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Introduction

Compounds labeled with carbon-14 or tritium have for decades been used in a vast number and wide range of applications, especially in the life sciences[1,2], including research and development of human and animal pharmaceuticals and crop protection agents. Notwithstanding new technological developments, and in some cases because of them, the value of these isotopes in various research areas continues to be great.

In studies of the interactions of small molecules (both synthetic and natural) with receptors, enzymes and other complex biological molecules and systems, compounds labeled with tritium are indispensable because they can be detected and quantified at the nanomolar level of concentration. The tritium label facilitates measurements of the affinity of labeled ligands to their cognate receptors, the densities of receptors in tissue preparations, and the development of high-throughput assays for assessment of the interactions of test compounds with receptors. Analogously, the activity of enzymes can be studied, and potential nonnative substrates screened, by use of a test substrate labeled in such a way as to signal (e.g., by release of radioactivity or change in chromatographic mobility of the substrate) the chemical transformation catalyzed by the enzyme.

Similarly, carbon-14-labeled compounds have no equal for assessment of their metabolism *in vitro* (such as with hepatocytes, cytochrome P450 subtypes or other enzyme or subcellular tissue preparations), or for *in vivo* characterization of their absorption, distribution, metabolism and excretion (ADME) in animals and humans[3], as they can be detected by several different methods and accurately quantified in complex biological matrices. One of the newer of these methods is accelerator mass spectrometry (AMS)[4], whose exquisite sensitivity allows the use of far smaller quantities of carbon-14 than standard ADME studies, therefore providing increased safety margins with regard to radiation exposure to human volunteers.

Compounds labeled with isotopes such as carbon-14 or tritium have also contributed to numerous advances in studies of biochemistry[5], biosynthetic pathways[6], enzyme mechanisms[7], elucidation of organic reaction mechanisms[8] and environmental sciences.

Preparation of Compounds Labeled with Tritium and Carbon-14 Rolf Voges, J. Richard Heys and Thomas Moenius

Clearly, the value of carbon-14 and tritium isotopes in research is dependent upon their incorporation into compounds of interest. This is made possible by the availability of a wide variety of preparative methods capable of furnishing study compounds possessing the desired isotope(s) in specific locations within the chemical structure and in suitable levels of enrichment.

The successful practitioner in standard (nonisotopic) synthetic organic chemistry needs to possess a broad knowledge of reactions and reagents, the ability to plan a practicable sequence of reactions starting from readily available starting materials and ending with the synthetic target, a facility in executing chemical laboratory operations efficiently and safely, and a working knowledge of analytical methods sufficient to ensure that the progress of a synthesis can be adequately assessed and to obtain information helpful in improving reaction parameters.

The synthesis of compounds labeled with isotopes requires the synthetic chemist to have additional expertise because the synthetic target must be assembled so as to contain one or more isotopic atoms. The preparation of compounds labeled with carbon-14 and tritium requires the ability to deal with a far smaller selection of starting materials compared with standard synthetic chemistry, the ability to plan reaction sequences that generate the correct chemical structures required isotopes in the appropriate positions, knowledge of the circumstances under which these isotopes' β^- emissions may lend additional instability to compounds and of ways to avoid or mitigate these effects. Moreover, work with tritium requires mindfulness of how this isotope's vulnerability to loss by exchange processes can be affected by its position in the chemical structure and by the conditions to which the compound is subjected. These are properties relevant not only to predictions about the stability and utility of the tritiated products, but also to the practicability of preparing them by tritium-for-hydrogen exchange when it would be advantageous to do so. Lastly, the fact that carbon-14 and tritium are unstable nuclei means that the practitioner must be well trained and familiar with the proper handling of radioactive materials.

Given all this, it is fair to say that organic synthesis with isotopes is a demanding specialty field within organic synthesis. This book is intended to be both a learning tool for scientists new to the field, and a continuing resource for radiochemical synthesis chemists throughout their careers. It is assumed that the reader has, at a minimum, a practical knowledge of synthetic organic chemistry and a good working knowledge of the chemistry laboratory.

The authors emphasize the importance of safe working practices and expect that readers make themselves familiar with, and take care to work at all times in accordance with, their national, local and institutional radiation safety protocols regarding carbon-14 and tritium, to maintain good practices of contamination monitoring, and are competent in the control and remediation of radioactive contamination. Some general guidelines have been published[9].

The organization of this book is as follows. The remainder of this chapter provides short accounts of purification, analysis and storage and stability of compounds labeled with carbon-14 and tritium, and descriptions of some common techniques and technologies unique to work with these isotopes. Chapter 2 discusses some strategies particularly appropriate for planning syntheses of compounds labeled with carbon-14 and tritium, an appropriate topic for inclusion because there are distinct differences vis-à-vis the ways nonisotopic synthetic problems are approached, and an appreciation of these differences is

key to effective work in the field. The discussion of one strategy unique to organic radiochemical synthesis, *reconstitution*, is considered worthy of its own chapter, and is elaborated in Chapter 10.

The main parts of the book are devoted to presentations and critical discussion of the use of building blocks, reactions and reagents. These sections are arranged in ways appropriate for each isotope: preparation of tritium-labeled compounds is in large part organized by methodological approach, while preparation of carbon-14-labeled compounds is organized by the various isotopically labeled building blocks. Though most labeling reactions with tritium involve incorporation of the isotope from tritium gas or tritiated water sources into the intact carbon frameworks of final products or late stage synthetic intermediates, sometimes the use of tritiated building blocks is more appropriate. The aim of planning is therefore to identify appropriate substrates and methods for introducing the label. Chapter 3 discusses methods of exchange labeling with tritium gas or tritiated water, and Chapter 4 presents methods of synthesis utilizing tritiated reagents and the relatively small number of readily available tritiated building blocks.

On the other hand, the preparation of compounds labeled with carbon-14 usually involves some amount of carbon framework construction, and a number of carbon-14-labeled building blocks are available for this purpose. Therefore the planning process for syntheses of carbon-14-labeled compounds involves evaluation of synthetic pathways and selection of building blocks, including one or more containing the carbon-14 label. Chapters 5–9 present the most frequently used carbon-14-labeled building blocks and discuss their use.

Finally, two chapters cover, in methodologically oriented fashion, the chemical synthesis of enantiomerically pure ^{3}H- and ^{14}C-labeled compounds (Chapter 11) and biological methods of preparation (Chapter 12).

This book is intended to be useful for the researcher in any of several ways. It can be used as a text, which by study in the entirety can bring a newcomer in this field up to a reasonable level of competence. It can be used by scientists faced with specific labeling tasks as a source of reference for comprehensive and critical information on the utility of particular methods, reagents and building blocks. And finally it is hoped that scientists working in the field will find that browsing the book will stimulate new ideas for labeling, provide reminders of methods that can be productively employed in future projects, or spark creative thinking for problem solving in the field.

At times throughout the book the authors have included examples using deuterium or carbon-13 (or even carbon-11) when, in their opinion, the methods are likely to be applicable to tritium and carbon-14, respectively. In particular we note the growing importance of synthesis of carbon-11-labeled compounds as the utility of positron emission tomography grows rapidly[10]; however, synthesis with short-lived isotopes such as carbon-11 and fluorine-18 is a subfield in itself and is covered elsewhere[11].

1.1 Physical Properties of Tritium and Carbon-14

The properties of tritium and carbon-14 are well suited for use as tracers in many life sciences and chemistry applications. Table 1.1 lists the important physical properties of the isotopes.

Table 1.1 *Physical properties of tritium and carbon-14*

	Tritium	Carbon-14
Half-life	12.3 years	5730 years
Specific activity	29.2 Ci/milliatom	62.4 mCi/milliatom
Maximum energy of radiation (β^-)	18.6 keV	156 keV
Mean energy of radiation	5.7 keV	56 keV
Decay product	$^3He^+$ (stable)	$^{14}N^+$ (stable)
Maximum penetration of radiation		
Air	ca 6 mm	ca 20 cm
Water	ca 6 μm	ca 250 μm
Glass/concrete	ca 2 μm	ca 170 μm

Tritium is prepared in a $^6Li(n,\alpha)^3H$-reaction by irradiation of appropriate lithium-6-enriched compounds (e.g. LiF) or alloys (Li–Al, Li–Mg) with a high flux of neutrons in a nuclear reactor. Some of the tritium evolves as $^3H_2$ gas from the target through recoil during the generation process, and the rest is retained in the solid from which it is liberated by chemical methods.

Carbon-14 is produced in a $^{14}N(n,p)^{14}C$ reaction, also in a nuclear reactor, by irradiation of solid beryllium or aluminum nitride or a saturated solution of ammonium nitrate for periods ranging from 1 to 3 years. Afterwards the target is dissolved in half-concentrated sulfuric acid and the effluent gases are oxidized over an appropriate catalyst. [^{14}C]Carbon dioxide resulting from this procedure is absorbed by an aqueous solution of sodium hydroxide and $Ba^{14}CO_3$ is precipitated by addition of aqueous barium hydroxide. Barium [^{14}C]carbonate is the standard chemical form for storage and commerce, and it is the universal starting material from which all other carbon-14-labeled compounds are prepared. Because of the omnipresence of environmental carbon, the isotopic purity of $Ba^{14}CO_3$ is normally in the range of 80–90%, corresponding to specific activities of 50–56 mCi/mmol. Material of higher specific activities up to 62 mCi/mmol is commercially available, but it is considerably more expensive and only needed in exceptional cases.

These isotopes emit low-energy β^- particle (electron) radiation that does not require shielding for worker safety, as the radiation cannot penetrate the skin. Only with large amounts of carbon-14 can detectable secondary X-radiation occur. This radiation, Bremsstrahlung[12], is produced when electrons are decelerated in the Coulomb fields of atomic nuclei. As the energy of Bremsstrahlung is proportional to both the energy of the electron and the atomic number of the matter through which it passes, it is very low for carbon-14 used or stored in normal laboratory vessels. Routine precautions must be taken, however, to avoid internal exposure to these isotopes through ingestion, inhalation, contact with open wounds, or topical contact with compounds that may be absorbed transdermally. This is easily accomplished by working in fume hoods or glove boxes when there is any possibility of airborne radioactivity, by wearing suitable gloves at all times and by refraining from eating, drinking or smoking in the laboratory. Monitoring of laboratory spaces, equipment and personnel for contamination is easily accomplished in the case of carbon-14 using thin-window Geiger counters; analogous monitoring for tritium can only be accomplished by using windowless gas proportional counting devices. Usually the most expedient method for monitoring of surfaces for removable tritium or carbon-14 contamination is by wiping

the surface with a moist cotton swab or filter paper disk and measuring the radioactivity on the wiper material by liquid scintillation counting. Internal exposure of personnel is most easily monitored by regular urine radioanalysis.

As carbon and hydrogen are fundamental components of every organic compound, they can be replaced with carbon-14 and tritium without changing compounds' chemical makeup. Therefore, the chemical and physical properties of compounds labeled with tritium or carbon-14 are very similar to those of their unlabeled counterparts. Metabolically, they behave the same with one exception: if a metabolic transformation involves oxidation at a carbon atom whose hydrogen has been replaced by tritium, that metabolic pathway may be slowed because of the greater energy that is required to break a carbon–tritium bond compared with a carbon–hydrogen bond (primary isotope effect), and in rare cases this can cause significant alterations in the ratio of two or more different metabolites ('metabolic switching'[13], see also Chapter 2). Also, in rare cases the small differences in polarity and/or pK_a caused by tritium isotopic substitution can become apparent during the use of especially sensitive separation methods, such as high performance liquid chromatography (HPLC), in which the retention times of labeled and unlabeled congeners may be different (see Section 1.3.1 below) (secondary isotope effect). Because of the small difference in mass between carbon-14 and carbon-12 these effects are very small, and in life sciences experiments they can usually be neglected.

The long half-lives of these isotopes rarely make it necessary to correct for natural decay; an exception is the long-term (> 1 year) storage of tritium-labeled compounds.

The range of specific activities available in compounds labeled with these isotopes is suitable for tracer applications extending from mass balance studies in drug metabolism research to detailed investigations of the interactions of small and medium-sized molecules with biological macromolecules such as receptors and enzymes, and from tracing of biosynthetic pathways to the elucidation of chemical reaction mechanisms. Both isotopes can be detected with high sensitivity by a variety of instruments readily available in life science laboratories (liquid scintillation counters, ^3H nuclear magnetic resonance (NMR) instruments, mass spectrometers, phosphorimagers[14]), allowing discrimination and measurement of labeled compounds in complex biological samples.

1.2 Purification

The methods suitable for purifying compounds labeled with tritium or carbon-14 are fundamentally the same as those for similar nonlabeled compounds on the same mass scale, which is typically in the tens to hundreds of milligrams for carbon-14-labeled compounds, and micrograms to a few milligrams for tritiated compounds. Books such as Microscale Manipulations in Chemistry title as appropriate[15] are useful guides to techniques and devices for manipulating small quantities of compounds. Besides small mass scales, the most important source of constraint on laboratory methods is the need to control the material so as to minimize exposure of the worker and contamination of the laboratory. Approaches to purifying any material should take into consideration the possibility of radiation damage to compounds, which can produce impurities different from those encountered in a corresponding unlabeled compound.

Chromatographic methods are by far the most useful ones for the purification of compounds labeled with carbon-14 and tritium. There is a variety of methods having medium to high resolving power, the most common of which are flash chromatography[16] and its automated cousins, closed column methods such as HPLC or medium pressure liquid chromatography (MPLC), the more recently emerging supercritical fluid chromatography (SFC), and the more classical planar techniques of preparative radial flow chromatography and thin layer chromatography (TLC). The choice of method depends on the equipment available, the mass scale, and the ease of separation of the impurities[17].

Another common purification method is recrystallization. It is operationally simple and can be done on quantities down to the milligram scale using conventional micro-scale techniques and apparatus. It is relatively easy to conduct the required manipulations so as to avoid the inadvertent dispersal of particulates. Compounds sensitive to radiation-generated oxygen radicals in solution can be protected by working under an inert gas atmosphere[18].

1.3 Analysis

Analytical characterization of tritium and carbon-14-labeled compounds used in life sciences usually includes the following aims:

(a) To provide evidence of chemical identity;
(b) To measure chemical, and if appropriate enantiomeric, purity;
(c) To measure radiochemical purity (and, rarely, radionuclidic purity);
(d) To determine the specific activity;
(e) To determine or confirm the site(s) of labeling within the molecule.

The analyses most pertinent to each compound are determined by its intended use and the method of its synthesis. The specifications or acceptable numerical limits will depend upon the intended use and the requirements of applicable local procedures, institutional standards or government regulations. The level of detail with which analytical procedures are prescribed, the skill and care with which they are conducted and the quality of data interpretation all vary significantly, according to the expertise of the analyst and local standards of practice. Such differences can be expected to result in correspondingly higher or lower risk to the success of the studies in which the compounds are used. The goal for all analytical measurements should be to minimize subjectivity.

The standards for thoroughness of analysis, degree of procedural rigor, etc. are generally flexible for compounds used in research and early drug discovery studies (where the setting of specifications may be relatively informal), somewhat more formal for studies such as ADME in animals (where some institutional specifications or standards of practice usually exist). The standards are highest for compounds intended for human radiolabel studies (where extensive and detailed prescriptions must be adhered to and formal oversight of procedures and independent review of written records and data are common).

1.3.1 Chemical Identity

Analyses pertaining to chemical identity are intended to provide evidence that the structure and, if appropriate, stereochemistry, of a compound are in accordance with that claimed.

NMR is a useful analytical method because it can provide quite detailed information about chemical structure; current widely available instruments are sensitive enough so that ^{1}H NMR analysis is feasible for all carbon-14-labeled compounds and all tritium-labeled compounds except the cases where very limited quantities of high specific activity samples are available. ^{13}C NMR should also be routinely run when possible, especially for carbon-14-labeled compounds (see below). 2D-NMR methods, which are within reach of most laboratories, are very powerful for assessment of structural details, and should be considered whenever 1D methods leave ambiguity. Mass spectrometric analysis is universally recommended: it can provide not only confirmation of molecular weight, but also data suitable for calculation of specific activity (see Section 1.3.4 below). Classical methods should not be discounted; for example, infrared analysis provides a detailed fingerprint when a reference standard is available for comparison. The potential for isotope-induced changes in NMR and IR spectra should also be recognized, but these changes are quantitatively predictable and need not detract from the quality of the analysis.

Matching of chromatographic retention times is an unreliable indicator of chemical identity, for two reasons. Firstly, it is not uncommon for closely related compounds to have indistinguishable retention characteristics, even in chromatographic systems of high resolving power. Neither is it uncommon that a byproduct or analog of the intended synthesis product is closely related to it, and therefore to the reference standard. Secondly, the higher the resolving power of the method, the more likely it is that the presence of isotopes can alter the retention characteristics of a compound, causing the labeled compound and its unlabeled reference standard to appear chromatographically nonidentical. This phenomenon, called isotopic fractionation[19] has been recognized for decades and has more recently been the subject of a review[20]. Furthermore, the pK_as of amines may be altered by substitution of tritium (or deuterium) for hydrogen on adjacent carbon atoms, resulting in a significant change in retention time if the HPLC mobile phase has a pH value near the pK_a of the amine[21].

1.3.2 Chemical (and Enantiomeric) Purity

Chemical purity is defined as the weight of the compound of interest contained in a sample of given weight, usually expressed in percent. For radiolabeled compounds, it is usually calculated arithmetically from measurements of the respective HPLC/UV peak areas obtained after multiple injections of known weights of a sample and of a reference standard of known chemical purity. In the absence of a suitable reference standard, it is only possible to establish a chemical purity *relative to* an available sample of the authentic compound. The ratio of the HPLC/UV peak area of the analyte to the total area of all peaks in an HPLC chromatogram is most definitely *not* a measure of the chemical purity, because such a measurement fails to take into account either the absorbance characteristics of the observed impurities or any impurities that do not absorb light at the wavelength of detection, nor do they account for any impurities not eluting from the column, or any solvents or inorganic salts that may be present in the sample.

Chemical purity measurements are often not performed on tritiated compounds of high specific activity. Such materials are usually prepared in submilligram quantities, and, even if it were feasible to weigh samples accurately enough to prepare solutions of known mass concentration, the required manipulations would increase the risk of decomposition of the compound (see Section 1.5). Fortunately, high specific activity tritiated compounds are

used in such small (mass) quantities that unlabeled impurities are unlikely to cause problems. The absence of major impurities can be assessed to a modest degree of certainty by HPLC analysis with UV detection.

Measurements of enantiomeric purity are most conveniently accomplished using chiral HPLC analysis against authentic samples of enantiomerically pure and racemic materials. Alternative methods include NMR with a chiral shift reagent and optical rotation measurements.

1.3.3 Radiochemical (and Radionuclidic) Purity

Radiochemical purity, in analogy to chemical purity, is the ratio of radioactivity contained in the compound of interest to the total radioactivity of the sample. Radiochemical purity is usually measured chromatographically in order to exploit its separation power, and HPLC with online radioactivity detection is the most preferred method because of its superior resolution and detail-rich radioactivity profiling. Prior to the advent of modern HPLC radiodetectors, the eluate stream was collected in fractions that were counted by liquid scintillation counting (LSC) in order to construct a histogram from which the radiochemical purity could be extracted. This method is sometimes used with extremely low specific activity samples; however, the newer technique of automated fraction collection in multiwell microplates followed by high-throughput solid scintillation counting allows a degree of fractionation high enough to approach the resolution of on-line detection[22].

Thin layer chromatography continues to be used in spite of its relatively lower resolving power compared with HPLC, and potential problems in quantitation, such as self-absorption of radiation within the layer, especially with tritium (ca 2 μm path length of β^- in solid materials, see Section 1.1 above). One advantage compared with HPLC radiodetection is that impurities not eluting through HPLC columns may be detected on the TLC plate. In this technique, radiometry is conducted after development and drying of the TLC plate; available methods are of two types, linear and two-dimensional. Linear methods are based on single- or multi-wire gas proportional counters sensitive to a narrow band parallel to the direction of plate development. Two-dimensional methods include film-based autoradiography, which is of relatively low quantitative power, and the more modern techniques based on phosphor imaging screens or high-resolution crossed-wire proportional counters and high sensitivity CCD cameras, both of which are supported by computerized measurement systems.

Since it is relatively common that compounds coelute or nearly coelute in even high-resolution chromatographic systems, it is always recommended that radiochemical purity analyses be carried out in two different chromatographic systems, as unlike one another as possible. For example, the combination of one reverse-phase HPLC or TLC method and one normal-phase HPLC or TLC method is usually recommended, but two reverse-phase HPLC analyses using different column types and mobile phases may also be acceptable.

It should be noted that these chromatographic–radiometric methods have inherent shortcomings that must be understood and taken into account both in the chromatography component and the radiodetection component of the assay.

The chromatographic part: in HPLC, there is the possibility that one or more radioactive components are not detected because they fail to exit the column by the end of the monitoring period. An effective check for this possibility is to measure the quantity of

radioactivity exiting the column and to compare it with the amount injected. In TLC, there is the possibility that one or more components may be lost or diminished through volatilization before scanning can be completed. Before running a TLC analysis, the absence of volatile components may be confirmed by measuring the radioactivity in a sample before and after drying *in vacuo*.

The radiometric part: there are two phenomena involved. First, unlike UV detectors for HPLC, which have virtually no baseline noise, in HPLC radiodetection and TLC radioscanning there is always a significant baseline noise, resulting from a combination of environmental radiation, detector noise and the decay statistics of low-level radioactivity. The presence of this noise makes it difficult to distinguish between baseline and minor radioactive impurities, and even more difficult to measure them accurately. The second is that radiodetector peaks of eluting components tend to be broader and to have more pronounced tails than the UV detector peaks for the same components. This reduction in resolution is caused by the additional internal volume of the in-line radiodetector cell and associated plumbing that the sample must travel through after it exits the UV detector. Resolution can be further degraded by suboptimal scintillant flow rates (for liquid scintillant radiodetector cells) and peak tailing characteristic of many solid scintillant cells. There is an unavoidable tradeoff between maximization of radiodetector resolution and sensitivity (signal-to-noise ratio). These characteristics of radiodetector performance may make it more difficult to recognize and accurately quantify impurities running close to the compound of interest, and more difficult to judge the point at which the tail of the peak of interest returns to baseline. The uncertainties involved make accurate and consistent interpretation difficult, and make the data reduction process vulnerable to subjective judgments and therefore person-to-person variability. It is not unusual for measurements by two scientists using the same instrumentation for the analysis of the same sample to vary by 2%, and by 1% between successive injections by the same person. It is therefore not surprising that it is sometimes difficult to be certain whether a 98% radiochemical purity level (typically specified for ADME studies) has been met. Most radiometric instruments allow the operator to establish settings for control of a variety of parameters for data processing, such as baseline correction, peak detection and peak deconvolution, but correctly establishing these parameters requires skill, and the same parameter settings may not be optimal for all analyses.

Radionuclidic purity is only of concern in the context of dual-isotope labeling, or if cross-contamination from a laboratory mishap is suspected. Radionuclidic purity is best measured by liquid scintillation counting; modern LSC instruments have detectors and analysis software designed to discriminate quantitatively between the different isotopes used in the life sciences, except at very low counting levels. Radionuclidic purity is entirely distinct from isotopic purity, or content, of compounds labeled with stable isotopes, such as deuterium or carbon-13. Such information may be very important to the utility of stable-labeled compounds such as internal standards for mass spectrometric quantitation assays[23].

1.3.4 Specific Activity

There are two ways in which specific activity is expressed, radioactivity per unit mass (e.g., mCi/mg) and radioactivity per molar unit (e.g., Ci/mmole or mCi/mmole).

The former, used more frequently for carbon-14-labeled compounds and low specific activity tritiated compounds, is most simply determined by preparing a solution of known

mass concentration and measuring the radioactivity of defined volumes by LSC. Solutions already made up for chemical purity determinations can be used for this assay.

The latter expression of specific activity is used almost always for high specific activity tritiated compounds, and often for carbon-14-labeled compounds. Measures of radioactivity per molar unit can be calculated from mass spectrometry data[24]. In this analysis, the distribution of isotopic species (e.g., $^3H_0$, $^3H_1$, $^3H_2$, ...) is determined by measuring the relevant peak intensities in the molecular ion envelope and correcting them for naturally occurring isotopes (e.g., ^{13}C, ^{34}S) present in the molecule; this can be done manually or by use of readily available computer algorithms. The contribution of each isotopic species to the total can be used to calculate the average number of isotopic atoms per molecule and thence, from the molar specific activity of the pure isotope, the molar specific activity of the compound.

Interconversion between expressions of radioactivity per unit mass and radioactivity per molar unit must take into account the fact that the former has to be corrected for the chemical purity of the sample, whereas the latter does not.

1.3.5 Position of Label

The importance of knowing the location of carbon-14 or tritium atoms within compounds depends primarily upon their intended use, but may be of interest also in studies of reaction mechanisms, molecular rearrangements and mechanisms of isotope exchange.

In most syntheses the location of carbon-14 atoms follows logically from the route of synthesis. However, there are cases in which the position of a label was altered by previously unrecognized or incompletely understood reaction pathways, as illustrated by the reactions of **1** to **2**[25] and **3** to **4**[26] in Figure 1.1. In these cases, the carbon-13 labels and ^{13}C NMR were

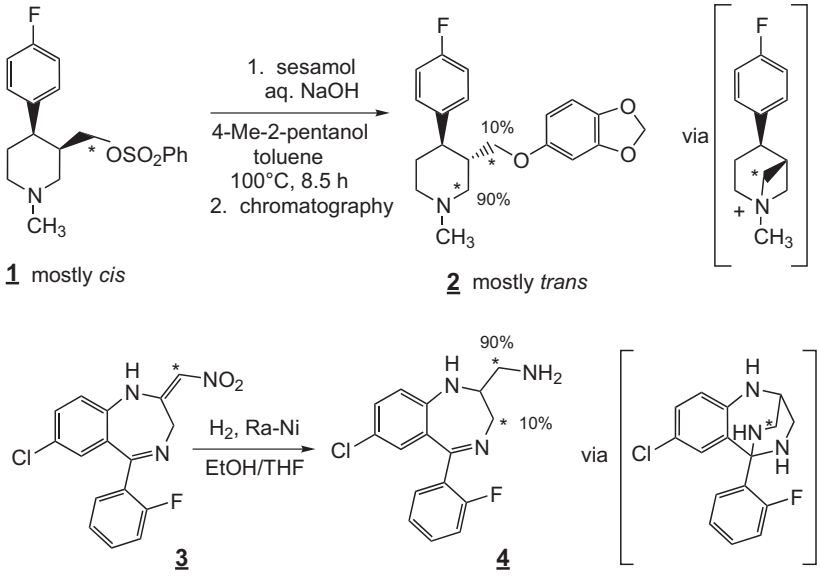


Figure 1.1 *Isotope scrambling in carbon-14 syntheses*

used to elucidate the phenomena. When it is of interest to confirm independently the location of carbon-14 atoms themselves within a structure, ^{13}C NMR spectroscopy can also be used for this purpose. As carbon-14 contains none of the 1.1% natural abundance carbon-13 isotope present in nonlabeled sites, ^{13}C NMR spectra measured under conditions where all atoms are relaxed between pulses (and all natural carbon sites therefore give signals of equal area or intensity), those carbon sites substituted with carbon-14 can be recognized by the diminution of the corresponding signals. As the extent of diminution is proportional to the carbon-14 isotopic enrichment at each site, the information can also be used in specific activity calculations.

Compounds labeled with carbon-14 biosynthetically may have complex patterns of isotope incorporation and/or low carbon-14 enrichment; in such cases compound prepared in carbon-13 pilot studies may be used as a surrogate for analysis.

For tritiated compounds, ^{3}H NMR is universally the method of choice for identification and quantitation of label distribution[27]. Tritium probes for NMR instruments have become widely available, the sensitivity of tritium detection is greater atom-for-atom than that for protium, and tritium chemical shifts are almost identical to those of protium. These factors make ^{3}H NMR an extremely powerful technique for quantitative determination of the distribution of tritium in samples of tritiated molecules down to the low mCi range in routine instruments, and as low as the tens of μCi range using high-field cryoprobe-equipped instruments[28]. Spectra recorded with and without suppression of protium–tritium coupling, and application of various 2D-NMR techniques[29] have been used to interpret complex ^{3}H NMR spectra. Even the measurement of $^{3}H_1$, $^{3}H_2$ and $^{3}H_3$ isotopomers of methylene and methyl groups can be accomplished through generally predictable peak multiplicities and geminal and vicinal isotope effects on chemical shifts[30].

1.4 Stability and Storage of Compounds Labeled with Tritium or Carbon-14

Because radiolabeled compounds are continuously exposed to their own radiation, they are often observed[31] to decompose much faster than their unlabeled counterparts. Therefore, the purity of radiolabeled compounds should either be measured by analysis shortly before use or by applying knowledge of stability data already accumulated for the same compound under the same storage conditions. However, in the latter case, the large batch-to-batch variability in stability often noted with radiolabeled compounds makes it risky to estimate purity levels of individual batches over time. Occasional observations of radioracemization[32] underline the need for appropriate purity controls to ensure the quality of compounds. It is common for isotope laboratories maintaining inventories of tracers to devote significant resources to purity monitoring and repurifications. One strategy sometimes used to mitigate these efforts is to store late synthetic intermediates rather than final products. This is most effective with intermediates that are more stable than final products and the transformation from one to the other is simple, and in cases where portions of the final product do not require frequent dispensing.

In any case, a good understanding of the nature of radiolytic decomposition and knowledge of ways to minimize its impact can be of great benefit[33,34]. Modes of decomposition have been summarized[35] as shown in Table 1.2.

Table 1.2 *Modes of decomposition of radiolabeled compounds*

Mode of decomposition	Cause	Mitigation
Primary (internal)	Isotopic decay	None at a given specific activity
Primary (external)	Interaction with radioactive emission	Dispersal of labeled molecules
Secondary	Interaction with molecules excited by the radiation	Dispersal of reactive molecules, Free radical scavenging, Cooling to low temperatures
Chemical	Chemical instability	Cooling to low temperatures

Primary (internal) decomposition is caused by disintegration of unstable nuclei within compounds and is of concern mainly for those labeled with short-lived isotopes such as sulfur-35, phosphorus-32 and phosphorus-33. Primary (internal) decomposition can be neglected for carbon-14-labeled compounds, as the half-life is very long (5730 years). In the case of tritium ($T_{1/2} = 12.3$ years), primary (internal) decomposition of approximately 5%/year can usually be ignored for mono-labeled compounds because the remaining molecular fragments are nonradioactive. However, compounds containing multiple tritium atoms or dual $^{3}H/^{14}C$ labels will produce radiolabeled impurities upon disintegration of one of the tritium atoms, at a rate proportional to the number of tritium atoms remaining in the molecule. For example, a compound containing four tritium atoms per molecule would be expected to generate impurities amounting to about $(4-1) \times 5\% = 15\%$ after 1 year of storage, from primary (internal) decomposition alone.

Primary (external) decomposition arises from direct interaction of the emitted (β^-) particles with nearby labeled molecules. The emission energy of even low-energy emitters such as tritium and carbon-14 is two to four orders of magnitude greater than the average energy of an organic bond (mean β^- energies for these isotopes = 5.7–56 keV or $8.3 \times 10^4 - 8.2 \times 10^5$ kcal/mol). Therefore, this interaction can easily lead to decomposition of the molecules impacted, which may number in the hundreds per β^- particle. Primary (external) decomposition is likely to be significant only for tritiated and carbon-14-labeled compounds in a neat state, i.e., a pure solid, bulk liquid or thin film. It is much more consequential for tritiated compounds for two reasons. First, the path length of tritium β^- particles is so short ($\sim 6\,\mu$m) that practically all their energy[36] is deposited within the bulk of the compound mass. Second, the radiation density within the bulk of tritiated compounds is usually very high, owing to the specific activities common for such compounds. In contrast, the longer path length of carbon-14 β^- emissions permits much of their energy to escape the mass of neat carbon-14-labeled compounds, and radiation densities are lower, in proportion to these compounds' lower specific activities. The combination of these two factors reduces the impact of primary (external) decomposition in carbon-14-labeled compounds. Moreover, crystal lattice energies can additionally contribute significantly to compound stability, so that compounds in crystalline form are often more stable than in their amorphous, liquid or glassy form.

For labeled compounds dissolved in a solvent or in contact with air, it is mostly solvent or air molecules that are activated, typically forming radicals. **Secondary decomposition** can result from the interaction of these radicals with labeled molecules. Usually this is by far the largest contributor to the decomposition of labeled compounds,

and the one that most needs to be controlled in order to extend the stability of compounds in storage.

Radiolytic decomposition is a multi-dimensional process, depending on a variety of parameters such as chemical structure, specific activity, impurities and storage conditions (formulation, solvent and storage temperature). Attempts have been made to calculate '*G*(-M)'values[*38], semiempirical measures that are meant to characterize the vulnerability of systems to damage by radiation, based on chemical structure. Unfortunately, as *G*(-M) values strongly depend on experimental data, and are importantly influenced by the impurity profile of individual batches, their practical value appears to be questionable[34]. General strategies for stabilizing radiolabeled compounds are usually effective and are discussed below, with the acknowledgment that it is difficult to predict in individual cases[32] what the effect of any particular parameter will be.

General aspects: One source of information on storage conditions for particular compounds, or compounds related to the compound of interest, is the catalogs of commercial radiochemical vendors. These companies have strong incentives to maximize the shelf-lives of their compounds, and they have had extensive experience with a variety of chemicals. As a rule, the use of highly pure solvents, tight container closures, controlled temperature and avoidance of unnecessary freezing–thaw cycles is recommended. Some discussion is provided below on three areas of consideration for stabilizing radiolabeled compounds.

(a) **Dispersal of radiolabeled molecules**

As discussed above, primary (external) decomposition is of concern mostly for tritiated compounds; it can be reduced by dispersal of the molecules, and dissolution in a solvent is the most commonly practiced method. Criteria for solvent selection to minimize reactive species are discussed in the next section. However, from a practical point of view the solvent should be chemically compatible with the compound, able to keep it in solution at the selected concentration and storage temperature, and either be removable with appropriate ease or be compatible with the intended use of the solute. The optimum concentration for a particular compound is a compromise between maximization of dispersal on the one hand and disadvantages of excessive dilution on the other. The disadvantages include increased vulnerability of the solute to chemical decomposition caused by dissolved oxygen or trace organic, acidic or metal contaminants in the solvent, and the impracticality of storing large volumes of solution.

Our own practical experience recommends concentrations of 0.5–10 mCi per mL of highly pure, deoxygenated solvent, and storage in several small quantities instead of one large one to avoid frequent opening. Radicals are readily formed upon interaction of radiation with oxygen, and compounds sensitive to these radicals are more prone to decomposition. Compounds containing divalent sulfur are highly sensitive; oxidation of amines has been documented; and data collected on the stability of MK0677[39] indicate the susceptibility of its benzyloxy moiety.

If the radiolabeled compound is to be stored in its natural physical state, it might be stabilized by dilution with unlabeled carrier to the lowest specific activity needed. While blending of a liquid radiolabeled compound and carrier can be simply

[*] *G*(-M) is defined as the number of molecules irreversibly changed per 100 eV of energy absorbed; see also Ref. 37.

accomplished by mixing, blending of labeled and unlabeled solids should be carried out by complete dissolution together and subsequent recrystallization of the blend, in order to avoid particle-scale inhomogeneity.

Traces of heavy metals can significantly catalyze the rate of decomposition. Therefore, for example, it is recommended to remove metal catalysts from crude products as soon as practicable after the reactions in which they have been used.

(b) **Minimizing reactive species**

For many years benzene either pure or in combination with methanol[40] was the solvent of choice for the storage of less polar radiochemicals. By virtue of its π-orbitals, benzene's ability to absorb energy and to stabilize excited states rationalized this choice. Nowadays toluene, which has equivalent stabilizing properties, is usually used instead of benzene because of the latter's high toxicity. Alcohols such as methanol or ethanol are commonly used for dissolution of more polar compounds. Both act as radical scavengers[41] and are widely observed to extend the shelf-life of a variety of compound types when used either as solvent or as a cosolvent. Other solvents such as pentane and ethyl acetate, being neither radical inhibitors nor promoters, are sometimes used either as solvents or cosolvents. In contrast, chloroform, dichloromethane[42], water and ether-type solvents tend to form radicals and therefore can destabilize radiolabeled compounds. Water tends to form highly reactive hydroxyl radicals in the presence of radiation, so water alone is a poor choice of solvent. However, addition of at least 2% ethanol to aqueous solutions effectively traps radicals, so effectively so that 95 : 5 aqueous ethanol is quite commonly used for the storage and shipment of polar compounds such as amino acids, sugars and nucleotides. Similarly, dichloromethane–methanol or water–methanol solvent mixtures may be suitable alternatives[43].

A significant advantage in using toluene or ethanol in storage solutions is their dual role as solvents and stabilizers. Other effective radical scavengers, such as dimethyl sulfide, 2-mercaptoethanol[38], and benzyl alcohol, are occasionally used as stabilizing additives to solutions of labeled compounds in various solvents, but usually only in cases where they are compatible with the intended studies as they are difficult to separate from the labeled solute.

(c) **Lowering the storage temperature**

As the decomposition of radioactive compounds by interaction with radicals or other activated molecules proceeds by chemical reactions with perceptible activation energies, the reaction rates are temperature dependent. Therefore, the lower the storage temperature the lower the rate of decomposition should be, and the optimum storage temperature is the lowest temperature practicable. This rule is well documented not only for solids and solutions, but also for frozen solutions[44].

If the storage temperature is lower than the freezing point of a solution, the solution should be frozen rapidly, for example by immersion in liquid nitrogen, rather than being placed in the freezer in the liquid state. Slow freezing of solutions can result in crystallization or precipitation of the solute, or cause migration of the solute towards the center of the volume (the last to freeze), thereby greatly increasing the local concentration of the compound and offsetting the benefit of molecular dispersion.

If liquid nitrogen storage is used, stringent precautions must be taken in packaging samples. The vial closures routinely used in organic chemistry laboratories do not

prevent the slow insinuation of nitrogen into vials, whether immersed in liquid nitrogen or stored in the −140 °C zone above it. They can then explode as they warm up after being removed from the storage facility. Plastic cryovials with silicone-ring seals are suitable for storage of samples in liquid nitrogen, but they are incompatible with many low-polarity organic solvents. Flame-sealed glass ampules are the most secure but they are fragile and troublesome to prepare. The use of 'cryo' class labeling materials or markers is recommended.

Freezers designed to maintain temperatures down to −140 °C are commercially available, but they are costly; therefore many radiochemistry laboratories compromise by storing stocks in more readily available −80 °C or −60 °C freezers.

1.5 Specialist Techniques and Equipment

Synthetic work with tritium- or carbon-14-labeled compounds differs in several ways from synthetic work with unlabeled materials, and this reality influences the way isotope scientists plan and conduct their work (see Chapter 2). Differences include:

- The need for more careful containment of materials, owing to the requirements of radiation safety and the desire to avoid the spread of contamination (subject to the specifics of national and institutional regulations);
- The use of smaller scales, especially with tritium;
- The higher likelihood that radioactive compounds will be unstable (discussed in general in Section 1.4 above, and mentioned for specific compounds where they are discussed throughout this book).

These differences impose greater constraints upon radiochemical synthesis work compared with those of 'regular' organic synthesis, constraints that can limit the feasibility of certain operations or significantly increase the difficulty of conducting them. Over the decades, various practices have been developed in efforts to manage or mitigate these constraints, usually through individual ingenuity and inventiveness. Regrettably, no thorough compilation of these "tricks of the trade" exists. While it is not the intention here to cover this subject comprehensively, some important aspects will be discussed. Other sources of information are available[45].

Methods and technologies for manipulation of reactants and reaction mixtures should take account of the potential volatility of radioactive components, including possible byproducts. A practical distinction can be made among components that are (a) gases at or near room temperature; (b) liquids whose boiling points (up to about 180–200 °C) render them suitable for manipulation by static vacuum transfer; (c) materials of low but significant volatility (liquids b.p. ∼200 to ∼280 °C and solids with a tendency to sublimate); and (d) nonvolatiles.

Fundamentally, the handling of nonvolatile materials need not differ significantly from that of unlabeled materials, except that special care should be taken against scattering of less dense solids by fume hood drafts or static buildup, which is a much more severe problem because of ionization induced by emitted β^- particles.

However, substantial practical efficiencies can be achieved by simplification of procedures. For example, many small-scale reactions can be conducted in inexpensive screw-cap

vials with 'flea'-sized magnetic stirring bars. Inert atmospheres and exclusion of moisture can be maintained by using septum stoppers and inert gas supplied through syringe needles. Heating is conveniently provided by an oil bath. Workups can be performed in the same or analogous vials, by separating layers using Pasteur pipets, removing solvents with gentle streams of nitrogen, etc. Utilizing such disposable supplies reduces the amount of glass washing required.

Materials that are gases at or near room temperature must unavoidably be handled in a closed vacuum line. A large number of glass vacuum line systems have been described for the manipulation of such materials[46], and extensive descriptions of vacuum line techniques are available[18,47]. As liquid nitrogen is generally used to condense materials in order to manipulate and transfer them on a vacuum manifold, tritium gas, [^{14}C]carbon monoxide and methane isotopomers, which have boiling points below 77 K, require different approaches. Commercial stainless steel vacuum systems have been developed for manipulation of tritium gas and its storage by reversible uptake on depleted uranium[48], supplanting the older Toepler pump technology[†]. The newer systems take advantage of the reversible, exothermic reaction of tritium gas with uranium metal to give U^3H_3[49]. Tritium gas can be expelled from the U^3H_3 storage bed with excellent control (equilibrium $^3H_2$ pressure = 1 atm (760 Torr) at 436 °C and $\sim 1.3 \times 10^{-3}$ Pa ($\sim 10^{-6}$ Torr) at 25 °C), and any excess of tritium gas is spontaneously reabsorbed by the bed as it cools.

There is as yet no analogous manifold available for handling ^{14}CO; however, a glass setup for its generation and use has been described[50]. Among the so-called 'condensible' gases (b.p. > 77 K), which are routinely manipulable using liquid nitrogen cooling, [^{14}C]carbon dioxide is usually prepared and used as needed on vacuum manifolds or simple glass vacuum assemblies, but a commercial steel $^{14}CO_2$ storage and handling manifold has recently become available[51]. Carbon-14-labeled gases purchased commercially are usually packaged in breakseal vessels with ground glass fittings that can be attached to the user's vacuum line and opened under controlled conditions.

Vacuum-transferrable volatile materials (b.p. up to about 180–200 °C) often encountered include (aside from those prepared as intermediates in the radiochemical laboratory) commercial building blocks such as [^{14}C]methyl iodide and other low-molecular-weight carbon-14-labeled alkyl halides, methanol, ethanol, benzene, acetic and haloacetic acids, acetyl and haloacetyl chlorides and dimethylformamide. These compounds are most appropriately handled on vacuum manifolds in the same way as gases, but some may, with proper experimental design, be used without such systems. In the latter case, it is strongly recommended that safety measures be taken against the possibility of the release of volatile radioactivity.

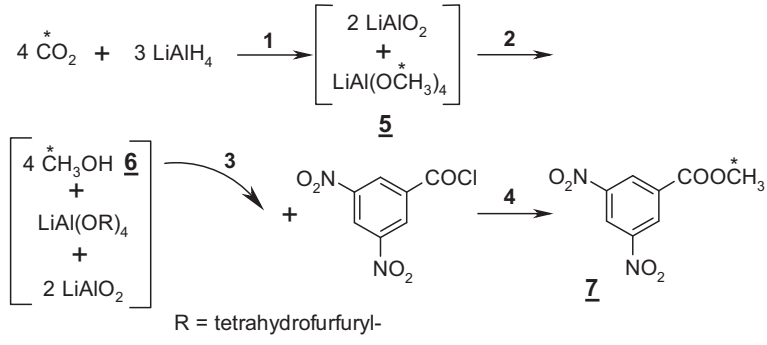
The most problematic materials can be those with low but significant volatilities. The main reason is that the potential for volatilization under particular conditions may not be evident or easily evaluated beforehand. For example, [^{14}C]benzoic acid can evaporate at a significant rate by sublimation on a rotary evaporator with a little warming, and some nonvolatile [^{14}C]aryl compounds, safely handled in the open, can gain significant volatility when substituted with fluoro groups. Experimental plans should be examined for possible

[†] The 'mercury air pump', August Toepler (1836–1912): lecturer, Academy Poppelsdorf, Bonn (1859–1864); chair of chemistry and chemical technology, Polytechnic Institute, Riga (1864–1868); professor, University of Graz (1868–1876); chair, Experimental Physics and director, Physical Institute, Dresden Technical University (1876–1900).

cases such as these, and if appropriate, modification of reaction conditions should be seriously considered. Tracer runs may be useful in developing appropriate methods.

A separate track in terms of intelligent handling of potentially problematic materials or reactions is the redesign of syntheses to make them more efficient—even if they do not involve volatile materials at all. There are two key drivers for synthesis redesign: reducing the need for manipulations of materials, and reducing the volatility of intermediates while preserving their relevant reactivity. Achievement of these goals has the added benefit of reducing the likelihood of radiation-induced decomposition (especially critical for polymerizable intermediates such as radiolabeled acrylates).

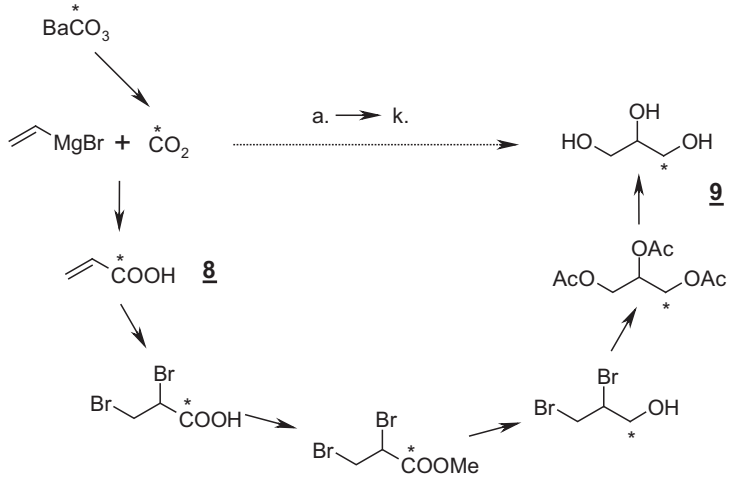
The reduction of manipulations can be accomplished through modification of experimental procedures so as to run as many reaction steps as possible in one pot, and/or to perform transfers of labeled intermediates from one reaction vessel into another without opening up the system, whether it be a complex vacuum manifold or a simple glassware assembly. Such tactics have been called 'telescoping' reactions[45a]. An example of a simple procedure is the preparation and use of ^{14}CO already mentioned. A more complex one, is illustrated for [^{14}C]methyl 3,5-dinitrobenzoate (**7**) in Figure 1.2[52]. Reduction of $^{14}CO_2$ with $LiAlH_4$ produces lithium tetramethoxyaluminate (**5**), which requires release of [^{14}C]methanol by addition of a proton source. Tetrahydrofurfuryl alcohol was selected for this purpose because of its relatively low volatility (b.p. 178 °C). The reduction itself was conducted in the solvent 2(3)-(tetrahydrofurfuryloxy) tetrahydropyran (b.p. 106–108 °C/6 mmHg) for the same reason. The [^{14}C]methanol (**6**) thus produced (94% yield) was expelled by purging the solution with a gentle stream of helium then trapped in a solution of 3,5-dinitrobenzoyl chloride and pyridine in ethyl acetate to generate the easily handled carbon-14-labeled ester, from which [^{14}C]methyl iodide can easily be prepared. This procedure is generally applicable for the synthesis of C1–C5 [^{14}C]alcohols that have reasonable volatility; higher alcohols are better separated from the solvent by fractional distillation (see Chapter 5, Section 5.1.2).



Reaction conditions: **1**. 2(3)-(tetrahydrofurfuryloxy)tetrahydropyran (T-solvent), $LiAlH_4$, 0 °C, 10 min; **2**. tetrahydrofurfuryl alcohol; **3**. He (50 mL/min), 110 °C; **4**. py, EtOAc; r.t., 16 h.

Figure 1.2 *Synthesis of [^{14}C]methyl 3,5-dinitrobenzoate*

A virtuosic example of telescoping[53], depicted in Figure 1.3, began with barium [^{14}C]carbonate and ended after six synthetic steps with [1-^{14}C]glycerol (**9**). It was carried out without any workups or isolations of intermediates; only the continuous extraction of [1-^{14}C]acrylic acid (**8**) might have been avoided. The overall yield of purified [1-^{14}C]glycerol at a specific activity of 10 mCi/mmol was 35%, based on $Ba^{14}CO_3$.



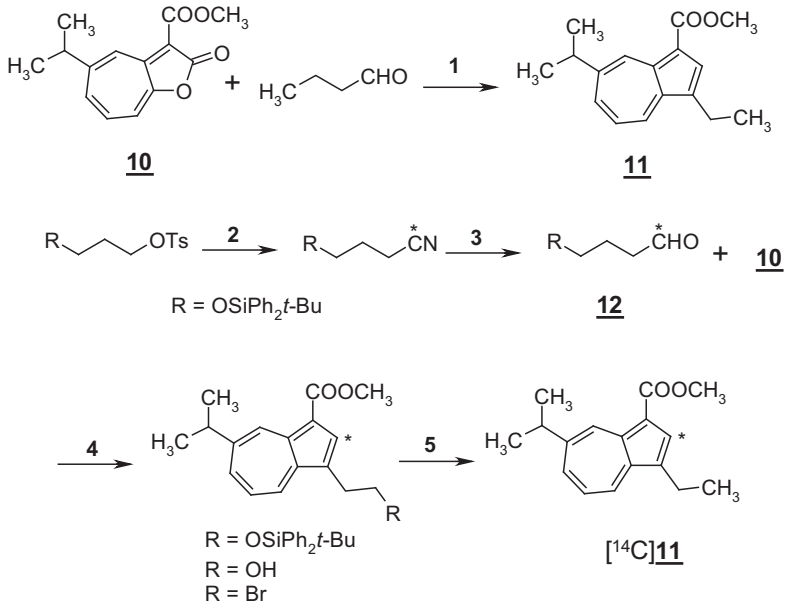
Reaction sequence: **a**. Et_2O; −20 °C, 20 min, **b**. add aq. H_2SO_4, **c**. continuous extraction with Et_2O (tr. hydroquinone), **d**. concentrate to 10 mL with N_2 stream, **e**. add Br_2; r.t., 1 h, **f**. add CH_2N_2/Et_2O; 0 °C, 3 h, **g**. add $AlCl_3/Et_2O$, $LiAlH_4$; −80 °C, 3 h, **h**. add wet Et_2O, **i**. add HOAc, NaOAc; reflux, 15 h, **j**. evap volatiles, **k**. add aq. NaOH; reflux, 6 h.

Figure 1.3 *'Telescoped' synthesis of [1-^{14}C]glycerol*

An alternative tactic for managing volatile intermediates is to 'devolatilize' them by covalently combining them with molecular entities that render the resulting compounds nonvolatile, preserves the reactivities of the radiolabeled moieties, and are removable after having served their purpose.

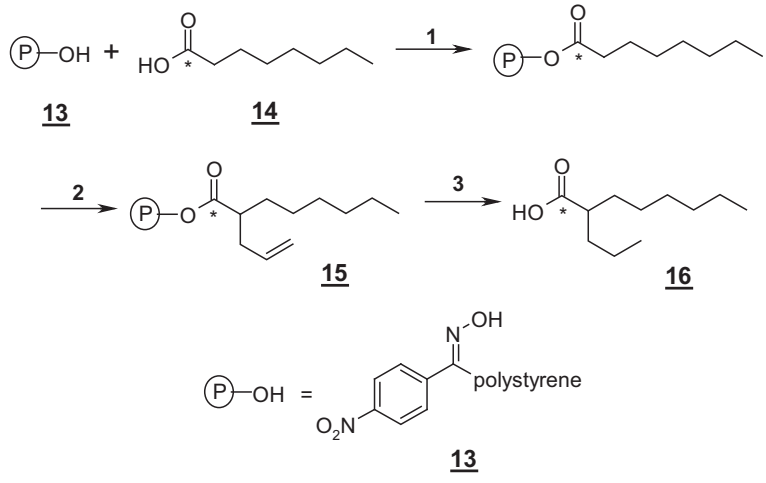
This principle can be illustrated by the synthesis of the carbon-14-labeled azulene derivative [^{14}C]**11**, an advanced intermediate in the preparation of an anti-inflammatory and anti-ulcerative drug candidate[54] (Figure 1.4). The route developed for the unlabeled compound utilized the condensation of butyraldehyde with the lactone **10**; substitution of [1-^{14}C]butyraldehyde in the reaction would lead to [^{14}C]**11** possessing the label at the desired position. However, rather than prepare, handle and utilize radiolabeled butyraldehyde (b.p. 75 °C), especially since the reaction is conducted at 78 °C, the investigators chose to utilize the synthetically equivalent but nonvolatile building block **12**. Condensation of **12** with the lactone partner proceeded similarly to that with butyraldehyde, then the oxygen function was removed in three high-yield steps as shown.

Another method of devolatilization is to use solid-phase synthesis methodologies. The preparation of 2-propyl[1-^{14}C]octanoic acid (**16**)[55] might serve as an example, even though the labeled substrate is not highly volatile. (Figure 1.5) [1-^{14}C]Octanoic acid (**14**) was



Reaction conditions: **1**. morpholine, EtOH; reflux, 7 h; **2**. $K^{14}CN$, 18-crown-6, MeCN; 90–100 °C, 9 h; **3a**. DIBAH, hexane/Et_2O; −25 °C, 2 h, **b**. 5% aq. H_2SO_4; 0 °C, 30 min; **4**. morpholine, EtOH; reflux, 8 h; **5a**. TBAF, THF; 20 °C, 1 h, **b**. PPh_3, CBr_4; 0 °C, 1.5 h then 20 °C, 30 min, **c**. Bu_3SnH, toluene; 100 °C, 2.5 h.

Figure 1.4 *Synthesis of methyl 3-ethyl-7-isopropyl[2-^{14}C]azulene-1-carboxylate.*



Reaction conditions: **1.** EDC, CH_2Cl_2; **2.** LDA/THF, −40 °C; allyl bromide; **3.** H_2, Pd/C, MeOH.

Figure 1.5 *Solid-phase synthesis of 2-propyl[1-^{14}C]octanoic acid*

attached to the oxime-functionalized polystyrene resin **13** then alkylated with allyl bromide to give the solid-phase supported 2-allyl derivative **15**. Exposure of **15** to mild catalytic hydrogenation reduced the allyl double bond and released the product (**16**) from the resin. Other examples, using carbon-14[56] and developed for use with labeled materials[57], have been published.

The devolatilization concept has been extended to certain commonly used isotope sources with the additional advantage of making them more stable or storable. [^{3}H/^{14}C]Methyl nosylate (**17**) has been developed[58] as a substitute for tritiated or carbon-14-labeled methyl iodide, both of which are relatively unstable and difficult to handle (Figure 1.6). Ester **17** is a nonvolatile, easily purifiable solid that is substantially less radiolytically sensitive than the corresponding methyl halides ([^{3}H]methyl nosylate at a specific activity of >80 Ci/mmol suffered no appreciable decomposition after storage at 39 mCi/mL for 14 weeks at 4 °C in hexane/ethyl acetate[58]). This derivative is reported[58] to possess similar reactivity to [^{3}H/^{14}C]methyl iodide in a variety of reactions, and to provide greater flexibility during use in synthesis.

9-Phenyl[9-^{2}H]fluorine (**18**), a nonvolatile solid with p$K_a \sim 18$, has been shown[59] to be a source of deuterium to quench carbanions; it was prepared in high isotopic content by Pd-catalyzed deuterio-debromination of 9-bromo-9-phenylfluorene using deuterium gas. A number of model compounds (exemplified by **19**–**22**) were labeled, in good chemical yields and excellent isotope incorporation, by metallation (*n*-BuLi, THF, −78 °C) and subsequent addition of **18** to the lithio species. The deuterium in **18** is acidic enough to undergo transmetallation with a wide variety of carbanions, but not so acidic as to be air- or moisture-sensitive. Although the analogous tritiated reagent has not been reported in the literature, it could be prepared analogously, appears likely to be stable on storage and could prove to be a good alternative to high specific activity tritiated water.

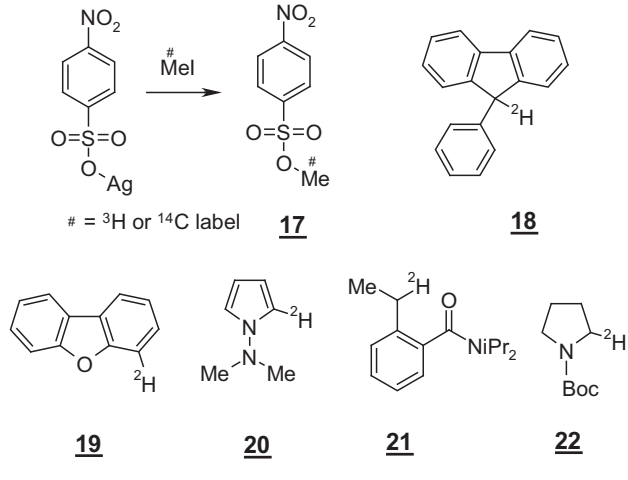


Figure 1.6 *Devolatilized sources of tritium/carbon-14 and deuterium*

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