SCIENTIFIC LETTER N°9

AtlanChim Pharma

Edito

Pour fêter les 12 ans d'AtlanChim Pharma, le **Professeur Jacques LEBRETON** a choisi de mettre en avant le marquage à froid dans sa 9ème lettre scientifique.

En plus de 12 ans de collaboration étroite avec ses clients, AtlanChim Pharma a su acquérir lors de la synthèse de plus de 200 composés marqués à froid (D, ^{13}C, ^{15}N et ^{34}S) une expertise forte. Les synthèses de composés marqués que nous ont confiées nos clients représentent pratiquement tous les types de structures organiques : des hétérocycles aux aminoacides chiraux en passant par les stéroïdes.

Ces synthèses allant de quelques étapes à plus de dix ont été réalisées avec succès à l'échelle du milligramme jusqu'à plusieurs grammes. Il est clair qu'à travers les nombreuses difficultés que nous avons rencontrées, surmontées ou contournées, nous avons accumulé un savoir-faire unique. Aujourd'hui, cette expertise nous permet de proposer à nos clients des solutions adaptées pour répondre à leur besoin en molécules marquées à froid, au-delà des molécules marquées standards des catalogues.

L'expertise de nos chimistes, présents pour certains depuis l'origine, s'enrichit chaque jour aussi bien à travers nos travaux dans le domaine du marquage, qu'à travers notre activité de synthèse à façon « classique » (plus de 1200 contrats en tout depuis 2004 !). La lecture régulière des travaux récents publiés dans la littérature dans ce domaine, ente autre, reste aussi pour notre équipe un atout essentiel et un travail de veille scientifique incontournable. Dans ce cadre, cette nouvelle lettre scientifique nous fait partager les récents travaux de nos collègues industriels et universitaires dans le domaine de la synthèse de produits marqués à froid.

Avant que vous ne plongiez dans la lecture de cette lettre scientifique, voici quelques nouveautés que nous voulions partager avec vous.

Notre groupe Atlanta a choisi de mettre en place les Bonnes Pratiques de Laboratoire, sur les études précliniques pour Atlantic Bone Screen et sur les services analytiques (validation de méthodes) pour Atlanchim Pharma. Cela vient compléter le système déjà en place assurant la maîtrise et la traçabilité de nos processus.

De plus, afin de compléter notre offre de service nous avons fait l'acquisition d'un système de purification automatisé avec détection UV (flash et semi-prep) et d'une U-HPLC couplée avec un détecteur UV et un détecteur d'aérosols chargés permettant de mesurer des molécules qui ne sont pas UV visible.

Enfin vous trouverez sur le modèle des lettres scientifiques précédentes, les portraits de deux membres de notre équipe : le Dr. Maud ANTOINE, notre chef de projet en charge du pôle analytique et le Dr. Kévin FOURMY qui a rejoint notre équipe cette année.

Bonne lecture !

Editorial

AtlanChim Pharma is celebrating its 12th birthday! For this special event, **Professor Jacques LEBRETON** has chosen to highlight (site specific) stable isotope labeling in his 9th scientific letter.

After 12 years of close collaboration with its customers, AtlanChim Pharma has synthesized more than 200 stable isotope labeled compounds (D, ^{13}C, ^{15}N et ^{34}S) and has thus gained a strong expertise in that field. From heterocycle to chiral amino acids through steroids, the labeled compounds that we have synthetized for our customers represent most of all types of organic structures.

These multi-step synthesis (from one step up to more than ten steps) have been successfully performed at lab-scale – from mg up to several gr scale. It is clear that throughout all the difficulties we have encountered, overcome or circumvented, we have accumulated a unique know-how. Therefore we can offer tailor made solutions beyond the standard molecules that can be found in catalogs.

With more than 1200 projects handled since 2004, the expertise of our chemists, some of them being part of the company since its creation, is constantly enriched throughout their work in the stable isotope labeling field as well as their experience in more classical synthesis. Regular reading of recently published studies is also a major asset and an essential scientific monitoring work. In this context, this new scientific letter makes us share the recent work of our industrial and academic colleagues in the field of stable isotope labeling.

Before you start reading this scientific letter, here are some news we wanted to share with you.

Our Atlanta group has chosen to implement Good Laboratory Practices on preclinical studies for Atlantic Bone Screen and on analytical method validation for AtlanChim Pharma. This complements the existing system ensuring traceability and control of our processes.

In addition, we acquired an automated purification system with UV detection (flash and semi-prep) and to complete our analytical services, a U-HPLC coupled with UV detector and charged aerosol detection to analyze molecules which are not UV visible.

Finally, you will find, like you did in our previous scientific letters, the portrait of two members of our team: Dr. Maud ANTOINE, our analytical specialist and Dr. Kevin FOURMY who joined us at the beginning of this year.

Happy reading everyone !

A JOURNEY TO THE SYNTHESIS OF ISOTOPICALLY STABLE LABELED COMPOUNDS*

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**This microreview is dedicated to my friend and colleague, Dr. Fabrice Dénès, on the occasion of his 40th birthday*

Over recent decades, the development of powerful analytical technology, such as high-performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS), has clearly increased the demand for isotopically stable labeled compounds.[1] These serve as accurate, precise and reliable internal reference standards for the identification and quantification of compounds of interest in drug development, particularly during clinical trials, pollutants in environmental systems, food analysis, etc. In this context, the internal standard should be a pure labeled analogue of the analyte, with the highest isotopic enrichment containing at least three more mass units to avoid a significant overlap of the respective mass signals due to the natural abundance of heavy isotopes. While isotopically labeled compounds appear as gold standards for the sensitive and precise quantification of traces in complex matrices, they are also precious research tools in the life sciences to study biosynthetic pathways, diagnose a disease, perform metabolic studies, etc. In addition, combined with high resolution liquid or solid-state NMR spectroscopy, the site-specific labeled molecules with stable isotopes such as ^{13}C, D, and ^{15}N are very useful probes to study the interactions of biomacromolecules, such as DNA, RNA oligosaccharides and proteins, with ligands, drugs or even other biomacromolecules. Moreover, NMR spectroscopy studies with site-specific labeled biomacromolecules can provide information on their conformations in solution. More recently, deuterated medicines have been identified as a new branch of Medicinal Chemistry to develop novel, highly differentiated drugs. The different points mentioned above will be illustrated through the synthesis of various site-specific labeled molecules. Despite the growing importance of isotopically stable labeled compounds, their preparation is challenging due not only to the poor commercial availability of the required labeled starting materials but also to their high price when they are available.[2]

The aim of this microreview is to provide the non-specialist reader with an overview of the current strategies most commonly used to prepare labeled compounds. It is organized according to the strategy used to introduce the stable isotope into the required labeled molecule.

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[1] (a) For a recent example, see: J. C. Precht, B. Ganchev, G. Heinkele, H. Brauch, M. Schwab, T. E. Mürdter, *Anal. Bioanal. Chem.* **2012**, 403, 301-308. (b) For a review, see: M. Jemal, Y. Q. Xia, *Curr. Drug Metab.* **2006**, *7*, 491-502.

[2] (a) For an excellent book on this topic, see: J. R. Hanson, The Organic Chemistry of Isotopic Labeling; The Royal Society of Chemistry: Cambridge, 2011. (b) R. A. Martinez, D. R. Glass, E. G. Ortiz, M. A. Alvarez, E. Juarez, S. N. Lodwig, C. J. Unkefer, *J. Label Compd. Radiopharm.* **2013**, *56*, 31-35 and cited literature.

Deuterium-hydrogen exchange

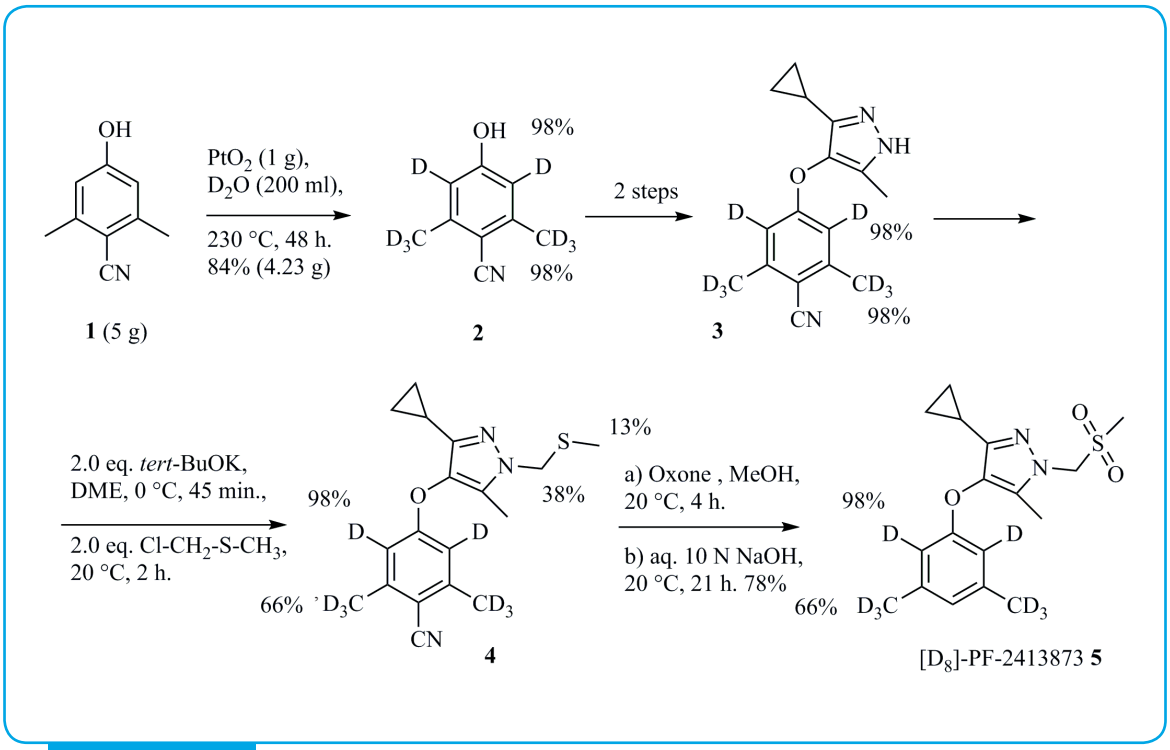
Compared to conventional synthetic approaches, post-synthetic H/D exchange is an easy and economical methodology to prepare multi-deuterated compounds. Since the pioneering work of Sajiki,[3] many deuterated compounds have been prepared by H/D exchange catalyzed by transition metals, mainly palladium and platinum, with D_2O as the deuterium source, under a hydrogen atmosphere.[4] To avoid handling gaseous hydrogen, Derdau and Atzrodt[5] significantly improved this methodology by using deuteride donors, such as $NaBD_4$, for pre-activation of the catalyst.

To support clinical trials of PF-2413873, a non-steroidal progesterone receptor antagonist for the treatment of secondary dysmenorrhea, the deuterated PF-2413873 **5** was prepared for use as a bioanalytical standard as outlined in Scheme **1**. The H/D exchange reaction of 4-cyano-3,5-dimethylphenol **1** using PtO_2 as catalyst in D_2O in a stainless steel autoclave at 230°C for 48 h provided the desired isotopomer **2** in good yield (84%, 4.23 g) with 98% deuterium incorporation. Two steps later, the intermediate **3** was treated with chloromethyl methylsulfide in the presence of potassium *tert*-butoxide to give the desired adduct **4** with some gain of deuterium in the sulfide side chain and concomitant significant loss of deuterium from the benzylic positions. After oxidation of the sulfide **4** with Oxone®, the acidic hydrogen atoms of the corresponding sulfone could be efficiently exchanged by treatment with excess sodium hydroxide in methanol to furnish partially deuterated PF-2413873 **5**. The reason for this loss of deuterium atoms was clearly identified and thus for the alkylation step a fully deuterated chloromethyl methylsulfide was used. Then, following the previous sequence, deuterated [D_8]-PF-2413873 **5** was obtained with correct deuterium incorporation.

[3] H. Esaki, N. Ito, S. Sakai, T. Maegawa, Y. Monguchi, H. Sajiki, *Tetrahedron* **2006**, *62*, 10954-10961 and cited literature.

[4] For excellent reviews in this field, see: (a) J. Atzrodt, V. Derdau, T. Fey, J. Zimmermann, *Angew. Chem. Int. Ed.* **2007**, *46, 7744-7765.* (b) N. Ito, T. Watahiki, T. Maesawa, T. Maegawa, H. Sajiki, *Synthesis* **2008**, 1467-1478. (c) W. J. S. Lockley, J. R. Heys, *J. Label Compd. Radiopharm.* **2010**, *53*, 635-644. (d) Y. Sawama, Y. Monguchi, H. Sajiki, *Synlett* **2012**, 959-972.

[5] V. Derdau, J. Atzrodt, *Synlett* **2006**, 1918-1922.

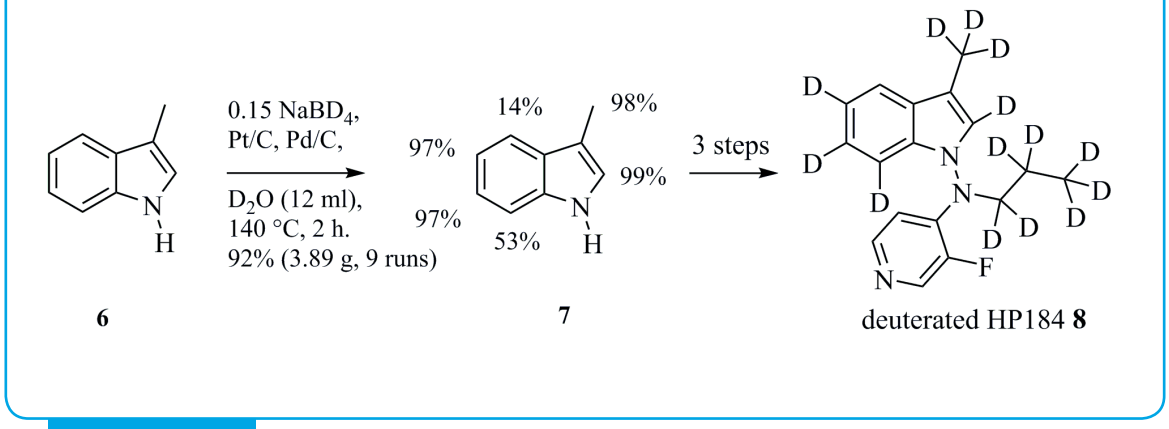


SCHEME 1

It is important to keep in mind that during the synthetic processes and/or analytical procedures back exchange occurs and deuterium atoms can be replaced by hydrogen atoms, affording a complex mixture of deuterated isotopomers: the more easily a deuterium atom can be introduced, the more easily it can be lost!

In the course of a complete study on the effects of deuteration on the metabolism and clearance of Nerispirdine (HP184) used for the treatment of multiple sclerosis, Schofield and Coll. described the preparation of deuterated HP184 **8**, as presented in Scheme 2.[6] To start the synthesis, commercially available 3-methylindole **6** was treated with $NaBD_4$ in D_2O using the synergistic effect of the palladium/C and platinum/C catalyst system in a sealed tube at 140°C for 2 h. Eight similar reaction runs were combined to give the desired deuterated 3-methylindole **7** in 92% yield (3.89 g). It should be pointed that positions 4 and 7 are resistant to exchange. From this latter material **7**, deuterated HP184 **8** was isolated in 28% overall yield following a known three-step sequence including an *N*-alkylation with commercially available fully deuterated *n*-1-bromopropane under microwave irradiation.

[6] J. Schofield, V. Derdau, J. Atzrodt, P. Zane, Z. Guo, R. van Horn, V. Czepczor, A. Stoltz, M. Pardon, *Bioorg. Med. Chem.* **2015**, *23*, 3831-3842.



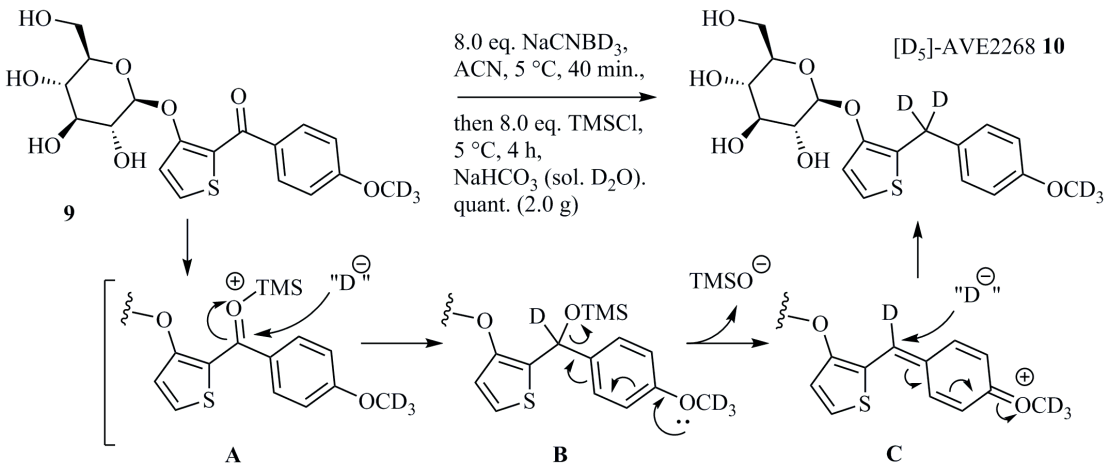
SCHEME 2

Deuterated hydrides

The incorporation of deuterium atoms can easily be performed by reducing various functional groups with inexpensive labeled hydrides, as presented in the following examples.

During their *in vivo* evaluation of sodium-glucose co-transporter 2 (SGLT2) inhibitors, such as AVE2268 for the treatment of type 2 diabetes mellitus (T2DM), Derdau and Coll. synthesized isotopically stable labeled [D_5]-AVE2268 **10** as described in Scheme 3.[7] As a key step, they used a methodology developed by Meleties and Box[8] for deoxygenation of aromatic aldehydes and ketones by reduction with $NaBH_3CN$ in combination with the mild electrophile TMSCl. This methodology was applied to the reduction of arylketone **9** with $NaBD_3CN$ to afford the desired deuterated compound **10** in quantitative crude yield. A proposed mechanism for this deoxygenation process is outlined in Scheme 3. Activation of the carbonyl group by TMSCl as silylated oxonium **A** followed by transfer of a hydride gave the corresponding silylether **B** intermediate. In some cases, the secondary alcohol side-products were isolated from desilylation of the parent silylether intermediate, such as **B**, during the aqueous work-up. Finally, the deoxygenated product was formed by a second hydride transfer on the intermediate **C**, which was provided by elimination of $TMSO^{\ominus}$ assisted by the methoxy group at the *para* position.

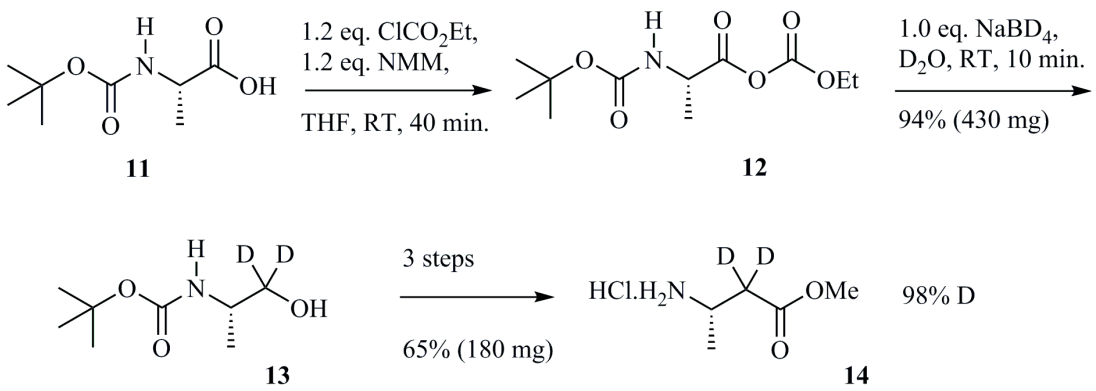
[7] V. Derdau, T. Fey, J. Atzrodt, *Tetrahedron* **2010**, *66*, 1472-1482.
[8] V. G. S. Box, P. Meleties, *Tetrahedron Lett.* **1998**, *39*, 7059-7062.



SCHEME 3

Non-proteinogenic ß-amino acids (one-carbon homologues of α-amino acids) have emerged as very promising tools in various fields of Medicinal Chemistry. Moreover, they often exhibit remarkable biological activity. ß -Amino acids are also present in important pharmaceuticals, such as the antitumor agent Taxol® and the antifungal jasplakinolide.[9] They can be incorporated into ß-peptide chains to induce new secondary and tertiary structures, thus bringing new biological properties. Deuterium labeling of proteins and peptides has been used successfully in quantitative proteomics analysis. In this context, 2,2-dideutero ß-amino ester **14** was prepared from (L)-*N*-Boc-alanine **11** as depicted in Scheme 4.[10] Activation of the carboxylic function of compound **11** as its corresponding mixed ethyl carbonic anhydride **12** formed *in situ* by treatment with ethyl chloroformate in the presence of *N*-methylmorpholine (NMM), followed by addition of a suspension of $NaBD_4$ in D_2O, gave the corresponding deuterated primary alcohol **13** in excellent yield. It is important to notice that such activation enabled a mild and chemioselective reducing agent to be used rather than the powerful $LiAlH_4$ used to reduce carboxylic acids directly into primary alcohols. Next, a homologation sequence was carried out by substitution of the corresponding iodide intermediate followed by nucleophilic displacement by cyanide and finally acid-catalyzed alcoholysis to give the corresponding protected 2,2-dideutero ß-amino ester **14**. Since the deuterium content percentage was maintained unchanged throughout this sequence, no exchange occurred even in the acid-catalyzed alcoholysis of the nitrile intermediate.

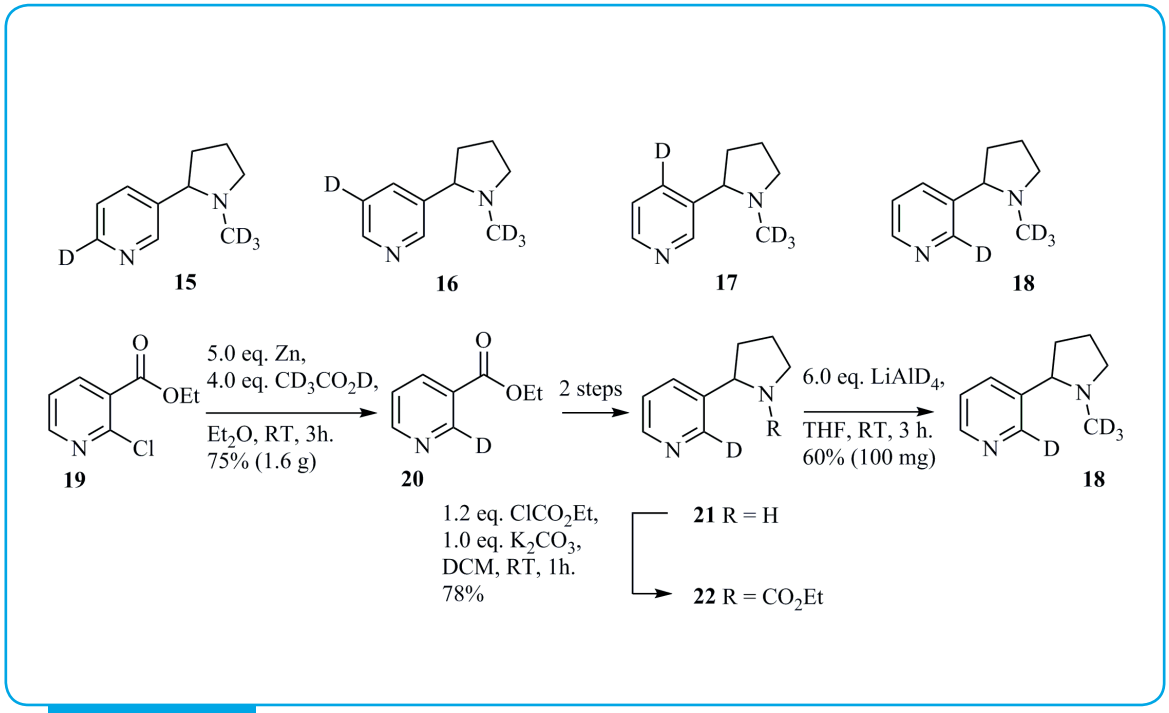
[9](a) P. W. Ford, K. R. Gustafson, T. C. McKee, N. Shigematsu, L. K. Maurizi, L. K. Pannell, D. E. Williams, E. D. de Silva, P. Lassota, T. M. Allen, R. Van Soest, R. J. Andersen, M. R. Boyd, *J. Am. Chem. Soc.* **1999**, *121*, 5899-5909. (b) M. Ege, K. T. Wanner, *Org. Lett.* **2004**, *6*, 3553-3556.
[10] A. Guaragna, S. Pedatella, V. Pinto, G. Palumbo, *Synthesis* **2006**, 4013-4016.



SCHEME 4

In our previous investigation to elucidate the mechanism of *N*-demethylation of nicotine in plants using the kinetic isotope effect, four racemic nicotine isotopomers **15-18**, doubly labeled with deuterium atoms, were synthesized, as presented in Scheme 5.[11] Our synthesis is highlighted by the preparation of [6-D-*N*-CD_3]nicotine **18**. Starting from commercially available ethyl 6-chloro nicotinate **19**, the corresponding 6-deuterated nicotinate derivative **20** was formed in 75% yield by deuterodehalogenation[12] using zinc metal and [D_4]-acetic acid in dry diethyl ether. Next, the deuterated material **20** was transformed into deuterated nornicotine **21** using an efficient one-pot procedure described by Jacob.[13] In the last stage, acylation of the latter compound **21** with ethyl chloroformate in the presence of K_2CO_3 afforded the corresponding *N*-ethylcarbamate **22** in 78% yield. This was then reduced with excess $LiAlD_4$ followed by hydrolysis of the reaction mixture with D_2O to give the expected racemic doubly-labeled nicotine **18** in 60% yield (100 mg) after purification by chromatography on silica gel. It is important to notice that for all isotopomers **15-18**, regioselective deuterium incorporation on the pyridine ring was up to 90% (90-93%) and *N*-CD_3 pyrrolidine deuteration ranged between 92 and 100%.

[11] W. Hatton, V. Silvestre, R. J. Robins, M. Mathé-Allainmat, J. Lebreton, *J. Label Compd. Radiopharm.* **2009**, *52*, 117-122.
[12] For recent work about deuterodehalogenation, see: C. S. Donald, T. A. Moss, G. M. Noonan, B. Roberts, E. C. Durham, *Tetrahedron Lett.* **2014**, *55*, 3305-3307 and cited literature.
[13] P. Jacob, *J. Org. Chem.* **1982**, *47*, 4165-4167.

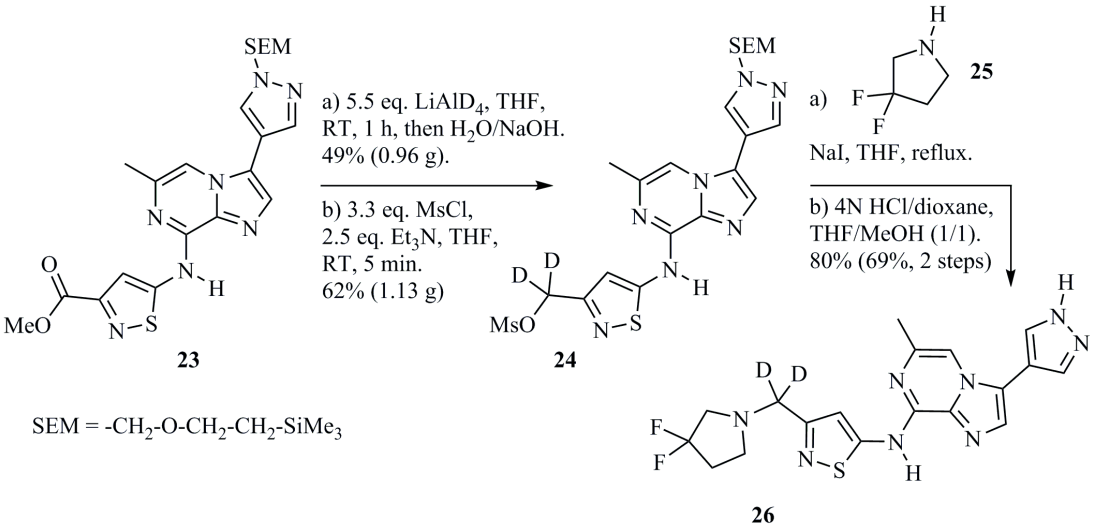


SCHEME 5

Aurora kinases play a crucial role in the cell cycle during mitosis, and are over-expressed in a variety of human tumors. In a complete study in this field, Kerekes and Coll. reported that the biological profile of the Aurora kinase inhibitor **26** was greatly improved, compared to its non-deuterated parent, by blocking oxidative metabolism at the benzylic position (see Scheme 6).[14] It is well-established that substitution of hydrogen atoms by deuterium atoms at metabolically labile sites often decreases the metabolism rate due to the kinetic isotope effect (C-D bond is stronger than C-H).[15] The preparation of compound **26** is outlined in Scheme 6. Treatment of methylester **23** with $LiAlD_4$ provided cleanly the corresponding deuterated primary alcohol which, after activation as mesylate intermediate **24** followed by nucleophilic displacement with 3,3-difluoropyrrolidine **25**, led to the deuterated target **26**, after cleavage of the SEM protecting group on the C-3 pyrazole.

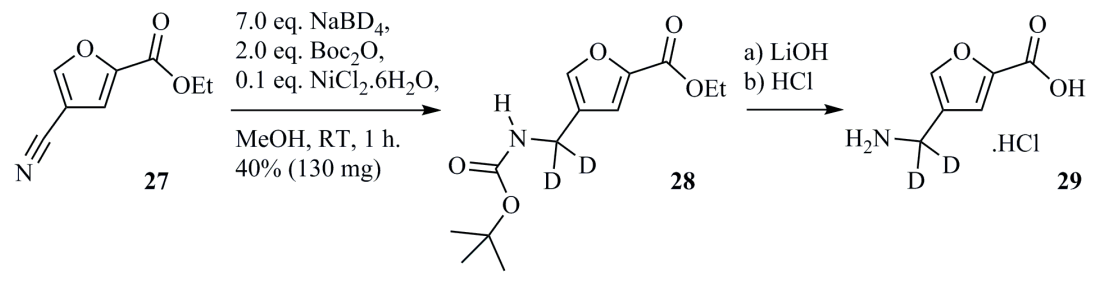
[14] A. D. Kerekes, S. J. Esposite, R. J. Doll, J. R. Tagat, T. Yu, Y. Xiao, Y. Zhang, D. B. Prelusky, S. Tevar, K. Gray, G. A. Terracina, S. Lee, J. Jones, M. Liu, A. D. Basso, E. B. Smith, *J. Med. Chem.* **2011**, *54*, 201-210.

[15] For an excellent review concerning the use of deuterium atoms in drug discovery, see: (a) T. G. Gant, *J. Med. Chem.* **2014**, *57*, 3595-3611. (b) For a recent example, see: R. Xu, M. Zhan, L. Peng, X. Pang, J. Yang, T. Zhang, H. Jiang, L. Zhao, Y. Chena, *J. Label Compd. Radiopharm.* **2015**, *58*, 308-312 and cited literature.



SCHEME 6

In the context of the development of new antiepileptic drugs, γ-amino butyrate aminotransferase (GABA-AT), which catalyzes the degradation of γ-amino butyrate (GABA) into succinic semialdehyde (SSA), is a valid target. Thus, much effort has been devoted to the synthesis and evaluation of various heteroaromatic compounds as potential substrates and inhibitors of GABA-AT. In a recent study in this field, a set of new heteroaromatic GABA analogues was evaluated and, to identify the rate-determining step for turnover, a kinetic isotope effect experiment was conducted using the deuterium-labeled inhibitor **29** (see Scheme 7).[16] The nitrile compound **27** was converted into the corresponding deuterated *N*-Boc amine **28** in modest yield by combining a large excess of $NaBD_4$ and 0.1 equivalent of $NiCl_2$, which is reduced *in situ* into NiB_2 acting as catalyst. It is worth noting that in this one-pot sequence, Boc_2O is necessary to trap the amine formed into the corresponding NH-Boc derivative **28** to prevent the well-known dimerization side-reaction leading to the symmetrical secondary amine.[17] The large amount of reducing agent is necessary for complete conversion presumably due to its instability in methanol. Removal of the protecting groups furnished the desired deuterium-labeled compound **29**.



SCHEME 7

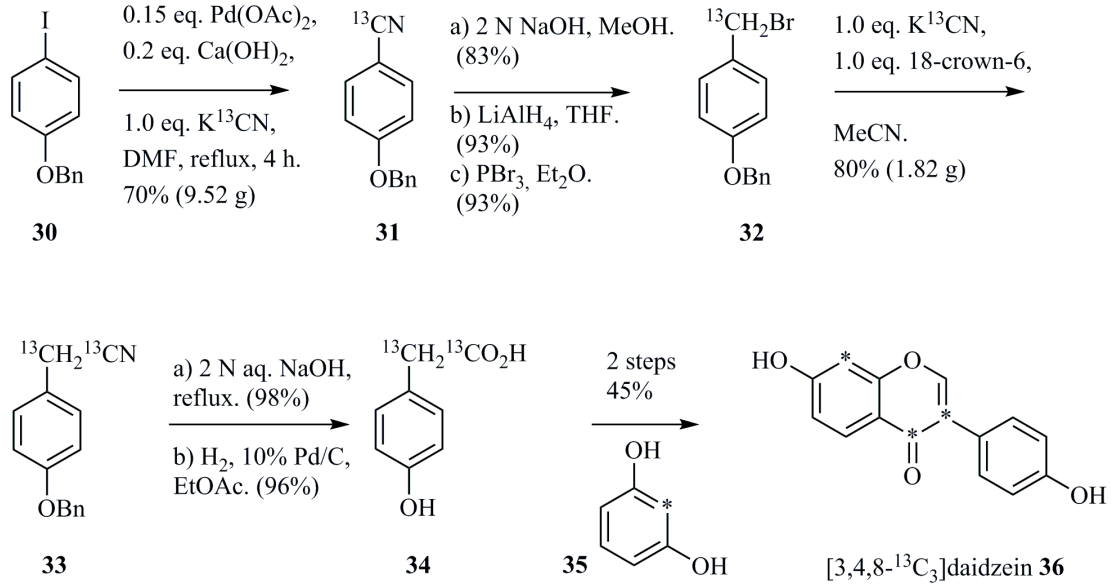
[16] D. D. Hawker, R. B. Silverman, *Bioorg. Med. Chem.* **2012**, *20*, 5763-5773.
[17] S. Caddick, A. K. de K. Haynes, D. B. Judd, M. R. V. Williams, *Tetrahedron Lett.* **2000**, *41*, 3513-3516.

Labeled potassium cyanide

Compounds containing a nitrile group are of significant interest in the field of Medicinal Chemistry through an increasing number of pharmaceutical products.[18] On the other hand, the nitrile group can also serve as a precursor of various other functional groups by simple and efficient well-known chemistry. The versatility of cyanide, as a one-carbon unit, in the preparation of isotopically labeled compounds is illustrated in the following examples involving palladium-catalyzed cross-coupling reactions or the Rosenmund-von Braun reaction from aryl halides, nucleophilic displacement or addition reactions. In this context, potassium cyanide is the most popular source of cyanide and is commercially available in three highly enriched isotopically labeled forms: $K^{13}CN$ (1 g/250 $), $KC^{15}N$ (1 g/1200 $) and $K^{13}C^{15}N$ (1 g/700 $).

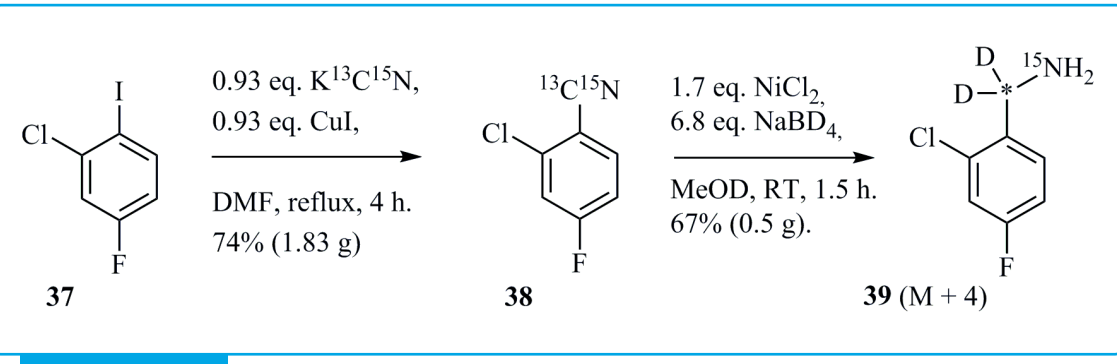
In recent years, many studies have been conducted to understand and evaluate the biological effects of dietary phytoestrogens and their impact on human health. Various analytical methods have been employed using an internal isotopically labeled standard to quantify the levels of phytoestrogens found in food and biological fluids. In this context, Botting and Coll. reported the first synthesis of multiple ^{13}C-labeled [3,4,8-$^{13}C3$]daidzein **36** as depicted in Scheme 8.[19] Palladium-catalyzed cyanation has attracted widespread interest. However, since potassium cyanide is the only available source of labeled cyanide, it is well-established that its anion inhibits the catalytic cycle by formation of unreactive palladium(II) cyano species, which cannot be reduced to catalytically active palladium(0) species. Nevertheless, co-catalysts like potassium hydroxide, sodium ethoxide, potassium carbonate, or calcium hydroxide facilitate the reduction of palladium(II) species. It is also interesting to note that palladium(II) acetate can serve as the catalyst without additional ligands. From 4-benzyloxyiodobenzene **30**, under optimized cyanation conditions using $K^{13}CN$ and a palladium(II) acetate catalyst in refluxing DMF containing calcium hydroxide, the ^{13}C-nitrile **31** was isolated in 70% yield (9.52 g). Following a classic three-step sequence, the latter benzonitrile derivative **31** was converted into benzyl bromide **32**, which was engaged, without purification due to its instability, in a nucleophilic substitution with $K^{13}CN$ in acetonitrile containing 18-crown-6 to give the desired M+2 labeled benzyl nitrile intermediate **33** in 80% yield (1.82 g) after purification on silica gel chromatography. Then, from this latter compound **33**, basic hydrolysis to the corresponding carboxylic acid followed by hydrogenolysis to cleave the benzyl ether furnished the precursor **34**, which by condensation with [2-^{13}C]resorcinol **35** (see Scheme 18 for its preparation) finally provided the target [3,4,8-$^{13}C3$]daidzein **36**.

[18] F. F. Fleming, L. Yao, P. C. Ravikumar, L. Funk, B. C. Shook, *J. Med. Chem.* **2010**, *53*, 7902-7917.
[19] M. F. Oldfield, L. Chen, N. P. Botting, *Tetrahedron* **2004**, *60*, 1887-1893.



SCHEME 8

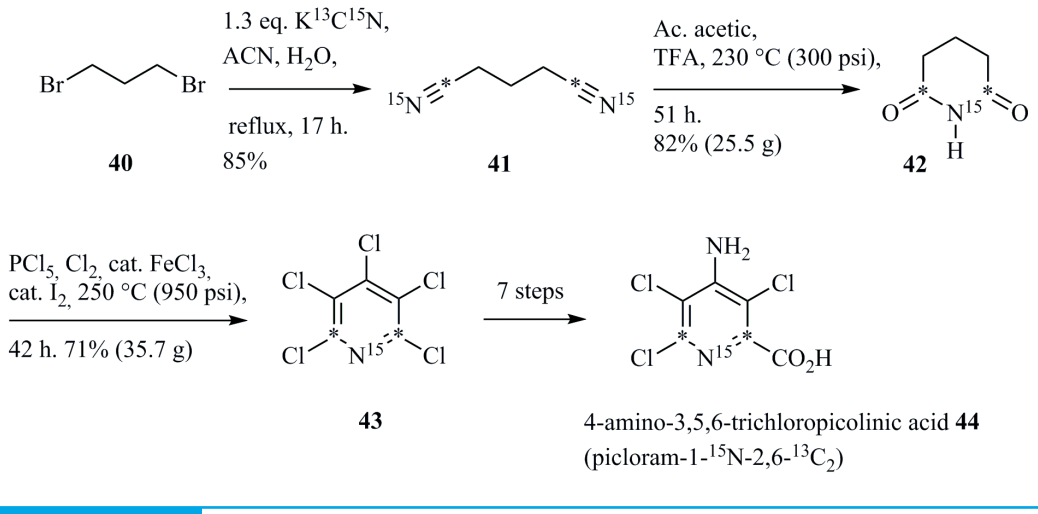
Alternatively, cyanation of aryl halides can be conducted with stoichiometric amounts of copper(I) cyanide using the Rosenmund-von Braun reaction as discussed in this next example. During the first kilogram-scale development of PF-03463275 at Pfizer, a GlyT1 inhibitor for the treatment of schizophrenia, the preparation of the M+4 isotopically labeled compound **39** as an internal standard for quantitative bioanalytical studies using mass spectrometry was achieved as presented in Scheme 9.[20] The key M+4 isotopically labeled benzylamine **39** was efficiently prepared from aryl halide **37** with stoichiometric amounts of copper(I) cyanide, formed *in situ* from $K^{13}C^{15}N$ and CuI as cyanating agent followed by reduction of the nitrile intermediate **38** with $NaBD_4$ in the presence of $NiCl_2$ (*vide supra*).



SCHEME 9

[20] M. A. Berliner, S. P. A. Dubant, T. Makowski, K. Ng, B. Sitter, C. Wager, Y. Zhang, *Org. Process Res. Dev.* **2011**, *15*, 1052-1062.

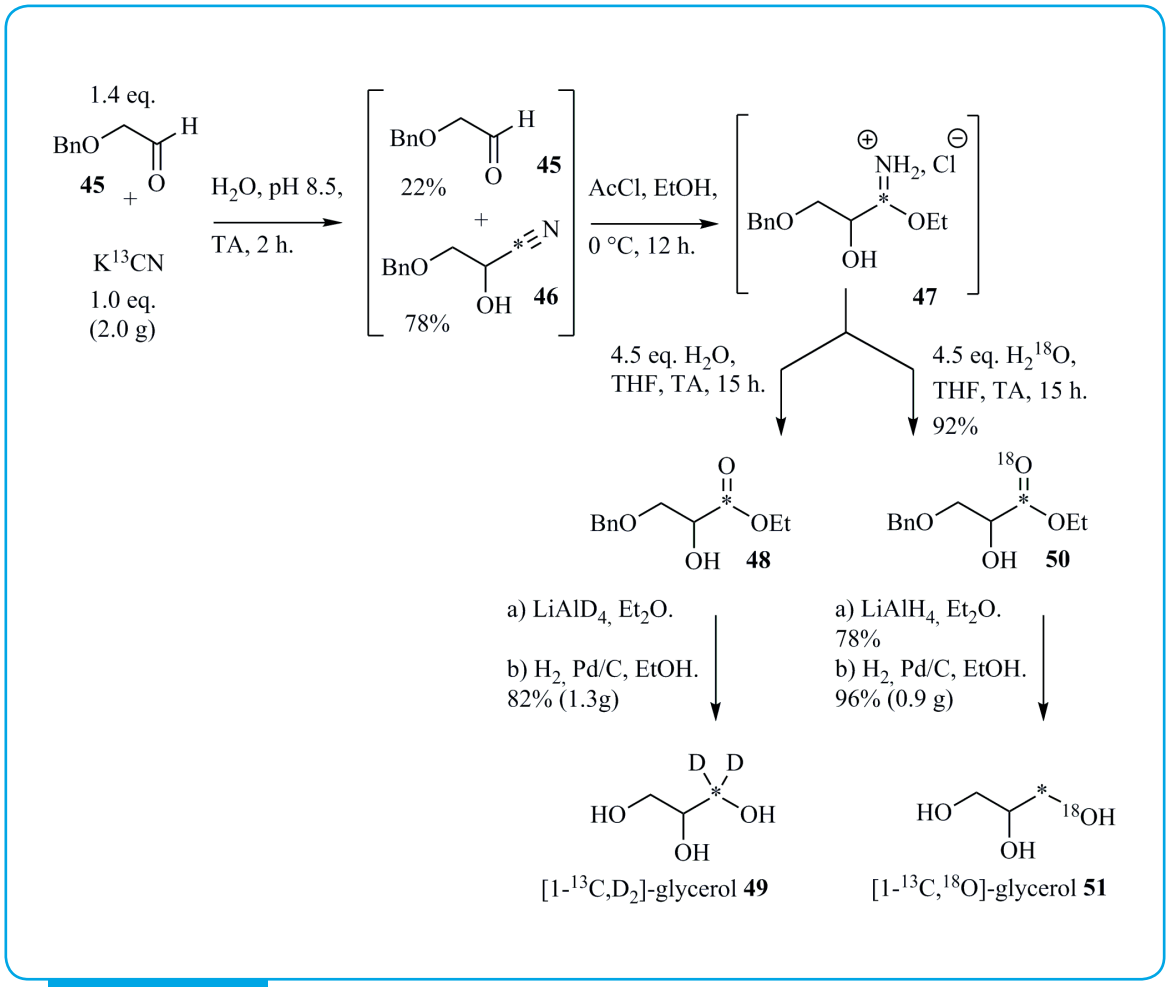
$KC^{15}N$ and $K^{13}C^{15}N$ are valuable labeled reagents for the preparation of ^{15}N-labeled amines, as well as ^{15}N-labeled heterocycles as presented in Scheme 10. In the course of registration studies concerning auxinic herbicides, which are widely used to control broadleaf weeds in cereal crops and turfgrass, Johnson and Coll. described a large-scale preparation of M+3 isotopically labeled pentachloropyridine **43**, as the key intermediate.[21] The commercially available 1,3-dibromopropane **40** was refluxed in a mixture of acetonitrile and water in the presence of a slight excess of $K^{13}C^{15}N$ (around 50 g!) to afford isotopically labeled glutaronitrile **41** in good yield (85%). Then, a mixture of glutaronitrile **41** in acetic acid and trifluoroacetic acid (10/1) was heated in a high-pressure high-temperature reactor system for 2 days at 230°C to give glutarimide-1-^{15}N-2,6-$^{13}C_2$ **42** in 82% yield after crystallization from ethanol. Next, using the same previous high-pressure equipment, isotopically labeled glutarimide **42** was treated with excess phosphorus pentachloride and chlorine in the presence of a catalytic amount of iron (III) chloride and iodine for 28 h at 250°C to yield pentachloropyridine-1-^{15}N-2,6-$^{13}C_2$ **43**, which was crystallized from methanol (71%, 35.7 g). This labeled material **43** served as the key intermediate in the preparation of an M+3 stable isotope of 4-amino-3,5,6-trichloropicolinic acid (picloram-1-^{15}N-2,6-$^{13}C_2$) **44** through a seven-step sequence.



SCHEME 10

A highly efficient synthesis of [1-^{13}C, D_2]- and [1-^{13}C, ^{18}O]-glycerol, **49** and **51** respectively, for the elucidation of biosynthetic pathways was published by Siskos and Hill[22] as presented in Scheme 11, based on nucleophilic addition of cyanide on the aldehyde function. Benzyloxyacetaldehyde **45** was reacted with $K^{13}CN$ to give a crude mixture containing the corresponding cyanohydrin **46** and the starting material **45** in 78% and 22% yields, respectively. The previous mixture was directly engaged in a Pinner reaction without any further purification to furnish the key imino ethyl ester **47** as its hydrochloride salt. This latter unstable and hygroscopic compound **47** was immediately treated with water, then the ethyl ester intermediate **48** formed was reduced with $LiAlD_4$ and hydrogenolysis of the benzyl protecting group led to [1-^{13}C, D_2]-glycerol **49** in 82% (1.3 g) overall yield for the three steps.

[21] P. L. Johnson, N. R. Pearson, B. Schuster, J. Cobb, *J. Label Compd. Radiopharm.* **2009**, *52*, 382-386.
[22] A. P. Siskos, A. M. Hill, *Tetrahedron Lett.* **2003**, *44*, 789-792.



SCHEME 11

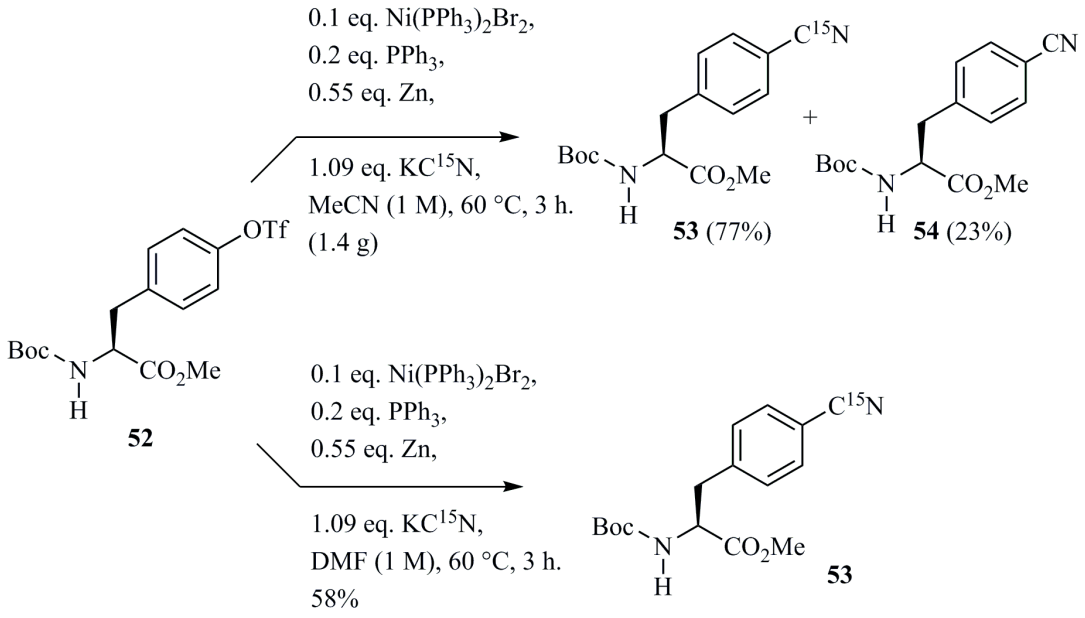
Treatment of the key imino ethyl ester **47** with $H_2{}^{18}O$ yielded [1-^{13}C, ^{18}O]-glycerol **51** using the previous sequence with $LiAlH_4$ instead of its deuterated isotopomer. This strategy offered the opportunity to insert the ^{18}O label efficiently as only the carboxylate oxygen atom, which was retained in the subsequent reduction step.[23]

To end this section on labeled potassium cyanide $KC^{15}N$, it should be pointed out that the use of acetonitrile as the solvent can be problematic in some cases. In the course of the preparation of *N*-(*tert*-butoxycarbonyl)-4-cyano-L-phenylalanine methyl ester **53** from its (trifluoromethyl) sulfonyl) parent **52** using a nickel-catalyzed cyanation reaction with potassium cyanide $KC^{15}N$ in acetonitrile, Brewer and Coll.[24] observed that the solvent could serve as a reservoir of the unlabeled cyanide leading to a mixture of isotopomers **53** and **54** in 77% and 23% yields, respectively (see Scheme 12). The same reaction carried out in DMF, instead of acetonitrile, with either $KC^{15}N$, $K^{13}CN$, or $K^{13}C^{15}N$ gave their corresponding isotopomers with high isotopic enrichment. This isotope scrambling has been previously reported in the literature as an exchange between labeled cyanide and the nitrile function of acetonitrile.[25]

[23] For an excellent review of synthetic methodologies to introduce oxygen 17 and 18 atoms, see: V. Theodorou, K. Skobridis, D. Alivertis, I. P. Gerothanassisa, *J. Label Compd. Radiopharm.* **2014**, *57*, 481-508.

[24] C. G. Bazewicz, J. S. Lipkin, K. A. Lozinak, M. D. Watson, S. H. Brewer, *Tetrahedron Lett.* **2011**, *52*, 6865-6868.

[25] M. Jay, W. J. Layton, G. A. Digenis, *Tetrahedron Lett.* **1980**, *21*, 2621-2624.

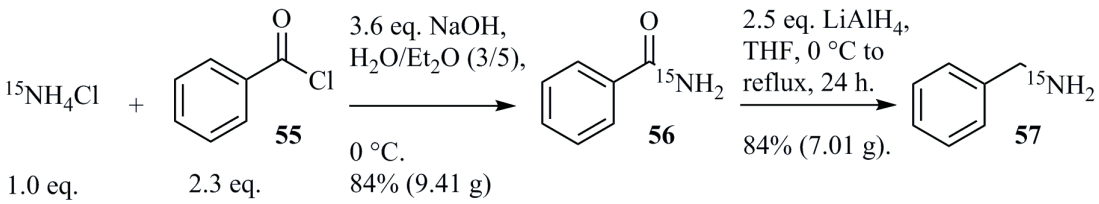


SCHEME 12

^{15}N-labeled NH_3, $NaNO_2$ or NH_4Cl

^{15}N-labeled ammoniac and sodium nitrate, as well as their derivatives, represent versatile nucleophile or electrophile sources of ^{15}N atoms, respectively.

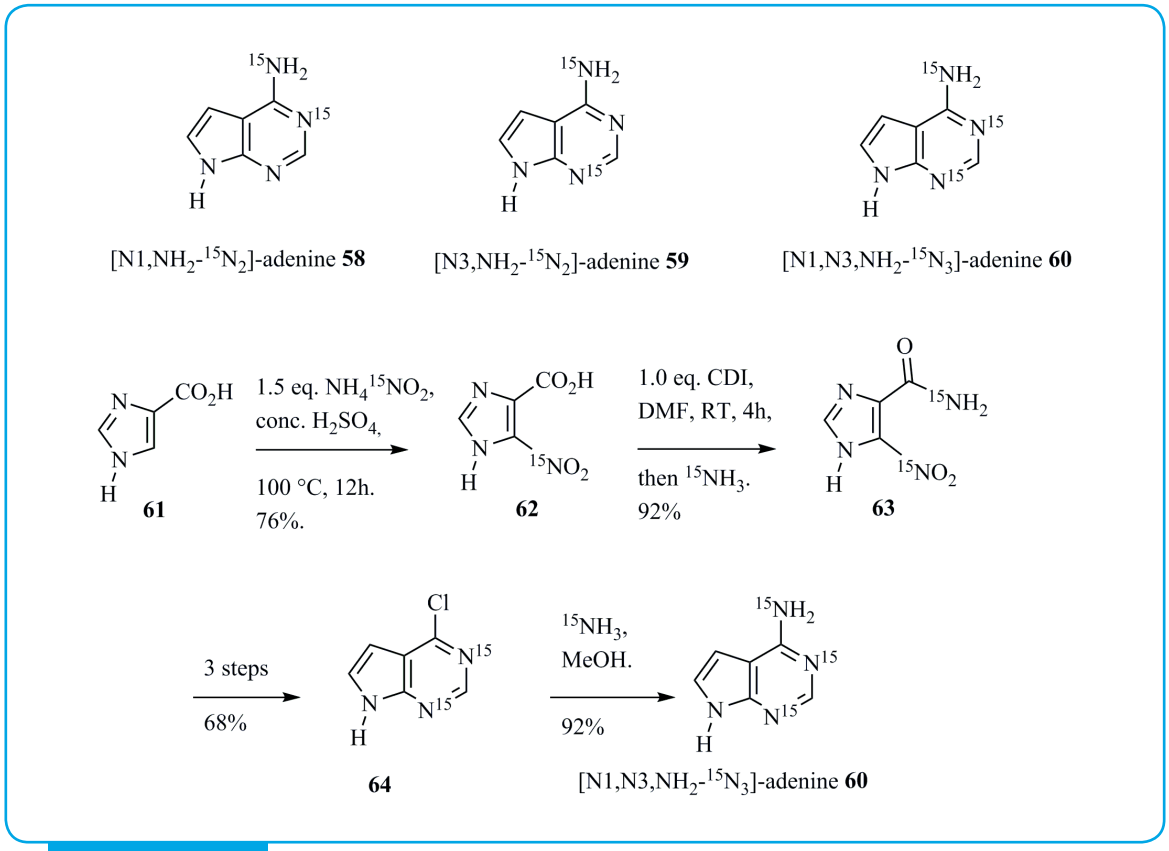
In the course of an ^{15}N NMR study of platinum-based anticancer compounds containing vicinal diamine moieties, a gram-scale synthesis of ^{15}N-labeled benzylamine from $^{15}NH_4Cl$ was used, as outlined in Scheme 13.[26] Benzoyl chloride **55** was treated with excess $^{15}NH_4Cl$ under a basic aqueous biphasic system to lead to the corresponding benzamide **56**, which was then reduced with $LiAlH_4$ to afford ^{15}N-labeled benzylamine **57** in 66% overall yield (7.01 g).



SCHEME 13

[26] G. Berger, M. Gelbcke, E. Cauët, M. Luhmer, J. Nève, F. Dufrasne, *Tetrahedron Lett.* **2013**, *54*, 545-548.

In the field of nucleic acids, ^{15}N NMR spectroscopy is a powerful tool to detect molecular interactions involved in various biological processes, such as interactions with proteins, ligands, or drugs. An efficient access of nucleosides with ^{15}N atoms at relevant sites is also a key issue. Cheng and Coll. published an elegant and efficient synthesis of [N1,NH_2-$^{15}N_2$]-, [N3,NH_2-$^{15}N_2$]-, and [N1,N3,NH_2-$^{15}N_3$]-labeled adenine, **58**, **59** and **60** respectively, illustrated through the preparation of this latter isotopomer as described in Scheme 14.[27] Nitration of the commercially available 4-imidazole carboxylic acid **61** using $NH_4{}^{15}NO_3$ in sulfuric acid to generate the reactive labeled ^{15}N-nitronium species provided the desired ^{15}N-nitroderivative **62** in 76% yield. It is worth noting that the solid nitrating agent $NH_4{}^{15}NO_3$ is cheaper and easier to handle than $H^{15}NO_3$. Next, the acid **62** was activated with 1,1'-carbonyldiimidazole (CDI) in DMF and the corresponding *N*-acylimidazole formed *in situ* was subsequently treated by bubbling $^{15}NH_3$, generated from $^{15}NH_4Cl$ and K_2CO_3. The desired doubly-labeled carboxamide **63** was isolated in 92% yield and engaged in a well-known three-step sequence to provide [N1,N3-$^{15}N_2$]-labeled 6-chloropurine **64** in 68% overall yield. In the final stage, aminolysis of **64** with $^{15}NH_3$ in methanol furnished [N1,N3,NH_2-$^{15}N_3$]-labeled adenine **60** in 92% yield.

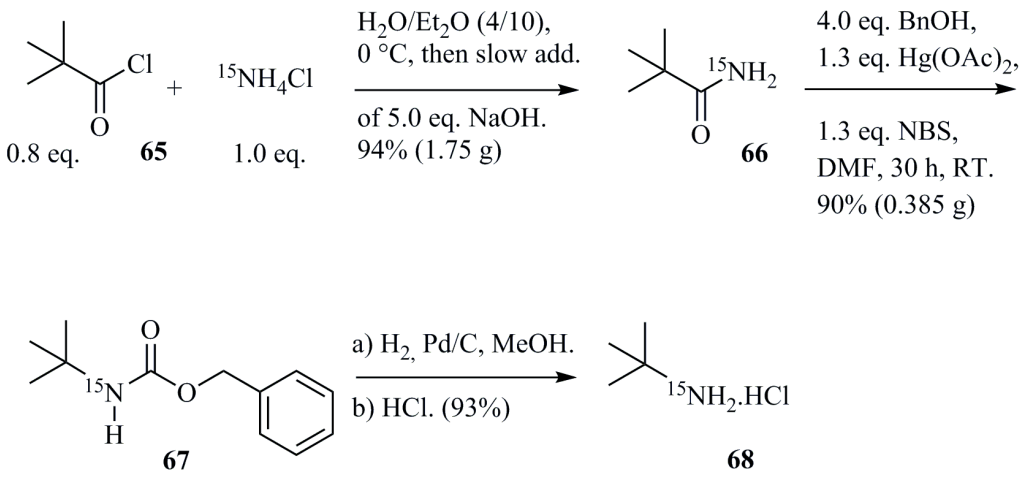


SCHEME 14

tert-Butylamines, like benzylamine (*vide supra*), are versatile and important tools for the preparation of various products with biological properties. Their corresponding ^{15}N-amine isotopomers are also key labeled starting materials for the preparation of biologically active compounds for use as analytical internal standards or for metabolic studies, as well as ^{15}N NMR studies. Wen and Coll. successfully explored the synthesis of ^{15}N-*tert*-butylamine **68** as its HCl salts, involving the Hofmann rearrangement as presented in Scheme 15.[28]

[27] M. L. Jain, Y.-P. Tsao, N.-L. Ho, J.-W. Cheng, *J. Org. Chem.* **2001**, *66*, 6472-6475.
[28] Y. Zhang, C. Lin, Z. Li, L. Qin, H. Wen, *J. Label Compd. Radiopharm.* **2010**, *53* 183-185.

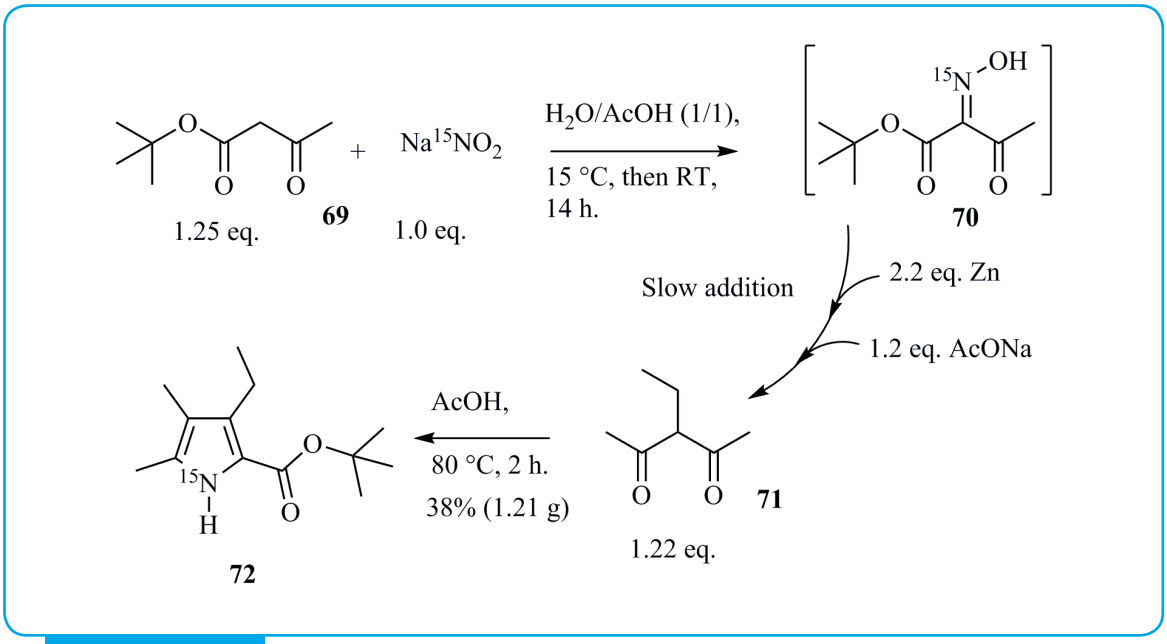
Pivaloyl chloride **65** was treated with one equivalent of $^{15}NH_4Cl$ under a basic aqueous biphasic system to afford the corresponding carboxamide **66**. This latter intermediate **66** was converted into the corresponding benzyl [^{15}N]N-*tert*-butylcarbamate **67**, having one less carbon, using a Hofmann rearrangement by treatment with *N*-bromosuccinimide (NBS), mercuric acetate and benzyl alcohol in DMF. Benzyl [^{15}N]N-*tert*-butylcarbamate **67** was isolated in 90% yield, and was then submitted to palladium catalytic hydrogenolysis followed by acidification with HCl gas to furnish [^{15}N]*tert*-butylamine hydrochloride **68** in 93% overall yield.



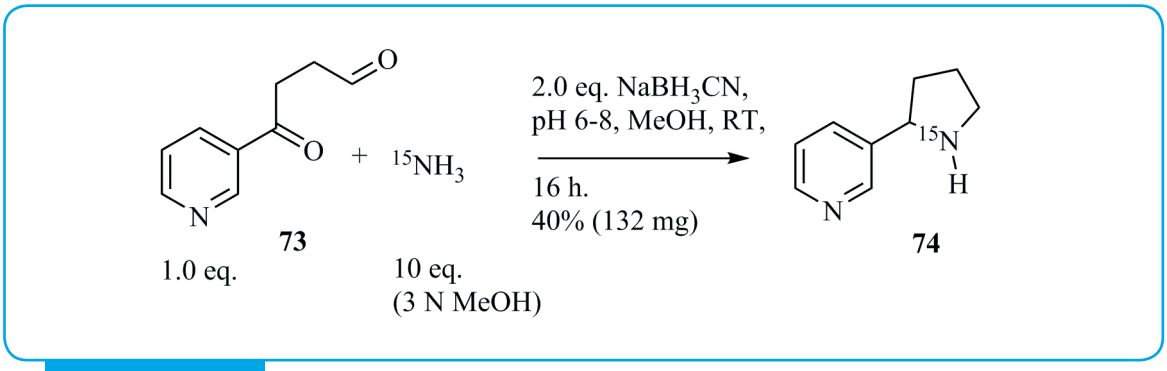
SCHEME 15

During a study concerning the analysis of etioporphyrins in oil shales and petroleum, a preparation of *per*-^{15}N-labeled etioporphyrins was developed from^{15}N-labeled pyrrole **72** as depicted in Scheme 16.[29] This labeled building block **72** was prepared using the Fischer-Fink pyrrole synthesis[30] from *tert*-butyl acetoacetate **69**. Treatment of *tert*-butyl acetoacetate **69** with $Na^{15}NO_2$ and acetic acid gave the corresponding labeled oxime **70**. The latter reaction mixture was simultaneously added with zinc dust and sodium acetate to a solution of 3-ethyl-2, 4-pentanedione **71** in acetic acid. The desired ^{15}N-labeled pyrrole **72** was then isolated in 38% overall yield from $Na^{15}NO_2$. In our previous study on the mechanism of *N*-demethylation of nicotine in plants, we also prepared ^{15}N-labeled nornicotine **74** as shown in Scheme 17.[31] Reductive aminocyclization of 1,4-ketoaldehyde **73** with sodium cyanoborohydride in the presence of a 3 N methanolic solution of $^{15}NH_3$ gave ^{15}N-labeled nornicotine **74** in 40% yield. The 1,4-ketoaldehyde **73** was obtained using a two-step sequence from 3-bromopyridine with 82% overall yield.

[29] T. D. Lash, S. Chen, *Tetrahedron* **2005**, *61*, 11577-11600.
[30] V. Chandrashaker, M. Taniguchi, M. Ptaszek, J. S. Lindsey, *Tetrahedron* **2012**, *68*, 6957-6967.
[31] G. Vo-Thanh, F.-X. Felpin, G. Nourrisson, M. Trierweiler, R. J. Robins, J. Lebreton, *J. Label Compd. Radiopharm.* **2001,** *44*, 881-888.



SCHEME 16

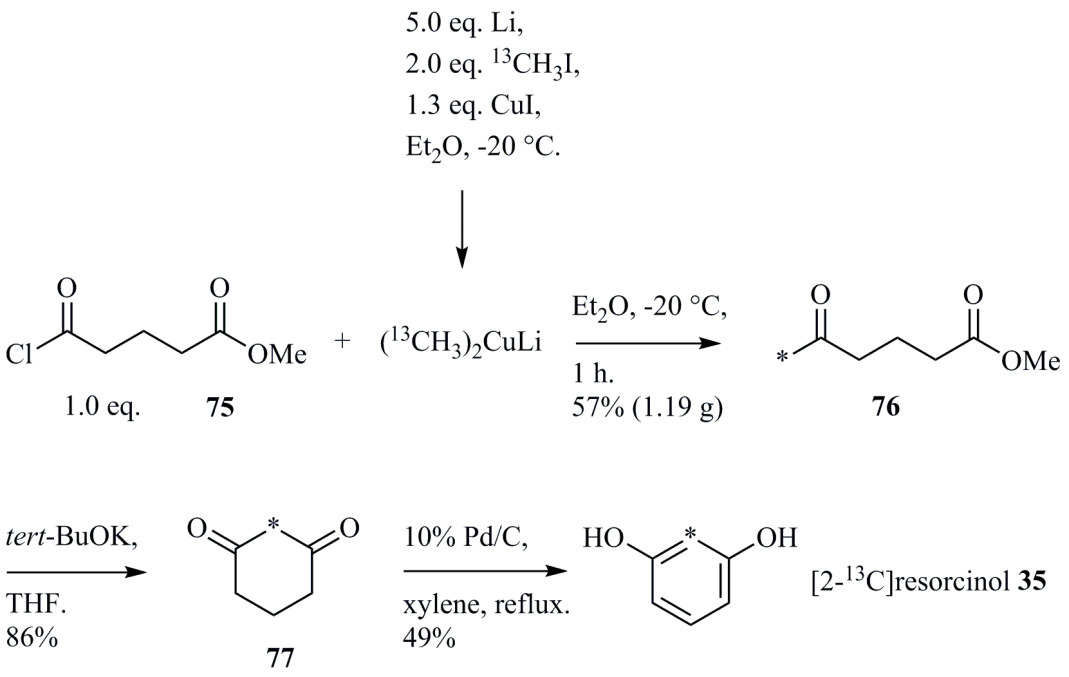


SCHEME 17

CH_3I

Methyliodide is an excellent electrophile whereas its corresponding lithium dimethylcuprate [$(CH_3)_2CuLi$] is an excellent nucleophilic reagent, which can be engaged in various types of transformations, such as substitution or conjugate addition reactions with the appropriate substrates.[32] More recently, the development of palladium-mediated cross-coupling using methyl iodide has significantly extended the scope of this reagent for the preparation of labeled compounds. During their investigations into the labeled synthesis of [3,4,8-$^{13}C_3$]daidzein **36** (see also Scheme 8), Botting and Coll. reported an optimized three-step synthesis of [2-^{13}C]resorcinol as shown in Scheme 18.[19] Treatment of commercially available methyl 5-chloro-5-oxopentanoate **75** with a solution of ($^{13}CH_3$)2CuLi, prepared from $^{13}CH_3I$, gave methyl 5-oxo-[6-^{13}C]hexanoate **76**, which was subsequently engaged in a cyclization reaction via an intramolecular Claisen condensation affording the cyclic diketone **77**, followed by an aromatization step, with concomitant loss of hydrogen, using a palladium catalyst on carbon in refluxing xylene.

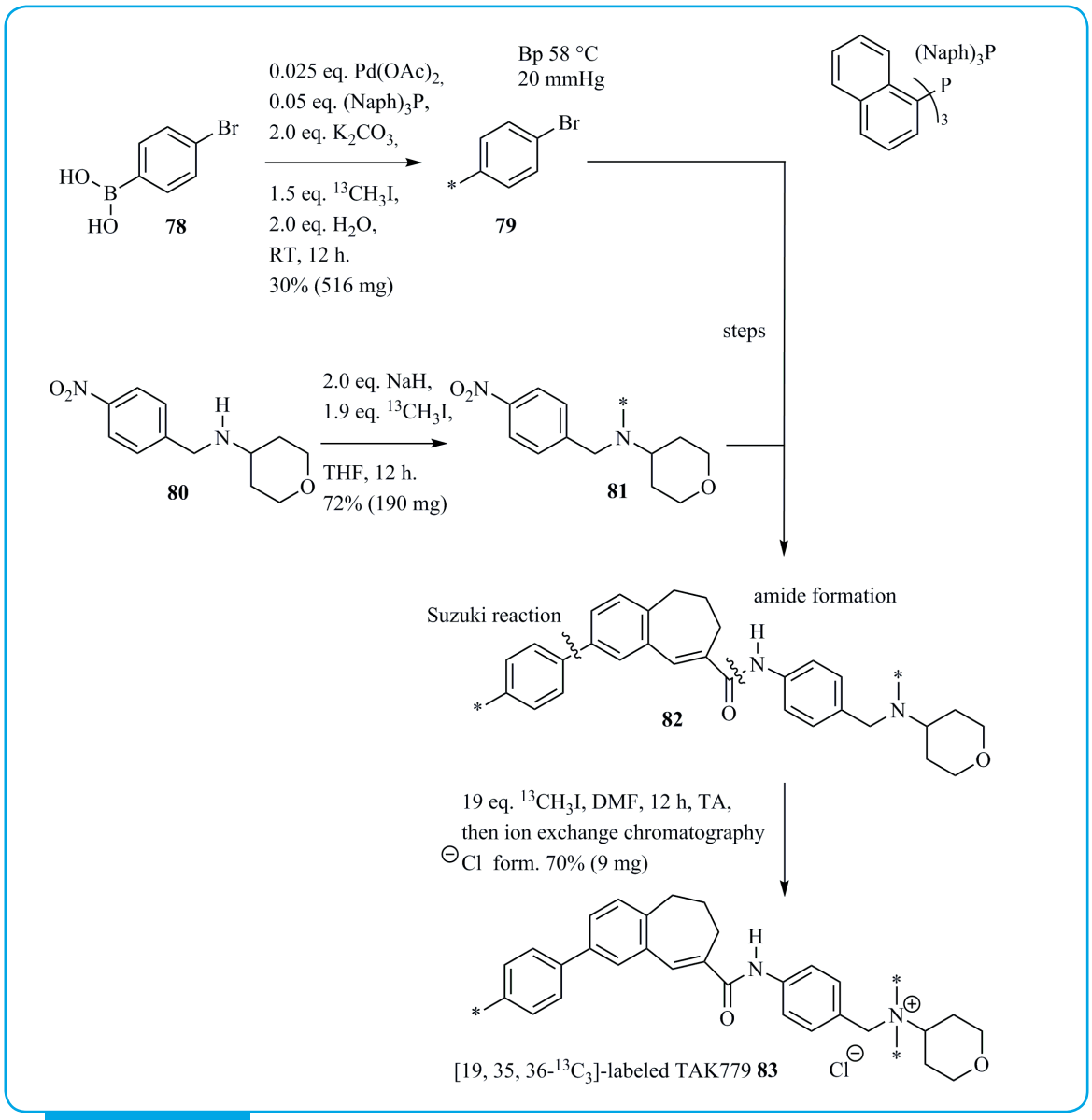
[32] For an excellent review in this field, see: N. Yoshikai, E. Nakamura, *Chem. Rev.* **2012**, *112*, 2339-2372.



SCHEME 18

[2-^{13}C]Resorcinol **35** was isolated in 25% (238 mg) overall yield. TAK779 was reported as the first small molecule to inhibit HIV-1 replication at the membrane fusion stage by blocking the interaction on the viral surface glycoprotein gp120 with the cysteine-cysteine chemokine receptor 5 (CCR5), a G protein-coupled receptor. To understand better the mode of interaction between TAK779 and CCR5, the isotopomer **83** was synthesized (see Scheme 19) as a molecular probe for solid-state NMR studies.[33] In this work, $^{13}CH_3I$ was used at three stages of the synthesis to afford [19, 35, 36-$^{13}C_3$]-labeled TAK779 **83**. First, a cross-coupling reaction of *para*-bromophenylboronic acid **78** with $^{13}CH_3I$ in the presence of palladium acetate and the sterically demanding tri-1-naphthylphosphine ligand gave the desired [$^{13}CH_3$]-labeled *para*-bromotoluene **79** in only 30% yield (516 mg) due to its volatility. It is clear that the volatility of the final products greatly complicates the work-up and the purification process, which is most relevant when the reactions are carried out on a small scale, as is very often the case in the synthesis of isotopically stable labeled compounds. This simple and practical protocol of palladium-catalyzed cross-coupling between boronic acids and methyl iodide developed by Gooßen[34] provided a very precious tool in the field of labeled compounds.[35] Later on in the synthesis, the secondary amine **80** was deprotonated with NaH followed by addition of $^{13}CH_3I$ to afford [$^{13}CH_3$]-labeled *N*-methylamine **81** in 72% yield. As the last step in the synthesis, treatment of [$^{13}C_2$]-labeled tertiary amine **82** with an excess of $^{13}CH_3I$ in DMF and subsequent counter-ion exchange gave the desired [$^{13}C_3$]-labeled TAK779 **83** in 70% yield.

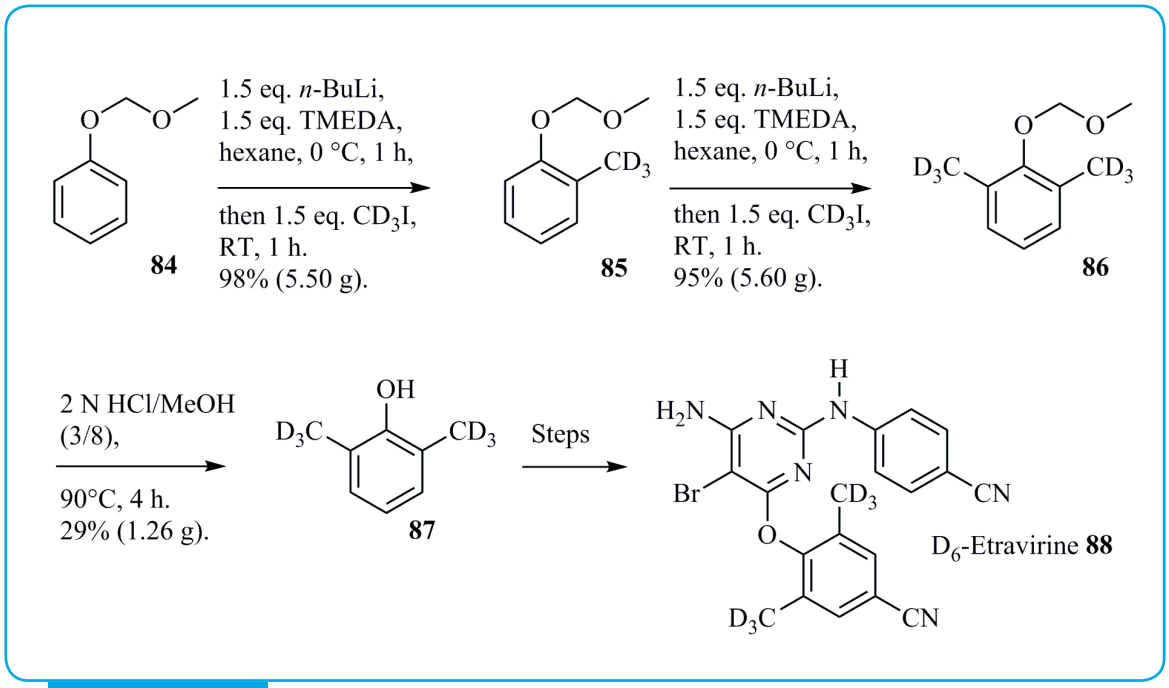
[33] H. Konno, S. Aimoto, S. O. Smith, K. Nosaka, K. Akaji, *Bioorg. Med. Chem.* **2009**, *17*, 5769-5774.
[34] L. J. Gooßen, *Appl. Organometal. Chem.* **2004**, *18*, 602-604.
[35] For a recent review on Pd-mediated cross-couplings using methyl iodide in the field of radiolabeling chemistry, see: H. Doi, *J. Label Compd. Radiopharm.* **2015**, *58*, 73-85.



SCHEME 19

During the clinical studies of etravirine, a human immunodeficiency virus type 1 (HIV-1) non-nucleoside reverse transcriptase inhibitor (NNRTI) approved in 2008 and marketed under the trade name Intelence™, the isotopomer **88** was prepared following an eight-step sequence from phenol as depicted in Scheme 20.[36] The key labeled phenol derivative **86** with six deuterium atoms was prepared using two consecutive *ortho*-lithiations followed by addition of CD_3I sequences. It should be emphasized that the methoxymethyl group and analogues act as an easily removed phenol-protecting group and an excellent directing metalation group, thus offering the opportunity to introduce various electrophiles exclusively at *ortho* position(s). Nevertheless, the low yield (29%) obtained for the acidic hydrolysis of the methoxymethyl group in **86** to afford the phenol derivative **87** is surprising in comparison with the high yields reported in the literature on close unlabeled substrates.[37]

[36] W. Wang, W. Wu, Z. Shen, L. Chen, *J. Label Compd. Radiopharm.* **2011**, *54*, 371-373.
[37] For a recent example of cleavage, see: E. Hyun Suh, Y. Liu, St. Connelly, J. C. Genereux, I. A. Wilson, J. W. Kelly, *J. Am. Chem. Soc.* **2013**, *135*, 17869-17880.



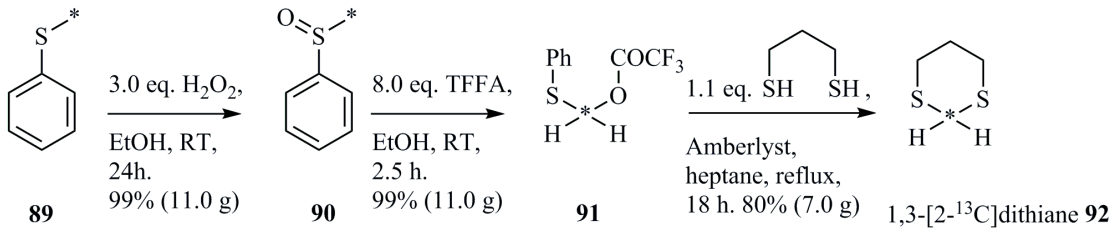
SCHEME 20

Preparation of important labeled reagents

Due to the small number of commercially available labeled reagents, significant efforts have been devoted to the development of efficient routes to isotopomers of the most important reagents.

Unlabeled 1,3-dithiane is a protected formaldehyde equivalent and its corresponding lithiated derivative is a formyl anion equivalent. Since the pioneering work of Seebach and Corey[38], this protected formaldehyde anion has been extensively used with success for the preparation of numerous compounds. An efficient gram-scale synthesis of 1,3-[2-^{13}C]dithiane **92** has recently been published by Martinez and Unkefer as outlined in Scheme 21.[39] Commercially available [^{13}C] methyl phenyl sulfide **89** (1 g/250 $) was oxidized by treatment with hydrogen peroxide to furnish [^{13}C]methyl phenyl sulfoxide **90** in excellent yield (99%, 11.0 g).[40] Then, this latter compound **90** was submitted to a Pummerer rearrangement in the presence of trifluoroacetic anhydride (TFAA) to give the mixed thioacetal **91**. After removal of the volatiles by distillation, this latter material **91** was treated with 1,3-propanedithiol to afford the desired 1,3-[2-^{13}C]dithiane **92** in 80% (7.0 g) yield for the two steps.

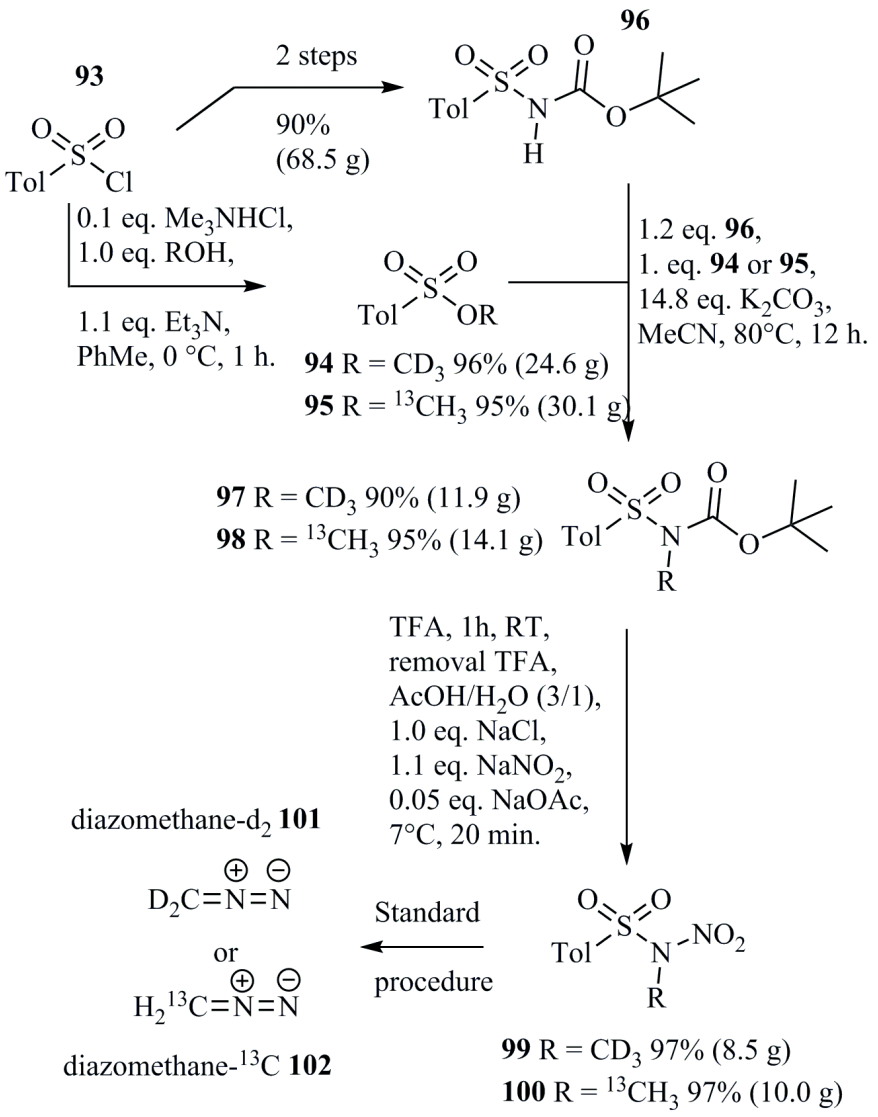
[38] D. Seebach, E. J. Corey, *J. Org. Chem.* **1975**, *40*, 231.
[39] R. A. Martinez, D. R. Glass, E. G. Ortiz, M. A. Alvarez, C. J. Unkefera, *J. Label Compd. Radiopharm.* **2014**, *57*, 338-341.
[40] For a preparation of the CD_3SPh isotopomer, see: E. Baciocchi, C. Chiappe, T. Del Giacco, C. Fasciani, O. Lanzalunga, A. Lapi, B. Melai, *Org. Lett.* **2009**, *11*, 1413-1416.



SCHEME 21

Diazomethane (CH_2N_2) is an extremely unstable and toxic reagent, requiring flame polished glassware for its preparation and use. Nevertheless, it is still widely used as a powerful reagent for various transformations including methyl ester formation from carboxylic acids or methylation of acidic heteroatoms as well as cyclopropanation. Diazomethane is a gas (boiling point of -23°C under atmosphere pressure) which is typically produced from *N*-methyl-*N*-nitroso-*para*-toluenesulfonamide (known as Diazald®, a registered trademark for Aldrich) and used as a solution in ether. Very recently, Manthorpe and Shields reported a robust and reliable multi-gram synthesis of *N*-methyl-D_3-*N*-nitroso-*para*-toluenesulfonamide **99** and *N*-methyl-^{13}C-*N*-nitroso-*para*-toluenesulfonamide **100** and their conversion into diazomethane-D_2 **101** and diazomethane-^{13}C **102**, respectively, from inexpensive labeled methanol as depicted in Scheme 22.[41] In this work, both isotopomers of methyl tosylate **94** and **95** were identified as easily handled, non-volatile and non-water-soluble electrophilic methyl sources. These labeled starting materials **94** and **95** were readily available from *para*-toluenesulfonyl chloride **93** and their corresponding methanol isotopomers. *N*-Boc-protected *para*-toluenesulfonamide **96**, commercially available (25 g/160 $) or easily prepared in two steps from *para*-toluenesulfonyl chloride **93**, was treated with labeled methyl tosylate **94** or **95** in the presence of a large excess of K_2CO_3 in refluxing acetonitrile to give the corresponding *N*-methylated compounds **97** and **98** in high yields after purification on silica gel chromatography. Finally, these labeled materials **97** and **98** were engaged in a deprotection/nitrosylation sequence to provide the required *N*-methyl-D_3-*N*-nitroso-*para*-toluenesulfonamide **99** and *N*-methyl-^{13}C-*N*-nitroso-*para*-toluenesulfonamide **100** in excellent yields. From these latter labeled intermediates **99** and **100**, the corresponding diazomethane-D_2 **101**[42] and diazomethane-^{13}C **102** were generated using a procedure adapted from the literature with a mini Diazald® apparatus from Sigma-Aldrich. Ethyl (triphenylphosphoranylidene)acetate **105** and triethyl phosphonoacetate **106** have gained much attention as two-carbon building blocks either with a single ^{13}C labeled at C-1 or C-2 or a doubly-labeled ^{13}C at C1,2 (see Scheme 23). From aldehydes, or less often from ketones, these reagents **105** and **106**, through Wittig or Horner-Wadsworth-Emmons olefinations, respectively, allow carbon chain elongation and further functionalization, offering a wide range of potential applications in organic synthesis. The preparation of the three isotopomers of **105** and **106** can be achieved from their corresponding ethyl bromoacetate isotopomers according to known procedures (Scheme 23, equations (a)[43] and (b)[44]).

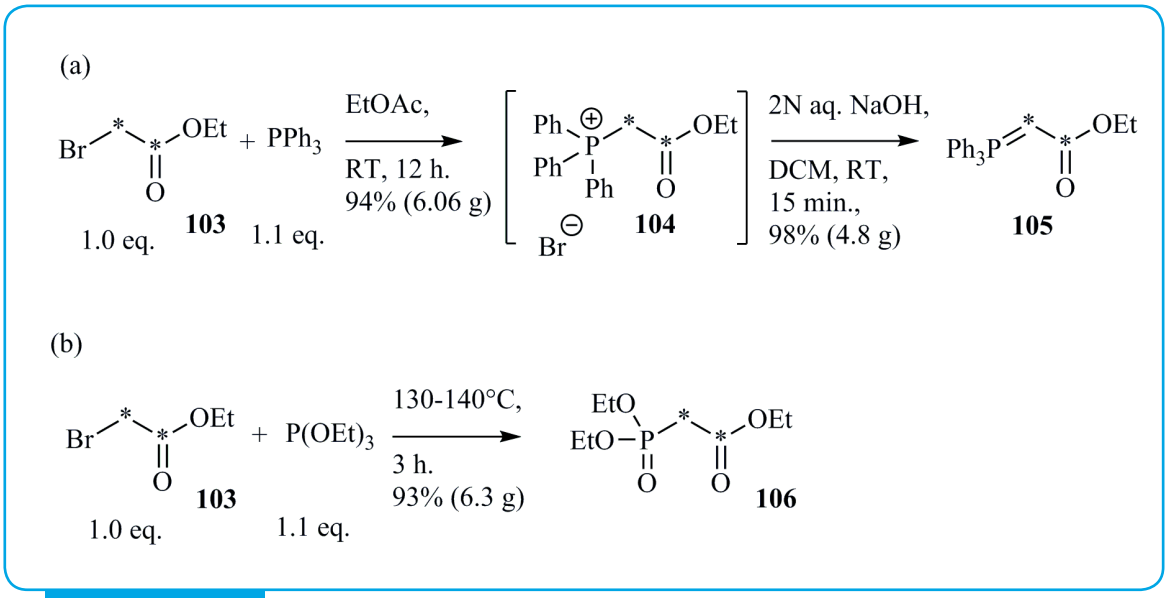
[41] S. W. J. Shields, J. M. Manthorpe, *J. Label Compd. Radiopharm.* **2014**, *57*, 674-679.
[42] For a recent application of diazomethane-D_2, see: K. Kleigrewe, E.-M. Niehaus, P. Wiemann, B. Tudzynski, H.-U. Humpf, *J. Agric. Food Chem.* **2012**, *60*, 8350-8355.
[43] S. Sadhukhan, Y. Han, G.-F. Zhang, H. Brunengraber, G. P. Tochtrop, *J. Am. Chem. Soc.* **2010**, *132*, 6309-6311.
[44] P. B. Shrestha-Dawadi, J. Lugtenburg, Eur. *J. Org. Chem.* **2003**, 4654-4663.



SCHEME 22

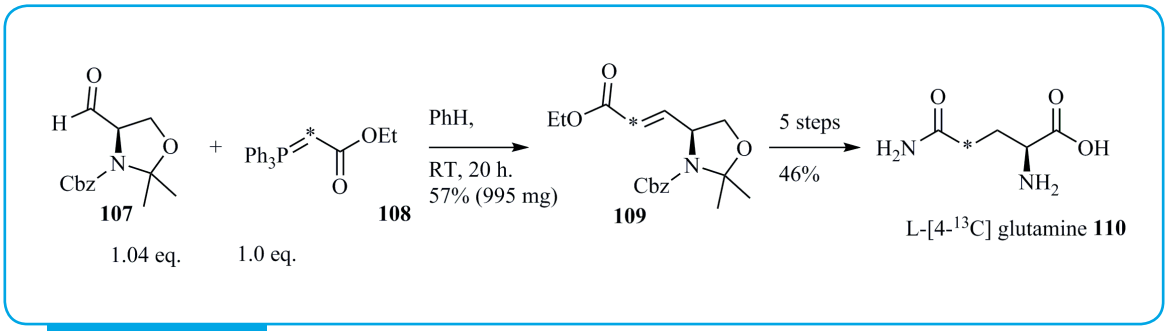
Commercially available sources of ethyl bromoacetate include all patterns of ^{13}C labeling (ethyl bromo[2-^{13}C2]acetate (1 g/300 $), ethyl bromo[1-^{13}C]acetate (5 g/1000 $) and ethyl bromo[1,2-^{13}C] acetate (5 g/750 $)). It should be noted that labeled ethyl bromoacetate can also be prepared from the corresponding labeled sodium acetate[45] or acetic acid[46] isotopomers. In this context, it is not surprising that each of the three ^{13}C isotopomers of **105** and **106** have been successfully employed in the synthesis of various isotopically labeled biological compounds as illustrated in the following Schemes 24 and 25.

[45] K. Takatori, A. Hayashi, M. Kajiwara, *J. Label Compd. Radiopharm.* **2004**, *47*, 787-795 and cited literature.
[46] A. F. L. Creemers, J. Lugtenburg, *J. Am. Chem. Soc.* **2002**, *124*, 6324-6334.



SCHEME 23

To study the neuron-astrocyte/glutamate-glutamine cycle, Takatori and Coll. have published an efficient synthesis of L-[4-^{13}C] glutamine **110** from D-serine as shown in Scheme 24.[47] In this synthesis, the Cbz analogue **107** of the Garner aldehyde, which has a Boc group as the *N*-protecting group, was used due to the better stability of the different synthetic intermediates toward acidic conditions in this synthesis. This D-(*N*-Cbz)-serinal **107** was derived from D-Cbz-serine.[48] Horner-Wadworsth-Emmons condensation of D-(*N*-Cbz)-serinal **107** with triethyl phosphonoacetate **108** led to the corresponding conjugated ester **109** in 51% yield, which was subjected to a set of known transformations affording L-[4-^{13}C] glutamine **110**. In the last step of this sequence, the *N*-Cbz protecting group was removed by hydrogenolysis. It should be mentioned that HPLC analysis of L-[4-^{13}C] glutamine **110** (99% ee) showed that no erosion of the enantiomeric purity occurred in the synthesis, especially for the Wittig olefination with the epimerizable aldehyde **107**.

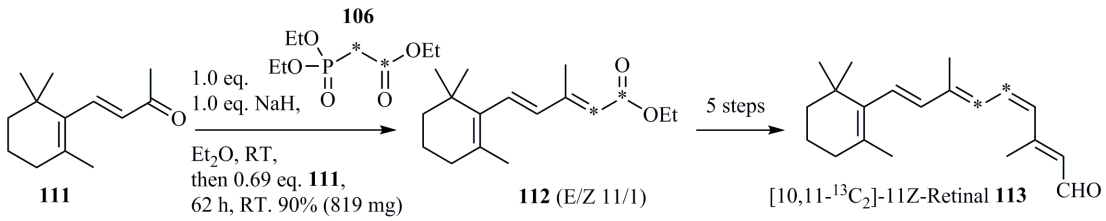


SCHEME 24

[47] K. Nagasawa, A. Kishida, M. Kajiwara, T. Kanamatsu, K. Takatori, *J. Label Compd. Radiopharm.* **2015**, *58*, 42-45.

[48] For the preparation of D-(*N*-Cbz)-serinal **107**, see: (a) P. L. Beaulieu, P. W. Schiller, *Tetrahedron Lett.* **1988**, *29*, 2019-2022. (b) J. A. Marshall, S. Beaudoin, J. Org. Chem. 1996, 61, 581-586.

A series of 11Z-retinal isotopomers with pairs of adjacent ^{13}C labels have been successfully prepared by Brown and Coll. at the University of Southampton in connection with solid-state NMR investigations of the rhodopsin chromophore and its retinal photointermediates.[49] A part of their synthetic efforts is highlighted by the synthesis of [10,11-$^{13}C_2$]-11Z-Retinal **113** as outlined in Scheme 25. Coupling the labeled phosphonate **106** with ß-ionone **111** provided the corresponding unsaturated ester **112** in 90% yield with high E-selectivity (E/Z 11/1). This latter labeled material **112** was then engaged in a five-step sequence to furnish the targeted [10,11-$^{13}C_2$]-11Z-Retinal **113** with a small contamination by other isomers, formed during the work-up and purification steps.



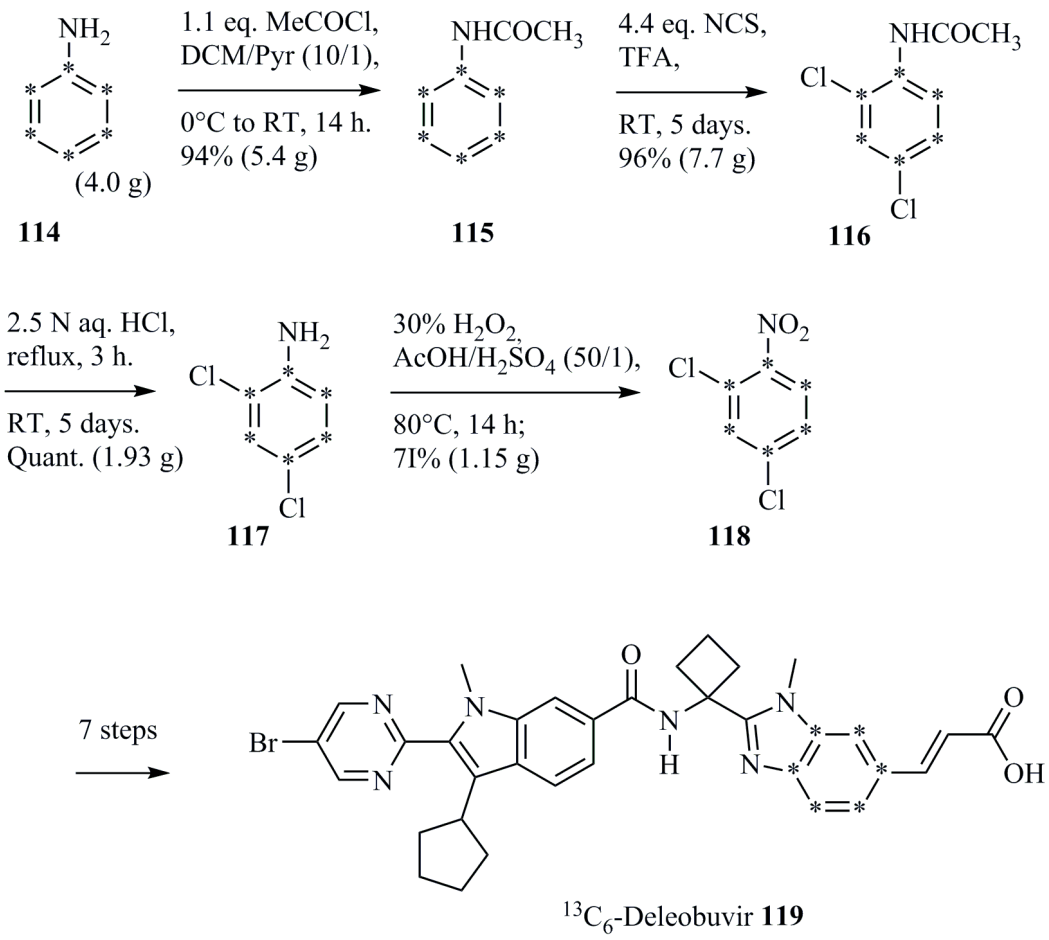
SCHEME 25

Labeled starting materials

In all the examples presented in this part, the synthesis was first performed with unlabeled material to optimize each step.

The published synthesis of unlabeled Deleobuvir, an oral non-nucleoside inhibitor of hepatitis C virus (HCV) NS5BRNA polymerase, developed by Boehringer Ingelheim, starts from the commercially available 2,4-dichloronitrobenzene (see Scheme 26).[50] However, since fully ^{13}C-labeled 2,4-dichloronitrobenzene **118** is not commercially available, the synthesis of $^{13}C_6$-Deleobuvir **119** started with $^{13}C_6$-aniline **114** (250 mg/400 $). Starting from 4.0 g of commercially available $^{13}C_6$-aniline **114**, $^{13}C_6$-Deleobuvir **119** (160 mg) was obtained through a nine-step sequence with 18% overall yield. The key compound fully ^{13}C-labeled 2,4-dichloronitrobenzene **118** was prepared following an original four-step sequence from $^{13}C_6$-aniline **114** with 65% overall yield. Subsequent chemical steps, involving aniline acetylation with acetyl chloride, direct chloration with *N*-chlorosuccinimide (NCS), cleavage of the acetyl-protecting group and oxidation of the aniline into the corresponding nitro derivative with 30% hydrogen peroxide, afforded fully ^{13}C-labeled 2,4-dichloronitrobenzene **118**. From $^{13}C_6$-Deleobuvir **119**, its two major acyl glucuronide metabolites were also synthesized using allyl-D-glucuronate.

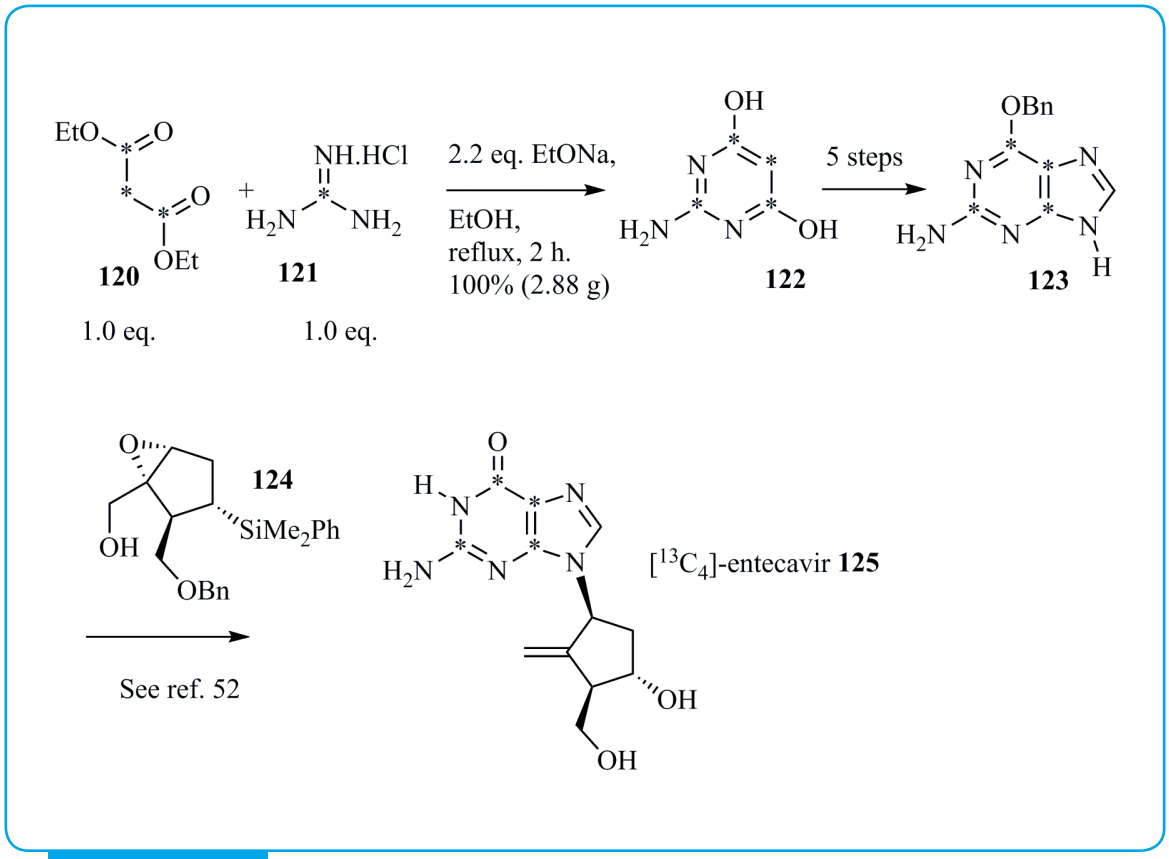
[49] N. J. McLean, A. Gansmuller, M. Concistre, L. J. Brown, M. H. Levitt, R. C. D. Brown, *Tetrahedron* **2011**, *67*, 8404-8410.
[50] B. Latli, M. Hrapchak, M. Chevliakov, G. Li, S. Campbell, C. A. Busacca, C. H. Senanayake, *J. Label Compd. Radiopharm.* **2015**, *58*, 250-260.



SCHEME 26

Entecavir was first approved in the United States in 2005 for the treatment of chronic HBV infection in adults. During the clinical phases, an isotopomer of entecavir was needed as an internal standard for mass spectrometry in support of bioassays. In this context, the synthesis of entecavir **125** labeled on the purine base was developed by Easter and Coll. from commercially available diethyl[1,2,3-$^{13}C_3$]malonate **120** and [^{13}C]guanidine HCl **121**, as presented in Scheme 27.[51] Condensation of diethyl[1,2,3-$^{13}C_3$]malonate **120** and [^{13}C]guanidine HCl **121** under known experimental conditions afforded the required labeled [2,4,5,6-$^{13}C_4$]pyrimidine-4,6-diol **122** in quantitative yield (2.88 g). Then, from this latter material **122**, the protected purine base **123** was prepared using an original five-step sequence. The key epoxide ring opening reaction on the chiral intermediate **124** with labeled 6-benzyloxy-2-aminopurine **123**, in the presence of LiOH as a base, gave the expected carbanucleoside[52], then functionalization of the sugar moiety and removal of the protecting groups furnished [$^{13}C_4$]-entecavir **125**.

[51] J. A. Easter, R. C. Burrell, S. J. Bonacorsi, *J. Label Compd. Radiopharm.* **2013**, *56*, 632-636.
[52] G. S. Bisacchi, S. T. Chao, C. Bachard, J. P. Daris, S. Innaimo, G. A. Jacobs, O. Kocy, P. Lapointe, A. Martel, Z. Merchant, W. A. Slusarchyk, J. E. Sundeen, M. G. Young, R. Colonno, R. Zahler, *Bioorg. Med. Chem. Lett.* **1997**, *7*, 127-132.

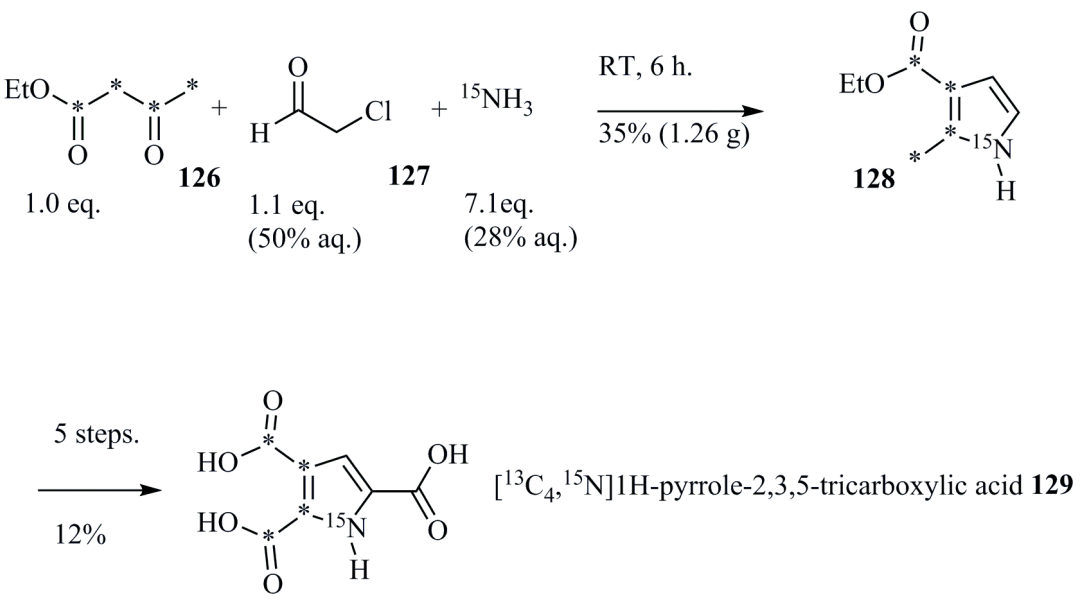


SCHEME 27

It is worth highlighting at this point that the synthesis of labeled nucleosides with a classic sugar moiety is readily accessible using well-established chemistry from commercially available [$^{13}C_6$]- α-D-glucose as published by Agrofoglio and Coll.[53] However, as presented in this example, for nucleoside analogues containing unusual and sophisticated sugar or carbocyclic moieties, the introduction of labeled pyrimidine or purine bases is more efficient.

A novel synthesis of [$^{13}C_4$,^{15}N]1*H*-pyrrole-2,3,5-tricarboxylic acid **129**, an important biomarker for melatonin metabolism, has been reported by Skaddam starting from [1,2,3,4-$^{13}C_4$]ethyl acetoacetate (1 g, 500 $) (see Scheme 28).[54] The construction of the 2,3-disubstituted pyrrole nucleus relied on a classic Hantzsch reaction involving the condensation of α-haloketones with 1,3-dicarbonyl compounds and amines. Thus, to a solution of [1,2,3,4-$^{13}C_4$]ethyl acetoacetate **126** in water was added chloroacetaldehyde **127**, followed immediately by a 6 N aqueous solution $^{15}NH_3$ to give the required [$^{13}C_4$, ^{15}N]-*N*-ethyl 2-methyl-1*H*-pyrrole-3-carboxylate **128**, which was isolated as a white powder in 35% yield (1.26 g). It is noteworthy that this modest yield was mainly due to the formation, in an equimolar amount, of the furan analogue *via* a competing Feist-Benary reaction, as shown during the optimization phase with unlabeled material. The desired [$^{13}C_4$,^{15}N]1*H*-pyrrole-2,3,5-tricarboxylic acid **129** was prepared in 12% overall yield from compound **128** following a five-step sequence.

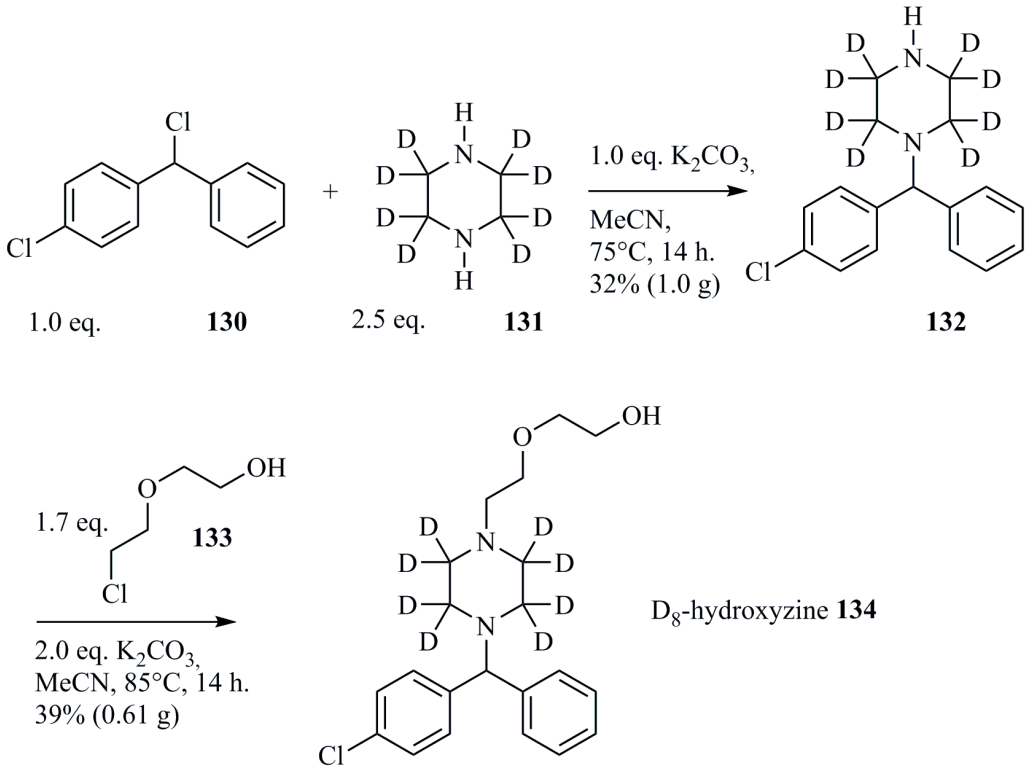
[53] Y. Saito, T. A. Zevaco, L. A. Agrofoglio, *Tetrahedron* **2002**, *58*, 9593-9603.
[54] M. B. Skaddan, *J. Label Compd. Radiopharm.* **2010**, *53*, 73-77.



SCHEME 28

An efficient synthesis of deuterium-labeled hydroxyzine **134**, used to treat anxiety disorders, has been reported by Vohra and Coll. as outlined in Scheme 29.[55] In a sealed reaction flask, 2.5 equivalents of commercially available deuterated piperazine **131** (1 g/350 $) was reacted with compound **130** in the presence of K_2CO_3 in acetonitrile at 85°C to give, after purification on silica gel column chromatography, the desired adduct **132** in around 30% yield (1.0 g) based on the labeled material **131**. The synthesis of unlabeled hydroxyzine was carried out using a large excess of inexpensive piperazine. In order to minimize dimer formation between piperazine **131** and the starting material **130**, this step was optimized and it was found that an increase in the number of equivalents of piperazine resulted in higher yields of the desired compound **132**. In terms of economic feasibility, 2.5 equivalents of deuterated piperazine **131** was the best compromise. In the final stage, condensation of intermediate **132** with chloroalcohol **133** provided D_8-hydroxyzine **134** in modest yield.

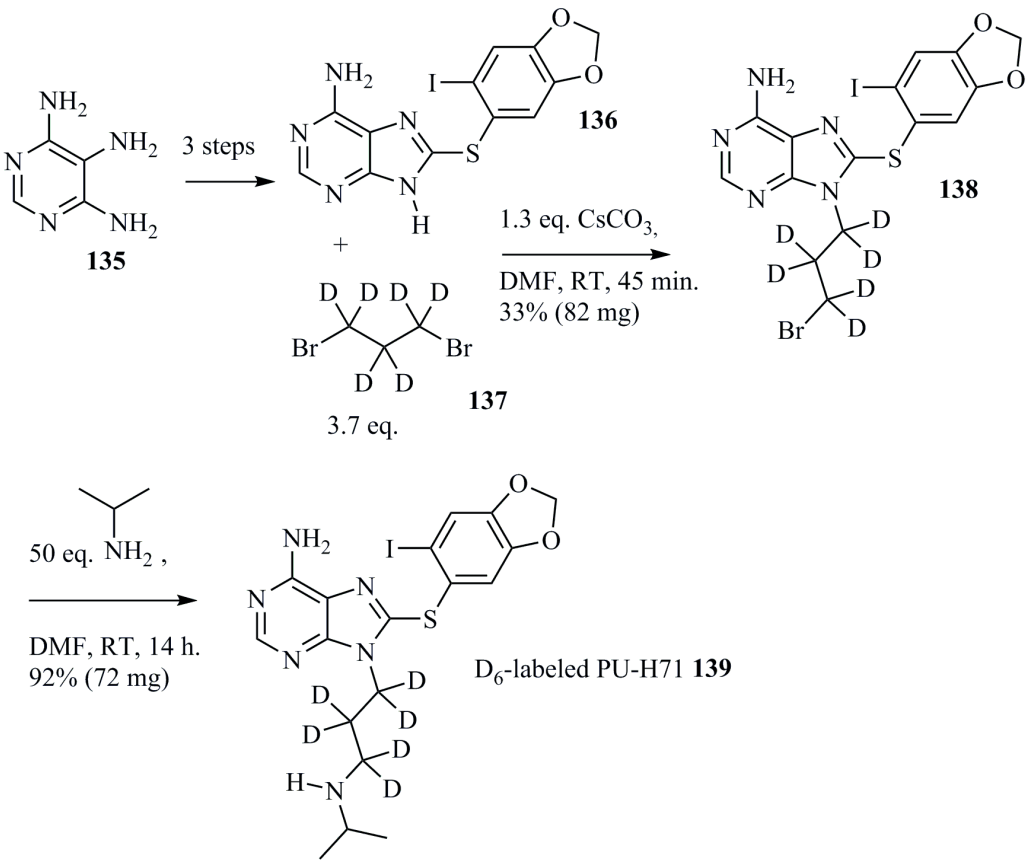
[55] M. Vohra, M. Sandbhor, A. Wozniak, *J. Label Compd. Radiopharm*. **2015**, *58*, 304-307.



SCHEME 29

PU-H71, a purine-scaffold compound, was identified as a potent inducer of apoptosis in breast tumor, B cell lymphoma, hepatocellular carcinoma and myeloma acting as a heat shock protein 90 (Hsp90) inhibitor.[56] From commercially available fully deuterated 1,3-dibromopropane **137**, an efficient synthesis of D_6-labeled PU-H71 **139** was developed in five steps from 2,4,5-triaminopyrimidine **135**.[57] This labeled compound **139** was used as an internal standard for LC-MS/MS analysis. The 8-arylsulfanyl adenine derivative **136**, prepared from **135** *via* a known three-step sequence[58], was first alkylated with 3.7 equivalents of fully deuterated 1,3-dibromopropane **137** (100 mg/1000 $) in the presence of Cs_2CO_3 in DMF to furnish the required intermediate **138** in modest yield (33%, 82 mg). Interestingly, during the preparation of the unlabeled analogue, dimer formation was not detected in this reaction when 5.0 equivalents of 1,3-dibromopropane was used; the desired compound was isolated in 75% yield. The resulting bromine derivative **138** was further alkylated in the presence of excess *iso*-propylamine to provide D_6-labeled PU-H71 **139** in 92% yield (72 mg).

[56] S. R. Ambatia, E. Caldas Lopes, K. Kosugi, U. Mony, A. Zehir, S. K. Shahd, T. Taldone, A. L. Moreira, P. A. Meyers, G. Chiosis, M. A.S. Moore, *Mol. Oncol.* **2014**, *8*, 323-336.
[57] T. Taldone, D. Zatorska, Y. Kang, G. Chiosis, *J. Label Compd. Radiopharm.* **2010**, *53*, 47-49.
[58] H. He, D. Zatorska, J. Kim, J. Aguirre, L. Llauger, Y. She, N. Wu, R. M. Immormino, D. T. Gewirth, G. Chiosis, *J. Med. Chem.* **2006**, *49*, 381-390.



SCHEME 30

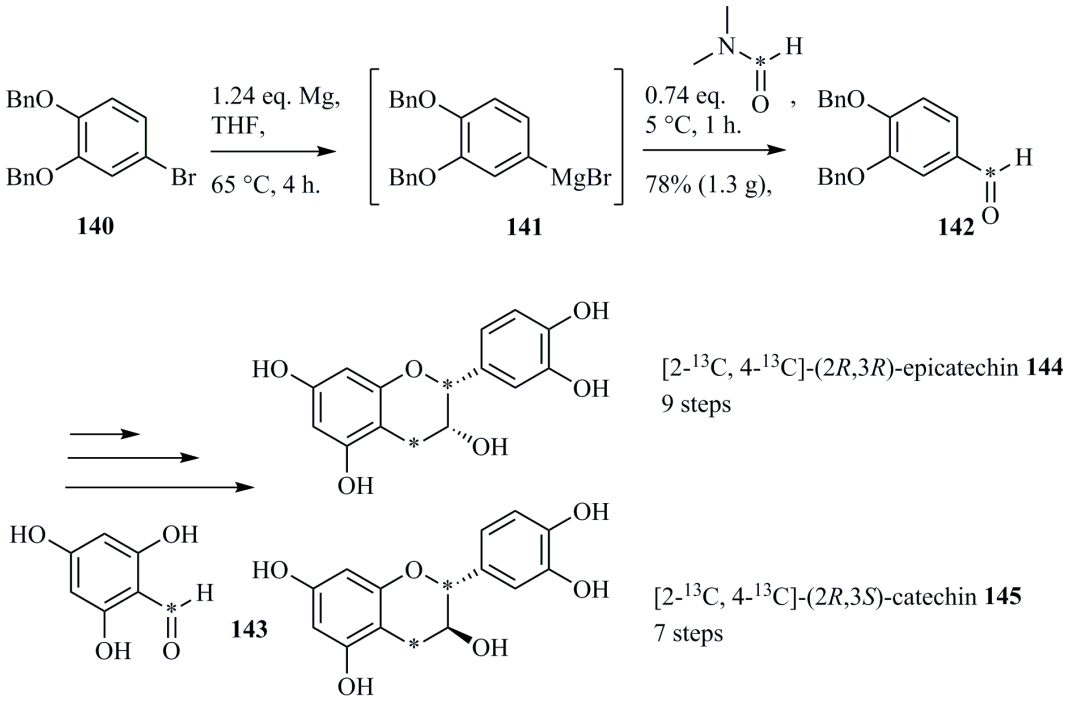
N,N-Dimethyl[^{13}C]formamide

Highly functionalized ^{13}C-labeled benzaldehydes are especially important, as they appear to be key intermediates in numerous syntheses of natural products and pharmaceutical drugs. As reported in the literature, *N,N*-dimethyl[^{13}C]formamide (DMF) (1 g/400 $) is an excellent source of ^{13}C to introduce the labeled formyl group in a single step, using either *ortho*-metalation, halogen metal exchange or a Vilsmeier-Haack formylation, as presented in Schemes 31 and 32.

In addition to the investigations already conducted *in vitro* and *in vivo* with flavanols, an important class of polyphenolic compounds found especially in foods and beverages, Sharma and Coll. achieved the synthesis of [2-^{13}C, 4-^{13}C]-*(2R,3S)*-catechin **145** and [2-^{13}C, 4-^{13}C]-*(2R,3R)*-epicatechin **144**, as depicted in Scheme 31.[59] It should be pointed out that the most common flavanols in plants are (-)-epicatechin and (+)-catechin, and regular consumption of flavanol-rich foods and beverages has been associated with health benefits, especially in the context of cardiovascular disease. The protected 4-bromo-catechol **140** was treated with magnesium metal in refluxing THF containing a catalytic amount of 1,2-dibromoethane to furnish the corresponding Grignard intermediate **141**.

[59] P. K. Sharma, M. He, L. J. Romanczyk, H. Schroeter, *J. Label Compd. Radiopharm.* **2010**, *53*, 605-612.

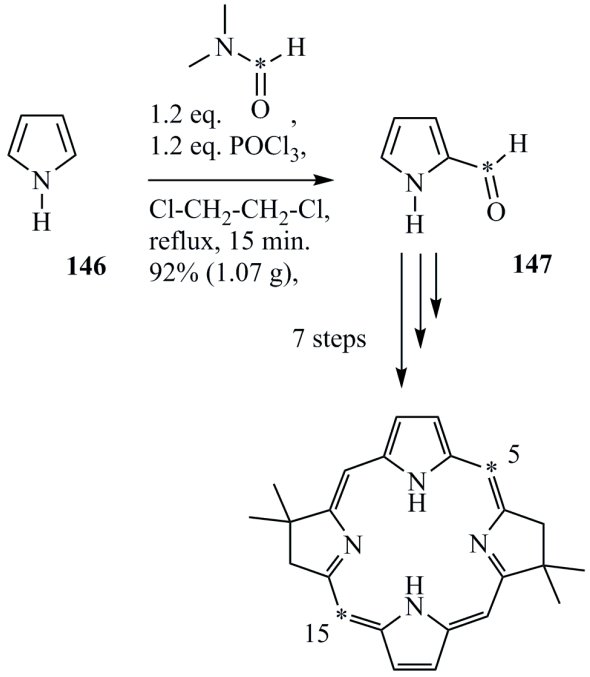
This was subsequently quenched with *N,N*-dimethyl-[^{13}C]-formamide to afford the required 4-*bis*(benzyloxy)benz-[2-^{13}C]-aldehyde **142** in 78% yield (1.3 g). From this latter labeled aldehyde **142**, the target molecules [2-^{13}C, 4-^{13}C]-(2*R*,3*S*)-catechin **145** and [2-^{13}C, 4-^{13}C]-(2*R*,3*R*)-epicatechin **144** were obtained following known seven- and nine-step sequences, respectively, involving a second ^{13}C-labeled benzaldehyde **143**.



SCHEME 31

Lindsey and Coll. have recently described an elegant strategy to prepare bacteriochlorins with the incorporation of pairs of ^{13}C or ^{15}N atoms in a site-specific manner, using inexpensive labeled starting materials such as *N,N*-dimethyl[^{13}C]formamide, $^{13}CH_3NO_2$, K^{13}CN and $CH_3{}^{15}NO_2$.[60] Thus, following a standard procedure, Vilsmeier-Haack formylation of pyrrole **146** (see Scheme 32) with a slight excess of *N,N*-dimethyl[^{13}C]formamide and $POCl_3$ in 1,2-dichloroethane yielded the pyrrole-2-[^{13}C]carboxaldehyde (5-^{13}Cformyl) **147** in 82% yield (1.07 g). The target bacteriochlorin-[^{13}C5,15] **148** was prepared in 7 steps from pyrrole-2-[^{13}C]carboxaldehyde **147**. In this impressive work, 24 bacteriochlorin isotopologues, each containing a symmetrical pair of ^{13}C or ^{15}N atoms in the inner core of the macrocycle, were prepared starting from simple commercially available labeled compounds.

[60] C.-Y. Chen, D. F. Bocian, J. S. Lindsey, *J. Org. Chem.* **2014**, *79*, 1001-1016.



bacteriochlorin-[^{13}C5,15]- **148**

SCHEME 32

Conclusion

The recent developments in organic chemistry applied to labeled molecule synthesis together with the pioneering work in this field have led to the efficient preparation of a broad range of compounds with the required isotopic purity as well as the desired number of mass units. Despite these important advances, a combination of industrial and academic research efforts, there are still many problems to be solved. The site-specific introduction of stable isotopes into molecules, involving a rational multi-step synthesis from suitable commercial labeled materials or reagents, mostly uses a different pathway from the one used for the unlabeled target. A time-consuming optimization of each step with unlabeled material is also necessary. The availability of more labeled starting materials and reagents at reasonable cost from suppliers or following efficient synthesis will greatly facilitate the preparation of the target molecules using their existing unlabeled parent synthetic pathway. In order to reach this goal, organic chemists should develop highly efficient tools as well as new approaches, particularly for introducing ^{13}C and ^{15}N atoms. However, this difficult but challenging and exciting task appears to be currently less attractive to academic chemists and various elegant syntheses of complex labeled molecules have been successfully conducted in pharmaceutical laboratories, as presented in this microreview.

The author would like to thank Professor François-Xavier Felpin for fruitful discussions and comments.

PORTRAITS

Dr. Maud ANTOINE
Chef de projet

Le **Dr. Maud ANTOINE**, chef de projet chez AtlanChim Pharma depuis 2010 est titulaire d'un Doctorat en chimie organique avec une spécialité en chimie thérapeutique réalisé à la faculté de pharmacie de Nantes. En charge du pôle analytique depuis 2013, Elle met au point les conditions de séparation en HPLC et réalise les purifications en phase inverse (flash et semi-préparative) des composés synthétisés par nos chimistes. Par ailleurs, elle est également responsable des projets d'isolement et de caractérisation d'impuretés ainsi que du développement et de la validation de méthodes analytiques en HPLC pour nos clients.

Dr. Maud ANTOINE, project manager at AtlanChim Pharma since 2010, holds a PhD in organic chemistry with a specialty in therapeutic chemistry obtained at the "Faculté de Pharmacie de Nantes". She has been managing the AtlanChim Pharma analytical department since 2013. She develops HPLC analytical conditions for separation and also purification (C18 flash and semi-prep) of compounds synthesized by our chemists. Furthermore, she is in charge of isolation and characterization of impurities as well as HPLC development and validation methods for our customers.

Dr. Kévin FOURMY
Chef de projet

Le **Dr. Kévin Fourmy**, est chef de projet chez AtlanChim Pharma depuis 2015. Après des études d'ingénieur en chimie organique et un master recherche à l'école Nationale Supérieure de Chimie de Clermont-Ferrand, Kévin a obtenu un Doctorat en chimie organométallique au Laboratoire de Chimie de Coordination de Toulouse en travaillant sur des complexes d'or et de platine. Il a ensuite complété sa formation par un contrat post doctoral sur le développement de méthodologies d'organo-catalyse à l'Institut de Chimie des Substances Naturelles dans le groupe d'Angela Marinetti. Actuellement, le Dr. Kévin FOURMY synthétise et optimise des contrats industriels de recherche en synthèse à façon de molécules complexes.

Dr. Kevin FOURMY joined AtlanChim Pharma at the beginning of 2015 as a project manager. After graduating from the "Ecole Nationale Supérieure de Chimie" of Clermont-Ferrand as an organic chemistry engineer, Kevin obtained a PhD in organometallic chemistry at the "Laboratoire de Chimie de Coordination" of Toulouse where he worked on gold and platinum complexes. He then did a postdoctoral position working on organo-catalytic reactions at the "Institut de Chimie des Substances Naturelles" in Angela Marinetti's group. Dr. Kevin FOURMY is now in charge of the synthesis and optimization of industrial contracts of research in custom-chemical synthesis of complex molecules.

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