies, **dFU-Me** required approximately a third of the time to achieve 80% repair relative to the **dU-Me** adduct.[3]

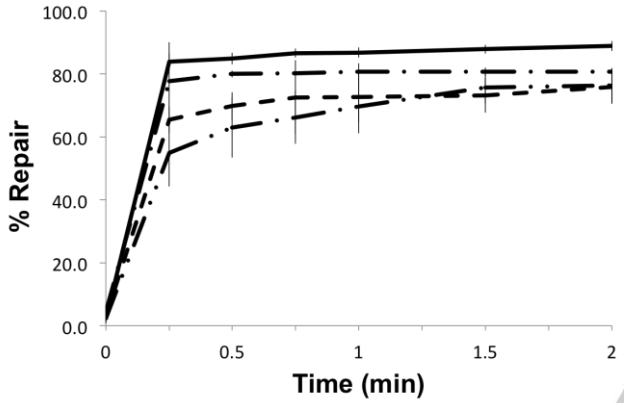


Figure 4. Time course repair assay of **dFU-Me** by OGT (—), Ada-C (—•—), hAGT (—••—), and hOGT (———) at 5-molar protein equivalence. Graphical illustration displays **dFU-Me** repair [%] over time (min).

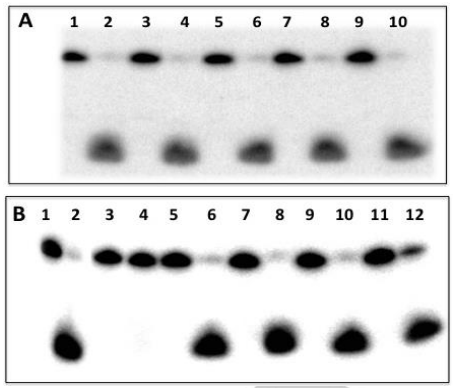


Figure 5. Repair of (**A**) **dFU** (2 pmol) and (**B**) **dFU-Me** (2 pmol) by hAGT (10 pmol), OGT (10 pmol), Ada-C (10 pmol),, and hOGT (10 pmol), for 2.5h at 37°C. Panel **A**; Lane 1, **dFU** DNA; lane 2, **dFU** + BclI; lane 3, **dFU** + hAGT; lane 4, **dFU** + hAGT + BclI; lane 5, **dFU** + OGT; lane 6, **dFU** + OGT+ BclI; lane 7, **dFU** + Ada-C; lane 8, **dFU** + Ada-C + BclI; lane 9, **dFU** + hOGT; lane 10, **dFU** + hOGT + BclL. Panel **B**; Lane 1, **dFU-Me** DNA; lane 2, **dFU-Me** + BclI; Lane 3, **dFU-Me**; lane 4, **dFU-Me** + hAGT; lane 5, **dFU-Me** + hAGT; lane 6, **dFU-Me** + hAGT + BclI; lane 7, **dFU-Me** + OGT; lane 8, **dFU-Me** + OGT+ BclI; lane 9, **dFU-Me** + Ada-C; lane 10, **dFU-Me** + Ada-C + BclI; lane 11, **dFU-Me** + 10 hOGT; lane 12, **dFU-Me** + hOGT + BclI.

The importance and role of fluorine in biological applications and drug regimens has been well documented.[26,30,33] Our study shows that the presence of the fluorine at the *C*5-position enhances repair susceptibility of methyl adducts at the O^4-position by hAGT with similar repair efficiency observed between hAGT and OGT. Moreover, elevated hAGT levels have been correlated to an increase in cellular resistance to certain chemotherapeutic agents. It is proposed that the presence of the fluorine at the *C*5 position weakens the ether O^4-C_α bond making it more susceptible to nucleophilic attack by Cys145 and therefore a good hAGT substrate. The presence of fluorine might also have a substantial effect on weak dipolar interactions and basicity of the active site.[26,34] Alternatively, hydrophobic interactions of the fluorinated substrate within the AGT active site may be occurring with computational studies currently underway to assess this hypothesis.[35] Crystallographic structures of the protein complexed with damaged DNA will aid in elucidating the critical interactions, which promote hAGT-mediated repair. Moreover, high-resolution structures of duplexes containing **dFU-Me** are currently being investigated *via* by a combination of molecular dynamics and high-field NMR experiments to gain insights on the impact of this modification on DNA structure. Studies are also currently underway with other **dFU** substrates to evaluate susceptibility towards AGT-mediated repair, which may find potential uses as AGT inhibitors in certain combinatory regimens.[20,21]

Conclusion

Our study describes the synthesis of a nucleoside and oligonucleotide containing **dFU-Me**. This was achieved via the formation of a convertible nucleoside and its incorporation into oligonucleotides by solid-phase synthesis in scales and purity sufficient for biochemical and biophysical studies. A decrease of 11 °C in the thermal stability was observed for duplexes containing this modification relative to the control duplex. The CD data revealed no major change in the global structure of DNA containing the methylated dFU insert relative to the control duplex. Repair of **dFU-Me** by the different AGTs occurred in less than 2 min, with the *E. coli* variants displaying significant repair proficiency (in less than 15 sec), which was in agreement with previous literature.[3,4] hAGT shows a remarkable increase in its repair efficiency towards **dFU-Me** compared to the **dU** and **T** analogues. Given the significant activity of hAGT towards repair of **dFU-Me**, this avenue of modification may provide leads in the design of hAGT inhibitors.

Experimental Section

Synthesis and characterization of nucleosides and oligonucleotides.

General Information:

5-fluoro-2'-deoxyuridine (compound **1**) was purchased from Berry Associates (Dexter, Michigan). "Fast deprotecting" 5'-*O*-dimethoxytrityl-2'-deoxyribonucleoside-3'-*O*-(β-cyanoethyl-N,N-diisopropyl)phosphoramidites and protected 2'-deoxyribonucleoside–CPG supports were purchased from Glen Research (Sterling, Virginia). Compounds **2** and **4** were prepared according to previous published procedures.[3,30,31] All other chemicals and solvents were purchased from the Aldrich Chemical Company (Milwaukee, WI) or EMD Chemicals Inc. (Gibbstown, NJ). Flash column chromatography was performed using silica gel 60 (230–400 mesh) purchased from Silicycle (Quebec City, QC). Thin layer chromatography (TLC) was carried out with precoated TLC plates (Merck, Kieselgel 60 F_{254}, 0.25 mm) purchased from EMD Chemicals Inc. (Gibbstown, NJ). All NMR spectra were recorded on a Varian 500 MHz NMR spectrometer at room temperature. ^{1}H NMR spectra were recorded at a frequency of 500.0 MHz and chemical shifts were reported in parts per million (ppm) downfield from tetramethylsilane. ^{13}C NMR spectra (^{1}H decoupled) were recorded at a frequency of 125.7 MHz and chemical shifts were reported in ppm with tetramethylsilane as a reference. ^{19}F NMR spectra were recorded at a frequency of 470.4 MHz and chemical shifts were reported in parts per million (ppm) downfield from trichlorofluoromethane. ^{31}P NMR spectra (^{1}H decoupled) were recorded at a frequency of 202.3 MHz and chemical shifts were reported in ppm with H_3PO_4 used as an external standard. High resolution mass spectrometry of all the modified nucleosides were acquired using an 7T-LTQ FT ICR instrument (Thermo Scientific) at the Concordia University Centre for Structural and Functional Genomics. The mass spectrometer was operated in full scan, positive ion detection mode. ESI mass spectra for oligonucleotides were obtained at the Concordia University Centre for Biological Applications of Mass Spectrometry using a Micromass Qtof2 mass spectrometer (Waters) equipped with a nanospray ion source. The mass spectrometer was operated in full scan, negative ion detection mode. T4 polynucleotide kinase (PNK) was obtained from New England BioLabs (NEB) [γ-^{32}P]ATP was purchased from PerkinElmer (Woodbridge, ON). BclI restriction enzyme was obtained from New England Biolabs (Ipswich, MA).

3'-*O*-(β-Cyanoethyl-*N,N'*-diisopropyl)-5'-*O*-(4,4'-dimethoxytrityl)- 5-fluoro -2'-deoxyuridine (3): DIPEA (0.19 mL, 1.12 mmol) was added to a solution of 5'-O-(4,4'-dimethoxytrityl)-5-fluoro-2'-deoxyuridine (0.204 g, 0.372 mmol) in THF (3.7 mL), followed by the dropwise addition of N,N-diisopropylamino cyanoethyl phosphonamidic chloride (0.2 mL, 0.893 mmol). After 30 min, the solvent was evaporated in vacuo and the crude product was taken up in EtOAc (40 mL). The solution was washed with 3% (w/v) solution of $NaHCO_3$ (2 x 50mL) and once with brine (50mL). The organic layer was dried over anhydrous Na_2SO_4 (~ 4g), and concentrated in vacuo. The crude product was purified by flash column chromatography using a hexanes/EtOAc (2:8) solvent system to afford 0.187g (67%) of a colorless foam. R_f (SiO_2 TLC): 0.87 (100% EtOAc). $\lambda_{max(MeCN)}$ = 268 nm. HRMS (ESI-MS) *m/z* calculated for $C_{39}H_{47}FN_4O_8P^+$: 749.3110; found 749.3104 $[M + H]^+$. ^{1}H NMR (500 MHz, d_6-acetone, ppm): 7.92-7.93 (d, J= 6.3 Hz, 0.5H; H6), 7.90-7.91 (d, J= 6.3 Hz, 0.5H; H6), 7.23–7.53 (m, 9H; Ar), 6.90-6.93 (m, 4H; Ar), 6.28-6.32 (m, 1H; H1'), 4.71-4.78 (m, 1H; H3'), 4.17-4.24 (m, 1H; H4'), 3.59-3.93 (m, 10H; NCH, $ArOCH_3$, CH_2OP), 3.39-3.50 (m, 2H; H5', H5''), 2.77-2.79 (t, 1H; CH_2CN), 2.65-2.67 (t, 1H; CH_2CN), 2.47-2.52 (m, 1H; H2'), 2.06-2.07 (m, 1H; H2''), 1.20-1.22 (m, 10H; CH_3), 1.13-1.14 (m, 2H; CH_3). ^{13}C NMR (125.7 MHz, d_6-acetone, ppm): 206.22, 158.82, 144.96, 135.65, 135.55, 130.12, 130.09, 128.07, 128.03, 127.81, 126.80, 126.77, 113.11, 86.67, 86.61, 85.42, 85.39, 85.08, 85.01, 63.29, 63.15, 58.71, 58.56, 54.64, 43.11, 43.01, 29.41, 29.25, 29.10, 28.94, 28.79, 28.64, 28.48, 24.02, 23.96, 23.93, 19.85, 19.79. ^{19}F NMR (470.4 MHz, d_6-acetone, ppm): -167.84, -167.85, -167.90, -167.91. ^{31}P NMR (202.3 MHz, d_6-acetone, ppm): 148.31, 148.34. IR (thin film); ν_{max} (cm^{-1}) = 3195, 3066, 2967, 2932, 2836, 2362, 2336, 2252, 1717, 1607, 1508, 1464, 1251, 1179, 1125, 829, 736.

5'-*O*-(4,4'-Dimethoxytrityl)-5-fluoro-*O*^{4}-methyl-2'-deoxyuridine (6): To a solution of triazole (0.28g, 4.07mmol) in anhydrous MeCN (6mL) at 0^{o}C under stirring, was added triethylamine (0.543mL, 3.89mmol). Then, a solution of 3'-O-(tert-butyldimethylsilyl)-5'-O-(4,4'-dimethoxytrityl) -5-fluoro-2'-deoxyuridine (0.300g, 0.45mmol) in anhydrous MeCN (6mL) was added to the triazole solution. After 40 min, an additional solution of triazole (0.28g, 4.07mmol), $POCl_3$ (0.084mL, 0.91 mmol) and triethylamine (0.543mL, 3.89mmol) in anhydrous MeCN (3mL) was added dropwise with stirring at 0^{o}C to the solution containing the nucleoside. This extra addition was repeated for a second time after 40 min. After 1 hour, the solvent was evaporated in vacuo, the crude was taken up in MeOH (7 mL) and a solution of NaOMe (0.086g, 1.6mmol) in MeOH (7mL) was added. After 30 min, an additional solution of NaOMe (0.086g, 1.6mmol) in MeOH (3mL) was added to the nucleoside. After 16 h, the solvent was evaporated in vacuo and the crude product was taken up in CH_2Cl_2 (40 mL), washed with a 3% (w/v) solution of $NaHCO_3$ (2 x 50mL), dried over anhydrous Na_2SO_4 (~ 4g), and concentrated in vacuo. The crude product was taken up in THF (4.5mL) and TBAF (1 M in THF) (0.543 mL, 0.54mmol) was added drop-wise. After 30 min, the solvent was evaporated in vacuo and the crude product was taken up in CH_2Cl_2 (40 mL), washed with a 3% (w/v) solution of $NaHCO_3$ (2 x 50mL), dried over anhydrous Na_2SO_4 (~ 4g), and concentrated in vacuo. The crude product was purified by flash column chromatography using a CH_2Cl_2/MeOH (49.5:0.5 → 49:1) solvent system to afford 0.189mg (74%) of a colorless foam. R_f (SiO_2 TLC): 0.63 (100% EtOAc). $\lambda_{max(MeCN)}$ = 283 nm. HRMS (ESI-MS) *m/z* calculated for $C_{31}H_{31}FN_2NaO_7^+$: 585.2008; found 585.2006 $[M + Na]^+$. ^{1}H NMR (500 MHz, $CDCl_3$, ppm): 8.05-8.06 (d, J= 5.6 Hz, 1H; H6), 7.21–7.41 (m, 9H; Ar), 6.83– 6.85 (m, 4H; Ar), 6.25-6.27 (m, 1H; H1'), 4.55-4.57 (m, 1H; H3'), 4.15-4.17 (m, 1H; H4'), 4.04 (s, 3H; OCH_3) 3.79 (s, 6H; $ArOCH_3$), 3.42 (m, 2H; H5', H5''), 3.18 (s, 1H; OH), 2.72-2.76 (m, 1H; H2'), 2.23-2.28 (m, 1H; H2''). ^{13}C NMR (125.7 MHz, $CDCl_3$, ppm): 162.68, 162.58, 158.63, 153.55, 144.31, 137.68, 135.72, 135.42, 135.25, 129.98, 129.96, 127.98, 127.93, 127.68, 127.43, 127.02, 113.31, 113.29, 87.10, 87.02, 86.59, 77.29, 77.03, 76.78, 63.11, 55.22, 55.07, 42.03. ^{19}F (470.4 MHz, in $CDCl_3$, ppm): -168.60, -168.62. IR (thin film); ν_{max} (cm^{-1}) = 3423, 2362, 2335, 1652, 1635, 1 506, 1497, 1403, 1251, 1176, 1035, 828.

3'-*O*-(β-Cyanoethyl-*N,N'*-diisopropyl)-5'-*O*-(4,4'-dimethoxytrityl)- 5-fluoro-*O*^{4}-methyl-2'-deoxyuridine (7): DIPEA (0.14 mL, 0.80 mmol) was added to a solution of (**5**) (0.150 g, 0.266 mmol) in THF (2.7 mL), followed by the dropwise addition of N,N-diisopropylamino cyanoethyl phosphonamidic chloride (0.14 mL, 0.64 mmol). After 30 min, the solvent was evaporated in vacuo, the crude product was taken up in EtOAc (40 mL), the solution was washed with a 3% (w/v) solution of $NaHCO_3$ (2 x 50mL) and once with brine (50mL). The organic layer was dried over anhydrous Na_2SO_4 (~ 4g) and concentrated

in vacuo. The crude product was purified by flash column chromatography using a hexanes/EtOAc (2:8) solvent system to afford 0.173g (86%) of a colorless foam. R_f (SiO_2 TLC): 0.11, 0.26 (1:1 hexanes/EtOAc). $\lambda_{max(MeCN)}$ = 283 nm. HRMS (ESI-MS) *m/z* calculated for $C_{40}H_{49}FN_4O_8P^+$: 763.3267; found 749.3104 $[M + H]^+$. ^{1}H NMR (500 MHz, $CDCl_3$, ppm): 8.15-8.16 (d, J= 6.3 Hz, 0.5H; H6), 8.12-8.13 (d, J= 6.3 Hz, 0.5H; H6), 7.23–7.53 (m, 9H; Ar), 6.89– 6.93 (m, 4H; Ar), 6.18-6.23 (m, 1H; H1'), 4.70-4.78 (m, 1H; H3'), 4.22-4.29 (m, 1H; H4'), 3.97-3.98 (s, 3H; OCH_3), 3.63-3.88 (m, 10H; NCH, $ArOCH_3$, CH_2OP), 3.43-3.52 (m, 2H; H5', H5''), 2.77-2.80 (t, 1H; CH_2CN), 2.60-2.70 (t, 1H; CH_2CN), 2.39-2.47 (m, 1H; H2''), 2.06-2.07 (m, 1H; H2'') 1.20-1.23 (m, 9H; CH_3), 1.12-1.14 (m, 3H; CH_3). ^{13}C NMR (125.7 MHz, $CDCl_3$, ppm): 205.21, 162.27, 162.17, 158.83, 152.36, 144.93, 137.15, 137.12, 135.62, 135.60, 135.51, 135.47, 135.21, 135.18, 130.11, 130.08, 128.06, 127.82, 127.81, 127.68, 127.65, 126.81, 126.79, 118.07, 117.94, 113.13, 113.11, 86.70, 86.65, 86.58, 86.51, 85.73, 85.70, 85.46, 85.41, 73.18, 73.05, 72.71, 72.58, 63.00, 62.80, 58.73, 58.67, 58.58, 58.52, 54.65, 54.63, 53.98, 43.13, 43.11, 43.03, 43.01, 40.36, 40.34, 40.17, 40.13, 29.42, 29.26, 29.11, 28.96, 28.80, 28.65, 19.90, 19.86, 19.84, 19.81. ^{19}F (470.4 MHz, d_6-acetone, ppm): -171.63, -171.64, -171.68, -171.70. ^{31}P NMR (202.3 MHz, d_6-acetone, ppm): 148.35, 148.21. IR (thin film); ν_{max} (cm^{-1}) =3071, 2966, 2869, 2836, 2362, 2335, 2252, 1683, 1652, 1607, 1541, 1506, 1457, 1401, 1336, 1252, 1179, 1116, 1035, 977, 829, 734.

Please see **Supplementary Information** for details concerning the synthesis, purification and characterization of oligonucleotides, NMR and MS spectra, UV thermal denaturation experiments, circular dichroism (CD) spectroscopy, repair assay details, and additional figures.

Acknowledgements

We are grateful to Dr. Anthony E. Pegg (Pennsylvania State University) for the plasmids encoding the wild-type hAGT, OGT and Ada-C genes. We thank Drs. Derek O'Flaherty and Anne Noronha for helpful discussions. This research was supported by grants from the Natural Sciences and Engineering Research Council of Canada (NSERC) and the Canada Foundation for Innovation (CFI). L.S. is the recipient of a Bourse de maîtrise en recherche from the Fonds de recherche du Québec - Nature et technologies (FRQNT), a Concordia Graduate Scholarship in Natural Sciences and Engineering Research and a graduate award from the NSERC CREATE training program in Bionanomachines.

Keywords: O^6-Alkylguanine DNA alkyltransferase, chemically modified oligonucleotides, DNA repair, DNA damage, direct repair.

References:

[1] S. S. Hecht, *Toxicology* **1998**, *11*, 560–603.

[2] P. D. Lawley, D. J. Orr, S. A. Shah, P. B. Farmer, M. Jarman, *Biochem. J.* **1973**, *135*, 193–201.

[3] F. P. Mcmanus, C. J. Wilds, *ChemBioChem* **2014**, *15*, 1966–1977.

[4] A. E. Pegg, *Chem. Res. Toxicol.* **2011**, *24*, 618–39.

[5] A. K. Basu, J. M. Essigmann, *Chem. Res. Toxicol.* **1988**, *1*, 1–13.

[6] D. J. Kalnik, M. W., Kouchakdjian, M., Li, B., Swann, P. F., and Patel, *Biochemistry* **1988**, 108–115.

[7] N. Shrivastav, D. Li, J. M. Essigmann, *Carcinogenesis* **2010**, 59–70.

[8] T. P. Brent, M. E. Dolan, R. Montesano, A. E. Pegg, P. M. Potter, B. Singer, J. A. Swenberg, D. B. Yarosw, *Biochem. J* **1988**, *85*, 1759–1762.

[9] G. T. Pauly, R. C. Moschel, *Chem. Res. Toxicol.* **2001**, *14*, 894–900.

[10] K. B. Altshuler, C. S. Hodes, J. M. Essigmann, *Chem. Res. Toxicol.* **1996**, *9*, 980–987.

[11] G. T. Pauly, S. H. Hughes, R. C. Moschel, *Carcinogenesis* **1998**, *19*, 457–461.

[12] S. R. Paalman, C. Sung, N. D. Clarke, *Biochemistry* **1997**, *36*, 11118–11124.

[13] A. E. Pegg, *Mutation Research* **2000**, 83–100.

[14] K. S. Srivenugopal, X. Yuan, H. S. Friedman, F. Ali-osman, *Biochemistry* **1996**, *35*, 1328–1334.

[15] H. Takinowaki, Y. Matsuda, T. Yoshida, Y. Kobayashi, T. Ohkubo, *Protein Science* **2006**, *15*, 487–497.

[16] G. W. Rebeck, S. Coons, P. Carroll, L. Samson, *Genetics* **1988**, 3039–3043.

[17] F. P. McManus, A. Khaira, A. M. Noronha, C. J. Wilds, *Bioconjugate Chem.* **2013**, *24*, 224–233.

[18] F. P. Mcmanus, D. K. O 'flaherty, A. M. Noronha, C. J. Wilds, *Org. Biomol. Chem* **2012**, *10*, 7078–7090.

[19] F. P. Mcmanus, Q. Fang, J. D. M. Booth, A. M. Noronha, A. E. Pegg, C. J. Wilds, *Org. Biomol. Chem* **2010**, *8*, 4414–4426.

[20] W. J. Bodell, T. Aida, M. S. Berger, M. L. Rosenblum, *Environmental Health Perspectives* **1985**, *62*, 119–126.

[21] Y. Cai, M. H. Wu, M. Xu-welliver, A. E. Pegg, S. M. Ludeman, M. E. Dolan, *Cancer Research* **2000**, *60*, 5464–5469.

[22] R. Loeber, E. Michaelson, Q. Fang, C. Campbell, A. Pegg, N. and Tretyakova, *Chem. Res. Toxicol.* **2010**, *21*, 612–626.

[23] L. Samson, S. Han, J. C. Marquis, *Carcinogenesis* **1997**, *18*, 919–924.

[24] E. M. Duguid, P. A. Rice, C. He, *Journal of Molecular Biology* **2005**, *350*, 657–666.

[25] P. Shah, A. D. Westwell, *Journal of Enzyme Inhibition and Medicinal Chemistry* **2016**, *6366*, 527–540.

[26] D. O. Hagan, H. S. Rzepa, *ChemComm* **1997**, 645–652.

[27] J. Wang, J. Luis, C. Pozo, A. E. Sorochinsky, S. Fustero, V. A. Soloshonok, H. Liu, *Chem. Rev.* **2014**, *114*, 2432–2506.

[28] L. Heidelberger, C., Chaudruri, N.K., Dannerberg, P., Mooren, D. and Griesbach, *Nature* **1957**, *4561*, 663–666.

[29] T. Dowler, D. Bergeron, A. Tedeschi, L. Paquet, N. Ferrari, M. J. Damha, *Nucleic Acids Research* **2006**, *34*, 1669–1675.

[30] Y. Xu, P. F. Swann, *Nucleic Acids Research* **1990**, *18*, 4061–4066.

[31] R. Stolarski, W. Egan, T. L. James, *Biochemistry* **1992**, *31*, 7027–7042.

[32] Q. Zhu, M. O. Delaney, M. M. Greenberg, *Bioorganic and Medicinal Chemsitry Letters* **2001**, *11*, 1105–1107.

[33] H. Bohm, D. Banner, S. Bendels, M. Kansy, B. Kuhn, M. Klaus, U. Obst-sander, M. Stahl, *ChemBioChem* **2004**, *5*, 637–643.

[34] P. Zhou, J. Zou, F. Tian, Z. Shang, *J. Chem. Inf. Model* **2009**, *49*,

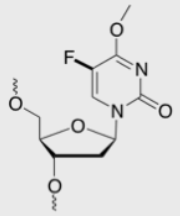
2344–2355.
[35] O. J. Wilkinson, V. Latypov, J. L. Tubbs, C. L. Millington, R. Morita, H. Blackburn, *Proceedings of the National Academy of Sciences* **2012**, 18755–18760.

Entry for the Table of Contents (Please choose one layout)

Layout 1:

FULL PAPER

This reports demonstrates that fluorine at the C5 position of 2'-deoxyuridine enhances the susceptibility of methyl adducts at the O^4 position to be removed by human AGT.



Lauralicia Sacre and Christopher J. Wilds [a]*

Page No. – Page No.

Title: Fluorine at the *C*5 position of 2'-deoxyuridine enhances repair of a O^4-methyl adduct by O^6-Alkylguanine DNA Alkyltransferases.

Layout 2:

FULL PAPER

((Insert TOC Graphic here; max. width: 11.5 cm; max. height: 2.5 cm))

*Author(s), Corresponding Author(s)**

Page No. – Page No.

Title

Text for Table of Contents

[a] *Lauralicia Sacre and Christopher J. Wilds**
Department of Chemistry and Biochemistry and Centre for Structural and Functional Genomics, Concordia University, 7141 Sherbrooke St. West, Montréal, Québec (Canada) H4B 1R6
E-mail: chris.wilds@concordia.ca

Supporting information for this article is given via a link at the end of the document.((Please delete this text if not appropriate))