Production of [^{11}C]cyanide and the synthesis of indole-3-[1-^{11}C]acetic acid for PET imaging of auxin transport in living plants

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Introduction

Since its first use by Kistiakowsky and Cramer in 1941 [1], [^{11}C]cyanide has been a useful and versatile radiochemical synthon for a wide variety of reactions. It has been used as a nucleophilic precursor for deoxyglucose [2–5], glucose [6], lactic [1, 7–10] and pyruvic [10, 11] acids, putrescine [12] and its enzymatic-precursor ornithine [13], fatty acids [14] and amines [15], spiroperidol [16], dimethyoxyphenethylamine [17], γ-vinyl-γ-aminobutyric acid [18], and [1-^{11}C]- [19–29], [4-^{11}C]- [30–32], and [5-^{11}C]-labeled [32, 33] amino acids. It has also been used to produce the electrophilic nitrilation precursor [^{11}C]cyanogen bromide [34], which has been used to label a variety of small molecules [35–38], polysacchirides [39] and proteins [40]. Most recently, the [^{11}C]cyanide precursor has found significant use in transition metal (Pd, Cu) catalyzed aryl nitrilation reactions [41–50]. In the vast majority of these synthesis procedures, ^{11}C is initially produced by cyclotron irradiation of a N_2 / O_2 gas target, producing [^{11}C]CO_2, which is then converted to [^{11}C]CH_4 over hot nickel catalyst, then combined with NH_3 and converted to [^{11}C]cyanide over hot platinum catalyst, most commonly citing two detailed literature references [20, 51]. However, despite its position as rote gas-phase product, the catalytic synthesis of [^{11}C]cyanide is difficult to optimize and often only perfunctorily discussed in the radiochemical literature.

Recently, [^{11}C]CN^- has also been used in the synthesis of plant hormones, such as indole-3-[1-^{11}C]acetic acid ([^{11}C]IAA) [52–54], for *in vivo* mechanistic studies of plant physiology using PET imaging. IAA is the principal phytohormone implicated in a wide variety of growth and development functions in plants, including stem elongation, response to light and gravity, and vasculature, lateral root, and organ formation [55]. The University of Wisconsin has expertise in cyclotron production and radiochemistry of ^{11}C and previous experience in the PET imaging of plants [56,57]. In the present work, we have worked to optimize [^{11}C]CN^- production for the synthesis of [^{11}C]IAA and the PET imaging of auxin transport in living plants.

Material and Methods

Compressed gases, including 99.999% He, 99.995% NH_3, and 99.9999% purity 90% N_2 / 10% H_2, from Airgas, platinum wire (99.99%, Strem Chemicals), ~60 mesh platinum sponge (99.98%, Alfa Aesar), gramine (99%, ACROS Organics), DMSO (silylation grade, Thermo Scientific), and other chemicals (at least ACS reagent grade) were used in this work. Preparative high-performance liquid chromatography (HPLC) was performed with an Rainin Instrument Co Inc HPXL solvent pump, Phenomenex Luna C18 250 x 10 mm (10 µm beads) column, Applied Biosystems model 785A absorbance detector, Carrol & Ramsey Associates model 105-S radiation detector, and PowerChrome model 280 eDAQ. Analytical HPLC was performed with a Waters system including two model 515 pumps, a Pump Control Module II, a model 2489 absorbance detector, an e-SAT/IN data acquisition module, a Carrol & Ramsey Associates model 105-S radiation detector, and a Phenomenex Kinetex C18 75 x 4.6 mm (2.6 µm beads) column.

[^{11}C]CH_4 was produced by irradiating 270 psi of 90% N_2, 10% H_2 with 30 µA of 16.1 MeV protons from a GE PETtrace cyclotron. After irradiation, the [^{11}C]CH_4 was converted to [^{11}C]CN^- by passing through a quartz tube containing 3.0 g of Pt wire and powder between quartz wool frits inside a 1000 ˚C Carbolite tube furnace. The constituents and flow rate of the [^{11}C]CH_4 carrier gas were varied in an effort to optimize the oven's catalytic production of [^{11}C]CN^- from CH_4 and NH_3. The following conditions were investigated:

i. Directly flowing irradiated target gas versus trapping, purging and releasing [^{11}C]CH_4 from a -178 ˚C HayeSep D column in He through the Pt furnace.
ii. Varying the amount of NH_3 mixed with the [^{11}C]CH_4 carrier gas prior to the Pt furnace.
iii. Varying the purity of the added NH_3 gas with the addition of a hydride gas purifier (Entegris model 35KF), reducing O_2 and H_2O impurities to <12ppb.

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iv. Varying the flow rate of He gas carrying trapped, purged and released [^{11}C]CH_4.

After flowing through the Pt furnace, the gas stream was bubbled through 300 µL of DMSO containing IAA precursor gramine (1mg), then passed through a 60cm x 5 cm column containing ascarite to absorb [^{11}C]CO_2, followed by a -178˚C Porapak Q column to trap [^{11}C]CH_4 and [^{11}C]CO, as shown in FIG. 1.

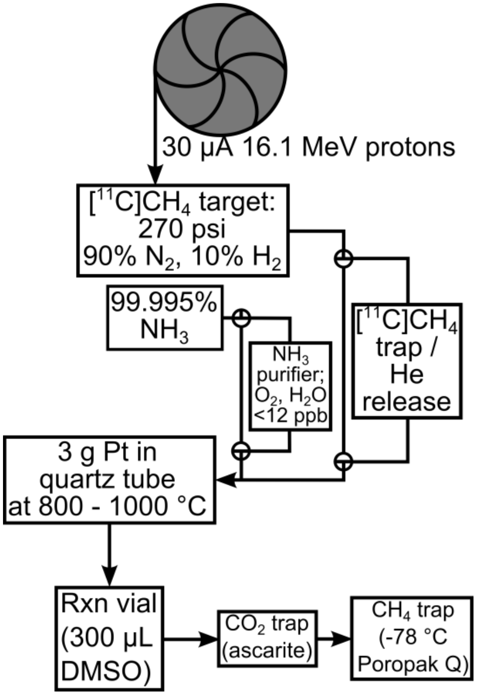

FIGURE 1. Simplified schematic of [^{11}C]CN^- production and synthesis system.

After bubbling, the DMSO/gramine vial was heated to 140 ˚C to react the gramine with [^{11}C]CN^-, forming the intermediate indole-3-[1-^{11}C]acetonitrile ([^{11}C]IAN), which was subsequently purified by solid phase extraction (SPE). The reaction mixture was diluted into 20 mL water and loaded onto a preconditioned (2 mL ethanol, 10 mL H_2O) Waters Sep-Pak light C18 cartridge, followed by rinsing with 5 mL 99 : 1 :: 0.1% HCl : acetonitrile and 10 mL 95 : 5 :: 0.1% HCl : acetonitrile, and eluted with 0.5 mL diethyl ether. The ether was subsequently evaporated under argon flow, followed by the hydrolysis of [^{11}C]IAN to [^{11}C]IAA with the addition of 300 µL 1 M NaOH and heating to 140 ˚C for 5 minutes.

After hydrolysis, the solution was neutralized with 300 µL 1 M HCl and purified using preparative HPLC with a mobile phase composition of 35 : 65 :: acetonitrile : 0.1% formic acid in H_2O and flow rate of 3 mL/min. The [^{11}C]IAA peak, eluting at 13 minutes, was collected and rotary evaporated to dryness, then again after the addition of 5 mL acetonitrile, followed by its reconstitution in 50 µL of water. Analytical HPLC was performed on the [^{11}C]IAA before and after this evaporation procedure with a linear gradient elution over 20 minutes of 10 : 90 - 30 : 70 :: acetonitrile : 0.1% formic acid and a 1 mL/min flow rate, eluting at 7.6 minutes.

Results and Conclusion

Optimization of the [^{11}C]CN^- gas phase chemistry was performed using two key metrics for measuring conversion yield. First is the fraction of total produced radioactivity that trapped in the DMSO/gramine solution (denoted %DMSO), and second, the fraction of DMSO/gramine-trapped activity that was able to react with gramine to form [^{11}C]IAN (denoted %IAN). The former of these metrics was measured by ionization chamber measurements of the reaction vial and ascarite and Poropak Q traps. Under certain conditions, significant losses were experienced in this step due to unconverted [^{11}C]CH_4 or through combustion, forming [^{11}C]CO_2 or [^{11}C]CO. The latter metric was measured by SPE or preparative HPLC of [^{11}C]IAN and experienced losses due to production of incomplete oxidation products of the CH_4-NH_3 reaction, such as methylamine. Total [^{11}C]CH_4 to [^{11}C]CN^- conversion yields (%CN^-) were reported by the product of the two metrics.

It was initially hypothesized that the irradiation of a 90% N_2, 10% H_2 target gas would produce sufficient in-target-hot-atom-produced NH_3 to convert [^{11}C]CH_4 to [^{11}C]CN^- in the Pt furnace. However, conversion yields were found to be low and highly variable, as shown in the top line of TABLE 1. While in disagreement with previous reports [51], this is likely as a result the batch irradiation conditions resulting in ammonia losses in the target chamber and along the tubing walls.

[^{11}C]CH_4 carrier gas composition	%DMSO	%IAN	%CN^-
~100 mL/min H_2/N_2.	(13±8)% (n=14)	(9 ±16)% (n=15)	(0.7 ±0.8)% (n=13)
~100 mL/min H_2/N_2, ~12 mL/min NH_3.	(37 ±10)% (n=17)	(22 ±9)% (n=17)	(8±3)% (n=17)
100 mL/min He, ~10 mL/min NH_3.	~2% (n=1)	n/a	n/a
40 mL/min He, ~10 mL/min NH_3.	~2% (n=1)	n/a	n/a
20 mL/min He, ~10 mL/min NH_3.	(40 ±18)% (n=5)	(44 ±21)% (n=5)	(16 ±7)% (n=5)
20 mL/min He,	(42	(59	(24

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~20 mL/min NH$_3$	±27)% (n=5)	±18)% (n=5)	±17)% (n=5)
20 mL/min He, 30 - 40 mL/min NH$_3$	(74±7)% (n=11)	(50 ±15)% (n=11)	(40 ±14)% (n=11)

Table 1. [^{11}C]CH$_4$ to [^{11}C]CN$^-$ conversion yields.

[^{11}C]CN$^-$ yields and reproducibility were improved when combining the target gas with a stream of anhydrous NH$_3$ gas flow with conversion yields reported in the second line of Table 1. However, these yields remained undesirably low, potentially as a result of the 10% H$_2$ carrier gas having an adverse effect on the oxidative conversion of [^{11}C]CH$_4$ to [^{11}C]CN$^-$. To remedy this, the irradiated target gas was trapped, purged, released in He and combined with NH$_3$ gas before flowing through the Pt furnace. Initial experiments using 99.995% anhydrous NH$_3$ gas resulted in very poor (<0.1%) [^{11}C]CN$^-$ yields as a result of nearly quantitative combustion forming [^{11}C]CO$_2$. Installation of a hydride gas purifier to reduce O$_2$ and H$_2$O impurities in NH$_3$ below 12 ppb improved yields for [^{11}C]CH$_4$ in He, but did not significantly affect those from [^{11}C]CH$_4$ in N$_2$/H$_2$ target gas.

Flow rates of He and purified NH$_3$ carrying [^{11}C]CH$_4$ through the platinum oven were optimized to maximize %CN$^-$ yield. Initial experiments determined that conversion yields were highly sensitive to [^{11}C]CH$_4$ carrier gas flow rate, with lower flow rates giving higher yields, as shown in lines 3 – 5 of Table 1. This result is in disagreement with Iwata *et al.* that report that [^{11}C]CN$^-$ yield decreases with flow rates below 100 mL/min [20]. The %CN$^-$ yield was also found to be dependent on the flow rate of NH$_3$ with higher flow resulting in higher yields, shown in lines 5 – 7 of Table 1. This trend was observed by Iwata *et al.*, but on a significantly different scale, where CN$^-$ yields plateaued above only 1% NH$_3$ concentration by volume. In the present work, the catalytic production of [^{11}C]CN$^-$ required a drastically higher fraction of the carrier gas to be NH$_3$. These notable discrepancies with previously published work imply that optimization of [^{11}C]CN$^-$ production yields is very sensitive to different experimental apparatuses, carrier gas compositions, and flow rates.

Full [^{11}C]IAA synthesis chemistries, including preparative HPLC, reconstitution in 50 µL of H$_2$O, and analytical HPLC, were performed utilizing [^{11}C]CN$^-$ produced through a variety of the above discussed carrier gas compositions, with the majority using ~100 mL/min H$_2$/N$_2$, ~12 mL/min NH$_3$. The decay-corrected to end-of-bombardment radiochemical yield for [^{11}C]IAA in 50 µL H$_2$O was (2.6±1.1)% of the total produced ^{11}CH$_4$ (n=14), with total synthesis time of 62±5 minutes. The subset of experiments utilizing the improved [^{11}C]CN$^-$ production conditions resulting from 20 mL/min He, 30 - 40 mL/min NH$_3$ carrier gas compositions showed only slightly improved radiochemical yields of (4±2)% (n=3). The most significant radiochemical losses were during the SPE procedure and in the reconstitution of the final [^{11}C]IAA product in 50 µL of H$_2$O. Representative results from 254 nm absorbance detectors and radiation detectors after preparative and analytical HLPC are shown in Fig. 2 and Fig. 3, respectively. The radioactive impurity eluting at 8 minutes from preparative HPLC was identified as indole-3-[1-^{11}C]acetamide, the incomplete hydrolysis product of [^{11}C]IAN. The only significant cold impurity co-eluting from preparative HPLC with [^{11}C]IAA was identified as indole-3-carboxylic acid, the hydrolysis product of gramine. Specific activities of [^{11}C]IAA were measured to be ~30 MBq/nmol, decay-corrected to end-of-bombardment.

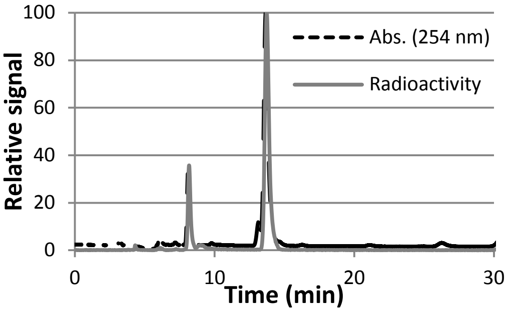

Figure 2. Relative absorbance and radioactivity eluting from preparative HPLC.

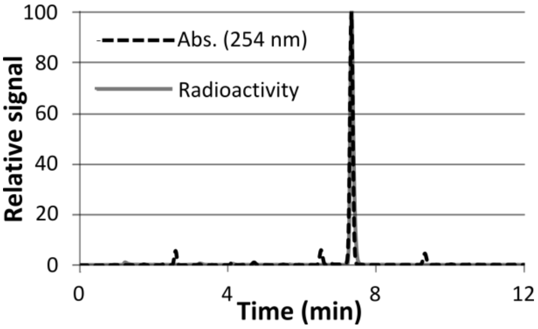

Figure 3. Relative absorbance and radioactivity eluting from analytical HLPLC.

This synthesis procedure allowed for the production of suitable quantities of [^{11}C]IAA for monitoring auxin distribution and transport in the model organism rapid-cycling *Brassica oleracea* (rc*Bo*) [58] using a Siemens microPET P4 scanner. In these experiments, 0.7 – 10 MBq of [^{11}C]IAA was administered as a bolus to the severed petiole of *rcBo* in 3 – 10 µL of H$_2$O at the start of a 90 minute PET scan. Transport was

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compared following administration to the first true leaf versus the final fully formed leaf in plants with and without exposure to the polar auxin transport inhibitor naphthylphthalamic acid (NPA). Results of these experiments will be reported in a forthcoming manuscript.

In conclusion, the Pt-catalyzed oxidative conversion of $[^{11}C]CH_4$ and NH_3 to $[^{11}C]CN^-$ is a challenging process to optimize and highly sensitive to carrier gas composition and flow rate. Optimization for our experimental conditions yielded several results which disagreed with previous reports. $[^{11}C]$IAA produced using $[^{11}C]CN^-$ is well suited for PET imaging of polar auxin transport in living plants.

References

1. R. D. Cramer, G. B. Kistiakowsky: *J. Biol. Chem.* **137**, pp. 549–555, 1941.
2. D. Van Haver, N. A. Rabi, M. Vandewalle, P. Goethals, C. Vandecasteele: *J. Label. Compd. Radiopharm.* **22**, pp. 657–666, 1985.
3. S. Stone-Elander, J. L. G. Nilsson, G. Blomqvist, E. Ehrin, L. Eriksson, B. Garmelius, T. Greitz, P. Johnström, I. Sjögren, L. Widén: Eur. J. Nucl. Med. **10**, pp. 481–486, 1985.
4. M. M. Vora, T. E. Boothe, R. D. Finn, P. M. Smith, A. J. Gilson: *J. Label. Compd. Radiopharm.* **20**, pp. 417–427, 1983.
5. R. R. Macgregor, J. S. Fowler, A. P. Wolf, C.-Y. Shiue, R. E. Lade, C.-N. Wan: *J. Nucl. Med.* **22**, pp. 800–803, 1981.
6. C.-Y. Shiue, A. P. Wolf: *J. Label. Compd. Radiopharm.* **22**, pp. 171–182, 1985.
7. K. Drandarov, P. A. Schubiger, G. Westera: *Appl. Radiat. Isot.* **64**, pp. 1613–1622, 2006.
8. M. B. Winstead, C.-I. Chern, T.-H. Lin, A. Khentigan, J. F. Lamb, H. S. Winchell: *Int. J. Appl. Radiat. Isot.* **29**, pp. 69–73, 1978.
9. P. Bjurling, B. Långström: *J. Label. Compd. Radiopharm.* **28**, pp. 427–432, 1990.
10. J. R. Ropchan, J. R. Barrio: *J. Nucl. Med.* **25**, pp. 887–892, 1984.
11. T. Takashashi, K. Någren, K. Aho: *Appl. Radiat. Isot.* **41**, pp. 1187–1191, 1990.
12. D. W. McPherson, J. S. Fowler, A. P. Wolf, C. D. Arnett, J. D. Brodie, N. Volkow: *J. Nucl. Med.* **26**, pp. 1186–1189, 1985.
13. Y.-S. Ding, G. Antoni, J. S. Fowler, A. P. Wolf, B. Långström: *J. Label. Compd. Radiopharm.* **27**, pp. 1079–1090, 1989.
14. T. Takahashi, T. Ido, K. Hatano, R. Iwata, H. Nakanishi: *Appl. Radiat. Isot.* **41**, pp. 649–654, 1990.
15. J. S. Fowler, B. M. Gallagher, R. R. MacGregor, A. P. Wolf: *J. Pharm. Exp. Ther.* **198**, pp. 133–145, 1976.
16. J. S. Fowler, C. D. Arnett, A. P. Wolf, R. R. MacGregor, E. F. Norton, A. M. Findley: *J. Nucl. Med.* **23**, pp. 437-445, 1982.
17. G. Mestelan, C. Crouzel, D. Comar: *Int. J. Nucl. Med. Biol.* **4**, pp. 185-193, 1977.
18. Z. Zhang, Y.-S. Ding, A. R. Studenov, M. R. Gerasimov, R. A. Ferrieri: *J. Label. Compd. Radiopharm.* **45**, pp. 199–211, 2002.
19. C. B. Smith, K. C. Schmidt, M. Qin, T. V. Burlin, M. P. Cook, J. Kang, R. C. Saunders, J. D. Bacher, R. E. Carson, M. A. Channing, W. C. Eckelman, P. Herscovitch, P. Laverman, B.-K. Vuong: *J. Cere. Blood Flow Met.* **25**, pp. 629–640, 2005.
20. A. R. Studenov, D. E. Szalda, Y.-S. Ding: *Nucl. Med. Biol.* **30**, 39–44, 2003.
21. R. Iwata, T. Ido, T. Takahashi, H. Nakanishi, S. Iida: *Int. J. Appl. Radiat. Isot.* **38**, pp. 97–102, 1987.
22. C. Halldin, K.-O. Schoeps, S. Stone-Elander, F.-A. Wiesel: *Eur. J. Nucl. Med.* **13**, pp. 288–291, 1987.
23. J. Sambre, C. Vandecasteele, P. Goethals, N. A. Rabi, D. Van Haver, G. Slegers: *Int. J. Appl. Radiat. Isot.* **36**, pp. 275–278, 1985.
24. J. R. Barrio, R. E. Keen, J. R. Ropchan, N. S. MacDonald, F. J. Baumgartner, H. C. Padgett, M. E. Phelps: *J. Nucl. Med.* **24**, pp. 515–521, 1983.
25. L. C. Washburn, T. T. Sun, B. L. Byrd, A. P. Callahan: *J. Nucl. Med.* **23**, 29–33, 1982.
26. U. Dunzendorfer, B. Schmall, R. E. Bigler, P. B. Zanzonico, P. S. Conti, J. R. Dahl, E. Kleinert, W. F. Whitmore: *Eur. J. Nucl. Med.* **6**, pp. 535–538, 1981.
27. L. C. Washburn, T. T. Sun, B. L. Byrd, R. L. Hayes, T. A. Butler: *J. Nucl. Med.* **20**, pp. 857–864, 1979.
28. M. C. Patel, R. D. Ganatara, J. B. Dhabhuwala, S. Mukherji: *Int. J. Appl. Radiat. Isot.* **29**, pp. 186–187, 1978.
29. R. L. Hayes, L. C. Washburn, B. W. Wieland, T. T. Sun, R. R. Turtle, T. A. Butler: *J. Nucl. Med.* **17**, pp. 748–751, 1976.
30. N. M. Gillings, A. D. Gee: *J. Label. Compd. Radiopharm.* **44**, pp. 909–920, 2001.
31. G. Antoni, H. Omura, M. Bergström, Y. Furuya, R. Moulder, A. Roberto, A. Sundin, Y. Watanabe, B. Långström: *Nucl. Med. Biol.* **24**, pp. 595–601, 1997.
32. G. Antoni, H. Omura, M. Ikemoto, R. Moulder, Y. Watanabe, B. Långström: *J. Label. Compd. Radiopharm.* **44**, pp. 287–294, 2001.
33. W. Qu, S. Oya, B. P. Lieberman, K. Ploessl, L. Wang, D. R. Wise, C. R. Divgi, L. P. Chodosh, C. B. Thompson, H. F. Kung: *J. Nucl. Med.* **53**, pp. 98–105, 2012.
34. G. Westerberg, B. Långström: *Appl. Radiat. Isot.* **48**, pp. 459–461, 1997.
35. G. Westerberg, B. Långström: *J. Label. Compd. Radiopharm.* **39**, pp. 525–529, 1997.
36. G. Westerberg, M. Bamford, M. J. Daniel, B. Långström, D. R. Sutherland: *J. Label. Compd. Radiopharm.* **38**, pp. 585–589, 1996.
37. G. Westerberg, B. Långström: *J. Label. Compd. Radiopharm.* **34**, pp. 545–548, 1994.
38. G. Westerberg, W. Kärger, H. Onoe, B. Långström: *J. Label. Compd. Radiopharm.* **34**, pp. 691–696, 1994.

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39. G. Westerberg, M. Bergström, S. Gustafson, U. Lindqvist, A. Sundin, B. Långström: *Nucl. Med. Biol.* **22**, pp. 251–256, 1995.
40. G. Westerberg, B. Långström: *Appl. Radiat. Isot.* **45**, pp. 773–782, 1994.
41. F. G. Siméon, J.-S. Liow, Y. Zhang, J. Hong, R. L. Gladding, S. S. Zoghbi, R. B. Innis, V. W. Pike: *Eur. J. Nucl. Med. Mol. Imaging* **39**, pp. 1949–1958, 2012.
42. I. Bennacef, C. A. Salinas, T. A. Bonasera, R. N. Gunn, H. Audrain, S. Jakobsen, N. Nabulsi, D. Weinzimmer, R. E. Carson, Y. Huang, I. Holmes, F. Micheli, C. Heidbreder, G. Gentile, T. Rossi, M. Laruelle: *Bioorg. Med. Chem. Lett.* **19**, pp. 5056–5059, 2009.
43. S. R. Donohue, V. W. Pike, S. J. Finnema, P. Truong, J. Andersson, B. Gulyás, C. Halldin: *J. Med. Chem.* **51**, pp. 5608–5616, 2008.
44. A. J. Airaksinen, J. Andersson, P. Truong, O. Karlsson, C. Halldin: *J. Label. Compd. Radiopharm.* **51**, pp. 1–5, 2008.
45. W. B. Mathews, J. A. Monn, H. T. Ravert, D. P. Holt, D. D. Schoepp, R. F. Dannals: *J. Label. Compd. Radiopharm.* **49**, pp 829–834, 2006.
46. J. Sandell, C. Halldin, H. Hall, S.-O. Thorberg, T. Werner, D. Sohn, G. Sedvall, L. Farde: *Nucl. Med. Biol.* **26**, pp. 159–164, 1999.
47. Y. Andersson, N. Tyrefors, S. Sihver, H. Onoe, Y. Watanabe, H. Tsukada, B. Långström: *J. Label. Compd. Radiopharm.* **41**, pp. 567–576 1998.
48. M. Ponchant, F. Hinnen, S. Demphel, C. Crouzel: *Appl. Radiat. Isot.* **48**, pp. 755–762, 1997.
49. Y. Andersson, B. Långström: *J. Chem. Soc. Perkin Trans. 1*, pp. 1395–1400, 1994.
50. Y. Andersson, M. Bergström, B. Långström: *Appl. Radiat. Isot.* **45**, pp. 707–714, 1994.
51. D. R. Christman, R. D. Finn, K. I. Karlstrom, A. P. Wolf: *Int. J. Appl. Radiat. Isot.* **26**, pp. 435–442, 1975.
52. M. Best, A. N. Gifford, S. W. Kim, B. Babst, M. Piel, F. Rösch, J. S. Fowler: *J. Label. Compd. Radiopharm.* **55**, pp. 39–43, 2012.
53. A. E. Reid, S. W. Kim, B. Seiner, F. W. Fowler, J. Hooker, R. Ferrieri, B. Babst, and J. S. Fowler: *J. Label. Compd. Radiopharm.* **54**, pp. 433–437, 2011.
54. Y. Xu, D. L. Alexoff, A. T. Kunert, W. Qu, D. Kim, M. Paven, B. A. Babst, R. A. Ferrieri, M. J. Schueller, J. S. Fowler: *Appl. Radiat. Isot.* **91**, pp. 155–160, 2014.
55. W. A. Peer, J. J. Blakeslee, H. Yang, A. S. Murphy: *Mol. Plant* **4**, pp. 487–504, 2011.
56. T. E. Barnhart, A. K. Converse, K. A. Dabbs, R. J. Nickles, K. Buckley, S. Jivan, T. J. Ruth, A. D. Roberts: *Appl. Radiat. Isot.* **58**, 21–26, 2003.
57. A. K. Converse, E. O. Ahlers, P. H. Williams, J. W. Engle, T. E. Barnhart, R. J. Nickles, and O. T. DeJesus: *J. Radioanal. Nucl. Chem.* **297**, 241–246, 2013.
58. P. H. Williams and C. B. Hill: *Science* **232**, 1385–1389, 1986.

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