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Review Article

Synthesis of isotopically labelled compounds at Schering-Plough, an historical perspective†

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Abstract: The use of isotopically labelled compounds at Schering-Plough (S-P) has substantially increased over the past 30 years. Since the formation of the S-P Radiochemistry section in the early 1970s, the group has expanded its role from supplying ^{14}C- and ^{3}H-labelled compounds for ADME studies for compounds in development, to providing ^{3}H-labelled compounds for drug discovery and also to provide stable isotope-labelled compounds that are used as LC/MS internal standards. Other isotopes used by the group have included ^{125}I and ^{35}S, which have been used to label both small molecules and proteins. Copyright © 2007 John Wiley & Sons, Ltd.

Keywords: tritium; deuterium; carbon-14; carbon-13; nitrogen-15; sulphur-35; iodine-125; synthesis

Introduction and early history

The Radiochemistry Section at the Schering-Plough (S-P) was formed in the early 1970s for supplying radiolabelled compounds for metabolism studies of compounds in development. Both ^{3}H- and ^{14}C-labelled compounds were used. High potency steroids were labelled with ^{3}H, and ^{14}C was the preferred isotope for other, less-potent compounds. ^{3}H labelling was frequently used, however, in cases where a ^{14}C synthesis was lengthy and difficult. In the early years, 'cold' synthetic procedures were usually developed at S-P and much of the subsequent 'hot' synthetic work was done at contract research organizations (CROs), such as Amersham Corp. and Midwest Research Institute.

Analytical chemistry was primitive by today's standards. Compound identity was confirmed by co-migration with authentic samples in multiple thin-layer chromatography (TLC) systems, occasionally augmented with mass spectrometry or infrared spectroscopy. TLC was the main technique for assessing radiochemical purity. Detection and quantitation of radioactivity on the plate were done using autoradiography and plate scrapping-liquid scintillation counting. High-performance liquid chromatography (HPLC) with a radioactivity flow detector and a Bioscan TLC plate scanner were introduced in the early and mid-1980s, respectively. Currently, HPLC with flow detectors are used for all labelled compound release. RadioTLC is used mainly as a tool for monitoring reactions and, occasionally, as a secondary assay for final products. The full range of modern spectroscopic methods – NMR, MS and LC–MS – is utilized extensively.

In the early 1990s, in response to a growing interest in obtaining early metabolism data to assist in discovery programs, the group set up an internal tritium labelling capability. With the move to LC/MS-based bioanalytical methods for measuring the concentrations of drug in biological matrices, the group began to supply stable isotope-labelled compounds to serve as internal standards. This paper will discuss the growth in the synthesis and applications of compounds labelled with radioactive and stable isotopes by the Schering-Plough Radiochemistry Section over the past 30 years.

Synthesis and analysis of tritium-labelled compounds

During the early years of the group, ^{3}H-labelled compounds were prepared for development programs when a ^{14}C synthesis was difficult or the specific activity needed was too high for ^{14}C. An example of the latter is the high potency steroid [^{3}H] mometasone furoate (**1**). Mometasone furoate is the active ingredient

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in S-P products including Elocon®, Asmanex® and Nasonex®. We synthesized [^{3}H]mometasone furoate many times over the years, often in Ci amounts, to support the extensive development program.[1] During the early years of the group, the entire synthesis was contracted out, but in subsequent years, as the size and capability of the group increased, only the initial tritium gas reduction and re-oxidation steps were contracted out and the ^{3}H-intermediate received was converted to the final compound in four steps, with the yields improving over the years by incorporating improvements from the process synthesis (Scheme 1).

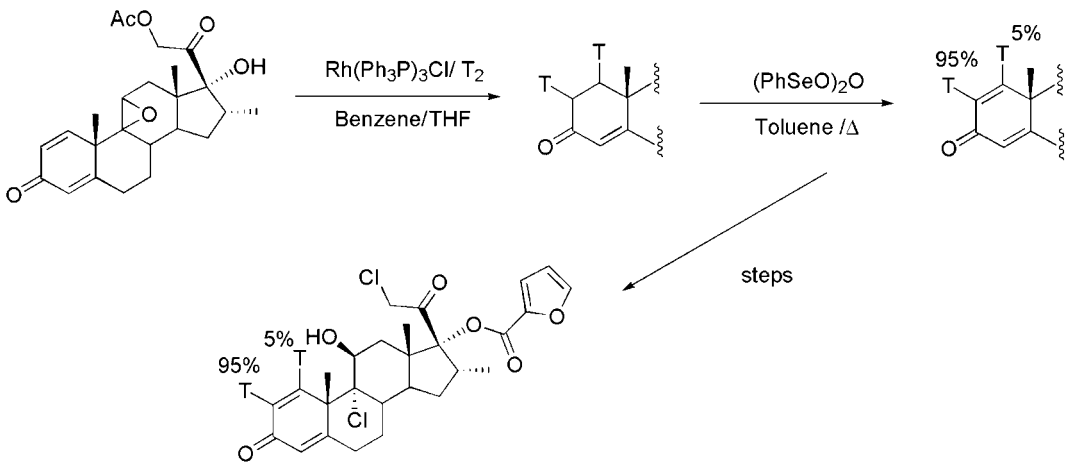
Radiochemistry started to provide ^{3}H-labelled compounds for early drug discovery use in 1987 and supplied 3–4 compounds each year for the next few years. Labelling methodology was worked out using deuterium, the labelling was contracted out to Amersham, and the crude product was returned for 'in house' purification and analysis. A few compounds were also prepared by the group during visits to the National Tritium Labelling Facility.[2]

In 1990, an increased emphasis was put on preparing compounds to assess ADME properties early in discovery. A ^{3}H nmr service was set up with Professor John Jones at the University of Surrey. The actual tritium labelling was still contracted to Amersham Corp. To decrease the time needed to supply compounds to the Drug Metabolism department, tritiated water exchange labelling capability was set up in 1991. Next, the group acquired a Trisorber in 1994 to bring a tritium gas capability in house and during the later 1990s, a ^{3}H nmr probe was purchased. As a result, over the past 15–20 years, the number of compounds prepared annually for supporting new drug discovery has increased nearly tenfold. Most ^{3}H compounds are now prepared in the 1–2 weeks time frame required for supporting fast moving discovery programs.

Most tritium-labelled compounds prepared by the group have made use of the many available hydrogen isotope exchange methods as opposed to the more traditional reduction methods. This approach has the advantage that in many cases the target molecule can be labelled by direct exchange, or failing that an advanced intermediate, which is available from the medicinal chemistry synthesis, can be exchange labelled and then converted to the target compound by a known route. This approach avoids the need for preparing unsaturated or halogenated precursors.

One of the earliest exchange methods employed by the group included the use of Bronstead or Lewis acid-catalysed exchange. An example of a Bronstead acid-catalysed exchange is [^{3}H]Sch 51048 (**2**), prepared by heptafluorobutyric acid-catalysed exchange with tritiated water of an advanced intermediate and converted to [^{3}H]Sch 51048 in a subsequent alkylation step[3] (Scheme 2). Metabolism data derived from this compound led to the identification of an active metabolite, which was ultimately advanced into development and is now marketed as Noxafil®. An example of a Lewis acid-catalysed exchange is [^{3}H]Sch 48973 (**3**) prepared by $AlCl_3$-catalysed exchange with tritiated water of 2,6-dichlorophenol, followed by a subsequent alkylation to form the target compound[4] (Scheme 3).

Base-catalysed exchange, although an old technique, has been used on a number of occasions by the group. A recent example involved the preparation of [^{3}H]Sch 414319 (**4**). In this case, the target compound was treated with 3 equivalents of *n*-butyl lithium and then quenched with tritiated water.[5]



[^{3}H]Mometasone Furoate (**1**)

Scheme 1

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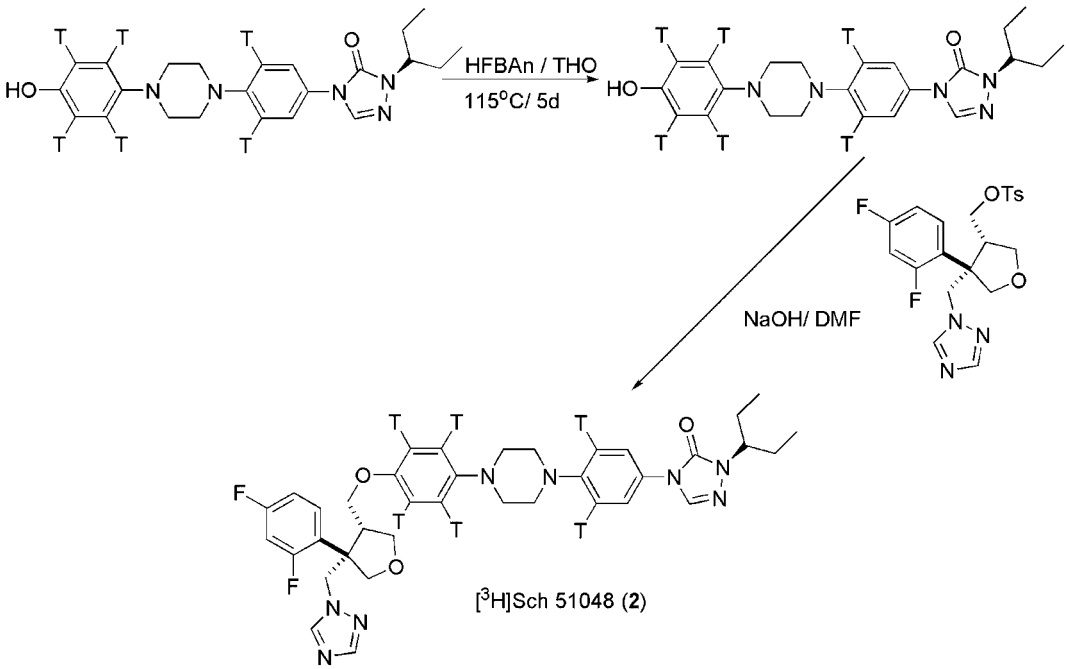
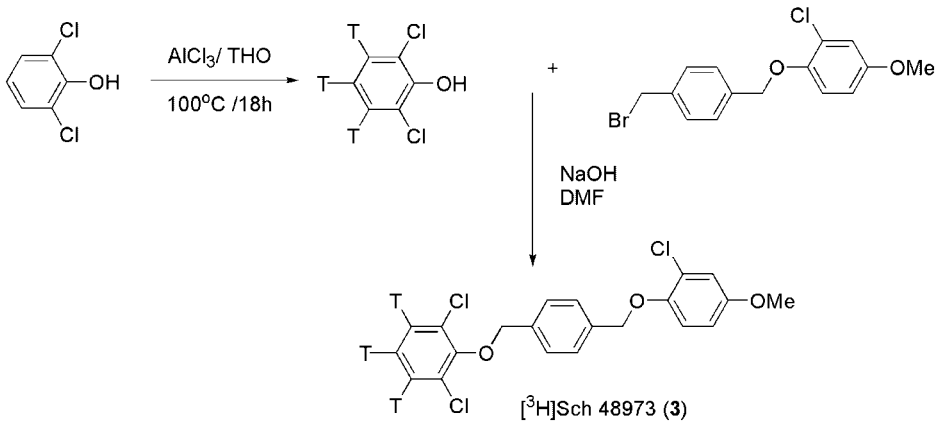


Scheme 2



Scheme 3

Metal-catalysed exchange has been used extensively to prepare ^{3}H-labelled compounds by the group. Among one of the oldest methods, Pt-catalysed exchange with tritiated water has proved to be an extremely versatile method and has been used by the group to label a very extensive range of structures. One such example was the preparation of [^{3}H]Sch 47949 (**5**), which was labelled by direct Pt-catalysed exchange of the target molecule with tritiated water. ^{3}H nmr analysis showed that the expected meta and para positions were labelled along with a small amount of incorporation in the benzylic site.[6] Similar methodology was also used to prepare [^{3}H]Sch 40120 (**6**) and [^{3}H]Sch 40853 (**7**) (Figure 1).[4,7]

Although versatile, Pt-catalysed exchange does suffer a significant drawback. Typically, the compound is exposed to high temperatures for several days, which often leads to degradation of the compound. In an attempt to address this issue, the group has conducted some initial research for using microwave activation as an alternative to thermal heating using Sch 388714 (**8**) as a model substrate.[8] Preliminary results have been encouraging and suggest that a similar degree of incorporation can be achieved using a 10 min microwave program compared with a 48 h thermal reaction, with similar degrees of compound degradation.

In addition to Pt, Raney Ni-catalysed exchange with tritiated water has also been used, with [^{3}H]Sch 14988

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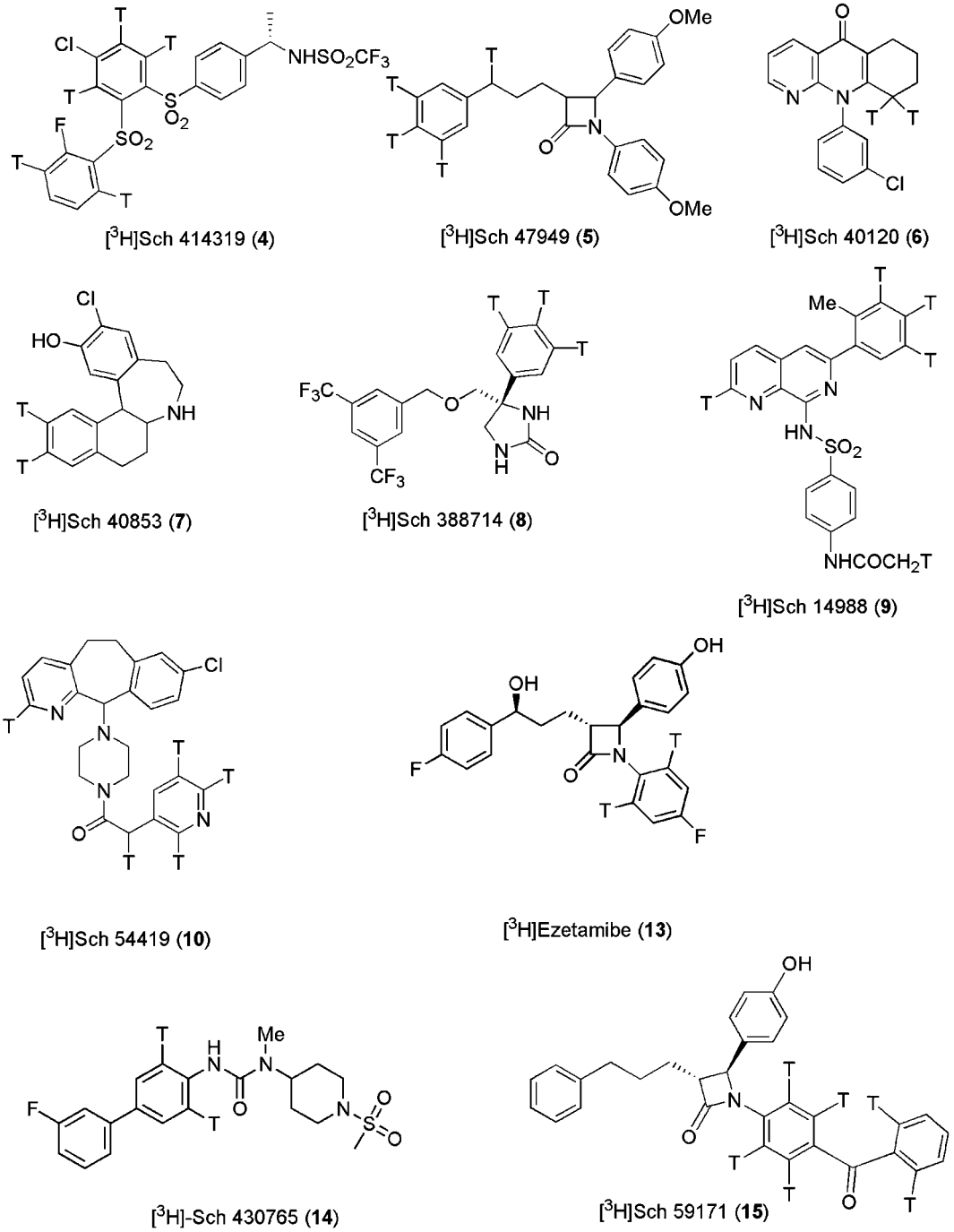

Figure 1 Structures of [^{3}H]-labelled compounds prepared by a single exchange step.

(**9**) and [^{3}H]Sch 54419 (**10**) labelled successfully using this method. ^{3}H nmr analysis showed a high degree of incorporation in the heterocyclic rings as would be expected for Raney Ni.[4]

Recent advances in the field of homogeneous metal-catalysed exchange have been extensively utilized by the group. In particular, the use of tris-triphenylphosphine ruthenium (II) chloride with tritiated water to

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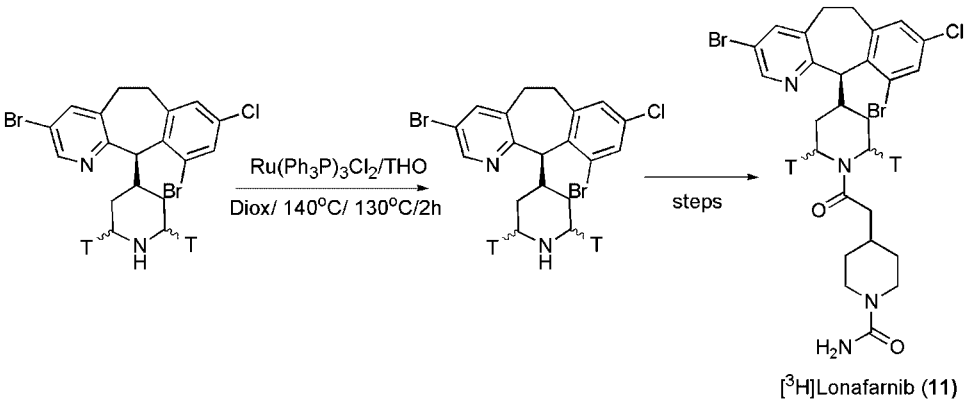
prepare ^{3}H-labelled piperidines and piperazines[9] has become one of the most commonly used labelling methods in the group, given that the piperidine and piperazine functionality are extensively found in pharmaceutical structures. Examples of compounds prepared using this route are [^{3}H]lonafarnib (**11**) and [^{3}H]Sch 211803 (**12**).[10,11] In each case, a piperidine intermediate with a free NH was tritiated and then converted to the target compound in one or more steps using the medicinal chemistry route. ^{3}H nmr analysis confirmed that the label was confined to the α-methylene protons adjacent to the free NH (Schemes 4 and 5).

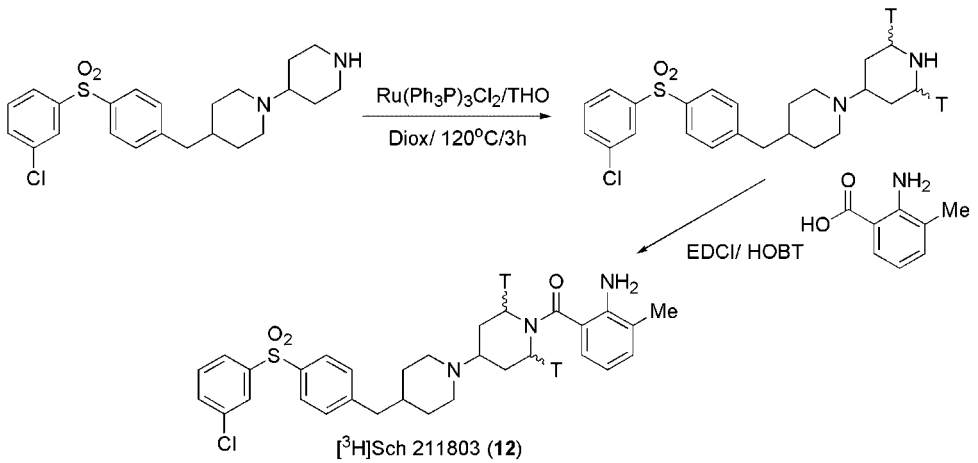
The development of homogeneous iridium catalysis by Heys[12] with tritium gas for tritium labelling by a cyclometalation mechanism has also had a significant impact on labelling methodology employed by the group. In particular, the commercially available Crabtree's catalyst has proved to be a powerful and convenient labelling technique for a wide range of compounds prepared by our group. As an early example, [^{3}H]ezetamibe (**13**) was prepared using Crabtree's catalyst and tritium gas at a specific activity of 28 Ci/mmol.[13,14] ^{3}H nmr analysis confirmed that the tritium was exclusively located in the ortho positions in the ring attached to the β-lactam nitrogen.

In addition to Crabtree's catalyst, the more active Ir (COD) dppe PF_6 catalyst has been synthesized and employed to label [^{3}H]Sch 430765 (**14**), which contains urea, a less effective directing group than amides and esters. Nevertheless, this more active catalyst gave product with a specific activity of 25 Ci/mmol.

The number of tritium-labelled compounds synthesized for new drug discovery continues to grow. Labelling methodology used by the group has continued to evolve and incorporate advances in tritium chemistry, such as the development of the iridium pentanedionate catalysts by Lockley and the preparation of '*in situ*' iridium catalysts by Ellames.[15–17] While hydrogen isotope exchange remains our primary focus,



Scheme 4



Scheme 5

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recent advances in iodination chemistry have led to the simple preparation of iodinated precursors, which are readily reduced with tritium gas and a catalyst.[18] We have made use of such methodology over recent months and will keep this method very much in mind for future projects.

High specific activity compounds

Tritium-labelled compounds for new drug discovery metabolism studies are typically prepared at modest specific activity. However, labelled compounds at high specific activity to support receptor binding studies have also been prepared. In the cases where the specific activity requirements can be met with tritium, these compounds are frequently prepared either via tritium gas reductions[6] or more commonly by iridium catalysed tritium gas exchange, such as in the case of [^{3}H]Sch 59171 (**15**), a benzophenone-containing photoaffinity probe, at a specific activity of >100 Ci/mmol.

^{3}H-methylations have also been extensively used by the group to prepare high specific activity ligands. Historically, these have been carried out by obtaining 80 Ci/mmol methyl iodide as a concentrated toluene solution as a custom preparation and reacting it with a suitable desmethyl precursor, such as in the case of [^{3}H]Sch 206272 (**16**) (Scheme 6), which employed the commercially available phase transfer catalyst 'Aliquat 336'.

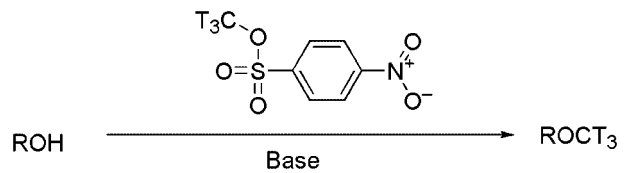
In 2003, Pounds reported on the development of [^{3}H]methyl nosylate as a radiochemical stable methylation reagent (Scheme 7).[19] [^{3}H]methyl nosylate is available at a specific activity of 80 Ci/mmol and does not have the volatility and instability issues of [^{3}H]methyl iodide. We now routinely keep an inventory of ^{3}H-methyl nosylate, and have found that even after a year's storage at −80°C, the material can be easily purified on silica and used in methylation reactions.

In addition to tritium, compounds labelled with other isotopes have been prepared by the group for discovery support. In the mid-1990s, several ^{125}I-labelled compounds were prepared, with the iodination steps contracted out to NEN. Following the development of the [^{35}S]methane sulphonic acid chemistry by Dean at Merck,[20] the group prepared its first ^{35}S-labelled methyl sulphonamide, [^{35}S]Sch 225336 (**17**) in 2001[5] (Scheme 8). Over the subsequent years, the preparation of [^{35}S]methane sulphonamides has increased substantially to the point that the group no longer works with ^{125}I.

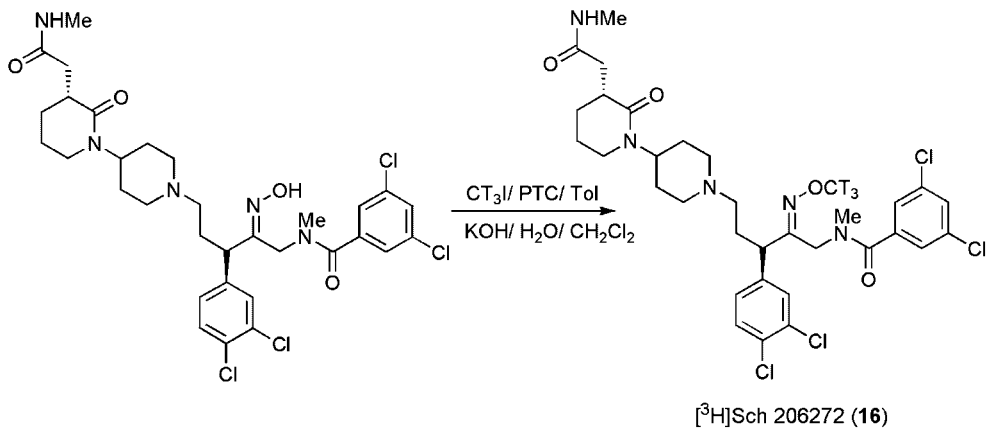
Preparation of stable isotope-labelled compounds

There was little demand for stable isotope-labelled compounds before 1996. In that year, [$^{13}C_3$]ribavirin (**18**) was synthesized (Scheme 9) as an internal standard for a quantitative bioanalytical LCMS/MS assay for ribavirin in biological fluids. In 1997, an absolute bioavailability study of ribavirin capsules was conducted using [$^{13}C_3$]ribavirin as an IV dose and [$^{13}C_6$]ribavirin as the internal standard.[21]

In 1998, to support the development of desloratadine (Clarinex®), a [D_4]desloratadine was prepared as an



Scheme 7



[^{3}H]Sch 206272 (**16**)

Scheme 6

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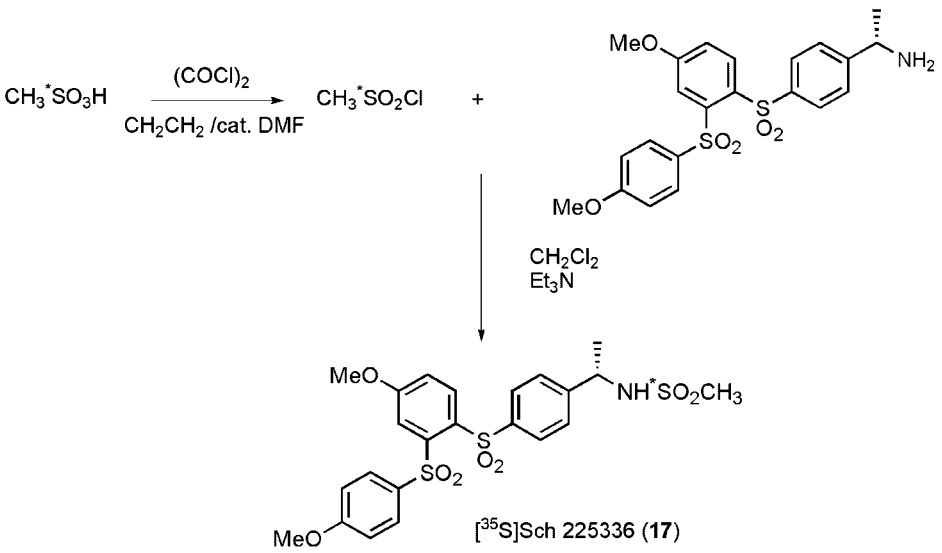
internal standard for the bioanalytical LCMS/MS assay. The key step in the synthesis of [D_4]desloratadine (**19**) involved preparation of D_4-*N*-benzyl-4-hydroxypiperidine from [D_2]formaldehyde via a Mannich-type cyclization (Scheme 10).[22]

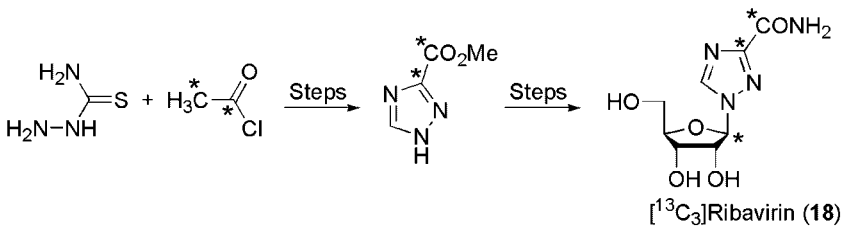
Sensitive LC/MS/MS bioanalytical assays are currently being developed for all compounds entering preclinical and clinical development. Radiochemistry started to synthesize stable isotope-labelled internal standards for all new compounds entering development and for some older compounds in the life cycle management stage. Our goal is to increase the molecular weight by at least three, using ^{2}H, ^{13}C and/or ^{15}N. Metabolic stability of the label is not a concern as it is for ^{3}H and ^{14}C compounds prepared for use in ADME studies.

$^{13}CD_3I$ has proved to be a useful reagent for preparing stable isotope internal standards. In one example, [$^{13}CD_3$]Sch 351125 (**20**) was prepared via the Grignard prepared from the labelled methyl iodide (Scheme 11).

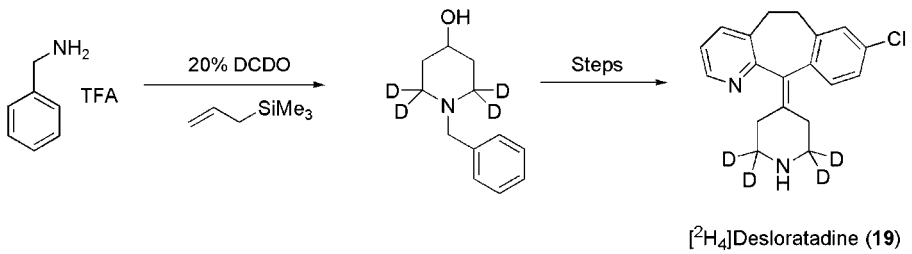
[$^{13}C_2^{15}N$]Lonafarnib[10] (**21**) is an example of an IS containing different isotopes derived from multiple labelled precursors, the labels being derived from



Scheme 8



Scheme 9



Scheme 10

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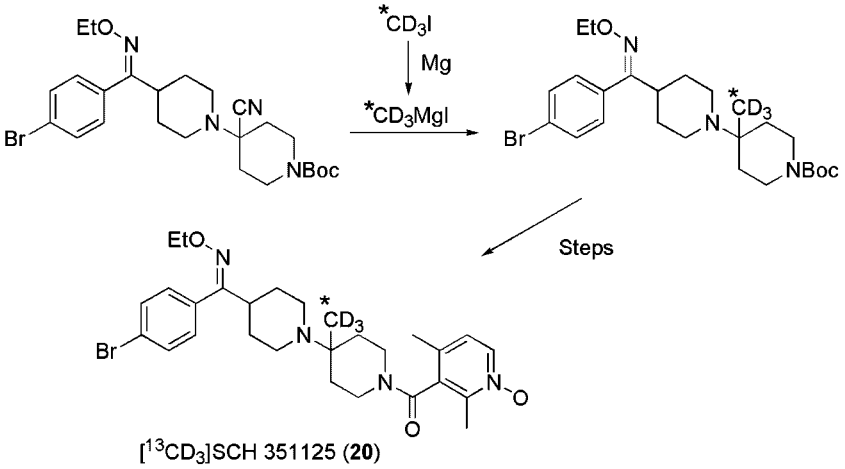
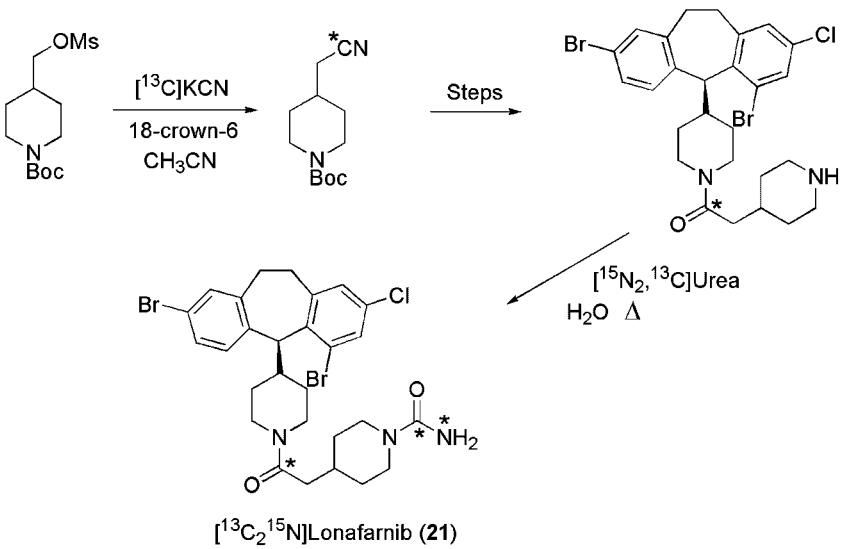
$K^{13}CN$ and $[^{15}N_2{}^{13}C]$urea. Key synthetic steps are shown in Scheme 12.

Aromatic rings uniformly labelled with ^{13}C are an attractive isotope source. An example from our labs is the synthesis of $[^{13}C_6]$Sch 414319[5] (**22**) from $[^{13}C_6]$fluorobenzene shown in Scheme 13. A majority of drugs contain aromatic rings, but limited access to uniformly labelled rings with more complex substitution patterns limits this approach.

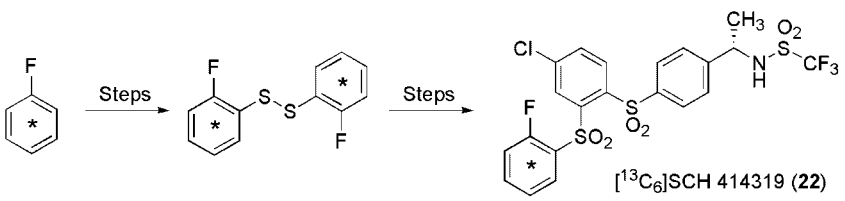
With the increasingly efficient HPLC/UPLC technology, the S-P Bioanalytical group and other groups have noted separation of highly deuterated internal stan-



Scheme 11



Scheme 12



Scheme 13

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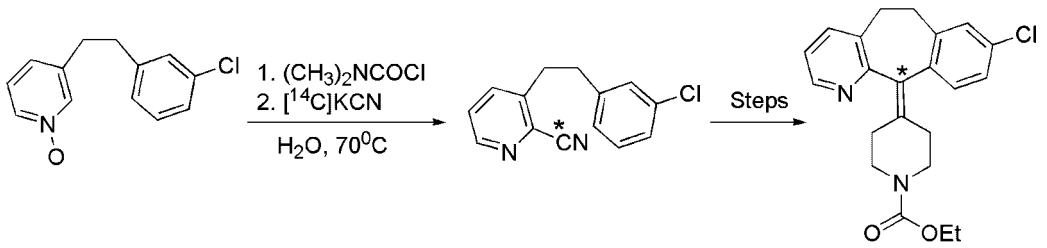
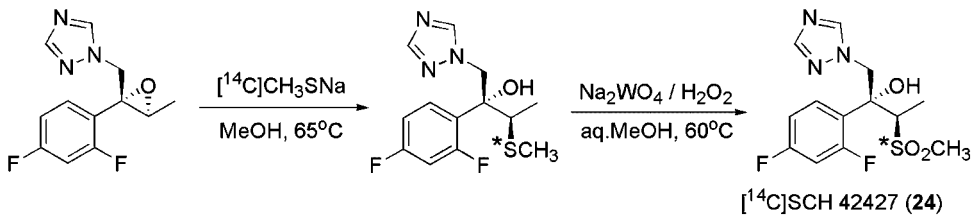
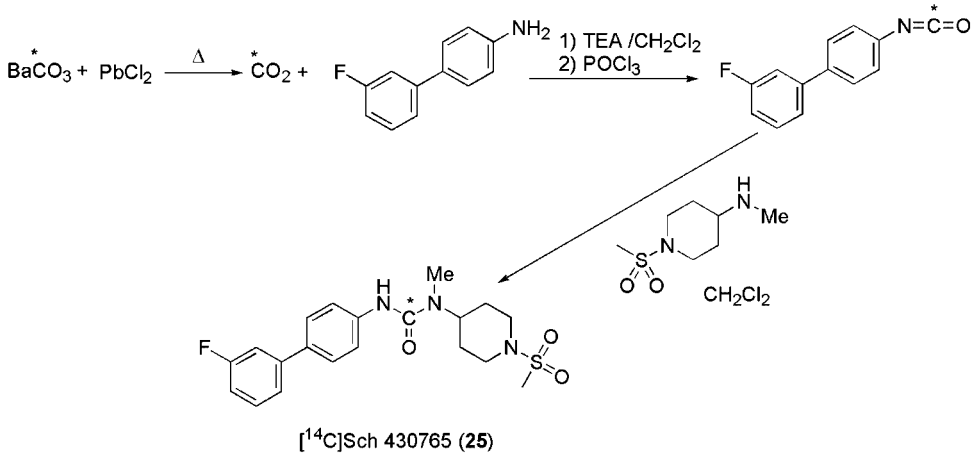


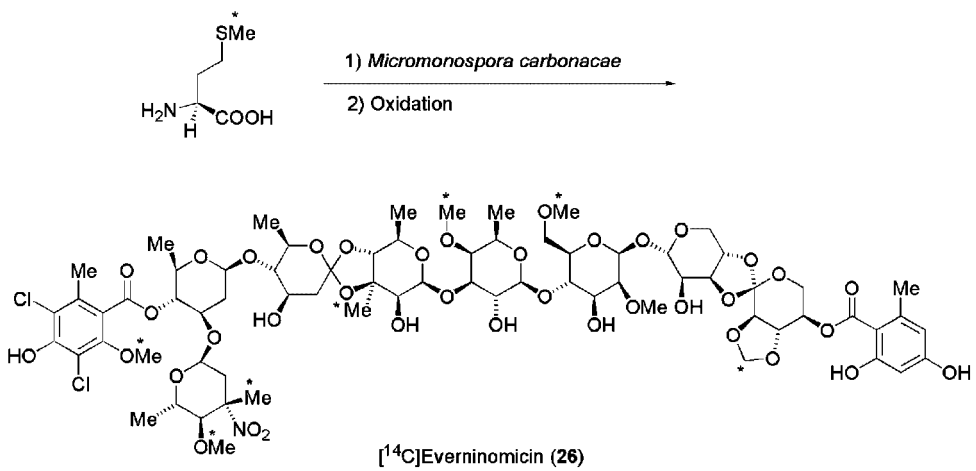
Scheme 14



Scheme 15



Scheme 16



Scheme 17

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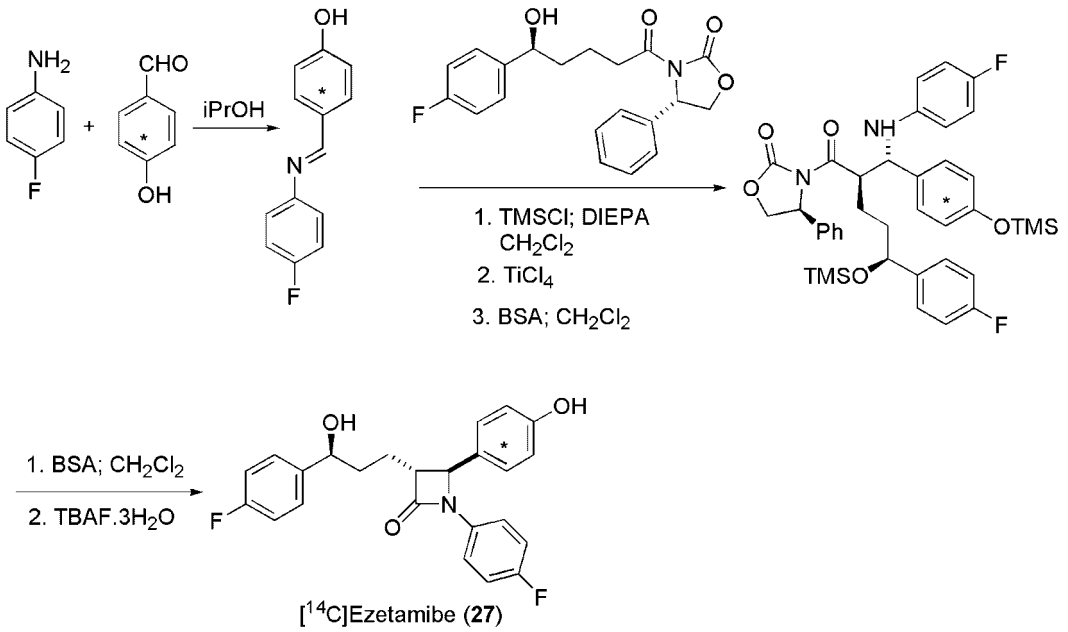
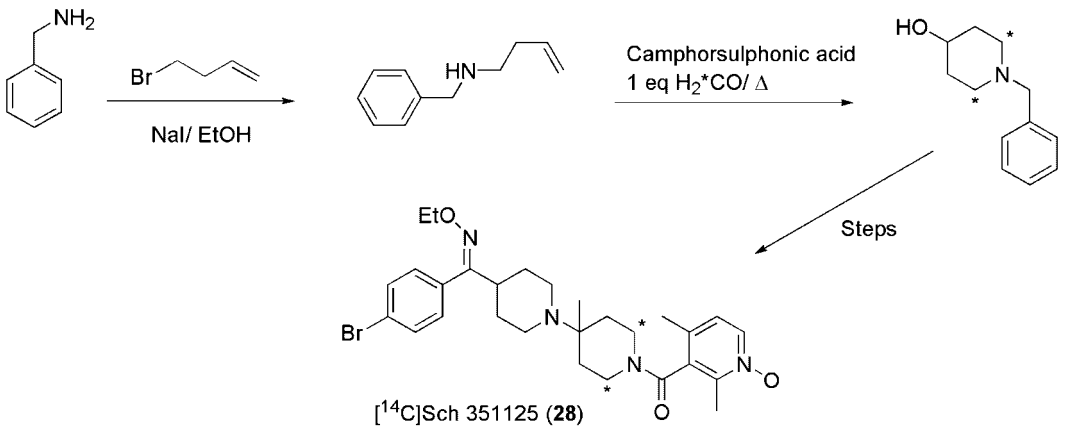


Scheme 18



Scheme 19

dards from the unlabelled analyte, which can affect the quality of the assay.[23] As a result, the group is avoiding the use of deuterium when preparing internal standards. The goal is to prepare compounds containing only ^{13}C and ^{15}N, with an enrichment of 3–4 mass units or higher.

>^{14}C-labelled compounds

As noted in the introduction, most early ^{14}C synthesis were contracted out; development of the synthesis was carried out 'in house' with unlabelled reagents. During the 1980s, the group increased in size and capability and more ^{14}C synthesis was done internally. By 1989, all ^{14}C synthesis was carried out internally and, except for ^{3}H-Sch 32088, all development compounds were now labelled with ^{14}C.

Advances in ^{14}C synthesis parallel advances in conventional organic synthesis methods. The major differences are (i) the restricted range and cost of ^{14}C starting materials and (ii) the rigorous additional safety requirements for manipulating radioactive compounds. Synthetic routes are often adapted from the routes developed by Medicinal Chemistry or Chemical Development. The label is incorporated as late in the synthesis as possible and it must be in a metabolically stable site. A few representative examples of ^{14}C syntheses carried out by S-P Radiochemistry follow.

The synthesis of [^{14}C]loratadine (Claritin®) (**23**) is an early example using [^{14}C]KCN as the isotope source as

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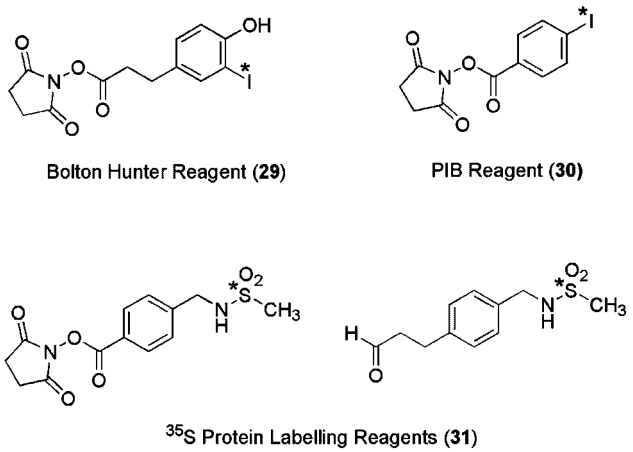

Figure 2 [^{125}I]-and [^{35}S]protein labeling reagents.

shown in Scheme 14. We use [^{14}C]KCN frequently in ^{14}C synthesis.

The 1991 synthesis of [^{14}C]Sch 42427[24] (**24**) (Scheme 15) is an example of a short synthesis where stereochemical control was important. Chiral HPLC was used for the first time in Radiochemistry for this synthesis.

Synthesis of [^{14}C]Sch 430765 (**25**) (Scheme 16) is an example using [^{14}C]CO_2 as starting compound. We use the improved Merck[25] lead chloride/ barium carbonate thermal generation methodology for generating [^{14}C]CO_2.

The development of the natural product everninomicin (**26**) in the mid-1980s was our first opportunity to prepare a labelled compound by fermentation (Scheme 17).[26] Based on fermentation experiments with [^{13}C]acetate and [^{13}C-S-methyl]methionine, radiolabelled methionine was used as a precursor. Both ^{3}H- and ^{14}C-labelled everninomicin were prepared with [^{3}H-S-methyl]methionine and [^{14}C-S-methyl]methionine as the labelled feedstocks. Stability of radiolabel experiments gave complete recovery of the ^{14}C dose, but incomplete ^{3}H dose recovery. [^{14}C]Everninomicin, although more technically challenging to prepare, was used for further metabolism studies. S-P discontinued natural products research and Radiochemistry has not prepared any other labelled compounds by fermentation.

^{14}C-labelled aromatics are the frequently used starting materials. An example is [^{14}C]ezetamibe (Zetia®) (**27**).[13] The label is introduced and two chiral centers set in the key aldol chiral reaction (Scheme 18).

4-Substituted piperidines are common fragments of drug molecules and attractive sites for labelling. We developed an efficient synthesis of *N*-Boc-4-hydroxy[^{14}C]piperidine from 1 to 2% aqueous ^{14}C-formaldehyde and have used this in the preparation of many labelled compounds, including [^{14}C]Sch 351125 (**28**) (Scheme 19).[27]

Labelled proteins

S-P was involved in protein therapeutics very early, with an approval for Intron® (interferon$_{\text{á2b}}$) in 1986. Our first attempt at preparing isotopically labelled interferon$_{\text{á2b}}$, in 1987, also brought us to the National Tritium Labelling Facility for the first time. We attempted thermal atom excitation labelling[28,29] and were not successful. We also tried an iodination/ tritium gas reduction sequence, also without success. [^{125}I]interferon was ultimately prepared at NEN using the ^{125}ICl method.

Our approach to iodinated proteins changed in 1995. Our interest was in assessing the tissue distribution of interferon and pegylated interferons. Iodination methods that label tyrosine and histidine residues, such as ^{125}ICl and Na^{125}I/chloramines-T, give products in which the label is lost rather easily *in vivo*. Conjugation reagents, which react with amino groups in proteins, give more stable labels *in vivo*. We labelled IFN using the commercially available Bolton–Hunter (**29**) and PIB reagents (**30**) and found the PIB reagent gave iodinated IFN with good label stability *in vivo*.[30] In 2004, we developed two ^{35}S conjugation reagents (**31**) and have successfully labelled over ten monoclonal antibodies and other proteins[31] (Figure 2).

Conclusion and the future

Since the early 1970s, the demand for isotopically labelled compounds at S-P has increased steadily. ^{3}H compounds are used extensively in discovery pro-

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grams. At S-P, ^{35}S has replaced ^{125}I when very high specific activity compounds are needed. The one area where isotope use is quickly decreasing is in high throughput screening programs because radiometric assays do not adapt well to the 386 and 1536-well plate format.

Compounds moving into development are routinely synthesized with both stable isotopes and ^{14}C. In addition to using internal standards in bioanalytical assays, stable isotope-labelled compounds are now used to aid metabolite identification,[32] a use that is increasing. Microdosing, made possible by accelerator mass spectroscopy detection,[32] will increase the need for ^{14}C compounds in early clinical research. S-P has microdose studies in the planning stage. Positron-emission tomography is increasing in importance in the clinical evaluation of new molecules and is dependent on the supply of new radiotracers.[33] At S-P, radiochemists are members of the teams that oversee these studies at dedicated PET centers. The future of labelled compound synthesis and application at Schering-Plough is bright.

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