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SYNTHESIS OF D-(6*R*)- AND D-(6*S*)-(6-$^2H_1$)GLUCOSE[1]

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Abstract—Chemical synthesis of D-(6*R*)- and D-(6*S*)-(6-$^2H_1$) glucose is described comprising (i) formation of (6-$^2H_1$)-3-*O*-benzyl-5,6-dideoxy-1, 2-*O*-isopropylidene-α-D-*xylo*-hex-5-ynofuranose from D-glucose; (ii) stereospecific reduction of the deuterated acetylene functionality to (*E*)- or (*Z*)-deuterated olefin; (iii) stereospecific *cis*-dihydroxylation of the deuterated olefin; and (iv) separation of stereoisomers based on the intrinsic chirality of D-glucose and subsequent deprotection.

The mechanisms and stereochemistry of enzyme reactions are of current interest in bio-organic chemistry. Essential to investigation of the stereochemistry of reactions are preparations of suitably labelled substrates with defined chirality and analytical methods for chirally-labelled reaction products. A number of approaches have so far been described for the syntheses of various compounds having chirally-labelled site(s) by the use of enzymatic and/or chemical reactions.[2]

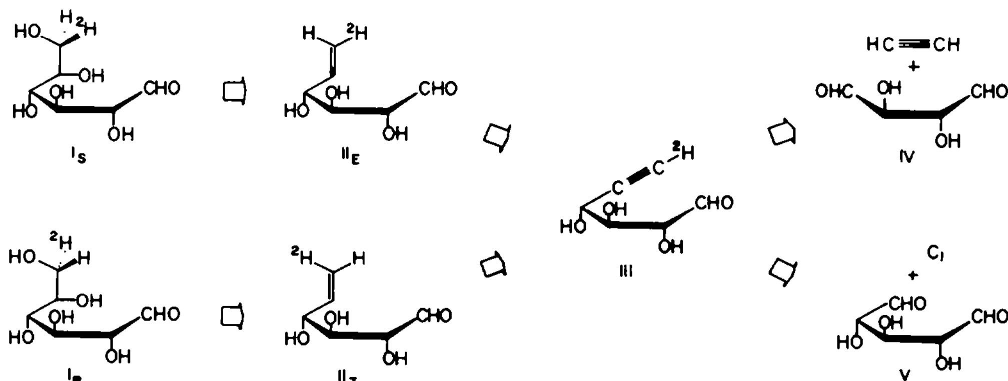
D-Glucose is among the most fundamental compounds for living organisms and is also a principal precursor of various organic natural products. The hydrogens on the prochiral centre of the C-6 hydroxymethyl group of D-glucose are obviously nonequivalent (diastereotopic) and the stereochemistry of enzymatic reactions involving this centre is quite intriguing from the biological as well as the chemical standpoints. These problems can be clarified only by using D-glucose or its derivatives labelled with isotopes (^{2}H and/or ^{3}H) at the C-6 position with defined stereochemistry. Those chirally labelled D-glucose derivatives have been prepared heretofore by the methods consisting of complex enzymatic reactions.[3,4] Since the amount of D-glucose being dealt with is minute, the enzymatic approaches are attainable only for the highly sensitive but rather time-consuming tritium labelling method. In contrast, deuterium NMR spectroscopy has recently become practical and is as convenient for analyzing deuterium-labelled sites as carbon-13 NMR spectroscopy in the ^{13}C tracing technique. One of the drawbacks of the ^{2}H-NMR technique is that the sensitivity is not high enough to detect the deuterium resonances in a small amount of sample. Consequently, chemical synthetic methodology which can supply an adequate quantity of the deuterium-labelled substrates is highly desirable to utilize this safe hydrogen isotope with good availability of materials.

This paper describes the first stereospecific chemical synthesis of D-(6*R*)- and D-(6*S*)-(6-$^2H_1$) glucose in detail,[1,5] the products of which have actually been utilized to the stereochemical studies on the biosynthesis of an aminocyclitol antibiotic ribostamycin.[6,7]

Previously, Horton and coworkers reported a synthesis of 6-*C*-deutero-D-glucose derivatives by a chemical approach,[8] which used sodium borodeuteride reduction of a 6-ulose intermediate prepared by photolysis of methyl 6-azido-6-deoxy-α-D-glucopyranoside. However, this method only gave rise to an unseparable mixture of (6*R*)- and (6*S*)-6-*C*-deutero-D-glucose derivatives.

Since no stereospecific reduction of D-glucose-6-ulose derivatives is yet known (in contrast to versatile reactions of simple deuterated aldehydes with chiral reducing agents[9]) a method of choice for the stereospecific introduction of deuterium to the C-6 prochiral hydroxymethyl group of D-glucose seemed to be an analogous method to the classical synthesis of chiral acetic acid by Cornforth *et al.*[10]

The synthetic plan is illustrated in Scheme 1. A



Scheme 1.

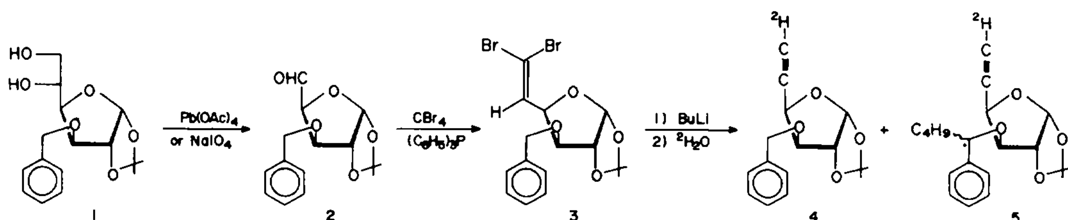
monodeuterohydroxymethyl group of I_R or I_S seems to be prepared by dihydroxylation of monodeuteroolefin II_Z or II_E, which may be derived from a deuteroacetylene **III** by sterospecific reduction. Thus, a common precursor to both D-(6*R*)- and D-(6*S*)-(6-$^2H_1$) glucose is a 5,6-yne derivative **III**, which can conceptually be obtained either by addition of acetylene to a L-*threo* C_4 precursor **IV** such as those derived from L-tartaric acid or by formation of an acetylene functionality from a D-*xylo* C_5 precursor **V** and C_1 unit. The former approach requires stereochemical control of two sites, i.e. the C-5 and C-4 positions of D-glucose, whereas the latter route needs only one site (C-5) of steric control. Therefore, the latter route was chosen in the present synthesis and a suitable precursor with D-*xylo* configuration was 3-*O*-benzyl-1,2-*O*-isopropylidene- α-D-*xylo*-pentodialdo-1,4-furanose **2**, readily available from D-glucose,[11] as shown in Scheme 2.

Further convenience of this route was that the unlabelled compounds corresponding to the intermediates are known and a precursor 3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-glucofuranose **1** can be a direct reference for assignment of the stereochemistry of the C-5 position at a later stage of the synthesis.

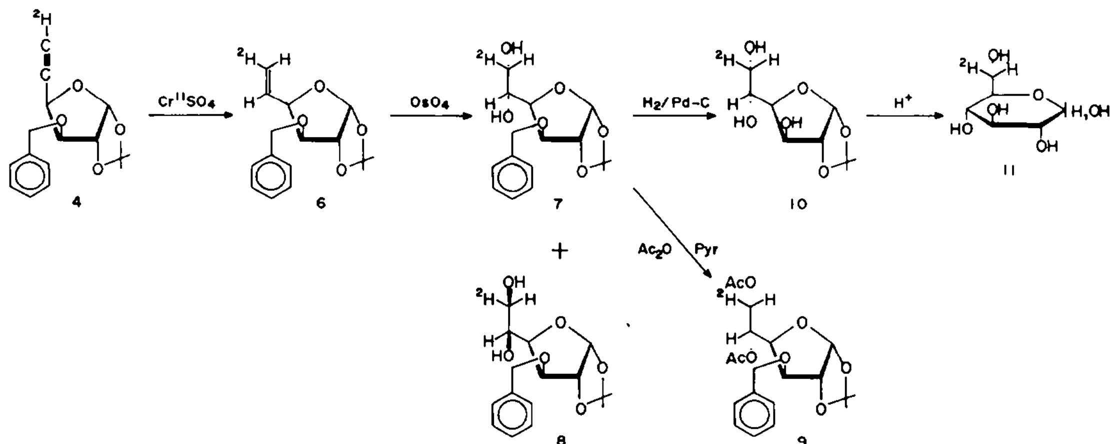
The precursory dibromo-olefin **3** was obtained as syrup from the aldehyde **2** by reaction with carbon tetrabromide in the presence of triphenylphosphine.[12] Subsequent treatment of **3** with two equivalents of n-butyllithium in THF at −70°, followed by quenching with deuterium oxide afforded the deuterated acetylene **4**,[13] together with an undesired n-butylated product **5** (*vide infra*). Care was taken to avoid possible exchange of deuterium with protium during work-up by immediate acidification of the reaction mixture. Formation of the byproduct **5** is presumably due to competitive deprotonation at the benzyl position and subsequent attack of this benzyl anion to n-butyl bromide generated *in situ* by the initial lithioolefin formation. A slightly deficient amount (1.7–1.8 mol equivalent) of the base was usually used to reduce the formation of **5** in the preparative operation.

Transformation of the deuteroacetylene **4** to D-(6*S*)-(6-$^2H_1$) glucose is illustrated in Scheme 3. Stereospecific reduction of **4** to the (*E*)-olefin **6** was affected by treatment with Cr(II) sulphate in aqueous DMF.[14] Some difficulties were encountered to isolate **6** from the reaction mixture. The n-butylated acetylene **5** was resistant to this reduction, probably because of the steric hindrance which inhibits simultaneous approach of two molecules of the reducing metal complex.

Dihydroxylation of the olefin **6** was achieved by oxidation with a catalytic amount of OsO_4 in the presence of *N*-methylmorpholine N-oxide. This oxidation is believed to proceed *via* stereospecific *cis*-addition of hydroxyl groups. In this instance, the reaction of **6** gave a mixture of (6*S*)-(6-$^2H_1$)-3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-glucofuranose **7** and its (6*R*)-(6-$^2H_1$)-L-*ido* isomer **8** in a ratio of 4:1 in favor of the desired product. These diastereoisomeric glycols were conveniently separated by chromatography. The configuration of the C-5 position of **7** was firmly assigned by comparison of its behaviour on TLC and the ^{1}H-NMR spectrum with



Scheme 2.



Scheme 3.

those of the non-deuterated standard **1** (*vide supra*). Thus the configuration of the deuterohydroxymethyl group was determined to be *S*. The deuterium enrichment of **7** was estimated to be 94% by mass spectrometry. The *pro R* proton on the C-6 position of **7** was observed in the ^{1}H-NMR spectrum at δ 3.66 ppm (1H d, J = 4.5 Hz), the coupling constant of which was consistent with the previous discussions by Horton.[8] Compound **7** was further derivatized to the crystalline diacetate **9**.

The protected D-glucose **7** was then deprotected to the free D-(6*S*)-(6-$^2H_1$) glucose **11**, first by the catalytic hydrogenation in the presence of 10% Pd–C to remove the benzyl group and by the subsequent acid hydrolysis.

As can be seen in Scheme 4, D-(6*R*)-(6-$^2H_1$) glucose was prepared similarly from **4** through (*Z*)-deuterated olefin **12**, which was obtained by the catalytic hydrogenation over Lindlar catalyst in the presence of quinoline while monitoring the reaction from time to time by TLC. During this reaction slight loss of deuterium was observed and the deuterium enrichment of **12** was estimated to be 90% by the integration of the ^{1}H-NMR spectrum. Prolonged hydrogenation resulted in the formation of undesirable (*E*)-olefin **6** as a byproduct. Earlier attempts to affect the stereospecific hydrogenation with $Pd/BaSO_4$ in pyridine or with Lindlar catalyst in EtOH also resulted in substantial contamination of non-deuterated olefin and (*E*)-olefin **6**. Although the reduction of **4** with an equimolar amount of potassium azodicarboxylate/acetic acid in MeOH underwent stereospecifically, the desired **12** was obtained in low yield as an unseparable mixture with a saturated product **13**, MS: *m/z* 264 (M^+-15); ^{1}H-NMR: δ 0.91 (br. t H-6) and 1.76 ppm (q, H-5).

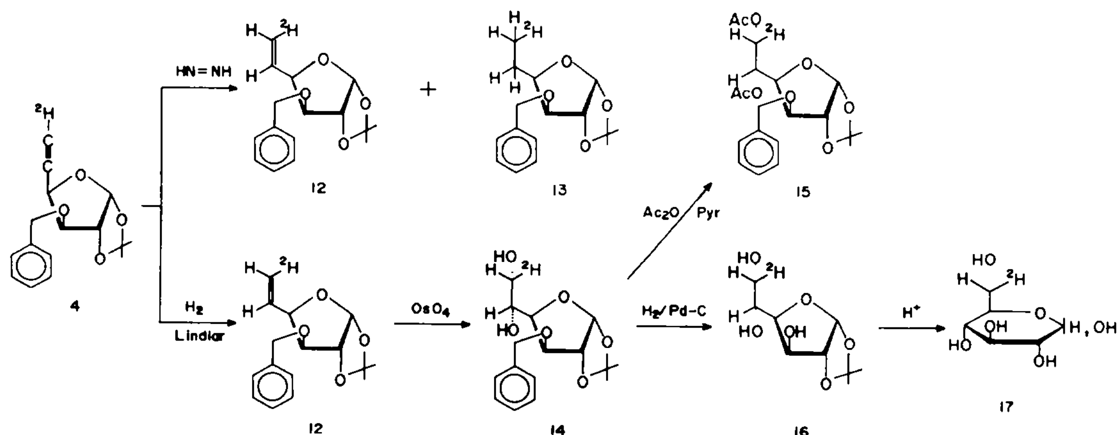
The (*Z*)-olefin **12** was further manipulated to D-(6R)-(6-$^2H_1$) glucose **17** by the above-mentioned sequence of reactions.

The 400 MHz ^{1}H-NMR spectra of these stereospecifically deuterated glucose **11** and **17** together with those of D-(6,6-$^2H_1$) glucose[15] and nonlabelled D-glucose are shown in Fig. 1. It is worth noting that all proton signals of D-glucose are now completely assigned including the protons of the C-6 prochiral hydroxymethyl group, i.e. the α-anomer: $\delta^{DSS}_{D_2O}$ 5.22 (d, J = 3.5 Hz, H-1), 3.52 (dd, J = 3.5 and 9.5 Hz, H-2), 3.70 (t, J = 9.5 Hz, H-3), 3.39 (t, J = 9.5 Hz, H-4), 3.82 (ddd, J = 2, 5 and 9 Hz, H-5), 3.75 (dd, J = 5 and 12.5 Hz, H-6*R*) and 3.82 ppm (dd, J = 2 and 12.5 Hz, H-6*S*); β-anomer: δ 4.63 (d, J = 8 Hz, H-1), 3.23 (dd, J = 8 and 9 Hz, H-2), 3.47 (t, J = 9 Hz, H-3), 3.38 (t, J = 9 Hz, H-4), 3.45 (ddd, J = 2, 5.5 and 9 Hz, H-5), 3.71 (dd, J = 5.5 and 12.5 Hz, H-6*R*) and 3.86 ppm (dd, J = 2 and 12.5 Hz, H-6*S*).

It now appears that the present preparative method facilitates the use of D-(6*R*)- and D-(6*S*)-(6-$^2H_1$) glucose for the stereochemical studies of wide variety of biochemical reactions. Furthermore, this approach seems to be useful for preparation of other compounds containing a stereochemically defined chiral methylene group. For instance, the deutero-olefin **6** and **12** were able to be epoxidized by peracid oxidation to 5,6-anhydro-D-*gluco*- and L-*ido*-hexofuranose derivatives with defined stereochemistry,[1] which could in turn be facile precursors for further transformation to compounds containing a chiral methylene functionality through the nucleophilic oxirane opening with inversion of configuration at the deuterated methylene group.

EXPERIMENTAL

Melting points were determined with a Mitamura Riken melting point apparatus and are uncorrected. Infrared spectra were taken on a Hitachi 260-10 spectrometer. Proton magnetic resonance spectra were recorded on a JEOL PS-100 or a FX-400 spectrometer using deuterochloroform as solvent. Chemical shifts are reported as δ values in parts per million relative to tetramethylsilane as an internal standard. Optical rotations were measured using a Perkin–Elmer Model 241 MC polarimeter. Electron inpact mass spectra were recorded on a Shimadzu LKB-9000S spectrometer at 70 eV by a direct inlet method. Analytical thin layer chromatography (TLC) was conducted on precoated TLC plates. Silica gel 60 F-254 (thickness 0.25 mm Merck, Art 5715). Silica gel columns for chromatography utilized silica gel 60, 70–230 mesh ASTM (Merck Art 7734). Elemental analyses were performed by the microanalysis laboratories of Institute of Applied Microbiology, the University of Tokyo, and of the Institute of Physical and Chemical Research.



Scheme 4.

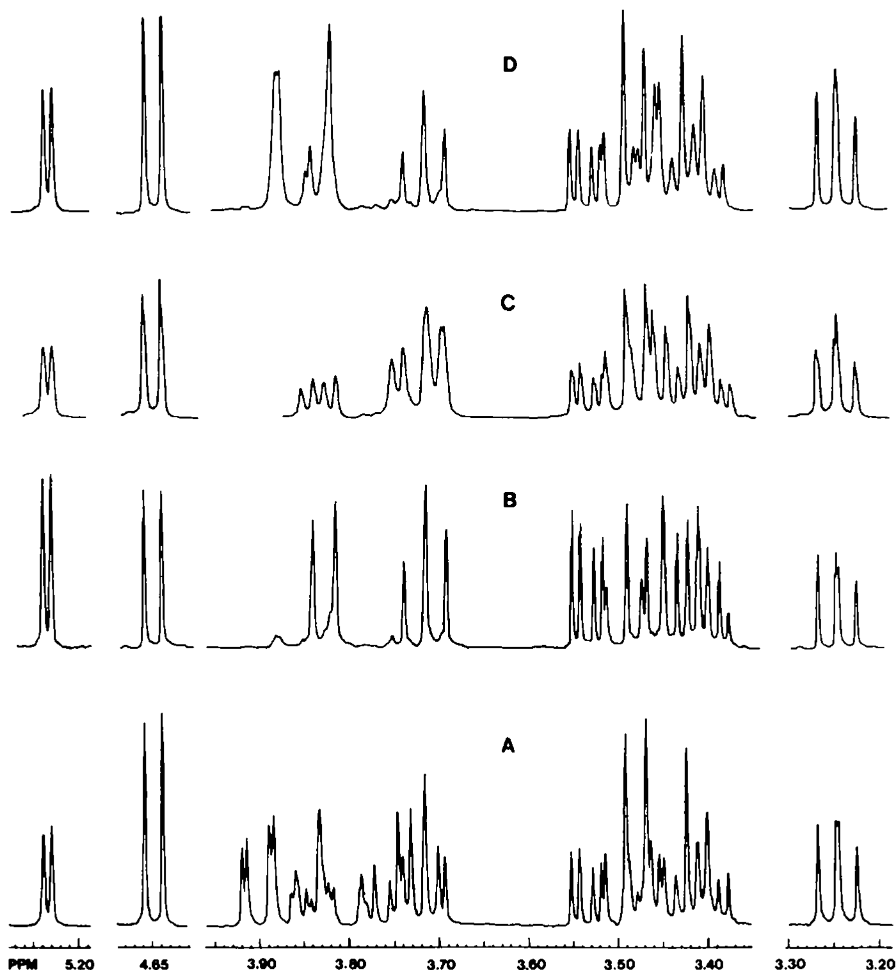


Fig. 1. 400 MHz ^{1}H-NMR spectra of the deuterium labeled and unlabeled D-glucose, Spectra were taken in deuterium oxide solvent and the chemical shift scale was standardized based on the HDO signal at δ 4.80 ppm (see text). Spectrum A: commercial D-glucose; Spectrum B: D-(6,6-$^2H_2$) glucose prepared by a literature method[15]; Spectrum C: D-(6*S*)-(6-$^2H_1$) glucose **11**; and Spectrum D: D-(6*R*)-(6-$^2H_1$) glucose **17**.

3-O-*Benzyl*-6,6-*dibromo*-5,6-*dideoxy*-1,2-O-*isopropylidene*-α-D-xylo-*hex*-5-*enofuranose* **3**

To a stirred mixture of 106 g (0.4 M) of triphenylphosphine and 67 g (0.2 M) of carbon tetrabromide in 100 mL of methylene chloride was added a soln of 25 g (0.09 M) of 3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-xylo-pentodialdo-1,4-furanose **2**, prepared by a known procedure,[11] dissolved in 65 mL of methylene chloride over a period of 30 min at 0°C. The mixture was then stirred for 30 min at the same temp and for 1.5 h at room temp. To the mixture was then added 800 mL of n-hexane with shaking and the resulting colourless soln was decanted. The dark brown residue was redissolved in 300 mL of methylene chloride and 1.5 L of n-hexane was added. The organic soln was decanted and combined with the first extract. Evaporation of solvent under reduced pressure gave a residue, which was purified by silica gel column chromatography using n-hexane–ether (4:1) as eluant to give 28.5 g (73% yield) of **3**, $[\alpha]_D^{25}$ −50.8° (*c* 1.0, $CHCl_3$), MS: *m/z* 417, 419, 421 (M^+ − 15) and *m/z* 353, 355 (M^+-HBr); ^{1}H-NMR: 1.34 (3H, s), 1.52 (3H, s), 4.02 (1H d, J = 3.2 Hz, H-3), 4.58 (1H d, J = 4.0 Hz, H-2), 4.50 (1H d, J = 13 Hz), 4.64 (1H d, J = 13 Hz), 4.78 (1H dd, J = 3.2 and 8.0 Hz, H-4), 5.92 (1H d, J = 4.0 Hz, H-1), 6.66 (1H d, J = 8.0 Hz, H-5) and 7.35 (5H, br.s).

(6-^{2}*H*)-3-O-*Benzyl*-5,6-*dideoxy*-1,2-O-*isopropylidene*-α-D-xylo-*hex*-5-*yno*-*furanose* **4**

To a stirred soln of 28.5 g (65.7 mM) of **3** dissolved in 70 mL of tetrahydrofuran under argon atmosphere was added dropwise 72 mL (112.5 mM) of 10% (w/v) n-butyllithium in n-hexane over a period of 2 h, during which time the temp of the mixture was maintained below −70°. After addition was completed, stirring was continued for 3 h at the same temperature. The mixture was then allowed to warm to room temp and further stirred for 1 h. To the resulting brown solution was added 10 mL of deuterium oxide (Merck 99.75 atom% enriched) and stirring was continued for 30 min. The mixture was then poured into 200 mL of ice-cold 1N HCl solution and organic solvent was removed by evaporation under reduced pressure. The residual aqueous suspension was extracted three times with 200 mL portions of ether and combined extract was washed with aqueous saturated sodium bicarbonate solution and brine, and then dried over anhydrous magnesium sulfate. Filtration and stripping of solvent gave brownish syrup, which was chromatographed on silica gel using n-hexane–ethyl acetate (10:1) as solvent to give, along with 4.5 g of **5**, 9.4 g (52%) of **4**, $[\alpha]_D^{25}$ + 7.0° (c 1.0), $CHCl_3$), MS: *m/z* 260 (M^+-15)); IR: ν_{max}^{neat} 2650 and 1980 cm^{-1}; ^{1}H-NMR: 1.30 (3H, s), 1.47 (3H, s), 4.00 (1H d, J = 3.2 Hz, H-3), 4.59 (1H d. J = 3.8 Hz, H-2),

4.73 (1H d, J = 12 Hz), 4.81 (1H d, J = 12 Hz), 4.83 (1H d, J = 3.2 Hz, H-4), 5.98 (1H d, J = 3.8 Hz, H-1) and 7.35 (5H, br.s). (Found: C, 70.16; H + D, 6.82. Calc. for $C_{16}H_{17}O_4D$: C, 69.80; H + D, 6.95%.)

(*E*)-(6-$^2H_1$)-3-O-*Benzyl-5,6-dideoxy-1,2-O-isopropylidene-α-*D-xylo-*hex-5-enofuranose* **6**

To a stirred solution of 5.0 g (18.2 mM) of **4** dissolved in 100 mL of dimethylformamide under argon atmosphere was added 250 mL of a chromous sulfate solution prepared by a literature procedure[14] at room temp and stirring was continued for 4 days. The reaction mixture was then saturated with solid ammonium sulfate and the whole was extracted three times with 150 mL portions of ether. Combined ethereal extract was washed with brine and dried over anhydrous magnesium sulfate. Filtration and stripping of solvent under reduced pressure gave yellowish syrup, which was chromographed on silica gel using n-hexane–ethyl acetate (10:1) as eluant. Fractions were collected and examined by TLC using the same solvent system. Appropriate olefin containing fractions were combined and stripped of solvent under reduced pressure gave 2.4 g of **6** as colourless syrup (48%) $[\alpha]_D^{25}$ −66.5° (c 1.3, $CHCl_3$); ^{1}H-NMR: 1.32 (3H, s), 1.50 (3H, s), 3.89 (1H d, J = 3.0 Hz, H-3), 4.56 (1H d, J = 12 Hz), 4.62 (1H d, J = 3.8 Hz, H-2), 4.64 (1H dd, J = 3.0 and 7.0 Hz, H-4), 4.65 (1H d, J = 12 Hz), 5.42 (1H d, J = 17 Hz, H-6), 5.96 (1H d, J = 3.8 Hz, H-1), 6.00 (1H dd, J = 7.0 and 17 Hz, H-5) and 7.32 (5H, br.s). (Found: C, 69.28; H + D, 7.66. Calc for $C_{16}H_{19}O_4D$: C, 69.29; H + D, 7.63%.)

(6S)-(6-$^2H_1$)-3-O-*Benzyl-1,2-O-isopropylidene-α-*D-*glucofuranose* **7**

To a stirred soln of 2.4 g (8.7 mM) of **6** dissolved in 10 mL of tetrahydrofuran were added 4.0 g (31.5 mM) of N-methylmorpholine N-oxide, 45 mL of a mixture of t-butyl alcohol-tetrahydrofuran–water (10:3:1) at room temp, and then unweighed catalytic amount of osmium tetroxide was added to the mixture, which was further stirred overnight at room temp. To the mixture was added 0.2 g of sodium bisulfite and 5 mL of water, and stirring was continued for 1 h. The mixture was filtered with an aid of Celite 545 and the brown precipitate was washed with 20 mL of acetone. Combined filtrate and washings was evaporated under reduced pressure to give brownish suspension, which was acidified with 1N hydrochloric acid and extracted five times with 40 mL portions of ethyl acetate. Combined extract was washed successively with 1N hydrochloric acid, saturated sodium bicarbonate solution and brine, and then dried over anhydrous sodium sulfate. Filtration and evaporation of solvent under reduced pressure gave 2.69 g of brownish syrup. The syrup was purified by flash chromatography[16] on silica gel 60 (Merck Art 9385, 230–400 mesh ASTM) using n-hexane–acetone (2:1) to give, along with 0.5 g (18.7%) of **8**, 2.0 g (74.8%) of **7** as colourless syrup, $[\alpha]_D^{26}$ −40.2° (c 1.2. $CHCl_3$); MS: *m/z* 296 (M^+-15) : *m/z* 295 = 100:6.6; ^{1}H-NMR: 1.30 (3H, s), 1.48 (3H, s), 3.23 (2H br.s, D_2O exchangeable), 3.66 (1H d, J = 4.5 Hz, *pro R* H-6), 3.95–4.30 (3H m, H-3, H-4 and H-5), 4.56 (1H d, J = 12 Hz), 4.58 (1H d, J = 3.9 Hz, H-2), 4.69 (1H d, J = 12 Hz), 5.92 (1H d, J = 3.9 Hz, H-1) and 7.33 (5H, br.s).

(6S)-(6-$^2H_1$)-5,6-O-*Diacetyl*-3-O-*benzyl*-1,2-O-*isopropylidene-α-*D-*glucofuranose* **9**

To a soln of 74.4 mg (0.24 mM) of **7** in 1 mL of pyridine was added 0.5 mL of acetic anhydride and the mixture was allowed to stand overnight at room temp. The mixture was poured into 15 mL of water and extracted three times with 30 mL portions of ether. Combined extract was washed with 1N hydrochloric acid, saturated sodium bicarbonate solution and brine, and then dried over anhydrous magnesium sulfate. Filtration and stripping of solvent gave a colourless syrup, which was crystallized from ether–n-hexane to give 71 mg (79%) of colourless rods **9**, mp 118.5–119°; ^{1}H-NMR: 1.33 (3H, s), 1.50 (3H, s), 1.91 (3H, s), 2.04 (3H, s), 3.95 (1H d, J = 3.2 Hz, H-3), 4.15 (1H d, J = 4.8 Hz, *pro R* H-6), 4.33 (1H dd, J = 3.5 and 8.6 Hz, H-4), 4.45 (1H d, J = 11.7 Hz), 4.61 (1H d, J = 3.6 Hz, H-2), 4.62 (1H d, J = 11.7 Hz), 5.33 (1H dd, J = 4.8 and 8.6 Hz, H-5), 5.92 (1H d, J = 3.6 Hz, H-1) and 7.32 (5H, br.s.). (Found: C, 60.59; H + D, 6.64. Calc. for $C_{20}H_{25}O_8D$: C 60.75; H + D, 6.88%.)

(6S)-(6-$^2H_1$)-1,2-O-*Isopropylidene-α-*D-*glucofuranose* **10**

Under hydrogen at atmospheric pressure, 469 mg of 10% palladium on activated carbon (Kawaken Fine Chemicals Co, Tokyo) was activated by magnetic stirring for 1 h in a mixture of ethyl alcohol-water-acetic acid (3:1:1) at room temp. To the mixture was added 1.8 g (5.8 mM) of **7** dissolved in 6 mL of ethyl alcohol and the whole mixture was stirred for 2 days under hydrogen at room temp. After disappearance of **7** was confirmed by TLC using chloroform-methyl alcohol (5:1) as solvent, the mixture was filtered with aid of Celite 545 and the catalyst was washed with ethyl alcohol. Combined filtrate and washings were evaporated to dryness under reduced pressure to give white precipitate, which was recrystallized from methyl alcohol–ether to yield 1.2 g (94%) of **10**, m.p. 159–161°. (Found: C, 48.50; H + D, 7.34. Calc. for $C_9H_{15}O_6D$: C, 48.84; H + D, 7.74%.)

D-(6S)-(6-$^2H_1$)-*Glucose* **11**

A mixture of 1.55 g (7 mM) of **10**, 20 mL of 0.1 N sulfuric acid was heated with stirring at 105° for 30 min. The reaction mixture was cooled to room temp and was neutralized by adding Amberlite IRA-410 (OH cycle) with stirring. The mixture was then filtered and the resin was washed with distilled water. Combined filtrate and washing was evaporated to dryness under reduced pressure. The colourless residue was taken in 6 mL of methyl alcohol, to which was added isopropyl alcohol until the mixture became turbid. The mixture was allowed to stand at room temp for a week to give 1.19 g (94%) of white crystalline precipitate **11**, m.p. 136-140° (Found: C, 39.37, H + D, 6.80. Calc for $C_6H_{11}O_6D$: C, 39.78; H + D, 7.23%.)

(Z)-(6-$^2H_1$)-3-O-*Benzyl-5,6-dideoxy-1,2-O-isopropylidene-α-*D-xylo-*hex-5-enofuranose* **12**

To a solution of 6.0 g (21.8 mM) of **4** in 50 mL of acetone were added 109 mg of 5% palladium on calcium carbonate (Lindlar catalyst; Kawaken Fine Chemicals Co, Tokyo) and 2 mL of freshly distilled quinoline, and the mixture was stirred magnetically under hydrogen atmosphere. The reaction was monitored by TLC using n-hexane–ether (4:1) as solvent. When the acetylene **4** was almost consumed, the mixture was filtered with aid of Celite 545 and the catalyst was washed with acetone. The filtrate and washings were combined and removed of solvent under reduced pressure. The residue was partitioned with 150 mL of ether and 150 mL of 1N hydrochloric acid, and layers were separated. The aqueous layer was further extracted three times with 150 mL portions of ether. The ethereal extracts were combined and washed with saturated sodium bicarbonate solution and brine, and then dried over anhydrous magnesium sulfate. After filtration and removal of solvent under reduced pressure, the syruppy residue was purified by silica gel column chromatography using n-hexane–ether (4:1) as eluant. Fractions were collected and examined by TLC as above. Appropriate olefin fractions were combined and evaporated to dryness to give 5.91 g (97%) of colourless syrup **12**, ^{1}H-NMR: 1.32 (3H, s), 1.50 (3H, s), 3.90 (1H d, J = 3.0 Hz, H − 3), 4.56 (1H d, J = 13 Hz), 4.62 (1H d, J = 3.8 Hz, H-2), 4.64 (1H d, J = 13 Hz), 4.64 (1H dd, J = 3.0 and 7 Hz, H-4), 5.32 (1H d, J = 10 Hz, H-6), (1H d, J = 3.8 Hz, H-1), 6.00 (1H m, H-5), 7.36 (5H, br.s).

Diimide reduction

To a stirred mixture of 1.75 g (6.4 mM) of **4**, 1.51 g (7.8 mM) of potassium azodicarboxylate[17] and 40 mL of methyl alcohol under nitrogen atmosphere was added drop-

wise 1.0 mL (17.5 mM) of glacial acetic acid and the mixture was stirred for 21 h at room temp. The reaction mixture was stripped of solvent under reduced pressure. The residue was partitioned between ethyl acetate and saturated sodium bicarbonate solution. Layers were separated and the aqueous layer was further extracted with ethyl acetate. Combined organic extract was washed with brine and dried over anhydrous magnesium sulfate. Filtration and evaporation of solvent under reduced pressure gave yellowish syrup, which was chromatographed on silica gel using n-hexane–ethyl acetate (10:1) as eluant. Fractions were collected and examined by TLC. Appropriate fractions were combined and evaporated to dryness to give 735 mg of the recovered acetylene **4** and 846 mg of a mixture of **12** and **13**. The component ratio of the latter mixture was estimated to be *ca.* 5:4 by the ^{1}H-NMR spectrum ($\delta_{H\text{-}3}$ 3.90 ppm for **12** and $\delta_{H\text{-}3}$ 3.80 ppm for **13**).

(6R)-(6-$^{2}H_{1}$)-3-O-*Benzyl*-1,2-O-*isopropylidene*-α-D-*glucofuranose* **14**

By the procedure described for the preparation of **7**, 5.91 g of **12** was subjected to the osmium tetroxide oxidation to give 3.34 g of **14**, $[\alpha]_{D}^{25}$ −38.9° (c 1.08, $CHCl_3$), ^{1}H-NMR: 1.30 (3H, s), 1.48 (3H, s), 2.64 (2H br, exchangeable), 3.77 (1H d, J = 3.0 Hz, *pro S* H-6), 3.95–4.20 (3H m, H-3, H-4 and H-5), 4.57 (1H d, J = 12 Hz), 4.60 (1H d, J = 3.9 Hz, H-2), 4.70 (1H d, J = 12 Hz), 5.96 (1H d, J = 3.9 Hz, H-1), and 7.36 (5H, br.s).

(6R)-(6-$^{2}H_{1}$)-5,6-O-*Diacetyl*-3-O-*benzyl*-1,2-O-*isopropylidene*-α-D-*glucofuranose* **15**

By the procedure described for the preparation of **9**, 25.5 mg of **14** was acetylated to give 29 mg (90%) of **15**, m.p. 118–119°. (Found: C, 60.83; H + D, 6.67. Calc. for $C_{20}H_{25}O_{8}D$: C, 60.75; H + D, 6.88%.)

(6R)-(6-$^{2}H_{1}$)-1,2-O-*Isopropylidene*-α-D-*glucofuranose* **16**

By the procedure described for the preparation of **10**, 3.34 g of **14** was hydrogenated to give 2.04 (86%) of **16**, m.p. 160–161°. (Found: C, 48.51; H + D, 7.40. Calc. for $C_{9}H_{15}O_{6}D$: C, 48.86; H + D, 7.74%.)

D-(6R)-(6-$^{2}H_{1}$) *Glucose* **17**

By the procedure described for the preparation of **11**, 2.0 g of **16** was hydrolysed to give 1.41 g (85%) of **17**, m.p. 140–143°. (Found: C, 39.74; H + D, 6.91. Calc. for $C_{6}H_{11}O_{6}D$: C, 39.78; H + D, 7.23%.)

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