Synthesis of ^{18}O-Labelled Alcohols from Unlabelled Alcohols

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The synthesis of primary, secondary and tertiary ^{18}O-enriched alcohols from readily available ^{16}O-alcohols *via* a Mitsunobu esterification and hydrolysis is described. The method is further exemplified in the labelling of the active pharmaceutical ingredient, Dropropizine and is shown to be tolerant of modern, separation friendly Mitsunobu reagents.

Stable isotope-labelled compounds have a variety of roles in organic chemistry and the biomedical sciences. For example, they are used to probe reaction mechanisms through the determination of kinetic isotope effects and isotope labelling experiments.[1] In pharmaceutical development, stable isotope-labelled compounds are widely used as internal standards for mass-spectrometry based analysis of pharmacokinetics and pharmacodynamics.[2] Additionally, isotope incorporation, notably deuterium, can improve the metabolic stability of active pharmaceutical ingredients.[3]

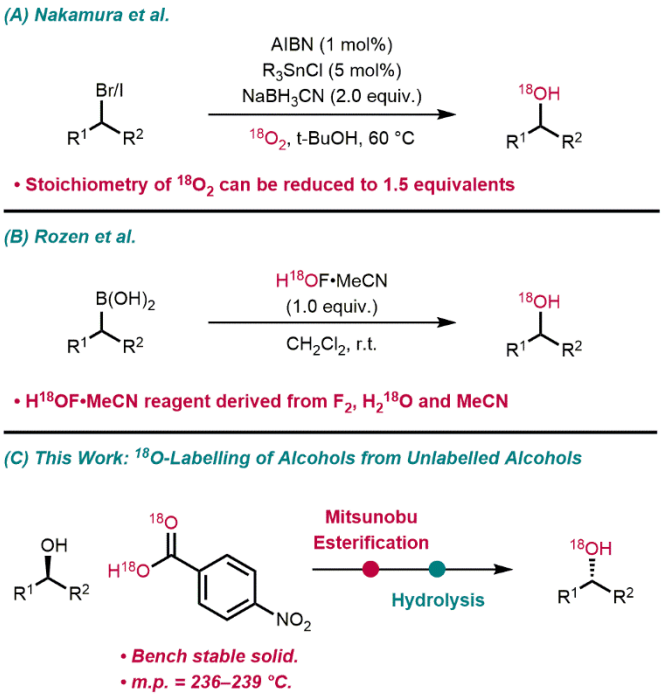

Figure 1 Approaches to the synthesis of ^{18}O-enriched alcohols. (A) Nakamura's tin hydride mediated synthesis from alkyl bromides and alkyl iodides. **(B)** Rozen's [H^{18}OF•MeCN] mediated oxidation of boronic acids. **(C)** This work: A Mitsunobu esterification-hydrolysis strategy using an ^{18}O-labelled carboxylic acid.

The synthesis of stable isotope-labelled active pharmaceutical ingredients is usually achieved by *de novo* syntheses using building blocks enriched with a specific stable isotope (most commonly ^{2}H, ^{13}C or ^{15}N). Although oxygen-containing functional groups are common in pharmaceutical molecules, compounds containing ^{18}O are rare,[4] due to the poor availability of ^{18}O-enriched building blocks.[2b] In most cases, ^{18}O-enriched alcohols are accessed using bespoke, substrate-specific syntheses.[5] To the best of our knowledge, only two *general* methods of accessing ^{18}O-enriched alcohols have been reported to date. Nakamura *et al.* disclosed a method for converting alkyl iodides and bromides to ^{18}O-enriched alcohols (Fig. 1A).[6] The reaction occurs through tin hydride-mediated generation of a radical, which is trapped with $^{18}O_2$ followed by reduction. In 2013, Rozen and co-workers reported the oxidative conversion of boronic acids into ^{18}O-enriched alcohols using the hypofluorous acid complex [H^{18}OF•MeCN] (Fig. 1B).[7] This unusual reagent is prepared from fluorine gas, ^{18}O-enriched water and acetonitrile.

Despite these encouraging reports we reasoned that a new method, *using readily available non-labelled alcohols as substrates*, would be of value and provide a general route to stereodefined ^{18}O-enriched alcohols for mechanistic studies.[8] Herein, we report a simple and practical protocol based on a Mitsunobu reaction[9] using an ^{18}O-enriched carboxylic acid followed by hydrolysis (Scheme 1C).

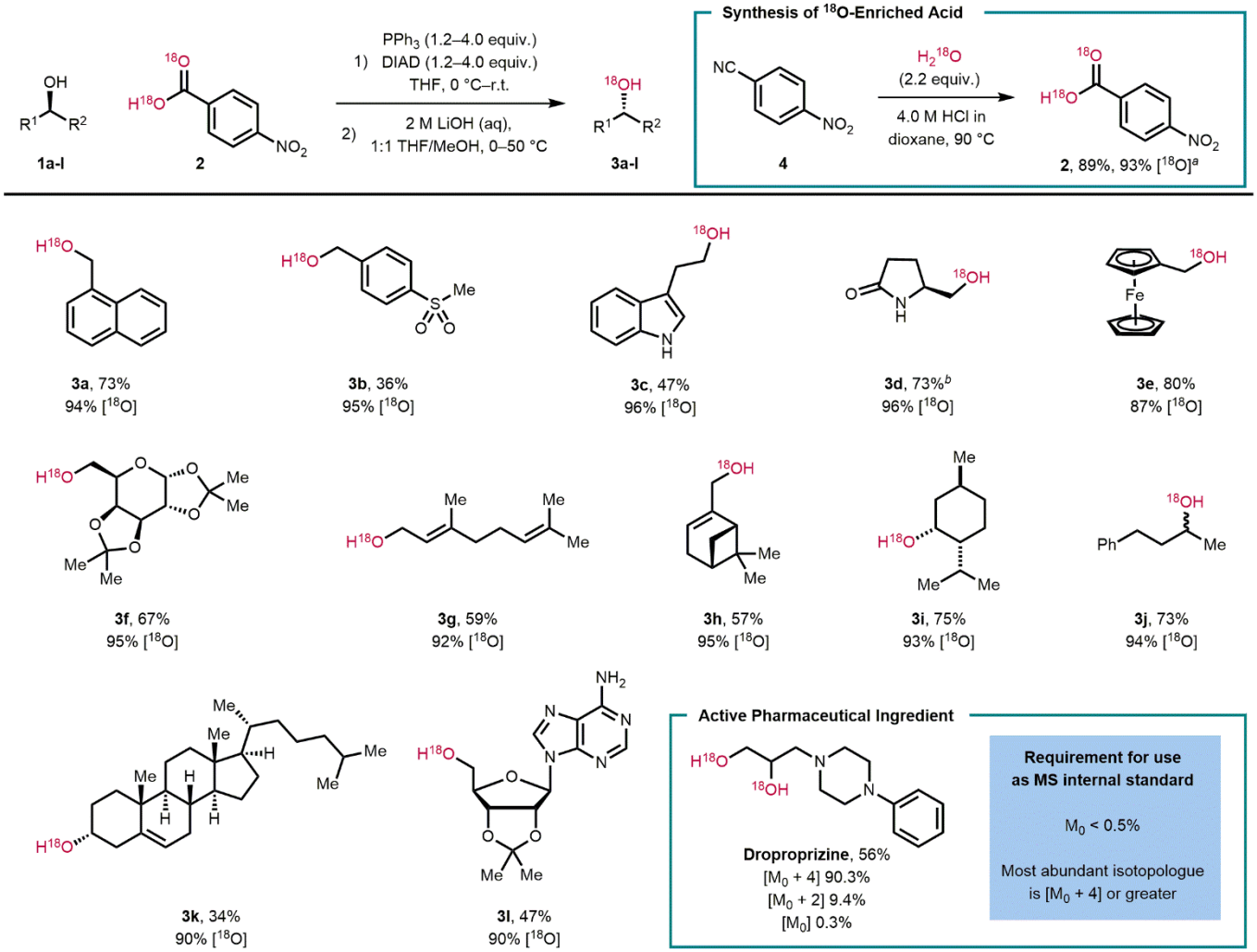

Figure 2 Substrate scope for the Mitsunobu esterification-hydrolysis mediated synthesis of ^{18}O-enriched alcohols. Yields are quoted as overall yield of the two-step process. ^{a} ^{18}O-enrichment is quoted as the mol% of sample enriched with two ^{18}O labels. ^{b} K_2CO_3 in MeOH/H_2O was used for the hydrolysis step.

We began by investigating the synthesis of 4-nitrobenzoic acid-[^{18}O]$_2$ (**2**) by an acid-mediated hydrolysis of 4-nitrobenzonitrile (**4**) using ^{18}O-enriched water. 4-Nitrobenzoic acid was chosen as a suitable carboxylic acid as it is a very commonly used in the Mitsunobu reaction.[10] A short optimisation sequence (see supplementary information, Table S1 and S2) revealed that the hydrolysis of 4-nitrobenzonitrile in 4 M HCl in dioxane afforded 89% of the corresponding doubly ^{18}O-labelled carboxylic acid with an isotopic purity of 93%. The only other isotopologue detected was the singly labelled acid, which accounted for the remaining 7%. In this protocol, 4-nitrobenzoic acid-[^{18}O]$_2$ was isolated by filtration and used directly in the Mitsunobu esterification step without further purification.

With a convenient method to access 4-nitrobenzoic acid-[^{18}O]$_2$ (**2**) in hand, we explored the scope of the labelling protocol with a range of alcohol substrates. Firstly, benzylic alcohols substrates were examined and both 1-naphthalenemethanol (**1a**) and sulfone-containing substrate **1b** were converted into the corresponding isotopologues with excellent levels of ^{18}O enrichment. Additionally, the ^{18}O isotopologues for indole-containing alcohol **1c** and amino acid derivative **1d** were accessed in moderate-to excellent yields and with high levels of ^{18}O enrichment. Ferrocenemethanol (**1e**) and sugar derivative **1f** were also efficiently labelled—the slightly lower level of ^{18}O enrichment for **1e** being possibly attributable to post hydrolysis S_N1-type hydroxyl exchange. The enrichment of monoterpenoids, namely geraniol (**1g**) and (1*R*)-(−)-myrtenol (**1h**) was also investigated, and the ^{18}O-enriched products (**3g** and **3h**) were isolated with good yields and excellent levels of ^{18}O incorporation. ^{18}O-enriched (±)-neomenthol (**3i**) was obtained when (±)-menthol (**1i**) was subjected to the reaction conditions, demonstrating the expected inversion of stereochemistry associated with the Mitsunobu esterification. Similarly, *epi*-cholesterol-[^{18}O] (**3k**) was obtained from natural cholesterol (**1k**). Finally, the ^{18}O-isotopologue of acetal-protected adenosine **1l** was produced with excellent ^{18}O enrichment albeit with a poor yield. Although the yields of different substrates were variable the ^{18}O enrichment for each example remained excellent throughout, ranging from 87–96%.

We next sought to extend this method to encompass active pharmaceutical ingredients. In the context of deuterium labelling, recent developments in hydrogen/deuterium exchange reactions have enabled straightforward access to deuterium-labelled pharmaceuticals.[11]

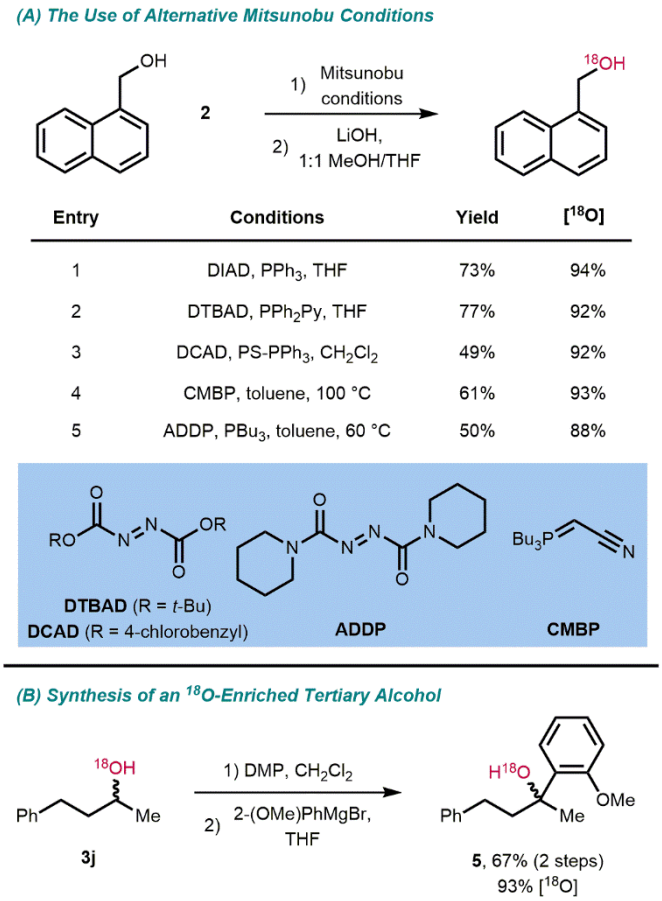

Figure 3 (A) The use of alternative, separation friendly Mitsunobu reagents in the Mitsunobu esterification-hydrolysis protocol. **(B)** The derivatisation of ^{18}O-enriched secondary alcohols into tertiary alcohol.

This approach, whereby certain hydrogen atoms of a pharmaceutical are exchanged for deuterium, offers a cost- and time-efficient alternative to *de novo* synthesis. We therefore wished to apply this principle to ^{18}O-enriched active pharmaceutical ingredients. Generally, for stable-isotope analogues of active pharmaceutical ingredients to serve as internal standards, the most abundant isotopologue is required to be at least four mass units heavier than the unlabelled isotopologue ([M_0 + 4]). In the context of ^{18}O labels, the most abundant isotopologue of an internal standard must contain a minimum of two ^{18}O labels. Furthermore, the internal standard should contain no greater than 0.5% of the parent, unlabelled isotopologue ([M_0]) in order to avoid cross-signal interferences.[12]

The cough suppressant, Dropropizine, was chosen as an appropriate example, since it contains two hydroxyl groups that are reactive towards a Mitsunobu esterification reaction. Using our standard protocol, we were able to obtain Dropropizine-[^{18}O]$_2$ in an overall yield of 56%. Mass spectrometry analysis of this sample indicated that the doubly-labelled isotopologue ([M_0 + 4]) accounted for 90.3% of the sample, the singly-labelled isotopologue ([M_0 + 2]) accounted for 9.4%, and only 0.3% of the unlabelled substrate ([M_0]) was present, thus demonstrating that our method can be effectively used as a means to access these mass spectrometry internal standards.

The classical Mitsunobu conditions (DIAD and triphenylphosphine), used in this study suffer from well-documented drawbacks, specifically the production of triphenylphosphine oxide and hydrazine by-products. Furthermore, DIAD is thermally unstable and its use should be avoided on scale.[13] Therefore we explored the use of second-generation Mitsunobu reagents[14] in our ^{18}O-labelling protocol.

Firstly, the use of di-*tert*-butyl azodicarboxylate (DTBAD) in conjunction with diphenyl-2-pyridylphosphine,[15] followed by hydrolysis, enabled the ^{18}O-enrichment of 1-naphthalenemethanol with comparable efficiency to the DIAD/triphenylphosphine system (Fig. 3A, Entry 2). Under these conditions, addition of hydrochloric acid coverts the hydrazine by-product into gaseous products and allows the phosphine oxide to be removed by phase-separation. Similarly, the use of di-(4-chlorobenzyl)azodicarboxylate (DCAD) developed by Lipshutz[16] with polystyrene-supported triphenylphosphine (PS-PPh$_3$), followed by hydrolysis, afforded 1-naphthalenemethanol-[^{18}O] with comparable ^{18}O enrichment (92%) but with a lower yield of 49% (Fig. 3A, Entry 3). Both the hydrazine and phosphine oxide by-products of this reaction are insoluble in dichloromethane and were separated from the reaction mixture by filtration.

Using Tsunoda's reagent (cyanomethylenetributylphosphorane, CMBP),[17] instead of DIAD and triphenylphosphine, afforded 1-naphthalenemethanol-[^{18}O] with excellent ^{18}O enrichment (Fig. 3A, Entry 4). Furthermore, the commonly used combination of 1,1'-(azodicarbonyl)dipiperidine (ADDP) and tributylphosphine (Fig. 3A, Entry 5),[18] followed by hydrolysis, also afforded 1-naphthalenemethanol-[^{18}O] albeit with reduced yield and isotopic enrichment. Overall, these experiments demonstrate that the use of the potentially hazardous

DIAD, and the chromatographic removal of the phosphine oxide and hydrazine by-products can be avoided in our Mitsunobu labelling protocol.

Finally, we turned our attention to the synthesis of ^{18}O-enriched tertiary alcohols, which are hard to prepare by direct Mitsunobu reactions.[19] Oxidation of ^{18}O-enriched alcohol **3j** to the corresponding ketone using Dess-Martin periodinane,[20] followed by a Grignard addition using 2-methoxyphenylmagnesium bromide afforded the tertiary alcohol **5** in a yield of 67% over two steps with minimal erosion of the ^{18}O enrichment (Fig. 3B).

In conclusion, an operationally simple synthesis of ^{18}O-enriched primary, secondary and tertiary alcohols has been developed. The Mitsunobu coupling approach allows abundant unlabelled alcohols to be used as substrates whilst also providing stereocontrol. The conditions were shown to be tolerant of a wide range of functional groups and were also applied to the synthesis of an isotopically labelled active pharmaceutical ingredient. Furthermore, we have demonstrated that alternative Mitsunobu reagents, which simplify purification, are compatible with this protocol. We envision that this method will be useful as a general and practical means of accessing a broad range of ^{18}O-enriched alcohols.

Conflicts of interest

There are no conflicts to declare.

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