Journal of Labelled Compounds and Radiopharmaceuticals
J. Labelled Cpd. Radiopharm. **XLI**, 993-1003 (1998)

SYNTHESIS OF TRITIUM-LABELLED DIAZINES AND THEIR ANALOGUES

Nykolai F. Myasoedov and Georgy V. Sidorov

Institute of Molecular Genetics, RAS, 123182 Moscow, Kurchatov Sq., Russia

SUMMARY

Some 40 diazines have been tritiated to high specific activities using a variety of labelling procedures such as catalytic hydrogen isotope exchange both in solution and the solid state, reduction and hydration. For purine derivatives it is shown that the solid state catalytic isotope exchange reaction is the most effective method. With pyrimidines this reaction is accompanied by a parallel hydration reaction of the 5,6-double bond to form a complex mixture of products. Identification and quantitative estimation of these products has been accomplished in terms of the reaction condition (solvent, nature of catalyst).

Key Words: tritium, catalytic hydrogenation, purines, pyrimidines, nucleosides, nucleotides, phytohormones, and terminators of DNA synthesis.

INTRODUCTION

Pyrimidine (1, Fig.1) is the most important compound among the diazines, since its derivatives, uracil (2), thymine (3) and cytosine (4), form part of ribo- and deoxyribonucleic 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

CCC 0362–4803/98/110993–11$17.50

Received 5 April 1998
Revised 20 July 1998
Accepted 27 July 1998

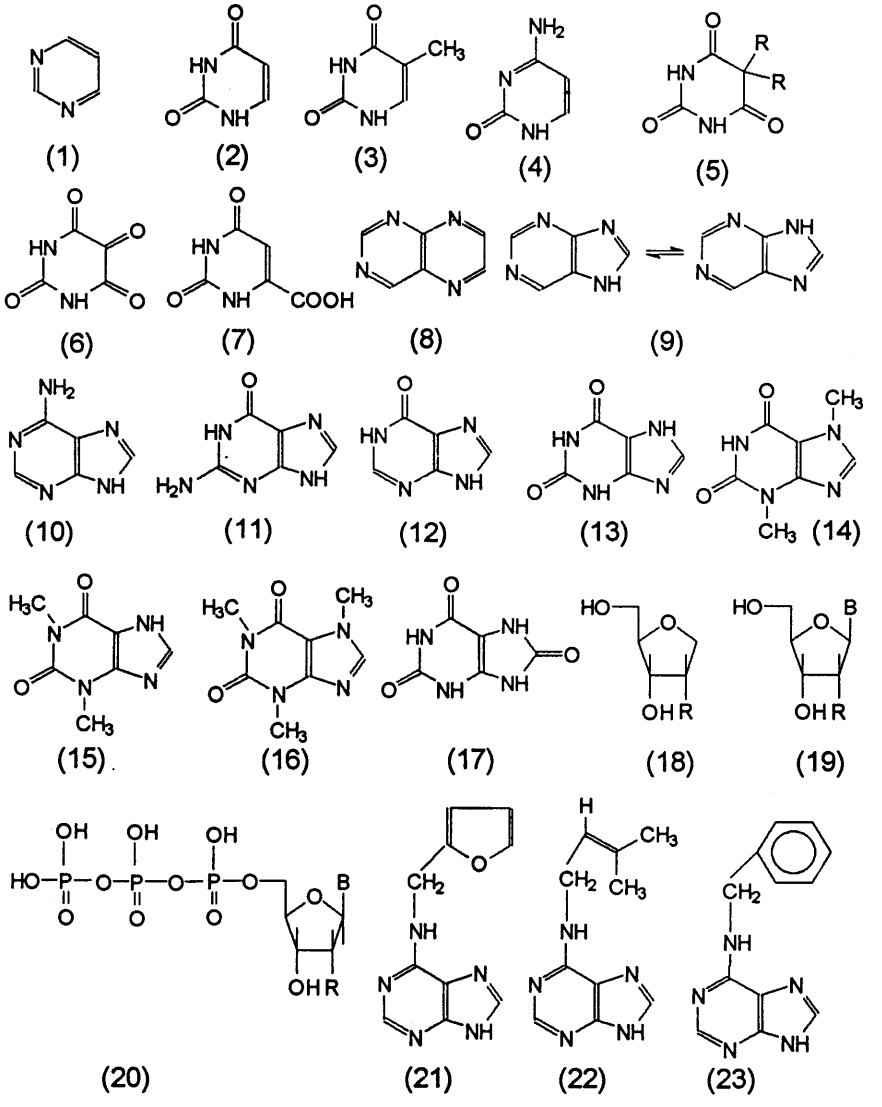

Figure 1. Some natural diazines. Pyrimidine (1), uracil (2), thymine (3), cytosine (4), barbiturates (5), alloxan (6), orotic acid (7), pteridine (8), purine (9), adenine (10), guanine (11), hypoxanthine (12), xanthine (13), theobromine (14), theophylline (15), caffeine (16), uric acid (17), ribose [R= -OH], 2'-deoxyribose [R= -H](18), ribo- and deoxyribonucleosides (19), nucleotides (20), kinetine (21), zeatine (22), benzylaminopurine (23).

form part of ribo- and deoxyribonucleic 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.

Ribose and 2'-deoxyribose (18) are bound to nitrogen bases by the N-glycoside bond. These compounds have been called ribo- and deoxyribonucleosides (19), and the phosphorous esters of nucleosides have been termed nucleotides. We have thus grouped the main natural diazines under the common name of "components of nucleic acids".

Other compounds of interest, belonging to diazines, are examples of the cytokinins and DNA synthesis terminators. Cytokinins are phytohormones (N^6-substituted adenine), for instance, kinetine (21), zeatine (22) and benzylaminopurine (23, Fig.1).

Analogues of nucleosides with the modified glycoside ring often appear to be DNA synthesis terminators, and they are potent inhibitors of retroviruses, including the human immunodeficit virus [1]. The tritium-labelled forms, are essential for studying the mechanism of action and metabolism of terminators of DNA synthesis. The present study assesses the most general methods of tritium incorporation into various diazines, using as examples the synthesis of tritium-labelled heterocyclic bases, nucleosides, and their natural and synthetic analogues as well as a number of nucleoside phosphates, oligo- and polynucleotides.

The data in Table 1 shows that in the majority of bases, almost complete substitution of hydrogen atoms by tritium is attained in the SSCH reaction with tritium gas.

The SSCH reaction has also proved to be effective for the synthesis of tritium labelled purine nucleosides, nucleotides, oligo- and polynucleotides (Table 2). It has been stated previously that the efficiency of palladium catalysts in the SSCH reaction with adenine nucleosides decreases in the following order: $Pd/CaCO_3 \geq Pd/BaSO_4 > Pd/Al_2O_3 > Pd/C$ [3].

The distribution of tritium between the heterocyclic base and the glycoside portion in nucleotides was also studied. For this purpose, chemical hydrolysis with subsequent

Table 1. Tritium-labelled heterocyclic bases obtained in the SSCH reaction using T_2 gas.

Compound	T °C	A_{mol}, Ci/mmol
Adenine	170	54.2
Guanine	210	24.9
Xanthine	180	25.1
Hypoxanthine	200	51.1
Thymine[a)]	140	85.0
Thymine[b)]	160	110
Thymine[c)]	170	112
Uracil	170	49
Benzyladenine	190	180
Theophylline	160	24.8
Aminotheophylline	160	5.8
Kinetin	160	160

a) from 5-formyluracil;

b) from 5-hydroxymethyuracil;

c) from 5-methyluracil

Table 2. Solid state catalytic hydrogenation of purines using T_2 gas.[2].

Compound	Cat.	Reaction conditions		A_{mol} Ci/ mmol
		t. °C	min	
Adenosine	F	230	30	84
2'-deoxyadenosine	F	200	30	74
Guanosine	F	210	30	47
2'-deoxyguanosine	F	210	30	42
ATP	B	150	60	12
ADP	B	125	60	13
AMP	B	150	60	19
GMP	B	230	30	29
2',5'-ApApAp	B	200	30	58
Poly-A	B	180	30	10.2 Ci/g

F = 5% $Pd/CaCO_3$

B = 5% $Pd/BaSO_4$

isolation of the respective base and determination of its A_{mol} was employed. The data in the study [7] indicate that over 90% of the tritium is retained at C-8 under the chosen conditions. The following results were obtained in the present study (compound - tritium ratio in the base): adenosine - 0.48; 2'-deoxyadenosine - 0.33; guanosine - 0.30; 2'-deoxyguanosine - 0.49. Thus, in the SSCH reaction of purine nucleosides, the heterocyclic part of the molecule accounts for less than half the tritium.

GMP is stable at temperatures up to 240 °C. Guanosine, resulting from the thermal destruction of GMP shows a higher A_{mol}. As was expected, the thermal degradation of AMP is greater than the nucleosides and GTP. One can hardly anticipate high A_{mol} values for this compound, since it almost completely decomposed. As in the case of GMP, the ATP thermolysis products (ADP and AMP), show higher A_{mol} values. A similar regularity was observed in the study of the reaction of SSCH with tritium of nucleic acids' bromo-substituted components [8]. The authors have attributed this phenomenon to the excitation of the molecule with the breakage of intermolecular bonds.

The thermal stability of the studied oligo- and polynucleotides is higher than that of ATP, which can possibly be accounted for by greater stabilisation on from the intermolecular phosphodiesteric bonds. 3',5'- OligoA appeared to be more labile. The following results were obtained on catalyst B at 110 °C (in brackets are given A_{mol} in Ci/mmol): 3',5'-ApA (9.2); 3',5'-ApApA (49.5); 3',5'-pApApAp (27.0). The isomer of 3',5'-ApApA, namely 2',5'-ApApA, at a ratio of 1:1 appears as one of the SSCH products. This fact was revealed chromatographically. Thus, the study of the SSCH reaction for a whole class of compounds, like the purines, allows us to conclude that this method is of practical and theoretical significance. Altering the solid phase composition, temperature, and the palladium catalyst support provides ample possibilities for variations in the yield and A_{mol} of the tritium labelled compounds. We should state that the role of the palladium catalyst support is crucial and has not so far been elucidated. The problem requires additional research. For instance, calcium carbonate is the most effective palladium support for purine bases and nucleosides, whereas barium sulphate has proved more effective for the rest of the compounds studied.

The main side reactions of the SSCH reaction of purines are thermal destruction of the initial compound and, as a consequence, the isomerization of compounds with a more complicated structure. As a result of these processes, compounds are produced, which are of practical significance. This is important, as destruction products show a higher value of A_{mol} than the initial compound.

Synthesis of tritium labelled non-natural analogues of nucleosides, DNA synthesis terminators, was performed by different methods (Table 3). Specifically labelled compounds were synthesised by using hydrogenolysis and isotope exchange reactions in solution. Generally labelled preparations were produced by the SSCH method. To produce compounds with the label in the carbohydrate part of the molecule, either precursors with

Table 3. Synthesis of tritium-labelled DNA synthesis terminators [4].

Item	Compound	Method of synthesis	Synthesis conditions		Mol. Activ. Ci/mmol
			Catalyst	Duration, min.	
1	[2',3'-^{3}H]2',3'-Dideoxyadenosine	Re-exchange at C8	0.1 N NaOH, 100°C, 10 h.		52.4
2	[8-^{3}H]2',3'-Dideoxyadenosine	LSHIE	B	180	23.2
3	[2',3'-^{3}H]2',3'-Dideoxythymidine	Hydrogenolysis	G	120	51.3
4	[2',3',8-^{3}H]2',3' Dideoxyadenosine	Hydrogenolysis	B	180	73.2
5	[2',3'-^{3}H]2',3'-Dideoxycytidinephosphonate	Hydrogenolysis	G	120	59.1
6	Thymidine([^{3}H]methyl)phosphonate	Hydrogenolysis	G	120	17.2
7	[2'-^{3}H]Thymidinephosphonate	Hydrogenolysis	G	120	18.5
8	[G-^{3}H]Azidothymidine	SSCH	B	120	30.0
9	[G-^{3}H]Azidothymidine-phosphonate	SSCH	B	160	6.3
10	[G-^{3}H]Acyclovire	SSCH	F	120	124
11	[G-^{3}H]Acyclovire phosphonate	SSCH	F	120	56
12	[G-^{3}H]5'-O-phosphonylmethylthymidine	SSCH	B	150	71

the unsaturated 2',3'-bond (3-5, Table 3) or corresponding haloid-substituted compounds (6 and 7, Table 3) were used. For instance, the reduction of 3'-dideoxy-2',3'-dehydrothymidine over palladium oxide smoothly led to [2',3'-^{3}H]dideoxythymidine. In the catalytic reduction of 2',3'-dideoxy-2',3'-dehydroadenosine, the insertion of tritium at the 2',3'- double bond was accompanied by isotope exchange at the C-8(H). This was consistent with the abnormally high molar activity of the produced compound (4, Table 3) 73.2 Ci/mmol, in agreement with the incorporation of approximately three atoms into the molecule, and from its tritium-NMR spectrum in which a signal at δ=8.25, corresponding to tritium at position 8 of adenine. After the label had been exchanged out of position 8, a preparation (1, Table 3) was obtained containing tritium only in the dideoxyribosyl residue. The catalytic heterogeneous isotope exchange reaction (LSHIE) was used for the introduction of tritium into the purine residue of 2',3'-dideoxyadenosine (2, Table 3), with virtually no label incorporated into the sugar residue.

In the SSCH reaction of azidothymidine-phosphonate (9, Table 3), like in the case of azidothymidine (8, Table 3), the highest yield was observed with a barium sulphate-based catalyst. It has been stated previously that for acyclovire (10, Table 3) the nature of the palladium catalyst support exerted no significant effect on the molar activity and, particularly, on the yield of the labelled compound. Similar results were established for acyclovire-phosphonate (11, Table 3). NMR experiments showed that about 90% of the tritium in [G-^{3}H]5'-O-phosphonylmethylthymidine (12, Table 3) was located in the 5-CH_3 group.

Fig. 2 shows the products of reduction of 5-hydroxymethyl-2'-deoxyUMP (hm-dUMP). In the reduction of the hm-dUMP (I) hydroxymethyl group, TMP (II) is formed which produces 5,6-dihydro-TMP (III) during subsequent reduction. In the hydrolysis of (III), 3-(N-2'-deoxy-5'-phospho-β-D-ribofuranosyl)ureidoisobytiric acid (IV) is formed. Depending on the reaction conditions (solvent, nature of catalyst), preferential reduction of the pyrimidine nucleus can proceed with formation of 5-hydroxymethyl-5,6-dihydro-2'-deoxyuridine-5'- monophosphate (V) which can produce in the hydrolysis 2'- hydroxy-3-(N^1-2'-deoxy-5'-phospho- β-D-ribofutanosyl)ureidobytiric acid (VI). Further reduction of the latter leads to the formation of the above-mentioned acid (IV). These results are essential for understanding the reactions taking place in the tritium hydrogenolysis of pyrimidine compounds. With regard to the catalyst and medium used (for reactions in solution), the direction of the reduction reaction may change, giving different products. For instance, in the reduction in 25% acetic acid over PtO_2, reduction of the hydroxymethyl group and the 5,6-double bond (I) occurs leading to the formation of TMP (II) and its 5,6-dihydroderivative (III). The 5,6- double bond is mainly restored over Rh/Al_2O_3 and Ru/Al_2O_3 catalysts, producing the respective 5,6-dihydroderivative (V) with a further formation of ureidoisobytiric acid derivative (VI). All the obtained data were analysed in the above-described way and summarised in Table 4.

It has been stated that low A_{mol} corresponds to high [^{3}H]TMP yields in the reaction in solution. The best results were obtained in the mixture of dioxane - acetic acid - water with the catalyst A. In these conditions, the A_{mol} of the resulting compound corresponds to the incorporation of one tritium atom with a degree of isotope substitution of 41%.

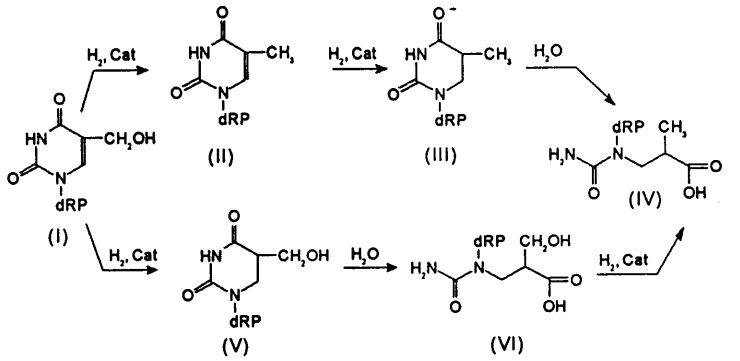

Figure 2. Preparation of tritium-labelled thymidine-5'-monophosphate (II). (I) hydroxymethyl-2'-deoxyUMP; (II) TMP; (III) 5,6-dihydro-TMP; (IV) 3-(N^1-2'-deoxy-5'-phospho-β-D-ribofuranosil)ureidoisobytiric acid; (V) 5-hydoxymethyl-5,6-dihydro-2'-deoxyUMP; (VI) 2'-hydoxy-3-(N^1-2'-deoxy-5'-phospho-β-D-ribofuranosil)ureidoisobytiric acid. dR - 2'-deoxyribose; P - monophospate [5].

Table 4. Composition of the product* mixture from the reduction of hm-dUMP by hydrogen (in brackets an approximate yield, %) in different solutions (duration of reaction 2 hours)

Solution	Catalysts				
	A	B	C	D	E
5% CH_3COOH	II (100)	I (50) II (50)	VI (100)	VI (100)	II (100)
25% CH_3COOH	I (30) II (70)	I (70) II 30)	I (30) V (20) VI (50)	I (40) V (20) VI (40)	II (50) III (50)
50% CH_3COOH	I (50) II (50)	I (80) II (20)	I (30) III (10) IV (60)	I (30) III (10) IV (60)	------
Dioxan-CH_3COOH-water (75:24:1)	I (90) II (10)	I (100)	III (3-5) V (3-5) IV and VI (80-90)	V (3-5) VI (90-95)	II (30) III (70)

* Structures of compound (I) – (VI) are on fig. 2.

The results of the SSCH reaction of HM-dUMP and TMP with gaseous tritium showed that extensive decomposition took place at temperatures above 170 °C. Less than 5% of the desired compound remained in the reaction products. If the reaction time was increased to over 30 minutes, accumulation of products of hydration of the 5,6-double bond (III) and derivatives of ureidoisobytiric acid (IV) take place (Fig.4). These products have a high A_{mol} and show properties close to those of the desired product. All this impedes further purification.

Comparison of the diverse approaches to the one-stage synthesis of [^{3}H]TMP shows that the reduction of HM-dUMP in solution, as compared with a similar solid state reaction, offers no advantage in terms of the yield and the A_{mol} of the desired product. In our opinion, the optimum choice is the production of tritium-labelled TMP by HM-dUMP reduction in the solid state at 170 °C (68.5 Ci/mmol; yield 10.2 %). Evidently, the application of the SSCH reaction to pyrimidine compounds is limited, because, side by side with the main reaction of isotope exchange, hydration of the 5,6-double bond proceeds with the formation of the products shown in Fig. 1. Therefore, catalytic reactions with gaseous tritium, such as isotope exchange (LSGIE) and dehalogenation (LSCD) in solution, were used for the synthesis of pyrimidine compounds (Table 5).

Table 5. Synthesis of some tritium labelled pyrimidines. Catalyst – B.

Item	Compound	Method of synthesis	Lab. compound	
			Yield, %	A_{mol} Ci/mmol
1.	Cytosine β-D-arabinoside	LSGIE	98.7	16.2
2	Cytosine β-D-arabinosid-5'-monophosphate	LSGIE	79.5	3.24
3	O^2,2'-cyclocytidine	LSGIE	93.6	15.7
4	O^2,2'-cyclocytidine-5'-monophosphate	LSGIE	90.3	4.32
5	6-Azauridine	LSCD	90.9	22.4
6	6-Azacytidine	LSCD	95.3	25.9

It follows from the acquired data that, under similar reaction conditions, the A_{mol} of nucleosides is considerably higher than that of nucleotides (2 and 4, Table 5). This fact is probably due to the particular features of the three-dimensional structure of these compounds, namely, their conformation in solution. Mobile nucleosides are likely to be more easily absorbed on the surface of the palladium catalyst, which contributes to the reaction being more fully accomplished.

EXPERIMENTAL

The solid state catalytic hydrogenations with gaseous tritium and the isolation of tritium-labelled heterocyclic bases have been performed as described in [2]. The reactions of liquid state catalytic heterogeneous isotope exchange with gaseous tritium and of hydrogenolysis, isolation and purification of tritium labelled purine bases have been conducted as described in [4]. The isolation, purification and identification of the compounds shown in Fig. 1 have been conducted as described in [5]. For the isolation and purification of compounds 1, 3, 5 and 6 (Table 5), chromatography on Sephadex G-10, 1.6 x 100 cm., elution with water, 25 ml/h were used. Compounds 2 and 4 (Table 5) were isolated on a DE-cellulose bicarbonate form column (26 x 360 mm). The elution was performed during 8 h, 0.05-0.15 linear TEAB gradient; pH 8.6; 100 ml/h.

The following catalysts were employed: A - 6% $Pd/BaSO_4$; B - 5% $Pd/BaSO_4$ (Fluka); C - 5% Rh/Al_2O_3 (Koch-Light); D - 5% Ru/Al_2O_3 (Koch-Light); E - PtO_2 (Merck); F - 5% $Pd/CaCO_3$ (Fluka); G - 5% Pd/Al_2O_3 from NPO GIPH, St. Petersburg, Russia. Catalyst (A) was prepared according to the procedure borrowed from [6]. UV spectra were recorded on a Specord M-40 spectrophotometer. Tritium NMR spectra were registered in D_2O on AS 250 NMR Bruker spectrometer. The radioactivity of the samples was measured

on a liquid scintillation counter using LĈ-8 scintillation cocktail (NPO "Monokristalreaktiv", Russia). Solvents were purified according to standard procedures.

In conclusion we are grateful to Professor J.R. Jones for various discussion concerning this work.

REFERENCES:

1. Kraevsky A.A., Beabealashvili R.Sh., Kukhanova M.K. - Itogy Nauki i Tehnyki, VINITI, Immunologia, **29** ,123 (1992)
Kukhanova MK, Kraevsky AA, ibid., 163

2. Sidorov G.V., Myasoedov N.F. - J.Lab.Comp.Radiopharm. **34**: 353 (1994)

3. Sidorov G.V., Myasoedov N.F. - Radiohimia **37**: 270 (1995)

4. Sidorov G.V., Myasoedov N.F., Yasko M.V. - J.Lab.Comp. Radiopharm. **34**: 34: 339 (1994)

5. Sidorov G.V., Myasoedov N.F. - Bioorganicheskaya khimia. **22**: 291 (1996)

6. Mozingo R. - Org.Syn. No 3: 685 (1955)

7. Sidorov G.V., Myasoedov N.F. - Radiokhimia **16**: 291 (1966)

8. Myasoedov N.F., Marchenkov N.S., Mikhailov K.S. - Organic compounds labelled by radioisotopes. Prague CKAE: 275 (1977)

