Synthetic Methods

Ultra-rapid Synthesis of ^{15}O-Labeled 2-Deoxy-D-glucose for Positron Emission Tomography (PET)**

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Positron emission tomography (PET) has gained importance in clinical diagnosis and in modern medicinal chemistry. In the former application, ^{18}F-labeled deoxyglucose ([^{18}F]FDG) is widely used for the analysis of glucose metabolism in vivo[1–5] and in the latter, PET is accelerating the pace of drug development.[6–8] Whereas efforts to develop PET tracer molecules has fallen largely in the area of ^{18}F labeling, development of a synthetic methodology suitable for more ubiquitous and natural elements is necessary, particularly for bioorganic and medicinal studies.[9] Oxygen is an attractive choice for labeling,[10] as it is ubiquitous in biologically active organic molecules. However, the 2-min half-life of the oxygen-15 isotope, which is much shorter than the half-lives of commonly used positron-emitting isotopes (^{11}C: 20 min; ^{13}N: 10 min; and ^{18}F: 110 min), has posed problems in synthesis, and the only successful target reported so far is ^{15}O-labeled butanol.[11] The short lifetime of ^{15}O is a potential advantage for repetitive PET measurements, as quicker and more reliable drug evaluation would be possible through the use of a variety of short-lived probes. Its potential disadvantages as a short-lived isotope have never been experimentally addressed, as suitably labeled molecules have not yet been accessible. In addition to the many potential applications of ^{15}O-labeled products, the synthesis of organic molecules with 2-min half-lives alone represents an exciting challenge in the efficiency of synthetic chemistry, in which yield and selectivity have been the central issues for a long time. Herein we report a single-step synthesis of 6-[^{15}O]-2-deoxy-D-glucose ([^{15}O]DG, **2**) from the corresponding iodide **1** as the proof-of-principle of our new ^{15}O-labeling methodology. Because of the mild and neutral nature of the radical conditions used, the synthesis can be performed without the protection of free hydroxy groups. This demonstrates that such labeling can be carried out as the last step in the synthesis of complex molecules, which would allow chemists to avoid carrying short-lived intermediates through a synthetic sequence.

Compound **2** was selected as the first target of our endeavor, as the initial rapid test of the labeled compound could be performed in an animal system with FDG as a reference standard. An attempted synthesis of an ^{15}O-labeled sugar from [^{15}O]H_2O (made from gaseous $^{15}O^{16}O$)[12] was published in a progress report in 1975; since then, no further mention of such an effort has been reported. Only several minutes are available for the synthesis and purification of **2**, which includes the time necessary for generation of ^{15}O from ^{15}N (a few minutes), mass transfer from the cyclotron ^{15}O generator to the chemical laboratory ($\approx$1 min), and transport from the chemical to the biological laboratory.

To handle the short-lived and radioactive compounds, the radical oxygenation reaction[13] that had been developed for ^{17}O and ^{18}O labeling of protected sugars (with air, alkyl halide, Bu_3SnH, and AIBN) was entirely remodeled (AIBN = azobis(isobutyronitrile)). The following nontrivial issues must be addressed for labeling with ^{15}O: 1) decreasing the reaction time from 10 h to a few minutes; 2) the lower rate of oxygenation that results from the low concentration $^{15}O^{16}O$ gas supplied (a mixture of O_2/N_2 (1.5:98.5), in contrast to the 1:4 ratio in air used previously); 3) a way to maximize the overall efficiency of the oxygen gas trapping (the absolute amount of ^{15}O supplied is too small ($^{15}O/^{16}O \approx 10^{-8}$:1) to be wasted in the synthetic operation); 4) the erratic induction time that is often observed in radical chain reactions; and 5) a variety of synthetic and operational issues related to radioactivity and automation. Given these limitations, we first had to forego use of the protected sugar that was employed in our ^{17}O and ^{18}O labeling studies, as there would be apparently no time for deprotection. As a technical approach, we discovered a mixture of $CF_3C_6H_5$/perfluorodecalin/2-butanol to be a good solvent for both molecular oxygen and unprotected sugar in a homogeneous solution.[14,15] Ordinary glassware was replaced with a special hot-air-jacketed reaction vessel equipped with a sintered glass bottom, through which oxygen gas was introduced as fine bubbles (Figure 1).

The synthesis of [^{15}O]DG (**2**) from 2,6-dideoxy-6-iodo-D-glucose (**1**) and putative intermediates of the reaction are shown in Scheme 1. The synthesis and purification were carried out with fully automated, computer-operated synthesis equipment.[16,17] The organic starting materials were simply introduced into the system, and after several minutes, a saline solution of the labeled sugar product **2** was retrieved for administration to test animals.

Figure 2 illustrates the synthetic operation system. Some details of the experimental procedure are described herein, as they are essential to reproducibility. Iodinated sugar **1** (274 mg, 1.00 mmol) in a 50-mL vial kept under nitrogen was first dissolved in 2-butanol (3.0 mL). The following were

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Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

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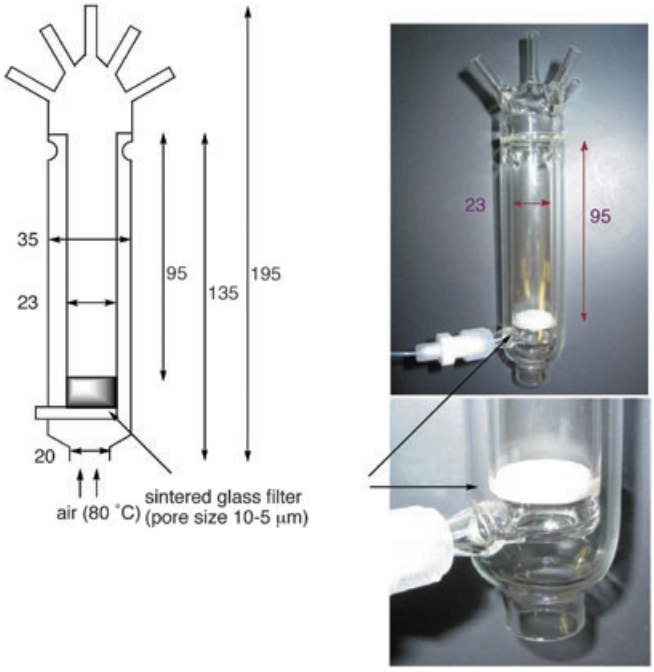


Figure 1. Reactor for the radical hydroxylation. The numbers refer to lengths in mm.

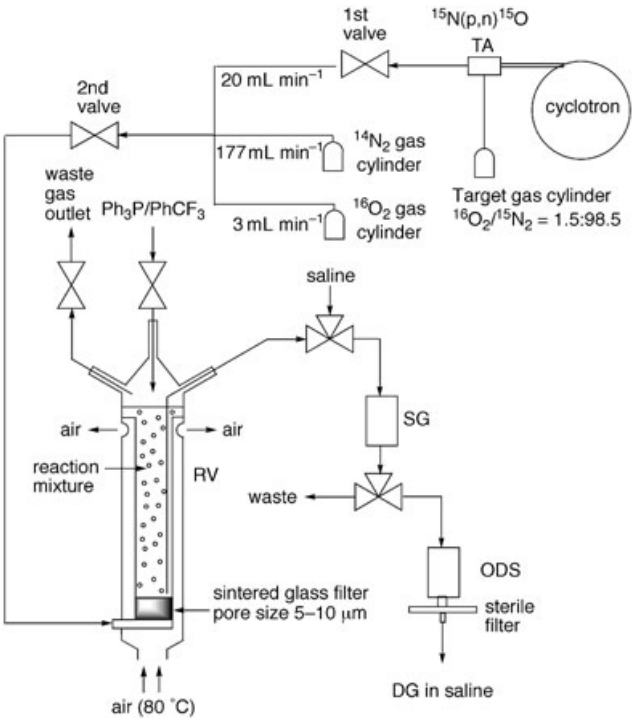
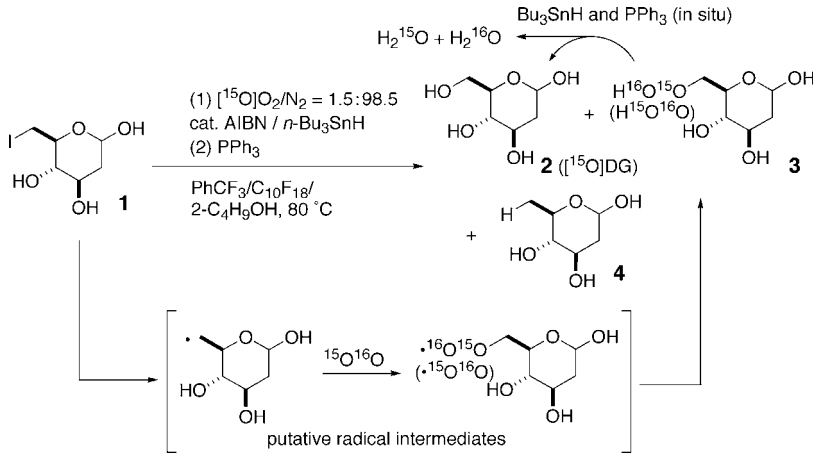


Figure 2. System diagram for synthesis of [^{15}O]DG (**2**): TA, target area (∅ 10 mm × 55 mm); RV, temperature-controlled reaction vessel (Figure 1); SG, Sep-Pak cartridge silica gel vac (3 cc) conditioned with $PhCF_3$ (10 mL); ODS, two Sep-Pak cartridges: C_{18} plus short in series conditioned with methanol (10 mL) and saline (10 mL); [^{15}O]O_2 reached RV without passing through soda lime and activated charcoal; valves are shown as triangle assemblies.

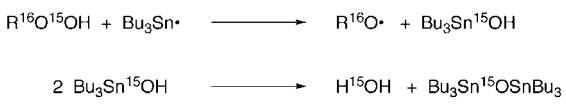
added to the solution in succession: α,α,α-trifluorotoluene (20.0 mL), perfluorodecalin (4.5 mL), and AIBN (4.0 mg, 0.025 mmol). Tributyltin hydride (807 μL, 3.00 mmol) was introduced with a microsyringe (100 μL) at room temperature, and the resulting homogeneous solution was transferred immediately with a syringe into the reaction vessel (RV) (Figure 1). The computer-controlled system was prepared for the following synthetic operations, and proton bombardment of the target gas was started at $t = 0$. At $t =$ 0.7 min, a supply of "cold" oxygen gas ($^{14}N_2 = 177$ mL min^{-1} and $^{16}O_2 = 3$ mL min^{-1}) was introduced to the reaction mixture simultaneously with the flow of temperature-controlled air (80 °C) in the RV jacket to initiate the radical chain reaction before the introduction of ^{15}O. Radical induction finished at $t = 2.2$ min, and the hydroxylation reaction started gradually to form [^{16}O]DG, peroxide **3,** and 2,6-dideoxy-D-glucose (**4**). At $t = 4.0$ min, as the rate of the chain reaction increased, the "hot" gas ([^{15}O]O_2/N_2) was released from the TA of the cyclotron (20 mL min^{-1}) through the first valve, mixed with cold O_2/N_2 gas through the second valve, and sent to the RV located in the room next to the cyclotron (200 mL min^{-1}). At $t = 4.7$ min, the "hot" gas arrived at the RV. At $t = 5.3$ min, the generation of ^{15}O in the cyclotron was stopped, yet the gas still continued to flow from the cyclotron. At $t =$ 6.0 min, both the "hot" gas [^{15}O]O_2/N_2 and the temperature-control (80 °C) air flows were stopped, for which the best timing was estimated reproducibly through a cold control experiment with the same reagents and setup. At $t = 6.1$ min, triphenylphosphine (262 mg, 1.00 mmol) in $C_6H_5CF_3$ (1.5 mL) was added to instantaneously convert any remaining hydroperoxide **3** into DG. At $t =$ 6.3 min, the reaction mixture was passed through the SG to allow retention of [^{15}O]DG on the silica gel and to elute most of the tin and phosphine compounds, as well as solvent. The adsorbed [^{15}O]DG was eluted with saline (3 mL), passed through the ODS to trap less polar contaminants, and finally a



Scheme 1. Rapid synthesis of 6-[^{15}O]-2-deoxy-D-glucose from iodinated sugar **1** and a suggested reaction mechanism.

sterile filter. Thus, at $t = 7.0$ min, [^{15}O]DG (**2**) was collected (0.04 mmol) in saline solution (3 mL). This sample was entirely free of tin and phosphine compounds, but contained 2,6-dideoxy-D-glucose (**4**, 0.04 mmol). As this latter compound does not contain ^{15}O, it does not affect the PET analysis. The mean radioactivity of the entire solution in the syringe for administration was reproducibly 0.7 GBq at $t =$ 8.0 min. The decay-corrected radiochemical purity was $\approx 70\,\%$, the remainder present as ^{15}O-labeled water (Scheme 1). The reproducibility depends critically on the duration of the induction period, which can be controlled through careful standardization of the chemical and instrumental details (Supporting Information). In experiments with "cold" $^{16}O_2$ gas, we synthesized, from 1.0 mmol starting compound (**1**), 0.27 mmol of the desired [^{16}O]DG and 0.71 mmol of 2,6-dideoxyglucose (**4**). The two products could be separated from each other readily by open chromatography purification (chloroform/methanol = 3:1).

The product-containing saline solution of [^{15}O]DG was a mixture of [^{15}O]DG and [^{15}O]H_2O (7:3; Supporting Information). The S_H2 reaction shown in Scheme 2[18] effects the



Scheme 2. Plausible mechanism for the formation of [^{15}O]H_2O.

production of [^{15}O]H_2O. Complete removal of this water remains a problem at this time but its resolution will be deferred, as it represents an issue of chemical engineering rather than one that is purely chemical. Taking into account the chemical yield in the cold control experiments, the mechanical loss during purification, the generation of 8 GBq of [^{15}O]O_2 at $t = 8.0$ min, and the fact that only half the ^{15}O in the $^{15}O^{16}O$ gas was incorporated into the sugar product (the remainder having gone into $H_2{}^{15}O$, $^{15}OPPh_3$, and $(Bu_3Sn)_2{}^{15}O$), we calculate that the yield of [^{15}O]DG based on hot oxygen gas was about 80 % before purification (Supporting Information).

The saline solution of [^{15}O]DG (3 mL) was administered to rats at $t = 8.1$–8.5 min. Figure 3a shows an image of a test animal based on the total gamma-ray capture in a planar positron imaging system (PPIS) between 15 and 30 min after injection. This image is essentially the same as that obtained with 2-deoxy-2-[^{18}F]fluoro-D-glucose ([^{18}F]FDG) (Figure 3b), which shows accumulation in the expected organs for the imaging of glucose metabolism: the heart, kidneys, and bladder. The image is different from that obtained by [^{15}O]H_2O imaging (Figure 3c), which indicates that the image in Figure 3a is the result of [^{15}O]DG rather than [^{15}O]H_2O. Taking advantage of the short lifetime of [^{15}O]DG, we were also able to perform sequential [^{15}O]DG–[^{15}O]H_2O–[^{18}F]FDG measurements at 5-min intervals to obtain similar images (Supporting Information). With these results in hand, we are currently developing new analytical protocols for ^{15}O-isotope metabolic analysis.[19]

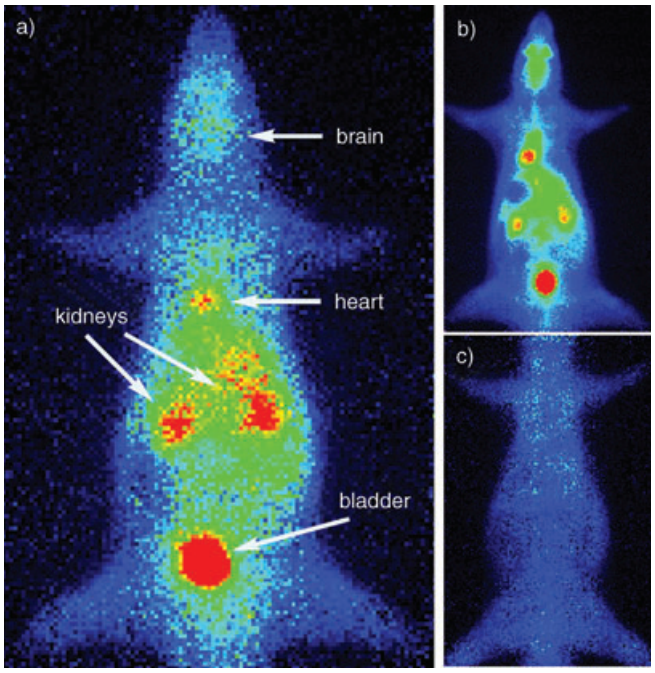


Figure 3. Representative planar positron images of the whole body distributions of 9-week-old male Wistar rats taken by PPIS with a) [^{15}O]DG (**2**), b) [^{18}F]FDG, and c) [^{15}O]H_2O; each image represents the accumulated images from 15 to 30 min after the beginning of intravenous injection of the tracer from a tail vein.

A practical quantity of an extremely short-lived radiopharmaceutical as complex as [^{15}O]DG has been synthesized for the first time. Particularly noteworthy is the simplicity of the oxygenation reaction,[13,20] which can be performed under full automation. The one-step synthetic operation is inherently simpler than ^{11}C labeling, which requires multiple-step operations that start with either ^{11}CO or $^{11}CO_2$.[9,21–23] With the proven synthetic merits of radical reactions in organic synthesis, the present method will be useful for labeling a variety of alcohols with ^{15}O, if the purification problems that depend on the specific compound at hand are set aside. This new ^{15}O-labeling methodology will allow investigations in both academic and industrial settings to focus on ^{15}O-labeled compounds of medicinal and bioorganic importance for the first time.

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