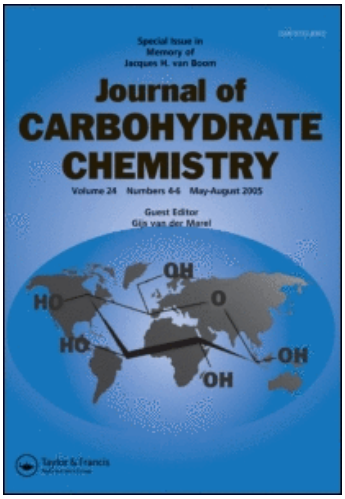

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REVIEW ARTICLE

STABLE ISOTOPICALLY-ENRICHED D-GLUCOSE: STRATEGIES TO INTRODUCE CARBON, HYDROGEN AND OXYGEN ISOTOPES AT VARIOUS SITES

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INTRODUCTION

For many years stable isotopes were confined to the chemical laboratory, where they were used primarily to study reaction rates,[1] reaction mechanisms,[2] and molecular conformation.[3,4] Recently, stable isotopically-enriched molecules have been playing increasing roles in biochemical studies, particularly as probes of *in vitro* biological metabolism[5,6] and of biomacromolecular structure.[7,8] These modern applications have been stimulated, in large part, by dramatic technical developments in nuclear magnetic resonance (NMR) spectroscopy[9] and mass spectrometry (MS).[10] Very-high field multinuclear high-resolution NMR in one-, two- and three-dimensions,[11,12] and various volume-selective forms of NMR derived from topical magnetic resonance (TMR) spectroscopy,[13] have become important analytical methods in the study of biological phenomena. TMR and related techniques such as ISIS (Image Selected In-Vivo Spectroscopy)[14] and Spatially Resolved Spectroscopy (SPARS)[15] are particularly attractive, as they permit high-resolution, volume-specific observations essential to non-invasive metabolic studies of intact organs and organisms. Magnetic resonance imaging (MRI)[16] is now a standard clinical tool for non-invasive diagnosis of disease, especially cancer, in humans. Clinical MRI is confined currently to the observation of water protons in cells, but will likely become multinuclear (e.g., ^{7}Li, ^{19}F, ^{23}Na, ^{31}P, ^{13}C) in the future.[17]

NMR structural characterization of biomacromolecules such as proteins and nucleic acids has been aided greatly by the incorporation of stable isotopes (^{2}H, ^{15}N, ^{13}C)[7,8] and the use of two- and three-dimensional NMR pulse sequences to edit complex spectra.[9] These stable isotope aided NMR methods[18] will influence profoundly the fields of chemistry and biology in the coming years, with perhaps the greatest impact on clinical medicine, where stable isotopes are expected to play an important role in the development of non-invasive and less traumatic methods of medical diagnosis. For these reasons, the development and refinement of methods to introduce stable isotopes such as ^{13}C, ^{2}H, ^{15}N and ^{17}O either selectively or uniformly into biomolecules remains an important and contemporary problem.

D-Glucose plays a central role in biological metabolism, being the preferred carbon source for most cells. D-[1-^{13}C]Glucose has been used in numerous NMR studies of biological metabolism,[19,20] probably because this labeled monosaccharide is available commercially at a reasonable cost. Considerably less work has been conducted with other singly-labeled glucoses such as D-[2-^{13}C]glucose and D-[6-^{13}C]glucose, mainly because these compounds have been prohibitively expensive and/or have not been commercially available. Multiply-labeled glucoses, likewise, are not common reagents in biological studies, despite their obvious advantages in studies of competing metabolic pathways and enzyme mechanisms. However, the fundamental aim of this review is to illustrate that current methods of glucose labeling with carbon, hydrogen and/or oxygen isotopes permit access to many isotopically-labeled forms of this hexose in good yield and in multi-gram quantities.

GENERAL STRATEGY

The labeling of carbohydrates with stable isotopes involves initial reactions to introduce the isotope, and subsequent reactions to modify the initial products to give the desired labeled end-products. The chemical reaction used often for label introduction is the cyanohydrin reduction reaction[21], a hybrid method based on the Kiliani (cyanohydrin) reaction[22a] and Kuhn reduction.[22b] This reaction is used to prepare aldoses labeled with stable isotopes of carbon, hydrogen and/or oxygen at C-1 (the anomeric carbon).

Reactions to modify aldoses labeled at C-1 are numerous and may be chemical or enzymic in nature. The most useful chemical transformation is the C2-epimerization reaction catalyzed by sodium molybdate, in which an aldose labeled at C-1 is converted into its C2-epimer labeled at C-2.[23] This reaction dramatically improves the synthesis of D-glucoses containing enrichments at non-terminal carbons. Due to their central role in D-glucose labeling, the cyanohydrin reduction and molybdate epimerization reactions are considered here in some detail; the subsequent chemical and enzymic transformations are treated more briefly.

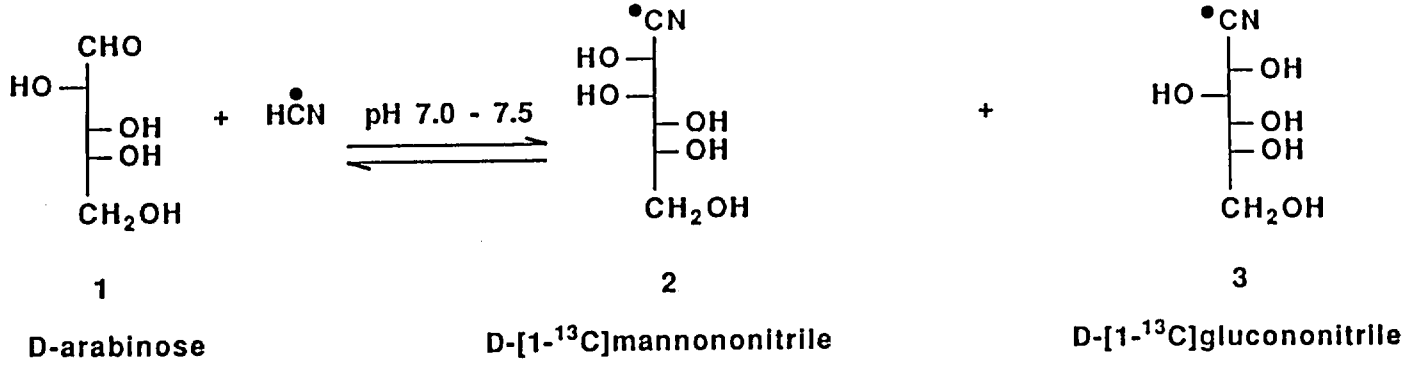
LABELING AT C-1: THE CYANOHYDRIN REDUCTION METHOD

Carbon labeling at C-1 of D-glucose is achieved by the cyanohydrin reduction method **(Scheme 1)**.[21,24-26] The parent aldose, D-arabinose **1**, is condensed with $K^{13}CN$ in aqueous solution at pH 7.0-7.5, generating a pair of C2-epimeric aldononitriles having the D-*manno* **2** and D-*gluco* **3** configurations. These nitriles, generated in a 7:3 *manno*:*gluco* ratio, are reduced without purification with H_2 and a $Pd/BaSO_4$ catalyst to give the corresponding C2-epimeric aldoses, D-[1-^{13}C]mannose **4** and D-[1-^{13}C]glucose **5**.

In order to get a > 95% conversion of D-arabinose **1** to the aldononitriles **2** and **3** a three-fold excess of cyanide is required.[26] In contrast, parent aldoses that cannot cyclize (e.g., glycolaldehyde, glyceraldehyde) or cyclize only to furanoses (e.g., D-aldotetroses) are converted almost quantitatively to nitriles with one equivalent of cyanide.[25] The solution pH of 7.0-7.5 allows rapid cyanide addition and retards aldononitrile hydrolysis to amides and acids.[27] The $Pd/BaSO_4$ catalyst favors the conversion of nitriles to aldehydes, whereas over-reduction to primary amines (1-amino-1-deoxyalditols) is favored with platinum catalysts.[26]

After cyanide condensation is complete, the solution pH is adjusted to 4.8, and the excess [^{13}C]cyanide is removed by aeration with N_2 and passage of the $H^{13}CN$-containing N_2 effluent through a KOH-ethanol trap.[24] Approximately 90% of the excess cyanide is recovered for use in new syntheses. Reduction of the nitriles is performed at pH 4.3 where aldehyde formation is favored; presumably the hydrolysis of the intermediate imine occurs more rapidly than reduction to the primary amine under these reaction conditions.[24] The reduction can be performed at atmospheric pressure or pressures up to 60 psi with essentially no change in yield (> 80% conversion of aldononitriles to aldoses). The entire process can be monitored conveniently by ^{13}C NMR spectroscopy **(FIG. 1)**.

After reduction is complete (~ 3 h), the catalyst is removed by filtration and the filtrate is treated batchwise with excess Dowex 50 x 8 (H^+) cation-exchange resin to remove by-product [1-^{13}C]-1-amino-1-deoxyalditols and inorganic cations. The D-[1-^{13}C]hexoses **4** and **5** are separated on a gravity-flow chromatographic



1

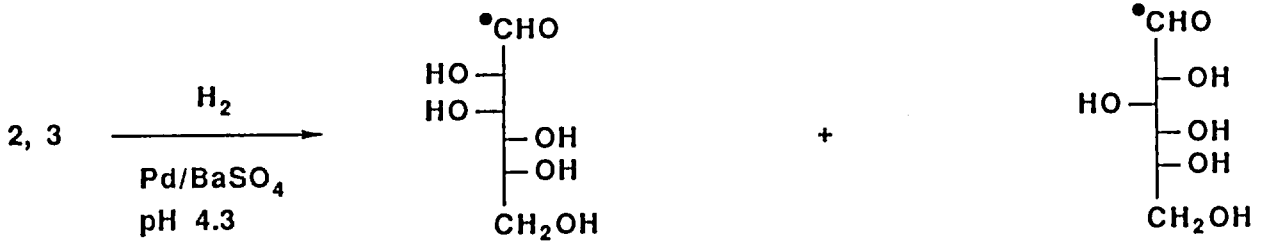
D-arabinose

2

D-[1-^{13}C]mannononitrile

3

D-[1-^{13}C]gluconononitrile



4

D-[1-^{13}C]mannose

5

D-[1-^{13}C]glucose

Scheme 1 :

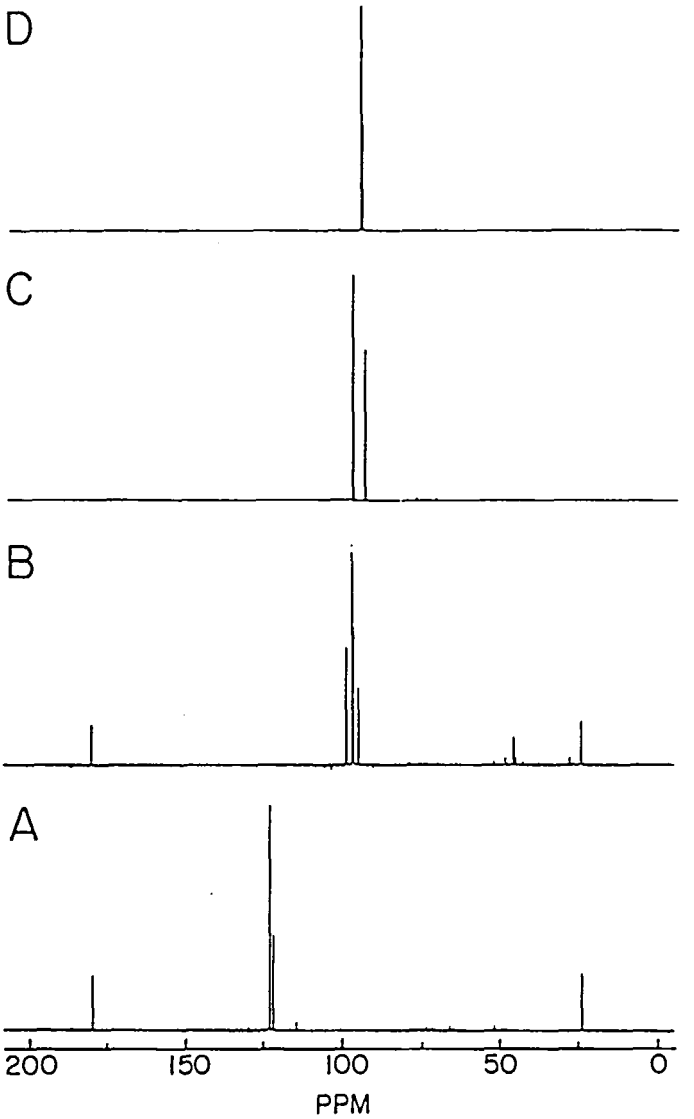


FIG. 1. Monitoring cyanohydrin reduction reactions by ^{13}C NMR. (A) The ^{1}H-decoupled ^{13}C NMR spectrum of a reaction mixture obtained after condensing D-arabinose **1** with $K^{13}CN$ under controlled solution conditions (see text). The signals at 120.8 and 121.9 ppm are the enriched C-1 carbons of D-[1-^{13}C]mannononitrile and D-[1-^{13}C]gluconononitrile, respectively. Signals at 22.4 and 178.6 ppm are due to acetic acid in the reaction mixture. (B) ^{13}C NMR spectrum of the reaction mixture in (A) after catalytic reduction with $Pd/BaSO_4$. The four new signals centered at 95 ppm are the enriched C-1 carbons of both pyranoses of D-[1-^{13}C]glucose **5** and D-[1-^{13}C]mannose **4**. The small signals at about 43 ppm arise from the enriched C-1 carbons of by-product [1-^{13}C]-1-amino-1-deoxyalditols. (C) and (D) ^{13}C NMR spectra of D-[1-^{13}C]glucose **5** (C) and D-[1-^{13}C]mannose **4** (D) obtained after purification by chromatography on Dowex 50 x 8 (200-400 mesh) (Ca^{+2}).

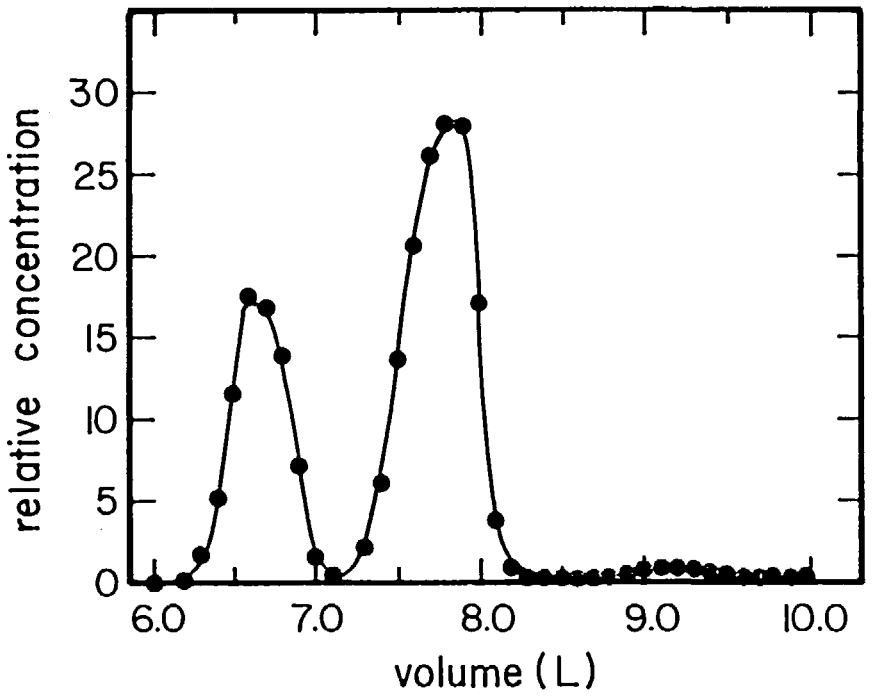


FIG. 2. Chromatographic separation of a cyanohydrin reduction mixture containing D-[1-^{13}C]mannose **4**, D-[1-^{13}C]glucose **5** and D-arabinose **1**. Approximately 20 g of syrup was loaded on a 15 cm x 100 cm column containing Dowex 50 x 8 (200-400 mesh) resin in the calcium form. The column was eluted with distilled water, and 10 mL fractions were collected at a flow rate of 1 mL/min. Fractions were assayed for reducing sugar with phenol-sulfuric acid.[29] Peak 1, **5**; Peak 2, **4**; Peak 3, unreacted **1**.

column containing Dowex 50 x 8 (200-400 mesh) resin in the calcium form[28] using deionized water as the eluent **(FIG. 2)**. Fractions containing **4** and **5** are pooled and concentrated, and α-D-[1-^{13}C]glucopyranose and α-D-[1-^{13}C]mannopyranose are obtained by crystallization from anhydrous methanol.

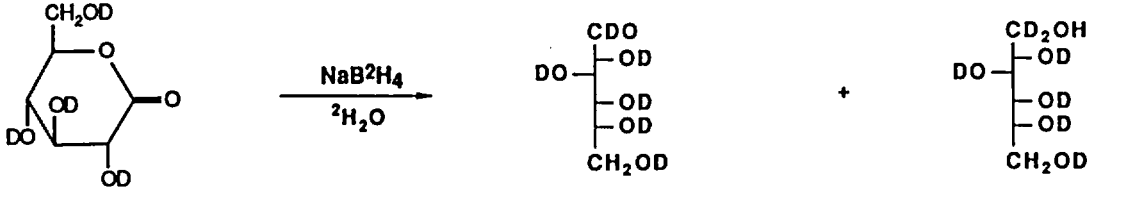
The above method to prepare **5** is easy to implement, has excellent reliability, and gives high yields with respect to incorporation of the ^{13}C isotope. Large-scale reactions (> 100 g of hexose) can be performed without complications, and the labeled precursor (K^{13}CN) is available at a reasonable cost commercially. The major shortcoming of the method is the generation of the by-

product, D-[1-^{13}C]mannose **4**. However, **4** can be converted to **5** by phenylboronic acid-alkali treatment, or to D-[2-^{13}C]glucose **6** by molybdate-catalyzed epimerization (see below).

Treatment of **4** with mild aqueous base (KOH) under anaerobic conditions generates an equilibrium mixture composed predominantly of **4**, **5** and D-[1-^{13}C]fructose **7**[30], and a small amount of these hexoses containing [^{13}C]-enrichment at C-6. The [6-^{13}C]-labeled by-products apparently arise from a symmetric 3,4-enediol intermediate generated by sequential enolization-ketolization down the carbon chain of the hexoses.[30] Inclusion of phenylboronic acid[31] in the reaction mixture eliminates this label scrambling,[32] thus making base isomerization an acceptable means to convert **4** into **5**. The mixture of **4**, **5** and **7** may be fractionated by chromatography on Dowex 50 x 8 (200-400 mesh) (Ca^{2+}), with **5** eluting first, followed in order by **4** and **7**. The recovered **4** and **7** can be recycled; **7** can also be converted to **5** enzymically (see below).

D-[1-^{2}H]Glucose **9** is prepared via cyanohydrin reduction by substituting $^{2}H_2$ for H_2 gas and conducting all reactions in $^{2}H_2O$ instead of H_2O.[33,34a] Failure to use $^{2}H_2O$ as the solvent results in decreased atom-% ^{2}H incorporation in the products due to ^{2}H-^{1}H exchange with water protons.[34a] Alternatively, **9** can be prepared from D-glucono-1,5-lactone **8** by reduction of the latter in $^{2}H_2O$ or H_2O with sodium borodeuteride at pH 4.5[34b] **(Scheme 2)** or with sodium amalgam.[35] In the former, D-[1-^{2}H]glucose **9** is the sole product; no over-reduction to the alditol, D-[1,1'-$^{2}H_2$]glucitol **10**, is observed. The borodeuteride method is the preferred route to prepare **9**.

Glucose enriched with oxygen isotopes (^{17}O, ^{18}O) at C-1 is prepared by H^+-catalyzed exchange of O-1 in [O]-labeled water[34a,36] **(Scheme 3)**. The exchange reaction proceeds via the *gem*-diol forms **11** and **12** **(Scheme 3)**. Consequently, aldoses whose aqueous solutions contain an appreciable amount of hydrate form at equilibrium (e.g., aldotetroses[37]) undergo O-1 exchange more readily than aldoses whose aqueous solutions contain minor amounts (e.g., aldohexoses). The rate and extent of exchange at O-1 can be monitored by ^{13}C NMR spectroscopy by observing of the oxygen isotope effect on the chemical shift of C-1.[38] Acid catalyses anomerization



8

D-glucono-1,5-lactone

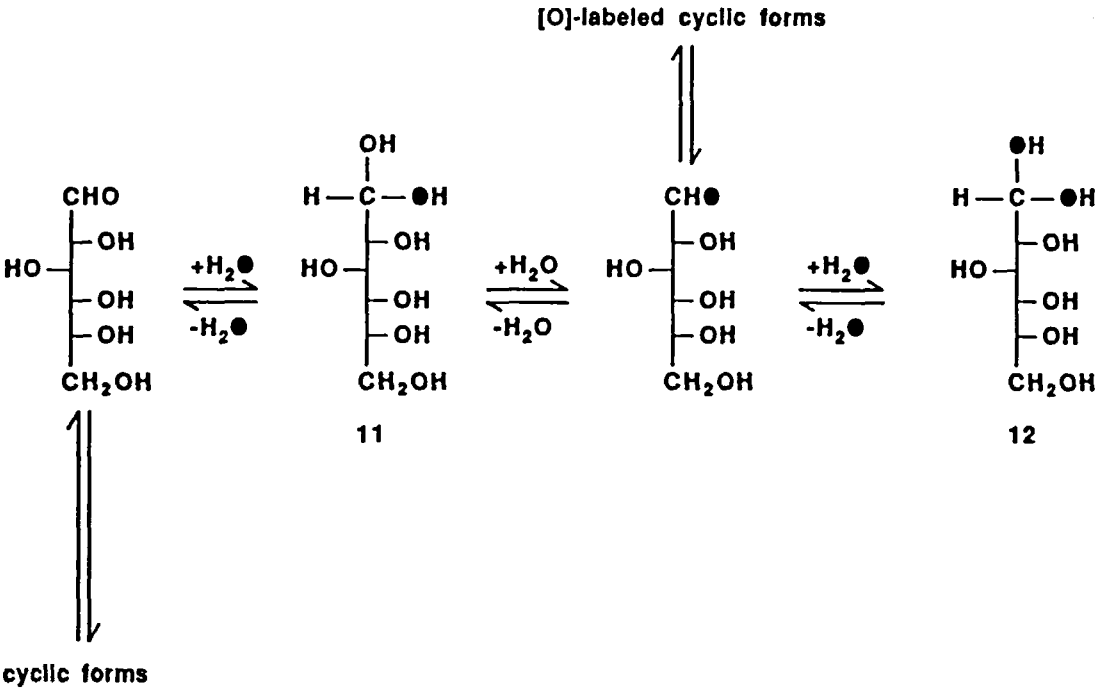
9

D-[1-^{2}H]glucose

10

D-[1,1'-$^{2}H_2$]glucitol

Scheme 2 :



Scheme 3 :

and carbonyl hydration/dehydration reactions, and thus accelerates oxygen exchange at O-1.

LABELING AT C-2: MOLYBDATE-CATALYZED EPIMERIZATION OF ALDOSES

D-[2-^{13}C]Glucose, D-[2-^{2}H]glucose and D-[2-^{17,18}O]glucose are prepared from D-[1-^{13}C]mannose, D-[1-^{2}H]mannose and D-[1-^{17,18}O]mannose, respectively, by molybdate-catalyzed aldose epimerization.[23,39] For example, treatment of D-[1-^{13}C]mannose (0.1 M in H_2O) with sodium molybdate (Na_2MoO_4, 5 mM) at pH 4.5 for 3 h at 95 °C gives a mixture of D-[1-^{13}C]mannose (35%) and D-[2-^{13}C]glucose (65%) **(FIG. 3)**, which are separated by chromatography on Dowex 50 x 8 (200-400 mesh) (Ca^{2+}). This interesting reaction inverts the C1-C2 fragment and transposes any labels at C-1 to C-2 of the corresponding C-2-epimer **(Scheme 4)**. Thus, D-[1-^{13}C,^{2}H]mannose

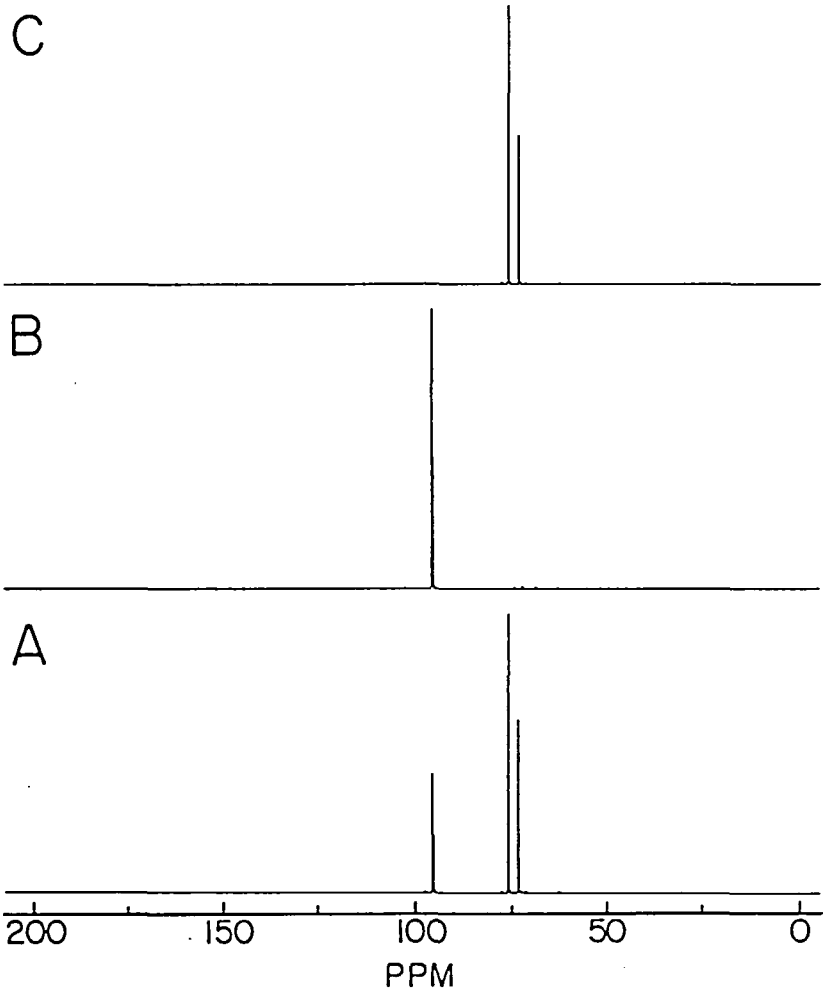
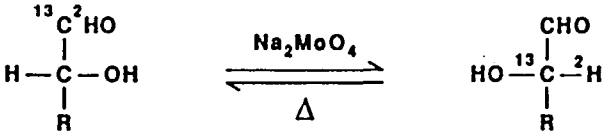


FIG. 3. The molybdate-catalyzed epimerization reaction. (A) The ^{1}H-decoupled ^{13}C NMR spectrum of a reaction mixture after treatment of D-[1-^{13}C]mannose **4** with Na_2MoO_4 for 3 h at 95°. Signals at 95.5 and 95.1 ppm are the enriched C-1 carbons of the α- and ß-pyranoses of **4**. Signals at 73.0 and 75.6 ppm are the C-2 carbons of the α- and ß-pyranoses of D-[2-^{13}C]glucose. (B) and (C) ^{13}C NMR spectra of the products generated in (A) after chromatography on Dowex 50 x 8 (200-400 mesh) (Ca^{+2}). Starting material **4** (spectrum B) and product D-[2-^{13}C]glucose (spectrum C) are obtained in pure form.



Scheme 4 :

can be easily converted to D-[2-^{13}C,^{2}H]glucose by this procedure. Epimerization reactions with D-[1-^{17,18}O]mannose are conducted in [O]-labeled water to avoid isotopic dilution through solvent exchange.[36] The D-[1,2-$^{17,18}O_2$]glucose generated during the reaction is converted to D-[2-^{17,18}O]glucose by final exchange with unlabeled water. A mechanism has been proposed to explain this transposition in which dimolybdate binds the acyclic aldehyde form of the aldose, forming a productive complex that confers stereospecificity to the reaction.[23]

Although D-[2-^{13}C]glucose is by far the primary product generated from the treatment of **4** with molybdate, a small amount of D-[1-^{13}C]glucose (< 1%) is also generated and presumably forms through the classical 1,2-enediol mechanism[40] involving fructose as an intermediate. Pentoses seem to be especially susceptible to these competing classical isomerization reactions when treated with sodium molybdate.[23] These side reactions can be minimized, however, by conducting the reaction in non-aqueous solvent (DMF) with dioxobis (2,4-pentanedionato-O,O')molybdenum (VI) ($MoO_2(acac)_2$) as the catalyst,[23] or by using anion-exchange resin in the molybdate form.[39]

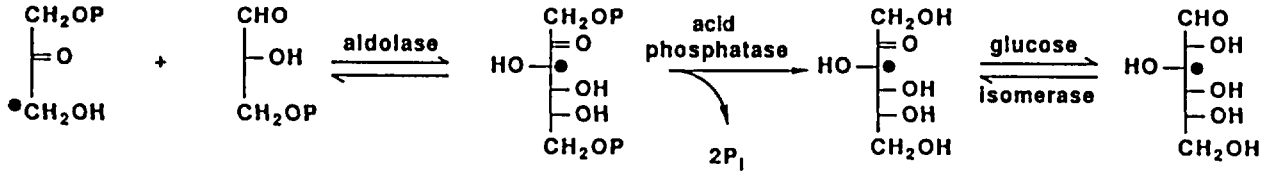
Recent studies[41-42] have revealed that Ni(II) catalyzes aldose epimerization with C1-C2 transposition analogous to that observed with sodium molybdate. Nickel(II), when complexed with diamines such as N,N,N',N'-tetramethylethylenediamine, serves as an efficient catalyst in methanol, with apparent equilibration reached in several minutes at 60 °C. Unlike the molybdate-catalyzed reaction, rearrange-ment is also observed at room temperature with Ni(II)-containing reagents.

D-[2-^{2}H]Glucose can be prepared from D-[1-^{2}H]mannose by molybdate-catalyzed epimerization, or by enzyme-mediated ^{1}H-^{2}H solvent exchange. In the latter method, D-fructose 6-phosphate is treated with phosphoglucoisomerase (E.C. 5.3.1.9) in $^{2}H_2O$ to give a mixture of D-[2-^{2}H]glucose 6-phosphate and D-[1*R*-^{2}H]fructose 6-phosphate.[43] The sugar phosphates are dephosphorylated with acid phosphatase (E.C. 3.1.3.2), and the deuterated glucose and fructose are separated by chromatography on Dowex 50 x 8 (200-400 mesh) (Ca^{+2}). In general, however, enzyme-mediated ^{1}H-^{2}H exchange reactions yield partial isotopic enrichments and/or low yields of the product. Interestingly, treatment of D-glucose with D-xylose (D-glucose) isomerase (E.C. 5.3.1.5) in $^{2}H_2O$ fails to produce D-[2-^{2}H]glucose[44], reflecting differences in these enzymes with respect to solvent exchange in the active site.

LABELING AT C-3

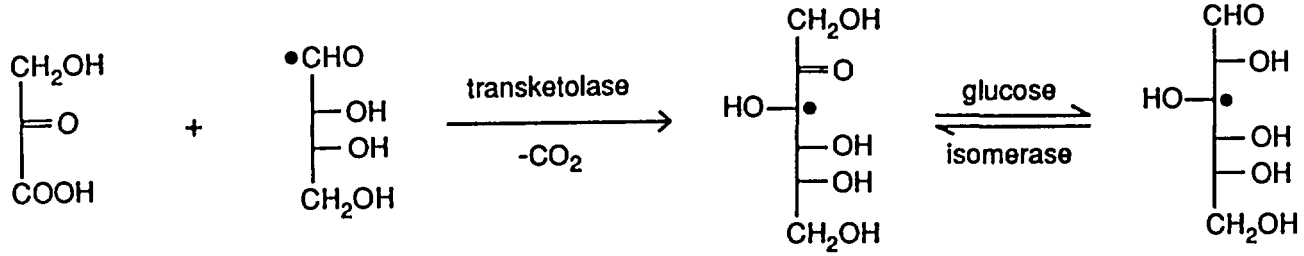
Three strategies can be applied to prepare D-[3-^{13}C]glucose **19**. In the first **(Scheme 5)** described by Goux[45a], [3-^{13}C]1,3-dihydroxy-2-propanone phosphate **15** ([3-^{13}C]dihydroxyacetone phosphate, DHAP) is condensed with D-glyceraldehyde 3-phosphate (G3P) **16** (or D-glyceraldehyde) in the presence of aldolase (E.C. 4.1.2.13), generating D-[3-^{13}C]fructose 1,6-bisphosphate (FBP) **17**. FBP is dephosphorylated with acid phosphatase, and the resulting D-[3-^{13}C]fructose **18** is converted to D-[3-^{13}C]glucose **19** enzymically with D-xylose (D-glucose) isomerase. The key enzyme in the preparation, enolpyruvate phosphate synthetase, is not available commercially, which may discourage the use of this procedure. This enzyme is required to convert [3-^{13}C]pyruvate, which can be obtained commercially at moderate cost, to [3-^{13}C]phosphoenolpyruvate (PEP); the latter is converted with the assistance of several glycolytic enzymes into [3-^{13}C]DHAP.

In another enzymic route, hydroxypyruvate and D-[1-^{13}C]erythrose are condensed with transketolase (E.C. 2.2.1.1), generating CO_2 and **18** which is converted to **19** with glucose isomerase **(Scheme 6)**.[45b] The advantages of this method are that phosphorylated intermediates are not required and that the enzyme (transketolase) is commercially available.



15	16	17	18	19
[3-^{13}C]DHAP	G3P	D-[3-^{13}C]FBP	D-[3-^{13}C]fructose	D-[3-^{13}C]glucose

Scheme 5 :



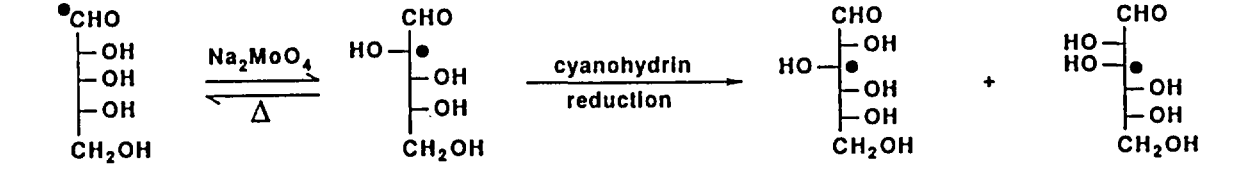
Scheme 6 :

An alternate chemical route (**Scheme 7**) involves the synthesis of D-[1-^{13}C]ribose **20** by cyanohydrin reduction, epimerization of **20** to D-[2-^{13}C]arabinose **21** by molybdate-catalyzed epimerization, and conversion of **21** to D-[3-^{13}C]glucose **19** (and D-[3-^{13}C]mannose **22**) by cyanohydrin reduction.[46] Compound **22** can be converted to **19** by molybdate-catalyzed epimerization to improve the overall yield of **19**. This chemical route is simple, and the reagents are relatively inexpensive and commercially available. The route shown in **Scheme 7** can also be used to prepare D-[3-^{2}H]glucose and D-[3-^{18}O]glucose from D-[1-^{2}H]ribose and D-[1-^{18}O]ribose, respectively; an alternate approach[47] to these labeled glucoses involves the reduction of 1,2;5,6-di-*O*-isopropylidene-α-D-*ribo*-hexafuranos-3-ulose, but yields of the desired *gluco* epimer are low (~ 10%; the *allo* product is favored).

LABELING AT C-4 AND C-5

D-Glucose labeled at C-4 and/or C-5 is prepared by the chemical and enzymic reactions[48] illustrated in **Scheme 8**. DL-Glyceraldehyde **23** is labeled at C-1 and/or C-2 by cyanohydrin reduction[21,25] and condensed with 1,3-dihydroxy-2-propanone phosphate (dihydroxyacetone phosphate, DHAP) (prepared in millimole quantities enzymically from dihydroxyacetone[48-49]) in an enzyme-catalyzed reaction with rabbit muscle aldolase.[50] Label scrambling due to triosephosphate isomerase (TPI) (E.C. 5.3.1.1), a common contaminant of commercial aldolase preparations, is avoided since DL-glyceraldehyde is not a substrate for TPI. The products, D-[4-^{13}C]fructose 1-phosphate **24** and L-[4-^{13}C]sorbose 1-phosphate **25**, are dephosphorylated with acid phosphatase, and the labeled fructose and sorbose are separated by chromatography on Dowex 50 x 8 (200-400 mesh) (Ca^{2+}). D-[4-^{13}C]Fructose **26** is converted to D-[4-^{13}C]glucose **27** by treatment with D-xylose (D-glucose) isomerase.

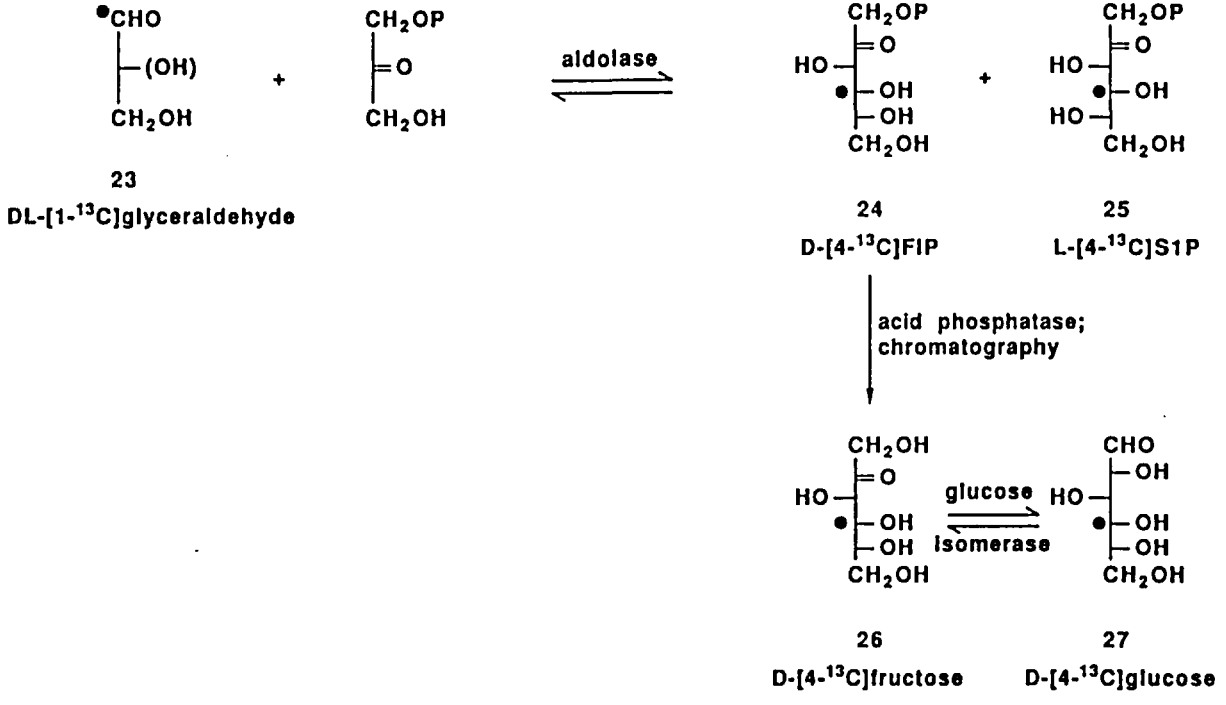
D-[4-^{13}C]Glucose (**Scheme 8**)[48] and D-[5-^{13}C]glucose are prepared using DL-[1-^{13}C]glyceraldehyde and DL-[2-^{13}C]glyceraldehyde, respectively, in the above protocol. Likewise, D-[4-^{2}H]glucose and D-[5-^{2}H]glucose are prepared from DL-[1-^{2}H]glyceraldehyde and DL-[2-



20 **21** **19** **22**

D-[1-^{13}C]ribose **D-[2-^{13}C]arabinose** **D-[3-^{13}C]glucose** **D-[3-^{13}C]mannose**

Scheme 7 :



23
DL-[1-^{13}C]glyceraldehyde

24
D-[4-^{13}C]FIP

25
L-[4-^{13}C]S1P

26
D-[4-^{13}C]fructose

27
D-[4-^{13}C]glucose

Scheme 8 :

^{2}H]glyceraldehyde, respectively. The method can also be modified to prepare D-[4-^{18}O]glucose and D-[5-^{18}O]glucose.

The generation of labeled L-sorbose reduces the yield of product by 50%. This problem can be alleviated if labeled D-glyceraldehyde (instead of the racemic mixture) is used in the synthesis. However, at the present time, a synthesis of D-glyceraldehyde that is suitable for isotopic labeling at C1 and/or C2 is not available.

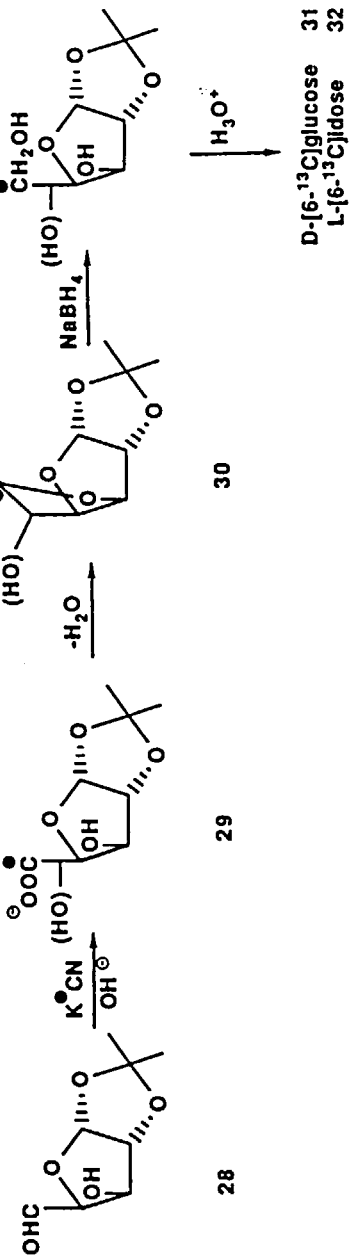
Whitesides and coworkers[49] have reported a preparative synthesis (10-500 mmol) of dihydroxyacetone phosphate (DHAP) involving phosphorylation of dihydroxyacetone by ATP, catalyzed by the enzyme, glycerol kinase (E.C. 2.7.1.30). Wong and coworkers[51] have suggested that the need for DHAP may be obviated by performing the aldolase reaction with dihydroxyacetone and the aldotriose phosphate in the presence of inorganic arsenate. In solution, dihydroxyacetone reacts reversibly with arsenate to form dihydroxyacetone arsenate, which mimics DHAP and is accepted as a substrate by aldolase. This modification eliminates the need for DHAP and enzyme-catalyzed dephosphorylation in the above synthesis.

D-[4-^{13}C]Glucose is alternatively prepared via the transketolase route **(Scheme 6)**[45b] starting from D-[2-^{13}C]erythrose. In this method the generation of L-[4-^{13}C]sorbose is avoided and the need of phosphorylated compounds is eliminated.

LABELING AT C-6

The classical approach[52,53] to D-[6-^{13}C]glucose **31** is shown in **Scheme 9**. Labeled cyanide is condensed with 1,2-*O*-isopropylidene-α-D-*xylo*-pentodialdo-1,4-furanose **28**, generating a mixture of [6-^{13}C]cyanohydrins which are hydrolyzed *in situ* to the corresponding [6-^{13}C]aldonates **29**. After lactonization, the [6-^{13}C]lactones **30** are reduced with $NaBH_4$ and deprotected to give the corresponding aldoses (D-[6-^{13}C]glucose **31**, L-[6-^{13}C]idose **32**). Compounds **31** and **32** are separated by chromatography on Dowex 50 x 8 (200-400 mesh) (Ca^{2+}), with **31** eluting first.

Recently,[54] an improved synthesis has been described (Scheme 10) that is based on the cyanohydrin reduction method described



Scheme 9:

above. Compound **28** is condensed with $K^{13}CN$, and the initially-formed [6-^{13}C]cyanohydrins **33** are reduced directly to the [6-^{13}C]dialdo derivatives **34**. Compounds **34** are treated with $NaBH_4$, and after deprotection, the products **31** and **32** are separated by chromatography on Dowex 50 x 8 (200-400 mesh) (Ca^{2+}). In comparison to **Scheme 8**, this modified protocol is faster to perform, lactonization is not required, and the yield of **31** is higher. Also, intermediates **34** can be used to prepare other labeled glucoses. For example, reduction of **34** with NaB^2H_4 should give D-[6-^{2}H]glucose (D-[6,6'-$^2H_2$]glucose is prepared by reducing 1,2-*O*-isopropylidene-α-D-glucurono-3,6-lactone with $LiAl^2H_4$, followed by deprotection.[55a] Stereospecific chemical syntheses of D-[6*R*-^{2}H]glucose and D-[6*S*-^{2}H]glucose have also been reported.[55b] Solvent exchange of **28** or **34** in [O]-labeled water prior to cyanide addition and reduction should lead to D-[5-^{17,18}O]glucose and D-[6-^{17,18}O]glucose, respectively. The by-product dialdo intermediate **34** having the L-*ido* configuration may also be used in the synthesis of L-[6-^{13}C]iduronic acid or L-[1-^{13}C]ascorbic acid.[54]

MULTIPLY-LABELED GLUCOSE

The methods described above for the preparation of singly-labeled glucoses can be extended to prepare multiply-labeled derivatives. In general, D-glucose containing multiple sites of [^{13}C]-, [^{2}H]- and/or [^{17,18}O]-enrichment can be obtained by an appropriate combination of the cyanohydrin reduction and molybdate epimerization reactions. For the purpose of illustration, only glucose labeled with ^{13}C at multiple sites is considered in the following discussion. The discussion would anyway remain very limited because in addition to the 19 singly-labeled (^{2}H, ^{13}C, ^{17,18}O) D-glucoses, more than half a million (524,268) multiply-labeled (^{2}H, ^{13}C, ^{17,18}O) isotopomers of D-glucose are possible. Uniformly [^{13}C]- or [^{2}H]-labeled glucose are not considered here; these compounds are normally prepared biosynthetically from $^{13}CO_2$ or ^2H_2O.[56,57]

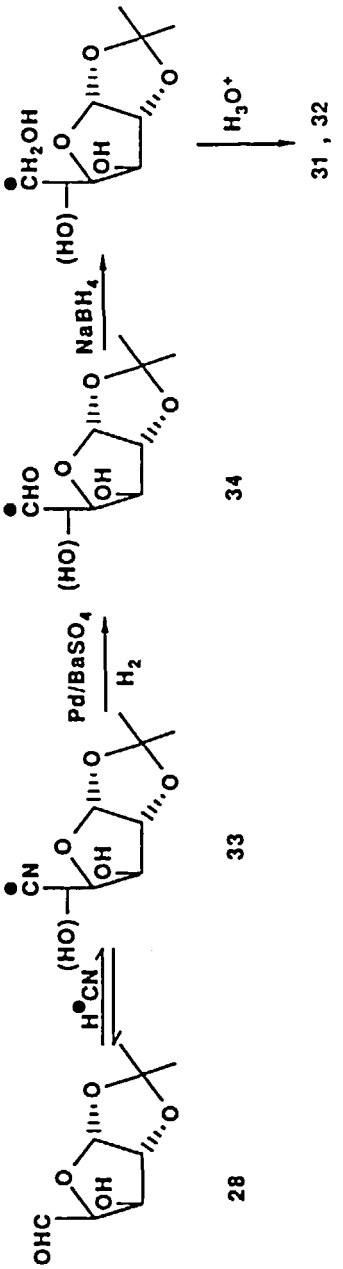
D-[1,2-$^{13}C_2$]glucose is prepared by two consecutive cyanohydrin reduction reactions starting with D-erythrose, and the molybdate-

catalyzed epimerization reaction can be applied to the by-product D-[1,2-$^{13}C_2$]mannose to increase the yield of D-[1,2-$^{13}C_2$]glucose.[46] D-[4,5-$^{13}C_2$]Glucose can be prepared from DL-[1,2-$^{13}C_2$]glyceraldehyde as outlined in **Scheme 8**; DL-[1,2-$^{13}C_2$]glyceraldehyde is prepared by two consecutive cyanohydrin reduction reactions with K^{13}CN starting from formaldehyde.[21,25] D-[1,3-$^{13}C_2$]Glucose is prepared by treating D-[1-^{13}C]ribose with molybdate to generate D-[2-^{13}C]arabinose, followed by cyanohydrin reduction with K^{13}CN. The by-product D-[1,3-$^{13}C_2$]mannose can be treated with molybdate to give D-[2,3-$^{13}C_2$]glucose.

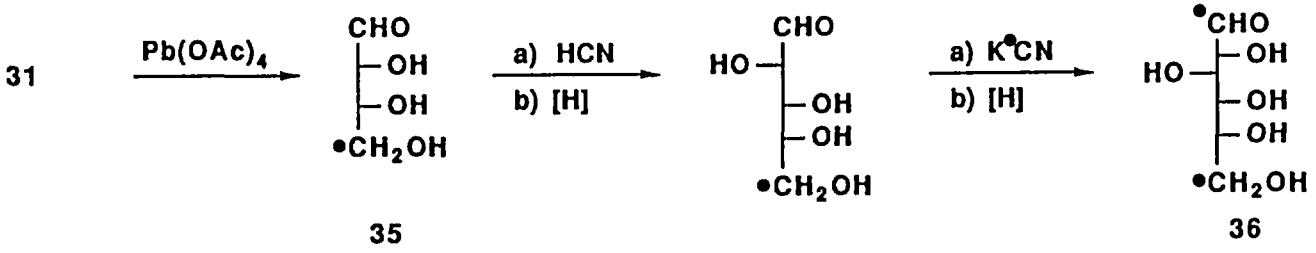
D-[1,6-$^{13}C_2$]Glucose is synthesized from D-[1-^{13}C]fructose 1,6-bisphosphate (FBP)[48,58] by simultaneous treatment with aldolase and triosephosphate isomerase, followed by enzyme-catalyzed dephosphorylation and isomerization to give D-[1,6-$^{13}C_2$]glucose. FBP labeled with 99 atom-% ^{13}C at C-1 will yield product FBP that is an isotopomeric mixture of four forms containing 49.5% atom-% ^{13}C at C-1 and C-6. For some applications, this label dilution, and the presence of isotopomers, can be tolerated. D-[2,5-$^{13}C_2$]Glucose can be prepared from D-[2-^{13}C]FBP in a similar fashion. D-[1-^{13}C]FBP and D-[2-^{13}C]FBP are prepared enzymically from D-[1-^{13}C]glucose and D-[2-^{13}C]glucose, respectively.[48]

If D-[1,6-$^{13}C_2$]glucose **36** containing 99 atom-% ^{13}C at both sites is required, D-[1-^{13}C]glucose **5** is converted to its 1,2:5,6-di-*O*-isopropylidene derivative,[59a] the exocyclic isopropylidene group is selectively hydrolyzed,[59b] and the substitution of C-6 is essentially carried out as described in **Scheme 10**. The same procedure is applied for the synthesis of D-[2,6-$^{13}C_2$]glucose and D-[3,6-$^{13}C_2$]glucose starting from D-[2-^{13}C]glucose and D-[3-^{13}C]glucose, respectively.

In an alternate synthesis of D-[1,6-$^{13}C_2$]glucose **36** **(Scheme 11)** containing 99 atom-% ^{13}C at both sites D-[6-^{13}C]glucose **31** is treated with lead tetraacetate to generate D-[4-^{13}C]erythrose **35**.[46] Compound **35** is converted to **36** by two consecutive cyanohydrin reduction reactions (the second with K^{13}CN). Compound **35** may also be used to prepare D-[2,6-$^{13}C_2$]glucose and D-[3,6-$^{13}C_2$]glucose by appropriate use of cyanohydrin reduction and molybdate epimerization reactions. D-[2,5-$^{13}C_2$]Glucose, D-[3,5-$^{13}C_2$]glucose and D-[1,5-



Scheme 10:



Scheme 11:

$^{13}C_2$]glucose labeled with 99 atom-% ^{13}C at both sites could be prepared from D-[5-^{13}C]glucose using the same strategy.

D-[4,6-$^{13}C_2$]Glucose can be prepared enzymically from DHAP and DL-[1,3-$^{13}C_2$]glyceraldehyde. The latter can be made from [^{13}C]formaldehyde (obtained commercially) by two consecutive cyanohydrin reduction reactions (the second with K^{13}CN). D-[5,6-$^{13}C_2$]Glucose is prepared similarly from DL-[2,3-$^{13}C_2$]glyceraldehyde. Both D-[4,6-$^{13}C_2$]glucose and D-[5,6-$^{13}C_2$]glucose can be treated with $Pb(OAc)_4$ to give D-[2,4-$^{13}C_2$]erythrose **37** and D-[3,4-$^{13}C_2$]erythrose **38**, respectively. Compounds **37** and **38** may be used to generate a wide variety of D-glucoses labeled with ^{13}C at three or more sites.

SUMMARY

This discussion has illustrated that reliable chemical and enzymic methods are currently available to prepare D-glucose labeled with stable isotopes at essentially any site in the molecule. Despite this fact, use of these compounds remains inhibited by their high cost and limited commercial availability. Cost is not likely to decline significantly until the economics of isotope enrichment, especially ^{13}C, improves, since a significant fraction of the total cost of synthesizing D-[1-^{13}C]glucose commercially derives from the cost of the [^{13}C]-labeled starting material, commonly K^{13}CN. However, new methods of ^{13}C enrichment that promise to be more cost effective are presently under development.[60] Additional cost reduction may be expected through further improvements in the chemical and enzymic processes for synthesis, and the implementation of automated, larger-scale manufacturing methods.[61] Despite these current limitations, however, the future promises increased applications of stable isotopes, especially in the fields of biochemistry and medicine.

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