Published in final edited form as:

Nucl Med Biol. 2013 January ; 40(1): 109–116. doi:10.1016/j.nucmedbio.2012.09.008.

Enhanced Radiosyntheses of [^{11}C]Raclopride and [^{11}C]DASB using Ethanolic Loop Chemistry

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Abstract

Introduction—To improve the synthesis and quality control of carbon-11 labeled radiopharmaceuticals, we report the fully automated loop syntheses of [^{11}C]raclopride and [^{11}C]DASB using ethanol as the only organic solvent for synthesis module cleaning, carbon-11 methylation, HPLC purification, and reformulation.

Methods—Ethanolic loop chemistry is fully automated using a GE TRACERLab FX_{C-Pro} synthesis module, and is readily adaptable to any other carbon-11 synthesis apparatus. Precursors (1 mg) were dissolved in ethanol (100 μL) and loaded into the HPLC loop. [^{11}C]MeOTf was passed through the HPLC loop and then the labeled products were purified by semi-preparative HPLC and reformulated into ethanolic saline.

Results—Both [^{11}C]raclopride (3.7% RCY; >95% RCP; SA = 20831 Ci/mmol; n = 64) and [^{11}C]DASB, both with (3.0% RCY; >95% RCP; SA = 15152 Ci/mmol; n = 9) and without (3.0% RCY; >95% RCP; SA = 10931 Ci/mmol; n = 3) sodium ascorbate, have been successfully prepared using the described methodology. Doses are suitable for human use and the described methods are now employed for routine clinical production of both radiopharmaceuticals at the University of Michigan.

Conclusions—Ethanolic loop chemistry is a powerful technique for preparing [^{11}C]raclopride and [^{11}C]DASB, and we are in the process of adapting it for other carbon-11 radiopharmaceuticals prepared in our laboratories ([^{11}C]PMP, [^{11}C]PBR28 *etc.*).

Keywords

positron emission tomography; carbon-11 radiochemistry; flow chemistry

1. Introduction

Loop chemistry, the radiochemical equivalent of flow chemistry and arguably the predecessor to microfluidics, is a particularly efficient strategy that has been widely used by our group, and others, for radiolabeling bioactive molecules with carbon-11. In order to conduct loop chemistry, a solution of the precursor is deposited as a thin film on the inside of an HPLC loop. The reaction then occurs by blowing [^{11}C]MeOTf (or [^{11}C]MeI) through the HPLC loop to generate the radiolabeled product. Following reaction, contents of the loop are then injected directly onto the HPLC column for immediate purification.

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Loop chemistry has the advantage of using a small amount of solvent; just enough to coat the HPLC loading loop with the precursor in mg scale. The advantage of such small amounts of reagents and solvents include more concentrated precursor solutions leading to improved methylation reactions. Moreover, the reduction in solvents and precursor associated with loop chemistry (when compared to more traditional solution phase reactions) offers simplified semipreparative HPLC due to the corresponding reduction in burden to the column and the chromatographic system. Historically, loop chemistry has always been carried out using volatile flushable solvents such as 2-butanone (methyl ethyl ketone, MEK), 3-pentanone, and tetrahydrofuran (THF), and we have used such approaches for years to prepare [^{11}C]DASB, [^{11}C]raclopride and [^{11}C]PiB for clinical use as previously described. Recently however, additional scrutiny over supply and disposal of solvents such as MEK, stemming from the environmental impact of their use in the dry cleaning industry, has made their routine use increasingly problematic [and expensive]. Furthermore, as the field of radiochemistry becomes increasingly regulated, the use of such solvents makes quality control (QC) of radiopharmaceuticals more challenging and time consuming. For QC purposes, we use the solvent classification system outlined by the *International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use* (ICH). Solvents are classified as Class I, II or III depending upon their toxicity. As Class I solvents (e.g. benzene, CCl_4) are the most toxic, and have single digit ppm dose limits, we never employ them in radiopharmaceutical syntheses. Contrastingly Class II (e.g. MeCN, DCM, DMF, THF) and Class III solvents (e.g. EtOH, DMSO, acetone, MEK) are widely used in radiopharmaceutical syntheses. Class II solvents are considered to have intermediate toxicity, reflected in residual solvent limits of, typically, a few hundred ppm per dose. Class III solvents on the other hand are generally considered safe; a fact reflected in the 5000 ppm dose limit suggested by ICH. We presently perform residual solvent analysis of all Class II and III solvents employed in a radiopharmaceutical synthesis (i.e. for module cleaning, synthesis and purification) for every dose we prepare. However, our radiopharmaceutical production program has seen continuous and rapid growth in recent years so that performing $2 - 3 \times$ fluorine-18 syntheses, $4 \times$ carbon-11 syntheses and $1 - 2 \times$ nitrogen-13 syntheses every day for clinical purposes is typical. As each synthesis requires 8 GC runs (blank injection – $3 \times$ reference standard injections – blank injection – $3 \times$ dose injections), and each run takes 20 – 30 minutes when post-run instrument cooling is factored in, this creates scheduling conflicts and a significant bottle neck in the clinical production and QC operations. Strategies to improve this work flow have been considered, and we were therefore particularly interested in the recent update to Chapter 823 of the U. S. Pharmacopeia, suggesting that residual solvent analysis can be reduced from a daily QC test to a periodic test (e.g. quarterly or annually), if only Class III solvents are employed in a given synthesis.

Reflecting all of these issues, we were desirous of phasing out Class II solvents from our carbon-11 radiopharmaceutical production program. As many of the radiopharmaceuticals in our clinical portfolio are prepared using loop methods, it was therefore necessary to identify alternative solvents with which to conduct carbon-11 loop chemistry. Historically, alcohol solvents such as ethanol have always been considered incompatible with methylating agents such as [^{11}C]MeOTf as, in general, alkyl perfluoroalkanesulfonate esters are particularly prone to solvolysis. Moreover, protic solvents are generally considered to retard $\text{S}_{\text{N}}2$ reactions because of unfavorable solvation of the nucleophilic component. However, many such assumptions are often based upon intuition rather than empirical evidence. In light of the remarkable results obtained by Chi and co-workers when conducting fluorination reactions with [^{18}F]fluoride in protic solvents, we were curious whether carbon-11 methylation reactions might also proceed in protic solvents. It was also postulated that the very small amounts of solvent employed in loop chemistry would greatly minimize the chance of solvolysis, and offer compatibility with [^{11}C]MeOTf. This did prove to be the

case, and herein we demonstrate that loop chemistry can indeed be conducted using ethanol. Proof of concept is demonstrated through fully automated loop syntheses of [^{11}C]DASB (**2**) and [^{11}C]raclopride (**4**), and ethanol as the only organic solvent for synthesis module cleaning, carbon-11 methylation, HPLC purification, and reformulation. In addition, the preparative HPLC methods used to purify [^{11}C]raclopride and [^{11}C]DASB enable elution of the radiolabeled products prior to their precursors, thus reducing precursor contamination and improving chemical purity and specific activity of the final doses. The high concentration of ethanol (80%) in the DASB mobile phase also proved to be sufficient to inhibit radiolysis of [^{11}C]DASB and eliminated the need to use sodium ascorbate throughout the synthesis and in the final formulation. Removing sodium ascorbate from the final formulation greatly simplifies quality control of [^{11}C]DASB, and improves confidence in determination of specific activity of the radiopharmaceutical, because the cold mass peak no longer gets caught in the tail of the large peak associated with sodium ascorbate.

2. Materials and methods

2.1 General considerations

Unless otherwise stated, reagents and solvents were commercially available and used without further purification: sodium chloride, 0.9% USP and Sterile Water for Injection, USP were purchased from Hospira; ethanol, USP was purchased from American Regent; ethanol for HPLC was purchased from Decon Laboratories, Inc.; DASB and raclopride precursors and reference standards were purchased from ABX Advanced Biochemicals; Shimalite-Nickle was purchased from Shimadzu; iodine was purchased from EMD; phosphorus pentoxide was purchased from Fluka; and molecular sieves were purchased from Alltech. Other synthesis components were obtained as follows: sterile filters were obtained from Millipore; sterile product vials were purchased from Hollister-Stier; C18 Sep-Paks and Porapak Q were purchased from Waters Corporation. Sep-Paks were flushed with 10 mL of ethanol followed by 10 mL of sterile water prior to use.

2.2 General procedure for production of [^{11}C]CH₃OTf

[^{11}C]CH₃OTf was prepared as previously described. Briefly, carbon-11 was produced, as [^{11}C]CO₂, via the $^{14}\text{N}(\text{p},\alpha)^{11}\text{C}$ nuclear reaction using a GE PETTrace cyclotron. [^{11}C]CO₂ (~3 Ci) was delivered to the TRACERlab FX_{C-Pro}, where it was reduced to [^{11}C]CH₄ (Ni/H₂(g), 350 °C). Subsequent reaction with iodine at 720°C provided [^{11}C]CH₃I, which was then passed through a silver triflate column, heated to 190°C, to yield [^{11}C]CH₃OTf.

2.3 Loop Synthesis of [^{11}C]DASB

To prepare [^{11}C]DASB, the TRACERlab FX_{C-Pro} synthesis module was configured as previously described, and loaded as follows: 2 mL steel HPLC Loop: *O*-desmethyl DASB (1.0 mg) in ethanol (100 μL); Vial 4: Sterile water for injection, USP (7 mL) $\pm$ sodium ascorbate for injection (50 μL); Vial 5: Ethanol (0.5 mL); Vial 6: 0.9% NaCl for Injection, USP (9.5 mL) $\pm$ sodium ascorbate for injection (50 μL); Round-bottomed Dilution Flask: Milli-Q Water (20–50 mL) $\pm$ sodium ascorbate for injection (100 μL). The precursor solution was loaded onto the HPLC loop (2 mL, steel) and conditioned with nitrogen gas for 20 seconds at 10 mL/min. [^{11}C]MeOTf was prepared according to the general procedure outlined above and passed through the HPLC loop at 40 mL/min for 3 minutes. Following reaction, the mixture was purified by semi-preparative HPLC (column: Phenomenex Luna CN, 250 $\times$ 10mm, mobile phase: 5 mM NaOAc in 80% ethanol, pH: 5.0, flow rate: 4 mL/min, typical trace: Figure 1). The product peak (t_{R} ~7 min) was collected into 20–50 mL of water. The solution was then passed through a C18 Sep-Pak (WAT054955), and washed with 7 mL sterile water. The product was then eluted with 0.5 mL of USP ethanol followed

by 9.5 mL of USP saline. This final formulation was then passed through a 0.22-micron filter into a sterile dose vial and submitted for QC testing.

2.4 Loop Synthesis of [^{11}C]Raclopride

[^{11}C]Raclopride was produced *via* the loop method using a TRACERlab FX_{C-Pro} synthesis module configured as previously described. The precursor, desmethyl-raclopride TBA salt (1 mg), was dissolved into ethanol (100 μL), loaded onto the 2 mL HPLC loop, and conditioned with nitrogen gas for 20 seconds at 10 mL/min. [^{11}C]MeOTf was prepared according to the general procedure outlined above and passed through the HPLC loop at 40 mL/min for 3 minutes. Following reaction, the mixture was purified by semipreparative HPLC (column: Phenomenex Luna NH₂, 250 $\times$ 10mm, mobile phase: 20 mM NH₄OAc in 10% ethanol, flow rate: 3 mL/min, typical trace: Figure 2). The radioactive peak was collected (t_R ~9 min) and diluted with 5.5 mL USP saline. This aqueous solution was filtered through a 0.22 μm sterile filter into a sterile dose vial and submitted for QC testing.

2.5 Quality control

Quality control for both radiopharmaceuticals was conducted using the guidelines outlined in the U.S. Pharmacopeia and described below. Results are reported in Table 1.

Visual Inspection—Doses are visually examined and must be clear, colorless and free of particulate matter.

Dose pH—The pH of the doses is analyzed by applying a small amount of the dose to pH-indicator strips and determined by visual comparison to the scale provided.

Chemical Purity and Radiochemical Purity / Identity—Chemical and radiochemical purity/identity are analyzed using an HPLC equipped with a radioactivity detector and a UV detector using the methods outlined below. Radiochemical purity for doses must be >95%, and identity is confirmed by comparing the retention time of the radiolabeled product with that of the corresponding unlabeled reference standard.

HPLC Analysis of [^{11}C]DASB—Column: Phenomenex Luna C18 5 μ , 100 $\times$ 2.0 mm; mobile phase: 35% MeOH : 65% 20 mM NH₄OAc; pH: 4.5; flow rate: 0.3 mL / min; oven: 30 $^{\circ}\text{C}$; UV: 254 nm; t_R = 5.5 min. [^{12}C]DASB non-radioactive reference standard was purchased from ABX Advanced Biochemicals (typical traces are shown in Figure 3).

HPLC Analysis of [^{11}C]Raclopride—Column: Phenomenex Luna C18 5 μ , 100 $\times$ 2.0 mm; mobile phase: 35% MeOH : 65% 20 mM NH₄OAc; pH: 4.5; flow rate: 0.8 mL / min; oven: 40 $^{\circ}\text{C}$; UV: 218 nm; t_R = 3.5 min. [^{12}C]Raclopride non-radioactive reference standard was purchased from ABX Advanced Biochemicals (a typical trace is shown in Figure 4).

Radionuclidic Identity—Radionuclidic identity is confirmed by measuring the half-life of radiopharmaceutical doses and comparing it to the known half-life of carbon-11 (20.8 min). Activities are measured using a dose calibrator and half-life is calculated using equation (1). Calculated half-life must be 18.4 – 22.4 min.

$$T_{1/2} = -\ln 2 (\text{Time Difference} / (\ln(\text{ending activity} / \text{starting activity}))) \quad (1)$$

Sterile Filter Integrity (Bubble Point) Test—Sterile filters from doses (with needle still attached) are connected to a nitrogen supply *via* a regulator. The needle is then submerged in water and the nitrogen pressure gradually increased. If the pressure is raised

above the filter acceptance pressure (typically 40 psi) without seeing a stream of bubbles, the filter is considered intact.

Bacterial Endotoxins—Endotoxin content in radiopharmaceutical doses is analyzed by a Charles River Laboratories EndoSafe® Portable Testing System and according to the US Pharmacopeia. Doses must contain <175 Endotoxin Units (EU).

Sterility—Culture tubes of fluid thioglycolate media (FTM) and tryptic soy broth (TSB) are inoculated with samples of the radiolabeled product and incubated (along with positive and negative controls) for 14 days. FTM is used to test for anaerobes, aerobes and microaerophiles whilst TSB is used to test for non-fastidious and fastidious microorganisms. Culture tubes are visually inspected on the 3rd, 7th and 14th days of the test period and compared to the positive and negative standards. Positive standards must show growth (turbidity) in the tubes, and dose / negative controls must have no culture growth after 14 days to be indicative of sterility.

3. Results

3.1. Loop Synthesis of [¹¹C]DASB

[¹¹C]DASB was prepared according to Scheme 1, and as outlined in Section 2.3. The total synthesis time was about 45 minutes. Typical yields of [¹¹C]DASB prepared using this method were 89 mCi at end of synthesis (3.0% based upon 3 Ci [¹¹C]CO₂, non-decay corrected, n = 9). The radiochemical purity was greater than 95% (Figure 3) and specific activity was 15152 Ci/mmol at end of synthesis. The preparative HPLC method utilized in these loop syntheses was the one reported by Gillings and colleagues, which reversed the order of elution of DASB product and precursor and improved chemical purity of the final doses. Doses of [¹¹C]DASB met all of the established release criteria, as summarized in Table 1 and outlined in Section 2.5.

3.2. Loop Synthesis of [¹¹C]Raclopride

[¹¹C]Raclopride was prepared according to Scheme 1, and as outlined in Section 2.4. The total synthesis time was about 30 minutes, and typical yields of [¹¹C]raclopride prepared using this method were 111 mCi at end of synthesis (3.7% based upon 3 Ci [¹¹C]CO₂, non-decay corrected, n = 64). The radiochemical purity was greater than 95% (Figure 4) and specific activity was 20831 Ci/mmol at end of synthesis. The semi-preparative HPLC system described permitted reversal of the retention order of product and precursor during chromatographic purification. This eliminated contamination of products resulting from precursor tailing into the product peak. Moreover, the semi-preparative HPLC solvent contained only 10% ethanol, and so no Sep-Pak reformulation was required. The product collected from HPLC was simply diluted with USP saline to make it less than 5% ethanol for injection, and submitted for QC testing. Doses complied with all release criteria, as summarized in Table 1 and outlined in Section 2.5.

4. Discussion

With the aim of phasing out Class II solvents, as well as MEK, from our carbon-11 radiopharmaceutical production program for the reasons outlined above, we considered alternative solvents with which to conduct carbon-11 loop chemistry. Whilst there are many Class III solvents to choose from that could achieve this goal, it quickly became apparent that, even for quarterly residual solvent analyses, GC methods would have to be developed for residual solvent analysis of any solvent chosen, except perhaps ethanol. We routinely

employ ethanol in HPLC mobile phases, as well as formulate radiopharmaceutical doses in 5 – 10% v/v ethanol, and so have reliable GC methods in place for its analysis.

[¹¹C]DASB and [¹¹C]raclopride were selected as the two radiopharmaceuticals for initial investigation of the ethanolic loop chemistry concept because, of all the radiopharmaceuticals labeled with carbon-11 using loop methods in this program, these two are by far the most commonly requested by nuclear medicine physicians at the University of Michigan. In initial attempts to prepare [¹¹C]raclopride, 1 mg of TBA precursor **3** was dissolved in 100 μL of ethanol and loaded into the HPLC loop. [¹¹C]MeOTf was then blown through the loop according to our standard protocol, 40 mL/min for 3 minutes. Analysis of the reaction mixture confirmed the production of [¹¹C]raclopride, and the notion that loop syntheses can be conducted using ethanol. The combination of the low amount of solvent, nucleophilicity of the raclopride precursor TBA salt, and relatively high flow rate of the [¹¹C]MeOTf combined to promote radiolabeling and minimize solvolysis of ethanol. As shown in the preparative HPLC trace (Figure 6), >85% of the radioactivity corresponded to [¹¹C]raclopride, and radioactive byproducts were limited. [¹¹C]Raclopride could then be purified using our standard, previously reported, semi-preparative HPLC method. Final dilution with saline provided formulated [¹¹C]raclopride.

Similar results were obtained for the corresponding synthesis of [¹¹C]DASB. In this case, the existing semi-preparative HPLC method (in which precursor eluted off first and occasionally tailed into the product peak) was replaced with the newly reported method from Gillings and colleagues, and a subsequent reconstitution into ethanolic saline. The new HPLC method is attractive as it actually reverses the elution order of DASB and the precursor, and so eliminates the chance of contamination resulting from precursor tailing into the product peak. This resulted in improved chemical purity of [¹¹C]DASB (less minor impurities presumably associated with residual precursor) and, interestingly, also appears to be reflected in improved specific activity. Using the old HPLC method, the average specific activity for [¹¹C]DASB was 5239 Ci/mmol (n = 10). Replacing the semi-preparative method with the new HPLC method resulted in 3-fold higher specific activities of 15152 Ci/mmol (n = 9).

At this stage, both [¹¹C]raclopride and [¹¹C]DASB were synthesized, purified and formulated using only ethanol. The only remaining solvent requiring elimination from the process was acetone, typically used during daily and weekly cleaning and drying of the synthesis module. Therefore the previously reported clean and dry cycles were modified to use only ethanol: clean (water), disinfect (70% ethanol) and dry (neat ethanol). Reflecting the decreased volatility of ethanol compared to acetone, the drying cycle was extended and the HPLC loop was blown dry with helium for 10 minutes, rather than the usual 5 minutes.

Following these initial positive results, simple proof-of-concept studies were demonstrated by preparing both radiopharmaceuticals multiple times. [¹¹C]Raclopride was routinely obtained in 3.7% radiochemical yield (n = 64, based upon [¹¹C]CO₂), higher than yields for the corresponding loop methylation conducted in MEK previously disclosed by this group (2.2%, n = 10, based upon [¹¹C]CO₂). This increase in yield is attributed to improved solubility of the TBA salt of raclopride precursor in ethanol, versus MEK. Similarly, [¹¹C]DASB was prepared in 3.0% radiochemical yield (n = 9, based upon [¹¹C]CO₂), comparable to previously reported yields obtained using MEK as the solvent (4.0%, n = 10, based upon [¹¹C]CO₂). Following purification and formulation, doses were submitted for QC testing (Table 1), which confirmed that the products were suitable for clinical use. Building on this, both of these synthesis methods are now employed to meet routine clinical supply of both [¹¹C]raclopride and [¹¹C]DASB.

Another aspect of the synthesis of [^{11}C]DASB with scope for improvement is handling the radiolytic decomposition of the product. We have previously investigated radiolysis of radiopharmaceuticals, and demonstrated that many aniline-containing species, including [^{11}C]DASB, are prone to radiolytic decomposition. Such decomposition results in radiochemical impurities in the final product, leading to batch failures because radiochemical purity (RCP) specifications are not met. Thus, when preparing [^{11}C]DASB using traditional methods, it is essential to add sodium ascorbate to the final formulation to inhibit radiolysis. However, a drawback of adding sodium ascorbate to final formulations is that the UV signal associated with ascorbate is so large that it overwhelms the analytical HPLC trace (Figure 3), making accurate analysis of cold [^{12}C]DASB to determine specific activity, and any other chemical impurities in the dose, problematic. Trapping of [^{11}C]DASB on the sep-pak is where the radiopharmaceutical is at its most concentrated, and we believe at its most vulnerable to radiolytic degradation. Considering this issue, we realized that the fairly high concentration of ethanol involved in reformulation, resulting from the change in HPLC mobile phase from our previous method (100 mM NH_4OAc in 30% MeCN, pH = 5.5), to that reported by Gillings and co-workers (10 mM NH_4OAc in 80% EtOH), might impart the same anti-oxidant protection previously imparted by sodium ascorbate during the critical reformulation step. Therefore, we repeated the synthesis of [^{11}C]DASB without any sodium ascorbate and found that our supposition about the presence of ethanol was correct. Concentrations proved to offer sufficient anti-oxidant protection so that [^{11}C]DASB was obtained in greater than 95% radiochemical purity (Table 1, $n = 3$). Moreover, doses were found to be stable up to 1 h after end-of-synthesis (data not shown), and HPLC analysis of levels of unlabeled [^{12}C]DASB present in doses was greatly simplified because of the lack of an ascorbate peak in the analytical HPLC traces (Figure 3).

5. Conclusions

The development of ethanolic loop chemistry has enabled the design and implementation of fully automated synthesis procedures for [^{11}C]raclopride and [^{11}C]DASB that employ ethanol as the only organic solvent for module cleaning, synthesis, purification, and reformulation. Doses are suitable for human administration and the described methods are now used for routine clinical production of both radiopharmaceuticals at the University of Michigan. In addition, we are in the process of adapting this new methodology for the synthesis of other carbon-11 radiopharmaceuticals prepared in our laboratories ([^{11}C]PMP, [^{11}C]PBR28 *etc.*). The elimination of all other organic solvents from the process simplifies production and QC as it eliminates the need to purchase, handle and properly dispose of other hazardous solvents (e.g. MEK), and relegates residual solvent analysis from a daily QC requirement to a quarterly or annual one. In addition, the reported semi-preparative HPLC methods permit reversal of retention orders of products and precursors during purification, eliminating precursor tailing into the product peak and leading to improved chemical purities and specific activities. The combination of all of these improvements reduces overall production times, and has provided [^{11}C]raclopride in higher yields and specific activities than our previously reported production methods. Similarly, the yields of [^{11}C]DASB are comparable to those previously communicated, but specific activity is about 3-fold higher and the need to include sodium ascorbate in the synthesis as an anti-oxidant to inhibit radiolysis is eliminated.

Acknowledgments

We thank the staff at the University of Michigan PET Facility for generation of the routine production data reported herein, and gratefully acknowledge the National Institutes of Health (NIH NS15655) for financial support of this research.

References

- Shao X, Hoareau R, Runkle AC, Tluczek LJM, Hockley BG, Henderson BD, Scott PJH. Highlighting the versatility of the Tracerlab synthesis modules. Part 2: fully automated production of [^{11}C]-labeled radiopharmaceuticals using a Tracerlab FX_{C-Pro}. *J. Label. Compd. Radiopharm.* 2011; 54:819–838.
- Shao X, Kilbourn MR. A simple modification of GE Tracerlab FX C Pro for preparation of [^{11}C]carfentanil and [^{11}C]raclopride. *Appl. Radiat. Isot.* 2009; 67:6002–6605.
- Wilson AA, Garcia A, Jin L, Houle S. Radiotracer synthesis from [^{11}C]-iodomethane: a remarkably simple captive solvent method. *Nucl. Med. Biol.* 2000; 27:529–532. [PubMed: 11056365]
- Wilson AA, Garcia A, Houle S, Vasdev N. Utility of commercial radiosynthetic modules in captive solvent [^{11}C]-methylation reactions. *J. Label. Compd. Radiopharm.* 2009; 52:490–492.
- Iwata R, Pascali C, Bogni A, Miyake Y, Yanai K, Ido T. A simple loop method for the automated preparation of [^{11}C]raclopride from [^{11}C]methyl triflate. *Appl. Radiat. Isot.* 2001; 55:17–22. [PubMed: 11339532]
- Ono M, Wilson A, Nobrega J, Westaway D, Verhoeff P, Zhuang ZP, Kung MP, Kung HF. ^{11}C -labeled stilbene derivatives as A β -aggregate-specific PET imaging agents for Alzheimer's disease. *Nucl. Med. Biol.* 2003; 30:565–571. [PubMed: 12900282]
- Studenov AR, Jivan S, Buckley KR, Adam MJ. Efficient in-loop synthesis of high specific radioactivity [^{11}C]carfentanil. *J. Label. Compd. Radiopharm.* 2003; 46:837–842.
- Wilson AA, Garcia A, Chestakova A, Kung H, Houle S. A rapid one-step radiosynthesis of the b-amyloid imaging radiotracer N-methyl- [^{11}C]2- (40-methylaminophenyl)-6-hydroxybenzothiazole ([^{11}C]-6-OH-BTA-1). *J. Label. Compd. Radiopharm.* 2004; 47:679–682.
- Lu SY, Hong J, Musachio JL, CF T, VE S, Wikström HV, Pike VW. Alternative methods for labeling the 5-HT_{1A} receptor agonist, 1-[2-(4-fluorobenzoylamino)ethyl]-4-(7-methoxynaphthyl)piperazine (S14506), with carbon-11 or fluorine-18. *J. Label. Compd. Radiopharm.* 2005; 48:971–981.
- Le Bars D, Mallevall M, Bonnefoi F, Tourvieille C. Simple synthesis of [1- ^{11}C]acetate. *J. Label. Compd. Radiopharm.* 2006; 49:263–267.
- Musachio JL, Hong J, Ichise M, Seneca N, Brown AK, Liow JS, Halldin C, Innis RB, Pike VW, He R, Zhou J, Kozikowski AP. Development of new brain imaging agents based upon cocaine-modafinil hybrid monoamine transporter inhibitors. *Bioorg. Med. Chem. Lett.* 2006; 16:3101–3104. [PubMed: 16621532]
- Reske SN, Blumstein NM, Neumaier B, Gottfried HW, Finsterbusch F, Kocot D, Möller P, Glatting G, Perner S. Imaging prostate cancer with ^{11}C -choline PET/CT. *J. Nucl. Med.* 2006; 47:1249–1254. [PubMed: 16883001]
- Cleij MC, Clark JC, Baron J-C, Aigbirhio FI. Rapid Preparation of [^{11}C]Flumazenil: Captive Solvent Synthesis Combined With Purification by Analytical Sized Columns. *J. Label. Compd. Radiopharm.* 2007; 50:19–24.
- Probst KC, Izquierdo D, Bird JL, Brichard L, Franck D, Davies JR, Fryer TD, Richards HK, Clark JC, Davenport AP, Weissberg PL, Warburton EA. Strategy for improved [^{11}C]DAA1106 radiosynthesis and in vivo peripheral benzodiazepine receptor imaging using microPET, evaluation of [^{11}C]DAA1106. *Nucl. Med. Biol.* 2007; 34:439–446. [PubMed: 17499734]
- Briard E, Zoghbi SS, Imaizumi M, Gourley JP, Shetty HU, Hong J, Cropley V, Fujita M, Innis RB, Pike VW. Synthesis and Evaluation in Monkey of Two Sensitive ^{11}C -Labeled Aryloxyanilide Ligands for Imaging Brain Peripheral Benzodiazepine Receptors In Vivo. *J. Med. Chem.* 2008; 51:17–30. [PubMed: 18067245]
- Jarkas N, Voll RJ, Williams L, Votaw JR, Owens M, Goodman MM. Synthesis and In Vivo Evaluation of Halogenated N,N-Dimethyl-2-(2'-amino-4'-hydroxymethylphenylthio)benzylamine Derivatives as PET Serotonin Transporter Ligands. *J. Med. Chem.* 2008; 51:271–281. [PubMed: 18085744]
- Donohue SR, Krushinski JH, Pike VW, Chernet E, Phebus L, Chesterfield AK, Felder CC, Halldin C, Schaus JM. Synthesis, Ex Vivo Evaluation, and Radiolabeling of Potent 1,5-

- Diphenylpyrrolidin-2-one Cannabinoid Subtype-1 Receptor Ligands as Candidates for In Vivo Imaging. *J. Med. Chem.* 2008; 51:5833–5842. [PubMed: 18800770]
18. Gómez V, Gispert JD, Amador V, Llop J. New method for routine production of L-[methyl- ^{11}C]methionine: in loop synthesis. *J. Label. Compd. Radiopharm.* 2008; 51:83–86.
 19. Studenov AR, Jivan S, Adam MJ, Ruth TJ, Buckley KR. Studies of the mechanism of the in-loop synthesis of radiopharmaceuticals. *Appl. Radiat. Isot.* 2004; 61:1195–1201. [PubMed: 15388110]
 20. ICH Harmonised Tripartite Guideline. Impurities: Guideline for Residual Solvents Q3C(R5). 2011:1–29.
 21. Chapter <823> Positron Emission Tomography Drugs for Compounding, Investigational, and Research Uses. USP 35 - NF 50. 2012:398–406.
 22. Effenberger F. Electrophilic Reagents - Recent Developments and Their Preparative Application. *Angew. Chem. Int. Ed.* 1980; 19:151–230.
 23. Lee S-S, Kim H-S, Hwang T-K, Oh Y-H, Park S-W, Lee S, Lee BS, Chi DY. Efficiency of Bulky Protic Solvent for $\text{S}_{\text{N}}2$ Reaction. *Org. Lett.* 2008; 10:61–64. [PubMed: 18052384]
 24. Oh Y-H, Im S, Park S-W, Lee S, Chi D-Y. $\text{S}_{\text{N}}2/\text{E}2$ Branching in protic Solvents: A Mechanistic Study. *Bull. Korean Chem. Soc.* 2009; 30:1535–1538.
 25. Lehel S, Madsen J, Gillings N. HPLC Methods for the purification of [^{11}C]-labelled radiopharmaceuticals: reversal of the retention order of products and precursors. *J. Label. Compd. Radiopharm.* 2009; 52:177–181.
 26. Scott PJH, Hockley BG, Kung HF, Manchanda R, Zhang W, Kilbourn MR. Studies into radiolytic decomposition of fluorine-18 labeled radiopharmaceuticals for positron emission tomography. *Appl. Radiat. Isot.* 2009; 67:88–94. [PubMed: 18951032]

C-11 Mel, Process: DASB on FXc, Batch No.: 030712DASB-V3, Operator: Admin, Start of Synthesis: 3/7/2012 12:23:49

Figure 1.
Preparative HPLC trace of [^{11}C]DASB

C-11 MeI, Process: RAC on FXc, Batch No.: 030912RAC-26, Operator: Admin, Start of Synthesis: 3/9/2012 13:30:40 , P

Figure 2.
Preparative HPLC trace of [^{11}C]Raclopride

UV Detector Response

UV Detector Ch1-254nm Results					
Retention Time	Area	Area %	Height	Width	S/N (ASTM)
5.533	11390	100.00	564	0.83	0.001057

UV Detector Ch1-254nm Results					
Retention Time	Area	Area %	Height	Width	S/N (ASTM)
1.667	49868	76.4	2034	0.733	4.83
2.275	7313	11.2	359	0.408	0.85
5.725	8098	12.4	382	0.983	48.78

Gamma Detector Response

Detector 3 Results		
Retention Time	Area	Area Percent
2.658	29219	0.926
5.691	3125612	99.074

RAD Results			
Retention Time	Area	Area %	Width
0.287	1910	0.0	0.26
0.393	2651	0.1	0.35
1.708	18635	0.4	0.43
2.048	48650	1.0	0.75
2.976	40394	0.8	1.11
6.161	4774113	97.6	2.11
7.671	4365	0.1	0.41

+ Sodium AscorbateNo Sodium Ascorbate

Figure 3.
Typical Analytical HPLC Trace for [^{11}C]DASB

UV Detector Response

UV Detector Ch1-218nm Results

Retention Time	Area	Area %	S/N (ASTM)	Width	Height
3.375	1454	100.00	0.003988	0.33	135

Gamma Detector Response

Bioscan RAD Results

Retention Time	Area	Area Percent
0.525	55077	3.099
1.500	2054	0.116
3.416	1720151	96.785

Figure 4.
Typical Analytical HPLC Trace for [^{11}C]Raclopride

Scheme 1.
Synthesis of $[^{11}\text{C}]\text{DASB}$ and $[^{11}\text{C}]\text{Raclopride}$

Table 1QC Data for [^{11}C]Raclopride and [^{11}C]DASB

Compound	[^{11}C]Raclopride (n = 64)	[^{11}C]DASB* (n = 9)	[^{11}C]DASB† (n = 3)
Yield (mCi, EOS)	111	89	62
Visual Inspection	clear, colorless	clear, colorless	clear, colorless
pH	5.0 – 6.0	5.0 – 6.0	5.0 – 6.0
Radiochemical Purity	> 95%	> 95%	> 95%
Specific Activity (Ci/mmol, EOS)	20831	15152	10931
Endosafe Test (EU/mL)	< 2.0	< 2.0	< 2.0
Sterility	Pass	Pass	Pass

* Method A: with sodium ascorbate;

† Method B: without sodium ascorbate