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AN IMPROVED, PRACTICAL SYNTHESIS OF 5-[^{2}H]-D-GLUCOSE

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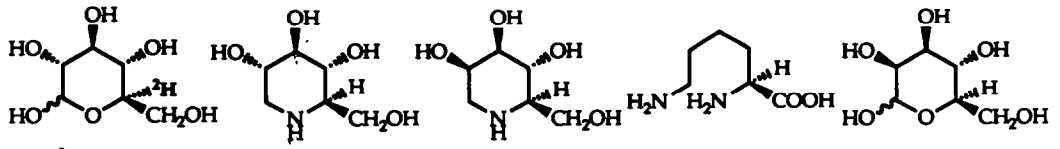
Abstract: A new, practical and stereoselective route to 5-[^{2}H]-D-glucose is described starting from 1,2,5,6-O-isopropylidene-α-D-glucose. 5-[^{2}H]-D-Glucose is produced exclusively with no evidence for the C5 epimer which has accompanied other synthetic methods. The strategy could be extended to include stereoselective tritiation at C5 of glucose for bioorganic studies.

Glucose is the precursor to an enormous range of primary and secondary metabolites, including acetate and several amino acids which themselves are precursors to more complex natural products.[1,2] Research in our laboratory, for example, is focused on the biosynthesis, in *Streptomyces* and *Bacilli* microorganisms, of the anti-Human Immunodeficiency Virus alkaloid, 1-deoxynojirimycin. Using detailed deuterium labelling experiments, we have shown that this alkaloid is produced from glucose and not from lysine.[3,4] Other important amino-sugars, such as the mannosidase inhibitor, 1-deoxymannojirimycin (cf. mannose), were found to originate from glucose in the same way by an unusual head-to-tail inversion of the sugar molecule.[3,4]



Often precursors labelled with the heavy isotopes of hydrogen can provide detailed information on a biosynthetic mechanistic process that is not available from experiments where ^{13}C or other isotopes are used as labels. This has made the efficient synthesis of deuterated or tritiated glucoses a primary goal, especially in view of their importance in biological processes. In a recent publication,[5] it was reported that penta-O-acetyl-β-5-[^{2}H]-D-glucose had been prepared from 3-O-benzyl-1,2-O-isopropylidene-6-O-trityl-α-D-xylofurano-5-ulose (**12**), however, no elaboration of the experimental method was included. In this *Letter*, we present a practical, gram-scale synthesis of 5-[^{2}H]-D-glucose (**15**) which has been completed in only seven steps from commercially available starting materials. This diastereomerically pure, deuterated glucose (**15**) is not available commercially and previous literature syntheses[6,7,8] of this compound had been relatively inefficient resulting in mixtures of diastereoisomers of limited practical use for bioorganic studies.

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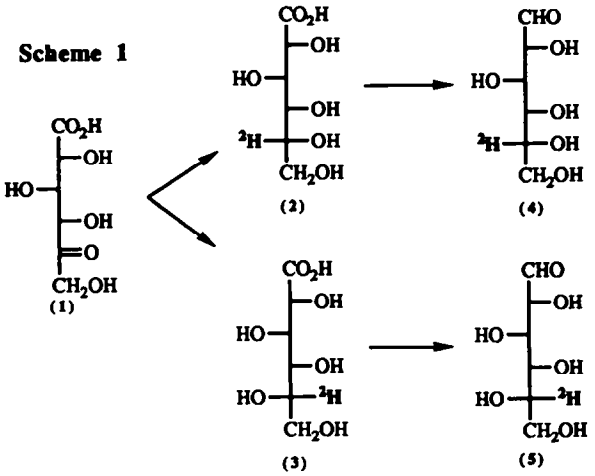
The first of these synthetic routes involves the NaB^2H_4 reduction of D-*xylo*-5-hexulosonic acid (**1**) (**Scheme 1**), produced from D-glucose using *Gluconobacter suboxydans*.[6] This reaction had been reported[6] to give equimolar quantities of the D-gluconic (**2**) and L-idonic acids (**3**), after which the acids were lactonised and further reduced to the corresponding aldoses (**4**) and (**5**) respectively.[7] In our hands, this method produced only trace quantities of 5-[^{2}H]-D-glucose, a result also reported in the literature.[7] An alternative strategy from Mackie and Perlin[8] (**Scheme 2**), was the CrO_3 oxidation, in low yield, of 1,2-O-isopropylidene-α-D-3,6-glucurono-lactone (**6**) to keto-lactone (**7**) and its subsequent reduction with NaB^2H_4.[8] This gave a 2:1 mixture of the *gluco* and *ido* lactones, (**8**) and (**9**) respectively, which required separation.

The new route to 5-[^{2}H]-D-glucose starts with the commercially available 1,2,5,6-O-diisopropylidene-α-D-glucose (**10**) which was smoothly converted into the protected furanose secondary alcohol (**11**) in 3 steps (35% overall yield) in accordance with literature precedent.[9] Alcohol (**11**) has been oxidised to ketone (**12**) using the conditions of Albright and Goldman (acetic anhydride/DMSO), although, in our hands, this gave the required product contaminated with impurities.[10] Swern oxidation (-78°C, oxalyl chloride/DMSO/NEt_3) of (**11**) gave ketone (**12**) in 70% recrystallised yield. Reduction of ketone (**12**) with $NaBH_4$ in CH_2Cl_2 : EtOH (1 : 1.25 v/v) led to the *gluco* diastereoisomer (**11**) exclusively, by ^{1}H NMR spectroscopy, although traces of a lower Rf component were detected by TLC (SiO_2, Et_2O : toluene, 1 : 9 v/v). As the absolute stereochemistry of (**11**) is known, the stereochemistry of the product derived from this reduction can be confidently assigned.

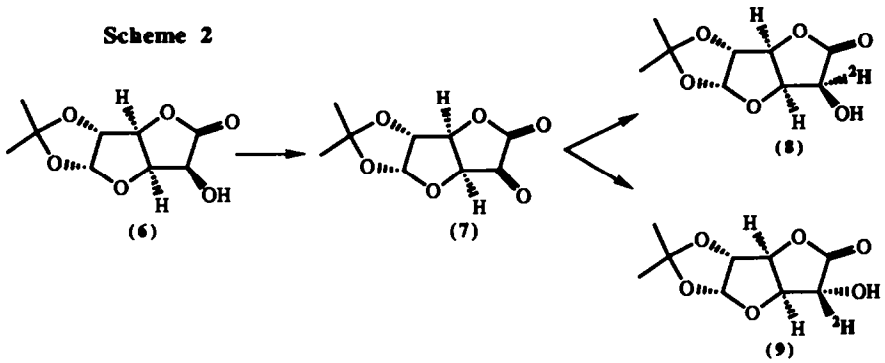
Replacement of the $NaBH_4$ with NaB^2H_4 allowed ready incorporation of deuterium to produce (**13**). In particular, the absence of a multiplet for ^{1}H on C5 around 4.17ppm and both protons on C6 displaying only a small geminal *J* value (9.3Hz) were diagnostic, and no vicinal $J_{H\text{-}D}$ was observed. Whereas, $J_{5\text{-}6}$ (5.5Hz) was observed in (**11**), the protio isotopomer of (**13**), and $J_{2\text{-}3}$ was appproximately 0Hz in these five-membered heterocycles. The origin of the stereoselectivity in this reduction has been alluded to in a synthesis of nojirimycin.[10] In this case, ketone (**12**) was converted into the hydroxylamine oxime and reduced with $LiAlH_4$ or Raney nickel in methanolic ammonia solution. After purification, a 9 : 1 *gluco* : *ido* diastereomeric ratio was observed, based on circular dichroism measurements, as a result of attack from the least hindered side of the C5 carbonyl group away from the C3 benzyl group. Further high stereoselectivities were observed, for the same reason, when (**12**) was reacted with MeMgI to give 3-O-benzyl-1,2-O-isopropylidene-5-C-methyl-6-O-trityl-α-D-glucofuranose exclusively.[11]

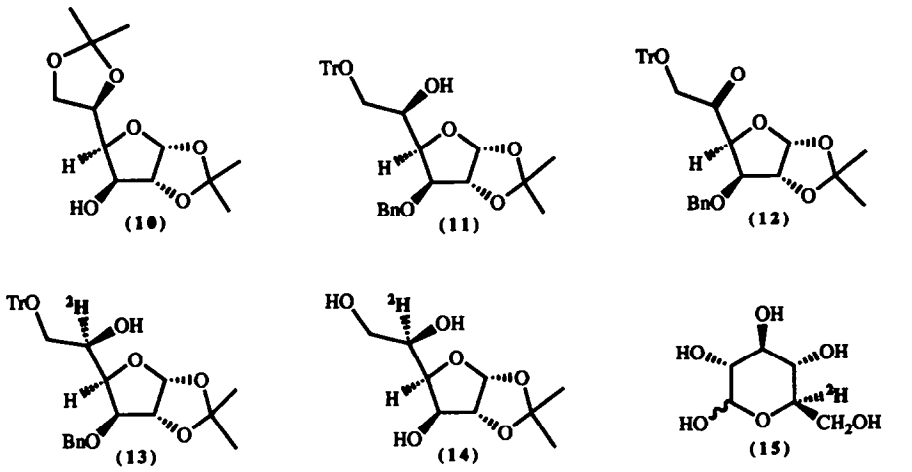
Deprotection of (**13**) by hydrogenolysis of the trityl and benzyl groups failed even at elevated hydrogen pressures (up to 100 atm). However, they were cleaved, in 86% yield, by dissolving metal reduction using lithium in anhydrous liquid ammonia to give (**14**). Hydrolysis of the acetonide moiety using aqueous sulfuric acid followed by purification using a mixed bed ion-exchange column, afforded 5-[^{2}H]-D-glucose (**15**) in 37% overall yield from (**11**) which co-eluted with authentic D-glucose by TLC (SiO_2, ^{n}BuOH : AcOH : EtOAc : H_2O, 1 : 1 : 1 : 1, v/v/v/v). Thus, the regioselectively deuterated glucose (**15**) [136864-16-9] has been prepared on a gram scale. ^{1}H NMR confirmed the position of the label in the isolated glucose (no C5 epimer could be detected) and the ammonia CI mass spectrum of the peracetyl derivative showed an enrichment of 92 atom % ^{2}H.[3]

Scheme 1



Scheme 2





Bn = benzyl Tr = trityl

An extension of the strategy presented here would be to tritiate at C5 of glucose. A previous synthesis of 5-[^{3}H]-D-glucose had not given the required stereoselectivity when the tritium isotope was introduced.[12] Hence, replacing the triphenylmethyl group of (**12**) with benzoyl and subsequent reduction of the ketone with NaB^3H_4 led to a 3 : 2 ratio of the epimeric alcohols having the L-idose and D-glucose configurations respectively. These were then successfully separated by preparative TLC.[12] 5-[^{3}H]-D-Glucose has also been prepared by an enzymatic route where D-glyceraldehyde-3-phosphate was isomerised in ^3H_2O catalysed by triose phosphate isomerase.[13] The tritium enriched glyceraldehyde-3-phosphate was then reacted with dihydroxyacetone phosphate in the presence of an aldolase enzyme to afford phosphorylated 5-[^{3}H]-D-glucose. The strategy presented in this *Letter* would achieve the same result chemically.

Experimental

Anhydrous tetrahydrofuran was obtained by distillation from sodium/benzophenone. Dichloromethane and triethylamine were distilled from calcium hydride. Oxalyl chloride was distilled before use and stored under nitrogen. Dimethyl sulfoxide was stored over activated 4Å molecular sieve and was not purified further. TLC was performed using glass backed SiO_2 TLC plates (Merck Kieselgel 60F-254) and compounds were visualised with 10% sulfuric acid in ethanol followed by heating with a hot air gun. Merck silica gel 60 (230-400 mesh ASTM) was used for flash chromatography.[14]

^{1}H NMR spectra were recorded at 220 MHz using a Perkin Elmer R34 or at 400MHz using a Bruker WH 400 spectrometer. Residual $CHCl_3$ (δ 7.23ppm) and CHD_2OD (δ 3.40ppm) were used as reference signals for samples in $CDCl_3$ and d_4-MeOH respectively. Mass spectra were recorded on a Kratos MS80 spectrometer using ammonia as the reagent gas for chemical ionisation.

3-O-Benzyl-1,2-O-isopropylidene-6-O-triphenylmethyl-α-D-*gluco*-hexofuranose(11)

(**11**) was synthesised from (**12**) using the same procedure as that outlined below , except that $NaBH_4$ was used instead of NaB^2H_4. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.31 (3H, s, Me), 1.49 (3H, s, Me), 2.67 (1H, br d, OH, 7.0), 3.23 (1H, dd, H6, 9.4, 5.5), 3.42 (1H, dd, H6, 9.4, 5.5), 4.03 (1H, d, H3, 3.1), 4.15-4.19 (1H, m, H5), 4.32 (1H, dd, H4, 7.6, 3.1), 4.45 (1H, d, $PhCH_2$, 11.6), 4.56 (1H, d, H2, 3.8), 4.61 (1H, d, $PhCH_2$, 11.6), 5.91 (1H, d, H1, 3.8), 7.20-7.45 (20H, m, aromatic H).

3-O-Benzyl-1,2-O-isopropylidene-6-O-triphenylmethyl-α-D-*xylo*-hexofurano-5-ulose(12)

Oxalyl chloride (2mL, 23mmol) was added to dichloromethane (50mL) at -78°C followed by the dropwise addition of dimethyl sulfoxide (3.4mL, 48mmol) over 5 min. The two reagents were stirred together for 10 min. at -78°C before (**11**) (11.2g, 20mmol) in dichloromethane (20mL) was added dropwise. Immediately following the addition, the reaction was warmed to -30°C whereupon the previously cloudy solution cleared. The solution was stirred at this temperature for a further 10 min. before triethylamine (14.6mL, 105mmol) was added. The reaction was allowed to reach room temperature slowly before water (50mL) and dichloromethane (50mL) were added. The organic layer was separated and washed with more water (2 x 50mL) and saturated brine (50mL). After drying ($MgSO_4$), the dichloromethane solution was concentrated *in vacuo* to give a pale yellow solid. Recrystallisation (cyclohexane) gave (**12**) (7.82g, 70%). TLC (10% diethyl ether : toluene) indicated that no alcohol was present.

(12): mp 160-162°C, lit.[15] 169-171°C, $[\alpha]^{23}_D$ = -9.7°C (c = 0.12, $CHCl_3$), ^{1}H NMR (400 MHz, $CDCl_3$) δ1.28 (3H, s, Me), 1.45 (3H, s, Me), 4.03 (1H, d, CH_2, 18.2), 4.09 (1H, d, CH_2, 18.2), 4.36-4.40 (2H, m, H3 or H4 and H6), 4.47 (1H, d, H6, 11.7), 4.50 (1H, d, H3 or H4, 3.6), 4.85 (1H, d, H2, 3.7), 5.92 (1H, d, H1, 3.6), 7.14-7.44 (20H, m, aromatic H), ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 26.3, 26.8, 68.2, 69.1, 72.4, 81.6, 83.4, 84.5, 87.1, 105.6, 106.0, 112.2, 126–128, 136.8, 143.2, 146.8, 202.9. Analysis: found; C 76.09, H 6.23% calc. for $C_{35}H_{34}O_6$; C 76.34, H 6.18%.

3-O-Benzyl-1,2-O-isopropylidene-6-O-triphenylmethyl-5-[^{2}H]-D-*gluco*-hexofuranose(13)

To a solution of **(12)** (7.76g, 14mmol) dissolved in dichloromethane (60mL) was added NaB^2H_4 (0.6g, 14mmol) dissolved in ethanol (75mL). When TLC (10% diethyl ether : toluene) showed that no starting material remained, the solvent was evaporated *in vacuo* and the residue was dissolved in dichloromethane (50mL). This solution was washed with water (75mL), dried ($MgSO_4$) and concentrated *in vacuo* to give **(13)** (7.5g, 96%). In identical experiments using $NaBH_4$, only the *gluco* isomer could be detected by ^{1}H NMR spectroscopy.

After reduction of **(12)** with NaB^2H_4, **(13)** shows: ^{1}H NMR (220 MHz, $CDCl_3$) δ 1.32 (3H, s, Me), 1.51 (3H, s, Me), 2.69, (1H, s, OH), 3.25 (1H, d, H6, 9.3), 3.45 (1H, d, H6, 9.3), 4.08 (1H, d, H3, 3.1), 4.37 (1H, d, H4, 3.1), 4.50 (1H, d, $PhCH_2$, 11.8), 4.60 (1H, d, H2, 4.0), 4.65 (1H, d, $PhCH_2$, 11.8), 6.08 (1H, d, H1, 4.0), 7.20-7.60 (20H, m, aromatic H).

1,2-O-Isopropylidene-5-[^{2}H]-α-D-*gluco*-hexofuranose(14)

This was prepared according to Inouye[10] with modification to the purification. Ammonia gas was condensed into a dry, round bottomed flask (250mL). The total volume of the liquid was approximately 100mL. After cooling to -78°C, the alcohol **(13)** (3.75g, 6.8mmol) in tetrahydrofuran (25mL) was added followed by lithium metal (200mg, 28mmol). As the lithium dissolved, the solution turned deep red. After 45 min. at -78°C, ammonium chloride (3.0g, 56mmol) was added in portions and the reaction was left to reach room temperature. Once the ammonia had evaporated, the remaining residue was washed repeatedly with tetrahydrofuran. This solution was filtered and concentrated to give an off-white oil. The crude product was taken up in water, washed with cyclohexane (2 x 40mL) and lyophilised. After purification (SiO_2, 5% methanol : ethyl acetate), **(14)** (1.3g, 86%) was obtained as an amorphous solid.

(14): ^{1}H NMR (220MHz, d_4-MeOH) δ 1.20 (3H, s, Me), 1.36 (3H, s, Me), 3.50 (1H, d, H6, 11.8), 3.68 (1H, d, H6, 11.8), 3.94 (1H, d, H3 or H4, 3.3), 4.14 (1H, d, H3 or H4, 3.3), 4.40 (1H, d, H2, 3.6), 5.81 (1H, d, H1, 3.6).

5-[^{2}H]-D-Glucose(15)

1,2-O-Isopropylidene-5-[^{2}H]-α-D-*gluco*-hexofuranose **(14)** (2.96g, 13.4mmol) was dissolved in water (40mL) and concentrated sulfuric acid was added until the pH was less than 2 (meter). The solution was then heated at 90°C for 1h after which time TLC (10% methanol : ethyl acetate) showed that all the starting material had hydrolysed. The pH of the solution was adjusted to 6.6 (meter) with 1M aqueous sodium hydroxide solution before being applied to a short mixed bed ion-exchange column. After eluting with water, the recovered eluent was lyophilised to yield **(15)** (1.54g, 64%) as a clear, colourless syrup.

(**15**): ^{1}H NMR (400 MHz, D_2O) δ 3.19 (1H, t, H2β, 8.0), 3.35 (2H, m, H4α and H4β), 3.44 (1H, t, H3β, 9.3), 3.48 (1H, dd, H2α, 9.6, 3.8), 3.65 (1H, t, H3α, 9.6), 3.67 (1H, d, H6β, 12.3), 3.70 (1H, d, H6α, 12.3), 3.79 (1H, d, H6α, 12.3), 3.84 (1H, d, H6β, 12.3), 4.61 (1H, d, H1β, 8.0), 5.18 (1H, d, H1α, 3.8) (α and β refer to the two anomers of glucose with the β anomer constituting approximately 60% of the mixture). The spectrum compared well with that of authentic D-glucose. H6 for both anomers displayed a doublet in the ^{1}H NMR spectrum and the H5 resonances for both anomers were absent. MS (peracetyl derivative) m/z 409 $(M + NH_4)^+$, 332 $(M + H - AcOH)^+$. $C_{16}H_{21}O_{11}{}^2H$ requires m/z 391.

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