INTRODUCTION OF TRITIUM INTO ORGANIC COMPOUNDS BY ISOTOPE EXCHANGE REACTIONS

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SUMMARY

Various isotope exchange reactions for the introduction of tritium into a range of organic compounds have been investigated. The influence of the catalyst, solvent and reaction conditions on the yield and specific activities of a number of steroids, phytohormones and sugars are reported. Heterogeneous hydrogen isotope exchange with gaseous tritium has also been used to label a number of nonpolar compounds. Similarly the potential of solid state hydrogen isotope exchange has been explored. The reaction mechanism is discussed as well as the influence of the reaction conditions on the specific activity and the label distribution.

Keywords: Tritium exchange, homogeneous, heterogeneous, solid state

Isotope exchange reactions are widely used for the introduction of the tritium label into organic compounds [1], especially into physiologically active compounds. This is due to the complexity of their structures which makes it difficult for them to be modified prior to the subsequent label incorporation. As a rule, physiologically active compounds, especially natural ones are available in limited quantities, and as a result one often has to perform tritium labelling of samples of several milligrams or even smaller quantities of the compound in question.

The following isotope exchange reactions are the most widely used for tritium introduction:

- homogeneous isotope exchange catalyzed by acids, bases, and soluble transition metal salts;
- heterogeneous isotope exchange, catalyzed by VIII group metals (Pt, Pd, Co, Ni, Ru, Rh, Ir);
- solid state isotope exchange, performed by heating of a mixture of an organic compound and the catalyst in an atmosphere of tritium.

The use of organic solvents allows one to bring down considerably the autoradiolysis of water as a result of a decrease in radioactivity per unit volume, whereas the application of anhydrous aprotic solvents (such as dioxan) makes it possible to use tritiated water with very high molar radioactivity levels, in fact almost as high as theoretical values. Studies of the impact of various heterogeneous catalysts, solvents, tritiated water mass percent, reaction conditions (time, temperature) upon the yield and molar radioactivity of certain steroids, phytohormones, carbohydrates were performed by us [2]. Catalysts, according to their activity in isotope exchange reactions between tritiated water and all the physiologically active compounds studied can be arranged in the following sequence: $Pd/Al_2O_3 > PdO > Pd/BaSO_4 > PtO_2$ (Fig.1).

This figure also shows the dependence of the molar radioactivity of certain steroids on the concentration of tritiated water and the isotope exchange temperature.

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Raising the reaction temperature results in an insignificant growth in molar radioactivity yet causes a considerable increase in the degree of decomposition. Studies of the isotope exchange reaction using phytohormones and steroids demonstrated that virtually identical results were

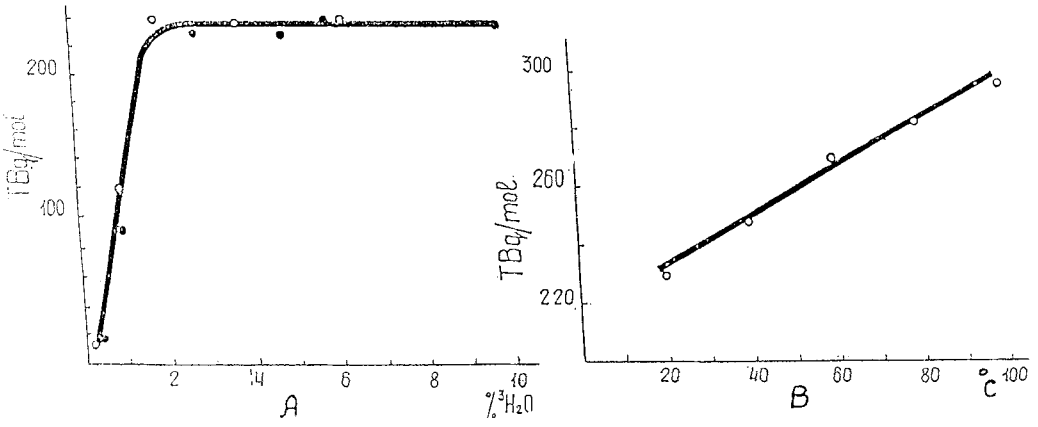


Fig.1. Dependence of steroids specific radioactivity on
A - tritiated water concentration (o - in ethanol; o - in ethyl acetate);
B - reaction temperature.

obtained with ethanol, dioxane, dimethylformamide and ethylacetate when used as solvents whereas chloroform produced results that were quite inferior. In a number of cases addition of triethylamine to the reaction mixture raised the molar radioactivity.

Figure 2 shows the isotope exchange kinetics for certain steroids in a number of solvents as well as the dependence of progesterone molar radioactivity on the steroid to catalyst ratio.

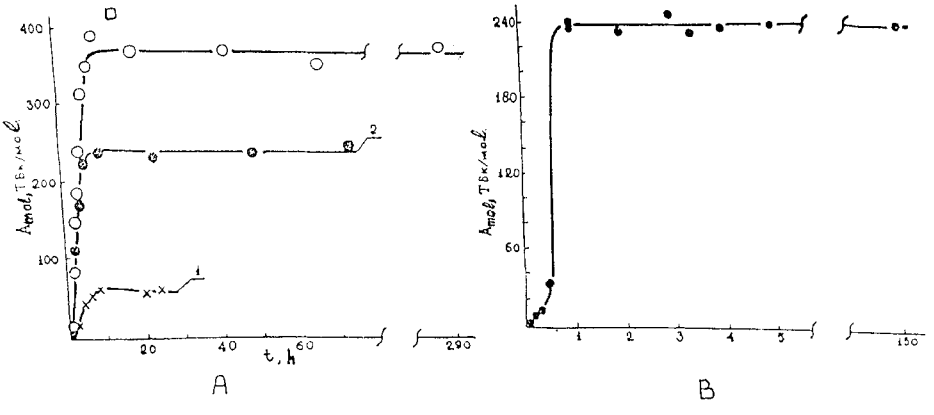


Fig.2. A - isotope exchange kinetics at 20°C. * - cholesterol in chloroform; ▪ - cholesterol in chloroform-methanol (9:1) mixture; ● - progesterone in ethylacetate; o - estriol in dioxane; B - progesterone specific radioactivity dependence on catalyst: steroid ratio (20°C,ethylacetate).

Table 1 presents the molar radioactivities of a number of compounds prepared by this method.

In our opinion this method is preferable to cases where compounds to be labelled contain groups that are easily hydrogenated or reduced.

Table 1. Molar radioactivities of preparations obtained through isotope exchange with high molar activity tritiated water.

Compound	Molar rad. TBq/mole
Progesterone	248
Estriol	400
Cholesterol	455
Cortisol	220
1,4,6-Androstatriene-3,17-dione	100
17-α-Methylandrosten-1,4-ol	330
17-α-Methylandrosten-5-diol-3,17	210
Benzyladenine	890
Indole-3-acetic acid	360
Fusicoccin-H	260
Ribose	260
2-Deoxyribose	240
Zeatin	72
Zeatin riboside	75

A method based on the use of gaseous tritium for heterogeneous catalytic isotope exchange has found wide application for the introduction of tritium into organic compounds. This method has been proposed by Evans in the early 1970's for incorporating labels into aromatic and unsaturated compounds [3]. Since then it has been used for the synthesis of compounds with a wide range of structures, as reflected in numerous publications [4]. However, the use of this method for the introduction of the tritium label into unsaturated compounds poses serious obstacles. This is due to the fact that isotope exchange with gaseous tritium is as a rule performed in the presence of palladium or platinum catalysts, which are also active hydrogenation catalysts. The next figure (Fig.3) demonstrates the correlation between the isotope exchange and hydrogenating activities of catalysts for methyl oleate labelling.

For this purpose the following series of catalyst samples has been prepared under identical conditions by treatment of 100 mg of 5% $Pd/BaSO_4$ with 5 mg (K), 10 mg (L), 15 mg (M), 20 mg 20 mg (N), and 30 mg (O) of lead (II) acetate in 1 ml of distilled water by stirring for 45 min at 80°C [5].

Partial inactivation of the catalyst with quinoline or performing the reaction in pyridine reduces the hydrogenation of unsaturated bonds, but also sharply reduces the heterogeneous isotope exchange with tritium gas [6].

Conditions for isotope exchange have to be chosen depending on the particular structures of the unsaturated compounds in question [7]. The effect of various isotope exchange catalysts, solvents and reaction time on molar radioactivity and preparation yield is shown in Tables 2 and 3 by reference to a number of different compounds.

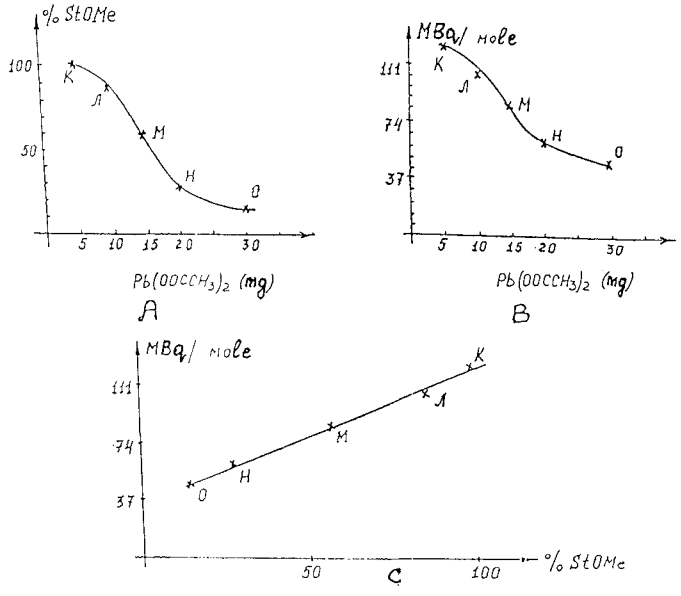


Fig.3. The correlation between the isotope exchange and hydrogenating activities of 5% $Pd/BaSO_4$ poisoned with lead acetate. Substrate - methyl oleate.
A - Degree of hydrogenation vs lead acetate quantity;
B - Specific radioactivity vs lead acetate quantity;
C - Correlation between degree of hydrogenation and specific radioactivity of methyl oleate.

From the data listed it follows that in cases of easily hydrogenated compounds the Lindlar catalyst is the most efficacious. It can be seen that yields of compounds are about 30-70% whereas the specific radioactivities are in the range of several hundreds TBq per mole. Lowering the tritium gas pressure (150 hPa or lower) makes it possible to use active hydrogenation

Table 2. Tritium label introduction into compounds of a lipid nature through heterogeneous isotope exchange (tritium pressure 400 hPa).

Initial compound	Reaction conditions	Yield %	Molar rad. TBq/mole
Steroids: β-sitosterol, 19-iodocholesterol,	5% $Pd/BaSO_4$, dioxane, 2-3 h	65-81	1.7-2.3
cholestan-3β-ol, cholestane-3β,5 α,6β-triol-3,6-diacetate)	5% PdO/Al_2O_3, dioxane, 2-3 h	78-93	0.5-0.8
Unsaturated fatty acids: dihomo-γ-linolenic, linoleic, tymnodonic	Lindlar cat., dioxane, 0.5-3h	25-36	25-146
Prostaglandins: $PGF_{2\alpha}$	5% PdO/Al_2O_3 dioxane, 1.5 h	30	59.2

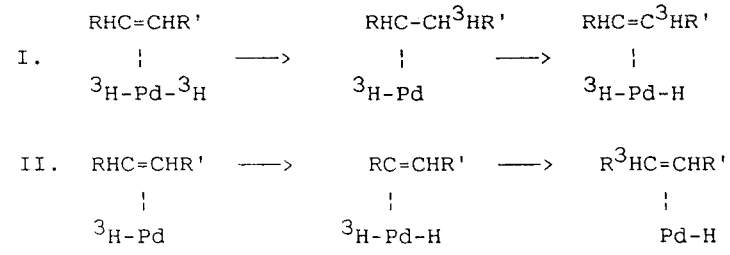
Initial compound	Reaction conditions	Yield %	Molar rad. TBq/mole
$PGF_{2\alpha}$	$LaNi_5$, $LaNi_4Cr$, dioxane, 22h	50-58	0.85-0.96
15-F-11,15-dideoxy--PGE_1 methyl ester	Lindlar cat., dioxan, 2h	80	130
15-Fluoro-15-deoxy- $PGF_{2\alpha}$ methyl ester	Lindlar cat., dioxane, 3h	59	93
Polyunsaturated phospholipids	5% $Pd/BaSO_4$, dioxane	80	0.3-3.0
	5% $Pd/BaSO_4$, ethyl acetate	70	0.3-3.6
Dipalmithoyl-phosphastidyl choline	5% $Pd/BaSO_4$, chloroform-methanol (2:1), 2.5h	65	14.8

catalysts (5% Pd/Al_2O_3 in the case of readily hydrogenated prostaglandin $F_{2\alpha}$). Active palladium catalysts can be used for label introduction into unsaturated compounds, containing double bonds with strong steric hindrance (steroids for example). If a compound contains easily hydrogenated double bonds that are active with regard to the Lindlar catalyst - copper and nickel based catalysts can be employed. However in this case the molar radioactivities of the preparations obtained are low, in fact two orders of a magnitude lower than with palladium catalysts.

Table 3. Introduction of the tritium label through heterogeneous isotope exchange into certain physiologically active compounds (tritium gas pressure 400 hPa).

Compound	Reaction conditions	Yield %	Molar rad., TBq/mole
Eleuterozid B	5% $Cu/CaCO_3$,dioxane, 4h	43	6.7
D-Digitoxose	5% $Pd/BaSO_4$, ethanol-water (3:7), 3h	50	200
Digoxin	5% PdO/Al_2O_3 ethanol-water (3:7), 3h	60	500
8-Methoxypsoralen	5% $Pd/BaSO_4$ dioxane-Et_3N (3:1), 3h	86	16.0
4,5',8-Tri-methylpsoralen	5% $Pd/BaSO_4$, chloroform-methanol-Et_3N(10:1:5),3h	72	4.3
Polyprenyl-phosphate()	5% $Cu/CaCO_3$, dioxane, 22 h 150 hPa	33	0.22
Epihid	5% $Pd/BaSO_4$,chloroform, or water or methanol, 3h	88	10.3-12.8
Saturated fatty acid methyl esters (stearic, palmitic, myristic, lauric)	10% $Pd/BaSO_4$, dioxane, 5 h	52-96	8.1-35.8

Studies of tritium incorporation into unsaturated compounds give ground for several conclusions. Unsaturated compound absorption is known to occur either due to double bond opening (associative absorption mechanism I) or due to a C-H bond split followed by a Pd-C bond formation (dissociative absorption mechanism II):



Three paths of tritium incorporation into organic compounds can be singled out: in the first instance double bond absorption on the catalyst does not occur, in the second - double bond absorption proceeds via a dissociative absorption mechanism (DM) with no double bond opening (resulting in high yields of labelled compounds with molar radioactivities up to 0.1 PBq/mol), in the third - an associative absorption mechanism (AM) prevails, resulting primarily in the formation of isomerisation and hydrogenation products.

For example (Table 2) labelled steroids are formed with yields of 65-80% and molar radioactivities close to those of saturated compounds labelled through isotope exchange. Labelled 15-fluoro-11,15-dideoxy PGE_2 methyl ester is formed with an 80% yield and a molar radioactivity 100 times exceeding that obtained with steroids, whereas PGE_2 under identical conditions is converted primarily into hydrogenation and isomerisation products.

In the case of label introduction into unsaturated compounds the label is predominantly incorporated at the double bond as well as allylic positions. The formation of cis-trans isomerisation or double bond migration products in the reaction mixture is due to intermediates that arise via an associative absorption mechanism [8]. Despite the problems that arise with label introduction into unsaturated organic compounds that were mentioned in this report, the heterogeneous isotope exchange method performed with gaseous tritium in a solution makes it possible to expeditiously obtain labelled preparations without their prior modification.

Solid state catalytic isotope exchange opens new opportunities in the synthesis of tritium labelled organic compounds. The Wilzbach method can be viewed as the first example of solid state isotope exchange; the isotope exchange reaction is known to be induced by tritium ß-fission. Publication [9] can be considered as the next step in the development of solid state isotope exchange reactions. In fact the application of catalysts to the Wilzbach method considerably increased the degree of label incorporation as well as its selectivity. Finally, our own research work [10-12] in solid state catalytic hydrogenation that allowed us to perform dehalogenation, hydrogenation, reduction and isotope exchange reactions lead us to the conclusion that in all cases we were dealing with a new process. The next stage in the studies of solid state reactions was based on the understanding of their mechanism, which includes the spillover of hydrogen activated on the catalyst, and its diffusion into the organic compound mass [13]. A diffusion model of the process has been developed [14]. The applicability of this model to certain reactions has been demonstrated: for example the solid state isotope exchange with thymine is accompanied by 5,6-double bond hydrogenation [18].

Figure 4 contains the scheme of solid state isotope exchange (SIE), which consists in activation of gaseous tritium, spillover of activated tritium into the organic compound layer, and solid state isotope exchange reactions resulting in the formation of the tritiated product.

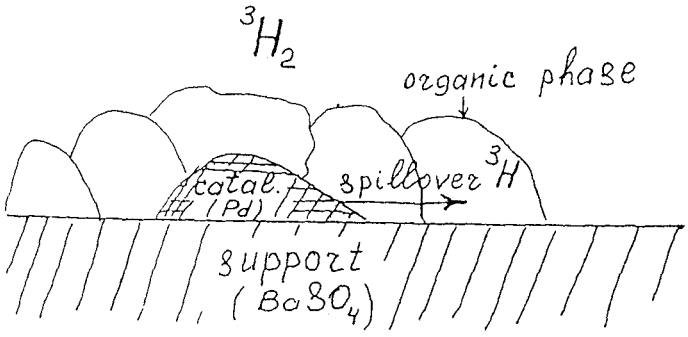


Fig.4. The reaction scheme of the solid state catalytic hydrogenation.

The degree of isotope exchange in solid state isotope exchange reactions depends on a number of factors:

- the nature of the metal-catalyst (Pd, Pt, Rh, Ru);
- the nature of the support ($BaSO_4$, $CaCO_3$, Al_2O_3, SiO_2, C);
- the compound to catalyst ratio;
- the reaction temperature (usually above 100°C).

The strongest effect on the degree of conversion in SIE reactions is that of temperature. The fact that the process takes place in the solid phase permits the temperature to be varied over a wide interval, up to the compounds melting point, or in certain cases even higher. This makes it possible to effect specifically both the degree of isotope exchange, and the label distribution. At temperatures within the 20-120°C interval the label is generally incorporated into the most reactive positions of the molecule [15]. Raising the reaction temperature causes an increase in the total label incorporation with reduced selectivity. At temperatures above 200°C a degree of exchange is achieved that is determined solely by the isotope forms ratio in the gaseous and solid phase [16]. Table 4 shows the pattern of tritium label distribution in valine at different temperatures.

The degree of isotope substitution dependence on temperature is presented in Table 5.

In cases when solid state isotope exchange is accompanied by side reactions (sublimation, compound destruction, catalyst poisoning) occurring at elevated temperatures the temperature dependence is of a more complex nature (Fig.5).

Tritium that has been activated as a result of its interaction with the catalyst at elevated temperatures is capable of migrating for considerable distances on a support. The solid state isotope exchange reaction can be performed efficaciously if a spatial separation of the catalyst and the organic compound is achieved. This goal is reached by mixing the inorganic support that has been coated with the organic substance in question and the solid catalyst. This technique of isotope exchange ensures a high chemical yield and catalyst protection from poisoning [17].

Table 6 shows the molar radioactivities and yields of several preparations, obtained in the manner described above.

Data reflecting the influence of the metal-catalyst upon the degree of hydrogen for tritium substitution are given in Table 7.

Figure 6 demonstrates typical solid state isotope exchange kinetic curves.

It follows from the Table that platinum and palladium based catalysts are the most active with regard to solid state isotope exchange. However, in the case of aliphatic compounds 5% Pt/C turned out to be the most active catalyst (Fig.5).

The next table (Table 8) demonstrates the effect of the support nature on the degree of solid state isotope exchange [18].

Table 4. Label distribution in valine at different temperatures.

Hydrogen position	Substitution at position			
	120°C	150°C	18O°C	210°C
C_α	-	0.016	0.27	0.69
C_β	-	0.012	0.18	0.69
C_τ	0.048	0.381	2.55	5.52
Specific radioactivity Ci/mmol	1.4	12	88	200

Table 5. Tritium label distribution between amino acid residues in peptides (degree of substitution %).

Peptide	Isotope exchange tempera-ture, °C	Molar radio-activity	VAL			GLY-1	GLY-2
			C_α	C_β	C_τ		
gly_1-gly_2-val	160	1739	0	0	2	65	14
gly_1-val_2-gly	160	1406	0	0	3	33	19
val-gly_1-gly_2	160	2590	26	8	23	21	12
val-gly_1-gly_2	180	7720	58	54	63	62	57

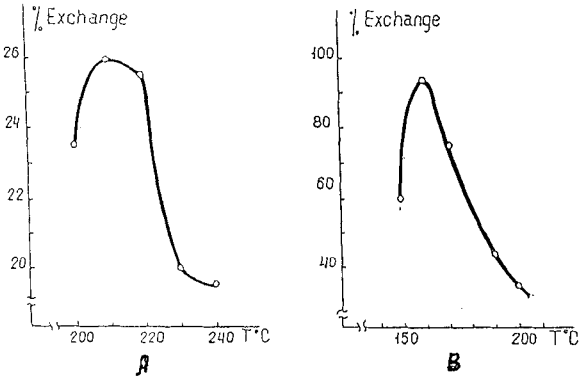


Fig.5. Degree of substitution vs temperature dependence for stearic acid for A - 5% Pd/C, B - 5% Rh/C.

Table 6. Tritium labelled organic compounds, synthesized through spatially separated SIE at 200°C.

Compound	Molar radioactivity, TBq/mole	Yield %
L-Alanine	2400	90
L-Phenylalanine	3030	35
L-methionine	3320	15
L-Histidine	4600	20
L-Tryptophan	4700	15
Tryptamine	2500	60
Indole acetic acid	3000	32
Biotin	1600	12

Table 7. The influence of the metal-catalyst nature on the degree of hydrogen for tritium substitution. The degree of hydrogen for tritium substitution in stearic acid (reaction time 20 min, compound to catalyst ratio 1:10).

Catalyst	Degree of substitution %	Isotope exchange temperature, °C
5% Pt/C	25.7	210
5% Pd/C	7.0	210
10% Pd/C	25.2	190
5% Rh/C	0.8	160

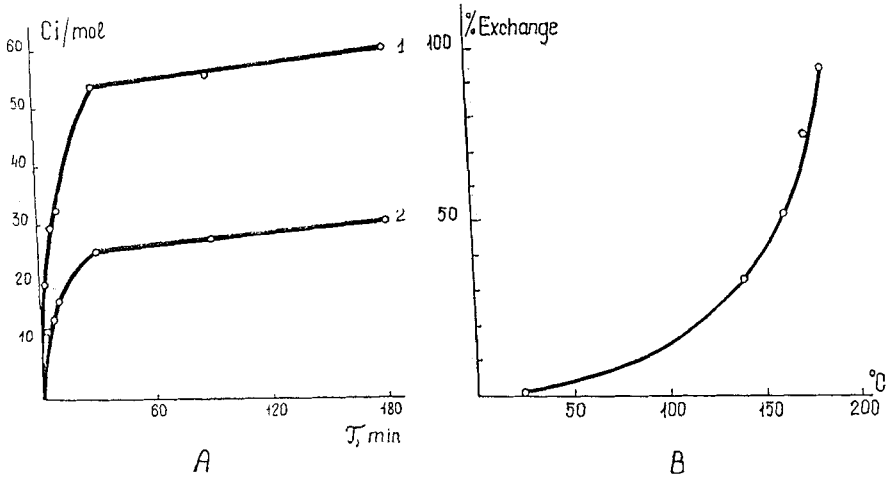


Fig.6. A. Kinetics of the tritium-SIE reaction with adenine. Tritium-hydrogen ratio 1:1000. (1) - total incorporation, (2) - incorporation at position 2.
B. The temperature dependence of the exchange degree for adenine. Reaction time 60 min.

Table 9 reflects the effect of the compound to catalyst ratio on the degree of solid state isotope exchange [23]. Solid state isotope exchange has been successfully used for tritium introduction into hundreds of physiologically active compounds.

The simplicity and general applicability of the method combined with the high degree of isotope substitution give sufficient ground to believe that it will find extensive use for tritium label introduction into organic compounds.

Table 8. The effect of catalyst support on the yield and molar radioactivity.

Compound	Molar radioactivity (PBq/mol) for		
	Pd/Al_2O_3	$Pd/BaSO_4$	$Pd/CaCO_3$
Guanine	0.63	0.83	0.61
Xanthine	-	0.93	0.17
Hypoxanthine	1.79	1.90	0.73
Acyclovir	-	4.35	4.58
Azidothymidine	-	0.09	0.56
Kinetin	4.60	2.50	1.48
Benzylaminopurine	4.48	7.66	2.30
Indolyl-3-acetic acid	-	1.2	2.67
Adenosine	4.26	2.26	2.97
Deoxy-guanosine	-	3.30	0.63
Deoxy-adenosine	-	2.81	2.01

Table 9. Synthesis of tritium labelled tryptophan through a reaction with the spatial separation of the substrate and catalyst under HTCIE conditions with gaseous tritium.

Support	Catalyst	Amino acid: support ratio	Temp. °C	Subst. degree	Yield %
$CaCO_3$	$Pd/BaSO_4$	1:10	160	0.25	90
- " -	- " -	1:20	160	0.29	79
- " -	- " -	1:40	160	0.43	60
- " -	- " -	1:80	160	0.68	51
- " -	- " -	1:10	180	0.74	31
- " -	- " -	1:20	180	1.26	27
- " -	- " -	1:40	180	1.46	25
- " -	- " -	1:80	180	3.38	20
- " -	- " -	1:80	190	3.67	22
- " -	- " -	1:80	200	4.47	15
$BaSO_4$	- " -	1:20	160	0.25	73
- " -	- " -	1:80	160	0.87	56
- " -	- " -	1:20	180	1.77	37
- " -	- " -	1:80	180	1.92	22
Al_2O_3	- " -	1:20	160	0.60	70
- " -	- " -	1:20	180	0.74	40
- " -	$Pd/BaCO_3$	1:20	160	1.03	66
- " -	$Pd/BaCO_3$	1:20	180	1.67	20

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