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Synthesis of tritium-labelled biologically important diazines

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Abstract. The methods for the introduction of tritium (by chemical synthesis and isotope exchange in both solution and solid state) into biologically active diazines, *viz*., nucleic acid components (heterocyclic bases, nucleosides, nucleotides), DNA synthesis terminators and cytokinins are considered. The bibliography includes 178 references.

I. Introduction

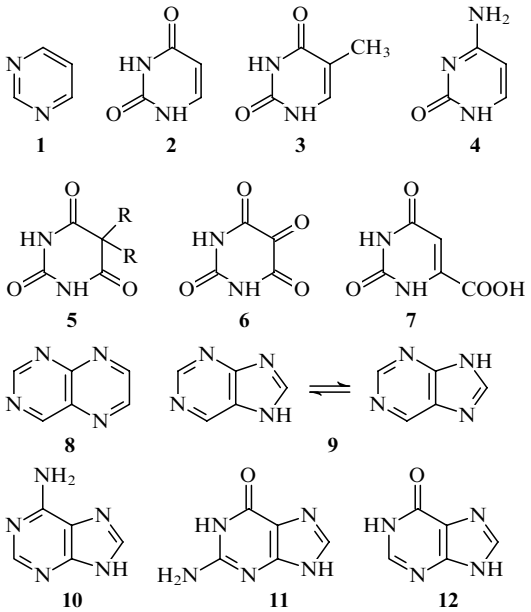
Recent progress in molecular biology, biochemistry, molecular genetics and experimental medicine are largely determined by the possibility of wide use of various radioactively labelled compounds. The application of such compounds has allowed direct studies of the mechanisms of chemical and biochemical reactions, as well as metabolic pathways in living organisms (*in vivo*) and in open systems (*in vitro*). Determination of quantitative characteristics of biochemical processes with the aid of labelled compounds is important for understanding of the essential features of processes occurring in living organisms and elucidation of the regulatory mechanisms underlying metabolism.

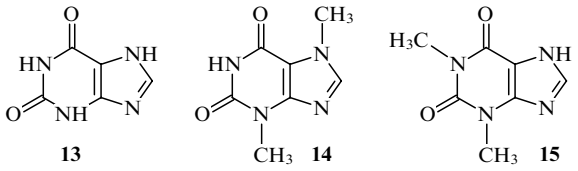
For over three decades now, researchers have been showing unremitting interest in tritium-labelled compounds. This is due to the valuable physical properties of tritium: its half-life period is sufficiently long for conducting experiments of virtually any duration; the low energy of tritium β-particles (E_{max} = 18.6 keV) determines low radiotoxicity of tritium-labelled compounds, which makes it possible to handle relatively large quantities of this isotope without special protective measures. Substitution of one tritium atom for hydrogen yields a compound with a molar radioactivity (A_{mol}) of 29.12 Ci mmol^{-1}. The use of such preparations and modern scintillation counters allows detection of $< 10^{-14}$ mol of a labelled compound.

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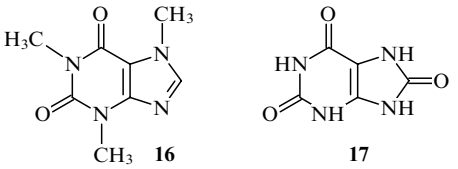
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Pyrimidine (**1**) is the most important compound among the diazines, since its derivatives, *viz*., uracil (**2**), thymine (**3**), and cytosine (**4**), form part of nucleic acids. Barbiturates (**5**), alloxan (**6**) and orotic acid (**7**) are also pyrimidine derivatives and contain no condensed rings. Among the polycyclic derivatives of pyrimidine, like pteridine (**8**), purine (**9**) deserves to be mentioned. Its derivatives, adenine (**10**) and guanine (**11**), also form part of ribo- and deoxyribo-nucleic acids. Other purine derivatives, such as hypoxanthine (**12**), xanthine (**13**), theobromine (**14**), theophylline (**15**), caffeine (**16**), and uric acid (**17**) are important natural compounds, which show biological activity. 2′-Deoxyribose (**18**) and ribose are linked to nitrogen bases through the N-glycosidic bond. These compounds have been called deoxyribonucleosides (**18a**) and ribonucleosides (**18b**), respectively, and the phosphorus esters of nucleosides have been termed nucleotides (**18c – 18e**). We have thus grouped the natural diazines under the common name of 'components of nucleic acids'. The number of such compounds for the main bases (**2 – 4**, **10**, **11**) of nucleic acids is more than 40.

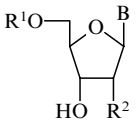




13 **14** **15**



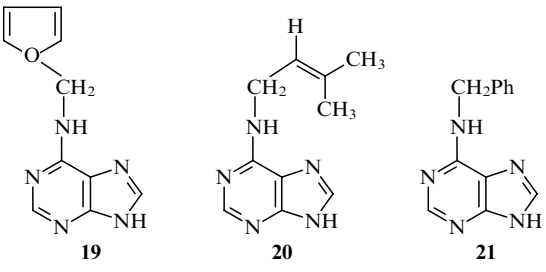
16 **17**



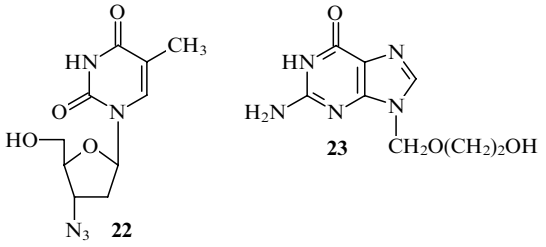
18a–e

18a: $R^1 = H$, $R^2 = H$;
18b: $R^1 = H$, $R^2 = OH$;
18c: $R^1 = H_2PO_3$, $R^2 = H, OH$;
18d: $R^1 = H_3P_2O_5$, $R^2 = H, OH$;
18e: $R^1 = H_4P_3O_7$, $R^2 = H, OH$.

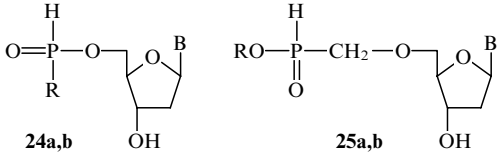
In recent years, considerable attention is being devoted to the so-called cytokinins, which are derivatives of 6-aminopurine, discovered in the 1950s. The cytokinins are natural substances that promote germination and blossoming of plants and retard their wilting. When used at concentrations $10^{-3}-10^{-5}$ mol litre^{-1}, they stimulate cell division. The principal cytokinins are kinetin (**19**), zeatin (**20**), benzylaminopurine (**21**) and a number of other compounds.



19 **20** **21**



22 **23** $CH_2O(CH_2)_2OH$



24a,b **25a,b**

24a: R = H;
24b: R = Me

25a: R = H;
25b: $R = H_2PO_3$

Analogues of nucleosides with the modified glycoside ring often appear to be DNA synthesis terminators. Azidothymidine (**22**), acyclovir (**23**), nucleoside phosphinates **24a** and nucleoside methylphosphinates **24b**, 5′-*O*-hydrohydroxyphosphoryl-2′-deoxynucleosides **25a** and the corresponding nucleotides **25b** may be cited as examples. They are potential inhibitors of retroviruses, including the HIV virus.[1,2]

This review considers methods used for the preparation of the above-mentioned compounds labelled with tritium. The main attention is centred on the general methods allowing synthesis of the widest set of compounds with maximum molar radioactivity.

II. Dehalogenation in solution

The reaction of catalytic hydrogen-for-halogen replacement (hydrogenolysis) has been known for a long time;[3] however, its use for the synthesis of tritiated compounds has come into practice relatively recently.[4,5] Catalytic dehalogenation is usually carried out in air-tight vessels with vigorous stirring of a suspension of a catalyst in solution of a halogenated compound under gaseous tritium. In this type of reaction, both polar and nonpolar solvents are employed. The tritium halide formed in this reaction must be neutralised since it poisons the catalyst, which leads to deceleration of the reaction.[6] The mechanism of tritium incorporation into the solvent under conditions of catalytic dehalogenation (catalysts PdO, $Pd/BaSO_4$, Raney nickel) has been considered.[7]

Catalysts of hydrogenation (mainly, supported Pd) are used for dehalogenation. The conditions for catalytic dehalogenation for the synthesis of tritium-labelled compounds have been published.[8,9] Examples of precursors and target compounds obtained by dehalogenation are given in Table 1.

Table 1. Results of halogen–tritium replacement in purines and pyrimidines.

Precursor	Target compound	A_{mol} (see [a])	Ref.
5-Iodocytidine	[5-^{3}H]cytidine	17.4	10
8-Bromoadenine	[8-^{3}H]adenine	15–20	11
2-Chloroadenine	[2-^{3}H]adenine	20–25	12
2,8-Dichloroadenine	[2,8-$^3H_2$]adenine	25–50	13
5-Iodoorotic acid	[5-^{3}H]orotic acid	15.6	13
5-Iododeoxyuridine	[5-^{3}H]deoxyuridine	26.4	13
5-Iodouracil	[5-^{3}H]uracil	15–20	14
5-Iodouridine	[5-^{3}H]uridine	15–20	14
5-Iodo-6-azauracil	[5-^{3}H]6-azauracil	15–20	15
5-Iodo-6-azauridine	[5-^{3}H]6-azauridine	15–20	15
8-Bromoadenosine	[8-^{3}H]adenosine	11.6–19	16, 17
8-Bromoguanosine	[8-^{3}H]guanosine	5.4–8	16, 17
8-Bromoinosine	[8-^{3}H]inosine	15	17
8-Bromo-AMP	[8-^{3}H]AMP	10	18, 19
8-Bromo-GMP	[8-^{3}H]GMP	15	20
5-Bromo-UMP	[5-^{3}H]UMP	10	21
8-Bromo-cAMP	[5-^{3}H]cAMP	17	22
5,6-Dibromouracil	[5,6-$^3H_2$]uracil	45–50	23, 24
5-Bromouracil	[5-^{3}H]uracil	20–30	25
6-Chlorouracil	[6-^{3}H]uracil	20–30	25
5-Bromocytosine	[5-^{3}H]cytosine	20–30	25
8-Bromo-substituted purine nucleotides	[8-^{3}H]purine nucleotides	20–30	25
5-Bromo-substituted pyrimidine nucleotides	[5-^{3}H]pyrimidine nucleotides	20–30	25

Notes. Abbreviations: AMP, adenosine 5′-monophosphate; GMP, guanosine 5′-monophosphate; UMP, uridine 5′-monophosphate; cAMP, adenosine 3′,5′-cyclomonophosphate.
[a] Dimension/ Ci mmol^{-1}.

Synthesis of a tritium-labelled compound **10** has revealed[11] that dehalogenation in aprotic solvents (dimethylformamide, dimethyl sulfoxide, dioxane) in the presence of catalysts (Pd black, Pd on carbon and on $BaSO_4$) has no advantages over the reaction in a protic solvent (aqueous–ethanolic solution): in both cases the maximum radioactivity is not attained. In the introduction of a tritium label into adenosine and guanosine, the rate of dehalogenation of the corresponding 8-bromo-compounds in 1 M KOH and 0.1 M HCl was higher than that in water; however, the

yields of labelled compounds and their molar radioactivity were larger in alkaline solution.[16] Table 1 presents data that characterise the synthesis of specific compounds. This reaction was not investigated as a method for the synthesis of tritiated diazines. In particular, the isotope effects of hydrogen, the ratio of reactants, the nature of reagents and the activity of catalysts have not been found to influence the yield and the molar radioactivity of the target compounds. Hydrogenolysis has been investigated as a method for the incorporation of a tritium label into diazines.[26–31] Hydrogenolysis of various compounds was conducted in the same solvents and with the same parameters such as tritium pressure, solvent, temperature, concentration of halogenated compound and reaction time. The basic characteristics of hydrogenolysis with tritium of bromo-substituted purines [26] and pyrimidines [27] are presented in Table 2.

Table 2. Basic characteristics of catalytic dehalogenation with tritium of 8-bromosubstituted purines and 5-bromo-substituted pyrimidines on supported Pd as the catalyst.[26, 27]

Labelled compound	Tritium concentration (%)	Yield (%)	A_{mol} / Ci mmol^{-1}
[8-^{3}H]Adenosine	85	19.5	9.8
[8-^{3}H]AMP	85	49.0	6.2
[8-^{3}H]AMP–TOA [a]	98	28.0	16.7
[8-^{3}H]cAMP	95	26.7	21.6
[8-^{3}H]dAMP	75	61.0	7.3
[8-^{3}H]Guanosine	98	45.4	12.7
[8-^{3}H]GMP	80	46.2	11.0
[8-^{3}H]GMP–TOA [a]	98	4.1	0.8
[8-^{3}H]cGMP	70	55.0	5.5
[8-^{3}H]dGMP	98	29.0	14.0
[5-^{3}H]Uracil	80	79.0	18.0
[5-^{3}H]Uridine	90	21.0	21.0
[5-^{3}H]Uridine	70	65.0	13.0
[5-^{3}H]UMP	90	96.0	3.7
[5-^{3}H]UMP–TOA [a]	98	80.0	19.0
[5-^{3}H]2′-Deoxyuridine	85	92.0	21.2
[5-^{3}H]dUMP	75	43.0	8.6
[5-^{3}H]cUMP	95	61.0	12.2
[5-^{3}H]CMP	70	34.0	1.1
[5-^{3}H]CMP–TOA [a]	70	89.0	9.3
[5-^{3}H]CMP–TOA [a]	98	74.0	25.1
[5-^{3}H]dCMP	95	74.0	18.0
[5-^{3}H]cCMP	95	54.0	18.1

Notes. Abbreviations: dAMP, 2′-deoxyadenosine 5′-monophosphate; cGMP, guanosine 3′,5′-cyclomonophosphate; dGMP, 2′-deoxyguanosine 5′-monophosphate; dUMP, 2′-deoxyuridine 5′-monophosphate; cUMP, uridine 3′,5′-cyclomonophosphate; CMP, cytidine-5′-monophosphate; dCMP, 2′-deoxycytidine-5′-monophosphate; cCMP, cytidine 3′,5′-cyclomonophosphate.

[a] Trioctylammonium salt (TOA).

The processes occurring in the hydrogen–palladium–water system with participation of hydrogen isotopes (protium and tritium) and their influence on the molar radioactivity (A_{mol}) of hydrogenolysis products have been investigated.[27] It was pointed out that one of the major reasons for the decrease in A_{mol} of the target compounds is the dilution of tritium with protium due to isotope exchange with the solvent protons and high values of protium–tritium partition coefficients (α_{H-T} reaches 12). The processes with different kinetics are analysed below.

1. The rate of the main reaction (dehalogenation, hydrogenation, isotope exchange) is much higher than that of tritium isotope exchange with the solvent (tritium is virtually undiluted with protium). Here, two extreme cases are possible: (*a*) if the contribution from the reaction of homomolecular isotope exchange may be disregarded, the molar radioactivity of the reaction product is equal to the A_{mol} of the initial tritium with consideration of the isotope effects of hydrogen on dissolution and adsorption on palladium (for this process $\alpha_{H-T} = 2.5$); (*b*) if an isotope equilibrium is established on palladium, it is necessary to take into account the reaction of monomolecular isotope exchange ($\alpha_{H-T} = 4.7$ or 3.6 for 50% tritium).

2. The rate of the main reaction is comparable or lower than the rate of isotope exchange of tritium with the solvent, *i.e.*, continuous dilution of tritium with protium takes place. Examination of this process requires consideration of the isotope equilibrium in the hydrogen–palladium–water system ($\alpha_{H-T} = 12$). Table 3 presents data that illustrate the effect of tritium concentration on the molar radioactivity of labelled products obtained by the reaction in an aqueous solution for different protium–tritium partition coefficients. The extreme values of A_{mol} (2.2 and 28.3 Ci mmol^{-1}) were calculated for the extreme cases. Hence, the increase in the molar radioactivity of purine and pyrimidine compounds is related to minimisation of the effect of hydrogen isotope partition on palladium in the course of catalytic dehalogenation in solution.

Table 3. Effect of isotopy on the molar radioactivity of labelled products of hydrogenolysis in aqueous solution.

[^{3}H] (%)	A_{mol} (Ci mmol^{-1}) for different α_{H-T} values			
	2.5	3.6	4.7	12
99	28.3	–	27.8	26.0
95	25.4	–	23.3	17.8
90	22.2	–	19.1	12.5
85	19.5	–	15.9	9.3
80	17.1	–	13.4	7.3
75	15.1	13.2	–	5.8
70	13.3	11.5	–	4.7
60	10.2	8.6	–	3.2
50	7.7	6.3	–	2.2

One of the ways of minimisation is enhancement of the rate of the main dehalogenation reaction, which can be achieved using more active palladium catalysts. The A_{mol} can increase with the greater palladium/bromo-substituted compound ratio. This is characteristic mostly of purine compounds.

Yet another method for the increase in molar radioactivity is the use of catalysts with a low (0.01%–0.05%) percentage of supported palladium.[31] In such catalysts, virtually no dissolution of the hydrogen isotopes occurs, and the hydride form is absent. If the protium–tritium partition coefficient is assumed to be equal to 2.5, the molar radioactivity of the dehalogenation product with 95% tritium must reach 25.4 Ci mmol^{-1}, which is consistent with the experimental data.[31] In practice, low-percentage supported palladium catalysts are rarely employed, since satisfactory yields of the target products are obtained at definite precursor/catalyst ratios in the reaction mixture (approximately 1 g of a catalyst per 1 mg of a precursor). This requires a larger volume of the solvent and, consequently, leads to a greater volume of the reaction tube, increased uptake of gaseous tritium, more difficult stirring of the reaction mixture, *etc*.

Gordeeva *et al*.[32] have described the synthesis of nucleoside 5′-triphosphates labelled with tritium in position 5 of the pyrimidine ring and in position 8 of the purine ring by catalytic dehalogenation of the corresponding bromo-substituted precursors.[32] The A_{mol} values of the compounds obtained are presented in Table 4. As a rule, the tritium-labelled nucleoside 5′-triphosphates have higher molar radioactivities than the nucleoside 5′-monophosphates prepared under similar conditions. These results correlate with the suggested [30] interrelation of such parameters as the sorption degree of the initial compounds on the catalyst and

Table 4. Results of catalytic dehalogenation of bromo-substituted nucleoside 5′-triphosphates with gaseous tritium.[32]

Initial compound	Catalyst	A_{mol} / Ci mmol^{-1}
8-Bromo-ATP	5% Pd/$BaSO_4$	17
8-Bromo-dATP	5% Pd/$BaSO_4$	15
	α-Pd	18
8-Bromo-GTP	5% Pd/$BaSO_4$	11
	α-Pd	19
8-Bromo-dGTP	5% Pd/$BaSO_4$	5
	α-Pd	18
5-Bromo-UTP	5% Pd/$BaSO_4$	17
	α-Pd	23
5-Bromo-dUTP	5% Pd/$BaSO_4$	12
	α-Pd	14
5-Bromo-CTP	5% Pd/$BaSO_4$	21
5-Bromo-dCTP	5% Pd/$BaSO_4$	18

Notes. Abbreviations: ATP, adenosine 5′-triphosphate; dATP, 2′-deoxyadenosine 5′-triphosphate; GTP, guanosine 5′-triphosphate; dGTP, 2′-deoxyguanosine 5′-triphosphate; UTP, uridine 5′-triphosphate; dUTP, 2′-deoxyuridine 5′-triphosphate; CTP, cytidine 5′-triphosphate; dCTP, 2′-deoxycytidine 5′-triphosphate.

the molar radioactivity, as well as the dependence of the latter on the ratio of the rates of dehalogenation and heterogeneous isotopic exchange of gaseous tritium with water.

Catalytic dehalogenation of respective bromo-substituted precursors has been employed for the synthesis of the following compounds:[33–40]

Compound	A_{mol} / Ci mmol^{-1}
[5-^{3}H]2′,3′-Dideoxycytidine	–
[8-^{3}H]ATP	–
[2-^{3}H]ATP	26.2
[5-^{3}H]dCTP	15.0
[6-^{3}H]Thymine	–
[2-^{3}H]Adenine	12.7
[2-^{3}H]Adenosine	–
[8-^{3}H]9-[(2-Hydroxyethoxy)methyl]guanine	25.0
[5-^{3}H]1-β-D-Arabinofuranosylcytosine	20.0
1-([3-^{3}H]Tetrahydrofuryl-2)-5-fluorouracil	29.7
[8-^{3}H]9-β-D-Arabinofuranosyladenine	20.0
5-Amino-7-(2[2′,3′,5′-$^3H_2$]phenylethyl)-2-(2-furyl)pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine	68.6

Tritium-labelled purine and pyrimidine nucleoside phosphates have been prepared by catalytic dehalogenation of respective bromo-substituted precursors with gaseous tritium.[41]

Baraldi *et al*.[42] have synthesised three tritiated inhibitors of reverse transcriptase by hydrogenolysis of bromobenzooxazole precursors. The molar radioactivity of the compounds obtained was 3 – 10 Ci mmol^{-1}.

In the process of dehalogenation, tritium can be incorporated not only into the heterocyclic base but also into the carbohydrate moiety of the molecule. Thus dehalogenation of the corresponding precursor has yielded [2′,3′-^{3}H]2′,3′-dideoxythymidine 5′-phosphate.[43]

The experimental procedure for the catalytic dehalogenation in solution is relatively simple and the products have rather high molar radioactivity. In these products, the tritium label is as a rule in a definite position determined by the replacement of the halogen atom by tritium. With a set of halogenated precursors at disposal, this reaction can be used for the synthesis of a series of the required tritium-labelled compounds. A disadvantage of this method is the necessity to synthesise special halogenated precursors. For example, in order to prepare [2-^{3}H]ATP it was necessary to carry out a six-stage synthesis of 2-bromo-ATP from guanosine.[35]

III. Catalytic dehalogenation without a solvent

The solvent-free method of catalytic dehalogenation has been proposed for the synthesis of ^{3}H-labelled uracil.[44] For this purpose, 5-iodouracil applied onto a Pd catalyst was treated with gaseous tritium under the conditions of a high-voltage and high-frequency discharge. This resulted in the formation of [5-^{3}H]uracil (A_{mol} = 16.1 Ci mmol^{-1}). Tritium-labelled thymine was prepared by the dehalogenation of 5-halogenomethyluracil with gaseous tritium over a Pd catalyst at 100 °C.[45] This method has been studied in detail in the synthesis of tritium-labelled components of nucleic acids and was called solid-state catalytic reductive dehalogenation (SCRD).[46] The SCRD reaction can be initiated in two ways, *viz*., by a high-voltage and high-frequency electric discharge [47] and heating from 50 to 150 °C.[48] The kinetics of SCRD has been investigated and its mathematical model has been proposed in which the catalyst was regarded as a consumable component of the reaction. The proposed model explains well the dependence of the yield and A_{mol} of the target compounds on the percentage of palladium on the support. Some kinetic characteristics of SCRD are presented in Table 5. The SCRD method was employed for the preparation of the following ^{3}H-labelled compounds:[46]

Compound	A_{mol} / Ci mmol^{-1}	Compound	A_{mol} / Ci mmol^{-1}
[5-^{3}H]Uracil	16.1	[8-^{3}H]2′-deoxyadenosine	16.4
[Methyl-^{3}H]thymine	3.1		
[8-^{3}H]Guanine	12.4	[8-^{3}H]AMP	4.9
[5-^{3}H]Cytidine [a]	16.3	[8-^{3}H]GMP	5.2
[8-^{3}H]Guanosine	6.7	[5-^{3}H]UMP	2.8
[8-^{3}H]Adenosine [a]	10.6	[5-^{3}H]CMP	5.9
[5-^{3}H]Uridine [a]	11.5	[8-^{3}H]dAMP	2.1
[5-^{3}H]2′Deoxyuridine [a]	18.5	[8-^{3}H]dGMP	1.6
		[8-^{3}H]dCMP	1.7
[5-^{3}H]Deoxycytidine [a]	33.0		

[a] Degradation products of the corresponding nucleotides.

If SCRD is carried out at elevated temperatures, some by-products are produced along with the target compounds due to the cleavage of the phosphoester and N-glycosidic bonds. The molar radioactivity of degradation products is higher than that of the target compound, which seems to be related to molecular excitation upon cleavage of these bonds. The results obtained indicate that the A_{mol} of the compounds does not reach its theoretical value on substitution of one halogen atom by tritium (29.12 Ci mmol^{-1}). This is explained [46] by dilution of the initial gaseous tritium by the mobile hydrogen present in the substance – catalyst system (hydrogen is capable of rapid isotope

Table 5. Kinetic parameters of SCRD of bromo-substituted components of RNA.[46]

Initial compound	Reaction rate constant (min^{-1} mmol^{-2}) at different temperatures (°C)					E/ cal mol^{-1}
	100	78	56	35	23	
8-Bromo-AMP	30.45	20.62	14.03	10.42	8.06	3380
8-Bromoguanosine	30.43	25.20	15.18	11.25	9.09	3670
5-Bromo-UMP	24.00	17.15	10.80	6.55	4.74	4760
5-Bromo-CMP	33.25	16.15	10.29	4.89	1.76	6470

exchange). In the solid-state process, some labile hydrogen is present in the compound to be labelled and in the catalyst itself.[46] It was found experimentally that 100 mg of 5% Pd/C catalyst contains 0.21 mmol of hydrogen capable of isotope exchange with tritium.

The SCRD process has not found wide practical usage for the synthesis of tritium-labelled diazines, since the reactions in solution are experimentally simpler and make it possible to obtain comparable, and often higher, A_{mol} values of the products. Nonetheless, the appearance of the SCRD method may be regarded as an important stage in the development of solid-state reactions involving gaseous tritium.

IV. Reactions of isotope exchange

There is a certain distinction between reactions of isotope exchange with tritium-containing solvents (including tritiated water) and catalytic reactions involving gaseous tritium. The reactions of catalytic isotope exchange are most often carried out using heterogeneous palladium catalysts,[49] homogeneous catalysts, such as trifluoromethanesulfonic acid,[50] rhodium trichloride and ruthenium acetylacetonate,[51] as well as catalysts composed of polymers [52, 53] and metals (Pt, Ni, Rh) applied to hydrophobic supports (Teflon, polyethylene, polystyrene).[54] The isotope exchange of activated aromatic protons in the $AcO^3H-3HCl$ solution has been investigated.[55] The catalytic role of metals and hydrogen ions has been discussed in Ref. 56.

1. Reactions with tritium-containing solvents

Reactions with a tritium-containing solvent, which is most often represented by tritiated water, are usually carried out in a strongly alkaline aqueous solution.[57] Werstiuk and Ju [58] have proposed a preparative method for the specific introduction of hydrogen isotopes into fused benzoheterocycles. This method is based on the isotope exchange with deuterated or tritiated water at a temperature above 145 °C. The methods for the preparation of heterocyclic and heteroaromatic compounds containing hydrogen isotopes have been reviewed.[59, 60]

The nucleic acid components exchange most readily the proton bound to the C(8) atom of the purine bases. The hydrogen bound to the C(2) atom is exchanged with a much greater difficulty. For example, by employing ^1H NMR spectroscopy (pD 6–7, 100 °C), Wong and Keck [61] have shown that the rate of isotope exchange of hydrogen at the C(8) atom of adenine is $\sim$ 1000 times as high as that at the C(2) atom.

Eidinhoff and Knoll [62] observed the exchange of the proton bound to the C(8) atom of adenine in the presence of a reduced platinum catalyst. The hydrogen isotope exchange has also been reported to occur on heating (> 90 °C) of purine and adenosine in D_2O.[63, 64] Such an exchange leads to the disappearance of the peak corresponding to signals for the protons at the C(8) atom in the ^1H NMR spectra of these compounds. IR spectroscopy of the isotope exchange in the purine base residues in the DNA macromolecule has also pointed to the preferential exchange of the C(8) hydrogen.[65]

Shelton and Clark [66] have used the reaction of isotope exchange for the incorporation of tritium into purine compounds. This reaction was conducted in an alkaline medium with tritiated water on heating to 100 °C. It was established that the guanine compounds were labelled more rapidly than the adenine ones. The exchange rate is increased in both bases as the pH is increased from 2 to 11. The conditions for tritium labelling are rather mild. This allowed one to apply them for the preparation of tritium-labelled nucleosides (adenosine, deoxyadenosine, and nucleotides), adenosine 3′,5′-cyclomonophosphate [67] and ATP.[68]

The kinetics of isotope exchange with water and the dependence of the rate constant of isotope exchange in ATP and GTP on pH have been ivestigated.[69] The kinetics of the incorporation of the tritium bound to the C(8) atom of purines of poly(A), poly(A)-poly(U) duplex, RNA, tRNA and DNA was studied by Gamble *et al.*[70] The reaction of tritium–hydrogen exchange has been used for the introduction of tritium into oligonucleotides,[71] oligodeoxyribonucleotides,[72] polyribonucleotides,[73] DNA,[74–77] $tRNA^{Ileu}$ and $tRNA^{Tyr}$ from yeast.[78] Drutsa [79] has proposed optimal conditions for the introduction of tritium into oligodeoxyribonucleotides: 2–4 h at 60 °C in tritiated water in an 0.3 M NaOH solution. Isotope exchange with tritiated water has been used for the preparation of an inhibitor of 5-lipoxygenation of arachidonic acid, which is an antiprostatic agent (A_{mol} = 19.8 Ci mmol^{-1}).[80] Mackenzie *et al.*[81] have investigated the isotope exchange of the hydrogen bound to the C(2) atom in ethyl 5-amino-1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)imidazole-4-carboxylate (pH 1.77–12.0, 65 °C).

The analysis of changes in the isotope exchange rate of protons at the C(8) atom of purine residues provided information about conformational changes in natural biopolymers such as polynucleotides [82–85] and RNA.[70, 86, 87] It has been established that the rate of hydrogen exchange at the C(8) atom in adenine, guanine and hypoxanthine in nucleic acids is comparable with the respective rates in nucleoside monophosphates.[88] In DNA, the protium–tritium exchange is approximately 2.4–2.8 times as slow as that in deoxynucleoside monophosphates.[89]

The study of the mechanism of isotope exchange with water in the C(8)H groups of purines has shown [87, 90, 91] that the exchange proceeds by the so-called ylide mechanism, *viz.*, not in neutral molecules, but in the protonated [at the N(7) atom] forms of purine derivatives and in zwitter-ions with a positive charge on the N(7) atom. Agranovich *et al.*[92] have studied the kinetics of protium–tritium exchange between water and the C(8)H group of purine bases, mono- and polynucleotides for different specific radioactivities of the medium over a wide temperature range (from 4 to 80 °C) and for different pH. It was shown that at high specific radioactivity of the medium, two non-competitive parallel exchange reactions occur simultaneously in the temperature interval studied: one follows the ylide mechanism and the other proceeds with participation of highly reactive radicals resulting from radiolysis of water under the action of β-radiation from tritium. The relative contribution from the radical exchange to the observed reaction rate decreases with higher temperature and lower specific radioactivity of the medium. In the case of polynucleotides and DNA, the isotope exchange follows basically the ylide mechanism at temperatures above 25 °C and in the case of nucleotide **18c** — above 37 °C (at a specific radioactivity of the medium of 1 Ci ml^{-1}). The increase in the medium acidity to pH 1 accelerates appreciably the radical exchange reaction, while the presence of β-mercaptoethanol decelerates it sharply. Changes in the observed hydrogen exchange rate in the C(8)H group of purine allow the estimation of variation of the pK_a of polynucleotides and nucleic acids caused by changes in the macromolecular conformation.[93]

The protons bound to the C(5) atom of pyrimidines are readily exchanged for tritium from the tritium-containing water in uracil photohydrates. The isotope exchange between 5,6-dihydro-6-hydroxyuridine 5′-phosphate and tritiated water with heating yields 5-tritium-substituted uridine 5′-phosphate.[94] Boer and Johns [95] have reported a method for the preparation of tritium-labelled 5,6-dihydro-6-hydroxyuridine 5′-phosphate on heating with tritiated water. Similar exchange was observed in the photohydrates of cytosine derivatives.[95] The reaction of hydrogen isotope exchange between uracil and tritiated and deuterated water proceeds only in the presence of Pt and Pd catalysts.[96, 97] In the absence of a catalyst, this reaction takes place only under very drastic conditions.[98–100] The exchange of C(5) hydrogen is markedly easier than that of the C(6) hydrogen. Wechter [101] has proposed conditions for isotope exchange allowing the selective deuteration of C(6) in pyrimidine nucleosides. This method consists in the synthesis of 5,6-dideuterio compounds and the reexchange of deuterium from position 6 under special conditions. An analogous method has been proposed by Rabi and Fox.[102] Isotope exchange of the hydrogen bound to the C(5) in pyrimi-

dines is accelerated in the presence of citrate buffer [103] and such compounds as 2-mercaptoethylamine, ethylamine, ethanolamine, sodium hydroxide,[104] hydrogensulfite anion,[105,106] methyl-, dimethyl- and trimethyl-amine hydrogensulfites, ethyl- and diethyl-amine, and imidazole,[107] cysteine and other sulfur-containing compounds, *e.g.*, 2-mercaptoethanol.[108,109] Under specific conditions, the reaction of nucleic acids with hydrogensulfite in the presence of tritiated water results in the transformation of the cytidine fragments into uridine fragments.[106] In all cases, the isotope exchange proceeds under mild conditions. Tritium is incorporated into uracil and cytosine derivatives in alkaline and acidic medium, respectively. These reactions can be used for the synthesis of tritium-labelled pyrimidine derivatives.

Hesk *et al.*[51] have described a method for homogeneous hydrogen isotope exchange under the action of $RhCl_3$ in dimethylformamide at 110 °C, which allows high regioselective introduction of tritium in the *ortho*-position of various aromatic acids, amides, amines and heterocyclic compounds. This study has also shown that in addition to $RhCl_3$, ruthenium acetylacetonate is another efficient catalyst of isotope exchange in the *ortho*-position of benzoic acids.

The reaction with tritium-containing solvents is a versatile method for the preparation of tritium-labelled compounds, diazines in particular. This method does not require synthesis of special precursors, and the conditions of tritium labelling are sufficiently mild to allow its use for the incorporation of this label into compounds of rather complex structure, *e.g.*, nucleic acids. When employing this reaction, the main attention should be paid to the choice of efficient homogenous or heterogeneous catalysts and solvents (tritium sources). A disadvantage of this method is a relatively low level of molar radioactivity of the target products. This is mainly determined by the specific radioactivity of the tritiated water used, which usually does not exceed 100–200 Ci ml^{-1} (3%–6% of its theoretical level). Tritiated water with higher specific radioactivity is unstable because of its autoradiolysis. Reactions of isotope exchange may be carried out with the tritiated water with very high specific radioactivity if it is mixed with an inert anhydrous organic solvent.[110] The isotope exchange with steroids has demonstrated [110] that the maximum A_{mol} is reached when a solution of ^3H_2O (2%–3%) in an inert solvent is used. This corresponds to a volume radioactivity of a solution of 65–100 Ci ml^{-1}. Solutions of highly active tritiated water were prepared by the oxidation of gaseous tritium over palladium oxide in an anhydrous organic solvent. Tritium-labelled benzylaminopurine has been obtained using highly active ^3H_2O (A_{mol} = 24 Ci $mmol^{-1}$).[111] The proposed method is laborious, while disadvantages such as relatively low levels of molar radioactivity (a few tens of Ci $mmol^{-1}$) and the necessity to use large volumes of tritiated water prevent its wide application for the synthesis of tritium-labelled components of nucleic acids. However, this method proves to be virtually the only one possible for compounds containing groups that can be readily reduced and/or hydrogenated (*e.g.*, steroids and pharmaceutical preparations [112]).

2. Catalytic liquid-phase heterogeneous isotope exchange with gaseous tritium

In 1974, two reports [113,114] were published simultaneously on the method of tritium labelling of organic compounds based on isotope exchange of gaseous tritium with hydrogen in the compound subject to labelling dissolved in an appropriate solvent in the presence of heterogeneous catalysts. Buchman *et al.*[113] have proposed a method of non-specific labelling with gaseous tritium in the presence of an activated platinum catalyst. The catalyst PtO_2 was more active than PdO in the isotope exchange reaction.[115] Evans *et al.*[114] conducted the isotope exchange reaction in a phosphate buffer in the presence of a $PdO/BaSO_4$ catalyst. Tritium was incorporated into carbohydrates, aliphatic and aromatic amino acids and nucleic acid components. Table 6 shows the conditions of isotope exchange reactions for the introduction of tritium into purine and pyrimidine compounds with this method. This is of a high practical value for the synthesis of tritium-labelled components of nucleic acids. Presumably, the isotope exchange proceeds by the free-radical mechanism and is facilitated for mobile hydrogen atoms, such as aromatic protons and hydrogen bound to the C(8) atom of purines. One could hardly expect high levels of molar radioactivity for the compounds with aliphatic protons, and it seems for protons combined with C(5) and C(6) of pyrimidines. Hanus *et al.*[116] have employed heterogeneous catalysts (PdO, PtO_2 and $Pd/BaSO_4$) in order to investigate the process of tritium incorporation into such compounds as dioxane, tetrahydrofuran, ethyl acetate, dimethylformamide, dimethylacetamide and acetic anhydride. It was shown that the proportion of tritium incorporated by the direct interaction with the solvent is negligibly small compared with that introduced in the presence of catalysts. The degree of tritium incorporation depends on the solvent purity. In extremely pure solvents, the tritium is present in the form of heavy water.

Table 6. Purine and pyrimidine compounds prepared by catalytic heterogeneous isotope exchange with gaseous tritium.[114]

Compound	Reaction conditions		A_{mol} / Ci $mmol^{-1}$
	pH	t/ min	
Arabinosyladenosine	8.2	240	5.6
Adenosine	8.2	120	12.0
cAMP	10.0	60	25.0
AMP	10.0	960	11.0
ATP	8.2	60	20.0
2′-Deoxyadenosine	8.2	210	17.0
2′-dATP	10.0	60	18.1
N^6,$O^{2'}$-Dibutyryl-cAMP	10.0	60	19.5
Guanosine	8.0	120	0.42
cGMP	8.0	20	0.88
GTP	8.2	30	1.5
IMP	8.0	5	1.2
	8.0	20	3.9
	8.0	60	2.7
	8.0	20	2.7
Nicotinamide	8.0	120	–
NAD	8.0	120	–
Puromycin	8.2	120	3.7
Puromycin aminonucleoside	8.2	120	13.7
Thymidine	8.0	60	5.0
	8.0	60	7.0
Uracil	8.0	60	9.0
	8.0	60	2.5

Notes. Abbreviations: IMP, inosine 5′-monophosphate; NAD, nicotinamide adenine dinucleotide.

The method of catalytic heterogeneous exchange with gaseous tritium has allowed synthesis of [^{3}H]acyclovir [39] and the following tritium-labelled compounds:[117–120]

Compound	A_{mol} / Ci $mmol^{-1}$
5-Azacytidine	17.4
5-Aza-2′-deoxycytidine	19.5
N^4-(5-Aminopentyl)cytidine	3.0
5-Methylcytidine	1.9
5-Methylcytosine	4.2
Arabinosyl-5-azacytosine	12.5
5-Hydroxy[6-^{3}H]uracil	0.35
5-Amino[6-^{3}H]uracil	2.4
1-β-D-Arabinofuranosyl[5,6-^{3}H]cytosine	12.0
9-[(2-Hydroxyethoxy)methyl][8-^{3}H]guanine	8.8
9-β-D-Arabinofuranosyl[8-^{3}H]adenine	1.0

Kaminskii *et al.*[38] have demonstrated that the use of AMP 1-*N*-oxide as the precursor facilitates significantly the tritium exchange

at the C(2) atom of the adenine ring. Introduction of tritium into adenine dinucleoside phosphates[121] and the P–H group of 2′-deoxyadenosine 5′-H-phosphonate has been described.[122]

The rate constants for liquid-phase catalytic heterogeneous protium–tritium exchange of [8-^{3}H] nucleotides of the adenine series with hydrogen[123] have been measured (the experimental procedure is given in Ref. 124). It has been established[125] that the exchange rate decreases in the following sequence: cAMP > ATP > ADP > AMP. An equation of the dependence of the rate constant for isotope exchange on pH has been deduced. At low concentrations of nucleotides, the reaction order with regard to nucleotide is 1, whereas at higher nucleotide levels it is close to 0.

Lacey *et al.*[126] have described a general method for specific introduction of tritium label into benzoimidazole carbamates (BIC), which includes catalytic isotope exchange in dioxane at 60 °C under gaseous tritium. The yield of labelled BIC was found to range from 8% to 70% with the molar radioactivity varying from 0.44 to 13.4 Ci mmol^{-1}. The use of gaseous tritium in the presence of a catalyst based on iridium complexes has made it possible to obtain high levels of molar radioactivity for benzophenones, quinolines, 2-aminopyridines, 2-aminobenzooxazole derivatives, benzoazepinone amides and complex benzamides.[127]

It is noteworthy that the reactions of catalytic dehalogenation and catalytic heterogeneous isotope exchange proceed under similar conditions. This fact should be taken into account in conducting catalytic reactions of hydrogenation, dehalogenation, *etc*. The tritium label can be introduced by both a chemical reaction and isotope exchange. For example, dehalogenation of monobromopapaverine on a palladium catalyst yielded a product with A_{mol} = 43 Ci mmol^{-1} (see Ref. 128). In this case, the percentages of the label due to the tritium substitution for bromine and the exchange of the methylene group proton with tritium were 49% and 46%, respectively, with 5% of the tritium introduced in other positions. A similar process takes place on dehalogenation of 2-halogeno-purines: tritium substitutes hydrogen at the C(2) atom owing to dehalogenation reaction and at the C(8) atom owing to isotope exchange.[38]

This reaction underlies the most versatile method used for the preparation of tritium-labelled compounds. As regards its general character, the method described is comparable to the isotope exchange with a tritium-containing solvent. The main advantage of this method consists in high levels of molar radioactivity of the compounds obtained. This is due to the fact that the isotope source is a high-percentage (> 95%) gaseous tritium whose molar radioactivity is higher than that of any tritium-containing solvent. A disadvantage of this method is that processes of dehalogenation, hydrogenation and reduction of various functional groups can take place under given reaction conditions.

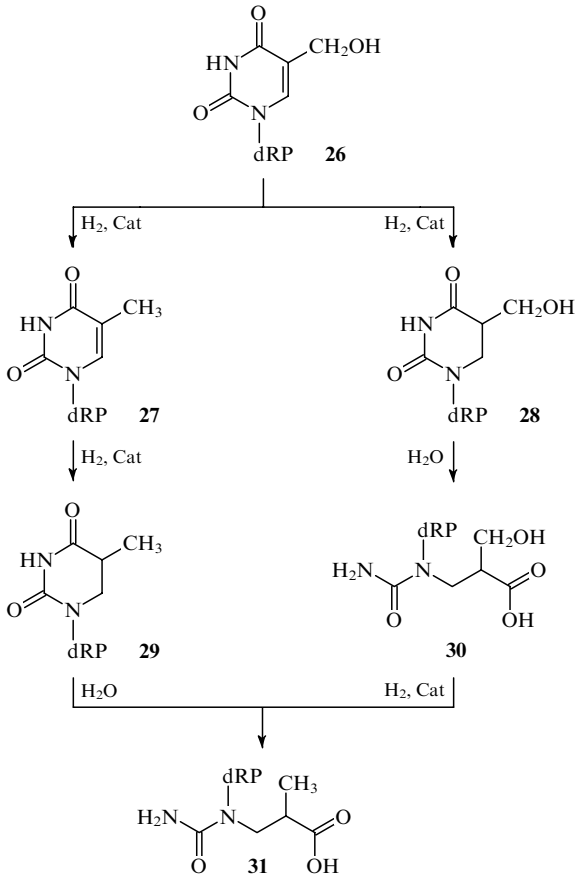
V. Reduction and hydrogenation

Processes of reduction and hydrogenation are used mostly for the introduction of tritium into the methyl group of thymine and its derivatives, and also into the carbohydrate moiety of nucleosides. Reduction is usually performed by means of catalytic reactions using gaseous tritium and tritium-labelled $NaBH_4$ and $LiAlH_4$. Coates *et al.*[129] have proposed to use lithium triethylborotritide (super-tritide) obtained by the reaction of LiT with Et_3B for the incorporation of tritium into various compounds by the reduction of certain functional groups.

Tritium-labelled thymidine (A_{mol} = 27.5 Ci mmol^{-1}) was prepared by the reduction of 5-hydroxymethyl-2′-deoxyuridine in the presence of rhodium on alumina as a catalyst.[130] Tritium-labelled 5-hydroxymethyl-2′-deoxyuridine was synthesised by the reduction of 5-formyl-2′-deoxyuridine in a phosphate buffer.[131]

Hydrogenation of 5-hydroxymethyl- and 5-formyluracil in the presence of a palladium catalyst yielded [132, 133] thymine with up to 2 tritium atoms in the methyl group. Successful application of Raney nickel for the reduction of 5-formyluracil to 5-hydroxymethyluracil has been reported.[134] Hydrogenation of 5-formyluracil in water with gaseous tritium over 1.5% Pd/$BaSO_4$ produced 5-hydroxymethyl[hydroxymethyl-^{3}H]uracil (A_{mol} = 64.8 Ci mmol^{-1}).[135] Further reduction of the latter with tritium over palladium in dry dioxane yielded [methyl-^{3}H]thymine.[136] In order to prepare thymine with the complete substitution of hydrogen by tritium in its methyl group, Yakovleva *et al.*[137] carried out catalytic reduction of 5-cyanouracil to aldimine followed by its hydrolysis to 5-formyluracil with subsequent catalytic reduction with gaseous tritium. Reduction of 5-formyluracil ethylene dithioacetal with tritium in dioxane over Raney nickel also led to the formation[138] of [methyl-^{3}H]thymine (A_{mol} = 11.6 Ci mmol^{-1}). Catalytic reduction of 5-formyluracil with gaseous tritium is described in Ref. 139.

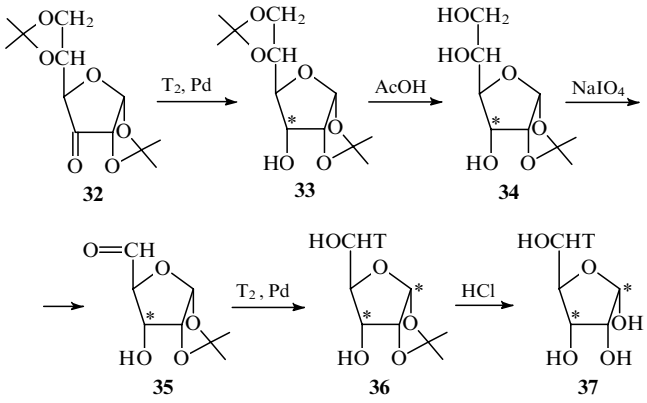
One-stage methods for the preparation of tritium-labelled thymidine 5′-monophosphate (TMP, **27**) have been reviewed.[140] The precursors of TMP were 5-hydroxymethyl-dUMP (**26**) and 5-formyl-dUMP. It was established that under specific conditions (depending on the nature of catalyst employed and the reaction medium) hydrogenation can affect the 5,6-double bond of the pyrimidine ring and lead to the formation of 5-hydroxymethyl-5,6-dihydro-dUMP (**28**), 5,6-dihydroTMP (**29**), 2′-hydroxy-3-(N^1-2′-deoxy-5′-phospho-β-D-ribofuranosyl)ureidoisobutyric acid (**30**), and 3-(N^1-2′deoxy-5′-phospho-β-D-ribofuranosyl)ureidoisobutyric acid (**31**).



The general method used for the synthesis of nucleosides labelled with tritium at the 5′-atom of the carbohydrate residue is based on the reduction of β-D-ribopentodialdo-1,4-furanosides of respective nucleic bases with gaseous tritium or sodium boro[^{3}H]hydride. This method was used for the preparation of compounds labelled at the 5′-position, such as 9-β-arabinofuranosyladenine,[141] thymidine,[138] 2′,3′-*O*-isopropylideneadenosine.[142] In order to prepare adenosine, guanosine and uridine labelled with tritium at C(5′), Akulov *et al.*[143] conducted the reduction reaction using gaseous tritium in the presence of a catalyst as well as sodium boro[^{3}H]hydride. Synthesis of [5′-^{3}H]3′-azido-3′-deoxythymidine (A_{mol} = 14 Ci mmol^{-1}) was carried out

by the oxidation of its precursor to 5′-aldehyde and subsequent reduction with NaB^3H_4.[144] Introduction of deuterium and tritium in the positions 5′ and 4′ of 2′-deoxynucleosides is described in Ref. 145. For this purpose, the corresponding 5′-aldehydes were prepared from deoxyguanosine, deoxycytidine and thymidine by the Moffatt method and then reduced with $NaBD_4$ to form 5′-deuterionucleosides. Heating of 5′-aldehydes in a D_2O-Py mixture (1 : 1) with subsequent reduction yielded 4′-deuteronucleosides. It is pointed out that the use of a similar procedure for the tritiation yields labelled compounds with low molar radioactivity. A recent US patent[146] describes a method of tritium introduction into oligodeoxyribonucleotides in which an immobilised oligonucleotide with a free 5′-hydroxy group is oxidised with an appropriate agent to aldehyde and then reduced, *e.g.*, with NaB^3H_4, to give a tritium-labelled 5′-hydroxymethyl group. Riboflavin (vitamin B_2) selectively labelled with tritium in the position 5′ was prepared[147] by the reduction of the 5′-aldehyde group with sodium boro[^{3}H]hydride. Synthesis of [ribose-2,3-^{3}H]2′,3′-dideoxycytidine phosphonate,[43] [ribose-2,3-^{3}H]2′,3′-dideoxyadenosine and [ribose-2,3-^{3}H]2′,3′-dideoxyinosine[33] was carried out by catalytic hydrogenation of the corresponding unsaturated analogues with gaseous tritium.

The key compound in the synthesis of tritium-labelled nucleotides and nucleosides is D-ribose. The use of tritium-labelled D-ribose and heterocyclic bases in enzymic reactions[12, 24, 148, 149] allows synthesis of nucleosides and nucleotides containing tritium in the ribose and heterocyclic moieties of the nucleoside and nucleotide molecules. We have proposed the following scheme for the synthesis of tritium-labelled D-ribose: reduction of 1,2:5,6-di-*O*-isopropylidene-α-D-ribohexofuranos-3-ulose (**32**) by tritium to 1,2:5,6-di-*O*-isopropylidene-α-D-allofuranose (**33**), oxidation of 1,2-*O*-isopropylidene-α-D-allofuranose (**34**) to 1,2-*O*-isopropylidene-α-D-ribopentodialdofuranose (**35**) and reduction of the latter to 1,2-*O*-isopropylidene-α-D-ribofuranose (**36**) combined with the hydrogen isotope exchange at the C(1) atom.



(The asterisks indicate the sites of tritium incorporation).

These consecutive reactions make it possible to synthesise the tritium-labelled D-ribose (**37**) with up to 3 tritium atoms.

VI. Solid-state catalytic hydrogenation

The method of solid-state catalytic hydrogenation (SCH) was proposed in 1967. Dehalogenation of 5-iodouracil may be regarded as an example of SCH reaction. Subsequent studies have shown that hydrogenation[150] and isotope exchange[151] occur under similar conditions. This method consists in the treatment of a solid mixture of the initial compound and catalyst with gaseous tritium. In order to intensify the process, an external supply of energy was provided. The optimum version of the initiation of this reaction proved to be heating of the reaction system. The solid-state reactions of isotope exchange have been further developed in the studies of Meshi and Takahashi.[152] The use of catalysts increases appreciably the extent of tritium incorporation and its selectivity. Zolotarev *et al.*,[150, 151] who investigated SCH in the reactions of hydrogenation, reduction and isotope exchange, have concluded that a new process took place in all cases. It has been demonstrated[153, 154] that the SCH is based on the spillover of hydrogen activated on the catalyst and its diffusion into the layer of organic compound. A diffusion model of this process has been proposed.[155] The applicability of this model was exemplified with solid-state isotope exchange in thymine accompanied by the hydrogenation of the 5,6-double bond.[156] The extent of isotopic substitution in the SCH reaction depends on the nature of the metal used as the catalyst (Pd, Pt, Rh, Ru) and support ($BaSO_4$, $CaCO_3$, Al_2O_3, SiO_2, C), the catalyst–compound ratio and the reaction temperature.

The application of the SCH method for the synthesis of biologically important diazines labelled with tritium gave good results. Solid-state catalytic hydrogenation of 5-formyluracil afforded thymine with the molar activity corresponding to the incorporation of three tritium atoms into the methyl group.[132, 133] Investigation of the hydrogenation kinetics of 5-formyluracil has shown[157] that starting from a definite temperature, the incorporation of tritium into thymine exceeds the stoichiometry corresponding to the reduction of the formyl group. This is explained by the fact that under given conditions, the reduction is accompanied by a parallel reaction of isotope exchange of the formyl group hydrogen with tritium. This leads to thymine with a virtually complete substitution of hydrogen by tritium in its methyl group ($A_{mol} \simeq 87$ Ci mmol^{-1}). The use of another precursor, *viz.*, 5-hydroxymethyluracil, a specially selected catalyst and more drastic reaction conditions have permitted synthesis of thymine[157] in which all tritium was substituted for hydrogen over all the C–H bonds ($A_{mol} > 100$ Ci mmol^{-1}), and the use of 5-hydroxymethyldeoxyuridine as the precursor has yielded tritium-labelled thymidine ($A_{mol} = 76$ Ci mmol^{-1}).[158]

Let us consider the basic regularities of the introduction of tritium label by the SCH method with synthesis of specific compounds as an example. It has been shown[157] that in the SCH reaction of tritium with purine bases the rate of isotope exchange is sharply increased in the temperature range 150–170 °C. This leads to a virtually complete substitution of hydrogen by tritium.

The results of synthesis of 11 alkylxanthines labelled with tritium using the SCH reaction are presented in Ref. 159. Analysis of the labelled compounds by ^{3}H NMR spectroscopy has revealed that the tritium label was incorporated not only in the C(8) position but also in the alkyl groups. The dependences of the yield of compound **22** prepared by SCH with tritium and of its molar radioactivity on temperature and the catalyst/compound ratio have been investigated.[160] The same dependences for compounds **19** and **21** were studied by Lushkina *et al.*[161] It has been found that for virtually all the compounds studied the higher temperature and the greater catalyst/compound ratio the lower the yield of specific products but the higher its molar radioactivity. Thus, the variation of reaction conditions [temperature, catalyst, catalyst/compound mass ratio and reaction time] opens wide possibilities for the synthesis of labelled compounds.

The main side reaction of SCH is degradation (thermolysis) of the initial compound. The products of the synthesis of tritium-labelled 2′,5′-oligoadenylate were found to include an isomerisation product, *viz.*, 3′,5′-isomer, which was separated by chromatography.[162] In the case of SCH of pyrimidines, hydrogenation of the 5,6-double bond of the heterocyclic ring may occur in parallel leading to the formation of compounds **28**–**31**.

The SCH method was employed for the introduction of a tritium label into compounds of a more complex structure such as polynucleotides and natural polymers,[162] *e.g.*, 15S RNA. The liquid- and solid-state catalytic isotope exchange with gaseous tritium in thymidine 5′-H-phosphonates, 2′-deoxyadenosine, and 2′-deoxy-5-hydroxymethyluridine has been investigated.[163] Analysis by ^{3}H NMR spectroscopy has revealed that tritium is incorporated into the P–H group. The solid-state method for the preparation of tritium-labelled DNA synthesis terminators

(**22**, **23**, azidothymidine phosphonate, acyclovir phosphonate, 3′-deoxythymidine) is described in Ref. 43. The tritium-labelled 5′-*O*-phosphonylmethylthymidine (A_{mol} = 71 Ci mmol^{-1}) was obtained by the solid-state reaction of isotope exchange at 150 °C.[164]

Table 7 lists the A_{mol} values of tritium-labelled purine and pyrimidine compounds prepared by the SCH method. The values presented are higher than those for these and analogous compounds labelled by other methods. For compounds of relatively simple structure, such as **2**, **3**, **10**–**13**, a virtually complete isotope substitution of hydrogen by tritium has been attained. Variation of the solid phase composition and temperature permits the directed modification of the yield and the molar radioactivity of specific compounds. The SCH method does not require synthesis of special precursors and offers the possibility of a greater degree of hydrogen substitution by tritium than do other methods. Degradation of thermally labile compounds, the occurrence of parallel reactions of hydrogenation, dehalogenation and reduction of various functional groups may be regarded as disadvantages of the SCH method. However, in many cases these disadvantages are not critical since a careful choice of reaction conditions makes it possible to minimise (as far as possible) the influence of side reactions and to synthesise compounds containing the groups (the 5,6-double bond of pyrimidines) susceptible to hydrogenation and even some thermally unstable compounds (oligo- and poly-nucleotides, RNAs).

Thus, the SCH method may be regarded as one of the most promising methods for the synthesis of tritium-labelled natural diazines, in particular, of the components of biologically important substances.

Table 7. Molar radioactivities of diazines obtained by SCH reactions.

Compound	T/ °C	A_{mol}/ Ci mmol^{-1}
Adenine	170	54.2
Guanine	210	24.9
Xanthine	180	25.1
Hypoxanthine	200	51.1
Thymine [a]	140	85.0
Thymine [b]	170	110.0
Thymine [c]	170	115.0
Alkylxanthines (11 compounds)	170	17–203
Uracil	170	49.0
Benzyladenine	190	180.0
Theophylline	160	24.8
Furfuryladenine	160	160.0
Adenosine	210	120.0
2′-Deoxyadenosine	190	84.0
Guanosine	210	69.0
2′-Deoxyguanosine	200	35.0
Thymidine [d]	160	76.0
3′-Azidothymidine	120	30.0
Acyclovir	210	125.0
2′,3′-Dideoxythymidine	120	66.0
3′-Azidothymidine phosphonate	120	63.0
Acyclovir phosphonate	120	56.0
2′,5′-ApApAp	200	58.0
3′,5′-ApA	110	9.2
3′,5′-ApApA	110	50.0
3′,5′-pApApAp	110	27.0
Poly-A (7–8 S)	180	10.2 [e]
Poly-U (12 S)	180	12.0 [e]
15 S RNA	180	12.1 [e]

[a] From 5-formyluracil; [b] from 5-hydroxymethyluracil; [c] from thymine; [d] from 5-hydroxymethyl-2′-deoxyuridine. [e] Specific radioactivity/ Ci g^{-1}.

Table 8. Characteristics of different methods used for the synthesis of biologically active diazines labelled with tritium.

Reaction	Reaction condition	A_{mol}/ Ci mmol^{-1}
Tritium incorporation by chemical synthesis		
Dehalogenation	in solution	15–45
	in solid state	5–20
Hydrogenation, reduction	in solution	15–40
	in solid state	40–90
Tritium incorporation by isotope synthesis		
Isotope exchange with:		
^{3}H-solvent	in solution	5–10
highly radioactive water	"	up to 25
gaseous tritium	"	5–25
	in solid state	20–120, up to 250

Comparative characteristics of different methods used for the synthesis of tritium-labelled biologically important diazines are presented in Table 8.

VII. Other methods of tritium labelling

A number of methods exist that did not find wide application for the synthesis of tritium-labelled natural diazines. Some pyrimidine derivatives labelled with tritium in the position 5 were prepared by heating (to 160 °C) the respective iodo-substituted compounds in DMSO with tritiated water.[165] The molar radioactivity of uracil, uridine, cytosine, cytidine and orotic acid thus obtained made up 30% of the A_{mol} level of [^{3}H]H_2O.[166] Electrolytic reduction of 5-bromouracil in tritiated water (mercury cathode, platinum anode) yielded [5-^{3}H]uracil.[167]

Decarboxylation of 5-substituted derivatives of orotic acid labelled with tritium in the carboxyl group produced [6-^{3}H]thymine [168] from 5-methylorotic acid, 5-fluoro[6-^{3}H]uracil [169] and 5-bromouracil [170] from the corresponding 5-halogeno-derivatives of orotic acid. The molar radioactivity of these products did not exceed 1 Ci mmol^{-1}. Heating of orotic acid supported on a Cu/$BaSO_4$ catalyst, which was partially oxidised in air, in the tritium atmosphere yielded [6-^{3}H]uracil (23 Ci mmol^{-1}).[171] β-D-Arabinofuranosyladenosine (A_{mol} = 0.43 Ci mmol^{-1}) was prepared by isotope exchange with UV-activated gaseous tritium.[172] Alkylation of [2,8-$^3H_2$]adenosine with 4-bromo-2-methylbut-2-ene in dimethylformamide gave N^6-(2-isopentyl)[2,8-^{3}H]adenosine, which was transformed into *trans*-[2,8-^{3}H]zeatin riboside (A_{mol} = 22 Ci mmol^{-1}) by allylic oxidation.[173] Heating (60 °C, 2 h) of N^6-(3-iodobenzyl)-N^9-[methyl(2,3-di-*O*-acetyl-β-D-ribofuranosyl)uronate]-2-chloroadenosine with tritium-labelled methylamine in methanol provided N^6-(3-iodobenzyl)-N^9-[*N*-methyl-(β-D-ribofuranosyl)uronamide] (A_{mol} = 29 Ci mmol^{-1}).[174] Azidothymidine was synthesised using tritium-labelled thymidine.[175] Deamination of [5-^{3}H]1-β-D-arabinofuranosylcytosine led to the formation of [5-^{3}H]1-β-D-arabinofuranosyluracil (A_{mol} = 20 Ci mmol^{-1}).[39] [8-^{3}H]9-β-D-Arabinofuranosyladenine (A_{mol} = 10 Ci mmol^{-1}),[39] [2-^{3}H]ade-nine and [2-^{3}H]adenosine [38] are the products of catalytic reductive desulfurisation of the corresponding SH-derivatives with gaseous tritium. The processes of tritium incorporation from the gas phase into thymidine and deoxyuridine in an aqueous solution have been considered.[176] Incorporation of tritium into these compounds resulted mainly from the attack by the $^3HeT^+$ species rather than the radiolysis induced by β-particles.

VIII. Conclusion

Nowadays, synthesis of tritium-labelled physiologically significant compounds may be regarded as an independent field of applied radiochemistry that makes use of the approaches and methods of bioorganic chemistry, biochemistry and enzymology. This is characterised by both the development of efficient techniques for tritium incorporation and organisation of the production of labelled substances. The above considerations suggest a number of inferences on the state-of-the-art of the synthesis of tritium-labelled diazines.

In the 1970s, rapid progress of molecular biology and molecular genetics increased dramatically the demand for tritium-labelled components of nucleic acids. This has stimulated the development of new methods and the improvement of existing techniques of their synthesis. Tritium-labelled compounds have played an important role in the studies of the structure and functions of nucleic acids. Catalytic dehalogenation in solution may be regarded as the general method for the synthesis of the tritium-labelled components of nucleic acids. The development of this method has allowed synthesis of compounds with a definite position of the tritium label and relatively high molar radioactivities (20–30 Ci mmol^{-1}). The need for the components of nucleic acids possessing high and superhigh molar radioactivity has stimulated the development of techniques for the preparation of purine and pyrimidine bases multiply labelled with tritium. These include reactions of catalytic dehalogenation (*e.g.*, of 2,8-dichloroadenine, 5-bromouracil and 6-chlorouracil) and solid-state catalytic hydrogenation (*e.g.*, synthesis of [2,8-^{3}H]adenine and [methyl-6-^{3}H]thymine from the respective unlabelled precursors). The use of the enzymes of purine and pyrimidine metabolism has opened a simple and reliable pathway for the preparation of a virtually complete set of nucleic acid components multiply labelled with tritium.

The recent years witness an ever-increasing interest in the tritium-labelled pharmaceutical preparations of rather complex structures. As regards diazines, these are mainly various DNA synthesis terminators, medicinal drugs in the stage of pharmaceutical assays and coenzymes. These and similar tritium-labelled compounds are most often synthesised by catalytic reactions of isotope exchange, in particular the isotope exchange with gaseous tritium in solution and in solid state. Such reactions may be considered as express-methods that do not require synthesis of special precursors. For example, tritium-labelled nicotinamide adenine dinucleotide (111 Ci mmol^{-1}) and coenzyme A (3.9 Ci mmol^{-1}) were prepared by the solid-state reactions of isotope exchange with gaseous tritium.[177] The application of the reactions of isotope exchange involving gaseous tritium is limited by the requirement that the initial compounds contain groups capable of easy reduction or hydrogenation and are thus susceptible to modification or substitution by tritium. In this case, the reaction of isotope exchange with highly active tritiated water is a promising method. The possibilities of the application of this method for the synthesis of tritium-labelled biologically active substances, including diazines, have been discussed.[178]

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