Research Article

Journal of Labelled Compounds and Radiopharmaceuticals

Received 1 April 2008, Revised 5 July 2008, Accepted 7 July 2008 Published online 27 August 2008 in Wiley Interscience

(www.interscience.wiley.com) DOI: 10.1002/jlcr.1535

Automated radiosynthesis of *N*-(4-[^{18}F]fluorobenzyl)-2-bromoacetamide: an F-18-labeled reagent for the prosthetic radiolabeling of oligonucleotides

Ingrid Koslowsky,[a] Soraya Shahhosseini,[b] John Wilson,[b] and John Mercer[b]*

The potential for radiolabeled antisense oligonucleotides to image gene expression combined with the enhanced resolution of positron-emission tomography justifies the continued interest in the development of oligonucleotides tagged with positron-emitting radionuclides. The radiolabeling of oligonucleotides is a multi-step process and may require handling large amounts of radioactivity initially. A previously reported method for radiolabeling oligonucleotides with *N*-(4-[^{18}F]fluorobenzyl)-2-bromoacetamide was adapted for use in a commercially available automated synthesis unit by linking two reaction trains. The yield of *N*-(4-[^{18}F]fluorobenzyl)-2-bromoacetamide ranged from 3 to 18% and the synthesis was completed within 1 h. Challenges in using this unit included the maintenance of anhydrous conditions for the effective reduction of 4-[^{18}F]fluorobenzonitrile. Preliminary results indicated that a mean yield of 36% could be obtained upon incubation of an oligonucleotide with *N*–(4-[^{18}F]fluorobenzyl)-2-bromoacetamide. The entire synthesis could be performed within 3 h.

Keywords: automated synthesis unit; antisense oligonucleotide; fluorine-18; positron-emission tomography.

Introduction

Following the first reported radiolabeling of an antisense oligonucleotide (asODN) in 1994,[1] there has been active interest in radiolabeling these biological molecules for imaging gene expression. AsODNs are short pieces of single stranded DNA or RNA, typically 13–25 nucleobases in length, with a sugar–phosphate backbone. The native phosphodiester backbone can be modified with various functional groups to increase the stability of the ODNs *in vivo*.[2] An asODN with a specific nucleotide sequence has the ability to hybridize with its complementary 'sense' mRNA that, in therapeutic concentrations, can inhibit specific gene expression. From a diagnostic/clinical perspective, ODNs can be tagged with a radioactive probe in order to detect upregulated gene expression in various diseases such as cancer. Our specific interest is to use ODNs as probes to measure early cellular response to drug or radiation treatment. This would be an invaluable tool for clinicians as it offers prognostic information regarding the success (or failure) of treatment, and ultimately, on patient outcome.

The enhanced detection and resolution that is available with positron-emission tomography (PET) has encouraged the development of PET labelled oligonucleotides. A variety of synthetic methods have been described for the labelling of ODNs with [^{18}F]fluoride. Pan *et al.*[3] reported the direct labelling of a native (phosphodiester) ODN with 5′-deoxy-5′-[^{18}F]-*O*-methylthymidine. Using a hexylamine linker ODNs have also been labelled with 4-([^{18}F]]fluoromethyl)phenyl isothiocyante[4], *N*-succinimidyl 4-[^{18}F]fluorobenzoate,[5,6] and the photosensitive compound, 3-azido-5-nitrobenzyl-[^{18}F]fluoride.[7] Toyokuni *et al.*[8] synthesized an [^{18}F]-labelled maleimide agent for conjugation to ODNs containing a 5′-hexylthiol linker. de Vries *et al.*[9,10] investigated a number of ^{18}F-labeled alkylating agents for their ability to radiolabel a monophosphorothioated nucleotide and a hexylthiol-reactive ODN. Their investigations included the alkylating agent *N*-(4-[^{18}F]fluorobenzyl)-2-bromoacetamide, the synthesis of which was originally described by Dollé *et al.*[11] in 1997. Use of *N*-(4-[^{18}F]fluorobenzyl)-2-bromoacetamide has since been extensively reported by the Orsay group and has been used to label the 3′-monophosphorothioated end of a native phosphodiester ODN,[12] a fully phosphorothioated ODN, 2′*O*-methyl-modified RNA, or hybrid phosphodiester methylphosphonate ODN;[13,14] the 5′-phosphorothioated 2′*O*-methyl-modified RNA;[15] 3′- or 5′ labelled RNA and DNA speigelmers;[16,17] and peptide nucleic acids.[18–20] A related bromoacetamide reagent has also been described by this group for the labelling of

[a]*Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, AB, T6G 2N8 Canada*

[b]*Edmonton PET Centre, Cross Cancer Institute, 11560 University Avenue, Edmonton, AB, T6G 1Z2 Canada*

**Correspondence to: John Mercer, Edmonton PET Centre, Cross Cancer Institute, 11560 University Avenue, Edmonton, AB, T6G 1Z2 Canada..*
E-mail: johnmerc@cancerboard.ab.ca

monophosphorothioated ODNs, modified RNAs,[21] and small interfering RNAs.[22]

The first step in the synthesis of *N*-(4-[^{18}F]fluorobenzyl)-2-bromoacetamide is the nucleophilic substitution reaction of [^{18}F]fluoride anion with a trimethylanilinium precursor. Thus, all reactions in this multi-step synthesis require the manipulation of radioactivity. High activities of [^{18}F]fluoride must be employed at the start of the synthesis in order to obtain a sufficient amount of radiolabeled oligonucleotide for biological studies. The issues of radiation safety and the complexity of the synthesis make it an ideal candidate for remote automated preparation using robotic systems or automated synthesis units (ASUs). The intent of this work was to modify a commercially available ASU (General Electric TRACERlab Fx_{FDG}) for the synthesis of *N*-(4-[^{18}F]fluorobenzyl)-2-bromoacetamide as described in the literature.

Results and discussion

Chemistry

*Synthesis of N-(4-[^{18}F]fluorobenzyl)-2-bromoacetamide ([^{18}**F**]-**4**)*

The multi-step synthesis of **[^{18}F]-4** reported by Dollé *et al.*[11] (Figure 1) was adapted to permit automation using the TRACERlab Fx_{FDG} ASU. This ASU incorporates two separate reaction trains, each designed for the synthesis of 2-[^{18}F]fluorodeoxyglucose (FDG). Both reaction trains and reactors were required for the preparation of **[^{18}F]-4**. A schematic diagram of the ASU modified for this synthesis is illustrated in Figure 2.

The synthesis of 4–[^{18}F]fluorobenzonitrile (**[^{18}F]-2**) was achieved by displacement of the quaternary ammonium leaving group of the precursor 4–cyano-*N,N,N*-trimethylanilinium trifluoromethanesulfonate (**1**) with [^{18}F]fluoride at elevated temperature in dimethyl sulfoxide (DMSO). Previous reports have used different reaction conditions for the synthesis of **[^{18}F]-2**. Dollé *et al.*[11] heated the precursor for 20 min at 120°C resulting in a yield of 60 to 85%. Kuhnast *et al.*[13] performed the reaction at 180°C for 20 min with similar yields. As 165°C is the maximum temperature to which the reactors in this ASU can be heated, the synthesis of **[^{18}F]-2** was performed at two temperatures, 130 and 160°C, for 10 or 20 min to determine the optimum temperature for this reaction. As seen in Figure 3, no difference in radiochemical purity (RCP) as determined by radio-thin layer chromatography (radio-TLC) or yield was observed when varying the temperature or length of reaction. Decay corrected radiochemical yields (with respect to [^{18}F]fluoride ion at end of bombardment and prior to the transfer of **[^{18}F]-2** to the C18 Sep-Pak cartridge) varied widely but typically ranged between 40 and 50%. However, fewer nonradioactive side products were observed by TLC at 254 nm at the lower temperature 130°C and shorter reaction time and these could be removed via Sep-Pak purification or ultimately by reverse phase high-pressure liquid chromatography (RP-HPLC) purification of **[^{18}F]-4**. Therefore, the reaction conditions of 130°C for 10 min were chosen for the synthesis of **[^{18}F]-2**. Upon completion of this step, water was added to reactor 1 to transfer the reaction mixture containing **[^{18}F]-2** to a C18 Sep-Pak cartridge. The cartridge was washed with additional water (via reactor 1) to remove unreacted reagents and water soluble side products.

The following step i.e. the conversion of the nitrile group of **[^{18}F]-2** to the amine of **[^{18}F]-3**, utilizes lithium aluminum hydride ($LiAlH_4$), and thus requires anhydrous conditions. Reactor 1 acts as a conduit for the transfer of anhydrous tetrahydrofuran (THF) and is used for the elution of **[^{18}F]-2** from the C18 Sep-Pak cartridge to reactor 2. Thus, toluene was used to azeotropically remove residual water from reactor 1 prior to the elution of **[^{18}F]-2** with THF. Residual water in the tubing and on the Sep-Pak cartridge was removed by passing the reaction mixture through a series of drying columns. The transfer of activity from reactor 1 to 2 resulted in the largest loss of radioactivity (based on readings from radioactivity detectors located under the reactors) with approximately 17 to 38% of the original activity transferred to reactor 2. Losses depended in part on the radiochemical yield of **[^{18}F]-2** (a higher yield reduced the amount of unreacted [^{18}F]fluoride in the waste with more **[^{18}F]-2** transferred to the drying cartridges), and in part on product retention on the molecular sieve drying column and cartridges. Decreasing the amount of molecular sieves or eliminating the sodium sulphate or silica cartridges increased the amount of radioactivity transferred to reactor 2; however, residual water carried into the reactor likely consumed a portion of the reducing agent, $LiAlH_4$, and ultimately resulted in a lower RCP and yield of **[^{18}F]-4**.

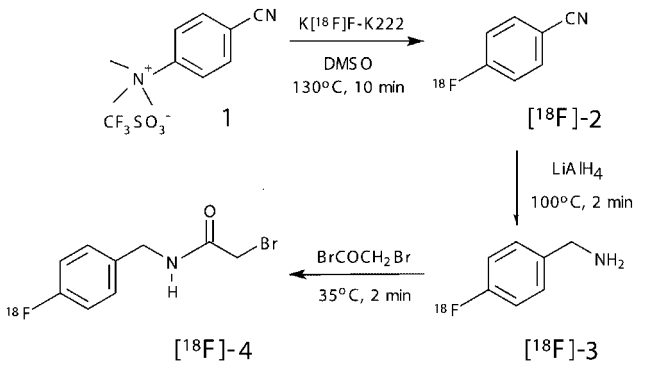

Figure 1. Synthesis of *N*-(4-[^{18}F]fluorobenzyl)-2-bromoacetamide ([^{18}F]-4).

In the conversion of **[^{18}F]-2** to 4-[^{18}F]fluorobenzylamine (**[^{18}F]-3**) reaction temperatures of 120–140°C were reported by Kuhnast *et al.*[13,15] In our system lower temperatures were used to prevent losses of volatile **[^{18}F]-2**. Trial reactions at longer reaction times and/or lower temperatures gave inconsistent results and did not improve the RCP or yield of **[^{18}F]-4**. The use of powdered, dry $LiAlH_4$ was generally observed to produce lower yields of **[^{18}F]-4** versus commercially available $LiAlH_4$ solution in THF. This may be due to inefficient mixing of powdered $LiAlH_4$ with the reaction mixture. The final step, the condensation with 2-bromoacetyl bromide, was quantitative. With the above modifications, a mean RCP of 80% of **[^{18}F]-4** was achieved with decay corrected radiochemical yields (before HPLC purification) ranging from 3 to 18% with a synthesis time of 1 h. The **[^{18}F]-4** reaction mixture was purified by RP-HPLC and a centre cut of the radioactive peak representing **[^{18}F]-4** was collected. A mean decay corrected yield of 3.8% ($n = 9$) was achieved after purification. Typically, 100–200 MBq of purified **[^{18}F]-4** could be obtained for radiolabeling ODNs from starting [^{18}F]fluoride of 10 GBq.

Radiolabeling of oligonucleotide (ODN)

Preliminary experiments have resulted in radiochemical yields of 36% (as determined by the fraction of radioactivity in the ODN

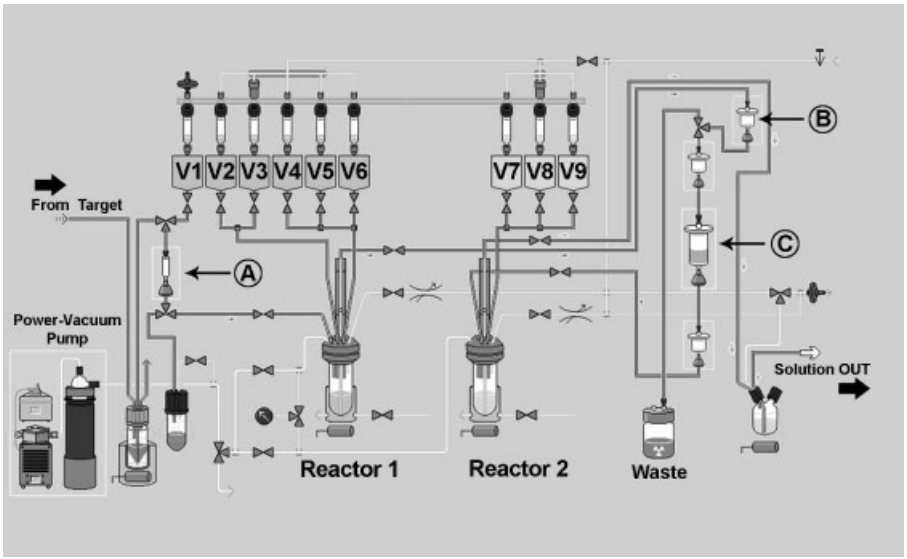

Figure 2. Schematic diagram of the reaction train for the synthesis of [^{18}F]-4 using the GE TRACERlab FX_{FDG} ASU. The content of the reagent vessels is as follows: V1: 0.8 mL Kryptofix eluant; V2: 0.8 mL acetonitrile; V3: 10 mg 4-cyano-*N,N,N*-trimethylanilinium trifluoromethanesulfonate in 0.8 mL DMSO (anhydrous); V4: 1 mL toluene; V5: 10 mL water; V6: 6 mL anhydrous THF; V7: 400 μL water; V8: 5 mL methylene chloride; V9: 10 μL bromoacetyl bromide 1.0 M solution diluted to 1 mL of methylene chloride; (A) ion exchange column for loading [^{18}F]fluoride; (B) C18 cartridge; (C) drying columns consisting of sodium sulphate cartridge (top), ground molecular sieves (centre), and silica cartridge (bottom).

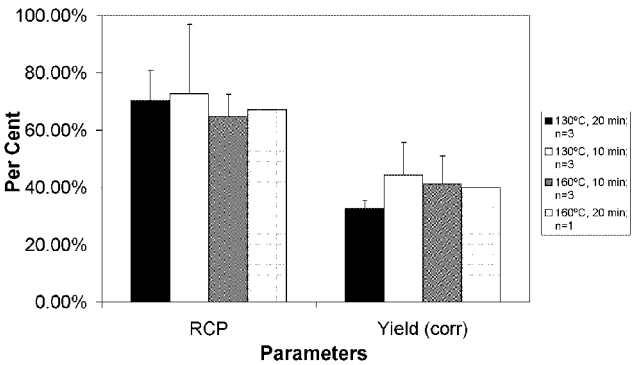

Figure 3. Effect of temperature and reaction time on the radiochemical purity (RCP) and yield (corrected for decay) in the synthesis of [^{18}F]-2. No significant difference in the RCP (determined by radio-TLC) and yield was observed between syntheses performed at 130 or 160°C at 10 or 20 min.

reaction mixture eluted as [^{18}F]-labeled ODN) when the reaction was carried out at atmospheric pressure in a vented vial. Lower yields and additional side products were observed when the reaction was performed in a sealed vial. The use of a Sephadex G-25 DNA grade mini column for purification negated the need for HPLC and provided a rapid purification with less loss of radioactivity. The disadvantage of purification with this method, however, is that unreacted ODN is co-eluted with the radiolabeled product resulting in lower specific activity. Preliminary results show similar yields of [^{18}F]ODN with 0.5–1 mg of ODN. Reducing the amount of starting material would improve the specific activity assuming that the yields remain the same. Currently, specific activities of 1.1 GBq/μmol (at end of synthesis) have been attained using the above method. Optimization of the reaction conditions is ongoing.

Experimental

General

Chemicals

Chemicals were purchased from Sigma-Aldrich (St. Louis, MO) or Caledon Lab (Canada) and were used without further purification. [^{18}F]fluoride was produced in-house using the Edmonton PET Center Cyclotron Facility.

Fully phosphorothioated, 20-mer oligonucleotides were purchased from the University Core DNA Services, Calgary, Canada: an asODN complementary to the 3′–untranslated region of human $p21^{WAF1}$ mRNA with the sequence 5′-TGT.CAT.GCT.GGT.CTG.CCG.CC-3′[23] and a random sequence oligonucleotide sequence (5′–CCG.GTG.AAC.GAG.CGA.GCA.CA-3′)[24]. The above oligonucleotides, with *N*–(4–fluorobenzyl)-2-bromoacetamide substituted in the 5′ position, were synthesized by the University Core DNA Services for use as reference compounds. Mass spectra and HPLC retention times for fluorinated and nonfluorinated ODNs are provided in Table 1.

Analytical methods

TLC was performed using MK6F Silica Gel 60Å, 250 μm plates (Whatman, UK) visualized under u.v. illumination at 254 nm. Heptane:ethyl acetate, 50:50 was used as the mobile phase for all TLC analyses. Radio-TLC was performed with an AR-2000 imaging scanner (Bioscan Inc, Washington, DC). Radio-TLC results were compared with corresponding TLC of nonradioactive reference compounds. The concentration of ODN was determined by u.v. spectroscopy at 260 nm using a Beckman DU 7400 spectrophotometer. RP-HPLC analyses and purifications were performed with a Beckman Coulter Inc system consisting of a Model 168 Diode Array u.v. module, 254 nm; a Model 126 analytical dual pump; a radioactivity detector (Ortec, TN): ACE *Mate*^{TM} Single Channel Analyzer; and a Whatman Partisil

Table 1. Mass spectrometry and HPLC analyses of 20-mer oligonucleotides[a]

Sequence	Calc mass (M)	Major peak observed	HPLC t_R (min)
5′-ps-TGT.CAT.GCT.GGT.CTG.CCG.CC	6477.2	6475.2	13.8
5′-FBBA-ps-TGT.CAT.GCT.GGT.CTG.CCG.CC	6642.4	6643.7	14.6
5′-ps-CCG.GTG.AAC.GAG.CGA.GCA.CA	6562.3	6561.2	13.7
5′-FBBA-ps-CCG.GTG.AAC.GAG.CGA.GCA.CA	6727.5	6733.2	14.4

[a]Fully phosphorothioated oligonucleotides.

10 ODS-3, 9.4 × 500 mm column (Whatman, UK) with guard column.

Chemistry

The method of Dollé *et al.*[11] was utilized for the synthesis of the nonradioactive, fluorinated reference compound, *N*–(4–fluorobenzyl)-2-bromoacetamide as well as synthesis of the precursor (**1**) with the exception that *N*–(4–fluorobenzyl)-2-bromoacetamide was recrystallized in cyclohexane (37% yield). TLC and NMR spectra were in agreement with that published earlier.[11]

Radiochemistry

Synthesis of N-(4-[^{18}F]fluorobenzyl)-2-bromoacetamide ([^{18}F]-4)

Aqueous [^{18}F]fluoride (4–15.3 GBq) was transferred to a GE TRACERlab FX_{FDG} automated synthesis unit (GE Healthcare, WS) and loaded onto a preconditioned Chromafix 30-PS-HCO_3 cartridge, (Machery-Nagel, Germany). The ^{18}F was then eluted into the reaction vessel (reactor 1) with the Kryptofix® eluant (Figure 2, V1) consisting of 16 mg of Kryptofix®222 (4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8.8.8]hexacosane) and 2.7 mg of potassium carbonate in 0.8 mL of a 77.5%/22.5% mixture of acetonitrile and water. The solvent was co-evaporated with 0.8 mL acetonitrile (Figure 2, V2) at 55°C over 6 min with a stream of nitrogen and reduced pressure. The contents were then heated to 95°C under full vacuum to remove residual water. The precursor (Figure 2, V3) was transferred to the reaction vessel (10 mg in 0.8 mL anhydrous DMSO) and the reaction proceeded at 130°C for 10 min. The temperature was reduced to 40°C followed by transfer of the mixture to a preconditioned Sep-Pak Plus® C18 cartridge (Waters Corp, Mass) with 10 mL water (Figure 2, V5). The cartridge was partially dried with nitrogen for 3 min. Residual water in reactor 1 was azeotropically removed with 1 mL toluene (Figure 2, V4) at 150°C for 2 min. 4–[^{18}F]fluorobenzonitrile (**[^{18}F]-2**) was then eluted from the C18 cartridge with 6 mL of anhydrous THF (Figure 2, V6) through a series of drying columns consisting of a 2.5 g sodium sulphate cartridge (International Sorbent Technology Inc, UK) followed by a column containing 4 g of ground molecular sieves (4 Å). The mixture was allowed to interact with the molecular sieves for 4–6 min before transferring the material via a SepPak silica cartridge (Waters Corp, Mass) to reactor 2 containing 1 mL of 1 M solution of $LiAlH_4$ in THF. Reduction of the nitrile group proceeded at 100°C for 2 min. The reaction mixture was cooled to 50°C and the solvent removed under reduced pressure and nitrogen. Water (400 μL; Figure 2, V7) was subsequently added to destroy the lithium aluminum complex followed by the addition of a 1:100 dilution of bromoacetyl bromide in methylene chloride (1 mL) (Figure 2, V9). The reaction proceeded for 2 min at 35°C. Methylene chloride (5 mL) (Figure 2, V8) was added and the soluble components were dispensed through a 1 μm glass fibre filter (Waters, MA) into a vented, 10 mL vial.

The clear, colourless **[^{18}F]-4** reaction mixture was concentrated to dryness under low heat and a stream of nitrogen. The residue was redissolved in methanol, reserving an aliquot for radio-TLC analysis, and the remainder purified with the following HPLC gradient system: acetonitrile 5% (*aq*)–95% over 20 min; flow rate: 6 mL/min; retention time (t_R): 13.3 min. The centre fraction of the peak representing **[^{18}F]-4** was collected to avoid the collection of nonradioactive side products formed during synthesis (t_R: 15.4 min) as well as unreacted reagents such as bromoacetyl bromide (t_R: 10 min) and concentrated to dryness using azeotropic distillation under a gentle stream of nitrogen. Radio-TLC of the reaction mixture was performed before and after purification by HPLC. The occasional sample of purified **[^{18}F]-4** was analyzed by RP-HPLC to verify radio-TLC results. The samples were co-spotted with nonradioactive *N*-(4-fluorobenzyl)-2-bromoacetamide (R_f 0.4).

Radiolabeling of oligonucleotide (ODN)

Lyophilized ODN (148 nmol, 1 mg) was reconstituted with 1 mL of phosphate-buffered saline (PBS) 0.1 M, pH 8 mixed with methanol, 1:1 (v/v) and added to **[^{18}F]-4**. The mixture was heated in a vented vial at 120°C for 30 min. The volume of the reaction mixture was maintained by the addition of 0.5–1 mL of 50% methanol midway through the reaction. Unreacted **[^{18}F]-4** was separated from the radiolabeled ODN with a NAP™-10 column (GE Healthcare, UK). The ODN was eluted with 1.5 mL PBS 0.01 M, pH 7.2. No further purification was performed. The concentration of ODN in the eluate was determined by u.v. spectroscopy at 260 nm. Analysis of the mixture was performed with radio-TLC (R_f [^{18}F]ODN: 0.0; **[^{18}F]-4**: 0.4) and by HPLC using the following gradient system: triethylammonium acetate/acetonitrile 95/5 to 90/10 over 3 min, followed by 90/10 to 75/25 over 7 min, with a washout phase of 50/50 for 10 min; flow rate: 6 mL/min; retention time: 14.6–14.9 min. The [^{18}F]ODNs eluted with the same retention time as the nonradioactive, fluorinated ODNs (Table 1).

Conclusions

The multi-step synthesis of **[^{18}F]-4** is challenging to perform using commercial ASUs designed for simpler syntheses. The synthesis of **[^{18}F]-4** as described in the literature cannot be performed in a single reaction vessel and synthesis units designed for clinically used products such as [^{18}F]FDG typically do not have two reaction trains. Thus, neither are these units designed to transfer materials in and out of the same vessel nor are sufficient reagent vessels available for a multi-step synthesis. A scrupulous cleaning or drying step of the reaction vessel may

be also required between steps in a synthesis. This complicates the possibility of reusing a reactor. The TRACERlab FX_{FDG} synthesis unit is unique in that it contains two reactors, which can be interconnected, with minor modifications, between the Sep-Pak cartridge and reactor 2. The challenge in the use of this ASU for the synthesis of **[^{18}F]-4** is the difficulty in eliminating water from **[^{18}F]-2** prior to its transfer to reactor 2 containing $LiAlH_4$, as well as the losses of radioactivity with each transfer. For example, the transfer from reactor 1 to 2 often resulted in the loss of up 75% of the radioactivity. In addition, purification of **[^{18}F]-4** by RP-HPLC adds time to the total synthesis as well as loss of product. Use of a normal phase HPLC system could improve the yield of purified **[^{18}F]-4** simply due to the faster removal of the organic mobile phase solvents as compared with those used in RP-HPLC. Modular research synthesis units for complex syntheses should allow improved flexibility in the synthetic design and allow more efficient transfer of solutions and reagents. Purification with an HPLC directly attached to the modular ASUs also reduces loss of product. Regardless of these limitations, sufficient *N*-(4-[^{18}F]fluorobenzyl)-2-bromoacetamide can be produced in ASUs designed for clinical products to permit radiolabeling of ODNs for our ongoing investigations with gene expression imaging in biological models.

Acknowledgement

The authors would like to thank the staff at the Edmonton PET Center for the synthesis of [^{18}F]fluorine. The authors would also like to thank the graphic artist, Ron Hildebrandt for his expert assistance in modifying the ASU schematic diagram. This work was supported, in part, through a grant from the Alberta Cancer Board Bridge and Pilot Fund.

References

[1] M. K. Dewanjee, A. K. Ghafouripour, M. Kapadvanjwala, S. Dewanjee, A. N. Serafini, D. M. Lopez, G. N. Sfakianakis, *J. Nucl. Med.* **1994**, *35*, 1054–1063.
[2] U. Haberkorn, W. Mier, M. Eisenhut, *Curr. Med. Chem.* **2005**, *12*, 779–794.
[3] D. Pan, S. S. Gambhir, T. Toyokuni, M. R. Iyer, N. Acharya, M. E. Phelps, J. R. Barrio, *Bioorg. Med. Chem. Lett.* **1998**, *8*, 1317–1320. DOI: 10.1016/S0960-894X(98)00239-X.
[4] E. Hedberg, B. Langstrom, *Acta Chem. Scand.* **1997**, *51*, 1236–1240.
[5] E. Hedberg, B. Langstrom, *Acta Chem. Scand.* **1998**, *52*, 1034–1039.
[6] J. Li, J. O. Trent, P. J. Bates, C. K. Ng, *J. Labelled Compds. Radiopharm.* **2006**, *49*, 1213–1221. DOI: 10.1002/jlcr.1136.
[7] C. W. Lange, H. F. VanBrocklin, S. E. Taylor, *J. Labelled Compds. Radiopharm.* **2002**, *45*, 257–268. DOI: 10.1002/jlcr.565.
[8] T. Toyokuni, J. C. Walsh, A. Dominguez, M. E. Phelps, J. R. Barrio, S. S . Gambhir, N. Satyamurthy, *Bioconjugate Chem.* **2003**, *14*, 1253–1259. DOI: 10.1021/bc034107y.
[9] E. F. J. de Vries, J. Vroegh, P. H. Elsinga, W. Vaalburg, *Appl. Radiat. Isot.* **2003**, *58*, 469–476. DOI: 10.1016/S0969-8043(03)00022-8.
[10] E. F. de Vries, J. Vroegh, G. Dijkstra, H. Moshage, P. H. Elsinga, P. L. Jansen, W . Vaalburg, *Nucl. Med. Biol.* **2004**, *31*, 605–612. DOI: 10.1016/j.nucmedbio.2004.02.002.
[11] F. Dollé, F. Hinnen, F. Vaufrey, B. Tavitian, C. Crouzel, *J. Labelled Compds. Radiopharm.* **1997**, *39*, 319–330. DOI: 10.1002/(SICI)1099-1344(199704)39:43.0.CO;2-7.
[12] B. Kuhnast, F. Dollé, S. Terrazzino, B. Rousseau, C. Loc'h, F. Vaufrey, F. Hinnen, I.. Doignon, F. Pillon, C. David, C. Crouzel, B. Tavitian, *Bioconjugate Chem.* **2000**, *11*, 627–636. DOI: 10.1021/bc990183i.
[13] B. Kuhnast, F. Dollé, F. Vaufrey, F. Hinnen, C. Crouzel, B. Tavitian, *J. Labelled Compds. Radiopharm.* **2000**, *43*, 837–848. DOI: 10.1002/1099-1344(200007)43:83.0.CO;2-2.
[14] B. Tavitian, S. Terrazzino, B. Kuhnast, S. Marzabal, O. Stettler, F . Dollé, J. Deverre, A . Jobert, F. Hinnen, B. Bendriem, C. Crouzel, L. Di Giamberardino, *Nat. Med.* **1998**, *4*, 467–470. DOI: 10.1038/nm0498-467.
[15] B. Kuhnast, F. Hinnen, R. Boisgard, B. Tavitian, F . Dollé, *J. Labelled Compds. Radiopharm.* **2003**, *46*, 1093–1103.
[16] B. Kuhnast, S. Klussmann, F. Hinnen, R. Boisgard, B. Rousseau, J. P. Fürste, B . Tavitian, F. Dollé, *J. Labelled Compds. Radiopharm.* **2003**, *46*, 1205–1219.
[17] R. Boisgard, B. Kuhnast, S. Vonhoff, C. Younes, F. Hinnen, J. Verbavatz, B . Rousseau, J. P. Furste, B. Wlotzka, F . Dollé, S. Klussmann, B. Tavitian, *Eur. J. Nucl. Med. Mol. Imaging* **2005**, *32*, 470–477. DOI: 10.1007/s00259-004-1669-8.
[18] B. Kuhnast, F. Dollé, B. Tavitian, *J. Labelled Compds. Radiopharm.* **2002**, *45*, 1–11. DOI: 10.1002/jlcr.522.
[19] B. Kuhnast, F. Hinnen, R. Hamzavi, R. Boisgard, B. Tavitian, P. E. Nielsen, F . Dollé, *J. Labelled Compds. Radiopharm.* **2005**, *48*, 51–61. DOI: 10.1002/jlcr.895.
[20] R. Hamzavi, F. Dollé, B. Tavitian, O. Dahl, P. E. Nielsen, *Bioconjugate Chem.* **2003**, *14*, 941–954. DOI: 10.1021/bc034022x.
[21] B. Kuhnast, B. De Bruin, F. Hinnen, B. Tavitian, F . Dollé, *Bioconjugate Chem.* **2004**, *15*, 617–627.
[22] T. Viel, B. Kuhnast, F. Hinnen, R. Boisgard, B. Tavitian, F . Dollé, *J. Labelled Compds. Radiopharm.* **2007**, *50*, 1159–1168. DOI: 10.1002/jlcr.1411
[23] W. Poluha, D. K. Poluha, B. Chang, N. E. Crosbie, C. M. Schonhoff, D. L. Kilpatrick, A. H . Ross, *Mol. Cell Biol.* **1996**, *16*, 1335–1341.
[24] H. Tian, E. K. Wittmack, T. J. Jorgensen, *Cancer Res.* **2000**, *60*, 679–684.