Synthesis of *N*-acetamido-4-[F-18]fluorodeoxyglucosamine analogues

Murthy Akula, Derek Cressy, Dustin Osborne

Department of Radiology, University of Tennessee Medical Center, Knoxville, USA.

INTRODUCTION:

Positron emission tomography (PET) is a powerful, noninvasive technique for investigating physiological parameters (blood-flow, glucose metabolism, receptor binding, and drug metabolism). Measurements using PET require the preparation of specific molecular imaging probes labeled with positron-emitting radioisotopes. In this regard, fluorine is particularly useful since it can replace hydrogen with minimal steric interference. Labeling pharmaceuticals with [^{18}F]fluorine often results in a fluorine-substituted analogue that can be used to probe biochemical processes while maintaining favorable interactions with the target. Alzheimers disease (AD) is a protein misfolding disease caused by accumulation of abnormally folded Amyloid β (Aβ) proteins in the brain. Kisilevsky's group[1] demonstrated that agents that can inhibit binding between heparin sulfate proteoglycan and the amyloid precursor are effective anti-amyloid compounds both *in vitro* and *in vivo*. Among the 4-deoxy-D-glucosamine analogs synthesized for *in vitro* study, 4-deoxy-peracetylated-D-glucosamine (**Figure 1**) exhibited anti-Aβ behavior in a mouse transgenic model of AD.

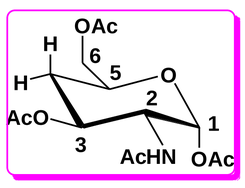


Figure-1

Encouraged by Kisilevsky's results, we synthesized the fluorine-18 labeled derivatives **1** and **2** (**Figure 2**), by replacing the hydrogen in the 4-deoxy-peracetylated-D-glucosamines to generate tracers for a PET imaging study of Alzheimer's disease(AD).

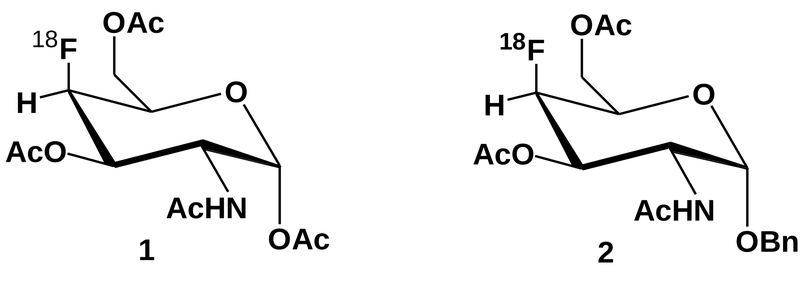
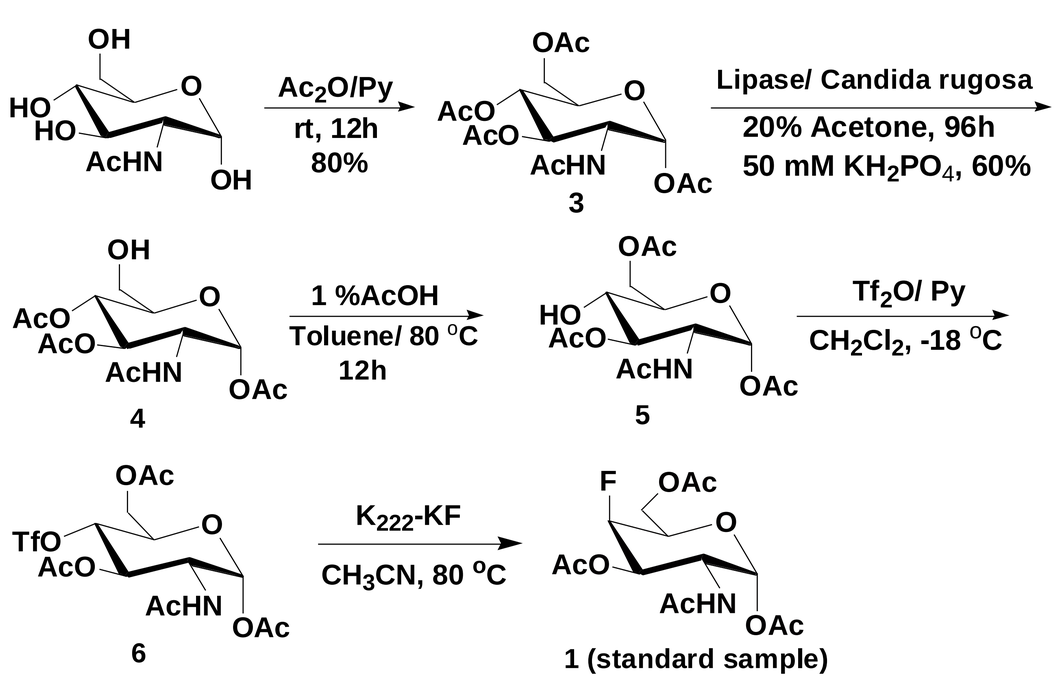


Figure-2

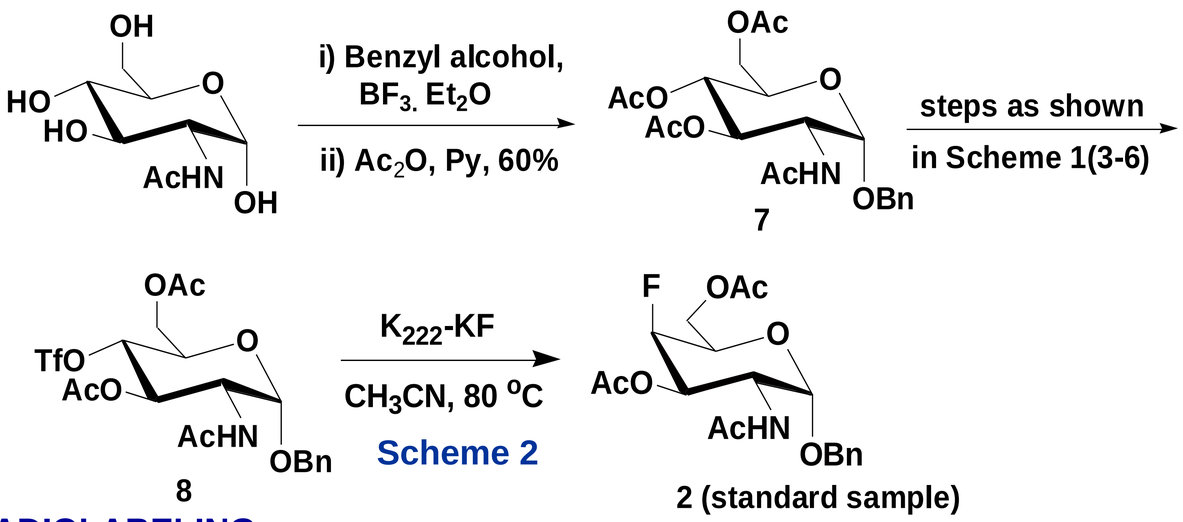
RESULTS and DISCUSSION:

During the investigation, we discovered a simple reaction sequence for the synthesis of radiofluorinated 4-fluoro-4-deoxy-1,3,6-triacetyl-D-glucosamine. (**1**). Starting from the commercially available *N*-acetyl-α-D-glucosamine, all hydroxyl groups were initially esterified using acetic anhydride and pyridine to form the peracetylated compound **3**. Then selective deprotection of the C-6 acetyl group to form **4** was achieved by enzymatic hydrolysis using lipase[2] from *candida rugosa*. Rearrangement of triacetate **4** to the desired 1,3,6-tri-*O*-acetyl derivative **5** was realized by treatment with a catalytic amount of acetic acid in toluene at 80 ^{o}C. The final triflate precursor (**6**) for the radiofluorination was obtained by reaction of **5** with trifluoromethane sulfonic acid anhydride catalyzed by pyridine (**Scheme 1).** Treatment of triflate **6** with K[F-18]F^{-}/$K_{2,2,2}$ in anhydrous acetonitrile afforded 4-fluoro-N-acetylgalactosamine derivative **1** in moderate yield.



Scheme 1

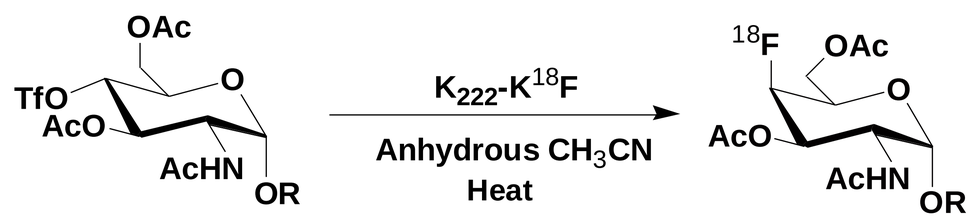
A similar synthetic route to a benzyl protected analog **2,** together with its radiolabeling precursor **8**, was also developed and is presented in **Scheme-2** .



Scheme 2

RADIOLABELING:

Radiofluorinated compounds **1** and **2** were synthesized (**Scheme 3**) using the NanoTek LF Microfluidic Synthesis System. The reaction conditions for radiolabeling were optimized in the Discovery Mode using NanoTek LF 1.4 Software[4]. Triflate (4 mg) **6** or **8** was dissolved in anhydrous acetonitrile (0.5 mL) and allowed to react with kryptofix-[^{18}F]fluoride (49 mCi) by mixing the reagents in the microreactor (2 m X 100 μm) at 100 ^{o}C or 80 ^{o}C, respectively. The [^{18}F]fluoride incorporation, measured by radio-TLC was 29% for precursor **6** and 94% for **8** [see Figure **3**; a and b]. Radiofluorinated compounds **1** and **2,** used for the imaging studies, were purified by HPLC using semipreparative HPLC (column: Econosphere C8, 10 μ, 10 x 250 mm; 5 mL/min. A: H_2O, B: CH_3CN; 0-2 min 98%A and 2%B;2-15 min 98%A-10%A; 15-25 min 10% A). The uncorrected isolated radiochemical yields were 10 % (compound **1**) and 48 % (compound **2**).



Scheme-3

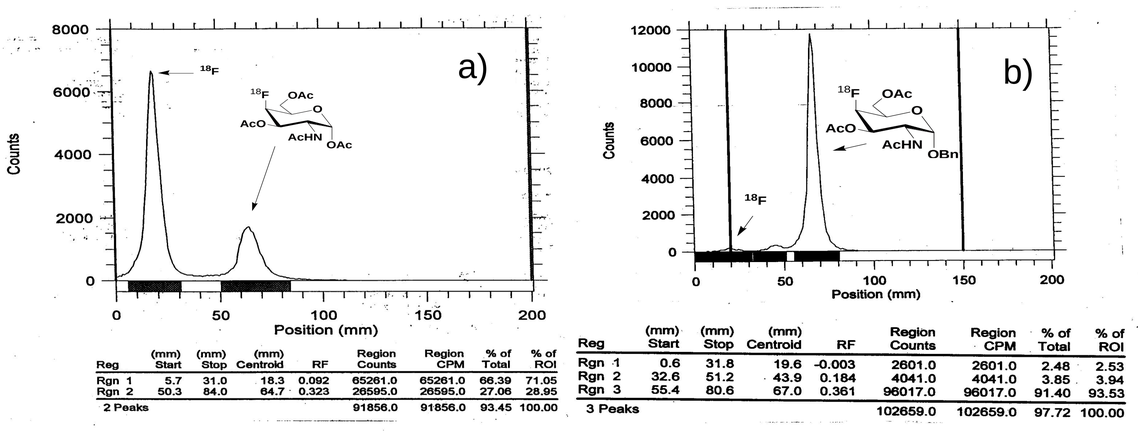


Figure 3: a) Radio-TLC of compound 1 b) Radio-TLC of compound 2

CONCLUSION:

Two novel radiofluorinated 4-deoxy amino sugar derivatives were synthesized successfully. Imaging studies in amyloid bearing mice are currently underway.

ACKNOWLEDGEMENTS:

We wish to thank the Department of Energy, the Robert H. Cole Foundation, and Physicians Education and Medical Research Fund (UTMCK) for support of this research.

REFERENCES:

1. Robert Kisilevsky et al., *Journal of Molecular Neuroscience* **2003,** *20*, 291-297.
2. Marco Filice et al, *ARKIVOC* **2006** (vii) 66-73**.**
3. David Chaplin at al., *J. Chem. Soc. Perkin trans 1*, **1992,** 235.
4. http://www.advion.com/biosystems/nanotek/nanotek-positron-emission-tomography.php

THE UNIVERSITY of TENNESSEE