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STABLE, ISOTOPICALLY SUBSTITUTED CARBOHYDRATES: AN IMPROVED SYNTHESIS OF (6-^{13}C)ALDOHEXOSES*

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ABSTRACT

1,2-*O*-Isopropylidene-α-D-*xylo*-pentodialdo-1,4-furanose (**1**) has been used as the parent aldose in the preparation of D-(6-^{13}C)glucose and L-6-^{13}C)idose *via* cyanohydrin reduction. The addition of K^{13}CN (pH 6.8, 5 min) to **1** yields D-*gluco* and L-*ido* cyanohydrins that are readily reduced with H_2 and Pd–$BaSO_4$, to give 1,2-*O*-isopropylidene-α-D-*gluco*-hexodialdo-1,4-furanose (**2**; ~65%) and 1,2-*O*-isopropylidene-β-L-*ido*-hexodialdo-1,4-furanose (**3**; 35%). Aldehydes **2** and **3** are reduced *in situ* with $NaBH_4$, the resulting alcohols are deprotected with aqueous acid, and the aldoses are chromatographed on Dowex 50 X-8 (Ca^{2+}) ion-exchange resin (200–400 mesh), to yield D-(6-^{13}C)glucose (**6**) and L-(6-^{13}C)idose (**7**). Molybdate epimerization of **6** and **7** yields D-(6-^{13}C)mannose and L-(6-^{13}C)gulose, respectively. A similar reaction scheme may be applied to methyl 2,3-*O*-isopropylidene-β-D-*ribo*-pentodialdo-1,4-furanoside to generate the remaining four (6-^{13}C)-aldohexoses. This route is considerably simpler than the traditional Kiliani–Fischer route, and higher yields are obtained.

INTRODUCTION

During the past ten years, an efficient chemical method for preparing (1-^{13}C)aldoses has been developed[1,2] that is based on the classical cyanohydrin (Kiliani) reaction[3] and Kuhn reduction[4]. This method, known as the cyanohydrin-reduction method (see Scheme 1), involves the condensation of a parent aldose with (^{13}C)cyanide at pH 7.0–7.5, to generate C-2-epimeric (1-^{13}C)cyanohydrins that are reduced *in situ* with H_2 and Pd–$BaSO_4$ to the corresponding C-2-epimeric (1-^{13}C)aldoses. Furthermore, these (1-^{13}C)aldoses can be converted in a single step into (2-^{13}C)aldoses by molybdate-catalyzed epimerization[5], providing a convenient route to aldoses labeled with ^{13}C at "internal" carbon atoms. By combining cyanohydrin reduction and molybdate epimerization, a wide range of (^{13}C)-labeled

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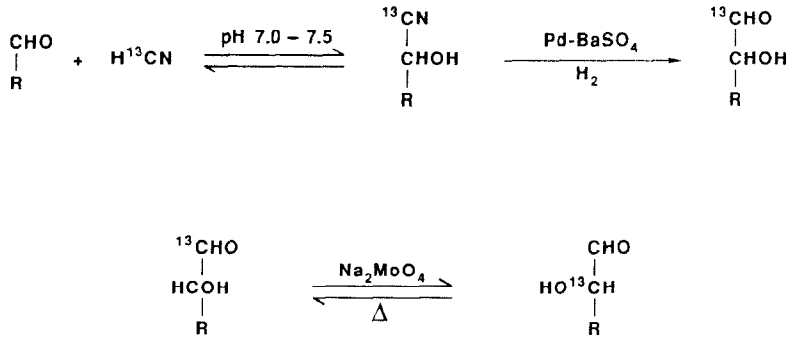


Scheme 1

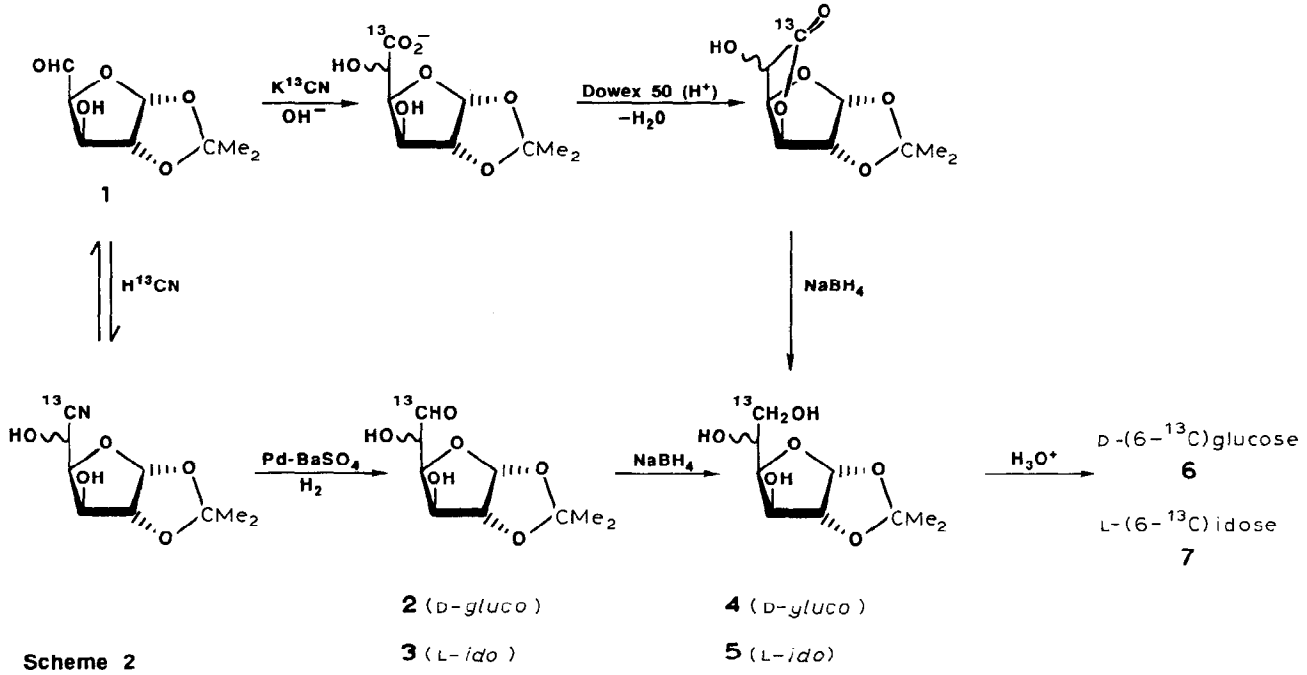
aldoses has become accessible[2] for use in chemical and biological (*e.g.*, metabolic) studies[6].

Methods for labeling aldoses with ^{13}C at the terminal hydroxymethyl group or penultimate carbon atom are not so well developed. For example, D-(6-^{13}C)glucose is usually prepared from 1,2-*O*-isopropylidene-α-D-*xylo*-pentodialdo-1,4-furanose (**1**) and $K^{13}CN$, as described many years ago by Sowden[7a] and later modified by Schaffer and Isbell[7b] (see Scheme 2). This route involves aldonate formation, followed by 6,3-lactonization, and reduction to the alcohol[7c]. We have developed an alternative faster route to D-(6-^{13}C)glucose that can be used more widely to prepare multigram quantities of (6-^{13}C)aldohexoses. In this improved route (see Scheme 2), **1** is treated with $K^{13}CN$ and the resulting C-5-epimeric (6-^{13}C)cyanohydrins are reduced with H_2 and Pd–$BaSO_4$ to the protected C-5-epimeric (6-^{13}C)dialdoses **2** and **3**. Compounds **2** and **3** are treated *in situ* with sodium borohydride, to give **4** and **5**, respectively. Deprotection affords D-(6-^{13}C)glucose (**6**) and L-(6-^{13}C)idose (**7**), which are separated by chromatography. Molybdate epimerization (Scheme 1) of **6** and **7** provides access to D-(6-^{13}C)mannose and L-(6-^{13}C)gulose. A similar reaction scheme starting with methyl 2,3-*O*-isopropylidene-β-D-*ribo*-pentodialdo-1,4-furanoside[8] could be employed to prepare D-(6-^{13}C)allose and L-(6-^{13}C)talose, and their C-2 epimers.

EXPERIMENTAL

Materials. — 1,2-*O*-Isopropylidene-α-D-glucofuranose was purchased from Aldrich Chemical Company. D-Ribose, sodium periodate ($NaIO_4$), 5% palladium-on-barium sulfate, and sodium borohydride ($NaBH_4$) were purchased from Sigma Chemical Company. Potassium (^{13}C)cyanide ($K^{13}CN$, 99 atom-% of ^{13}C) was obtained from Cambridge Isotope Laboratories. All solvents were reagent grade, and used without purification.

Instrumentation. — ^1H-Decoupled, ^{13}C-n.m.r. spectra were recorded at 75 MHz with a Nicolet NT-300 Fourier-transform n.m.r. spectrometer equipped with


OHC
OH
CMe2
1
K13CN
OH−
13CO2−
HO
Dowex 50 (H+)
−H2O
13C
H13CN
NaBH4
13CN
Pd-BaSO4
H2
13CHO
13CH2OH
H3O+
D-(6-13C)glucose
6
L-(6-13C)idose
7
2 (D-gluco)
3 (L-ido)
4 (D-gluco)
5 (L-ido)

Scheme 2

a 293B pulse programmer and quadrature-phase detection. Spectra were recorded at 25°, and carbon chemical shifts are reported in p.p.m. relative to external β-D-(1-^{13}C)glucopyranose in $^{2}H_2O$ (97.4 p.p.m.).

Preparation of the parent dialdose. — 1,2-*O*-Isopropylidene-α-D-*xylo*-pentodialdo-1,4-furanose (**1**) was prepared by periodate oxidation of 1,2-*O*-isopropylidene-α-D-glucofuranose (25 g) as described by Wolfrom and Thomas[9], with the following modifications. The dry CH_2Cl_2 extract was evaporated to a syrup *in vacuo* at 30°, and a solution of the syrup in methanol (40 mL) was evaporated to a syrup *in vacuo*. Addition and evaporation of methanol were repeated 3–4 times to remove most of the formaldehyde generated during the $NaIO_4$ oxidation. After the final evaporation, the syrup was dissolved in water (~25 mL), and the solution was stored at 4°; crystallization of **1** (probably a dimer[10] of **1**) occurred during 5–7 days, giving a first crop of crystals (~15 g; yield, 70%). Further crystallization from water gave an additional 1 g of **1**.

D-*(6-*^{13}*C)Glucose* (**6**) *and* L-*(6-*^{13}*C)idose* (**7**) *(Scheme 2).* — A solution of compound **1** (15 g, 80 mmol) in H_2O (150 mL) was added to a stirred solution of $K^{13}CN$ (6.3 g, 96 mmol) in H_2O (20 mL) adjusted to pH 6.8 with acetic acid. A 500-mL, two-necked, round-bottomed flask was used for the reaction, with a pH electrode placed in one neck to permit monitoring of the pH. After 5 min at pH 6.8, the pH of the mixture was adjusted to 4.4 with acetic acid, and the mixture was treated with N_2 to remove and trap[11] the excess of $H^{13}CN$. The pH of the solution (containing the C-5-epimeric (6-^{13}C)cyanohydrins) was adjusted to 4.3 with HOAc, and the cyanohydrins reduced at atmospheric pressure with pre-reduced 5% Pd–$BaSO_4$ (5.0 g) and H_2. After uptake of H_2 was complete (~8 h), the suspension was filtered, the pH of the filtrate (containing **2** and **3**) was adjusted to 10.7 with 2M KOH, and $NaBH_4$ (3.0 g, 80 mmol) was added batchwise, with stirring. After 4 h at room temperature, the solution was treated batchwise with Dowex 50 X-8 (H^+) ion-exchange resin (20–50 mesh), the resin removed by vacuum filtration, and the filtrate evaporated to a syrup *in vacuo* at 30°. The syrup was dissolved in methanol (100 mL) and the solution was evaporated to a syrup *in vacuo* at 30°. Addition and evaporation of methanol were repeated twice more (to remove methyl borate), giving a mixture of **4** and **5** as a white solid (16 g).

To effect deprotection, this solid (16 g) was dissolved in 25mM H_2SO_4 (300 mL), and the solution was heated under reflux for 0.5 h at 100°, cooled, the acid neutralized by batchwise addition of $BaCO_3$ with efficient stirring, and the $BaSO_4$ precipitate and excess of $BaCO_3$ were removed by vacuum filtration through glass-fiber filter-paper (Whatman GF/B). The resulting clear, slightly yellow filtrate was separately treated batchwise with an excess of Dowex 1 X-8 (HCO_3^-) resin (20–50 mesh) and of Dowex 50 X-8 (H^+) ion-exchange resin (20–50 mesh), the resins were removed by vacuum filtration, and the filtrate was evaporated *in vacuo* at 30° to a syrup containing **6** and **7** (57 mmol by hypoiodite oxidation[12]). A solution of the syrup in de-ionized H_2O (35 mL) was applied to a column (15 × 100 cm) of Dowex 50 X-8 ion-exchange resin (200–400 mesh) in the calcium form[13], and the column

was eluted with de-ionized water. Fractions (20-mL) were collected at the rate of 4 mL/min, and assayed for reducing sugar with phenol–sulfuric acid[14a]. D-(6-^{13}C)Glucose (**6**) was eluted first (fractions 1–45), followed by L-(6-^{13}C)idose (**7**) in fractions 120–160; compounds were identified by their characteristic C-6 chemical shifts[14b,c]. The fractions containing each labeled hexose were pooled, and evaporated to syrups at 30° *in vacuo*: D-(6-^{13}C)glucose (**6**; 8 g, 44 mmol); L-(6-^{13}C)-idose (**7**; 4 g, 22 mmol); total yield of hexoses (**6** + **7**) from **1**, 66%. D-(6-^{13}C)-Glucose (**6**) crystallized from methanol; a first crop of crystals (5.5 g, 30 mmol) was obtained. ^{1}H-Decoupled, ^{13}C-n.m.r. spectra of both (6-^{13}C)hexoses (enriched and unenriched regions) in $^{2}H_2O$ were recorded. These spectra were compared with those of authentic D-glucose[14c] and D-idose[14b] previously obtained. Based on these comparisons, the (6-^{13}C)hexoses produced were unambiguously identified, and their purity was estimated to be >95%.

Chromatographic separation of **2** *and* **3** *(Scheme 2).* — A mixture containing **2** and **3** (~5 mmol) was applied to a column (4.2 × 110 cm) of Dowex 50 X-8 (Ca^{2+}) ion-exchange resin[13] (200–400 mesh), and eluted with de-ionized water (1 mL/min; 15-mL fractions). Two well resolved peaks, detected with phenol–sulfuric acid[14a], were eluted: Peak 1 (fractions 82–92) contained the C-5 epimer displaying enriched-carbon signals at 105.3 and 100.6 p.p.m. and peak 2 (fractions 123–137) gave signals at 103.2, 97.6, and 91.5 p.p.m. Reduction, and deprotection of the dialdose that eluted first (peak 1) gave L-(6-^{13}C)idose, permitting assignment of peak 1 to compound **3**, and, therefore, of peak 2 to compound **2**.

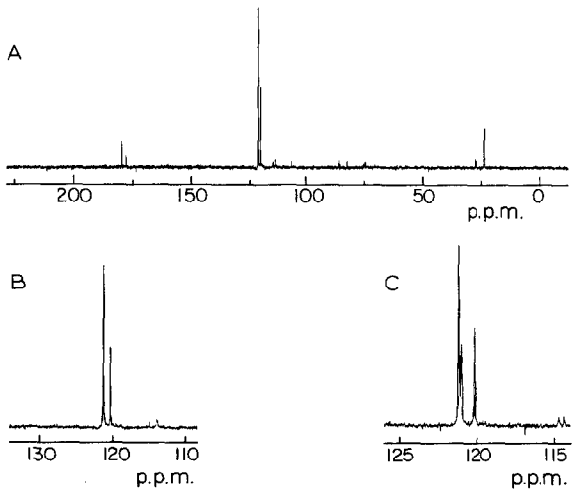


Fig. 1. (A) The ^{1}H-decoupled ^{13}C-n.m.r. spectrum (75 MHz) of the reaction mixture after addition of H^{13}CN to a purified preparation of **1**, showing the formation of C-5-epimeric (6-^{13}C)aldononitriles (signals at 121.2 and 120.2 p.p.m.; expanded region in spectrum B). The enriched signals at ~178 p.p.m. are probably due to cyanohydrin hydrolysis products (*e.g.*, amides and lactones). Spectrum C shows the same region as in B after addition of cyanide to a preparation of **1** containing formaldehyde as a contaminant. The additional signal at 121.0 p.p.m. is due to contaminating (1-^{13}C)glycolonitrile.

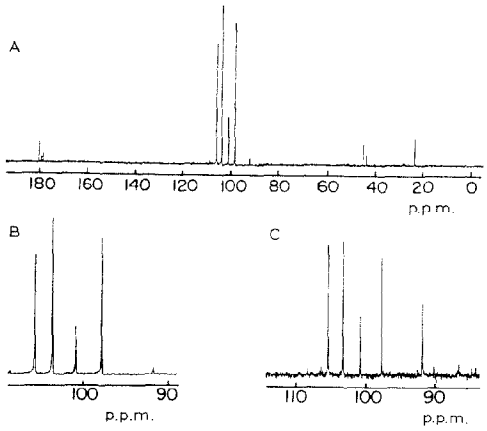


Fig. 2. (A) The ^{1}H-decoupled ^{13}C-n.m.r. spectrum (75 MHz) of the reaction mixture after Pd–$BaSO_4$ reduction of the C-5-epimeric (6-^{13}C)aldononitriles, showing the production of dialdoses **2** and **3**. Signals at 105.3 and 100.6 p.p.m. (expanded spectrum in B) are due to compound **3** (β- and α-furanose), and those at 103.2 and 97.6 p.p.m. are due to compound **2** (α- and β-furanose) (see text). Reaction by-products (amines generated by "over-reduction") give signals at ~43 p.p.m., and are produced in low yield under the given reaction conditions (see Experimental section). Spectrum C is the same region as in B, obtained after reduction of (6-^{13}C)aldononitriles containing (1-^{13}C)glycolonitrile (Fig. 1, spectrum C). The additional signal at 91.6 p.p.m. is due to contaminating (1-^{13}C)glycolaldehyde.

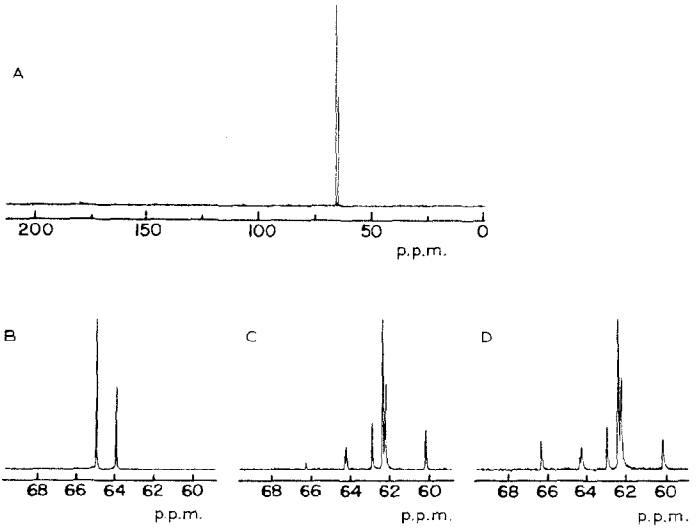


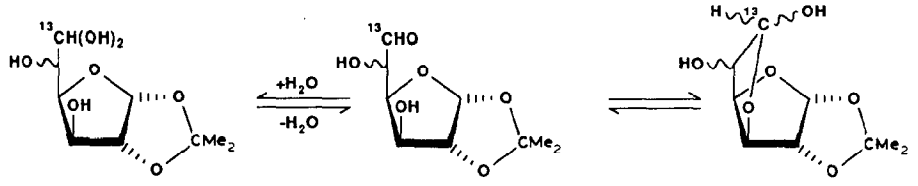
Fig. 3. (A) The ^{1}H-decoupled ^{13}C-n.m.r. spectrum (75 MHz) of compounds **4** and **5** obtained after $NaBH_4$ reduction (expanded region in spectrum B). Spectra C and D were respectively obtained after 0.5 h and 1 h of acid hydrolysis of **4** and **5**, showing the production of compound **6** (signals at 62.4 and 62.3 p.p.m.) and **7** signals at 64.4, 64.3, 63.0, and 60.2 p.p.m.). The signal at 66.4 p.p.m. increases in intensity as hydrolysis times are increased, and it probably arises from 1,6-anhydro-β-L-(6-^{13}C)idopyranose.

RESULTS AND DISCUSSION

The procedure for preparing D-(6-^{13}C)glucose (**6**) and L-(6-^{13}C)idose (**7**) described herein is shorter, and easier to perform, than the Schaffer–Isbell method[7b], and the yields of products are higher (see Figs. 1–3). In addition, the procedure does not require 6,3-lactonization, thereby permitting access to the (6-^{13}C)aldohexoses (*allo, talo*) derived from a furanose precursor having the *ribo* configuration (*e.g.*, methyl 2,3-*O*-isopropylidene-β-D-ribofuranoside).

The improved procedure provides access to such hexodialdose derivatives as **2** and **3**. The ^{13}C-n.m.r. spectra of these compounds in $^{2}H_2O$ (see Fig. 2) show that they exist in solution mainly as cyclic hemiacetals formed by reaction of O-3 with the carbonyl group containing C-6, forming a *cis*-fused, di-furanose ring system (see Scheme 3). The dialdoses **2** and **3** can be separated by chromatography (see Experimental section), which should facilitate their use in future syntheses (see later). From inspection, the labeled hemiacetal carbon atom of **2** is contained in a furanose ring having the *lyxo* configuration, whereas that of **3** is part of a furanose ring having the *xylo* configuration. It is interesting that the anomeric distributions observed for **2** and **3** are inconsistent with previous correlations[15,16] with furanose-ring configuration. The application of these rules (derived for simple furanoses having the *lyxo* and *xylo* configurations) would have resulted in the incorrect assignment of peak 1 to compound **2**; that is, the larger difference in anomeric proportions observed for that compound eluted in peak 1 is more consistent with a furanose ring having the *lyxo* configuration, leading to an erroneous assignment to compound **2**. Apparently, the behavior of *cis*-fused furanoses is significantly different from that of simple furanoses with respect to the effect of ring configuration on anomeric proportions.

Compounds **2** and **3** may prove to be useful intermediates in the synthesis of (6-^{13}C)hexuronic acids and L-(1-^{13}C)ascorbic acid. After purification by chromatography, compound **2** could be converted into biologically important D-(6-^{13}C)glucose by procedures described herein, or converted into D-(6-^{13}C)glucuronic acid by oxidation[17] with O_2–PtO_2 (Adams' catalyst) prior to deprotection. Compound **3** could also be oxidized, and the product deprotected, to give L-(6-^{13}C)iduronic acid, which may be useful in studies of glycosaminoglycan structures. Compound **3** may also be used in the synthesis of biologically important L-(1-^{13}C)ascorbic acid using



Scheme 3

a route described by Bakke and Theander[18]. Because the stereochemistry at C-5 is ultimately lost in this synthesis, the (by-product) *ido* epimer **3** may be used instead of **2** in this preparation. Alternatively, compounds **2** and **3** may serve as parent aldoses in cyanohydrin-reduction reactions, providing a route to (6-^{13}C)heptoses, or reduced with NaB^2H_4 to give mono-*C*-6-deuterated aldohexoses not readily accessible by other methods.

A prerequisite to the practical application of our improved method is a preparation of **1** that is free from contamination by formaldehyde (from $NaIO_4$ oxidation). This by-product must be removed from **1** before addition of cyanide is attempted, as otherwise some of the cyanide will be consumed by formaldehyde (as well as by **1**), giving **2, 3**, and (1-^{13}C)glycolaldehyde after palladium reduction (see Figs. 1 and 2). This is undesirable, as it results in lower yields and a more-complex purification problem. We found that repeated evaporation of methanol from syrupy **1** removes most of the formaldehyde, and facilitates the crystallization of pure **1**.

The aldononitriles produced from the reaction of cyanide with **1** were found to be readily hydrolyzed under mildly alkaline conditions (pH $\geq$8), giving amides (see Fig. 1). This rate of hydrolysis appears to be enhanced over that observed for nitriles derived from simple aldose precursors[19], presumably because ring-closure (involving O-3) to form imido-1,4-lactones[19] is more favored in the bicyclic system. The practical implications of this behavior are that the pH of the solution and the reaction time are critical to minimizing nitrile hydrolysis and maximizing yields. The conditions used herein were chosen to meet these criteria.

The conditions used to hydrolyze the protected alcohols **4** and **5** were found to affect the yield of (6-^{13}C)hexoses **6** and **7** significantly. For example, hydrolysis with 0.12M H_2SO_4 for 2 h produced a substantial proportion (>20%) of a by-product derived mainly from L-(6-^{13}C)idose; although the identity of this by-product was not established with certainty, it is most probably 1,6-anhydro-β-L-(6-^{13}C)idopyranose[20]. Use of dilute acid (25mM) and shorter reaction times (0.5 h) substantially lowers the formation of this by-product (see Fig. 3).

Although a detailed protocol for the use of methyl 2,3-*O*-isopropylidene-β-D-ribofuranoside instead of **1** as the parent aldose is not given herein, it was found that the cyanide condensation reaction cannot be performed in aqueous solution, because of the limited solubility of this compound in water. The problem was circumvented by using 1:4 (v/v) methanol–water for the addition of cyanide and the cyanohydrin reduction, with no apparent effect on the yields. The dialdose derivatives are soluble in water, and subsequent reactions ($NaBH_4$ reduction, deprotection) can be performed in this solvent.

Using the protocol described, (6-^{13}C)aldohexoses having the D-*gluco*, L-*ido*, D-*manno*, and L-*gulo* configurations can be prepared. The ^{13}C-n.m.r. spectrum of D-(6-^{13}C)glucose prepared in this way is shown in Fig. 4. The remaining four (6-^{13}C)hexoses (D-*allo*, L-*talo*, D-*altro*, and L-*galacto*) could be prepared by extension of the method to methyl 2,3-*O*-isopropylidene-β-D-ribofuranoside as the parent al-

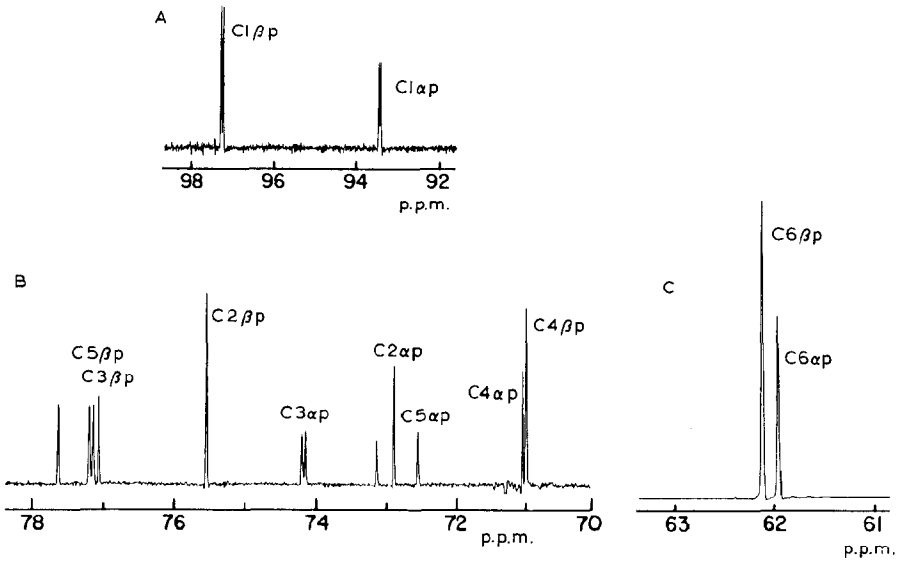


Fig. 4. The resolution-enhanced, ^{1}H-decoupled ^{13}C-n.m.r. spectrum (75 MHz) of D-(6-^{13}C)glucose (**6**) in $^{2}H_2O$, showing the C-1 atoms (spectrum A), the C-2–C-5 atoms (spectrum B), and the enriched C-6 atoms (spectrum C). Several ^{13}C–^{13}C couplings can be observed: α-pyranose, $^{1}J_{C\text{-}6,C\text{-}5}$ 43.3, $^{3}J_{C\text{-}6,C\text{-}1}$ 3.3, and $^{3}J_{C\text{-}6,C\text{-}3}$ 3.7 Hz; β-pyranose, $^{1}J_{C\text{-}6,C\text{-}5}$ 43.2, $^{3}J_{C\text{-}6,C\text{-}1}$ 4.1, and $^{3}J_{C\text{-}6,C\text{-}3}$ 4.2 Hz.

dose, followed by molybdate epimerization of the purified products. The general approach described herein should provide a complete set of (6-^{13}C)aldohexoses for chemical and biological studies. It should be noted that L-(6-^{13}C)galactose would be obtained from the D-ribofuranose precursor just mentioned. The more biologically useful D-(6-^{13}C)galactose could be prepared from the corresponding L-ribofuranose derivative.

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