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Research Article

The synthesis of a new probe for PET imaging reporter gene HSV1-tk: 2-amino-6-[^{18}F] fluoro-9- (4-hydroxy-3-hydroxymethylbutyl) purine (6-[^{18}F]fluoropenciclovir)

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Summary

The one step radiosynthesis of 2-amino-6- [^{18}F]fluoro-9-(4-hydroxy-3-hydroxymethyl-butyl) purine (6-[^{18}F]fluoropenciclovir) **6** is reported. Radiolabeled product 6-[^{18}F]fluoropenciclovir **6** was prepared by radiofluorination of compound **4** with [^{18}F]KF and isolated by a silica Sep-Pak cartridge. The radiochemical yield of compound **6** was 45–55% decay corrected (d.c.) in six runs with radiochemical purity >98% and the radiosynthesis time was 35–42 min from end of bombardment (EOB). Copyright © 2006 John Wiley & Sons, Ltd.

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Key Words: fluorine-18; synthesis; penciclovir; radiofluorination

Introduction

The noninvasive PET imaging of reporter gene herpes simplex virus type 1 thymidine kinase (HSV1-tk) *in vivo* using corresponding reporter probes could provide valuable information for monitoring gene therapy of cancer.[1–4] Many of radiolabeled nucleoside derivatives have been under investigation as probes for PET imaging reporter gene HSV1-tk. However, the short half-life of ^{11}C

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and potential for de-iodination of ^{124}I are less than optimal for imaging purposes. The ^{18}F-labeled nucleoside derivatives should be more advantageous.[5] The ^{18}F-labeled compounds, which include mainly pyrimidine nucleoside derivatives 2′-deoxy-2′-[^{18}F]fluoro-1-ß-D-arabinofuranosyluracil and its 5-substituted nucleosides, and acylguanosine derivatives such as 8-[^{18}F]fluoroganciclovir (FGCV), 8-[^{18}F]fluoropenciclovir (FPCV), 9-[(3-[^{18}F]fluoro-1-hydroxy-2-propoxyl) methyl]guanine (FHPG) or 9-(4-[^{18}F] fluoro-3-hydroxymethylbutyl) guanine (FHBG), are currently investigated as probes of PET imaging reporter gene HSV1-tk.[6–11] The chemical structures of these probes were shown in Figure 1. Comparing the probes, although ^{18}F-labeled pyrimidine nucleoside derivatives were better than acylguanosine derivatives in sensitivity, the routine radiosynthesis of pyrimidine nucleosides

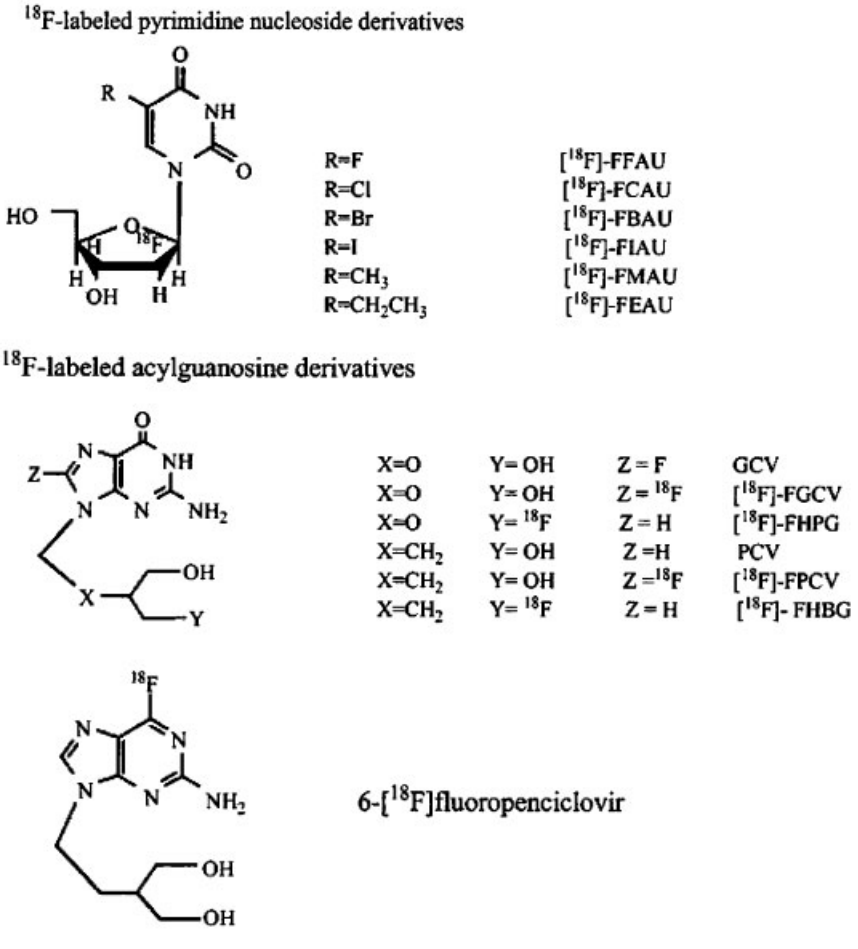

Figure 1. ^{18}F-labeled compounds as probes for PET imaging reporter gene HSV1-tk

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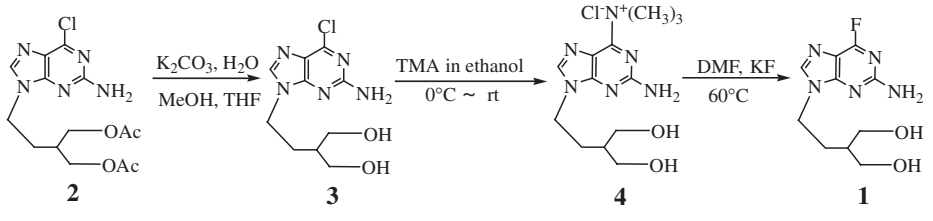
were multi-step and time-consuming. As to acylguanosine derivatives, although the radiosynthesis of FPCV and FGCV were electrophilic reactions and low yields, and nucleophilic fluorinated penciclovir analogues (including FPCV and FHBG) had many advantageous characteristics over fluorinated ganciclovir (including FGCV and FHPG). The improved pharmaceutical characteristics of penciclovir led to investigate its further potential use as a probe for PET imaging the reporter gene HSV1-tk.[12] Exploring the active site of Herpes Simplex Virus Type 1 Thymidine Kinase (HSV1-tk) with penciclovir showed that 6-position of the purine ring was pertinent to activity of Penciclovir.[13,14] And the esters of 2-amino-6-fluoro-9- (4-hydroxy-3-hydroxymethylbutyl) purine as anti-viral agents brought the encouraging results.[15] To develop more sensitive and selective probes for PET imaging reporter gene HSV1-tk, in this article, we reported the one step radiosynthesis of 6-[^{18}F]fluoropenciclovir **6** for PET imaging reporter gene HSV1-tk.

Results and discussion

Chemistry

The reference compound 6-fluoropenciclovir **1** was synthesized from intermediate 2-amino-6-chloro-9- (4-acetoxy-3-acetoxy-methylbutyl) purine **2** as shown in Scheme 1.[15,16]



Scheme 1. Synthesis of 6-fluoropenciclovir (1)

2-amino-6-chloro-9-(4-acetoxy-3-acetoxy-methylbutyl) purine **2** was hydrolyzed to 2-Amino-6-chloro-9- (4-hydroxy-3-hydroxymethylbutyl) purine **3** by using potassium carbonate in water solution, and then was converted to the corresponding trimethylammonium chloride **4** by treatment with trimethylamine in ethanolic solution.[16]

The compound **4** was reacted with potassium fluoride in DMF solvent to yield 2-amino-6-fluoro-9- (4-hydroxy-3-hydroxymethylbutyl) purine **1** with the yield of 87.3%. This reaction was complicated by competition with Hofmann degradation, in which the trimethylammonio-group was degraded to a dimethylamino-group under high temperature of more than 60°C as shown in Scheme 2. The affect the temperatures had on the synthesis of compound **1**

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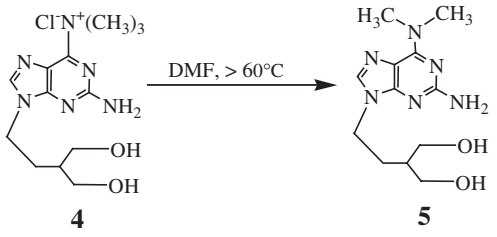

Scheme 2. Degradation of 2-amino-9-(4-hydroxy-3-hydroxymethylbutyl) -*N*, *N*, *N*-trimethyl-9H-purin-6-aminium chloride (4)

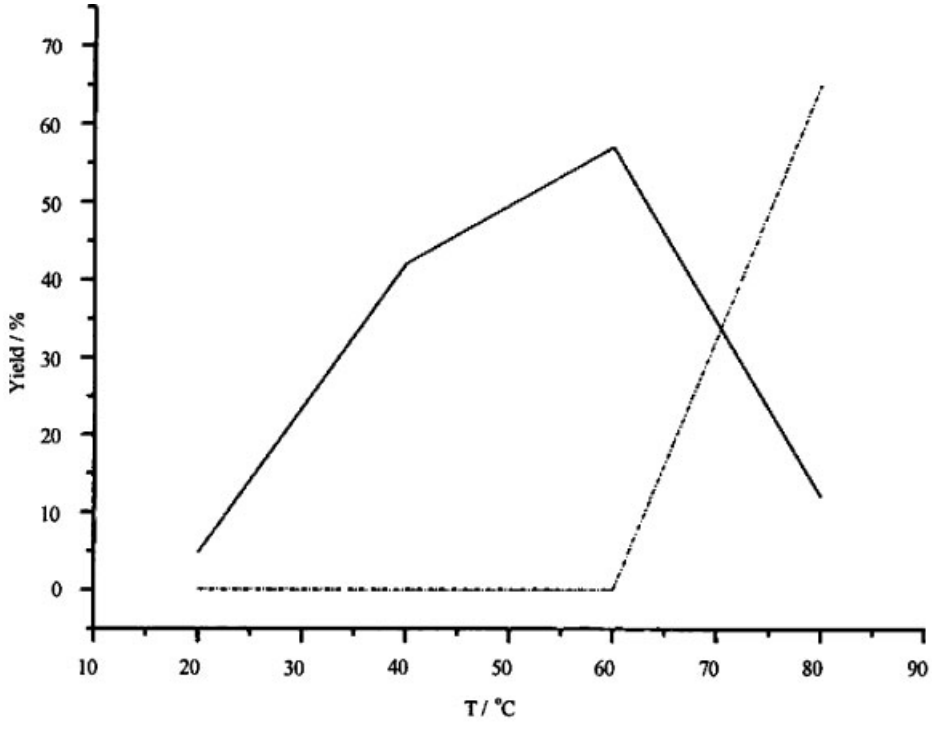

Figure 2. The yield (%) of 2-amino-6-fluoro-9- (4-hydroxy-3-hydroxymethylbutyl) purine (1) and 2-amino-6-dimethylamino-9-(4-hydroxy-3-hydroxymethylbutyl)purine (5) under different temperatures; — Comp. 1, —Comp. 5; the yield (%) is indicated as compound 1 or 5 was reacted for an hour under different temperatures

or degradation of compound **4** were investigated as indicated in Figure 2, and it was found that the desired compound **1** was prepared in good yield when temperature was at 60°C, but the yield of compound **1** was decreased due to formation of 2-amino-6-dimethylamino-9-(4-hydroxy-3-hydroxymethylbutyl) purine **5** when temperature was more than 60°C.

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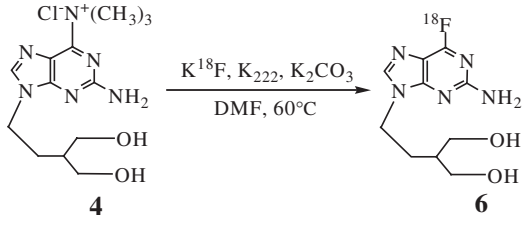
Radiochemistry

The radiosynthesis of 6-[^{18}F]fluoropenciclovir **6** was based on one step reaction as shown in Scheme 3. The nucleophilic displacement of trimethylammonio-group as a route to fluoropurine derivatives appeared to be a better fluorination method.[17,18] ^{18}F-labeled 6-fluoropurine and 6-fluoro-ß-D-ribofuranosylpurine were prepared by nucleophilic displacement of the trimethylammonio-group with $^{18}F^-$.[19] So we substituted *O*6-position of the purine ring of compound **4** to 6-[^{18}F]fluoropenciclovir by nucleophilic radiofluorination with K^{18}F in one step. The trimethylammonium chloride precursor **4** was labeled by conventional nucleophilic substitution with K^{18}F/K2.2.2 in DMF solvent at 60°C for 15 min to afford the radiolabeled compound **6**. The crude product was purified by a silica Sep-Pak cartridge. The radiochemical yield of 6-[^{18}F]fluoropenciclovir was 45–55% in six runs and the synthesis time was 35–42 min from end of bombardment (EOB). Radiochemical purity of 6-[^{18}F]fluoropenciclovir was determined by analytical HPLC as indicated in Figure 3. Comparison HPLC chromatogram of the final



Scheme 3. Synthesis of 6-[^{18}F]fluoropenciclovir (6)

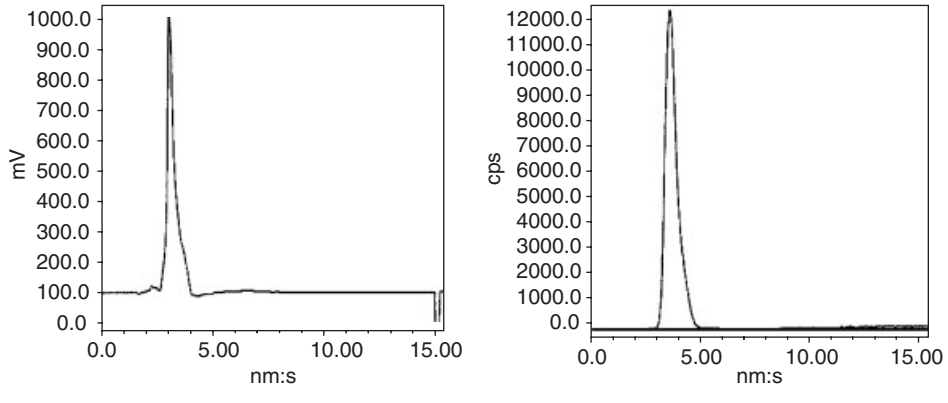


Figure 3. HPLC chromatogram of 6-[^{18}F]fluoropenciclovir (6), co-injected with standard 6-fluoropenciclovir (1): C_{18} analytical column; solvent system 90% MeCN/H_2O; flow rate 0.9 ml/min

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product with the reference compound 6-fluoropenciclovir showed that both the radiolabeled and standard 6-fluoropenciclovir were co-eluted at 3.4 min, the radiochemical purity of the 6-[^{18}F]fluoropenciclovir was $>98\%$.

Experimental

Materials and methods

2-Amino-6-chloro-9-(4-acetoxy-3-acetoxy-methylbutyl)purine was obtained as gifts from Changzhou Kony Pharmaceuticals Co., Ltd (Changzhou, China). No-carrier-added [^{18}F]F^- was supplied by Amershan-Kexing pharmaceuticals Co., Ltd (Shanghai, China), and was produced via ^{18}O (p, n) ^{18}F reaction using enriched [^{18}O]H_2O on a cyclotron-30 (IBA, Belgium). The other solvents and reagents were commercially available and used without further purification unless stated. Compounds **3**, **4**, **5** and reference compound 6-fluoropenciclovir **1** were prepared using the following method which was developed in our laboratory.

High performance liquid chromatography (HPLC) analysis was performed on a Dionex system equipped with a P680 pump, PDA-100 photodiode array detector and a NaI(Tl) scintillation detector. The column used for identification of reference compound and radioactive products was μ Bondapak^{TM} C_{18} analytical column (3.9 × 300 mm) and eluted with CH_3CN: H_2O (9:1, v/v) at a constant flow rate of 0.9 ml/min. Analytical thin layer chromatography (TLC) was carried out on precoated plate (silica gel 60 F_{254}), and spots were visualized with ultraviolet light or a Bioscan AR-2000 radioanalyzer. The silica Sep-Pak cartridge for solid phase extraction was purchased from Waters Corporation. Flash column chromatography was carried out on silica gel (200–300 mesh) with common flow of the solvent system with CH_3OH: CH_2Cl_2 (1:9, v/v). Melting points (m.p.) were determined on a WRS-1A melting points apparatus and were uncorrected. ^{1}H and ^{19}F NMR spectra were recorded on a Bruker AC-500 (500 MHz) instrument. Electron impact mass spectra (EI-MS) were obtained on MicroMass GCT CA 055 spectrometers. Infrared spectra (IR) were recorded on Avatar 370 FT-IR Thermo Nicolet.

2-Amino-6-fluoro-9-(4-hydroxy-3-hydroxy-methylbutyl) purine **1**

Compound **4** (165 mg, 0.5 mmol) dissolving in DMF (5 ml) was reacted with anhydrous KF (240 mg, 5 mmol) at 60°C until the TLC analysis showed no significant amount of starting material remained. The reaction mixture was cooled to room temperature and filtered. The filtrate was evaporated to dryness *in vacuo*. The crude product was purified by column chromatography and was eluted with 10% MeOH/CH_2Cl_2. The desired product white solid (111 mg) was obtained in 87.3% yield; m.p. 173.5–175.4°C; IR (KBr): 3320,

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3310, 2930, 1660, 1570, 1410, 1220, 1030, 783, 625 cm^{-1}; ^1H NMR (DMSO-d_6): $\delta = 8.10$ (s, 1H, C_8H), 4.15–4.05 (t, 2H, 4′H–CH_2, $J = 7.42$ Hz), 3.45–3.30 (m, 4H, OCH_2), 1.80–1.70 (m, 2H, CH_2CH), 1.50–1.35 (m, 1H, CH_2CH); ^{19}F NMR (DMSO-d_6): δ–74.12 (s, 1F); MS m/z (%): 255 (M^+, 26), 224 (27), 153 (100).

2-Amino-6-dimethylamino-9-(4-hydroxy-3-hydroxymethylbutyl)purine **5**

Compound **4** (165 mg, 0.5 mmol) dissolving in DMF (5 ml) was heated and stirred at 80°C several hours until the TLC analysis showed no significant amount of starting material remained. The reaction mixture was cooled to room temperature and filtered. The filtrate was evaporated to dryness *in vacuo*. The crude product was purified by column chromatography and eluted with 10% MeOH/CH_2Cl_2. The desired product (95.2 mg) was obtained in 68%. IR (KBr): ν 3360, 2930, 1940, 1590, 1410, 1210, 1040, 783 cm^{-1}; ^1H NMR (DMSO-d_6): $\delta = 7.70$ (s, 1H, C_8H), 6.70 (s, 2H, NH_2), 4.58–4.52 (m, 2H, OH), 4.02–3.97 (t, 2H, 4′H–CH_2, $J = 7.57$ Hz), 3.55 (s, 6H, CNH_3), 3.45–3.26 (m, 4H, OCH_2), 1.70–1.65 (m, 2H, CH_2CH), 1.50–1.40 (m, 1H, CH_2CH); MS m/z (%): 280 (M^+, 96), 263 (40), 155 (100).

2-Amino-6-[^{18}F] fluoro-9-(4-hydroxy-3-hydroxymethylbutyl) purine **6**

2-Amino-6-[^{18}F]fluoro-9-(4-hydroxy-3-hydroxymethylbutyl) purine was synthesized using no-carrier-added [^{18}F]F^- in the form of potassium fluoride containing potassium carbonate (1–2 mg) and K2.2.2 (10–12 mg) in aqueous solution. This [^{18}F]KF solution was added to a V-vial. Water was azeotropically evaporated from this mixture using HPLC grade acetonitrile (3×0.5 ml) in an oil bath at 90–100°C under a stream of nitrogen. This anhydrous K^+/K2.2.2. pair complex was dissolved dry DMF (1.0 ml). Then the precursor compound **4** (3–5 mg) was added the DMF solution containing $^{18}F^-$.The reaction mixtures were heated for 15 min at 60°C. After the reaction mixture was cooled, the crude product was passed through a silica Sep-Pak cartridge to remove K2.2.2. and unreacted fluoride. The silica Sep-Pak cartridge was eluted with 50% MeOH/CH_2Cl_2 (4.0 ml) to yield the pure product compound **6**. The solvent was evaporated at 90–100°C under a stream of nitrogen, and then the pure product was dissolved in PBS solution. The mixture was sterile-filtered through a 0.22 μm filter and collected into a sterile vial. The identification of the final product compound **6** was carried out by analytical HPLC and compared with the reference compound, it was found that radiochemical purity of compound **6** was >98% and the radiochemical yield was 45–55% in six runs. The synthesis time was 35–42 min from EOB.

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Conclusion

We have developed an one step synthetic scheme for the preparation of 2-amino-6-[^{18}F]fluoro-9-(4-hydroxy-3-hydroxymethylbutyl) purine (6-[^{18}F] fluoropenciclovir) as a new agent for PET imaging reporter gene HSV1-tk. The radiochemical yield of 6-[^{18}F]fluoropenciclovir was 45–55% decay corrected (d.c.) in six runs with radiochemical purity $>98\%$ and the synthesis time was 35–42 min from EOB.

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References

1. Gambhir SS, Barrio JR, Herschman HR, Phelps ME. *Nucl Med Biol* 1999; **26**: 481–490.
2. Yaghoubi SS, Barrio JR, Dahlbom M, Iyer M, Namavari M, Satyamurthy N, Goldman R, Herschman HR, Phelps ME, Gambhir SS. *J Nucl Med* 2001; **42**: 1225–1234.
3. Wiebe LI, Knaus EE. *Curr Pharmaceutical Des* 2001; **7**: 1893–1906.
4. Urbrain JC. *J. Nucl Med* 2001; **42**: 106–109.
5. Alauddin MM, Conti PS, Fissekis JD. *J Label Compd Radiopharm* 2002; **45**: 583–590.
6. Alauddin MM, Conti PS. *Nucl Med Biol* 1998; **25**: 175–180.
7. De Vries EFJ, Van Dillen IJ, Van Waarde A, Willemsen ATM, Vaalburg W, Mulder NH, Hospers GAP. *Nucl Med Biol* 2003; **30**: 651–660.
8. Namavari M, Barrio JR, Toyokuni T, Gambhir SS, Cherry SR, Herschman HR, Phelps ME, Satyamurthy N. *Nucl Med Biol* 2000; **27**: 157–162.
9. Alauddin MM, Conti PS, Fissekis JD. *J Label Compd Radiopharm* 2003; **46**: 285–289.
10. Alauddin MM, Shahinian A, Park R, Tohme M, Fissekis JD, Conti PS. *Nucl Med Biol* 2004; **31**: 399–405.
11. Buursma AR, Rutgers V, Hospers GAP, Mulder NH, Vaalburg W, De Vries EFJ. *Nucl Med Commun* 2006; **27**: 25–30.
12. Iyer M, Barrio JR, Namavari M, Bauer E, Satyamurthy N, Nguyen K, Toyokuni T, Phelps ME, Herschman HR, Gambhir SS. *J Nucl Med* 2001; **42**: 96–105.
13. Pilger BD, Perozzo R, Alber F, Wurth C, Folkers G, Capozza L. *J. Biol Chem* 1999; **274**: 31967– 31973.

14. Champness JN, Bennett MS, Wien F, Visse R, Summers WC, Herdewijn P, De Clercq E, Ostrowski T, Jarvest RL, Sanderson MR. *Proteins* 1998; **32**: 350–361.
15. Kim DK, Lee N, Kim HT, Im GJ, Kim KH. *Bioorg Med Chem* 1999; **7**: 565–570.
16. Cai HC, Yin DZ, Zhang L, Wang YX. *J Fluor Chem* 2006; **128** (in press, available online 3 May, 2006).
17. Kiburis J, Lister JH. *J Chem Soc (C)* 1971; **23**: 3942–3947.
18. Gurvich V, Kim HY, Hodge RP, Harris CM, Harris TM. *Nucleos Nucleo* 1999; **18**: 2327–2333.
19. Irie T, Fukushi K, Ido T. *J Appl Radiat Isot* 1982; **33**: 445–448.

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