https://www.scirp.org/journal/ns Natural Science, 2024, Vol. 16, (No. 3), pp: 19-23

Synthesis of 4-[F-18]Fluoro-4-Deoxy-*N*-Acetyl-1,3,6-Tri-*O*-Acetylglucosamine, a Potential Brain Imaging PET Agent

Murthy Akula[1], Derek Cressy[1], Lee Collier[2], George Kabalka[1], Dustin Osborne[1]

[1]Department of Radiology, The University of Tennessee Medical Center, Knoxville, USA; [2]Advion Biosciences, Ithaca, USA

Correspondence to: Murthy Akula, makula@utmck.edu

Keywords: N-Acetylglucosamine, Amyloidogenesis, Lipase-*Candida rugosa*

Received: December 30, 2023 **Accepted:** March 26, 2024 **Published:** March 29, 2024

ABSTRACT

The synthesis of a promising brain imaging agent 4-[F-18]fluoro-4-deoxy-N-acetyl-1,3,6-tri-O-acetylglucosamine, 2, was successfully accomplished from commercially available N-acetyl glucosamine in 5 steps. The non-decay corrected radiochemical yield and purity were found to be 31% ± 4% (n = 3) and >98% respectively. The total reaction time for radio labelling step was 50 min.

1. INTRODUCTION

Positron emission tomography (PET) is a powerful noninvasive technique for investigating physiological parameters such as blood flow, glucose metabolism, receptor binding and drug metabolism. Measurements using PET require the preparation of specific molecular imaging probes labeled with positron emitting isotopes like ^{11}C ($T_{1/2}$ = 11 min), ^{74}Br ($T_{1/2}$ = 25 min), ^{124}I ($T_{1/2}$ = 2.4 days) or ^{18}F ($T_{1/2}$ = 110 min). Of these PET isotopes F-18 is widely used since it has optimal half-life and can be conveniently produced by cyclotron from O-18 water in large amounts of activity up to 4 Curies. F-18 is particularly useful since it can replace hydrogen with minimal steric interference. Labelling pharmaceuticals with F-18 often results in fluorine substituted analogues that can be used to monitor biochemical processes while maintaining favorable interaction with the target. Extra cellular fibril protein deposits such as amyloid proteins stain specifically and have distinct structural characteristics. Also these deposits are known to cause disorders such as Alzheimer's disease (AD), joint destruction during extended hemodialysis, and adult onset diabetes. More than 20 different amyloids have been identified [1] and all these proteins have primary defining protein and a common set of structural constituents [2] such as heparan sulfate proteoglycan (HSPG). It has been reported that the interaction between these common structural components and amyloid protein play a significant role in amyloidogenesis. Amyloid-associated glycosaminoglycans (GAGs) HSPG have been shown to have subtle changes [3-6] in structure when binding with amyloidogenic proteins. Kisilevsky's *et al.* [7] have reported that agents that can inhibit binding between heparan sulfate proteoglycan and amyloid protein are effective anti-amyloid agents both *in vivo* and *in vitro*.

Among several glucosamine analogues that were tested as anti-amyloid agents, peracetylated-4-deoxyglucosamine (Figure 1) exhibited anti-Ab property in a mouse transgenic model of AD. So far the only known F-18 labelled glucosamine analogue is [F-18]FAG for imaging tumors in mice bearing hepatomas [7]. The same compound was later used to selectively image bacterial infection instead of non-bacterial inflamation [8].

2. RESULTS AND METHODS

Based on these previously reported observations, we wish to report the synthesis of the title compound **1** as a potential PET agent for imaging brain of AD. The requisite triflate precursor **6** was prepared in four steps [Scheme 1] starting from the commercially available *N*-acetylglucosamine in overall yield of 28.8%. Accordingly *N*-acetylglucosamine, 2, was acetylated using acetic anhydride in pyridine at room temperature for 24 hr to obtain *N*-acetyl-1,3,4,6-treta-*O*-acetylglucosamine, **3**, in 80% yield. The tetraacetate **3** was subjected to selective enzymatic hydrolysis with Lipase-*Candida rugosa* in 20% acetone in water and 50 mM phosphate buffer at room temperature for 4 days to afford 6-deacetylated triacetate **4** in 60% yield. The triacetate **4** was rearranged with acetyl migration from 4th position to 6th position using a catalytic amount of acetic acid in toluene by heating at 80˚C to obtain *N*-acetyl-1,3,6-tri-acetylglucosamine, **5**, in 80% yield. *N*-Acetyl-1,3,6-tri-*O*-acetyl-4-trifuoromethylsulfonylglucosamine, **6**, was obtained from the tri acetate **5** in 75% yield by treating **5** with triflic anhydride and pyridine in dichloromethane at −40˚C. Nucleophilic displacement of triflate **6** by $^{18}F^-$ was carried out by treating anhydrous complex of krypto-fix-potassium carbonate-[F-18]fluoride in acetonitrile at 80˚C in 31% radiochemical yield [Scheme 2].

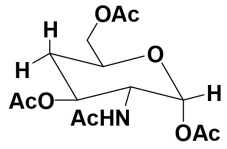
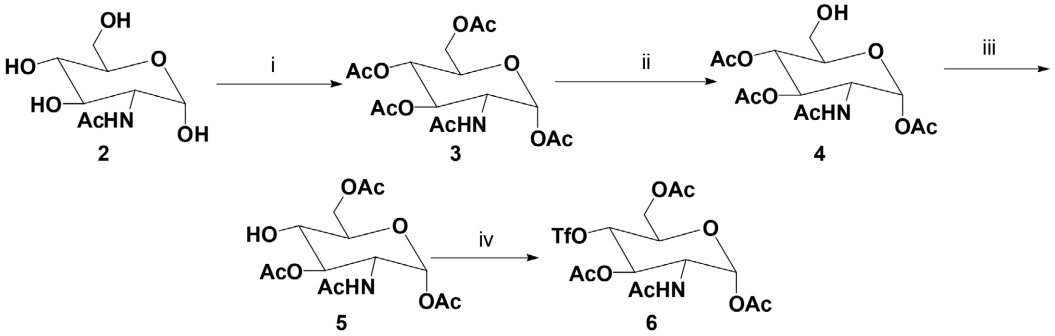
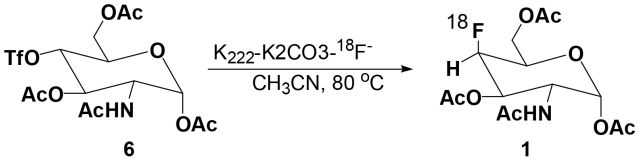


Figure 1. Peracetylated glucosamine.



reagents: i, Ac_2O, pyridine; ii, Lipase/candida rugosa, 20 % acetone, 50 mM KH_2PO_4; iii, 1 % AcOH, toluene; iv, Tf_2O, pyridine, CH_2Cl_2

Scheme 1. Synthesis of triflate precursor 6.



Scheme 2. Radiofluorination of precursor 6.

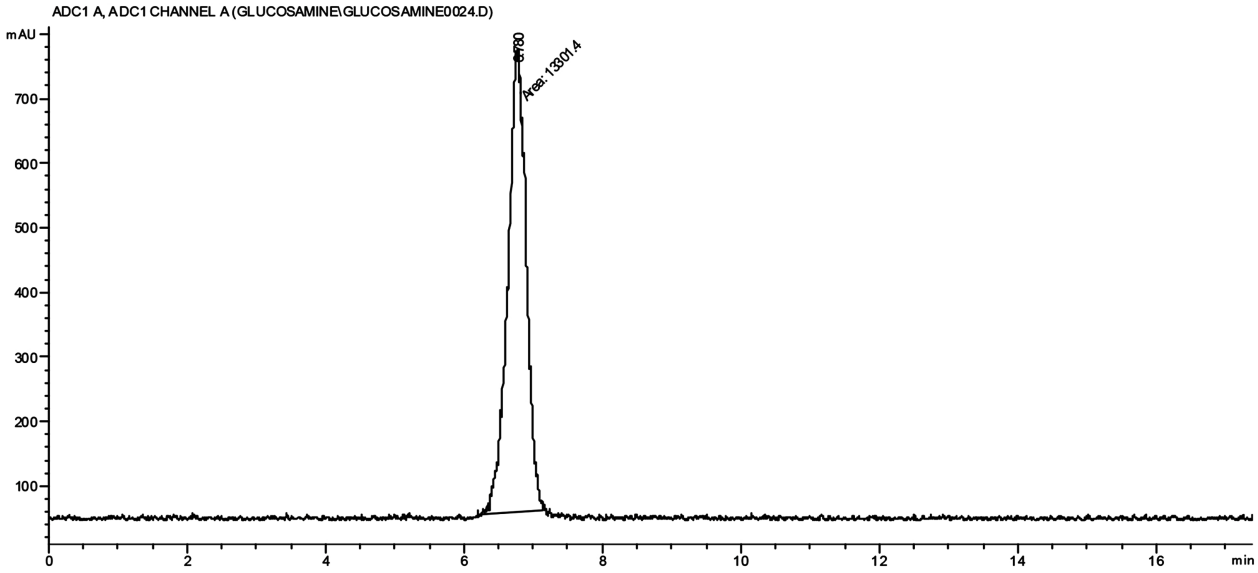


Figure 2. **Analytical HPLC chromatogram of compound 1.**

The crude product was purified by reverse phase semi-preparative HPLC (column: Econosphere C8, 10 m, 10 × 250 mm, 5 mL/min, A: water, B: acetonitrile ; 0 - 2 min 98% A and 5% B; 2 - 15 min 90% B and 10% A; 15 - 25 min 10% A) to obtain 15.6 mCi (31.2%; E. O. S) of (4-[F-18]Fluoro-4-deoxy-*N*-acetyl-1,3,6-tri-*O*-acetylglucosamine The peak between 8 - 9 min was collected and tested the purity of the final product (R_t = 6.78 min) using analytical HPLC with the same gradient elution as semi-prep [**Figure 2**].

3. CONCLUSION

4-[F-18]Fluoro-4-deoxy-*N*-acetyl-1,3,6-tri-*O*-acetylglucosamine, 1, was synthesized in 5 steps starting from *N*-acetylglucosamine in 5 steps in 31% radiochemical yield (E. O. S) and >98% radiochemical purity. The total time for the radiolabeling was 50 min. The animals studies to evaluate the potential binding affinity of this tracer with amyloid proteins is currently underway.

4. EXPERIMENTAL

All reagents and solvents were purchased from Acros Chemicals or Sigma-Aldrich and were used as received. Column chromatography was performed using silica gel (60 Å, 230 - 400 mesh, Sorbent Technologies, USA). Analytical thin-layer chromatography was performed using 250 μm silica plates (Analtech, Inc., Newark, DE). ^{1}H-NMR and ^{13}C-NMR spectra were recorded at 300 MHz or 500 MHz respectively. Chemical shifts for ^{1}H-NMR and ^{13}C-NMR spectra were referenced to the residual protons of the deuterated solvents or to TMS. High Resolution Mass Spectrometry was performed using a JEOL AccuTOF™ DART Mass Spectrometer. No-carrier-added [^{18}F]F^-, produced from recycled [^{18}O] water, was obtained from PETNet (Knoxville, TN). Thin-layer chromatography visualization was performed with radiation detectors using a BioScan AR-2500 radio-TLC reader and Win Scan 1.3 software. All radio-TLC plates were developed using 5% methanol in chloroform. Analytical radio-HPLC analyses were performed on an Agilent 1200 series instrument employing a 254 nm UV detector and a Econosphere C_8 column, 10 μ, 4.6 × 250 mm. F-18 labelling was performed Advion NanoTek Microfluidic Synthesis System controlled by NanoTek LF 1.4 Software. Semipreparative HPLC was performed on Econosphere C_8 column, 10 μ, 10 × 250 mm using PerkinElmer 200 series with Total Chrome software.

***N*-Acetyl-1,3,4,6-treta-*O*-acetylglucosamine 3**. *N*-Acetylglucosamine, **2**, (8 g, 36.4 mmol) was reacted with acetic anhydride (34 ml, 36.4 mmol) in pyridine (35 ml) at room temperature for 12 hr. The reaction

mixture was evaporated under vacuum to give a syrup that was crystallized using ethanol to obtain *N*-acetyl-1,3,4,6-treta-*O*-acetylglucosamine **3** (10.0 g, 80%). m. p. 131˚C - 133˚C (lit., m. p. 137˚C [9], 134˚C - 135˚C [10]); $[a]_D$ +86 (c 1.00, $CHCl_3$) [lit., +87.4 [11] (c 1.07, $CHCl_3$); d_H (300 MHz; $CDCl_3$), 6.03 (1H, d, *J* = 3.6, 1-H), 5.46 (1H, d, *J* = 9.0, NH), (1H, d, *J* = 3.6, 1-H), 5.04 (2H, m, 3-H, 4-H), 4.41 (1H, ddd, *J* = 10.6, 9.0, 3.6, 2-H), 4.19 (1H, dd, *J* = 3.9, 12.5 6_a-H), 4.02 (1H, d, *J* = 2.3, 12.5, 6_b-H), 3.93 (1H, ddd, *J* = 2.3, 3.9, 9.6, 5-H),2.14 (3H, s), 2.07 (3H, s), 2.05 (3H, s), 2.02 (3H, s) and 1.92 (3H, s); d_C 171.3,170.2, 169.5, 168.7, 168.1, 90.5, 70.4, 69.6, 67.4, 61.2, 51.0, 22.7, 20.5, 20.4 and 20.2.

***N*-Acetyl-1,3,4,-tri-*O*-acetylglucosamine 4**. *N*-acetyl-1,3,4,6-treta-*O*-acetylglucosamine **3**, (8 g, 20.0 mmol) was suspended in K_2HPO_4 buffer (pH = 5, 50 mM, 100 m) and 20% acetone (50 ml). To this suspension, lipase-*Candida rugose* (0.3 g) was added and stirred at room temperature for 94 hr. The solution was evaporated under reduced pressure and the residue was extracted with ethyl acetate (2 × 50 ml) and ethanol (2 × 50 ml). The combined extracts were removed under vacuum to yield a syrup that was crystallized from ethanol to obtain *N*-acetyl-,1,3,4,-tri-*O*-acetylglucosamine **4** (4.3 g, 60%). m. p. 108˚C (134˚C - 135˚C [12]); $[a]_D$ +54 (c 1.00, $CHCl_3$), [lit., +57 (c 1.07, $CHCl_3$) [11]; d_H (300 MHz; $CDCl_3$), 6.13 (1H, d, *J* = 3.4 Hz, 1-H), 5.72 (1H, d, *J* = 9.0, N-H), 5.24 (1H, dd, *J* = 9.5, 10.9 Hz, 3-H), 5.04 (1H, t, *J* = 9.4 Hz, 4-H), 4.43 (1H, ddd, *J* = 10.6, 9.0, 3.6, 2-H), 3.75 (1H, ddd, *J* = 2.3, 4.3, 10.1, 5-H), 3.62 (1H, dd, *J* = 2.2, 12.6 Hz, 6_a-H) and 3.57 (1H, d, *J* = 4.3, 12.6 Hz, 6_b-H), 2.15 (3H, s), 2.10 (3H, s), 2.04 (3H, s), and 1.89 (3H, s); d_C 171.3,170.2, 169.5, 168.7, 168.1, 90.5, 70.4, 69.6, 67.4, 61.2, 51.0, 22.7, 20.5, 20.4 and 20.2.

***N*-Acetyl-1,3,6-tri-*O*-acetylglucosamine 5**. *N*-Acetyl-1,3,4,-tri-*O*-acetylglucosamine **4** (3.9 g, 12 mmol) was added to toluene (60 ml) and heated to 80˚C. Glacial acetic acid (15, v/v; 0.75 ml) was added to the toluene solution and heated at 80˚C for 24 hr. The solvent was taken off under vacuum to furnish a brown syrup that was dissolved in ethyl acetate and filtered through silica. Ethyl acetate (100 mL) was removed under reduced pressure and the residue was crystallized from CH_2Cl_2 to yield *N*-acetyl-1,3,6-tri-*O*-acetylglucosamine **5** (3.09 g, 80%), m. p. 158˚C [lit., 160˚C - 161˚C [13]]; $[a]_D$ +59 (c 1.00, $CHCl_3$) [lit., +62 (c 1.07, $CHCl_3$); d_H (300 MHz; $CDCl_3$), 6.10 (1H, d, *J* = 3.5 Hz, 1-H), 5.90 (1H, d, *J* = 8.9 Hz, N-H), 5.06 (1H, dd, , *J* = 9.1, 10.9 Hz, 3-H), 4.48 (1H, dd, *J* = 2.1, 12.4 Hz, 6_a-H), 4.31 (1H, ddd, *J* = 11.0, 9.0, 3.5, 2-H), 4.20 (1H, d, *J* = 2.2, 12.4 Hz, 6_b-H), 3.84 (1H, dt, J = 9.4, 2.2, 3.5 Hz, 5-H), 3.61 (1H, dd, *J* = 9.1, 9.6 Hz, 4-H), 2.13 (3H, s), 2.10 (3H, s), 2.08 (3H, s) and 1.92 (3H, s); d_C 171.9, 171.6, 170.1, 169.1, 90.7, 72.4, 67.7, 62.3, 51.0, 22.8, 20.7, 20.6 and 20.4.

***N*-Acetyl-1,3,6-*O*-triacetyl-4-trifluoromethylsulfonylglucosamine 6**. *N*-Acetyl-1,3,6-tri-*O*-acetylglucosamine **5** (3.0 g, 8.7 mmol) was dissolved in dichloromethane (40 ml) and pyridine (4 ml) under nitrogen atmosphere. The solution was cooled to −40˚C using dry ice-acetonitrile bath. Trifllic anhydride (1.5 ml, 8.9 ml) The product which was crystallized from ethanol to obtain *N*-acettyl-1,3,6,-tri-*O*-acetyl-4-trifluoromethylsulfonylglucosamine, **6** (5.38 g, 75%); d_H (500 MHz; $CDCl_3$), 6.68 (1H, d, *J* = 9.7 Hz, N-H), 6.21 (1H, d, *J* = 3.7 Hz, 1-H), 5.49 (1H, t, *J* = 9.7, 3-H), 5.20 (1H, t, *J* = 9.7 Hz, 4-H), 4.62 (1H, dt, *J* = 3.7, 9.7, 2-H), 4.35 (1H, dd, *J* = 3.6, 12.4, 6_a-H), 4.24 (2H, m, 5-H, 6_b-H), 2.17 (3H, s), 2.15 (3H, s), 2.12 and 2.00 (3H, s); d_C 171.9, 171.6, 170.1, 169.1, 90.7, 72.4,. 67.7, 62.3, 51.0, 22.8, 20.7, 20.4.

4-[F-18]Fluoro-4-deoxy-*N*-acetyl-1,3,6-tri-*O*-acetylglucosamine: Radiofluorination was carried out on Advion Nanotek LF Synthetic Flatform. Cyclotron produced $[^{18}F]F^-$ (50 mCi) water was passed through a ORT ion exchange cartridge to trap fluoride and remove water. The isotope was released using a solution of kryptofix (10 mg) and potassium carbonate (1 mg) in acetonitrile (0.4 mL) and water (0.1 mL). The released [F-18]fluoride-kryptofix-potassium carbonate complex was thoroughly dried azeotropically using acetonitrile (3 × 0.3 mL). Triflate precursor **6** (2 mg) was dissolved in anhydrous acetonitrile (1 mL) and added to the isotope complex and the solution was transferred to a vial in a reactor module and heated at 100˚C for 15 min. The reaction mixture was diluted with water (4 mL) and was injected into the 5 mL HPLC loop (Perkin Elmer 200 series, Total Chrome software) and loaded onto column column: Econosphere C8, 10 m, 10 × 250 mm, 5 mL/min, A: water, B: acetonitrile; 0 - 2 min 98% A and 2% B; 2 - 15 min 90% B and 10% A; 15 - 25 min 10% A). The product collected at 8 - 9 min was diluted with water (60 mL) and was passed through C_{18} Sep-Pak cartridge to trap the tracer and remove acetonitrile and the product was eluted with ethanol (3 mL) to obtain the final pure product 15.6 mCi (31%).

CONFLICTS OF INTEREST

The authors declare no conflicts of interest regarding the publication of this paper.

REFERENCES

1. Westermark, P. (1997) Classification of Amyloid Fibril Proteins and Their Precursors: An Ongoing Discussion. *The Journal of Protein Folding Disorders*, **4**, 216-218. https://doi.org/10.3109/13506129709014387
2. Kisilevisky, R., Fraser, P.E. and Benson, M. (1997) AßT Amyloidogenesis: Unique, or Variation on a Systemic Theme. *Critical Reviews in Biochemistry and Molecular Biology*, **32**, 361-404. https://doi.org/10.3109/10409239709082674
3. Lindhal, B., Eriksson, L. and Lindhal, U. (1995) Structure of Heparan Sulfate from Human Brain with Special Regards to Alazheimer's Disease. *Biochemical Journal*, **306**, 177-184. https://doi.org/10.1042/bj3060177
4. Lindhal, B., Eriksson, L., Spillman, D., Caterson, B. and Lindhal, U. (1996) Selective Loss of Cerebral Keratan Sulfate in Alzheimer's Disease. *Journal of Biological Chemistry*, **271**, 16991-16994.
5. Lindhal, B. and Lindhal, U. (1997) Amyloid-Specific Heparin Sulfate in Human Liver and Spleen. *Journal of Biological Chemistry*, **272**, 26091-26094. https://doi.org/10.1074/jbc.272.42.26091
6. Lindhal, B., Westling, C., Gimenez-Gallego, G., Lindhal, U. and Salmivirta, M. (1999) Common Binding Sites for β-Amyloid Fibrils and Fibroblast Growth Factor-2 in Heparan Sulfate from Human Cerebral Cortex. *Journal of Biological Chemistry*, **274**, 30631-30635. https://doi.org/10.1074/jbc.274.43.30631
7. Fujiwara, T., Kubota, K., Sato, T., Matsuzawa, T., Tada, M., Iwata, R., Itoh, M., Hatazawa, J., Sato, K., Fakuta, H. and Ido, T. (1990) N-[^{18}F]Fluoroacetyl Glucosamine: A Potential Agent for Cancer Diagnosis. *Journal of Nuclear Medicine*, **31**, 1654-1658.
8. Martinez, M.E., Kiyoma, Y., Noriki, S., Inai, K., Mandap, K.S., Kobayashi, M., Mori, T., Tokunaga, Y., Tiwari, V.N., Okazawa, H., Fujibiashi, Y. and Ido, T. (2011) New Radiosynthesis of 2-Deoxy-2-[^{18}F]Fluoroacetamido-D-Glucopyranose and Its Evaluation as Bacterial Infections Imaging Agent. *Nuclear Medicine and Biology*, **38**, 807-817. https://doi.org/10.1016/j.nucmedbio.2011.02.006
9. Kisilevsky, R., Szarek, W.A., Ancsin, J., Bhat, S., Li, Z.J. and Marone, S. (2003) Novel Glycosaminoglycan Precursors as Anti-Amyloid Agents. *Journal of Molecular Neuroscience*, **20**, 291-297. https://doi.org/10.1385/JMN:20:3:291
10. Inoue, Y., Onodera, K., Kitaoka, S. and Ochiai, H.H. (1957) An Acyl Migration in Acetohalogenoglucosamines. *Journal of the American Chemical Society*, **79**, 4218-4222. https://doi.org/10.1021/ja01572a062
11. Galemmo, R.A. and Horton, D. (1983) Preparative Routes to Methyl 2-Acetamido-2,6-Dideoxy-α-Glucopyranoside. *Carbohydrate Research*, **119**, 231-240. https://doi.org/10.1016/0008-6215(83)84058-0
12. Burger, P.J., Noshed, M.A. and Anderson, L. (1983) A Convenient Preparation of 2-Acetamido-2,6-Dideoxy-D-Glucose, Some of Its Alkyl Glycosides, and Allyl 2-Acetamido-2,6-Dideoxy-α-D-Galactopyranoside. *Carbohydrate Research*, **119**, 221-230. https://doi.org/10.1016/0008-6215(83)84057-9
13. Chaplin, D., Crout, D.H.G., Borneman, S., Kutchinson D.W. and Khan, R. (1992) Conversion of 2-Acetamido-β-D-Glucopyranose into 2-Acetamido-Beta-D-Galactopyranos Using a Biotransformation to Generate a Selectively Deprotected Substrate for S_N2 Inversion. *Journal of the Chemical Society, Perkin Transactions*, **1**, 235-237. https://doi.org/10.1039/P19920000235