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Synthesis of D-[1-^{11}C]Mannitol and its Enzymatic Oxidation to D-[1/6-^{11}C]Fructose

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D-[1-^{11}C]Mannitol was synthesised from [^{11}C]methyl iodide using a Wittig reaction in combination with an asymmetric dihydroxylation (AD) followed by acid hydrolysis of the protecting groups. Unreacted triphenylphosphine was quenched by the addition of an oxidant, *N*-methylmorpholine *N*-oxide, prior to the AD. In the synthesis of D-[1-^{11}C]fructose, tris(hydroxymethyl)aminomethane buffer was used as the solvent and D-mannitol dehydrogenase and nicotinamide adenine dinucleotide were added after pH adjustment. The enzyme was denatured and the solution was filtered prior to injection into a semi-preparative HPLC-system. Starting from 9.8 GBq [^{11}C]methyl iodide, the yield was 0.37 GBq D-[1-^{11}C]mannitol and 0.12 GBq D-[1-^{11}C]fructose in 60 and 70 min, respectively. The radiochemical decay-corrected yield was 29% in the synthesis of D-[1-^{11}C]mannitol and 15% for the synthesis of D-[1-^{11}C]fructose. The radiochemical purities exceeded 97% for the two ^{11}C-labelled monosaccharides. A mixed ^{11}C/^{13}C-experiment was performed in order to confirm the labelling positions with ^{13}C nuclear magnetic resonance spectroscopy.

The nuclides used in positron emission tomography (e.g., ^{11}C, $t_{1/2}=20.3$ min) allow *in vivo* and *in vitro* studies when incorporated in a biologically active compound. So far these nuclides have rarely been used in *in vitro* experiments, however the high sensitivity, compared with ^{14}C and ^{3}H, makes them interesting candidates for such experiments. Furthermore, the short half-lives of the positron-emitting nuclides make it possible to perform experiments where, for instance, a ^{14}C and a ^{11}C labelled compound are used in combination.

During the study of transport processes in absorptive epithelial monolayers, labelled D-mannitol (^{14}C, ^{3}H) has been used to asses the integrity of the monolayer.[1] The transport of D-mannitol across epithelial monolayers is limited to the paracellular route,[2] i.e., through the tight junctions between the cells. D-Mannitol labelled with ^{11}C allowed samples to be taken for measurement of the radioactivity with shorter time intervals, compared with ^{14}C-labelled D-mannitol.[3] New drugs, labelled with a positron-emitting nuclide, may thus be evaluated using this technique for studying transport properties over epithelial monolayers before they are applied in more advanced *in vivo* models.

D-Glucitol (sorbitol) accumulation is believed to cause complications in diabetes mellitus. An increased concentration of D-glucitol in the lens has been associated with lens fibre swelling that precedes cataract formation.[4] D-Glucitol is formed by the action of the enzymes aldose reductase on D-glucose and sorbitol dehydrogenase on D-fructose. These enzymes are both present in the retina.[5] By use of labelled D-fructose the activity of sorbitol dehydrogenase could be investigated. Furthermore, having available ^{11}C-labelled D-glucose and D-fructose would allow comparisons of the affinity of different glucose transporters for the two monosaccharides. This could be of interest in studies of tumour cells expressing different D-glucose transporters.

In this paper the synthesis of D-[1-^{11}C]mannitol **(4)** (Scheme 1) and D-[1/6-^{11}C]fructose **(5)** (Scheme 2) are described. Synthesis of the corresponding ^{11}C-labelled alkene **(2)** was performed in a two step procedure and asymmetric dihydroxylation (AD) was performed directly on the crude alkene. Purification of the ^{11}C-labelled diols on a solid phase extraction column and hydrolysis of protecting groups followed by injection on a semi-preparative HPLC system yielded D-[1-^{11}C]mannitol. Alternatively, enzymatic oxidation (mannitol dehydrogenase) prior to the HPLC-purification yielded D-[1/6-^{11}C]fructose.

Experimental

General. [^{11}C]Carbon dioxide was produced by the ^{14}N(p,α)^{11}C nuclear reaction using a target containing

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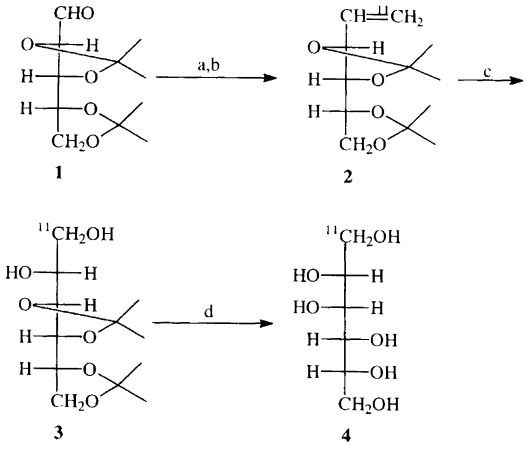
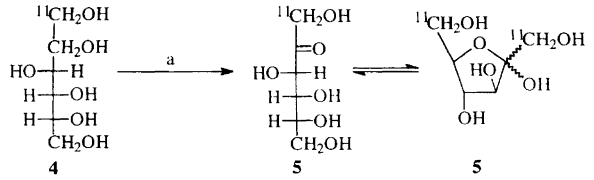


Scheme 1. Synthesis of [1-^{11}C]-D-mannitol: (a) $PPh_3{}^{11}CH_3I$, epichlorohydrin, 150 °C, 6 min; (b) 4-methylmorpholine *N*-oxide 150 °C, 4 min; (c) OsO_4, 4-methylmorpholine *N*-oxide, DHQ-MQ, ambient temp., 7 min; (d) HCl, 85 °C, 5 min.



Scheme 2. Enzymatic synthesis of [1/6-^{11}C]-D-fructose: (a) D-mannitol dehydrogenase and NAD^+, 7 min, ambient temp.

99.95% nitrogen gas (AGA Nitrogen 6.0) and 0.05% oxygen gas (AGA Oxygen 6.0), bombarded by 17 MeV protons on a Scanditronix MC-17 cyclotron at the Uppsala University PET Centre. [^{11}C]Methyl iodide was prepared according to a method presented previously.[6]

Solid phase extraction (SPE) columns used were Astec SiOH (3 ml, 500 mg of packing material). *o*-Dichlorobenzene, 4-methylmorpholine *N*-oxide, hydroquinine 4-methyl-2-quinolyl ether (DHQ-MQ) and epichlorohydrin were purchased from Aldrich, Sweden. *o*-Dichlorobenzene and epichlorohydrin were purified by distillation. Tetrahydrofuran was dried by distillation over sodium–benzophenone under a nitrogen gas atmosphere. The precursor aldehyde, 2,3:4,5-di-*O*-isopropylidene-D-arabinose **(1)** was synthesised according to a published method.[7] An authentic sample of D-mannitol was purchased from Lancaster. D-Fructose, D-mannitol dehydrogenase (EC 1.1.1.67 from actinobacillus sp.), nicotinamide adenine dinucleotide (NAD^+) and tris(hydroxymethyl)aminomethane (Tris) were purchased from Sigma. The enzyme was dissolved in 20 mM potassium phosphate buffer containing 0.1 mM dithiothreitol and pH-adjusted to 7.5 using 2 M KOH. The final concentration of the enzyme was 0.25 units/μL. NAD^+ was dissolved in 20 mM potassium phosphate buffer, yielding a solution containing 100 mM NAD^+.

Analytical HPLC was performed using a Beckman 126 pump, a Beckman 166 UV-detector or an Erma Optical 7510 RI detector in series with a β^+ flow detector. The column used for analysing 3,4:5,6-di-*O*-isopropylidene-D-[1-^{11}C]-*arabino*-hex-1-enitol **(2)** and 3,4:5,6-di-*O*-isopropylidene-D-[1-^{11}C]mannitol was a Beckman ultrasphere C-18, (250 × 4.6 mm), eluting with 10 mM ammonium formiate pH 3.5 (A) and acetonitrile–water (50/7) (B), 2 ml min^{-1}. A Gilson 231 autosampler was used for injecting the analytical samples. D-[1-^{11}C]glucitol and D-[1-^{11}C]mannitol **(4)** were analysed on an Interaction USP L-19 Ca^{2+} cation exchange column (250 × 4 mm) eluting with water, 0.6 ml min^{-1} at 85 °C. D-[1/6-^{11}C]Fructose **(5)** was analysed on a Dionex ion chromatograph using a CarboPac PA-1 column eluted with 0.15 M NaOH, 1 ml min^{-1}. An electrochemical detector (pulsed amperiometric detection program; 0.00 s–+0.10 V, 0.50 s–+0.10 V, 0.51 s–+0.60 V, 0.59 s–+0.60 V, 0.60 s––0.60 V, 0.65 s––0.65 V) in series with a β^+ flow detector was used. A Jones Chromatography Nucleosil NH_2 column (250 × 4.6 mm) was also used for analysis of D-[1-^{11}C]mannitol **(4)** and D-[1/6-^{11}C]fructose **(5)**, eluting with water (5%) and acetonitrile–water (50:7) (95%), 1.0 ml min^{-1}.

Semi-preparative HPLC was performed on a Nucleosil (250 × 10 mm) NH_2 column with a Beckman 126 pump equipped with a Beckman 166 UV detector in series with a β^+ flow detector, eluting with acetonitrile–water (9:1) and a flow of 4.5 ml min^{-1}. Synthia,[8] a semi-automated chemistry system, was used for synthesis of 3,4:5,6-di-*O*-isopropylidene-D-[1-^{11}C]-*arabino*-hex-1-enitol **(2)**, injection into the analytical and the semi-preparative HPLC systems, fraction collection and evaporation.

^{13}C NMR spectra were recorded on a Varian Gemini 200 instrument. Deuteriated water was used as the solvent, with sodium 4,4-dimethyl-4-silapentane sulfonate as internal standard.

Liquid chromatography–mass spectrometry (LC–MS) was performed on a Fisons VG Quattro using negative electrospray. The chromatographic system and conditions used were as described for the Nucleosil NH_2 analysis.

*Synthesis of 3,4:5,6-di-*O*-isopropylidene-*D*-[1-^{11}C]-arabino-hex-1-enitol* **(2)**. [^{11}C]carbon dioxide was transferred in a stream of nitrogen into 300 μl 0.2 M lithium aluminium hydride in tetrahydrofuran. The tetrahydrofuran was distilled off and 1 ml 57% hydroiodic acid was added. The [^{11}C]methyl iodide formed was transferred in a stream of nitrogen (30 ml min^{-1}) to a 0.8 ml vial containing 4 mg (0.017 mmol) triphenylphosphine and 3 mg (0.013 mmol) 2,3:4,5 di-*O*-isopropylidene-D-arabinose **(1)** in 300 μl *o*-dichlorobenzene. The mixture was heated at 150 °C for 2 min after which 70 μl (0.9 mmol) epichlorohydrin were added and the reaction mixture was heated for another 4 min. Unreacted triphenylphosphine was quenched over 4 min by the addition of 5 mg 4-methylmorpholine *N*-oxide (0.04 mmol) in 70 μl dimethyl formamide–water (25:1). The alkene eluted after 8.7 min eluting with 55% B on the C-18 system described under General.

*Synthesis of 3,4:5,6-di-*O*-isopropylidene-*D*-[1-^{11}C]-mannitol* (**3**). After quenching the unreacted triphenylphosphine the reaction mixture was transferred to a 3 ml tube containing 200 µl acetone, 50 µl water, 5 mg (0.04 mmol) 4-methylmorpholine *N*-oxide, 7 mg (0.015 mmol) DHQ-MQ and 65 µl (0.01 mmol) OsO_4 (4% in water). The AD was performed at ambient temperature for 7 min with occasional agitation. The mixture was taken up in 2.5 ml dichloromethane and loaded onto a 3 ml Si-OH SPE column. After washing with 0.6 ml dichloromethane the labelled diol mixture was eluted in 1 ml acetonitrile–dichloromethane (4:1). 3,4:5,6-di-*O*-isopropylidene-D-[1-^{11}C]mannitol eluted after 3.8 min eluting with 65% B on the C-18 system described under General.

Synthesis of D-*[1-^{11}C]mannitol* (**4**). The ^{11}C-labelled diol was transferred to a hydrolysis vessel preloaded with 100 µl 6 M HCl. Hydrolysis of the protecting groups and removal of the organic solvents were performed at 85 °C for 5 min with a nitrogen flow of 140 ml min^{-1}. The vessel was rinsed with 1 ml of acetonitrile–water (9:1) and injected into the semi-preparative HPLC system described under General. The fraction containing D-[1-^{11}C]mannitol was collected after 9.3–10.7 min of elution and was transferred to a rotary evaporator where the solvents were removed under reduced pressure. Phosphate buffer (5 ml, pH 7.4) was added and the solution was transferred to a septum-equipped vial using a flow of helium. Using a Ca^{2+} cation exchange column D-[1-^{11}C]mannitol eluted after 16.2 min. D-[1-^{11}C]-mannitol eluted after 4.8 min on the NH_2 system.

Synthesis of D-*[1/6-^{11}C]fructose* (**5**). Removal of the solvents and hydrolysis of the protecting groups were performed as described in the synthesis of [1-^{11}C]mannitol. 1 ml 0.1 M TRIS buffer was used for rinsing the hydrolysis vessel and the solution was pH-adjusted to 7.4–8.0 by dropwise addition of 6 M KOH. NAD^+, 100 µl (10 µmol), and 25 µl (6.25 units) of D-mannitol dehydrogenase were added. The proteins were denatured after 7 min of reaction at ambient temperature using 50 µl 6 M HCl. The solution was filtered and injected into the semi-preparative HPLC system as described. After collection of the fraction containing D-[1-^{11}C]fructose (8.3–9.0 min) the acetonitrile was removed by evaporation and the final product was formulated as described for D-[1-^{11}C]mannitol. Using the same procedure as described above, D-[1/6-^{11}C]-fructose was also synthesised with another enzyme, sorbitol dehydrogenase. D-[1/6-^{11}C]Fructose was analysed on the Dionex system and an NH_2 column as described under General. Retention times were 4.7 and 5.3 min, respectively.

Synthesis of D-*[1-^{11}C/^{13}C]mannitol and* D-*[1/6-^{11}C/^{13}C]fructose.* (^{13}C)Methyl iodide (4 µl, 63.8 µmol) was added to the vessel used for trapping [^{11}C]methyl iodide and the reactions were performed as described above. The fraction containing the product was collected on the semi-preparative HPLC and left to decay before work-up and ^{13}C NMR analysis.

Results and discussion

In the Wittig reaction a carbon–oxygen double bond is converted into a carbon–carbon double bond.[9] This reaction has been used to synthesise ^{11}C-labelled alkenes from [^{11}C]methyl iodide[10] and from hydrogen [^{11}C]cyanide.[11,12] A method has been developed for proton abstraction from the ^{11}C-labelled phosphonium salt in which the use of strong bases is avoided through the addition of an epoxide generating an equimolar amount of base *in situ*.[10,13] The two-step alkene synthesis produced the Wittig salt in 87–90% radiochemical yield and the alkene (**2**) in 91–94% radiochemical yield (calculated from the ^{11}C-labelled Wittig salt). After the Wittig salt had been formed, neat epichlorohydrin was added which provided the alkene in a total reaction time of 6 min. When [^{11}C]methyl iodide was trapped in *o*-dichlorobenzene containing all the reagents necessary for the Wittig reaction, in a one-pot synthesis, the yield of the alkene decreased and the amount of labelled side products increased. The best solution was therefore to trap [^{11}C]methyl iodide in *o*-dichlorobenzene containing triphenylphosphine and 2,3:4,5-di-*O*-isopropylidene-D-arabinose (**1**).

AD[14] is a well established method for producing *cis*-diols from alkenes. Performing AD directly on the crude alkene mixture was not possible owing to the presence of unreacted triphenylphosphine in the reaction mixture which acts as a powerful poison towards OsO_4. Therefore, unreacted triphenylphosphine was quenched by the addition of an oxidant, 4-methylmorpholine *N*-oxide, which allowed the AD to be performed on the crude alkene. The reaction time of the AD was longer than the reaction on the purified diol. However the overall result favoured the direct oxidation of the alkene. The ^{11}C-labelled diols were synthesised in good yields (80–85%) and within a time-frame (7 min) suitable for rapid labelling synthesis. The ratio of D-[1-^{11}C]mannitol to D-[1-^{11}C]glucitol was 93:7. The alkene could be purified with respect to triphenylphosphine using either SPE or semi-preparative HPLC-purification. In order to keep the total reaction time as short as possible semi-preparative HPLC was not a realistic alternative. Furthermore, the diol had to be purified prior to the enzymatic oxidation, which would result in two purifications, increasing the reaction time and losses of activity during handling of the reaction mixture.

Two SPE methods for purifying the labelled diol prior to the enzymatic oxidation were examined. The best results were obtained when the reaction mixture, containing the protected ^{11}C-labelled diol was taken up in dichloromethane and loaded onto an SiOH SPE column. Lipophilic compounds were not retained on the column

while the diol was, and the product was eluted off the column using acetonitrile–dichloromethane, leaving the polar impurities on the SiOH-matrix. Rapid and efficient purification of 3,4:5,6-di-*O*-isopropylidene-D-[1-^{11}C]mannitol (**3**) was crucial when performing the enzyme catalysed oxidation. An alternative procedure was to deprotect the ^{11}C-labelled diols prior to the purification. However, deprotecting the diol prior to the SPE purification required water for elution of the product from the column (SiOH or NH_2 SPE). This produced a fraction containing the labelled product and polar impurities (most likely epichlorohydrin, 4-methylmorpholine *N*-oxide and methylmorpholine) decreasing the radiochemical yield in the enzymatic oxidation.

HCl was added to the acetonitrile–dichloromethane fraction and evaporation of the solvents and hydrolysis of the protecting groups were performed at 85 °C.

Chemoselective oxidation of a secondary alcohol in the presence of a primary alcohol could be achieved either by chemical or by enzymatic means. There are methods for chemoselective oxidations of diols described in the literature.[15] However, these methods often require good control of the stoichiometry, which made those methods less suitable when working with trace amounts of the labelled 1,2-diol. Enzymes, on the other hand, exhibit a high degree of chemoselectivity, minimising formation of side products and are easy to remove from the reaction mixture at the end of synthesis. After reacting with one substrate molecule enzymes are restored to their active state by the action of a cofactor. The commercially available, NAD-dependent enzyme D-mannitol dehydrogenase was used for converting D-[1-^{11}C]mannitol (**4**) into D-[1/6-^{11}C]fructose (**5**). Another commercially available enzyme, sorbitol dehydrogenase was also used in the synthesis of D-[1/6-^{11}C]fructose. The yield using this enzyme was equivalent to using D-mannitol dehydrogenase.

D-[1-^{11}C/^{13}C]Mannitol and D-[1/6-^{11}C/^{13}C]fructose were analysed by ^{13}C-NMR spectroscopy and LC–MS in order to verify the labelling position and the identity of the products. The peaks obtained in the ^{13}C NMR spectra were in accordance with the spectra obtained for the reference compounds [64.2 (mannitol), 64.4 and 64.9 (fructose) ppm, respectively]. Masses obtained in the LC–MS analysis were *m/z* 181 and 182 for D-mannitol and *m/z* 179 and 180 for D-fructose, which correspond to the expected masses for ^{12}C and ^{13}C in the respective compounds.

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