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Very High Isotope Incorporation in the C-1 Position of Glucose by Exchange with Deuterium or Tritium Gas

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Summary

The experimental conditions which control the exchange from deuterium or tritium gas into the C-1 proton position of glucose in aqueous solution have been studied in detail, with a view to determining the factors which maximize exchange into glucose, but minimize exchange into the solvent water. The favoured conditions for producing glucose with close to 100% isotope labelling in the C-1 position, and with negligible formation of labelled byproducts, were a reaction time of 6 to 8 hours, a temperature of 60 °C, and a pH of 7 or higher. $Pd/BaSO_4$ was the preferred catalyst and slow pre-addition of the deuterium or tritium gas to the catalyst bed was essential to maximize the subsequent isotope exchange into the substrate.

Key Words: Glucose, tritium gas, deuterium gas, palladium catalyst, tritium NMR, deuterium NMR

Introduction

Exchange between tritium gas and some particular classes of organic substrates in aqueous solution,[1] when catalyzed by palladium on barium sulfate, has been widely used as a means of tritiation of the substrates to high specific activity. An essential feature of the procedure is that conditions are chosen such that the tritium exchanges into the substrate in preference to the solvent water. It thus differs in concept from alternative techniques[2,3] where exchange with the isotopic solvent is the method of tritiation of a substrate.

While the method was originally described by Evans *et al.*[1,4] some years ago, and a variety of carbohydrates, benzylic compounds and nucleosides were successfully labelled, no detailed

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study of the influence of many of the various experimental parameters on the efficiency of the labelling process has been published. Their study[1,5] did show that a pH of at least 7 is necessary, and room temperature conditions are favoured, for clean labelling of carbohydrates. Akulov and coworkers[6,7] recently extended the method to the labelling of some disaccharides. Buchman *et al.*[8,9] described a similar technique, but exchange with the solvent was involved and their procedure more closely resembled conventional heterogeneous metal catalyzed exchange.[3,10]

Regular use of the gas exchange procedure as outlined by Evans[1] for the labelling of carbohydrates and some other compounds[11,12] has shown that the level of incorporation of tritium into the organic substrate varies considerably for reasons which have remained obscure. It was not known whether the low tritium incorporation was the result of preferential entry of tritium into the aqueous solvent, overall slower exchange, or some poisoning of the catalyst by a product of the reaction.

In the typical reaction mixture, the mole ratios of reactants are such that the protons of the aqueous solvent are in large excess over the tritium gas, which exceeds the organic substrate. Thus a high specific activity is attainable in the organic substrate if, and only if, exchange between water and tritium gas is relatively slow and the reaction can be stopped well short of isotopic equilibrium. At equilibrium, virtually all the tritium would appear in the solvent.

Thus, the normal experimental procedure with a substrate such as glucose[1] has allowed the exchange reaction to proceed for 2 hours at room temperature, under neutral pH conditions. These conditions were chosen, not only to produce high specific activity glucose, but also to maximize the specificity of the label to the C-1 position in the molecule and minimize formation of any other labelled byproducts. The essential objectives of the labelling process are high specific activity (1 tritium atom per molecule), high positional specificity, and high radiochemical purity.

This paper reports an assessment of the following factors on the above objectives: reaction temperature, reaction time, pH of solution, and type of Pd catalyst. In the latter stage of the study it became apparent that the rate of addition of tritium or deuterium gas to the catalyst may significantly affect the results, and this parameter also was explored. It was considered important, in gaining an understanding of the exchange process, that the rate of exchange into solvent water as well as the organic substrate be measured and the exchange and analysis procedures were designed to that end. The bulk of the work was performed with deuterium rather than tritium for ease of manipulation and conservation of tritium.

Experimental

Catalysts

Most of the work was performed with 5% $Pd/BaSO_4$ supplied by Aldrich Chemical Company Inc. (batches 0816PK and 05128EP). These were designated as catalysts A and B. A number of other catalyst were tested for comparison purposes and were designated as follows:

Catalyst A:	5% $Pd/BaSO_4$	Aldrich-50g bottle, 0816PK
Catalyst B:	5% $Pd/BaSO_4$	Aldrich-10g bottle, 05128 EP
Catalyst C:	10% $Pd/BaSO_4$	Engelhardt
Catalyst D:	10% $Pd/BaSO_4$	Manufacturer Unknown
Catalyst E:	5% $Pd/CaCO_3$	Aldrich
Catalyst F:	5% Pd/Alumina	Aldrich

D-Glucose

D-glucose, anhydrous AR grade, *ex* Mallinckrodt Inc., was used. ^1H NMR analysis of a fresh aqueous solution showed it to be almost entirely α-D-glucose which equilibrated over 24 hours to the thermodynamic α,β mixture (38:62).[13] Glucose solutions for the exchange reactions were prepared at least 24 hours in advance in order that an equilibrium mixture was used.

Solvent

Deuterium depleted water, *ex* Cambridge Isotope Laboratories, was used in all deuterium exchange experiments (specification: 2-3 ppm ^2H). ^2H NMR analysis confirmed the deuterium isotope abundance to be <3 ppm. Deionized water, of natural isotopic abundance, was used in the tritiation experiments.

Deuterium Gas

Deuterium gas (AIRCO Grade 2.5) was used as supplied.

Deuterium Exchange Procedure

Glucose solution (buffered by the addition of the appropriate ratio of sodium phosphate salts (0.05 M)) was placed in a glass reaction vessel with septum sealed side arm and a rotatable spoon in which the catalyst was held above the solution. The size of this vessel was chosen such that the required volume of D_2 gas at 1 atmosphere pressure was enclosed by the stopcock which isolated the vessel from the vacuum system. Samples for analysis could be removed by syringe *via* the septum. The actual scale of an experiment was selected on the basis of the number of samples to be extracted during the exchange cycle, and the size of the vessel and reagent quantities were chosen such that their mole ratios were constant. In single sample experiments, the standard

quantities of reagents were as follows: glucose (21 mg, 0.116 mmole), water (0.5 mL, 27.8 mmole), catalyst (50 mg), D_2 gas (*ca.* 35 mL at 730 mm, 1.41 mmole).

This ratio of reagents would lead to a deuterium abundance of 4.8% in each exchangeable position if the deuterium was randomized by exchange between the deuterium gas, the C-1 hydrogen of glucose, and the water hydrogen atoms. In addition, only a small fraction of the total deuterium in the system would appear in the glucose at equilibrium, since the mole ratio of the glucose was very small (*ca.* 240 moles of water for each mole of glucose).

Aqueous glucose solutions were degassed by three freeze, pump, thaw cycles and pumping continued until the pressure was less than 50 millitorr. Deuterium gas was then admitted to the reaction vessel until the pressure was close to 1 atmosphere while the glucose solution remained frozen in liquid nitrogen. Reagents were mixed by rotation of the spoon to dispense the catalyst followed by rapid warming of the flask to the selected temperature, and the reaction was allowed to proceed with continuous stirring.

Samples (*ca.* 0.3 mL) of reaction solution were withdrawn by gas tight syringe and divided into two parts (*ca.* 0.25 mL and 0.05 mL respectively) by passage through a Millipore micro-filter into two pre-weighed sample vials and the mass of the solutions determined.

Analysis of Deuterated Samples

Deuterium Content of Glucose – The larger of the two sample parts (0.25 mL), designated Part A, was evaporated to dryness in a stream of nitrogen at room temperature, deuterium depleted water was added and re-evaporated. Depleted water (0.25 mL), dioxane (2.15 mL) and a precise aliquot of standard DMSO-d_6 solution (0.010 mL) were added to the dry glucose and the solution transferred to a 10 mm NMR tube for analysis of the D content of glucose.

Deuterium Content of Water – Dioxane (2.30 mL) was added to the smaller part of the sample (0.05 mL, Part B), and the solution was transferred to a 10 mm NMR tube for analysis of the D content of the solvent water.

NMR Analyses – The ratios of solvents in the above samples were chosen to provide for adequate separation of all peaks of interest in the ^2H NMR spectra, obtained with a Bruker AF300 instrument operating at 46 MHz. The D content of the C-1 position of glucose was measured by integration relative to the DMSO-d_6 internal reference in sample part A. The D content of the water was determined by integration relative to the natural abundance D content of the added dioxane (independently confirmed by comparison with added C_6D_6 to be 0.016 atom%) in sample part B.

Chemical shifts (non referenced) in these samples, and in pure water were as follows:

	Part A (10% water, 90% dioxane)	Part B (2% water, 98% dioxane)	Water
α-C-1	5.20	5.17	5.20
β-C-1	4.59	4.52	4.61
dioxane	3.74	3.70	3.57
water	3.86	3.37	4.76
DMSO	2.71		2.67

The %D in the glucose and the water were calculated using the measured mass of samples part A and part B with the assumption that all glucose remained in solution. The question as to the completeness of recovery of glucose from the reaction solution in the presence of the Pd/BaSO4 catalyst was considered. "Low" labelling results would be obtained if a significant quantity of the glucose was adsorbed on the catalyst. Therefore, experiments were performed in which 5,6-^{3}H-glucose was added to the initial glucose solution. Filtered samples from the exchange mixture were assayed by liquid scintillation counting and this showed quantitative recovery (>99%, 4 samples) of the glucose was achieved in the normal work-up procedure.

Additional Comments on ^{2}H NMR Analysis Procedures

In the exploratory stages of the study, attempts were made to obtain satisfactory analyses for C-1 deuterons in glucose and in water on single samples of similar composition to sample A. In these spectra water was poorly resolved from dioxane and while the water/dioxane resolution could be improved by increasing the water concentration, interference with the C-1 peaks of glucose then resulted. Hence the two sample method above was adopted.

Attempts were also made to use the internal natural abundance of D in dioxane as the quantitative reference for C-1 deuterons in glucose. This approach required comparison of a small glucose peak with a very much larger dioxane peak, and the integrals were not accurate. The use of DMSO as an additional reference material at a D level similar to that in the glucose was much more satisfactory.

It was possible to delete the inclusion of dioxane in sample part A, and to perform the ^{2}H NMR analysis on a solution in pure ^{2}H-depleted water. This method appeared to be at least as accurate as use of 10% in dioxane samples, but had the disadvantage that larger quantities of depleted water were consumed, *viz.* 2.4 mL per NMR sample. The repeated evaporation to dryness of sample Part A, with addition of depleted water, ensured that no detectable water peak was present in the ^{2}H NMR spectrum to interfere with the glucose peak integration.

Tritium Exchange Procedure and Analysis

The procedure adopted for the tritium exchange experiments followed closely that described above, using the quantities of reagents specified for single sample reactions. The amount of tritium gas enclosed in the reaction flask (*ca.* 35 mL at 730 mm) was about 69 Ci. This was metered slowly onto the catalyst such that the pressure did not rise above 2 mm in the first 15 minutes. At the completion of exchange and after removal of tritium gas, D_2O (10 mL) was added to the reaction mixture and a sample (0.3 mL) of the resulting solution was removed for ^3H NMR analysis (Bruker AF300, 320 MHz). This permitted determination of the ratio of tritium in glucose to that in water. The remainder of the reaction mixture was filtered, evaporated to dryness and redissolved in D_2O (0.5 mL) for further ^3H and ^1H NMR analysis in a 3 mm tube, and for assay by liquid scintillation counting.

Results and Discussion

Reaction Time and Temperature of Deuterium Exchange

Deuterium exchange reactions were performed at both room temperature and 60 °C for various reaction times, and typical results are summarized in Table 1

Table 1. Influence of Reaction Temperature and Time on Deuteration

Temp. °C	Time h	Catalyst	%D C-1-glucose	%D water	%D gas
25	4.0	A	30.7	0.27	93.3
60	4.0	A	52.4	0.76	82.9
25	2.0	D	16.8	0.73	84.9
60	2.0	D	68.4	0.62	85.1
25	2.0	B	17.8	0.11	97.1
60	2.0	B	96.7	0.40	88.0
25	1.0	B	8.0	0.09	97.9
25	2.0	B	17.8	0.11	97.1
25	4.0	B	19.6	0.15	96.3
25	12.0	B	41.7	0.35	91.5
25	21.0	B	54.9	0.80	82.1
60	0.5	A	12.7	0.13	96.9
60	1.0	A	15.8	0.20	95.5
60	2.0	A	38.1	0.46	89.5
60	4.0	A	52.4	0.76	82.9
60	9.0	A	81.8	1.40	69.2
60	22.0	A	73.0	3.04	37.9

All experiments listed in Table 1 were performed with $Pd/BaSO_4$ as catalyst. Values for %D in glucose and water were derived from ^2H NMR measurements. The %D in the gas is the deuterium content calculated to have remained in the gas phase from the %D values for glucose and water, on the assumption that all protons lost from these compounds had entered the gas phase. Hence, incorporating the mole ratios and the number of exchangeable hydrogens for each compound leads to the formula: $\%D_{gas} = 100 - [(19.7 \times \%D_{water}) + (0.0411 \times \%D_{glucose})]$.

The first three pairs of results listed are those for reactions where conditions were identical within each pair except for the reaction temperature. Clearly there was a substantial increase in the extent of deuteration of both glucose and water on raising the temperature from 25 °C to 60 °C. While some scatter in exchange rates is apparent, as is typical of heterogeneous phase catalytic reactions, the results show that the ratio of the rates of entry of isotope into glucose and water remains the same order of magnitude (*ca.* 10^2) with the increase in temperature.

The results listed in the final two sections of the table show the progress of exchange with time in both glucose and water at 25 °C and 60 °C respectively. Exchange appears to continue with time in both glucose and water, no catalyst poisoning being apparent. At 60 °C the glucose deuteration approached the deuterium gas isotope abundance at 4 to 9 hours, and a subsequent drop in the glucose D level was observed through back exchange in the 22 hour sample.

pH of Solution

A comparison of some typical deuterium exchange reactions performed at 25 °C under phosphate buffered conditions over the pH range 5 to 9, together with a non-buffered example (pH = nil), is presented in Table 2.

Table 2. Influence of pH on Deuteration

pH	Time h	Catalyst	%D C-1-glucose	%D water
5	2.0	A	0.4	1.03
5	4.5	A	1.8	2.00
7	2.0	A	14.8	n.d.
9	2.0	A	29.1	0.79
9	4.0	A	53.4	1.07
nil	2.0	A	1.1	1.19
7	2.0	B	17.8	0.11
7	4.0	B	19.6	0.15
8.5	2.0	B	18.0	0.20
8.5	6.0	B	67.2	0.42

The exchange into glucose was facile over the pH range 7 to 9, and possibly favoured at pH 9 compared with pH 7. In contrast, at pH 5, the exchange into glucose was very slow, while exchange into water appeared to be enhanced under this slightly acidic condition. It appears that acidic conditions disfavour the labelling of glucose, but not the solvent water, in support of conclusions reached by Evans *et al.*[1]

Catalyst Type

The bulk of the study was performed with $Pd/BaSO_4$ as catalyst, since this has been the standard catalyst for such purposes since the early publication of the technique, but some additional experiments were conducted to test the efficacy of Pd/alumina and $Pd/CaCO_3$. The results for these alternative forms of palladium catalysts are presented in Table 3.

Table 3. Influence of Catalyst Type on Deuteration

Catalyst	Temp. °C	Time h	%D C-1-glucose	%D water
F (Pd/alumina)	25	2.0	40.1	0.73
E ($Pd/CaCO_3$)	60	4.0	104.0	1.36
A ($Pd/BaSO_4$)	25	2.0	8.9	1.10
B ($Pd/BaSO_4$)	25	2.0	17.8	0.11
D ($Pd/BaSO_4$)	25	2.0	16.8	0.73
A ($Pd/BaSO_4$)	60	4.0	52.4	0.46
B ($Pd/BaSO_4$)	60	2.0	103.0	0.40
C ($Pd/BaSO_4$)	60	2.0	83.2	0.93
D ($Pd/BaSO_4$)	60	4.0	101.0	0.62

Pd/alumina and $Pd/CaCO_3$ both showed facile exchange into glucose, comparable with that of the most active of the $Pd/BaSO_4$ catalysts. However the purity of the labelled glucose was lower with these catalysts, as discussed below. Exchange was also relatively fast into water with both these alternative catalysts. %D figures slightly in excess of 100% reflect the accuracy of the measurements, considered to be ±5%.

Several typical results for different sources of $Pd/BaSO_4$ catalyst also are included in Table 3. While the 60 °C results for glucose were too close to equilibrium to reliably show relative activities, the four $Pd/BaSO_4$ catalysts results in general showed that the three catalysts designated B, C and D were similar in their activity, but Catalyst A was of a somewhat lower activity.

Rate of Addition of D_2 to Catalyst

It became obvious during the study that the results were also influenced by the rate of addition of the deuterium gas to the catalyst. Furthermore, the most active catalysts appeared to be those which changed colour slowest on addition of the deuterium gas. Therefore two modes of addition of the deuterium gas were explored, designated as "fast D addition" and "slow D addition" modes. In the fast addition mode the deuterium was rapidly admitted to the degassed catalyst until the pressure reached close to 1 atmosphere. In the slow addition mode the deuterium gas was added slowly such that a pressure of about 5 to 10 mm was attained in about 10 minutes. This pressure was held for several minutes before being raised to 1 atmosphere over a period of about 15 minutes. In this latter mode, very slow blackening of the initially grey catalyst bed occurred. Results for these two modes of deuterium addition are compared in Table 4.

Table 4. Influence of Rate of D_2 Addition to the Catalyst

Conditions	Time h	%D C-1-glucose	%D water
Catalyst A, fast D addition	2.5	8.9	1.08
Catalyst A, fast D addition	8.0	24.8	2.63
Catalyst A, slow D addition	2.0	29.5	0.31
Catalyst A, slow D addition	6.0	83.4	0.70
Catalyst B, fast D addition	2.0	17.8	0.11
Catalyst B, fast D addition	4.0	19.6	0.15
Catalyst B, slow D addition	2.0	24.6	0.24
Catalyst B, slow D addition	6.0	60.2	0.29

It is clear that this parameter has a major influence on the deuteration of glucose. The inferior catalyst, Catalyst A, yielded results comparable to the best of the other catalysts and the more active Catalyst B also showed a substantial increase in activity.

It was noted in preliminary experiments that Catalyst A underwent the fastest visible color change on the fast addition of deuterium gas. This reaction was observed to be strongly exothermic and it is likely that the rapid addition procedure caused considerable heating of the catalyst bed. Hence, the explanation for this "rate of deuterium addition" effect may lie in the temperature attained by the catalyst, with deactivation as a result of catalyst sintering.

Purity of Deuterium Labelled Glucose

A selection of the deuterium labelled glucose samples were dissolved in depleted water alone and their ^2H NMR spectra examined carefully to assess the presence of deuterated impurities. In every sample so examined, a small quantity of impurity with chemical shift centered at 3.60 to 3.65 ppm was observed at the levels reported in Table 5. The impurity is expressed as a percentage of the D which appears in the C-1 positions of glucose in the same sample. The chemical shift of the impurity peaks lies between that of the groups of peaks observed for the protons of glucose, and is therefore assumed to not represent exchange into an alternative position in glucose itself, but instead, deuterium incorporation into a derivative of glucose. Glucose metabolism studies with tritiated glucose containing the same impurity have shown that this impurity is not consumed during metabolism of the glucose, confirming that the impurity was not glucose itself.[14]

Table 5. Yields of Deuterated Impurity

Conditions	Time h	Temp. °C	%D C-1-glucose	%D water	Impurity (% of C-1)
Catalyst A ($Pd/BaSO_4$)	9.0	60	81.8	1.40	4
Catalyst A ($Pd/BaSO_4$)	22.0	60	73.0	3.04	4
Catalyst B ($Pd/BaSO_4$)	2.0	60	96.7	0.40	3
Catalyst B ($Pd/BaSO_4$)	4.0	60	103.5	0.86	4
Catalyst B, slow D addition	2.0	25	24.6	0.24	3
Catalyst B, slow D addition	6.0	25	60.2	0.29	3
Catalyst B ($Pd/BaSO_4$)	6.0	25	67.2	0.42	3
Catalyst C ($Pd/BaSO_4$)	2.0	60	83.2	0.93	8
Catalyst D ($Pd/BaSO_4$)	4.0	60	101.6	1.29	4
Catalyst E ($Pd/BaCO_3$)	4.0	60	104.0	1.36	27
Catalyst F (Pd/alumina)	6.0	25	76.8	2.22	15

With all the $Pd/BaSO_4$ catalysts the deuterium content of the impurity was in the range 3% to 8% of glucose. There appeared to be no consistent difference between 25 °C and 60 °C reactions, and longer exchange times did not appear to lead to an increase in the relative amount of impurity. In contrast, $Pd/CaCO_3$ (Catalyst E) and Pd/alumina (Catalyst F) yielded substantially higher deuterated impurity, *viz.* 27% and 15% respectively. It is concluded that $Pd/BaSO_4$ is the most suitable catalyst for yielding cleanly and specifically labelled compounds by this deuterium gas exchange procedure.

Tritium Exchange Reactions

Four tritium exchange reactions with glucose were carried out using conditions judged to be favourable on the basis of the deuterium experiments. In each case the tritium gas was added slowly to the $Pd/BaSO_4$ (catalyst B) and solutions were buffered at a pH of 7. Results are summarized in Table 6. The final two reactions listed in the table differed only in the rates of tritium addition to the catalyst. While this rate of addition was considered to be slow in each case (the tritium pressure was raised to <2 mm in 15 minutes) it was somewhat faster in the fourth reaction.

In all samples substantial labelling of the glucose was observed and only traces of labelled impurities, too low to estimate reliably from the ^3H NMR spectrum, were formed. The ^3H NMR spectra confirmed that all glucose activity was in the C-1 position. The α/β ratio of the two tritiated anomers was consistently about 35:65, in contrast with the accepted equilibrium value for glucose of 38:62,[13] possibly reflecting a small isotope effect in the equilibrium between the two species.

Table 6. Tritium Labelling of Glucose

Time h	Temp. °C	Glucose Activity Ci/mmole	%T C-1-glucose %/mole	%T water %/mole H	Impurity Activity % of C-1
6.0	25	6.4	22.3	0.80	<2
4.0	60	18.3	63.6	0.13	<2
6.0	60	22.5	78.2	0.22	<2
6.0	60	23.8	82.8	0.58	<2

In support of the deuterium study, higher levels of exchange were observed at 60 °C but the approach to equilibrium after 6 hours reaction time (75-80%) appeared to be consistently lower than that for deuterium exchange (100%) under equivalent conditions. The extent of exchange of tritium into water varied somewhat between samples and was about two orders of magnitude less (per mole of substance) than exchange into glucose. The reason for the variation between samples was not apparent, but it was noted that the experiments in which the $Pd/BaSO_4$ darkened fastest during tritium addition were those which yielded the higher water exchange.

Conclusions

It has been demonstrated that it is possible to produce glucose-1-^{3}H with close to full tritiation of the C-1 position by tritium gas exchange if appropriate reaction conditions are selected. It is assumed that these selected conditions would apply to the high specific activity labelling of other substrates previously shown to be amenable to this useful labelling technique.[1]

The results have revealed the following features of these deuterium and tritium labelling reactions:

1. The rate of catalyst reduction with deuterium or tritium gas is critical to the catalytic activity. Slow addition of gas over a period of several minutes is necessary to maintain high catalyst activity and to minimize the isotope incorporation into the solvent water. This has probably been the least controlled parameter in previous experiments with this catalytic system.
2. $Pd/BaSO_4$ is the most successful catalyst for clean labelling of glucose in the C-1 positions. There is little difference between the various sources of $Pd/BaSO_4$ when optimum conditions are chosen.
3. A choice of pH conditions in the region of 7 to 9 is necessary, and slightly basic conditions may be advantageous over neutral conditions.
4. An exchange temperature of 60 °C appears to ensure a high degree of labelling in glucose without serious loss of isotope to the solvent water.
5. The time span of the exchange reaction should be in the region of 6 to 8 hours to maximize labelling at 60 °C. There appears to be no justification for stopping the reaction at 2 hours, as has been the common and recommended procedure in the past.
6. Low exchange results for glucose under less favourable conditions are usually not the result of preferential incorporation of the isotope into the solvent water, but are the result of low catalytic activity towards both glucose and water exchange.
7. The level of incorporation of isotope into the observed impurity is independent of time and temperature, and remains a very minor product, under the range of conditions studied.

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References

1. Evans E.A., Sheppard H.C., Turner J.C. and Warrell D.C. — J. Labelled Compd., 10: 569-587 (1974)
2. Garnett J.L. and Long M.A. — "Catalytic Exchange Methods of Hydrogen Isotope Labelling", in Isotopes in the Physical and Biomedical Sciences, Vol. 1; Labelled Compounds (Part A), E. Buncel and J.R. Jones (Eds.), Elsevier: Amsterdam, 86-121 (1987)
3. Williams P.G. — "Tritium NMR Studies of Hydrogen Isotope Exchange Reactions", in Isotopes in the Physical and Biomedical Sciences, Vol. 2; Isotopic Applications in NMR Studies, E. Buncel and J.R. Jones (Eds.), Elsevier: Amsterdam, 55-98 (1991)
4. Evans E.A. — Tritium and Its Compounds, Second Edition, Halsted Press, a Division of John Wiley & Sons, Inc., London (1974)
5. Elvidge J.A., Jones J.R., Mane R.B., Chambers V.M.A., Evans E.A. and Warrell D.C. — J. Labelled Compd. Radiopharm., 15: 141-151 (1978)
6. Akulov G.P., Kayumov V.G., Snetkova E.V. and Kaminskii Y.L. — Radioisotopy, 29: 128-134 (1988)
7. Akulov G.P., Snetkova E.V., Kaminskii Y.L., Kudelin B.K. and Efimova V.L. — Radiokhimiya, 33: 74-75 (1991)
8. Buchman O., Pri-Bar I. and Shimoni M. — J. Labelled Compd. Radiopharm., 14: 155-159 (1978)
9. Buchman O., Azran J., Shimoni M. and Pri-Bar I. — Synth. Appl. Isot. Labelled Compd. 1988, Proc. Int. Symp., T.A. Baillie and J.R. Jones (Eds.), Elsevier: Amsterdam, 129-134 (1989)
10. Williams P.G., Than C., Rabbani S., Long M.A. and Garnett J.L. — J. Labelled Compd. Radiopharm., 36: 1-14 (1995)
11. Williams P., Morimoto H., Gehring K.B., Nikaido H., Carson P., Un S., Klein M. and Wemmer D.E. — Synth. Appl. Isot. Labelled Compd. 1988, Proc. Int. Symp., T.A. Baillie and J.R. Jones (Eds.), Elsevier: Amsterdam, 487-492 (1989)
12. Landvatter S.W., Heys J.R. and Senderoff S.G. — J. Labelled Compd. Radiopharm., 24: 389-396 (1987)
13. Angyal S.J. — Adv. Carbohydr. Chem. Biochem., 42: 15-68 (1984)
14. Newmark R.D., Un S., Williams P.G., Carson P.J., Morimoto H. and Klein M.P. — Proc. Natl. Acad. Sci. U.S.A., 87: 583-587 (1990)