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The use of metal-catalysed hydrogen isotope exchange in the contract supply of tritiated compounds

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The metal-catalysed tritium exchange labelling methods available to a contract labelling facility, and the principles that dictate the selection of the most appropriate method are described.

Keywords: tritium; exchange; metal; contract

Introduction

A contract labelling facility, offering tritium labelling, is potentially faced with a wide variety of challenges from its customers. The compounds that are to be labelled do not fall into a neat group related by structure or functionality; rather they cover the whole spectrum of chemistry.

Some customers can provide precursors that facilitate tritium labelling, such as a halogenated or unsaturated precursor (for tritio-reduction with tritium gas), or a desmethyl version of the target molecule (for methylation with tritiated methyl iodide). This gives some advantages in obtaining a product of high specific activity. If the reaction does not go to completion, the unreacted starting material can normally be separated from required product and so does not interfere with the specific activity.

However, in many cases the only starting material available to the contract manufacturer is the target molecule itself. In these circumstances the options available for labelling are either chemical transformation followed by labelling reaction (such as halogenation followed by tritiodehalogenation, or oxidation followed by reduction with sodium borotritide), or a direct exchange reaction on the target molecule itself.

Obtaining a high specific activity via isotopic exchange can be more challenging as an inefficient exchange process will leave unlabelled material in the product, leading to a lower specific activity product.

This article will cover the use of metal catalysts in facilitating such direct exchange reactions and will describe the strategy employed by a contract labelling facility in determining the appropriate labelling methodology for a target molecule. Practical examples will be given to demonstrate the use of this approach.

Determining strategy in metal-catalysed hydrogen exchange labelling

When a contract labelling facility receives a request for tritium labelling, the chemist has a variety of exchange methods available. The main methods are:

1. Metal-catalysed hydrogen exchange in solution using a soluble catalyst (usually iridium based) and tritium gas.
2. Metal-catalysed hydrogen exchange in solution using an insoluble catalyst (usually palladium, platinum or rhodium based) and tritium gas.
3. Metal-catalysed hydrogen exchange in the solid phase using a suitable catalyst and tritium gas.
4. Metal-catalysed hydrogen exchange in solution, using either neat tritiated water or a tritiated water/co-solvent mixture.

The selection of the best technique to use will depend on the demands of the customer, principally in terms of specific activity requirements. This is usually the major challenge as issues of yield, while related to specific activity, can usually be solved by repeated reactions, and purity can be addressed by chromatographic techniques. It is important to note that in a contract facility these labelling reactions are normally performed only once, so there is no drive to produce an optimised process – the aim is to produce the required quantity and quality of material, at the required specific activity, in an appropriate timescale. So, while we always hope for a high-quality exchange labelling with few by-products, a multi-component mixture containing some of the desired product at the desired specific activity is preferable to a 'clean' exchange labelling that does not meet the required specific activity. This is particularly true given the power of modern chromatographic techniques to separate complex mixtures.

The selection will also depend on the structure of the molecule to be labelled. If it contains a functionality, for example a reducible double bond, that will react under the exchange conditions then that may limit exchange possibilities. The use of tritiated water, rather than tritium gas, as labelling reagent may then be required.

The solubility of the molecule can also have an influence on the techniques that would be considered by the contract laboratory. This is often unknown to the contract supplier at the

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initiation of the project and may only become apparent as a limiting factor during experimental work, necessitating a revision of the proposed labelling strategy.

Restrictions on the possible positions of labelling, defined by customer requirements, can also influence the labelling strategy. Exchange labelling projects are normally accepted on the basis that the contract laboratory has a free hand to exchange tritium for any non-labile hydrogen in the molecule. While the labelling positions obtainable from exchange reactions can often be predicted (more so with some techniques than others), there is always some unpredictability and the possibility of non-specific exchange always exists. This can be investigated by tritium NMR analysis. However, this provides only retrospective information, in that the analysis is carried out post preparation and purification. It also relies on:

- A suitable NMR and tritium probe being available to the contract facility.
- A fully authenticated and assigned reference NMR of the cold material being available from the customer (or performed by the contract facility).
- Having sufficient material to perform the tritium-NMR.
- The client being willing to pay for the experimental work.

Many clients pursuing an exchange labelling strategy are willing to accept a general labelling and may indeed be grateful for non-specific exchange which boosts the specific activity obtained.

Available metal-catalysed hydrogen exchange methodologies

The labelling of compounds with tritium was first documented in extensive detail in 1966 by the seminal work of Evans[1] and has recently been updated by Voges *et al.*[2]

Metal-catalysed hydrogen exchange in solution using a soluble catalyst (usually iridium based) and tritium gas

Cyclooctadienylyiridium(I) phosphine complexes are frequently utilised in this regard and are reviewed in this special edition by Salter.

This methodology is often the first choice for a contract labelling facility, as it offers the potential for high specific activities, reasonable predictability as to the labelling position, and moderately clean reactions. The standard catalyst we have used for these reactions is the Crabtree catalyst (Figure 1), which has the advantages of being reasonably inexpensive, commercially available and stable at room temperature storage for months, or even years.

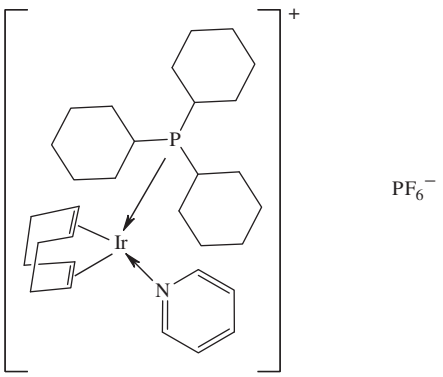


Figure 1. Crabtree catalyst, (1,5-cyclooctadiene)(pyridine) (tricyclohexylphosphine) iridium(I) hexafluorophosphate, CAS 64536-78-3.

The use of this catalyst for deuterium exchange was reported by the Schering Plough group of Hesk *et al.*[3] in 1995 and its potential use in tritium labelling was recognized at the time.

We have used other iridium-based catalysts, primarily Salter's catalyst (Figure 2), which was developed by Salter as part of a collaborative project between Amersham and Novartis.[4] This catalyst utilises a Josiphos-type ligand and can complement the Crabtree catalyst, giving different selectivity and better specific activity.

However, this approach relies on the presence of a suitable functional group in the molecule to direct the label into a particular position. For example, a carbonyl group next to an aromatic ring should result in *ortho*-tritiation. Moreover, such reactions are best suited to target compounds which are soluble to a reasonable degree in dichloromethane solvent.

A typical labelling procedure as carried out in our laboratories is given below:

The substrate (2–5 mg) and catalyst (5–15 mg) are dissolved in dichloromethane and stirred under an atmosphere of tritium gas (5–10 Ci) for 4–16 h. Labile tritium is removed by repeated evaporations to dryness with a protic solvent, usually ethanol. Experience has shown that decomposition can occur if the catalyst is not removed from the crude product. Often a convenient way to partially purify the crude material is to pass it through a short silica column, or a Sep-Pak silica cartridge.

Using this labelling method we have achieved excellent specific activities for many compounds. An example is tritiated paclitaxel (often called Taxol) (Figure 3).

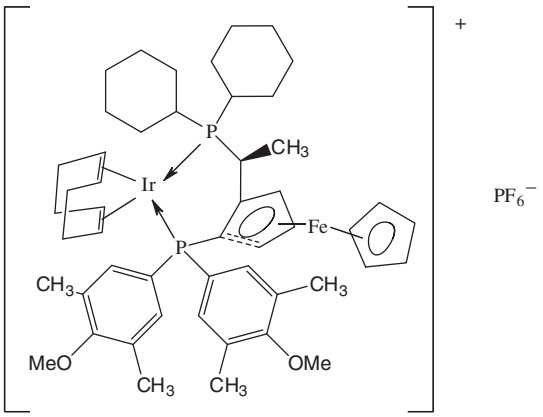


Figure 2. Salter's catalyst, (1,5-cyclooctadiene)((R)-1-{(SP)-2-[bis(4-methoxy-3,5-dimethylphenyl)phosphino]ferrocenyl}ethyldicyclohexylphosphine) iridium(I) hexafluorophosphate.

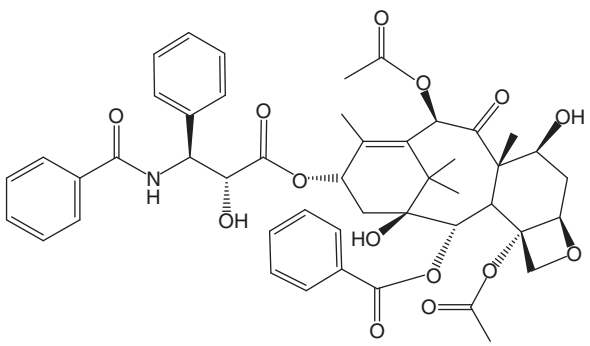


Figure 3. Paclitaxel (Taxol).

This complex, naturally occurring molecule is difficult to synthesise so is an ideal candidate for tritium exchange labelling. As a contract labelling facility we have attempted to label this compound several times over many years using a variety of techniques. The most successful technique used was a tritiated water exchange reaction at elevated temperature which resulted in a very crude product that required extensive purification and low specific activities, in the range 1–5 Ci/mmol. However, the method of choice now is to use a homogenous catalyst, Crabtree catalyst, as reported by Shu and Heys.[5] The compound is now labelled using similar conditions to the generic conditions described above, and gives a much purer product directly from the tritiation, with specific activity orders of magnitude higher at 50–70 Ci/mmol.

In addition to compounds containing directing functionalities the Crabtree catalyst has been shown to give some surprising results, including labelling the formyl protons of aldehydes. An example from the work at Amersham is 3,5-di-*tert*-butyl-4-hydroxybenzaldehyde[6] (Figure 4). The strategy was intended to label ortho to the aldehyde functionality on the aromatic ring but actually labelled exclusively in the formyl position.

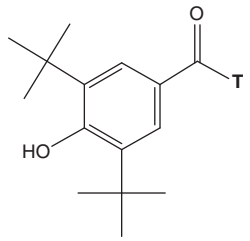


Figure 4. 3,5-Di-*tert*-butyl-4-hydroxybenzaldehyde.

3,5-Di-tert-butyl-4-hydroxybenzaldehyde (10 mg) and Crabtree catalyst (10 mg) in dichloromethane (2 ml) were stirred under an atmosphere of tritium gas (10 Ci) at room temperature for 16 h. Labile tritium was removed by repeated evaporation to dryness from ethanol. After purification by column chromatography on silica (hexane:ethyl acetate), analysis by mass spectrometry showed a singly labelled product at 23 Ci/mmol.

The tritium NMR of the product (Figure 5) showed that the molecule was exclusively labelled in the formyl position as shown, with no labelling on the aromatic ring, because of the steric effects of the bulky *tert*-butyl groups.

At Amersham Biosciences a series of experiments was carried out to investigate the utility of this type of exchange.[7] The results are summarised in Table 1, in all cases the ring labelling was exclusively *ortho* to the directing group.

Consideration needs to be given to a few limitations with this type of exchange:

1. Unhindered double or triple bonds are prone to reduction.
2. The target compound needs to have some solubility in dichloromethane. There are various techniques to improve solubility such as derivatisation (for example, acids can easily be converted to esters for the labelling procedure) and in the case of salts, use of the compound as the free acid or base.
3. Use of other co-solvents to aid solubility is reported in the literature, but in our experience even a small amount tends to have a detrimental effect on the exchange process.
4. Reduction of the carbonyl to the corresponding benzylic alcohol was not observed in the examples above.

There is extensive literature on the preparation and use of homogeneous catalysts for tritium exchange, mainly based around iridium. A wide variety of ligands have been examined, but we have found that for the majority of tritium exchanges we

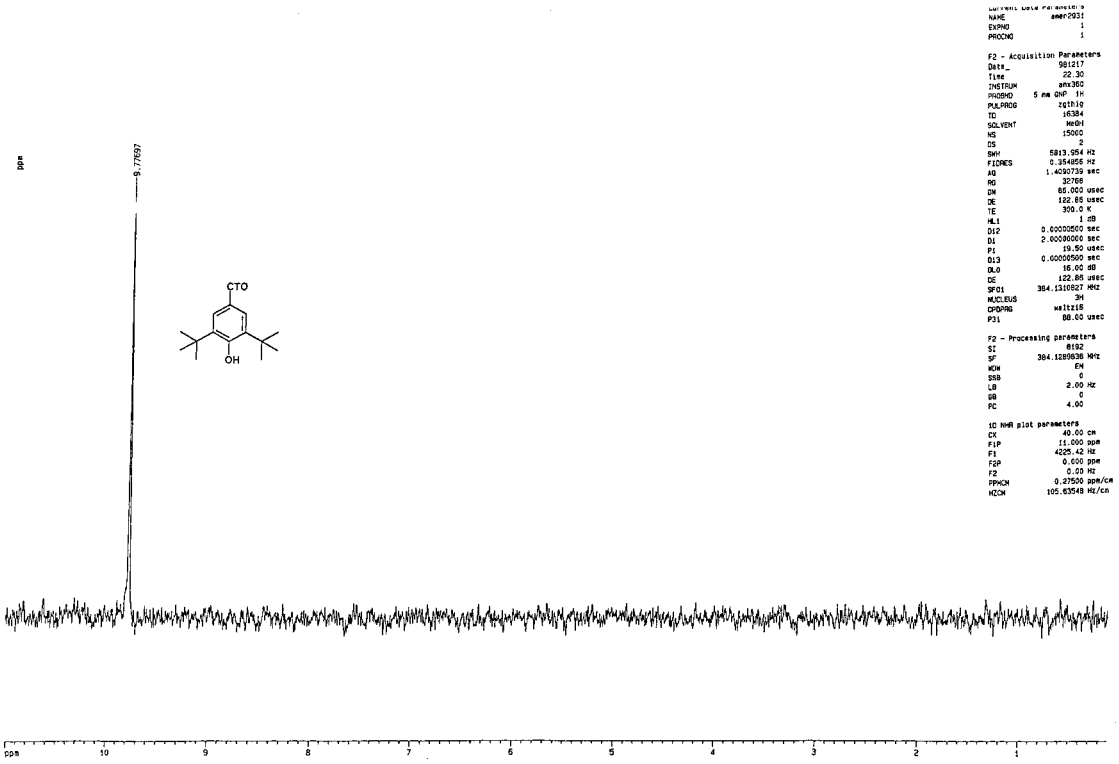
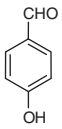
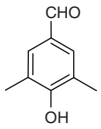
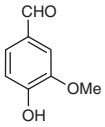
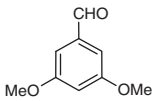
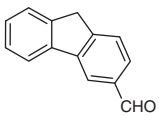
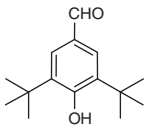
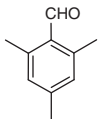
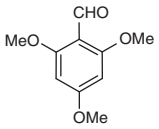


Figure 5. Tritium NMR of 3,5-di-*tert*-butyl-4-hydroxy[formyl-^{3}H]benzaldehyde.

Table 1. Tritium labelling of aromatic aldehyde compounds

Aldehyde	Specific activity (Ci/mmol)	Ring label (%)	Formyl label (%)
	74	77	23
	68	67	33
	52	56	44
	22	30	70
	50	60	40
	24	0	100
	8	0	100
	13	0	100

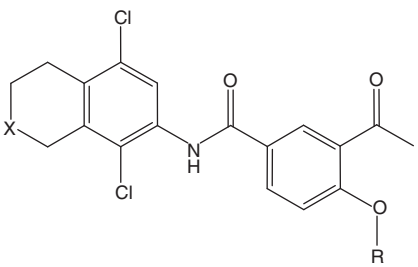


Figure 6. Proprietary compound, partial structure.

have been required to perform, Crabtree catalyst or the easily prepared Salter's catalyst have been sufficient.

However this field continues to develop, and for the applicability of more recently developed carbene catalysts, see the contribution from W. Kerr and G. Nilsson in this Special Issue. An example of the different selectivity between Crabtree catalyst and Salter's catalyst is shown in the labelling of the proprietary compound, partial structure depicted in Figure 6:

Tritium exchange using Crabtree catalyst would be expected to work on this compound. However, the reaction mixture obtained using Crabtree catalyst was complex and did not contain any of the required labelled product. In contrast, using the same conditions with Salter's catalyst gave the required product after purification with a specific activity of 23 Ci/mmol.

Metal-catalysed hydrogen exchange in solution using an insoluble catalyst (usually palladium, platinum or rhodium based) and tritium gas

This methodology, when it works, has the advantage of relatively clean labelling and very simple work-up, since the catalyst can simply be filtered from the tritiation mixture. The catalysts we employ in these labellings are usually palladium on charcoal, palladium oxide on barium sulphate, platinum on charcoal, or rhodium on charcoal. However the specific activities obtained are usually lower than those obtained with the soluble catalyst approach, and the scope of such reactions is limited: the

approach has been used primarily to label benzylic hydrogen, the 8-position of purines/pyrimidines, and the anomeric positions of sugars.[8]

An example of the labelling in the 8-position of a purine is puromycin dihydrochloride (Figure 7), formerly an Amersham catalogue item at 2–10 Ci/mmol.

Puromycin dihydrochloride (15 mg) and 10% palladium oxide on barium sulphate (50 mg) in aqueous phosphate buffer (3 ml) were stirred under an atmosphere of tritium (10 Ci) for 2 h at room temperature. The catalyst was removed by filtration and labile tritium was removed by repeated evaporations to dryness from water. The typical specific activities vary using different batches of catalyst but are generally in the range 2– 10 Ci/mmol.

This labelling method is intolerant of reducible functionalities, further limiting its use. We have used it to best effect in molecules rich in benzylic hydrogen, such as tocopherol (Figure 8), or derivatives of tocopherol, where very respectable specific activities can be achieved:

Tocopherol (6 mg) and 10% palladium on charcoal catalyst (10 mg) in acetic acid (3 ml) were stirred under an atmosphere of tritium (5 Ci) for 2 h at room temperature. The catalyst was removed by filtration and labile tritium was removed by repeated evaporations to dryness from ethanol. The typical specific activities vary significantly and are in the range 30– 70 Ci/mmol.

A similar type of exchange that has been used with some success is exchange into the protons α to the pyridine nitrogen, using rhodium on charcoal as catalyst, as reported in the work of Alexakis *et al*.[9] We discovered this when attempting to fully reduce a pyridine ring with tritium gas, under forcing conditions, using rhodium on charcoal as catalyst – rather than reducing the ring, we found a fully intact aromatic ring but an exchange-labelled starting material!

An example is the proprietary compound (Figure 9), partial structure showed below:

The substrate (3 mg) and rhodium on charcoal catalyst (20 mg) in dimethylformamide (2 ml) were stirred under an atmosphere of tritium gas (10 Ci) for 2 h at room temperature. The catalyst was removed by filtration and labile tritium was removed by repeated evaporations to dryness from ethanol. Achievable specific activities by this method vary but are respectable at 5– 15 Ci/mmol. More forcing conditions were not attempted as this specific activity was acceptable to the customer.

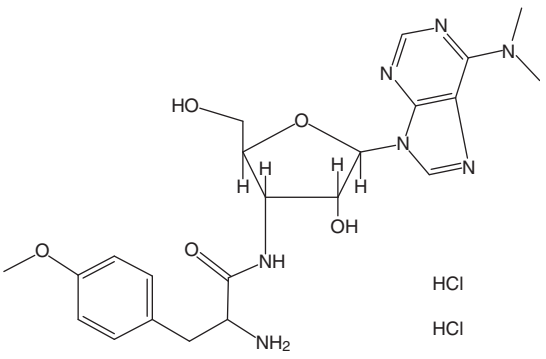


Figure 7. Puromycin dihydrochloride.

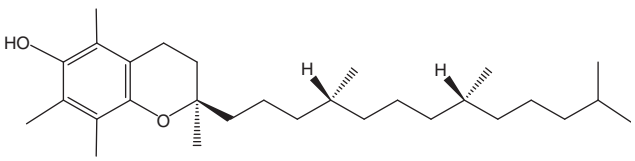


Figure 8. Tocopherol.

Metal-catalysed hydrogen exchange in the solid phase using a suitable catalyst and tritium gas

This method is the subject of another contribution to this Special Issue (See Nagaev, *et al*.). This labelling method uses no solvent, but employs an intimate mixture of substrate and catalyst (usually ground together using a mortar and pestle) which is exposed to tritium gas in a sealed glass tube. This tube is then shaken and heated (in a dedicated shaker/oven) for a set period of time, prior to work-up. Our catalyst of choice would usually be a palladium-based catalyst such as palladium black or palladium on charcoal. The work-up involves opening the tube in a controlled manner, dissolving/suspending the reaction mixture of substrate/catalyst in a suitable solvent, and filtering off the catalyst. The advantages and disadvantages of this method, in our experience, are that it is very unpredictable. It can work spectacularly well to give outstanding specific activities, but these are sometimes difficult to reproduce. Further, the lack of predictability can make it unattractive to a contract supplier.

There are many variables in this labelling technique, which could affect the viability of the labelling and the specific activity obtained:

- Ratios of substrate/catalyst/tritium gas.
- Degree of mixing (it is very hard to reproduce, characterise or standardise the mixing procedure).
- Temperature of reaction.
- Pressure generated inside the tube (dictated by a combination of tube size, quantity of gas used, and reaction temperature).
- Time of exchange.

Of these, the temperature and time of the reaction have the most effect. We find that the longer and hotter the reaction, the higher the specific activity that can be obtained. However, the same factors cause increased decomposition of the substrate, so it is necessary to achieve a balance between the two.

Consequently, the desired product is frequently obtained as a minor component in the reaction mixture. However, since the compound can often be isolated by chromatographic techniques, this may be an acceptable approach if it achieves the desired specific activity.

The following constitutes our first approaches to labelling via this technique:

- A room temperature exchange over a prolonged period of time, usually 24 h.
- An exchange at 100°C for a shorter period, usually 1 h.

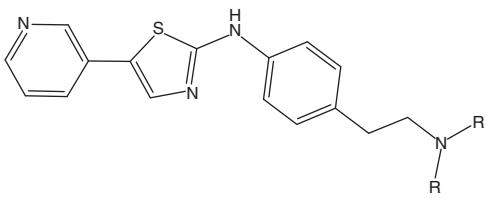


Figure 9. Proprietary compound.

- An exchange as hot as possible (to a point close to the melting point of the substrate) for a short period of time, 5 min.

Analyses of the degree of labelling and the amount of decomposition from these conditions can then be used to deduce an improved set of conditions for a further labelling experiment. These experiments can be sequential (with the potential to save both effort and radioactivity if the experiment works well on an early attempt) or in parallel (to give more information in a shorter time but at the risk of wasted effort and radioactivity).

An example of an excellent specific activity obtained using this method is tritiated gabapentin (Figure 10):

Gabapentin (3 mg) and palladium black (9 mg) were gently ground together using a mortar and pestle and transferred to a glass tube. The tube was evacuated and then sealed under an atmosphere of tritium gas (20 Ci). The tube was heated at 200°C for 20 min. The tube was opened under controlled conditions, the reaction mixture suspended in ethanol and filtered to remove catalyst. Labile tritium was removed by repeated evaporations to dryness from ethanol. These extreme labelling conditions were found to cause formation of the cyclic amide, which was then opened by acid hydrolysis (c.HCl, reflux, 3.5 h). After purification the specific activity was determined by mass spectrometry, and was found consistently to be in the range of 150–200 Ci/mmol.

However, if successful tritium labelling does not occur as a result of the experiments required for the standardised approach above, then there is a significant cost in terms of wasted time and radioactivity. Hence this methodology carries some financial risk and, hence, is not ideal for a contract organisation.

Metal-catalysed hydrogen exchange in solution, using either neat tritiated water or a tritiated water/co-solvent mixture

This labelling method has the advantage over tritium gas methods in tolerating reducible functionalities. It has several disadvantages however: it requires the generation and subsequent disposal of significant quantities of tritiated water and generally leads to products of relatively low specific activity, though there are some important exceptions. In most cases the tritiated water is required at the maximum specific activity in order to produce a high specific activity product, the handling of which can require specialist vacuum manifold techniques.

The reaction is normally done by dissolving the substrate, and catalyst if appropriate, in a minimum quantity of an aprotic solvent, in a glass tube. The tritiated water (normally around 10–20 Ci, 0.15–0.3 mmol, less than 10 ul) is then distilled into the tube on a vacuum manifold, the tube flame-sealed, and heated if appropriate. Work-up is achieved by opening the tube, pumping out the volatile components, removing catalyst by filtration if appropriate, and finally removing labile tritium. This labelling technique can be used to replace protons that are exchangeable under catalytic conditions, sometimes using a metal-based catalyst to promote the exchange, sometimes using acid or base to achieve this end. Direct non-metal-catalysed acid/base exchanges are outside the scope of this article.

An example for this type of metal-catalysed exchange is the cardiac glycoside Ouabain (Figure 11), formerly an Amersham catalogue item:

Ouabain (10 mg) and 10% platinum on charcoal (20 mg) were combined in a glass tube and dissolved/suspended in dimethylformamide (0.3 ml). Tritiated water at full specific activity (50 Ci) was distilled in under vacuum and the tube was flame sealed. The tube was shaken for 16 h at 80°C. The base of the tube was then frozen using liquid nitrogen while the tube was opened. Volatile components were pumped into the back trap of a vacuum manifold, and the residue was dissolved/suspended in ethanol and filtered to remove catalyst. Labile tritium was removed by repeated evaporations to dryness from ethanol. The crude product required extensive HPLC purification. Achievable specific activities by this method were in the range of 15–50 Ci/mmol.

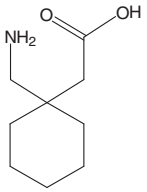


Figure 10. Gabapentin.

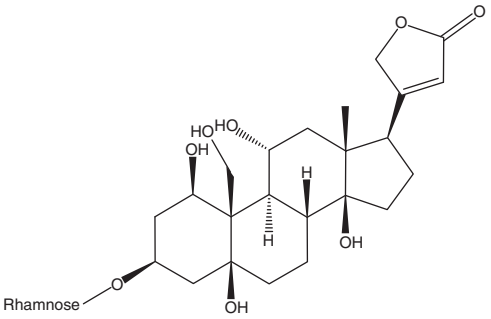


Figure 11. Ouabain.

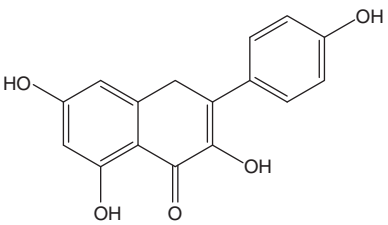


Figure 12. Kaempferol.

While the role of the metal catalyst is unclear, it is certainly beneficial to the exchange as experience shows that lower specific activities (in the range of 5–10 Ci/mmol) were obtained using the same conditions but without the metal catalyst.

Another example is the natural flavonoid kaempferol (Figure 12). A tritiated water exchange using acid catalysis gave no incorporation, so a metal-catalysed exchange was attempted:

Kaempferol (5 mg) and 5% Rhodium on aluminium oxide (10 mg) were combined in a glass tube and dissolved/suspended in dimethylformamide (0.25 ml). Tritiated water at full specific activity (10 Ci) was distilled in under vacuum and the tube was flame sealed. The tube was shaken for 16 h at 70°C. The base of the tube was then frozen using liquid nitrogen while the tube was opened. Volatile components were pumped into the back trap of a vacuum manifold, and the residue was dissolved/suspended in ethanol and filtered to remove catalyst. Labile tritium was removed by repeated evaporations to dryness from ethanol. Mass spectrometry showed up to three tritiums to have been exchanged into the molecule at a specific activity of 12 Ci/mmol.

Conclusions

There exists a range of techniques open to the contract laboratory to satisfy the needs of the customer, particularly those of specific activity. The selection of the best technique can be made using considerations of structure, specific activity requirement, and past experience (intuition?), but tritium labelling on complex molecules is not an exact science and remains unpredictable and capable of surprises!

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