Coliva A, Monterisi C, Apollaro A, Gatti D, Penso M, Gianolli L, Perani D, Gilardi MC, Carpinelli A.

Synthesis optimization of 2-(4-N-[11C]methylaminophenyl)-6-hydroxybenzothiazole ([11C]PIB), β-amyloid PET imaging tracer for Alzheimer's disease diagnosis.

Appl Radiat Isot. 2015 Nov;105:66-71. Epub 2015 Jul 17.

doi: 10.1016/j.apradiso.2015.07.003.

Synthesis optimization of 2-(4-N-[11C]methylaminophenyl)-6-hydroxybenzothiazole ([^{11}C]PIB), β-amyloid PET imaging tracer for Alzheimer's Disease diagnosis

A. Coliva[a], C. Monterisi[b], A. Apollaro[b], D. Gatti[c], M. Penso[d], L. Gianolli[c], D. Perani[a,b], M.C. Gilardi[b,c], A Carpinelli[b,c*].

a Vita-Salute S. Raffaele University, Milan, Italy

b CNR-IBFM, Milan, Italy

c Nuclear Medicine Department, IRCCS Ospedale San Raffaele, Milan, Italy

d CNR-ISTM, Milan, Italy

Highlights

- Good yields and specific activity of [11C]PIB were obtained by reaction parameters optimization
- Improvement of the HPLC purification
- Quality parameters and drug formulation allows a readily application in clinic

* Corresponding author: Assunta Carpinelli, CNR-IBFM c/o Department of Nuclear Medicine, Ospedale San Raffaele, Via Olgettina 60, 20132 Milano, Italy

Phone: +39 02 2643 3630. E-mail: carpinelli.assunta@hsr.it

Abstract

[^{11}C]PIB is the most used amyloid plaques-specific positron-emitting radiotracers. The radiosynthesis of this compound, carried out by methylation of its precursor with [^{11}C]methyl triflate in 2-butanone, has been improved optimizing the initial concentration and the purification method. Two HPLC methods were compared: good radiochemical yields, specific activities, and chemical purity above 98% were achieved by using as eluant acetonitrile/citrate and formulation in 10% ethanol.

Keywords:

Cyclotron; Alzheimer's disease; [^{11}C]MeOTf; [^{11}C]PIB, β-amyloid

Introduction

Alzheimer's disease (AD) is a very common neurodegenerative disorder characterized by progressive impairment of cognitive functions and the formation of amyloidal plaques in the brain (Tiraboschi et al, 2004), neuronal loss and neurofibrillary tangles. It has been shown that the underlying pathology of Alzheimer's disease precedes the onset of clinical symptoms by many years (Bateman et al, 2012), and the definitive diagnosis of Alzheimer's disease is considered possible only by post-mortem histopathology (Ni et al, 2013). A probable diagnosis in living patients is possible through the detection of neuropathological lesions either by means of brain imaging, or through suitable CSF biomarkers (Klunk, 1998). Amyloid plaques represent a specific target for selective radiotracers in early and differential diagnosis of AD (Klunk, 2011; Villemagne et al, 2011; Chételat et al, 2013). Amyloid imaging has been proposed firstly by Mathis et al.(2002) employing derivatives of thioflavin-T labeled with carbon-11. In particular, 6-OH-BTA-1 (Pittsburgh Compound B-PIB) showed a very rapid brain entry, rapid brain clearance of nonspecifically bound and free tracer, and specific, prolonged binding to amyloid-targets (Bacskai et al, 2003). [11C]PIB, visualizing fibrillar Aβ, created new challenge for early detection of brain pathology *in vivo* and recently also for treatment monitoring (Nordberg et al, 2010).

The original radiosynthesis of [^{11}C]PIB was carried out by Mathis et al. (2003) through N-methylation of the methoxymethyl-protected phenol precursor with [^{11}C]methyl iodide, followed by acid deprotection (Figure 1, path *a*). Recently, a different strategy, which employs in the methylation step the more reactive [^{11}C]methyl trifluoromethanesulfonate ([^{11}C]MeOTf) has been published (Mathis et al, 2003; Solbach et al, 2005; Wilson et al, 2004) (Figure 1, path *b*). The use of

[^{11}C]MeOTf allowed the direct radiolabelling of the unprotected phenolic precursor, thus enhancing the radiochemical yield.

Figure 1

When, by following a literature procedure,(filippa) we started to work on the radiosynthesis of [11C]PIB very poor results were obtained in terms of radiochemical yields and specific activity. Starting from these first attempts, several aspects were investigated and improved to the extent possible. To this regard, target gas mixture containing high purity (99.9999%) N60 nitrogen and oxygen was chosen in order to exclude water and other contaminants, which may affect specific activity. Moreover, hydrogen and helium implied in the process, whose purity could affect the overall quality of the intermediate [^{11}C]CH_3I, were passed through two gas purifier traps before using. 2-Butanone dried over 4 Å molecular sieves gave the best results as a reaction solvent. On the contrary, when non-dried solvents were employed the overall results was adopted because results were unsatisfying (data not shown). Furthermore, a general revision of the whole preparation process was made, with particular attention to the reaction solvent and precursor amounts and to the semi-preparative purification conditions.

By this work, we aimed to optimize and validate a fast and fully automated method for the production of [^{11}C]PIB giving high yields and good specific activity.

Materials and Methods

All analytical grade chemicals and solvents were purchased commercially and used without further purification, except for 2-butanone, as described before. Loading target gas N_2 (0.5% O_2) was supplied by Gruppo SAPIO (Milan, Italy). Sublimated iodine was

purchased from Sigma-Aldrich (Saint Louis, MO, USA). The Ni catalyst (Shimalite Ni reduced, 80/100 mesh) was purchased from Shimadzu (Kyoto, Japan). During the early set up of the method, the powder of silver triflate impregnated carbon was prepared in our laboratory dissolving 1g of silver trifluoromethanesulfonate in 150 mL of anhydrous diethyl ether. To this solution 2 g of Graphpack C (C 80/100 mesh) were added and the suspension was stirred under reduced pressure in the dark to leave the ether dry off slowly. When the ether was evaporated, the resulting powder was dried for at least 2 hours under vacuum (0.2 Torr). The dry powder was stored into a dark glass bottle. Afterwards, the reagent was provided by ABX GmbH, (ABX-Advanced Biochemical Compounds, Radeberg, Germany) that prepared it following the same procedure.

The precursor 6-OH-BTA-0 [2-(4′-Aminophenyl)-6-hydroxybenzothiazole, purity > 95%], 6-MeO-BTA-0 [2-(4'-aminophenyl)-6-methoxybenzothiazole], the reference compound of a potential radioactive by-product, and 6-OH-BTA-1 (2-[4'-(methylamino)phenyl]-6-hydroxybenzothiazole hydrochloride) reference compound were obtained from ABX GmbH, (ABX-Advanced Biochemical Compounds, Radeberg, Germany). 2-Butanone (Chromasolv®, for HPLC $\geq$ 99.7%), acetonitrile, acetone, ethanol, ascorbic acid, and trisodium citrate dihydrated were purchased from Sigma Aldrich (Saint Louis, MO, USA). Sterile ethanol, water, and sodium citrate for final formulation were supplied by S.A.L.F (Cenate Sotto, BG, Italy). tC18 plus cartridges were purchased from Waters (Waters Associated, Milford, MA, USA). Millex-GP filters were obtained from Millipore (Bedford, MA, USA). Semi-preparative Eclipse XDB-C18, 5 μm, 9.4 mm×250 mm, and analytical Eclipse C18, 5 μm, 4.8 mm×250 mm HPLC columns were supplied by Agilent (Agilent Technologies, Santa Clara CA, USA). C18 LUNA, 10 μm, 10 mm×250 mm, HPLC column was supplied by

Phenomenex (Torrance, CA, USA). GLC capillary column (ELITE-1301, 1 μm, 0.32 mm×30 m) was purchased from Perkin Elmer (Waltham, MA, USA).

The automatic synthesis module TracerLab FX-C Pro and the remote control software were purchased from General Electric Medical System (Uppsala, Sweden). Semi-preparative HPLC equipment was a Sykam pump S1021 (Fuerstenfeldbruck, Germany). Analytical HPLC consists of a 515 pump, a 486 Tunable Absorbance UV detector (operated at 350 nm) and a NaI-radiodetector Flowcount FC 3200 (Eckert & Ziegler Radiopharma Inc. Hopkinton, MA, USA). Gas-chromatography was performed using a Clarus 500 model (Perkin Elmer, Waltham, MA, USA) with Flame ionization detector (FID). pH was measured using a 827 pH LAB model (Metrohm, Herisau, Switzerland). Radionuclide identification was performed using a NaI (Tl) gamma detector 3M3/3-X (Ortec, Advanced Measurement Technology, Inc, South Illinois Avenue, Oak Ridge, TN, USA). Mass spectra were recorded using a LCQ Advantage spectrometer equipped with an ESI source (Thermo Finnigan LLC, San Jose, California, USA).

Fully automated radiosynthesis

The synoptic of the synthesis module is represented in Figure 2.

Figure 2

Preparation of the synthesis module

All glassware and tubing of the module were rinsed with pure water, acetone or ethanol and then dried using a stream of helium. During the preparation of the synthesis, vessels were filled as follows:

Vial 1: HPLC eluent to quench the reaction and dilution before HPLC injection.

Vial 4: water for washing the SPE cartridge.

Vial 5: absolute ethanol for elution of the SPE cartridge.

Vial 6: 0.01M tri-sodium citrate with 0.1% ascorbic acid for final rinsing of the SPE cartridge and dilution of the eluate.

Round bottom flask: 0.01M tri-sodium citrate for dilution of the HPLC fraction after peak cutting and pH adjustment.

A tC18 cartridge was conditioned with 5 mL of ethanol followed by 20 mL of sterile water, dried and connected to its dedicated position. Vials 4,5,6, the round bottom flask, and the SPE cartridge were used only for tests performed following Method B for purification (see paragraph *"Purification of [^{11}C]PIB"* for description).

The reactor was filled with 0.5-1 mg of 6-OH-BTA-0 dissolved in 0.3-0.5 mL of 2-butanone few minutes before starting the synthesis and then placed into the heating block.

The AgOTf-oven contains a tube filled with about 500 mg graphitized carbon impregnated with silver triflate. MeI Trap-oven contains about 300 mg of Porapak Q.

Stynthesis of [^{11}C]methyl triflate ([^{11}C]MeOTf)

No carrier added [11C]CO_2 was produced by the 14N(p,α)11C nuclear reaction at a IBA Cyclone 18/9 MeV cyclotron (IBA Molecular, Louvain la Neuve, Belgium). Target was loaded with N60 (99.9999 % purity) N_2 containing 0.5% of O_2 and irradiated for 45 min with a 35 μA beam currents. [^{11}C]CO_2 was delivered into the automatic synthesis module TracerLab FXC-Pro and [^{11}C]CH_3I was produced via the gas-phase conversion pathway (Larsen et al, 1997). [^{11}C]CO_2 was trapped at room temperature in a column containing 4 Å molecular sieves (powder) and Ni-catalyst, and then converted into [^{11}C]CH_4 under hydrogen at 350 °C. [^{11}C]CH_3I was obtained by reaction of [^{11}C]CH_4 with I_2 at 720 °C in a gas recirculating process system. [^{11}C]methyl iodide was

trapped at room temperature onto the Porapak Q and then recovered by heating the adsorbents at 190 °C under a moderate helium flow. [^{11}C]MeOTf was thereafter generated by reaction of [^{11}C]CH_3I with silver triflate in an online flow-through process at 200 °C under helium gas flow.

Synthesis of [11C]PIB

[^{11}C]MeOTf was bubbled into the reactor cooled at 13 °C containing a precursor 6-OH-BTA-0 (0.5-1 mg) solution in 2-butanone (0.3-0.5 mL). The reactor was then heated at 75 °C for 2 min, and the reaction was quenched by adding 1.2-1.4 mL of HPLC mobile phase. This solution was injected into the HPLC by passing through the fluid detector. Methylation takes place at 75 °C within 2 min~~utes~~. HPLC injection occurred passing through the fluid detector after quenching the reaction by adding 1.2-1.4 mL of HPLC mobile phase.

Purification of [11C]PIB

The crude mixture was purified by semi-preparative HPLC. The apparatus was equipped with an UV detector (λ = 280nm) and a NaI radioactivity detector placed in series. To prevent autoradiolysis, ascorbic acid was added to both the mobile phase and the final formulation. Two different chromatographic conditions were investigated.

Method A: the product was eluted on the C18 LUNA column with ethanol/0.01 M tri-sodium citrate (40/60, v/v), 0.1% ascorbic acid, as mobile phase, and a flow rate of 6 mL/min. The collected pure product, was diluted with saline and sterilized by filtration on Millex GP 0.22 µm filters, without further treatments.

Method B: elution was performed on C18 Eclipse XDB column with acetonitrile/0.01 M-trisodium citrate (40/60, v/v), containing 0.1% of ascorbic acid at pH 3.5. Flow rate was 4.5 mL/min. The [11C]PIB fraction was collected and diluted with 30 mL of tri-sodium citrate 0.01M. To change the solvent and to concentrate the product, this solution was then loaded on a tC18 SPE cartridge. After washing with 10 mL of sterile water, the product was recovered with 1.2 mL of ethanol followed by 3.8 mL of 0.01M tri-sodium citrate containing 0.1% of ascorbic acid. The final solution (total volume 5 mL) was then transferred to a shielded laminar air-flow hot cell and, there, filtered through a Millex-GP filter (0.22 μm) into a sterile vial pre-filled with 7 mL of 0.01M tri-sodium citrate (ascorbic acid 0.1%), so reaching a final volume of 12 mL.

Quality control of [^{11}C]PIB

Chemical and radiochemical purities of the labelled compound [^{11}C]PIB were determined by analytical HPLC. The instrument was equipped with a UV detector (λ = 350 nm) coupled to a radioactivity flow detector. Separation was achieved on the C18 Eclipse Plus column with CH_3CN/tri-sodium citrate 0.01 M (50/50; v/v) as mobile phase and at a flow rate of 1 ml/min. The analytical method was validated as regards linearity, specificity, accuracy, repeatability, robustness and limit of quantification and detection. The products eluted as follows:

- precursor 6-OH-BTA-0 RT_1 = 3.8 min;
- target product [11C]PIB RT_2 = 5.8;
- by-product 6-[11C]MeO-BTA-0 RT_3 = 8.9 min

as confirmed by comparison with standard samples.

The radiochemical purity was calculated as the percentage of the total radioactivity that was bound to [^{11}C]PIB.

Residual solvents and ethanol content were analyzed and quantified using a GLC validated method using an ELITE-1301 capillary column, connected to a FID and a split/splitless capillary injector, ~~and~~ using helium as ~~a~~ carrier (T_0 = 40 °C, T_{end} = 200 °C, rate = 20 °C/min). The retention times were as follows: RT(EtOH) = 3.29 min, RT(MeCN) = 4.16 min, RT(2-butanone) = 6.17 min.

pH was measured using a pH-meter equipped with a glass electrode. Radionuclidic purity was assessed measuring physical half-life and recording the corresponding gamma spectrum with a NaI (Tl) gamma detector.

LAL test for the determination of the bacterial endotoxin content was performed using Endosafe® PTS or MCS instrument (Charles River Laboratories) after dilution of the product in a proper buffer to adjust pH. Sterility was performed by an external partner (Eurofins Biolab S.r.l, Vimodrone, Milan, Italy) following a validated procedure.

Results and discussion

During the process optimization, several aspects were improved respect the starting conditions, as the target gas purity, the transporting gases moisture, Ag-triflate catalyst preparation and reaction solvent anhydrification. Some parameters, instead, were investigated and optimized as the solvent volume, the precursor amount and the purification strategy.

Radiosynthesis of [^{11}C]PIB

Synthesis time, elapsed from trapping of methane on CH_4Trap to collection of the final compound, was about 30 min. Semi-preparative purification followed Method B, as before described in the paragraph "*Purification of [11C]PIB*". Elution of the product from SPE cartridge with 1.2 mL of ethanol followed by 3.8 mL of trisodium citrate 0.01M was nearly quantitative (≥ 95% of product recovered). Sterilization on Millex GP filters caused a considerable product loss (about 30%), but preliminary tests (data not shown) with Millex GV filters suggested the possibility to reduce it to about 5%.

We investigated also the influence on radiochemical yield and specific activity of both the precursor amount (0.5-1 mg) and the reaction solvent volume (0.3-0.5 mL). Results concerning radiosynthesis of [11C]PIB are summarized in Table 1.

Table 1

Radiochemical yields are calculated as the fraction of the activity, decay corrected, related to the product using [^{11}C]MeOTf activity as starting value. Reduction of the precursor amount improved specific activity, while did not affect the radiochemical yield. On the other hand, an increase of the reaction yield is correlated to a diminished volume of the solvent. Finally, radiochemical purity observed was always > 99%, independently from the conditions applied.

Semi-preparative purification of [11C]PIB

In Table 2 are reported the results concerning the semi-preparative purification. The supposed time saving and reduction of product loss by applying Method A was disregarded because of the increase of the product retention time and of the broad peak profile, with respect to that achieved with Method B. In addition, by applying Method A the collected volume, even if slightly variable, was higher, and very diluted solutions of

the product were obtained. This negative aspect was worsened by the lower activity detected.

Table 2

Finally, chemical purity of the product was lower by Method A than by Method B. An example of semi-preparative and analytical chromatographic profiles with the two methods are illustrated in Figure 3.

Figure 3

The only residual solvent present using Method A is 2-butanone and its content in the final solution is lower than the Limit of Detectability (LOD) of the analytical method. Using Method B, the tC18 cartridge step eliminates acetonitrile, despite its massive use in the HPLC purification, and only traces of this solvent were detectable in the final solution (0,033 ± 0,018 mg/mL). Also the content of 2-butanone is negligible (< 0.0125 mg/mL, LOD).

Product obtained via Method A, because of the elevated ethanol amount (40%), should be diluted with saline to be injectable (EtOH 10%).

Quality control of [11C]PIB

In absence of a specific monograph in the Pharmacopoeia relative to [11C]PIB, we have adopted the quality requirements summarized in Table 3 and derived from the general monograph “Radiopharmaceutical preparations”, while chemical purity and specific activity limits were inferred from literature data.

Table 3

Some tests, evidenced with an asterisk, are required for release: appearance, chemical and radiochemical purity, radiochemical identity, radionuclidic purity, half-life, pH and

bubble point test for filter integrity. Residual solvents analysis, ethanol quantification and endotoxin content are usually performed within 24 hours, with the exception of sterility.

In all performed runs, notwithstanding the amount of precursor or the volume of the solvent, quality controls satisfied acceptance criteria. Radiochemical identity was confirmed by comparison of the retention time of [11C]PIB with that of the standard (5.9 ± 1.7%). Chemical purity was generally over 98% (98.4 ± 2.2% with 1 mg of precursor in 0.5 mL of 2-butanone, 99.7 ± 0.1 % with 1 mg in 0.3 mL and 99.5 ± 0.2% with 0.5 mg in 0.3 mL) and the precursor was the only impurity present (0.0015 ± 0.001 nmol/Vmax). Residual solvents present in the final formulation were far below the limits referred in the Pharmacopoeia (Acetonitrile 0,033 ± 0,018 mg/mL, 2-butanone < 0.0125 mg/mL), as quantified by a specific validated method of analysis, while ethanol was considered an excipient and quantified by GLC by comparison with a reference standard. Microbiological controls were always negative for microbial growth and pyrogen content.

Because of the lacking of a specific Pharmacopoeia monograph, an Investigational Medicinal Product Dossier has been prepared and submitted to the Competent Authorities in order to be authorized to carry out clinical studies.

Conclusions

The method that we have developed optimizes the literature described procedures for the radiosynthesis and the purification of [^{11}C]PIB with a commercial automated synthesis module. Results here reported demonstrate that the method is feasible and reliable and that the process is completed in a short time. The reduction of the precursor amount, together with the optimization of the purification procedure, allows to obtain a

product with a very high chemical purity, good radiochemical yield and specific activity. The radiotracer produced is compliant to all requirements needed to be injectable and so it can be proposed for clinical studies.

Acknowledgements

This work was supported by the EU FP7 INMIND Project (FP7-HEALTH-2011) grant agreement no. 278850.

References

Tiraboschi, P., et al, 2004. The importance of neuritic plaques and tangles to the development and evolution of AD. Neurology 62(11), 1984–1989.

Bateman, R.J., et al., 2012. Clinical and biomarker changes in dominantly inherited Alzheimer's disease. N Engl J Med. 367(9), 795-804.

Ni, R., Gillberg, P.G., Bergfors, A., Marutle, A., Nordberg, A., 2013. Amyloid tracers detect multiple binding sites in Alzheimer's disease brain tissue. Brain. 136(Pt 7), 2217-2227.

Klunk, W.E., 1998. Biological markers of Alzheimer's disease. Neurobiol Aging 19(2), 145-7.

Klunk, W.E., 2011. Amyloid imaging as a biomarker for cerebralb-amyloidosis and risk prediction for Alzheimer dementia. Neurobiol Aging 32 (Suppl 1), S20–S36.

Villemagne, V.L., et al, 2011. Longitudinal assessment of Ab and cognition in aging and Alzheimer disease. Ann Neurol. 69, 181–192.

Chételat, G., et al, 2013. Amyloid imaging in cognitively normal individuals, populations and preclinical Alzheimer’s disease. Neuroimage Clin. 2, 356–365.

Mathis, C.A., et al, 2002. A lipophilic thioflavin-T derivative for positron emission tomography (PET) imaging of amyloid in brain. Bioorg. Med. Chem. Lett. 12, 295-298.

Bacskai, B.J., et al, 2003. Four-dimensional multiphoton imaging of brain entry, amyloid binding, and clearance of an amyloid-β ligand in transgenic mice. PNAS; 100 (21), 12462–12467.

Nordberg, A., et al, 2010. The use of PET in Alzheimer disease. Nat Rev Neurol. 6, 78–87.

Mathis, C.A., et al, 2003. Synthesis and evaluation of 11C labeled 6-substituted 2-arylbenzothiazoles as amyloid imaging agents. J. Med. Chem. 46, 2740-2754.

Solbach, C., et al, 2005. Efficient radiosynthesis of carbon-11 labelled uncharged thioflavin T derivatives using [11C]methyl triflate for b-amyloid imaging in Alzheimer's disease with PET. Appl Radiat Isotop. 62, 591–595.

Wilson, A.A., et al, 2004. A rapid one-step radiosynthesis of the b-amyloid imaging radiotracer N-methyl-[11C]2-(40-methylaminophenyl)-6-hydroxybenzothiazole ([11C]-6-OH-BTA-1). J. Label. Compd. Radiopharm. 47, 679-682.

Philippe, C., et al, 2011. Optimization of the radiosynthesis of the Alzheimer tracer 2-(4-N-[11C]methylaminophenyl)-6-hydroxybenzothiazole([11C]PIB). Appl. Radiat. Isot. 69, 1212-1217.

Larsen, P., et al, 1997. Synthesis of [C-11]iodomethane by iodination of [C-11]methane. Appl. Radiat. Isot. 48, 153–157.

Figures

Figure 1: synthesis scheme: a) $[^{11}C]CH_3$ approach; b) $[^{11}C]$MeOTf approach.

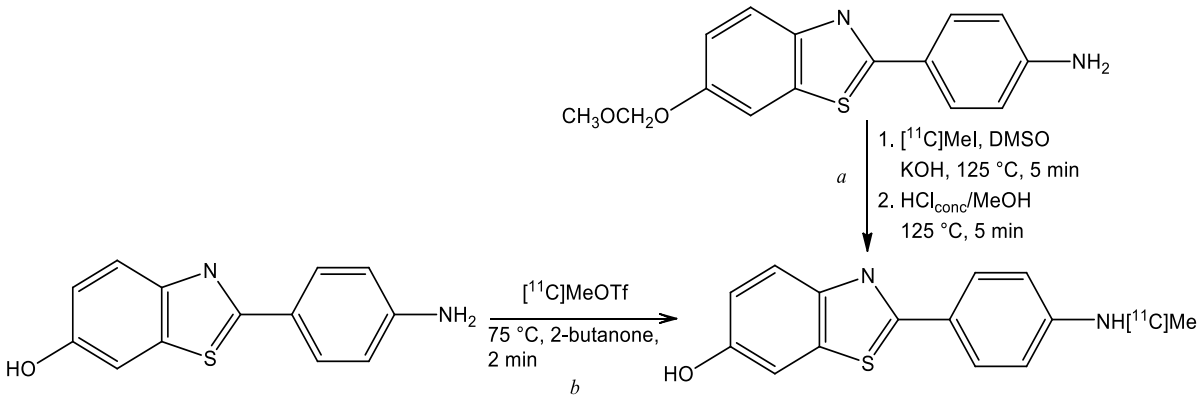


Figure 2: Synoptic of the module set-up of the commercial automated synthesizer TracerLab FxC-Pro. CO2 bypass still designed on the synoptic is not actually used for the synthesis of [^{11}C]PIB, but it should be useful in case of labelling of Grignard reagents.

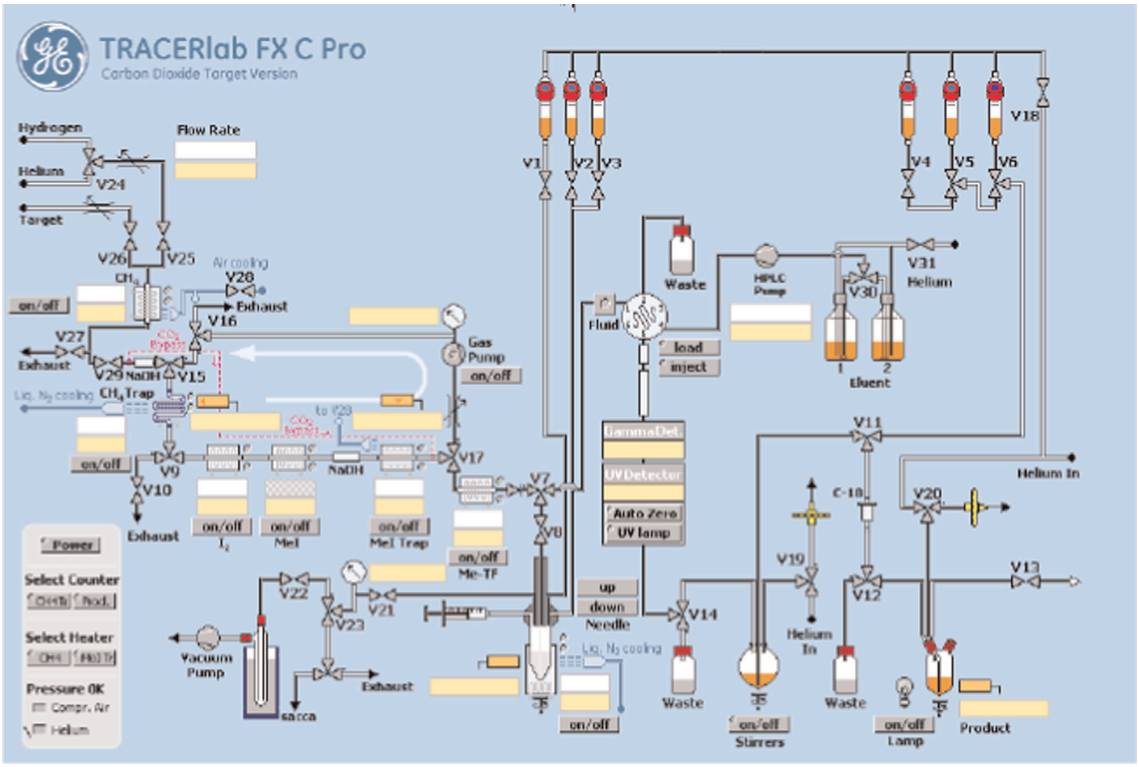
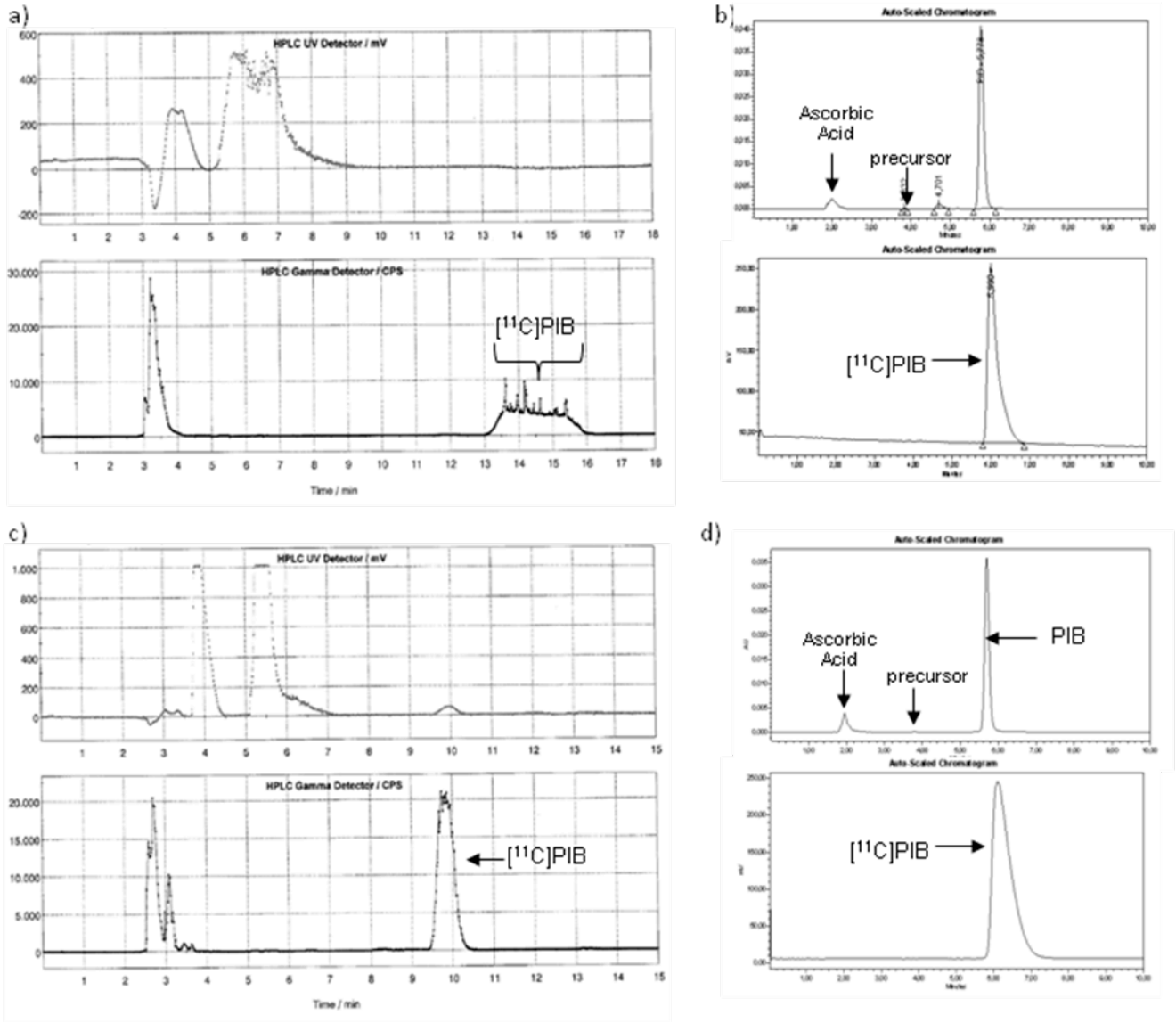


Figure 3: Comparison of semi-preparative and analytical chromatographic traces using Method A and Method B. a) Semipreparative chromatogram (UV and radioactive) using Method A; b) Analytical chromatogram (UV and radioactive) of product purified using Method A; c) Semipreparative chromatogram (UV and radioactive) using Method B; d) Analytical chromatogram (UV and radioactive) of product purified using Method B.

Method A: EtOH/0.01M citrate (40/60, v/v), 0.1% ascorbic acid, rate 6 mL/min;

Method B: CH_3CN/0.01M citrate (40/60, v/v), pH 3.5, 0.1% ascorbic acid, rate 4.5 mL/min.



Tables

Table 1: Reaction conditions vs yield, specific activity and radiochemical purity

Precursor quantity (mg)	0.5	1	1
Solvent volume (mL)	0.3	0.3	0.5
Number of experiments	5	4	10
Yield (GBq)[a]	1.82 ± 0.48	2.92 ± 0.55	2.21 ± 0.64
Yield (%)[b]	35.7 ± 6.4	50.3 ± 6.8	37.5 ± 7.8
Specific Activity (GBq/μmol)[c]	107.3 ± 55.5	63.8 ± 30.4	44.4 ± 18.5
Radiochemical purity (%)	>99	>99	>99

[a] EOS
[b] yield was calculated using triflate activity as starting activity and corrected for decay
[c] calculated on HPLC quantification

Table 2: Results obtained with two different methods employed for chromatographic semipreparative purification.

Condition	Synthesis time (min)	Yield d.c[a]. (%)	Retention time (min)	Product volume (mL)	Chemical purity (%)	Radiochemical purity (%)
Method A (n=14)	32 ± 3	28.6 ± 21.7	13-16	13.5 ± 4.2	90.4 ± 13.9	> 99
Method B (n=14)	32 ± 2	37 ± 9	9-11	12[b]	98.4 ± 2.1	> 99

[a]Yield calculated using triflate activity as starting activity and decay corrected
[b]Volume obtained after SPE on tC18 cartridge
Method A: EtOH/0.01M citrate (40/60, v/v), 0.1% ascorbic acid
Method B: CH_3CN/0.01M citrate (40/60, v/v), pH 3.5, 0.1% ascorbic acid

Table 3: Specifications of [^{11}C]PIB

Test		Specifications
Appearance of solution*		Clear, Colorless
Radiochemical Purity*		≥ 95%
Radiochemical identity*		$RT_{[^{11}C]PIB} = RT_{std} \pm 10\%$
Chemical Purity*	Precursor	≤ 2.89 nmol/Vmax
	PIB	≤ 27.3 nmol/Vmax
Residual Solvents	Acetonitrile	≤ 0.41 mg/mL ≤ 4.1mg/Vmax
	2-butanone	≤ 5 mg/mL ≤ 50 mg/Vmax
Ethanol quantification		≤ 10% (w/v)
Radionuclidic Purity*		511 ± 5 KeV
Half-life*		20.4 minuts ± 5%
Specific Activity		≥ 11.1 GBq/μmol
pH*		4-8
Microbiological Purity	Bubble point*	≥ 3500 mbar
	LAL-test	≤ 175/Vmax IU/ml
	Sterility	Absence of microorganisms