

The Synthesis of High-Specific-Activity UDP-[6-³H]Galactose, UDP-N-[6-³H]Acetylgalactosamine, and Their Corresponding Monosaccharides

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Tritiated uridine-5'-diphosphogalactose (UDP-[³H]Gal) has been widely used to study oligosaccharide biosynthesis and structure. It can be synthesized either chemically or enzymatically using galactose oxidase to oxidize the hydroxyl moiety at C-6 to an aldehyde (6-*aldo*-UDP-Gal), which is then reduced back to the alcohol with tritiated sodium borohydride. Although the enzymatic approach is simple and efficient, there are several problems associated with it. First, incomplete oxidation to the aldehyde reduces the final specific activity. Second, if the galactose oxidase is not removed from the 6-*aldo*-UDP-Gal prior to reduction, the resulting UDP-[6-³H]Gal can be reoxidized to 6-*aldo*-UDP-[6-³H]Gal. We present evidence for the occurrence of this compound in one commercially obtained preparation of UDP-[6-³H]Gal. Finally, if an excess of 6-*aldo*-UDP-Gal is used for good yield, it is necessary to quench the reduction with nonradioactive borohydride, again reducing the final specific activity. We have devised a rapid, inexpensive, and efficient synthesis of UDP-[6-³H]Gal that circumvents all of these problems. Galactose oxidase is used to produce 6-*aldo*-UDP-Gal and the completeness of this reaction is confirmed on polyethyleneimine (PEI) cellulose TLC plates. The 6-*aldo*-UDP-Gal is purified on silica gel 60 TLC plates. This purified compound is then reduced with tritiated sodium borohydride, with the aldehyde present in excess. Unreacted 6-*aldo*-UDP-Gal is then purified away from the product UDP-[6-³H]Gal by chromatography on PEI cellulose. Radiochemically pure UDP-[6-³H]Gal with a specific activity of 10 Ci/mmol was obtained using the above scheme. This same strategy has been extended to the synthesis of UDP-N-[6-³H]acetylgalactosamine. The tritiated monosaccharides of these sugar nucleotides are also easily obtained by mild acid hydrolysis

and passage through cation- and anion-exchange chromatography columns. © 1992 Academic Press, Inc.

Radiolabeled sugar nucleotides and monosaccharides have played important roles in studying oligosaccharide biosynthesis and structure. The sugar nucleotide UDP-galactose is the galactose donor used by a variety of galactosyltransferases. In the most common transfer reaction galactose is added to terminal GlcNAc² residues by UDP-galactose:D-glucose 4- β -galactosyltransferase (galactosyltransferase: EC 2.4.1.22). Radiolabeled UDP-galactose is therefore used to characterize galactosyltransferase enzymes (1) and to assess cell fractionation in which galactosyltransferase is considered a marker enzyme of the Golgi apparatus (2). UDP-[³H]galactose, in conjunction with exogenously added galactosyltransferase, has also been used to *in vitro* label oligosaccharides containing terminal GlcNAc residues (3-5). The monosaccharide [³H]galactose is taken up by growing cells and incorporated into newly synthesized glycoproteins and glycolipids. Hence it has been used to study oligosaccharide biosynthesis in tissue culture cells (6,7).

The success of such investigations relies on the use of high-specific-activity, radiochemically pure UDP-[6-³H]galactose and [³H]galactose. Although these reagents are commercially available, they are generally expensive and may contain significant amounts of radioactive impurities. Previously described syntheses of UDP-[6-³H]galactose either have involved relatively cumbersome procedures or specialized equipment or have resulted in relatively low-specific-activity product

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² Abbreviations used: PEI, polyethyleneimine; GlcNAc, N-acetylglucosamine; PCA, perchloric acid.

(8,9). We describe here a synthesis of high-specific-activity, radiochemically pure UDP-[6-³H]galactose that is quick and inexpensive and that relies on readily available materials. We have also synthesized UDP-*N*-[6-³H]acetylgalactosamine using a similar approach. The tritiated monosaccharides of these sugar nucleotides are easily obtained by mild acid hydrolysis and passage over cation- and anion-exchange chromatography columns.

METHODS AND MATERIALS

Materials

Uridine-5'-diphosphogalactose, uridine-5'-diphospho-*N*-acetylgalactosamine, galactose, *N*-acetylgalactosamine, UDP-galactose:D-glucose 4- β -galactosyltransferase (galactosyltransferase), galactose oxidase (type V, from *Dactylium dendrites*), catalase (bovine liver), and polyethyleneimine (PEI) cellulose TLC plates were from Sigma Chemical Co. Paper chromatography medium (No. 1 grade) was from Whatman, and silica gel 60 TLC plates were from EM Sciences. NaB³H₄ was from New England Nuclear and had a specific activity of 75 Ci/mmol. All other chemicals were of reagent grade and were purchased from commercial sources.

Analytical Procedures

The concentrations of uridine-5'-diphospho-*N*-acetylgalactosamine and uridine-5'-diphosphogalactose were determined by absorbance at 262 nm, where the extinction coefficient of uridine was taken as 10,000 liters/mol·cm. Descending paper chromatography was used to assess the breakdown of the nucleotide sugars. Papers were developed in ethanol:1 M ammonium acetate, pH 3.9 (7:3 by volume), as described by Paladini and Leloir (10). Monosaccharides were analyzed using Dionex HPLC by the method of Hardy (11) where 18 mM NaOH was the eluent. The flow rate was 1 ml/min and fractions were collected every 0.33 min.

Transfer of [³H]Galactose from UDP-[³H]Galactose to *N*-Acetylglucosamine by Galactosyltransferase

Galactosyltransferase was autogalactosylated as described (4) and used at a concentration of 0.4 Unit/ml in a final volume of 50 μ l containing 100 mM Na cacodylate, pH 6.5, 40 mM MnCl₂, 86 mM 2-mercaptoethanol, 0.5 mM UDP-galactose, 4 mM ATP with and without 80 mM acceptor GlcNAc. The reaction was allowed to proceed for 20 min at 37°C and stopped by the addition of 1 ml of 10 mM EDTA, and the solution passed over a Dowex AG1 \times 8 column equilibrated in 5% sodium borate and washed with water (12). The product *N*-[³H]-acetylglucosamine passes through the column, whereas UDP-[³H]galactose, [³H]galactose 1-phosphate, and

[³H]galactose are all retained by the column and subsequently eluted with 0.9 M NaCl, 0.1 M HCl. The percentage of label incorporated into product was determined by liquid scintillation counting.

Transfer of [³H]Galactose from UDP-[³H]Galactose to Ovalbumin by Galactosyltransferase

The conditions of the transfer reaction using ovalbumin as the acceptor are identical to those above for GlcNAc except that 2 mg of ovalbumin was added to each reaction in place of 80 mM GlcNAc. The reactions were incubated for 20 min at 37°C and then stopped by the addition of 950 μ l of 8% (v/v) perchloric acid (PCA). The solution was centrifuged at 12,000g for 10 min and the supernatant was removed and saved. The pellets were surface washed with 1 ml of 8% PCA, resuspended in 1 M NaOH, and neutralized with an equal volume of 1 M HCl. The PCA supernatants and resuspended pellets were subjected to liquid scintillation counting, and the percentage label added to ovalbumin was determined. The negative controls contained 80 mM GlcNAc as a nonprecipitable competitor. Typically, 2–4% of the label was associated with the PCA pellet in the negative controls.

Reduction of Commercially Obtained UDP-[³H]Galactose

The UDP-[6-³H]galactose (10 μ l) was added to 100 μ l of 200 mM Hepes, pH 8.0. To 40 μ l of this was added either 0.4 μ l of 10 mM NaOH or 0.4 μ l of 10 mM NaOH with 1 M NaBH₄. The reaction was allowed to proceed for 30 min at room temperature at which time another 0.4 μ l was added. The reaction was quenched after a total incubation time of 1 h by adding 5 μ l of acetone.

Reoxidation of Synthesized UDP-[³H]Galactose by Galactose Oxidase and Catalase

To the synthesized UDP-[6-³H]galactose, 3 μ Ci in 100 μ l of 100 mM Na₂PO₄, pH 6.5, was added 500 Units of catalase and 8 units of galactose oxidase. The reaction was allowed to proceed at room temperature and aliquots were removed at the described times and frozen on dry ice. These aliquots were then split into four parts: one was subjected to liquid scintillation counting, a second was lyophilized and then subjected to liquid scintillation counting, and the final two were spotted on PEI cellulose TLC plates and developed in either water or 1 M LiCl. The TLC plates were sprayed with EN³HANCE autoradiography surface enhancer and autoradiographed. The plates were then cut into thirds containing respectively the origin, the middle, and the solvent front. These were then subjected to liquid scintillation counting.

RESULTS AND DISCUSSION

*Synthesis of UDP-[6-³H]Galactose and
UDP-N-[6-³H]Acetylgalactosamine*

Galactose oxidase was used to oxidize the 6-hydroxyl group of the sugar moiety of UDP-galactose to an aldehyde (9). Catalase is added to degrade the hydrogen peroxide that is produced. The resulting 6-aldo-UDP-Gal is subsequently reduced back to the original alcohol with tritiated NaBH₄, resulting in the introduction of a tritium label. Typically, 2–5 mg of sugar nucleotide was oxidized in a volume of 100 μl of 25 mM Na₂HPO₄, pH 7.8, containing 25 units of galactose oxidase and 500 Units of catalase. The reaction was allowed to proceed overnight at room temperature in the dark in an uncapped tube. The resulting 6-aldo-UDP-Gal was purified using thin-layer chromatography. The mixture was spotted as a line on a plastic-backed silica gel 60 TLC plate and developed in butanol:ethanol:water (2:5:3 by volume). The plate was air-dried and a hand-held uv light was used to locate the uridine-containing compounds. Another TLC plate was spotted separately with only galactose oxidase or catalase and similarly developed. Both enzymes remained at the origin as determined by staining the plate with iodine vapor.

The fastest migrating uv-absorbing material is the aldehyde. The strip of TLC plate containing this region was cut out, placed on end, and redeveloped in water. The aldehyde migrates with the solvent front when the TLC plate is developed in water and hence becomes concentrated at the top of the strip. The small region of TLC plate containing the aldehyde was then cut out with scissors and placed in a 500-μl microfuge tube in which a slit had been cut in the bottom. Water (33 μl) was used to wet the silica, and the 500-μl tube was placed in a 1.5-ml microfuge tube and centrifuged for 3 min at 6500 rpm. The piece of silica plate was similarly washed two more times as above and the combined water eluents were removed from the 1.5-ml tube.

The resulting aldehyde was analyzed for purity on PEI cellulose TLC plates. Inspection under uv light indicated that UDP-galactose, UDP, and UMP all remained at the origin of the PEI cellulose plate when developed in water and that all ran with the solvent front upon development in 1 M LiCl. The silica gel-purified oxidation product was quantitatively retained at the origin upon development of the TLC plate in either water or 1 M LiCl. We therefore concluded that the material eluted from the silica plate is essentially pure oxidized UDP-galactose. Since the final specific activity of the UDP-[6-³H]galactose is dependent upon the percentage of the original UDP-galactose that is oxidized, it is important that the purified product of the oxidation remain quantitatively at the origin of the PEI cellulose TLC plates developed in both water and 1 M LiCl.

It should be noted that the 6-aldo-UDP-Gal could not be eluted intact from the PEI cellulose TLC plate even with 10 M LiCl and may be covalently attached. However, the aldehyde of galactose appeared to migrate with the solvent front upon development of the PEI TLC plate in 1 M LiCl. It has been previously shown that periodate-oxidized ATP also remains at the origin of PEI cellulose TLC plates upon development in salt (13). One possible explanation for these observations is that the 6-aldo-UDP-Gal interacts with the PEI cellulose through the formation of a Schiff's base with the imine group of the plate, but that this interaction requires the presence of an adjacent negative charge.

The silica gel TLC-purified nucleotide sugar aldehyde was applied to a 3-ml QAE Sephadex column equilibrated in water in order to remove any free, oxidized galactose that may result from the chemical degradation of 6-aldo-UDP-Gal. The column was washed with water and the aldehyde was eluted with 1 M NaCl. This was desalted on Sephadex G-10 developed in water and concentrated by lyophilization. The resulting purified material was dissolved in 100 μl of 100 mM Hepes, pH 8.0, and reduced with 25 mCi NaB³H₄ dissolved in 25 μl of 10 mM NaOH for 10 min in a fume hood approved for volatile tritium. After 10 min, another 25 mCi of NaB³H₄ in 25 μl of 10 M NaOH was added, and the reduction allowed to proceed for another 50 min. The reduction was performed at pH 8 to minimize the base-catalyzed hydrolysis of the 6-aldo-UDP-Gal and UDP-[6-³H]Gal. It has been our experience that the NaB³H₄ sold in 500-mCi aliquots and shipped in sealed ampoules is most reactive and that the reduction should be performed immediately after opening the ampoule. The reduction was quenched by the addition of 10 μl of acetone and the reaction mixture was again applied to QAE Sephadex. The column was extensively washed with water and eluted with 1 M NaCl. The resulting UDP-[6-³H]galactose was desalted on Sephadex G-10 and applied to a 3-ml PEI cellulose column in order to remove any unreduced aldehyde. The PEI cellulose column was washed with water until there was no more radioactivity in the run through. The UDP-[6-³H]galactose was eluted with 1 M NaCl and again desalted. The use of PEI cellulose to remove unreduced aldehyde eliminates the need to quench the reaction with nonradioactive NaBH₄ and ensures the maximum final specific activity of the product.

The specific activity of UDP-[6-³H]galactose was calculated by using the absorbance of uridine at 262 nm to determine the molar concentration, and scintillation counting was used to determine the amount of radioactivity present. UDP-[6-³H]galactose (7.5 mCi of 10 Ci/mmol) was obtained from 5 mg of UDP-Gal and 50 mCi NaB³H₄. The final desalted UDP-[6-³H]galactose was then stored at -20°C in 70% ethanol.

UDP-*N*-[6-³H]acetylgalactosamine was similarly synthesized. The silica gel TLC-purified aldehyde of UDP-*N*-acetylgalactosamine was analyzed by PEI cellulose TLC. The uv-absorbing material remained at the origin when the PEI cellulose TLC plate was developed in water but there was also uv-absorbing material at the solvent front when the plate was developed in 1 M LiCl. This is probably due to the less efficient oxidation by galactose oxidase but could also be due to the presence of contaminating UMP/UDP resulting from the breakdown of the starting sugar nucleotide. Regardless, the final yield from 50 mCi of NaB³H₄ was 12.5 mCi of UDP-*N*-[6-³H]acetylgalactosamine having a specific activity of 6.0 Ci/mmol. This was also stored in 70% ethanol at -20°C.

Proof of the Products

UDP-[6-³H]galactose and UDP-*N*-[6-³H]acetylgalactosamine were subjected to descending chromatography on Whatman No. 1 paper developed in 7:3 ethanol:1 M ammonium acetate, pH 3.9. Approximately 90% of the radioactivity from the UDP-[6-³H]galactose migrated with authentic UDP-galactose, showing little breakdown of the label. Greater than 98% of the UDP-*N*-[6-³H]acetylgalactosamine was intact, presumably due to the greater stability of the *N*-acetyl derivatives of the sugar nucleotides (14).

Aliquots of both radioactive nucleotides were subjected to mild acid hydrolysis by 10 mM HCl at 100°C for 1 h. The hydrolysate was passed over anion- and cation-exchange columns (2 ml each, equilibrated in water). The run through fraction was lyophilized, resuspended in water, and subjected to monosaccharide analysis on a Dionex CarboPac HPLC column. Nonradioactive internal standards were monitored with a pulsed amperometric detector. Fractions were collected and analyzed by liquid scintillation counting. A single peak of radioactivity (Fig. 1) comigrating with the galactose standard was obtained from the hydrolysis of UDP-[6-³H]galactose. Similarly a single peak of radioactivity (Fig. 1) comigrating with standard *N*-acetylgalactosamine was obtained from the hydrolysis of UDP-*N*-[6-³H]acetylgalactosamine.

Comparison with Commercially Obtained UDP-[³H]galactose

In the course of these investigations, we found that the majority of the radioactivity in one lot of commercially obtained UDP-[6-³H]Gal behaved the same as 6-aldo-UDP-Gal on PEI cellulose. On a conventional paper system developed in 7:3 ethanol:1 M ammonium acetate, pH 3.9, 70–80% of the label migrated as UDP-Gal, with or without reduction. However, approximately 70% of the radioactivity remained at the origin of the TLC plate upon development in 1 M LiCl. Reduction with sodium borohydride resulted in greater than 90%

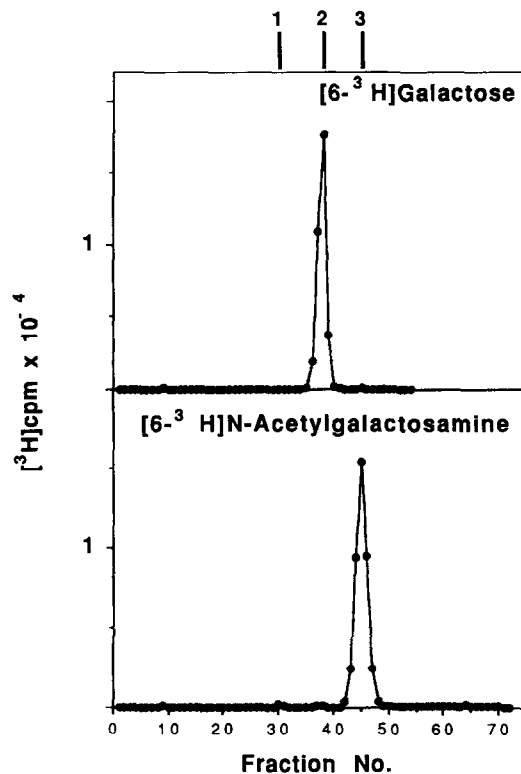


FIG. 1. HPLC analysis of radioactive monosaccharides obtained from the hydrolysis of UDP-[6-³H]galactose and UDP-*N*-[6-³H]acetylgalactosamine. The mild acid hydrolysates were purified and analyzed on a Dionex CarboPac HPLC column as described under Methods and Materials. The flow rate was 1 ml/min and fractions were collected every 0.33 min. The standards labeled 1, 2, and 3 are GalNH₂, Gal, and GalNAc, respectively.

of the radioactivity migrating with the solvent front upon development in 1 M LiCl (see Table 1). Additionally, treatment of this material with catalase did not liberate tritiated water, indicating that the radioactivity was not in hydrogen peroxide. Taken together, these data indicate that one commercially obtained lot of UDP-[6-³H]Gal contained a large percentage of 6-aldo-UDP-[6-³H]Gal, indistinguishable from UDP-[6-³H]Gal on paper chromatography. The manufacturer of this preparation does not routinely remove the galactose oxidase and catalase prior to the reduction of the 6-aldo-UDP-Gal with NaB³H₄. Thus, we believe that their UDP-[6-³H]galactose had been reoxidized by the galactose oxidase, resulting in the formation of 6-aldo-UDP-[6-³H]Gal. We then compared UDP-[6-³H]galactose obtained from other suppliers and also a second batch from the manufacturer that produced the 6-aldo-UDP-[6-³H]Gal. In all cases, greater than 98% of the radioactivity remained at the origin of the PEI cellulose TLC plate upon development in water. Approximately 95% of the radioactivity from each of these other batches ran with the solvent upon development of the PEI cellulose TLC plates in 1 M LiCl (data not shown).

TABLE 1
Behavior of UDP-[6-³H] Galactose Preparations during Chromatography on PEI Cellulose TLC Plates

	H ₂ O			1 M LiCl		
	Origin	Middle	Front	Origin	Middle	Front
Synthesized UDP-[6- ³ H]galactose	94.9%	2.5	2.6	0.5%	7.3	92.2
Commercial UDP-[6- ³ H]galactose	99.0	0.7	0.3	70	7.7	22.6
NaBH ₄ reduced commercial UDP-[6- ³ H]galactose	98.5	0.4	1.1	5.3	2.5	92.2

Note. PEI cellulose TLC plates were spotted with either synthesized or commercially obtained UDP-[6-³H]galactose and developed in water or 1 M LiCl. The chromatograms were sprayed with EN³HANCE autoradiography surface enhancer and autoradiographed. Radioactivity was found to be at either the origin or the solvent front depending on the sample and developing conditions. The TLC plate was then cut into three equal sections containing the origin, the middle of the plate, or the solvent front. These were then subjected to liquid scintillation counting and the percentage of radioactivity in each section was calculated. NaBH₄ reduction was performed as described under Methods and Materials.

Thus, although 6-ald-UDP-[6-³H]Gal is a potential impurity in commercially obtained UDP-[6-³H]Gal, it is not necessarily present in the UDP-[6-³H]Gal obtained from every supplier or in every lot from a given supplier. The only simple test available for 6-ald-UDP-[6-³H]Gal is PEI cellulose chromatography, and we recommend that UDP-[6-³H]Gal used in applications requiring high radiochemical purity be assayed for the presence of 6-ald-UDP-[6-³H]Gal by this method.

Reoxidation by Galactose Oxidase

The product UDP-[6-³H]galactose behaved the same as the starting UDP-Gal on PEI cellulose. It remained at the origin of the PEI cellulose TLC plates when developed in water but migrated at the solvent front when developed in 1 M LiCl. This was determined by both autoradiography and by liquid scintillation counting (see Table 1). If the product UDP-[6-³H]galactose was retreated with galactose oxidase and catalase, there was a time-dependent increase in the percentage of radioactivity remaining at the origin of the PEI cellulose TLC plate developed in 1 M LiCl, with 40% of the radioactivity remaining at the origin following a 5-h incubation (see Fig. 2). Thus, the UDP-[6-³H]galactose could be reoxidized by galactose oxidase to yield radioactive 6-ald-UDP-Gal. If both of the hydrogen atoms at C-6 are equivalent, one would expect 50% of the label to be released as water on reoxidation. However, while a trend toward a decrease in bound radioactivity was seen on prolonged oxidation, the 50% level was never achieved. Similar results were noted when the radioactivity was subjected to lyophilization after the incubation with galactose oxidase and catalase (data not shown). It is possible that either the two hydrogen atoms are not equivalent and galactose oxidase is stereoselective or there is an isotope effect where the proton at C-6 is preferentially removed over the tritium atom. This postulated isotope effect would be a substrate isotope effect similar to that described for UDP-glucose 4-epimerase (15) and distinct from the described solvent isotope effect previ-

ously described for galactose oxidase (16). This matter deserves further investigation.

Transfer of [6-³H]Galactose to Exogenous Acceptor

The transfer of [6-³H]galactose from UDP-[6-³H]galactose to GlcNAc and ovalbumin by galactosyltransferase was performed as described under Methods and Materials, using both the synthesized and commercially obtained impure UDP-[6-³H]galactose. The percentage of the label incorporated into product is shown in Table 2. The synthesized UDP-[6-³H]galactose was a very good donor for the transferase reaction. The commercially obtained UDP-[6-³H]galactose was utilized much less effectively by galactosyltransferase, indicating that 6-ald-UDP-galactose is not a donor for the transfer

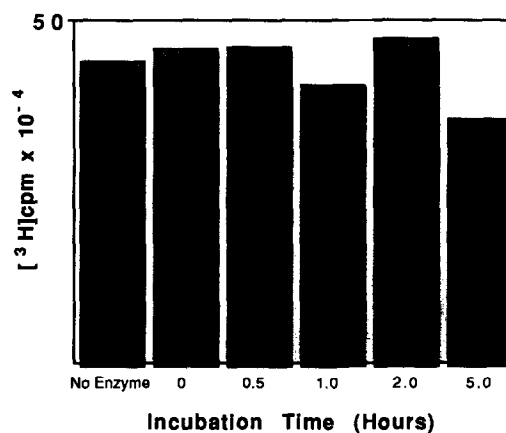


FIG. 2. Time course of reoxidation of synthesized UDP-[6-³H]galactose by galactose oxidase and catalase. UDP-[6-³H]galactose was reoxidized with galactose oxidase and catalase as described under Methods and Materials. Aliquots were removed at the indicated times, spotted on PEI cellulose, and developed in either water or 1 M LiCl. The TLC plate was then cut into three equal sections containing the origin, the middle of the plate, or the solvent front and these were then subjected to liquid scintillation counting. Greater than 95% of the radioactivity remained at the origin when the plate was developed in water. The radioactivity at the origin (■), middle (▨), and solvent front (□) following development in 1 M LiCl are shown.

TABLE 2
UDP-[6-³H]Galactose Preparations as Donors
for Galactosyltransferase

	<i>N</i> -Acetylglucosamine acceptor	Ovalbumin acceptor
Synthesized UDP-[6- ³ H]galactose	30%	69%
Commercial UDP-[6- ³ H]galactose	4.2	7

Note. Galactosyltransferase reactions were performed as described under Methods and Materials. The percentage of radioactivity transferred to added acceptor in each reaction is shown. The background has been subtracted.

reaction. This aldehyde may actually be an inhibitor of the enzyme. Further, if 6-aldo-UDP-[6-³H]Gal is present in transfer reactions aimed at identifying acceptors of galactose, it is possible that the aldehyde may form a Schiff's base with the primary amino groups of other proteins in solution and lead to spurious autoradiographs.

Generation of Radiolabeled Monosaccharides

The monosaccharides [6-³H]galactose and *N*-[6-³H]-acetylgalactosamine can be generated from their corresponding sugar nucleotides on a preparative scale by treatment with mild acid followed by passage over both anion- and cation-exchange columns as described above for the analysis of monosaccharides. We prefer to generate the radiolabeled monosaccharides from the nucleotide sugar for several reasons. First, the negative charge of the UDP moiety facilitates subsequent purification. Second, the uridine moiety can be used to determine specific activity. Finally, the quality of the radiolabeled sugar nucleotide can be assessed with a functional assay such as transfer to exogenous acceptor by galactosyltransferase.

SUMMARY

Radiolabeled monosaccharides and sugar nucleotides have been widely used to study oligosaccharide biosynthesis and structure. High specific activity and high radiochemical purity are essential in these experiments since often only small quantities of sample are available for study. Although these radiochemicals are available commercially, they are generally quite expensive. We have presented a method for the rapid, efficient synthesis of high-specific-activity UDP-[6-³H]galactose, UDP-*N*-[6-³H]acetylgalactosamine, and their corresponding monosaccharides. The procedure is inexpensive and utilizes technologies available to any research laboratory.

An important aspect of the presented synthesis of UDP-[6-³H]galactose is the use of PEI cellulose both in assessing the completeness of the oxidation reaction and in removing any unreacted aldehyde after the reaction with NaB³H₄. The final specific activity of the product is dependent on both of these steps. PEI cellulose can also be used to assess the radiochemical purity of the UDP-[6-³H]galactose obtained from any source.

It should be possible to use the enzyme UDP-galactose 4-epimerase to extend the synthesis of UDP-[6-³H]galactose to UDP-[6-³H]glucose. This enzyme catalyzes the epimerization of the sugar residue and yields a mixture of UDP-galactose and UDP-glucose at equilibrium. The resulting UDP-[6-³H]glucose could then be separated from the UDP-[6-³H]galactose by TLC as described (17). UDP-galactose 4-epimerase also acts on UDP-*N*-acetylgalactosamine and therefore it should be possible to make UDP-*N*-[6-³H]acetylglucosamine from UDP-*N*-[6-³H]acetylgalactosamine, where the separation of the two is achieved using *Griffonia simplicifolia* I lectin (18).

REFERENCES

1. Witsell, W. L., Casey, C. E., and Neville, M. C. (1990) *J. Biol. Chem.* **265**, 15,731-15,737.
2. Fleischer, B., Fleischer, S., and Ozawa, H. (1969) *J. Cell Biol.* **43**, 59-79.
3. Whiteheart, S. W., Passaniti, A., Reichner, J. S., Holt, G. D., Haltwanger, R. S., and Hart, G. (1989) in *Methods in Enzymology* (Ginsburg, V., Ed.), Vol. 179, pp. 82-95, Academic Press, San Diego, CA.
4. Holt, G. D., and Hart, G. W. (1986) *J. Biol. Chem.* **261**, 8049-8057.
5. Thilo, L. (1983) in *Methods in Enzymology* (Fleischer, S., and Fleischer, B., Eds.), Vol. 98, pp. 415-421, Academic Press, San Diego, CA.
6. Cummings, R. D., and Kornfeld, S. (1984) *J. Biol. Chem.* **259**, 6253-6260.
7. Swamy, M. J., and Sweeley, C. C. (1989) *Biochem. Biophys. Res. Commun.* **162**, 1188-1193.
8. Kirkwood, S. (1972) in *Methods in Enzymology* (Ginsburg, V., Ed.), Vol. 28, pp. 296-299, Academic Press, San Diego, CA.
9. Fudem-Goldin, B., Voulalas, P., and Orr, G. A. (1988) *J. Biochem. Biophys. Methods* **11**, 199-202.
10. Paladini, A. C., and Leloir, L. F. (1952) *Biochem. J.* **51**, 426-430.
11. Hardy, M. R. (1989) in *Methods in Enzymology* (Ginsburg, V., Ed.), Vol. 179, pp. 76-82, Academic Press, San Diego, CA.
12. Bretz, R., and Stauble, W. (1977) *Eur. J. Biochem.* **77**, 181-192.
13. Easterbrook-Smith, S. B., Wallace, J. C., and Keech, D. B. (1976) *Eur. J. Biochem.* **62**, 125-130.
14. Nunez, H. A., and Barker, R. (1976) *Biochemistry* **15**, 3843-3847.
15. Nelsestuen, G. L., and Kirkwood, S. (1970) *Biochim. Biophys. Acta* **220**, 633-635.
16. Kosman, D. J., and Driscoll, J. J. (1988) *Prog. Clin. Biol. Res.* **274**, 251-267.
17. Merril, C. R., Das, A. K., LaPolla, R. J., and Prissofsky, I. (1976) *Anal. Biochem.* **72**, 606-613.
18. Piller, F., Eckhardt, A. E., and Hill, R. L. (1982) *Anal. Biochem.* **127**, 171-177.