SYNTHESIS OF RADIOACTIVE ^{11}C MOLECULES FOR MEDICAL RESEARCH ON A MICRO-SCALE : A THEORETICAL AND PRACTICAL APPROACH

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SUMMARY

It is often necessary to obtain certain ^{11}C radiopharmaceuticals with a high specific activity (> 1000 Ci/mmole), especially when these are intended for medical purposes and are either toxic or used for "in vivo" specific receptor binding studies. The amounts of labelling agent must be very small (concentrations often below to 10^{-3} M) and some synthesis are difficult or impossible under these conditions.

- This article shows how the specific activity of the labelling agent A_1^* affects the synthesis yield of the radiopharmaceutical $A_1^{*\prime}$ in the reaction :

$$\nu_1 A_1^* + \nu_2 A_2 \rightleftharpoons \nu'_1 A_1'^* + \nu'_2 A_2'$$

- A specific activity increase can improve, reduce or have no effect on the yield according to the respective values of ν_1, ν_2, ν'_1, ν'_2.

- Different ways to shift an equilibrium unfavourable to labelling are also given : reaction temperature, concentrations, addition of starting products, removal of final products.

- The reaction kinetics are important since the half-life of carbon 11 is 20 mn, but this parameter is a complex function of concentrations which can only be determined by experiment. It seems however that in special cases the percentage of radioacti-

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vity incorporated in a given time is independent of specific activity.

- The effect of impurities, which can be present in concentrations of the same order of magnitude as those of the labelling agent is also discussed.

- Each theoretical development is illustrated by concrete examples studied in the laboratory.

- The conclusion is that theoretically it should not be impossible to synthesize strictly carrier-free ^{11}C radiopharmaceuticals in certain favourable cases (if it were known how to prepare such carrier-free labelling agents).

Key Words: ^{11}C radiopharmaceuticals, Synthesis yield, specific radioactivity

INTRODUCTION

Of the radioisotopes used for labelling organic molecules, ^{11}C offers particular advantages. This radioisotope can be used for human in vivo investigation because of its short half-life (T = 20,4 mn) and its positron disintegration which leads to gamma radiation detectable by external counting.

According to the disintegration law $\frac{dN}{dt} = -\lambda N$ the radioactivity corresponding to a given amount of isotope, or specific radioactivity, can be calculated. For ^{11}C the theoretical S.R.A. is 10^4 Ci/µmole. The amount of ^{11}C radioactivity generally handled for labelling is of the order of 0,1 to 1 Ci which corresponds theoretically to 10 to 100 picomoles of carbon. Actually in spite of all precautions taken to avoid dilution of the ^{11}C with stable carbon the specific activity obtained at present is lower by several powers of 10 than the theoretical value : a few Ci/µmole at the end of bombardment (1, 2). The quantity of carbon involved in reality is therefore about a fraction of a µmole and the concentration of radioactive precursor is less than M/1000. This raises problems unusual in organic synthesis and certain reactions are not feasible. Since in practice the reaction volumes cannot be reduced beyond certain limits it is sometimes necessary to add carrier. However this is often detrimental to the medical use of the radiopharmaceutical (when the labelled product is highly toxic or when it is used for in vivo specific receptor binding studies).

The aim of this work is to study the influence of the labelling agent concentration on the organic synthesis yield and to discuss some ways to shift the balance when the yield is poor. Moreover because of the half-life of ^{11}C the time taken to reach this equilibrium is also an important factor which needs to be mentioned.

INFLUENCE OF THE LABELLING AGENT CONCENTRATION ON THE SYNTHESIS YIELD

The labelling agent is normally in a relatively simple chemical form :

^{11}CO, $^{11}CO_2$, $^{11}CH_4$, $H^{11}CN$, $H^{11}CHO$, $I^{11}CH_3$, $^{11}CH_3OH$, etc...

and is required to react with a precursor of the radiopharmaceutical. Let us consider the general reaction, taking place in a homogeneous medium :

$$\nu_1 \; A^*_1 + \nu_2 \, A_2 + \nu_i A_i \rightleftharpoons \nu'_1 \; A'^*_1 + \nu'_2 \, A'_2 + \ldots \nu'_i \, A'_i$$

with ν_1, ν_2,... ν'_1... ν'_i the different coefficients of the chemical equation, A^*_1 the labelling agent and A'^*_1 the radiopharmaceutical expected.

When the components are in sufficiently dilute solution and behave ideally the equilibrium condition is expressed by (3) :

$$\frac{C'^{\nu'_1}_1 \cdot C'^{\nu'_2}_2 \cdot C'^{\nu'_i}_i}{C_1^{\nu_1} \quad C_2^{\nu_2} \quad C_i^{\nu_i}} = K_c (V_o)^{-\Delta\nu} \qquad (1)$$

where C_i is the molar concentration of component i,

V_o the volume of the solution,

K_c the equilibrium constant referred to the molar fractions

and $\Delta\nu = \nu'_1 + \nu'_2 + \nu'_i - \nu_1 - \nu_2 - \nu_i$.

In the gas phase, with perfect gases the expression becomes (3) :

$$\frac{C'^{\nu'_1}_1 \cdot C'^{\nu'_2}_2 \cdot C'^{\nu'_i}_i}{C_1^{\nu_1} \quad C_2^{\nu_2} \quad C_i^{\nu_i}} = K_p (RT)^{-\Delta\nu} \qquad (2)$$

where K_p is the equilibrium constant referred to the partial pressures,

T the absolute temperature

and R the perfect gases constant.

The labelling yield is defined by the ratio :

$$\eta \frac{A'^{*}_{1}}{A^{*}_{1}} = \frac{\text{radioactivity in } A'^{*}_{1} \text{ at equilibrium}}{\text{radioactivity introduced by } A^{*}_{1}} = \frac{C'_1/\nu'_1}{C_1^{o}/\nu_1} \qquad (3)$$

with C_1^{o} = concentration of component 1 before the reaction.

When the original system contains none of the final products, but only initial components of concentration C_i^{o} and inert gases or solvents we reach the relationship (4) :

$$\frac{\left(\frac{C_1^{o}\,\eta}{\nu_1}\right)^{\Sigma\nu'} \cdot \nu'_1{}^{\nu'_1} \ldots \nu'_i{}^{\nu'_i}}{C_1^{o\nu_1}\,(1-\eta)^{\nu_1} \cdot \left(C_i^{o} - C_1^{o}\,\eta\,\frac{\nu_i}{\nu_1}\right)^{\nu_i}} = K_c (V_o)^{-\Delta\nu} \qquad (4)$$

$$\text{or } K_p (RT)^{-\Delta\nu}$$

with $\Sigma\nu' = \nu'_1 + \nu'_2 + \nu'_i$.

Let us see what happens to this general expression in simple cases illustrated by examples.

1) Reactions of type $A^{*}_1 + A_2 \rightleftharpoons A'^{*}_1 + A'_2$

Here we have $\Delta\nu = 0$ ($\nu_1 = \nu_2 = \nu'_1 = \nu'_2 = 1$) and expression (4) is reduced to :

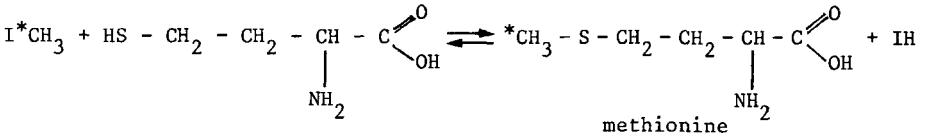
$$\frac{\eta^2}{1-\eta} = K_c \left(\frac{C_2^{o}}{C_1^{o}} - \eta\right)$$

When the specific activity of the labelling agent is very high, C_1^{o} becomes very small compared with C_2^{o} and the yield η is close to unity. Improving the specific activity increases the yield in this type of synthesis.

Examples are the labelling of methionine, diazepam or flunitrazepam by the action of methyl iodide on the corresponding non-methylated derivative. The labelling agent concentrations are below M/1000.

a) Methionine (5, 6)

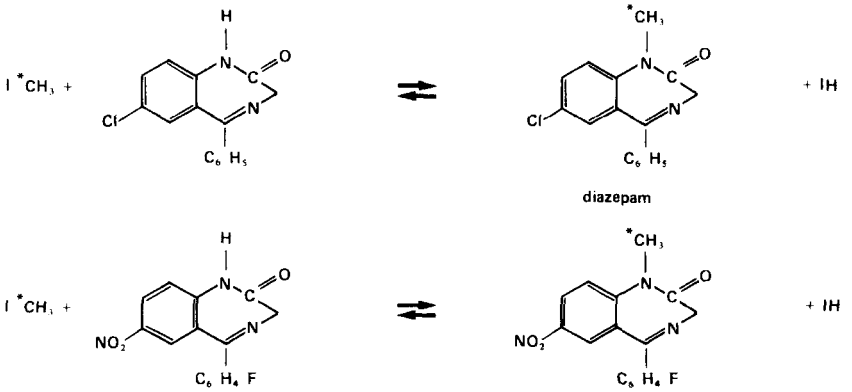
Homocystein (in thiolactone form, 2 µmoles) is dissolved in a water-acetone mixture (150/200 µl) containing sodium hydroxide (70 µmoles, added for reasons given below). The quantity of radioactive methyl iodide lies between 0,1 and 0,3 µmole. The reaction is performed at 70° for 7 mn and is practically quantitative.



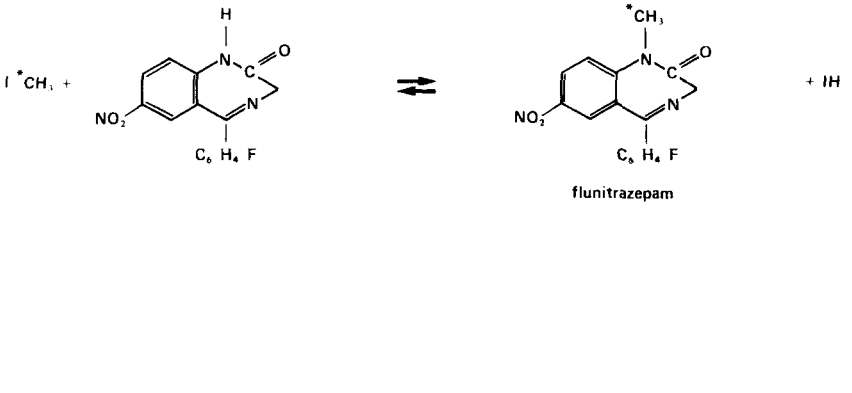
methionine

b) Diazepam, flunitrazepam (7)

1 µmole nordiazepam or norflunitrazepam in 240 µl acetone reacts for 8 mn at 90°C with 0,1 to 0,3 µmole ICH_3 in the presence of 8 µl 10 N sodium hydroxide.



diazepam



flunitrazepam

In both cases the concentration of sodium hydroxyde in large excess is constant and the reaction may be considered as being of the $A_1^* + A_2 \rightleftharpoons A_1^{*'} + A_2'$ type.

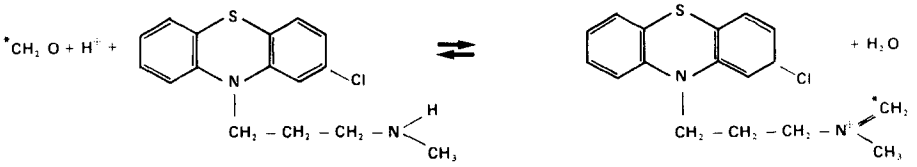
2) Reactions of type $A_1^* + A_2 \rightleftharpoons A'^*_1$

In this case $\Delta\nu = -1$ ($\nu_1 = \nu_2 = \nu'_1 = 1$) and ④ becomes :

$$\frac{\eta}{1-\eta} = K_c V_o (C_2^o - \eta C_1^o)$$

When C_1^o is small enough the second term is reduced to $K_c V_o C_2^o$ and the yield becomes independent of the specific activity of the labelling agent.

An illustration of such a case is the reaction of ^{11}C formaldehyde on monodesmethyl chlorpromazine (or on monodesmethylimipramine, monodesmethylchlorimipramine, nor nicotine) (6).



The pH and water concentration are constant during this reaction, given the quantities involved. They therefore play no part in the yield equation and an $A^*_1 + A_2 \rightleftharpoons A'^*_1$ reaction may be assumed.

The ^{11}C formaldehyde concentration is less than M/1000. 1 µmole of monodesmethyl derivative reacts for 7-10 mn with 0,1 to 0,3 µmole labelled formaldehyde in an acetonitrile-water mixture (200/50 µl) containing 2 µl acetic acid (to acidify the solution) and 1 µmole sodium cyanoborohydride (to reduce the imine formed into methyl derivative, which is the final product used).

3) Reactions of type $A_1^* \rightleftharpoons A'^*_1$

$\Delta\nu = 0 \quad (\nu_1 = \nu'_1 = 1)$

$$\frac{\eta}{1-\eta} = K_c$$

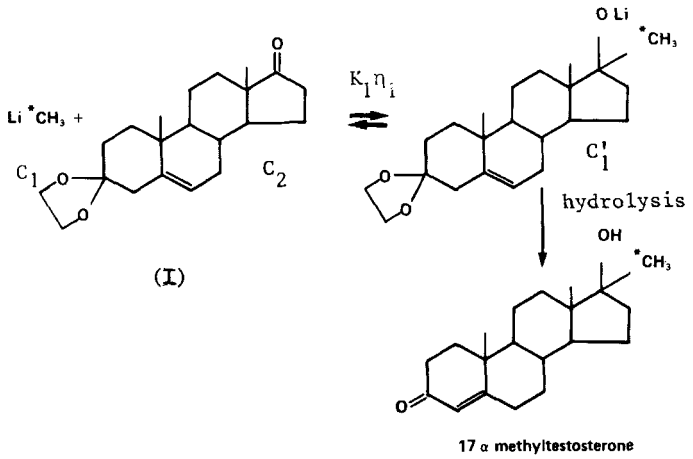
The labelling yield is not dependent on any concentration.

An example is the synthesis of methyl iodide from methanol (5, 6)

$$^{*}CH_3OH + IH \rightleftharpoons {}^{*}CH_3I + H_2O$$

0,1 to 0,3 µmoles of ^{11}C methanol react with 200 µl of a heated 67 % hydriodic acid solution under reflux. A constant nitrogen current brings in the methanol and carries off the methyl iodide formed. The operation lasts about 5 mn. The hydriodic acid and water concentrations are constant and we arrive at an $A_1^* \rightarrow A'^*_1$ type reaction.

Another example is the addition reaction of methyl lithium on 3.3 ethylene dioxy Δ^5 androsten-17 one (I) for the labelling of 17 α methyltestosterone.



When the solution is saturated in initial product I, the type of reaction is $A_1^* \rightleftharpoons A'^*_1$.

Fig. 1 shows in this case that yield is roughly independent of concentration of labelling agent.

Four µmoles of methyl lithium in diethyl ether react with I in powder (10 mg) for 10 mn at ambient temperature.

The solvent is then evaporated and hydrolysis is carried out by heating 3 mn at 70°C with 500 µl ethanol and 200 µl HCl 2 N.

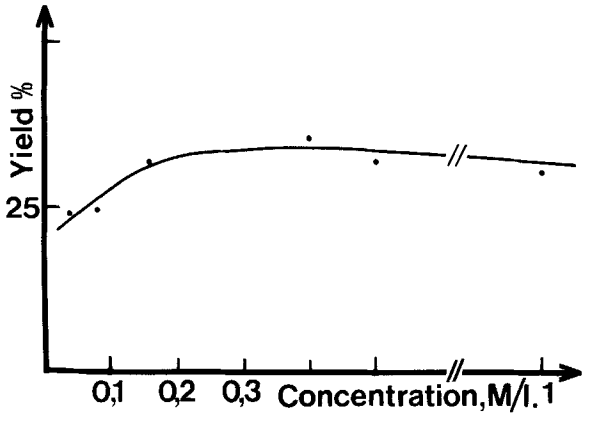


Fig.1. Influence of concentration of labelling agent in the synthesis of 17 α methyltestosterone using methyl lithium.
Reaction mixture : $LiCH_3$, 4 µmoles, dissolved in diethyl ether.
Initial component in powder.
Reaction conditions : 10 mn at ambient temperature. The solvent is evaporated and hydrolysis is carried out by heating 3 mn at 70°C with 500 µl ethanol and 200 µl HCl 2N.

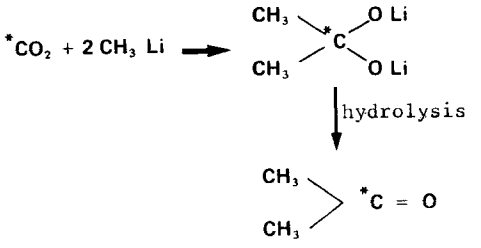
4) Reactions of type $A_1^* + 2A_2 \rightleftharpoons A'^*_1$

$$\nu_1 = \nu'_1 = 1 \qquad \nu_2 = 2 \qquad \Delta\nu = -2$$

$$\frac{\eta}{1-\eta} = K_c V_o^2 (C_2^o - 2C_1^o \eta)^2$$

Here again when C_1^o is small enough the yield is independent of the specific activity of the labelling agent.

The synthesis of acetone by methyl lithium is a relevant example (8)



$^{11}CO_2$ is brought by a current of nitrogen into a cooled solution (-20°C) of methyl lithium in anhydrous ether (5 µmoles in 100 µl). The ether is evaporated, then after hydrolysis by 50 to 100 µl water the radioactivity is carried off by a nitrogen current.

5) Reactions of type $2A_1^* + A_2 \rightleftharpoons A'^*_1$

$2A_1^* \rightleftharpoons A'^*_1$

In the first case, we have $\Delta\nu = -2$ ($\nu_1 = 2 \quad \nu_2 = \nu'_1 = 1$) and we get :

$$\frac{\eta}{(1-\eta)^2} = 2K_c V_o^2 C_1^o \left(C_2^o - \frac{C_1^o \eta}{2}\right)$$

Since C_2^o is limited by the solubility of component A_2 in the solvent the yield tends toward zero as the specific activity of A_1^* increases.

When the solution is saturated with A_2 the type of reaction is

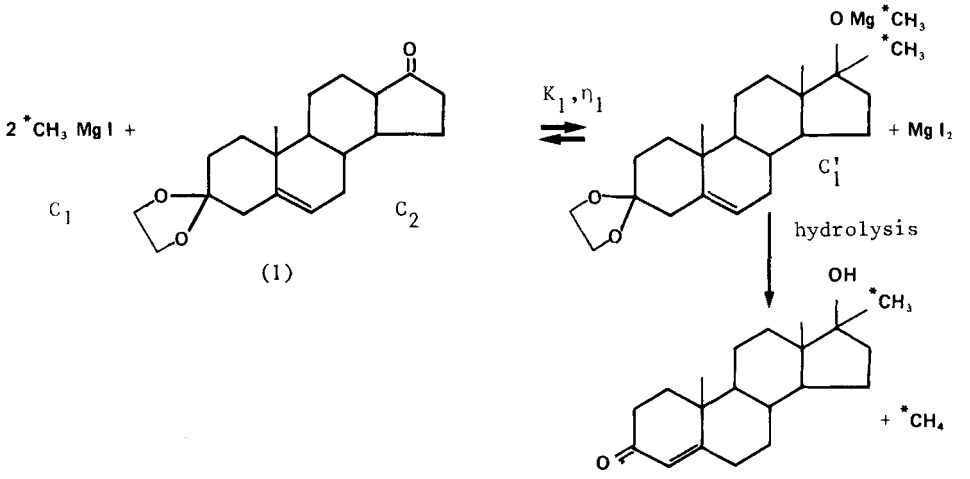
$$2A_1^* \rightleftharpoons A'^*_1$$

$\Delta\nu = 1 \quad (\nu_1 = 2 \quad \nu'_1 = 1)$

$$\frac{\eta}{(1-\eta)^2} = 2K_c V_o C_1^o$$

The conclusion is the same as above.

An example is the addition reaction of methylmagnesium iodide on (I). The reaction scheme is that proposed by Ashby (9, 10), with two molecules of methylmagnesium iodide.



Product I is present in large excess, at saturation in the reaction solvent, magnesium iodide is insoluble and the concentration of these two compounds may therefore be considered as constant and not involved in the yield equation.

After hydrolysis the final yield is reduced by a factor of 2 because loss of radioactive methane.

Fig. 2 shows that the labelling yield is close to zero when the specific activity of the reagent is high.

Methylmagnesium iodide (1 µmole in ether) reacts with I in powder for 5 mn at ambient temperature.

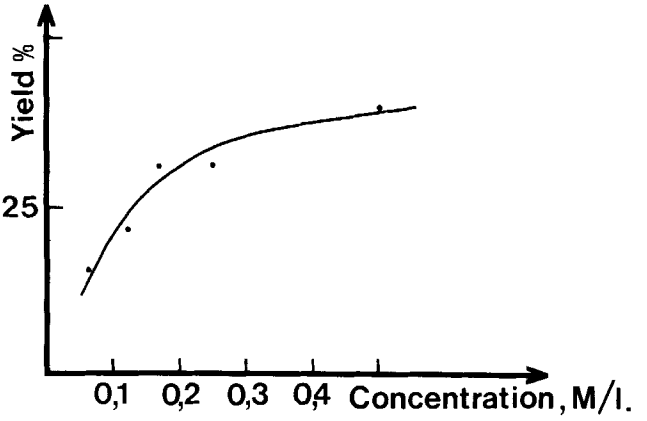
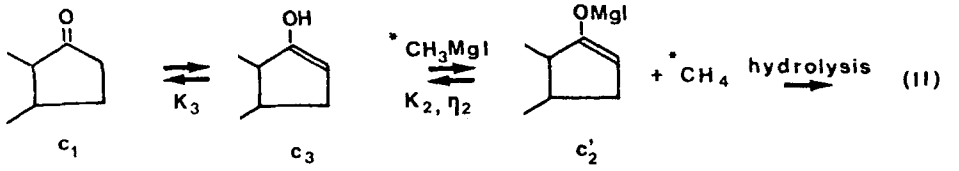


Fig.2. Influence of concentration of labelling agent in the synthesis of 17 α methyltestosterone using methyl magnesiumiodide. Reaction mixture : ICH_3, 1 µmole, in anhydrous diethyl ether. Magnesium powder mixed with solid initial component (1/1). Reaction conditions : 5 mn at ambient temperature. Hydrolysis is carried out by heating 3 mn at 70°C with 700 µl ethanol and 100 µl HCl 2N.

Moreover this synthesis is made inapplicable under these conditions with ^{11}C because of the competition between the addition reaction and enolization (11).



If we call η_1 the addition yield on the ketone (I), η_2 that of the reaction on the enol (II) we can write :

$$2C'_1 = \eta_1 C_1^o$$

$$C'_2 = \eta_2 C_1^o$$

$$\frac{\eta_1}{\eta_2} = \frac{2C'_1}{C'_2}$$

with $K_1 = \frac{C'_1}{C_1^2}$ (concentrations of MgI_2 and of compound I are constant)

$$C'_1 = K_1 C_1^2$$

$K_2 = \frac{C'_2}{C_1 C_3}$ (methane being volatile, its concentration in the solution is low and constant)

$$C'_2 = K_2 C_1 C_3$$

$$\frac{C_3}{C_2} = K_3 \text{ (enolization)}$$

$$C_2 = C_2^o \text{ (saturation with compound I)}$$

$$C_3 = K_3 C_2^o$$

$$\frac{\eta_1}{\eta_2} = \frac{2 K_1 C_1^2}{K_2 C_1 C_3} = \frac{2 K_1 C_1}{K_2 K_3 C_2^o}$$

$$C_1 = C_1^o - 2C'_1 - C'_2 = C_1^o (1 - \eta_1 - \eta_2)$$

$$\frac{\eta_1}{\eta_2} = \frac{2 K_1 C_1^o}{K_2 K_3 C_2^o} (1 - \eta_1 - \eta_2)$$

The proportion of addition reaction on ketone (leading after hydrolysis to 17 α methyltestosterone) thus decreases as the methyl magnesium iodide concentration tends towards zero. Labelling becomes very difficult when the concentration falls below M/10.

Remark : Competition due to enolization is not so important when methyl lithium is used.

In this case we have : $A_1^* \rightleftharpoons A_1'^*$

$C_1 \; K_1 \eta_1 \; C'_1$

$K_1 = \frac{C'_1}{C_1}$ (concentrations of MgI_2 and of compound I are constant)

$$C'_1 = K_1 C_1$$

$$C_1' = \eta_1 \, C_1^{\,o}$$

$$\eta_1 = \frac{K_1 \, C_1}{C_1^{\,o}}$$

$$K_2 = \frac{C_2'}{C_1 \, C_3}$$ (methane is volatile and its concentration is constant).

$$C_2' = \eta_2 \, C_1^{\,o}$$

$$\eta_2 = \frac{K_2 \, C_1 \, C_3}{C_1^{\,o}}$$

$$\frac{C_3}{C_2} = K_3 \text{ (enolization)}$$

$$C_3 = K_3 \, C_2^{\,o}$$

$$\eta_2 = \frac{K_2 \, K_3 \, C_1 \, C_2^{\,o}}{C_1^{\,o}}$$

$$\frac{\eta_1}{\eta_2} = \frac{K_1}{K_2 \, K_3} \cdot \frac{1}{C_2^{\,o}}$$

The ratio of yields of the two reactions is independent of concentration of labelling agent.

6) Reactions of type $2A_1^* + A_2 \rightleftharpoons A_1'^* + A_2'$

$\Delta\nu = -1 \quad (\nu_1 = 2 \quad \nu_2 = \nu_1' = \nu_2' = 1)$

Expression ④ becomes : $\left(\frac{\eta}{1-\eta}\right)^2 = 4 \, K_c \, V_o \, (C_2^{\,o} - \eta \, C_1^{\,o})$

As the specific activity of the labelling agent increases the yield tends towards a limit dependent on the initial product concentration.

The example n°5 could relate to this kind of reaction if the synthesis were carried out in a solvent capable of dissolving the magnesium salt.

7) Reactions of type $2A_1^* \rightleftharpoons A_1'^* + A_2'$

$\Delta\nu = 0 \quad (\nu_1 = 2 \quad \nu_1' = \nu_2' = 1)$

$$\frac{\eta}{1-\eta} = 2\sqrt{K_c}$$

The synthesis yield is independent of all concentrations. Cannizzaro reactions, dismutations belong to this category.

Table I sums up the influence of the labelling agent concentration on the different reaction yields, the concentration of the initial product remaining constant.

Table I.

	$\nu_1 = 1$		$\nu_1 = 2$	
	Reaction	Yield	Reaction	Yield
$\Delta\nu = 0$	$A_1^* + A_2 \rightleftharpoons A'^*_1 + A'_2$ $A_1^* \rightleftharpoons A'^*_1$	$\nearrow 1$ $\rightarrow \neq 0$	$2A_1^* \rightleftharpoons A'^*_1 + A'_2$	$- \neq 0$
$\Delta\nu = -1$	$A_1^* + A_2 \rightleftharpoons A'^*_1$	$\rightarrow \neq 0$	$2A_1^* + A_2 \rightleftharpoons A'^*_1 + A'_2$ $2A_1^* \rightleftharpoons A'^*_1$	$\rightarrow \neq 0$ $\searrow 0$
$\Delta\nu = -2$	$A_1^* + 2A_2 \rightleftharpoons A'^*_1$	$\rightarrow \neq 0$	$2A_1^* + A_2 \rightleftharpoons A'^*_1$	$\searrow 0$

$\nearrow 1$: the yield tends towards 1 when A_1^* decreases.

$\rightarrow \neq 0$: the yield η tends towards a limit $0 < \eta < 1$, depending on the initial concentration of A_2, the mass constant coefficient and the solution volume.

$\searrow 0$: the yield tends towards 0
$\Delta\nu = \nu'_1 + \nu'_2 - \nu_1 - \nu_2$

DISPLACEMENT OF EQUILIBRIA

When the yield is inadequate it can be improved by varying the reaction parameters : temperature, pressure or volume, concentration of reagents.

1) Temperature

The effect of temperature on the reaction yield is studied beforehand with non radioactive compounds. A temperature rise promotes the development of an endothermic reaction (Le Chatelier's law) and vice-versa.

Sometimes an optimum is observed, due to the appearance of decomposition reactions at high temperature. Fig. 3 gives an example of this study in the case of o-methyl bufotenine synthesis (12).

2) Pressure, dilution

In gaseous systems, a rise in pressure favours reactions involving a volume

reduction ($\Delta\nu < 0$). Dilution with an inert component improves the yield when $\Delta\nu$ is positive (as in dissociation, ionisation, etc...).

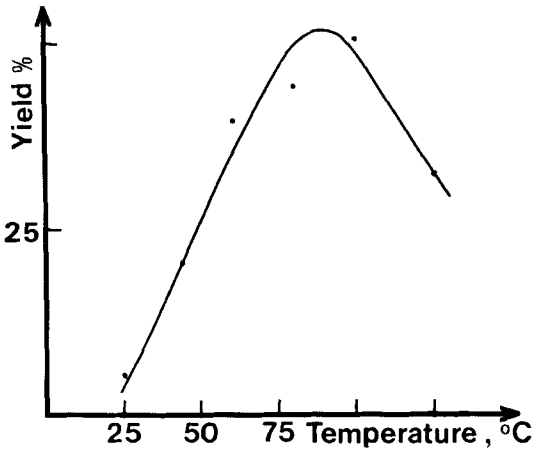


Fig.3. Influence of temperature on the synthesis of o-methyl-bufotenine.
Reaction mixture : methoxytryptamine : 2 µmoles
formaldehyde : 2 µmoles
sodium cyanoborohydride : 5 µmoles
acetic acid : 2 µl
acetonitrile : 300 µl
water : 100 µl

3) Compositions

In certain cases an equilibrium may be shifted by addition of an initial product (other than the labelling agent) or removal of a final product.

a) addition of an initial product

The balance is shifted in the direction of final product formation if the following inequality prevails :

$$\Delta\nu + \frac{\nu_k}{x_k} > 0 \qquad (13)$$

where x_k represents the molar fraction of the initial component k ($x_k < 1$).

Examples :

a_1) In the cases of methionine, diazepam and flunitrazepam synthesis (see "Influence of the labelling agent concentration on the synthesis yield", § 1), the inequality is respected since $\Delta\nu = 0$, $\nu_2 = 1$ and x_k is positive. It is always advisable therefore to use an excess of initial product.

a_2) The same applies to the synthesis of chlorpromazine, imipramine or

chlorimipramine (see "Influence of the labelling agent concentration on the synthesis yield", § 2)

$\Delta\nu = -1 \qquad \nu_2 = 1$

$$-1 + \frac{1}{x_2} > 0$$

The inequality is still satisfied since $x_2 < 1$.

Addition of the nor-derivative moves the balance in the labelling direction, as shown on fig. 4, with chlorimipramine (14).

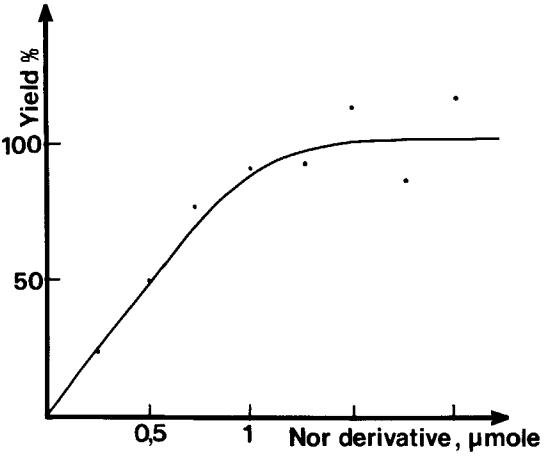


Fig.4. Influence of the quantity of initial component on the synthesis of clomipramine.
Reaction mixture : formaldehyde : 0,5 µmole
formic acid : 1 µmole
dimethyl formamide : 500 µl
nor-derivative : monodesmethylclomipramine.
Reaction conditions : 5 mn at 110°C.

a_3) Addition of methyl lithium in the synthesis of acetone (see "Influence of the labelling agent concentration on the synthesis yield", § 4) should be beneficial since we have :

$\Delta\nu = -2 \qquad \nu_2 = 2$

and the inequality $-2 + \frac{2}{x_2} > 0$ still applies since $x_2 < 1$.

In fact the reverse is true and the acetone yield actually decreases beyond a certain reagent concentration (fig.5, ref. 8). This is because in large excess an extra molecule of methyl lithium reacts to give ter butyl alcohol a phenomenon which can be explained as follows :

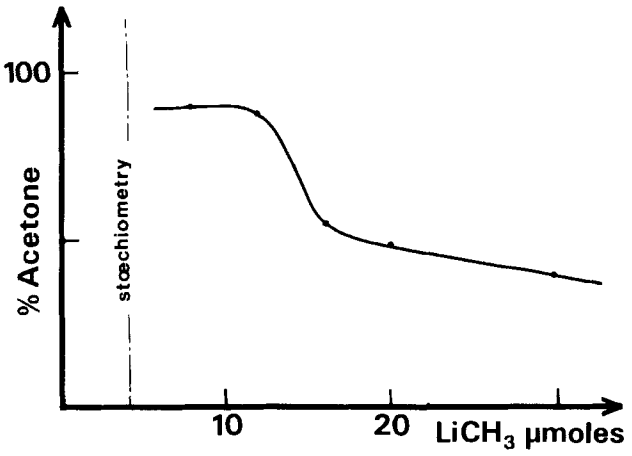
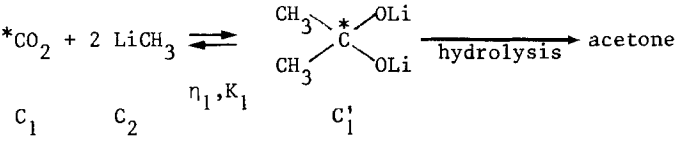
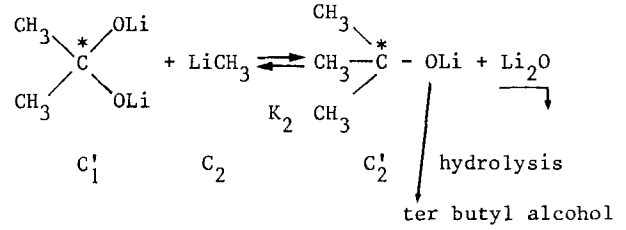


Fig.5. Influence of reagent excess.
Synthesis of acetone with CO_2 and $LiCH_3$.
Percentage of acetone is expressed as $\frac{\text{acetone} \times 100}{\text{acetone} + \text{ter butyl alcohol}}$
Reaction mixture : $LiCH_3$ in 100 µl anhydrous diethyl ether.
Reaction conditions : CO_2 is trapped in $LiCH_3$ solution, the solvent is evaporated and 100 µl water added. The solution is heated and the acetone is transferred by current of nitrogen.





Let η_2 be the overall yield of ter butyl alcohol.

Lithium oxide is insoluble in the reaction solvent (diethyl ether), its concentration is very low and constant.

$$K_2 = \frac{C_2'}{C_1' C_2} = \frac{\eta_2 C_1^{\circ}}{\eta_1 C_1^{\circ} . C_2} = \frac{\eta_2}{\eta_1} C_2$$

$$C_2 = C_2^{\circ} - 2 C_1' - 3 C_2' = C_2^{\circ} - 2C_1^{\circ}\eta_1 - 3C_1^{\circ}\eta_2$$

C_2 # C_2^o with a large excess of methyl lithium

$$\frac{\eta_2}{\eta_1} = K_2\, C_2^o \quad \text{or} \quad \eta_1 = \frac{\eta_2}{K_2\, C_2^o}$$

The acetone yield decreases as the quantity of methyl lithium rises.

b) Removal of a final product

For the equilibrium to shift in favour of labelling we must have :

$$\frac{\nu' k'}{x_{k'}} - \Delta\nu > 0$$

where $x_{k'}$ represents the molar fraction of a final product ($x_{k'} < 1$).

When $\Delta\nu$ is negative or zero the expression is still verified.

b_1) This applies for instance to the synthesis of methionine, diazepam or flunitrazepam ($\Delta\nu = 0$, see "Influence of the labelling agent concentration on the synthesis yield", § 1). The presence of sodium hydroxide is aimed at neutralizing the hydriodic acid formed and the yield is considerably improved (table II).

Table II.

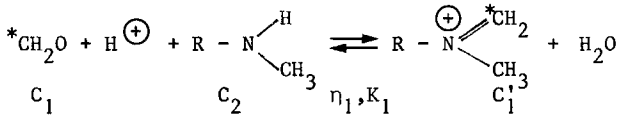
	Without NaOH	With NaOH
Methionine yield, % *	8	80
Flunitrazepam yield, % **	0	81

* Conditions described in "Influence of the labelling agent concentration on the synthesis yield", § 1a) Homocystein thiolactone has been first hydrolysed in basic solution for 5 mn at 70°C and then neutralised.

** Conditions described in "Influence of the labelling agent concentration on the synthesis yield", § 1b).

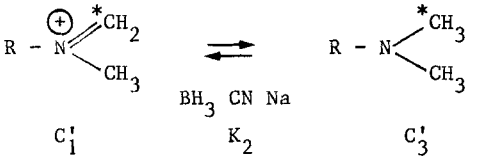
b_2) Chlorpromazine and similar molecules are synthetized in two stages (Influence of the labelling agent concentration on the synthesis yield", § 2).

→ formation of an imine :



Here, the $H^{\oplus}$ et water concentrations are constant.

→ reduction of the imine by sodium cyanoborohydride :



C_1' K_2 C_3'

Let η_2 be the overall labelling yield

$$\eta_2 = \frac{C_3'}{C_1^{\,o}}$$

$$K_1 = \frac{C_1'}{C_1 C_2}$$

$$C_1^{\,o} = C_1' + C_1 + C_3' = \eta_1 C_1^{\,o} + C_1 + \eta_2 C_1^{\,o}$$

$$C_2^{\,o} = C_2 + C_1' + C_3' = C_2 + \eta_1 C_1^{\,o} + \eta_2 C_1^{\,o}$$

$$K_1 = \frac{\eta_1 C_1^{\,o}}{(1-\eta_1-\eta_2) C_1^{\,o} \cdot (C_2^{\,o} - (\eta_1+\eta_2) C_1^{\,o})}$$

To simplify the calculation, it will be assumed that the sodium cyanoborohydride concentration C_3 is in excess with respect to that of the other components and remains constant (it is in fact 3 to 10 times higher than that of formaldehyde).

$$\frac{C_3'}{C_1'} = K_2 = \frac{\eta_2}{\eta_1}$$

$$K_1 = \frac{\frac{\eta_2}{K_2}}{(1-(1+\frac{1}{K_2})\eta_2)\ (C_2^{\,o} - (1+\frac{1}{K_2})\eta_2 C_1^{\,o})}$$

When the specific activity of the labelling agent is sufficiently high we have :

$$K_1 = \frac{\eta_2}{K_2\ (1 - (1+\frac{1}{K_2})\ \eta_2)\ C_2^{\,o}}$$

$$K_1 K_2 C_2^{o} - K_1 K_2 \left(1 + \frac{1}{K_2}\right) C_2^{o} \eta_2 = \eta_2$$

$$\eta_2 = \frac{K_1 K_2 C_2^{o}}{1 + K_1 K_2 \left(1 + \frac{1}{K_2}\right) C_2^{o}}$$

If K_2 is large, as in the case of reactions with sodium cyanoborohydride (15) the yield is close to unity.

b_3) Methyl iodide synthesis (by bubbling of methanol vapours in a refluxing solution of hydriodic acid) is improved by carrying off the product as it forms. Methanol, which has a higher boiling point, is recondensed or remains in the hydriodic acid solution.

- To sum up, an equilibrium unfavorable to labelling may be shifted by playing on the reaction temperature or on the pressure in the gas phase, by adding initial products when $\Delta\nu + \frac{\nu_k}{x_k}$ is positive or by removing final products when $\frac{\nu'_k}{x'_k} - \Delta\nu'$ is positive. Application of this rule implies an exact knowledge of the reaction scheme.

REACTION KINETICS

Given the short half life of ^{11}C the time taken for the equilibrium to be established is an important factor. Unfortunately thermodynamics, which can predict the state of equilibrium of systems, are uninformative on the fundamental question of time (16), while in general the rates and affinities of chemical reactions are not related. The problem can only be solved by experience.

For instance when experiment has shown that a reaction of the type :

$$\underset{C_1}{A_1^*} + \underset{C_2}{A_2} \rightleftharpoons \underset{C'_1}{A_1'^*} + \underset{C'_2}{A_2'}$$

obeys the Van't Hoff's law, we can write :

$$v = -\frac{dC_1}{dt} = k\, C_1\, C_2$$

The labelling agent fraction incorporated per unit time is :

$$-\frac{dC_1}{dt} \cdot \frac{1}{C_1^{o}} = \frac{kC_1C_2}{C_1^{o}} \qquad C_1 = C_1^{o} - \eta_t C_1^{o} = C_1^{o}\,(1-\eta_t)$$

η_t = yield at time t.

$$C_2 = C_2^{o} - \eta_t C_1^{o} \qquad -\frac{dC_1}{dt} \cdot \frac{1}{C_1^{o}} = k(1-\eta_t)\,(C_2^{o} - C_1^{o}\,\eta_t)$$

When C_1^{o} is very small, we have : $-\frac{dC_1}{dt} \cdot \frac{1}{C_1^{o}} = k(1-\eta_t)\,C_2^{o}$

The percent incorporation at radioactivity per unit time is a function of the initial product concentration and of the progress of the reaction but is independent of the specific activity of the labelling agent.

This theoretical example shows that the reaction kinetics can be favourable even at very high specific activities.

On the other hand, Långström et al (17) have recently studied the evolution of the radiochemical yield versus time in the particular case of bimolecular reactions and they have shown that under certain conditions the influence of the rate constants is the determinant factor. The kinetic and thermodynamic aspects are therefore complementary to each other.

CONCLUSION

In conclusion it is possible, using the simple rules announced above, to foresee how the specific activity of the labelling agent will affect the yield and to eliminate certain unfavourable cases automatically, when the reaction scheme is known. However, to calculate the yield itself it is necessary to know the mass action constant.

These rules also show by which parameters the equilibrium may be shifted when the labelling percentage is inadequate.

As for the kinetics they can only be determined by experiment, but it is worth noting that in certain cases the time needed to reach equilibrium may be independent of specific activity.

The examples quoted here show that many syntheses are possible with a fraction of a µmole of labelling agent and calculations suggest that the same would be true in certain cases for much smaller amounts.

However any reagent or receptacle contains impurities at concentrations perhaps much the same as those of the labelling agent if the specific activity is very high. What is likely to happen if impurities and labelling agent react ? Here again it all depends on the reaction scheme.

- For a synthesis of the type :

$$\underset{C_1}{2A_1^*} + \underset{C_2}{A_2} \rightleftharpoons \underset{K_1,\eta_1\ C_1'}{A_1^{*\prime}}$$

with the impurity A_3 reacting as follows :

$$\underset{C_1}{A_1^*} + \underset{C_3}{A_3} \rightleftharpoons \underset{K_2,\eta_2\ C_2'}{A_2^{*\prime}}$$

We can write (see "Influence of the labelling agent concentration on the synthesis yield", § 5)

$$\frac{\eta_1}{\eta_2} = \frac{2K_1}{K_2}\,\frac{C_1\,C_2}{C_3} = \frac{2K_1}{K_2}\,\frac{C_2}{C_3}\,C_1^{\,o}\,(1-\eta_1-\eta_2)$$

$$= \frac{2K_1\left(C_2^{\,o} - \frac{\eta_1}{2}\,C_1^{\,o}\right)\cdot C_1^{\,o}\,(1-\eta_1-\eta_2)}{K_2\,(C_3^{\,o} - \eta_2\,C_1^{\,o})}$$

If the specific activity is such that $C_1^{\,o}$ is negligible compared with $C_3^{\,o}$ (and even more compared with $C_2^{\,o}$) the useful yield tends towards 0.

$$\frac{\eta_1}{\eta_2} = C_1^{\,o}\,\frac{K_1}{K_2}\,\frac{C_2^{\,o}}{C_3^{\,o}}\,(1-\eta_1-\eta_2)$$

- Conversely when the reaction scheme is as follows :

$$\underset{C_1}{A_1^*} + \underset{C_2}{A_2} \rightleftharpoons \underset{K_1,\eta_1\ C_1'}{A'^*_1}$$

$$\underset{C_1}{A_1^*} + \underset{C_3}{A_3} \rightleftharpoons \underset{K_2,\eta_2\ C_2'}{A'^*_2}$$

We have (see "Influence of the labelling agent concentration on the synthesis yield", § 5)

$$\frac{\eta_1}{\eta_2} = \frac{K_1}{K_2}\frac{C_2}{C_3} = \frac{K_1}{K_2}\frac{(C_2^{\,o} - \eta_1 C_1^{\,o})}{(C_3^{\,o} - \eta_2 C_1^{\,o})}$$

If $C_1^{\,o}$ is negligible with respect to $C_2^{\,o}$ and $C_3^{\,o}$ we get :

$$\frac{\eta_1}{\eta_2} = \frac{K_1}{K_2}\frac{C_2^{\,o}}{C_3^{\,o}}$$

The $\frac{C_2^{\,o}}{C_3^{\,o}}$ ratio is high (A_3 is an impurity of A_2) and the useful synthesis is not perturbed.

There hence seems to be no theoretical reason why the synthesis of strictly carrier free ^{11}C radiopharmaceuticals should not be possible under certain favourable conditions (if the appropriate labelling agents could be obtained), which would offer a special advantage when the products are highly toxic or are to be used for "in vivo" specific receptor binding.

With this in view laboratory research is in progress to improve the specific activity.

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