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Research Article

Synthesis of 1-(2′-deoxy-2′-fluoro-β-D-arabinofuranosyl)-[*Methyl*-^{11}C]thymine ([^{11}C]FMAU) *via* a Stille cross-coupling reaction with [^{11}C]methyl iodide

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Summary

1-(2′-deoxy-2′-fluoro-β-D-arabinofuranosyl)-[*methyl*-^{11}C]thymine ([^{11}C]FMAU) [^{11}C]-**1** was synthesised *via* a palladium-mediated Stille coupling reaction of 1-(2′-deoxy-2′-fluoro-β-D-arabinofuranosyl)-5-(trimethylstannyl)uracil **2** with [^{11}C]methyl iodide in a one-pot procedure. The reaction conditions were optimized by screening various catalysts and solvents, and by altering concentrations and reaction temperatures. The highest yield was obtained using $Pd_2(dba)_3$ and P(*o*-tolyl)$_3$ in DMF at 130°C for 5 min. Under these conditions the title compound [^{11}C]-**1** was obtained in 28 ± 5% decay-corrected radiochemical yield calculated from [^{11}C]methyl iodide (number of experiments = 7). The radiochemical purity was > 99% and the specific radioactivity was 0.1 GBq/μmol at 25 min after end of bombardment. In a typical experiment 700–800 MBq of [^{11}C]FMAU [^{11}C]-**1** was obtained starting from 6–7 GBq of [^{11}C]methyl iodide. A mixed ^{11}C/^{13}C synthesis to yield [^{11}C]-**1**/(^{13}C)-**1** followed by ^{13}C-NMR analysis was used to confirm the labelling position. The labelling procedure was found to be suitable for automation.

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Introduction

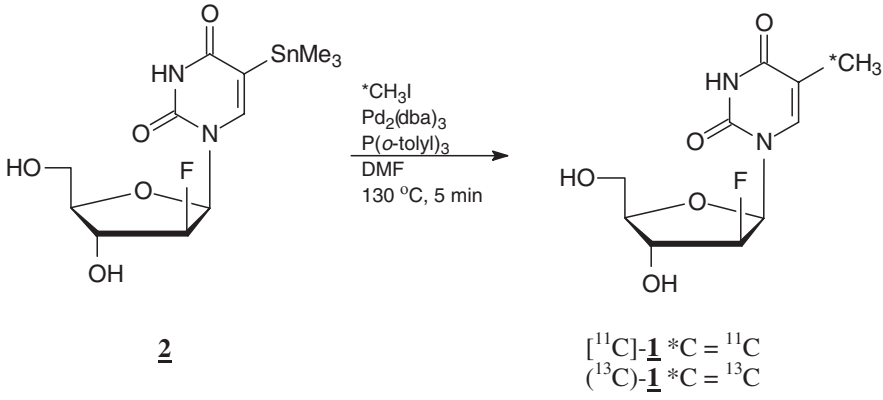
^{11}C or ^{18}F-labelled 1-(2′-deoxy-2′-fluoro-β-D-arabinofuranosyl)thymine ([^{11}C]- or [^{18}F]FMAU) have been proposed as potential tracers for imaging cell proliferation *in vivo* using Positron Emission Tomography.[1–4] [^{11}C]FMAU has previously been synthesized in two different ways, both involving multi-step syntheses. The first and most commonly applied method involves reacting the dianion of 1-(3′,5′-*O*-bis-(tetrahydropyranyl)-2′-fluoro-β-D-arabinofuranosyl)uracil with [^{11}C]methyl iodide, followed by subsequent deprotection of the hydroxyl groups using HCl in methanol.[3] The reported yields have been variable ranging from 10 to 50% and a specific radioactivity of up to 3.7 GBq/μmol. The second method is based on enzymatic reactions in a set of three bioreactors utilizing [^{11}C]methanol and 1-(2′-fluoro-2′deoxy-β-D-arabinofuranosyl)uridine.[5] The reported yield of the labelled product has been low, only around 4%.

The Stille reaction has been shown to be an efficient radiolabelling route for the introduction of [^{11}C]methyl groups on aromatic or vinylic substrates.[6] The method is a palladium-mediated coupling reaction between an organostannane and [^{11}C]methyl iodide forming a new ^{11}C–C bond. The reason for using this reaction is that the reaction is tolerant to a variety of functional groups in both the halide and the stannane, and the reaction takes place under neutral conditions.

In this paper we describe a fully automated one-step, one-pot labelling method based on the Stille reaction for the synthesis of [^{11}C]-**1**.

Results and discussion

The synthesis is conducted by a one-pot procedure in which [^{11}C]methyl iodide is reacted with a trimethylstannyl precursor in the presence of $Pd_2(dba)_3$ (dba = dibenzylideneacetone) and P(*o*-tolyl)$_3$ in DMF (Scheme 1). Under these mild reaction conditions, there is no need for protection of the hydroxyl groups. A number of parameters that may influence the radiochemical yield were investigated, such as the solvent,



2

[^{11}C]-**1** *C = ^{11}C
(^{13}C)-**1** *C = ^{13}C

Scheme 1. Synthesis of [^{11}C]/(^{13}C)-FMAU *via* a Stille coupling with [^{11}C]/(^{13}C)methyl iodide

reaction temperature, and concentration of the stannane and the catalyst.[7] The choice of ligand for the catalyst depends on a number of parameters such as steric and electronic factors, solvent, air sensitivity, thermo-stability and availability. The steric and electronic factors are often described as the cone angle (for monodentate ligands) or bite angle (for bidentate ligands) and pK_a, respectively.[8] The *in situ* generation of the catalyst from $Pd_2(dba)_3$ and a ligand is convenient since the type and amount of ligand can easily be varied.

The results from the investigation of different ligands are presented in Table 1. Using $Pd_2(dba)_3$ in combination with P(*o*-tolyl)$_3$ (entries 1–3), [^{11}C]-FMAU was produced in approximately 30% decay-corrected radiochemical yield, independent of the number of equivalents of the phosphine. This was somewhat surprising knowing from previous reports that the ratio of the Pd and ligand is important.[9] In our case the halide concentration is very low, and naturally this will effect the course of the reaction. The reason for the success of using P(*o*-tolyl)$_3$ is probably the very large cone angle (194°) for this ligand which would result in the release of steric strain in the transmetallation step, combined with a basicity comparable to that of PPh_3 resulting in rapid oxidative addition.[10]

The use of the catalyst Pd[P(*o*-tolyl)$_3$]$_2$ resulted in the formation of product, but in slightly lower yield (entry 4). Using other monodentate ligands like tri-2-furylphosphine (TFP), triphenylarsine ($AsPh_3$) or triphenylphosphine (PPh_3) (entries 5–8) resulted in no product

Table 1. Effect of different ligands[a]

Entry	Pd source	Ligand	Ratio Pd:L	Yield (n)[b]
1	$Pd_2(dba)_3$	P(o-tolyl)$_3$	1 : 2	28 ± 5 (7)
2	$Pd_2(dba)_3$	P(o-tolyl)$_3$	1 : 4	29 (1)
3	$Pd_2(dba)_3$	P(o-tolyl)$_3$	1 : 6	27 (1)
4[c]	Pd[P(o-tolyl)$_3$]$_2$	—	1 : 2	22 ± 7 (4)
5	$Pd_2(dba)_3$	TFP	1 : 4	0 (2)
6	$Pd_2(dba)_3$	$AsPh_3$	1 : 4	0 (2)
7	$Pd_2(dba)_3$	PPh_3	1 : 2	0 (1)
8	$Pd_2(dba)_3$	PPh_3	1 : 4	0 (2)
9	$Pd_2(dba)_3$	dppp	1 : 1	0 (2)
10	$Pd_2(dba)_3$	—	—	0 (1)

[a] All reactions were performed using 1 μmol $Pd_2(dba)_3$, 4–12 μmol ligand (except for entry 4) and 2 μmol stannane **2** in 300 μl DMF at 130°C for 5 min.
[b] Isolated decay-corrected radiochemical yield counting from [^{11}C]methyl iodide. Number of experiments in parentheses.
[c] 4 μmol Pd[P(o-tolyl)$_3$] was used.

Table 2. Effect of microwave heating[a]

Entry	Power (W)	Time (min)	Yield (n)[b]
1	100	3	19 (1)
2	70	3	27 (1)
3	50	3	18 (1)

[a] All reactions were performed using 1 μmol $Pd_2(dba)_3$, 4 μmol ligand and 2 μmol stannane **2** in 300 μl DMF.
[b] Isolated decay-corrected radiochemical yield counting from [^{11}C]methyl iodide. Number of experiments in parentheses.

formation at all. The bidentate ligand 1,3-bis(diphenylphosphino)propane (dppp) was thought to improve the reaction rate by the facilitated reductive elimination from the *cis*-PdRR'L_2 complex formed.[11,12] However, no product was obtained under these conditions (entry 9). The Stille reaction has also been reported to proceed successfully under 'ligandless' conditions.[13] In our investigation the use of $Pd_2(dba)_3$ alone resulted in no product formation (entry 10).

Microwave heating can enhance the rate of the Stille reaction as compared with thermal heating.[14] Operating at 100 W for 3 min gives the product in 19% radiochemical yield (Table 2, entry 1). Suspicion of fast decomposition of the catalyst under these harsh conditions prompted us to use a lower power. When running the reaction at 70 or 50 W the yield was comparable to that using conventional heating or

lower (entries 2–3). Even though the reaction time was improved using microwave heating, it was not considered a realistic option for automated synthesis because at present the microwave oven is not integrated with our robotic system.

The effects of catalyst and substrate concentration were also investigated. A small increase in the palladium concentration does not affect the radiochemical yield, but doubling the concentration of palladium or lowering it tenfold resulted in decreased yields. The yield of the product is not improved by the use of more stannane, but using half the amount resulted in less product being formed.

The solvent is another important factor for the reaction rate. Polar aprotic solvents are known to be favourable for the Stille reaction.[15] Moreover, these solvents are excellent for trapping the [^{11}C]methyl iodide (>90% trapping in DMF). We have previously obtained good results in our group using DMF[6b] and therefore this was the solvent of choice. Reaction using DMSO yielded no product whereas a 1:1 mixture of DMF and DMSO gave rise to a radiochemical yield of 8%.

The effect of the temperature was finally investigated. Previous results show that high temperatures are sometimes necessary in this type of reaction.[6b] At first, 130°C was used in the reaction which resulted in the highest yields. Lowering of the reaction temperature to 100°C resulted in a reduced radiochemical yield (14 ± 2%). In order to try to reduce the reaction temperature further without decreasing the radiochemical yield a method was applied where potassium carbonate and copper(I) chloride is added to the reaction mixture and heated to 65°C. The method has been reported to reduce both reaction times and temperatures in the Stille reaction,[16] but only gave 6% radiochemical yield when applied to our system.

To determine the position of the label, a mixed ^{11}C/^{13}C synthesis was performed (Scheme 1). After ^{11}C decay, the product (^{13}C)-**1** was analysed by ^{13}C-NMR. One signal was observed ($\delta = 12.2$ ppm) which corresponds to the methyl signal of the authentic reference compound.

During the development of the synthesis the starting radioactivity used was around 6–7 GBq of [^{11}C]methyl iodide, resulting in a decay-corrected radiochemical yield of 30%. An interesting observation was made when using larger batches of [^{11}C]methyl iodide in the reaction. When the starting radioactivity was increased to 10–12 GBq, the radiochemical yield dropped to 19–24% ($n = 3$). Further increase up to 20–30 GBq resulted in even lower yields, approximately 15% ($n = 2$). The amount of radioactivity in the final product was practically the

same in all cases, regardless of the starting radioactivity. The phenomenon has been observed previously in our research group when the Stille reaction was employed. So far, we have no explanation but we speculate that it might be due to radiolysis of the stannane.

The mass of the product was fairly constant regardless the batch and the total amount was 6 ± 0.3 μmol ($n = 2$). The specific radioactivity of [^{11}C]FMAU varies with the amount of radioactivity, from 0.1 GBq/μmol for a 7 GBq batch of [^{11}C]methyl iodide up to 0.8 GBq/μmol for a full batch (30 μAh bombardment). From previous work in our group, specific radioactivities of 100 GBq/μmol for tracers synthesized *via* the Stille reaction have been reported.[16] The reason for the low specific radioactivity in this case might be explained by a side reaction occurring when using trimethylstannanes rather than tributylstannanes.[6c] Possibly, a cross-coupling between one of the methyl groups on the tin and the nucleoside part can occur yielding FMAU and thus lowering the specific radioactivity. From our experience, trimethyl stannanes give higher yields than their tributyl counterparts[6a] and consequently we decided to use **2** in the coupling reaction despite the side-reaction. In the biological studies planned with [^{11}C]FMAU the specific radioactivity obtained is sufficient.

Experimental

General

[^{11}C]Carbon dioxide was produced by the reaction ^{14}N(p,α)^{11}C by the Scanditronix MC-17 cyclotron at the Uppsala University PET Centre using a gas target containing nitrogen (AGA, Nitrogen 6.0) and 0.05% oxygen (AGA, Oxygen 6.0) bombarded with 17 MeV protons. An automated synthesis system, Synthia,[17] was used for the production of [^{11}C]methyl iodide, heating of the reaction mixture, dilution, HPLC injection, fraction collection and sterile filtration. [^{11}C]methyl iodide was synthesized according to the previously published procedure.[18]

HPLC was performed using a Beckman 126 gradient pump and a Beckman 166 variable wavelength UV-absorbance detector in series with a β^+-flow detector. Data collection was performed using the Beckman System Gold chromatography software package for semi-preparative and analytical LC. Semi-preparative LC was performed at room temperature using a Beckman Ultrasphere ODS C_{18} (5 μm,

250 × 10 mm i.d.) and analytical LC using a Beckman Ultrasphere ODS C_{18} (5 μm, 250 × 4.6 mm i.d.). Mobile phases were saline (NaCl, 9 mg/ml) (A), 99.5% ethanol (B), 25 mM KH_2PO_4, pH 4.7 (C) and acetonitrile:water 50:7 (D). In the analysis of the ^{11}C-labelled compound, unlabelled reference substance was added in the HPLC runs, using UV-absorbance detection at 254 nm.

^{13}C-NMR spectra were recorded in DMSO-d_6 on a Varian Unity 400 (400 MHz) spectrometer at 25°C using the solvent as internal reference ($\delta = 39.5$ ppm). LC-MS was performed using a Fisons Platform mass spectrometer equipped with pneumatically assisted electrospray ionization (ESI^+), a Beckman 126 solvent delivery module, and a Beckman 166 variable wavelength UV absorbance detector in series with a β^+-flow detector and a CMA 200 autosampler.

Microwave experiments were performed using a MicroWell 10 single-mode microwave cavity producing constant irradiation (2450 MHz) from Personal Chemistry AB, Uppsala, Sweden. The reaction vessels used were heavy-walled borosilicate tubes with screw-cap vials equipped with teflon-coated rubber septa. Tubes with narrow inner diameter were used creating a high sample height to ensure more efficient energy absorption.

The determination of tin and palladium residues in the product fraction was performed by inductively coupled plasma time-of-flight mass spectrometry (ICP-TOFMS) using a Renaissance spectrometer (LECO, USA). The instrument was used with standard settings for all operational parameters and calibrated towards standards from Spectrascan (Referensmaterial AB, Ulricehamn, Sweden). The isotopic masses used were Pd (104, 105, 106 *m/z*) and Sn (116, 118, 119, 120 *m/z*). All mass to charge ratios gave the same results indicating no isobaric overlaps. The detection limit is about 15 ng/ml for both elements in the actual sample type.

Sterile filtration was performed using a Dynagard ME filter, 0.22 μm pore size purchased from AB Göteborgs termometerfabrik, Västra Frölunda, Sweden. *N,N*-Dimethylformamide 99.8%, dimethylsulfoxide 99.9%, 1.0 M lithium aluminum hydride in THF, hydroiodic acid (57%), tris(dibenzylideneacetone)dipalladium(0), tri-*o*-tolylphosphine, triphenylphospine, tri(2-furyl)phosphine, triphenylarsine and 1,3-bis (diphenylphosphino)propane, copper(I) chloride and potassium carbonate were purchased from Sigma-Aldrich Sweden AB, Stockholm, Sweden and were used without further purification. Ethanol (99.5%) was purchased from Kemetyl AB, Haninge, Sweden; Sodium chloride

(9 mg/ml) in sterile water from Fresenius Kabi Norge AS, Halden, Norway; acetonitrile (for HPLC, gradient grade) from Riedel-de Haën and KH_2PO_4 (p.a.) from E. Merck AB, Stockholm, Sweden.

Bis(tri-*o*-tolylphosphine)palladium (0) was a kind gift from Dr Hisashi Doi, Uppsala University. 1-(2′-deoxy-2′-fluoro-β-D-arabinofuranosyl)-5-trimethylstannyluracil **2** was purchased from Chemische Laboratorien Dr Christoph Mark, Worms, Germany. (^{13}C)Methyl iodide (99% ^{13}C) was purchased from Larodan Fine Chemicals AB, Malmö, Sweden. 1-(2′-deoxy-2′-fluoro-β-D-arabinofuranosyl)thymine was prepared by the published method.[19]

1-*(2′-Deoxy-2′-fluoro-β-*D*-arabinofuranosyl)-[methyl-^{11}C]thymine ([^{11}C]-FMAU) (^{11}C)-1*

A solution of tris(dibenzylideneacetone)dipalladium(0) (0.9 mg, 1 μmol), tri(*o*-tolyl)phosphine (1.2 mg, 4 μmol) and 1-(2′-deoxy-2′-fluoro-β-D-arabinofuranosyl)-5-(trimethylstannyl)uracil **2** (0.8 mg, 2 μmol) in DMF (300 μl) in a 0.8 ml oven-dried septum-equipped vial was prepared at room temperature. The solution was purged with nitrogen gas for 10 min, whereby the colour changed from purple to yellow. [^{11}C]methyl iodide was dried by passing it through a Sicapent® tower before trapping it in the solution at room temperature. The reaction mixture was heated at 130°C for 5 min and the addition of 500 μl of H_2O was followed by injection onto the semi-preparative LC column. The reaction vessel was then washed with 800 μl of H_2O, which was also injected. A gradient program was used starting with a mobile phase of A-B (92:8, v/v) at a flow of 6.0 ml/min. After 12 min, the mobile phase composition was changed to 20:80 over 1 min and was kept there for 9.5 min (to elute lipophilic by-products). The product was collected at approximately 6.6 min. Sterile filtration of the collected fraction into a sterile vial was performed using a Dynagard filter. A sample was analysed by analytical HPLC for radiochemical purity using solvents C-D (95:5, v/v) at a flow of 2.0 ml/min. A gradient was used: from 95:5 to 92:8 over 8 min, then to 20:80 over a 4 min period and maintained for an additional 3 min.The retention time of [^{11}C]-FMAU was 6.5 $\pm$ 0.1 min and the radio peak co-eluted with the authentic reference compound. The identity was determined by electrospray mass spectrometry using single ion monitoring. The ions *m/z* 261 and *m/z* 283 were detected which represent $[M+H]^+$ and $[M+Na]^+$, respectively. The mobile phases used were acetonitrile-50 mM formic acid (95:5, v/v) at a

flow rate of 20 µl/min. The product solution was analysed for metal residues using inductively coupled plasma time-of-flight mass spectrometry. 30 ng/ml of tin was detected, but no palladium residues could be found (<15 ng/ml). The amount of tin is below the LC_{50} for trimethylstannanes, which is reported to be <1 µg/ml for acute toxicity.[20]

The synthesis was performed using the fully automated Synthia system[17] in order to minimize the radiation exposure, reduce the total synthesis time and to increase the reproducibility of the synthesis.

1-(2′-Deoxy-2′-fluoro-β-D-arabinofuranosyl)-[methyl-^{13}C]thymine ([^{13}C]-FMAU) (^{13}C)-1

The synthesis was performed as described for [^{11}C]-**1** with the exception that a solution of (^{13}C)methyl iodide (20% v/v in octane, 5 µl, 17 µmol) was added to the reaction mixture just after the trapping of [^{11}C]methyl iodide was finished. The product fraction with a retention time of 6.3 min was collected. After decay of the ^{11}C, the solvent was removed *in vacuo*, the residue was dissolved in 0.7 ml DMSO-d_6 and the resulting solution was filtered.

^{13}C-NMR (DMSO-d_6): $\delta = 12.2$ ppm (CH_3).

Conclusion

A fully automated, highly reproducible one-pot procedure for the synthesis of 1-(2′-deoxy-2′-fluoro-β-D-arabinofuranosyl)-[*methyl*-^{11}C] thymine ([^{11}C]-FMAU) [^{11}C]-**1** has been developed. The product was obtained in 27 ± 5% decay-corrected isolated radiochemical yield at 25 min after end of nuclide production using $Pd_2(dba)_3$ and P(*o*-tolyl)$_3$ to generate the active catalyst *in situ* and using DMF as solvent.

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References

1. Lu L, Samuelsson L, Bergström M, Sato K, Fasth K-J, Långström B. *J Nucl Med* 2002; 43 (in press).

2. Mangner TJ, Klecker R, Andersson L, Shields A. *J Label Compd Radiopharm* 2001; **44(**S1**)**: S912–S915.
3. Conti PS, Alauddin MM, Fissekis JR, Schmall B, Watanabe KA. *Nucl Med Biol* 1995; **22**: 783–789.
4. Alauddin MM, Conti PS, Fissekis JD. *J Label Compd Radiopharm* 2002; **45**: 583–590.
5. Hughes JA, Hartman NG, Jay M. *J Label Compd Radiopharm* 1995; **36**: 1133–1145.
6. (a) Andersson Y, Cheng A, Långström B. *Acta Chem Scand* 1995; **49**: 683–688; (b) Björkman M, Andersson Y, Doi H, et al. *Acta Chem Scand* 1998; **52**: 635–640; (c) Suzuki M, Doi H, Björkman M, Långström B, Watanabe Y, Noyori R. *Chem Eur J* 1997; **3**(12): 2039–2042; (d) Karimi F, Björkman M, Långström B. *J Label Compd Radiopharm* 2001; **44**(S1): S1013; (e) Lange O, Forngren T, Sandell J, Dollé F, Långström B. *J Label Compd Radiopharm* 2001; **44**(S1): S413–S415; (f) Sandell J, Yu M, Emond P, et al. *J Label Compd Radiopharm* 2001; **44**(S1): S170–172.
7. Farina V, Roth GP. Recent advances in the Stille reaction. In *Advances in Metal-Organic Chemistry*, Liebskind LS (ed.). JAI Press: Greewich, UK, 1996; 1–53.
8. Tolman CA. *Chem Rev* 1977; **77**(3): 313–348.
9. Fauvarque J-F, Pflüger F, Troupel M. *J Organomet Chem* 1981; **208**: 419–427.
10. Farina V, Krishnan B. *J Am Chem Soc* 1991; **113**: 9585–9595.
11. Csákai Z, Skoda-Földes R, Kollár L. *Inorg Chim Acta* 1999; **286**:93–97.
12. Brown JM, Guiry PJ. *Inorg Chim Acta* 1994; **220**:249–259.
13. Beletskaya IP. *J Organomet Chem* 1983; **250**: 551–564.
14. Larhed M, Hallberg A. *J Org Chem* 1996; **61**: 9582–9584.
15. Scott WJ, Stille JK. *J Am Chem Soc* 1986; **108**: 3033–3040.
16. Björkman M, Doi H, Resul B, et al., *J Label Compd Radiopharm* 2000; **43**: 1327–1336.
17. Bjurling P, Reineck R, Westerberg G, et al. *Proceedings of the Sixth Workshop on Targetry and Target Chemistry* Vancouver, 1995; 282–285.
18. Långström B, Antoni G, Gullberg P, et al. *J Nucl Med* 1987; **28**:1037–1040.
19. Howell HG, Brodfuehrer PR, Brundidge SP, Benigni DA, Sapino C Jr . *J Org Chem* 1988; **53**: 85.
20. N-class database 2002. http://www.kemi.se/nclass/ [May 2002] Published by the Nordic council of Ministers and European Chemicals Bureau.

