SCIENTIFIC LETTER N°12

1
Edito

ATLANCHIM PHARMA

2-14
Science

Most recent advances in the preparation of labeled substances with D, ^{13}C, ^{15}N, ^{18}O and ^{34}S atoms.

Edito

Depuis plus de 15 ans, AtlanChim Pharma vous accompagne dans vos projets de synthèse à façon, d'isolements d'impuretés et de marquage à froid en adoptant une approche résolument orientée client, tout en vous apportant une grande réactivité. Ainsi au travers de leur forte expertise scientifique, nos Chefs de Projet seront en mesure d'appréhender vos attentes et vos besoins, et communiqueront régulièrement avec vous tout au long de votre projet.

Cette 12ème Lettre Scientifique intitulée « Most recent advances in the preparation of labeled substances with D, ^{13}C, ^{15}N, ^{18}O and ^{34}S atoms » fait suite à notre dernier webinaire du 27 Novembre 2018 et complète la Lettre Scientifique N°9 de Décembre 2015 sur les composés marqués.

Nous profitons de cet édito pour vous inviter à nous rencontrer lors de la 55ème édition du RICT qui se déroulera du 3 au 5 Juillet 2019 à la Cité des Congrès de Nantes (France). AtlanChim Pharma exposera lors de ce salon et nous serons ravis d'échanger avec vous sur vos besoins en chimie fine.
https://www.rict2019.org/

Le professeur Jacques Lebreton ainsi que toute l'équipe d'AtlanChim Pharma vous souhaite une agréable lecture.

Editorial

Since 15 years, Atlanchim Pharma collaborates to your projects of customs synthesis, stable isotope labeling and isolation of impurities, with a customer-oriented approach and a high reactivity. Thanks to their strong scientific expertise, our Project managers will be able to understand your expectations and needs and will regularly communicate with you throughout your project.

This 12th Scientific Letter entitled "Most recent advances in the preparation of labeled substances with D, ^{13}C, ^{15}N, ^{18}O and ^{34}S atoms" follows our last webinar on November 27th 2018 and completes our 9th Scientific Letter from December 2015 on labeled compounds.

We take the opportunity of this editorial to invite you to meet us at the 55th edition of the RICT which will take place from July 3rd to 5th 2019 at the Cité des Congrès in Nantes (France). AtlanChim Pharma will be exhibiting at this event and we will be happy to discuss with you about your needs in fine chemistry.
https://www.rict2019.org/

Professor Jacques Lebreton, as well as all the Atlanchim Pharma's team wish you a pleasant reading.

Most recent advances in the preparation of labeled substances with D, ^{13}C, ^{15}N, ^{18}O and ^{34}S atoms.

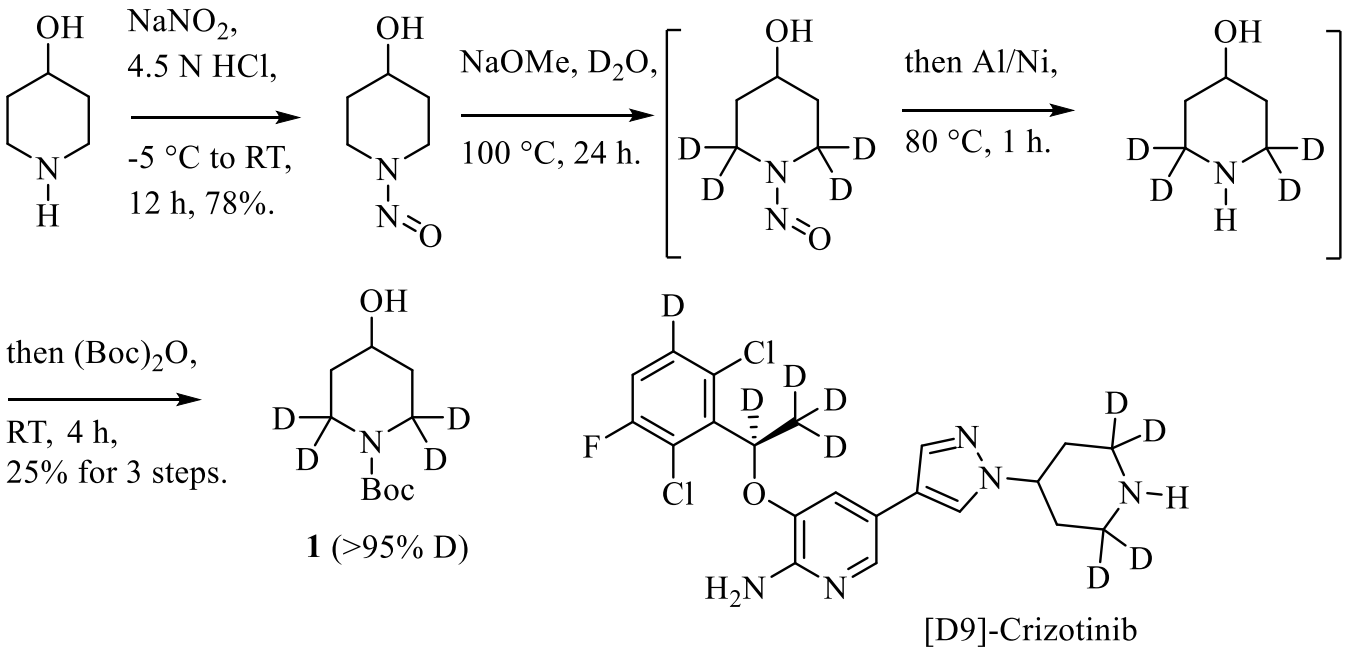
Jacques Lebreton
jacques.lebreton@atlanchimpharma.com

To complete our AtlanChimPharma Scientific Letter N°9, December, 2015 "A journey to the synthesis of isotopically stable labeled compounds", we would like to share with you some interesting and very recent work on the synthesis of labeled compounds.

Deuterium-hydrogen exchange

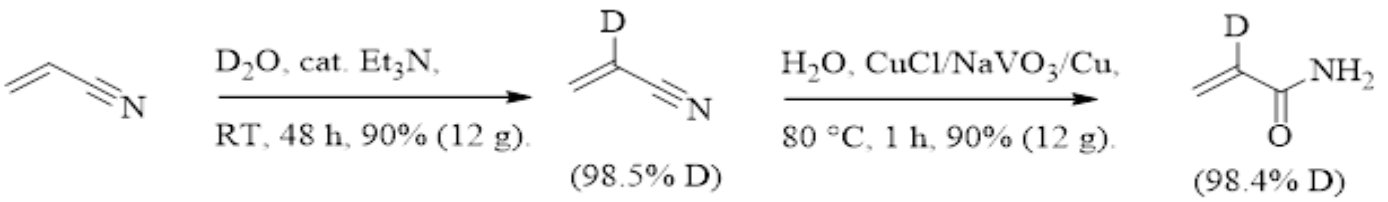
The use of *N*-nitroso-amine intermediates to exchange a-hydrogen atoms of amine function is an efficient process to prepare selectively a-deuterated amines as reported in Scheme 1[1]. Base catalyzed (NaOD formed *in situ* from MeONa in D_2O) hydrogen/deuterium isotope exchange on *N*-nitroso derivatives led to the corresponding a-deuterated nitrosamines which after reduction of the nitroso group with aluminum-nickel (Al-Ni) alloy[2] in alkaline solution (*in situ* generation of Raney-Ni) gave the fully a-deuterated amines[3]. This efficient method was used with success to obtain the 4-[2,2,6,6-D_4]-hydroxypiperidine **1** (Scheme 1) which is a key intermediate to prepare racemic [D9]-Crizotinib as internal standards in the drug clinical study. Crizotinib is the first anaplastic lymphoma kinase (ALK) inhibitor for the treatment of patients with advanced non-small cell lung cancer (NSCLC) marketed by Pfizer under the trade name Xalkori (first approved August 26, 2011).

[1]W. Ao, Y. Li, Y. Zhang, *J. Label. Compd. Radiopharm*. **2018**, *61*, 1036-1042.
[2L]L. K. Keefer, G. Lunn, G. *Chem. Rev*. **1989**, *89*, 459-502.
[3]For previous examples, see: (a) M. Pacchioni, A. Bega, A. C. Fabretti, D. Rovai, A. Cornia, *Tetrahedron Letters* **2002**, *43*, 771-774. (b) R. N. Loeppky, H. Xiong, *J. Label. Compd. Radiopharm*. **1994**, *34*, 1099-1110.



SCHEME 1

A synthesis of 2-*d*-acrylamide has been published based on hydration of acrylonitrile in D_2O followed by an efficient hydrolysis of nitrile using a 3-component catalytic system $CuCl/NaVO_3/Cu$ in H_2O without the loss of deuterium at C2 by exchange, as reported in Scheme 2.[4]

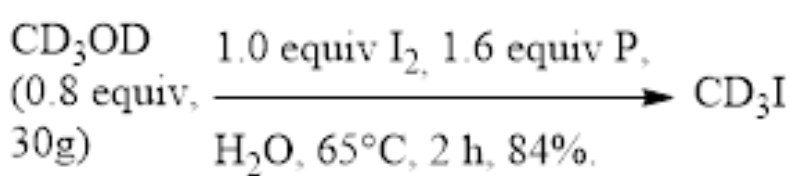


SCHEME 2

[4]A. V. Salin, R. Z. Musin, *J. Label. Compd. Radiopharm*. **2018**, *61*, 595-598.

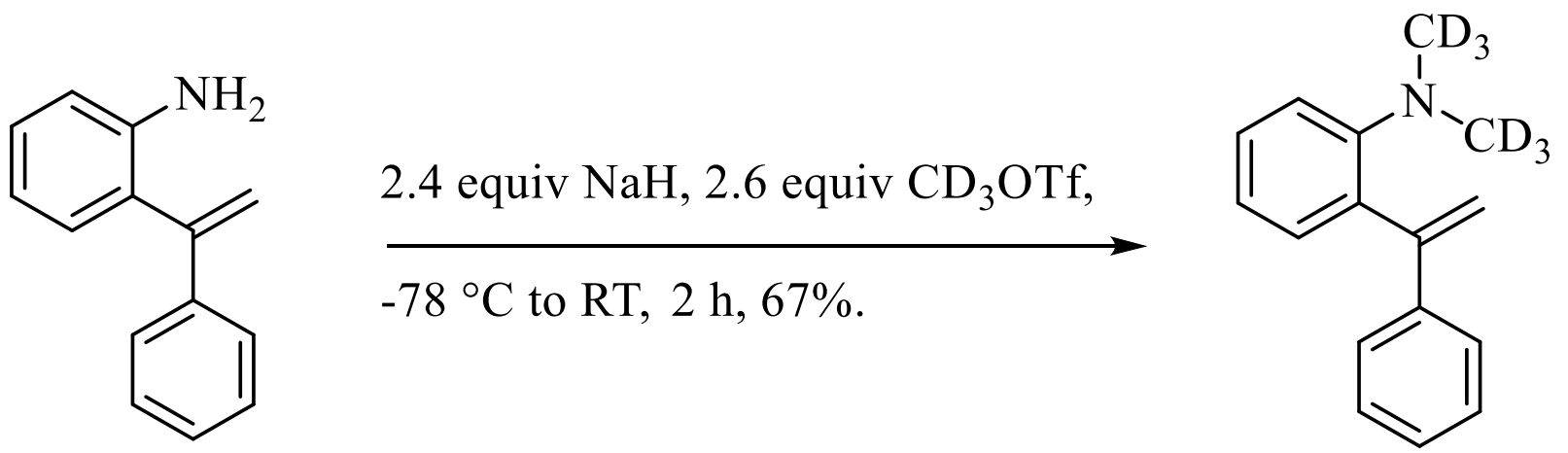
Labeled starting reagent: Methyl iodide.

Methyl iodide is one of the most popular labeled reagent and is commercially available in three highly enriched isotopically labeled forms: $^{13}CH_3I$, $^{13}CD_3I$ and CD_3I. In the other hand, these labeled reagents could be prepared in large scale from their corresponding methanol isotopologues, as shown in Scheme 3.[5]



SCHEME 3

Methyl iodide is an excellent electrophile and most frequently used with carbanions or enolates as well as in *N*-, *O*- and *S*-methylation reactions. It should be pointed out that methyl triflate is more reactive compared to methyl iodide, and also less volatile, as illustrated in Scheme 4.[6]

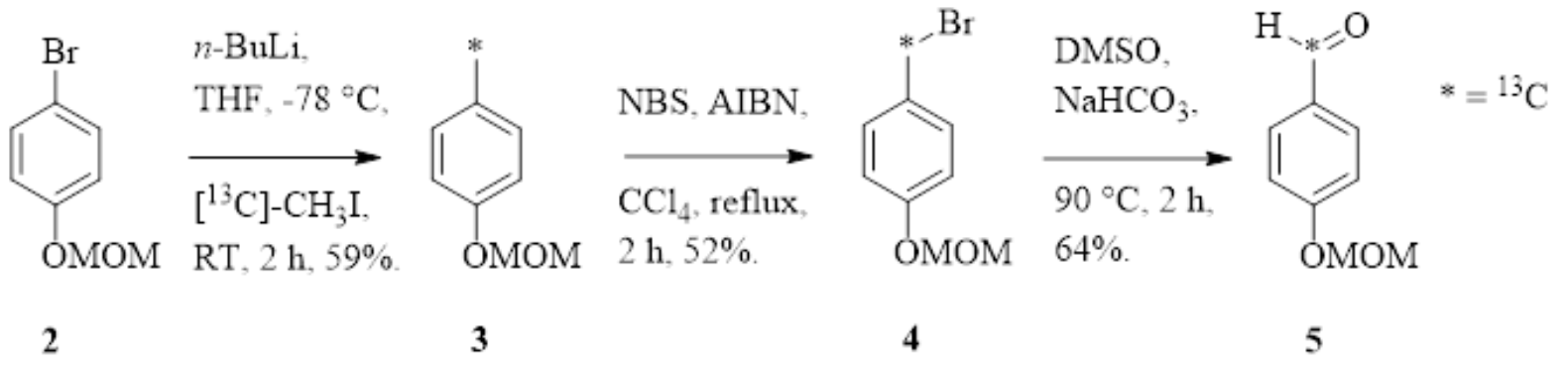


SCHEME 4

[5]Y. Tu, J. Zhong, H. Wang, J. Pan, Z. Xu, W. Yang, Y. Luo, *J. Label. Compd. Radiopharm.* **2016**, *59*, 546-551.

[6]T. Torigoe, T. Ohmura, M. Suginome, *Angew. Chem. Int. Ed*. **2017**, *56*, 14272-14276.

An interesting sequence to prepare [^{13}C]-benzaldehyde derivative **5** from their corresponding [^{13}C]-labeled methyl precursor **3** has been developed by Blakemore[7] based on a Wohl-Ziegler radical bromination followed by a Kornblum oxidation of the resulting bromobenzyl intermediate **4** as presented in Scheme 5. The key labeled [^{13}C]-**3** was prepared in 59% yield from bromobenzene **2** by bromine-lithium exchange followed by alkylation of the resulting carbanion with [^{13}C]-methyl iodide.

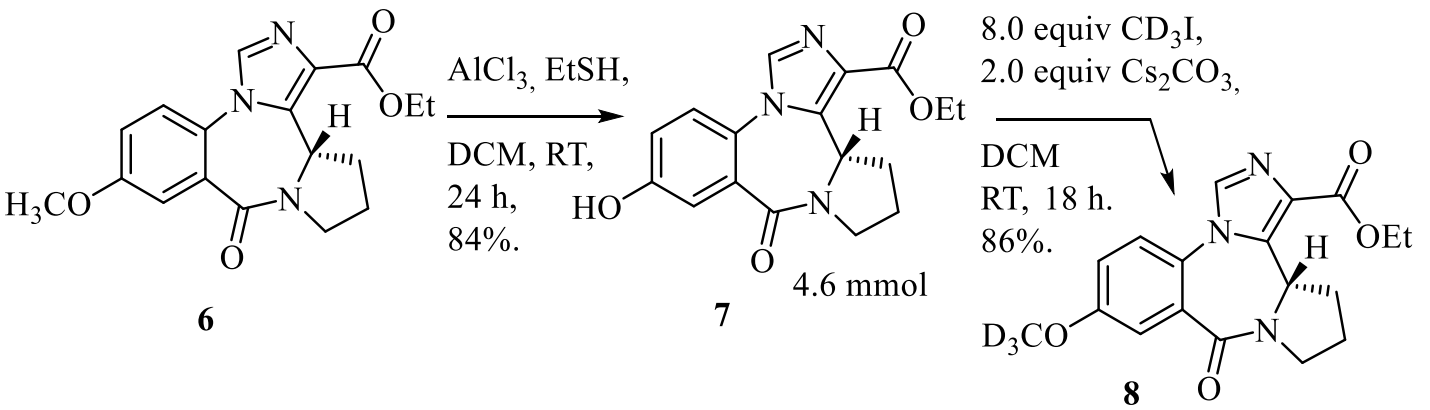


SCHEME 5

As part of the development of new treatments for asthma, various imidazobenzodiazepine derivatives, such as compound **6**, have been prepared and evaluated as a selective $\alpha4\beta3\gamma2$ $GABA_AR$ ligand. A deuterated more metabolically stable ligand **8** has been obtained following a demethylation-alkylation sequence as depicted in Scheme 6.[8] The imidazobenzodiazepine ester **6** was chemoselectively *O*-demethylated using $AlCl_3$ and EtSH to provide the corresponding phenol **7** which was then re-alkylated with CD_3I leading to the desired deuterated compound **8** in good overall yield.

[7]D. C. Ellinwood, M. F. El-Mansy, L. S. Plagmann, J. F. Stevens, C. S. Maier, A. F. Gombart, P. R. Blakemore, *J. Label. Compd. Radiopharm*. **2017**, *60*, 639-648.

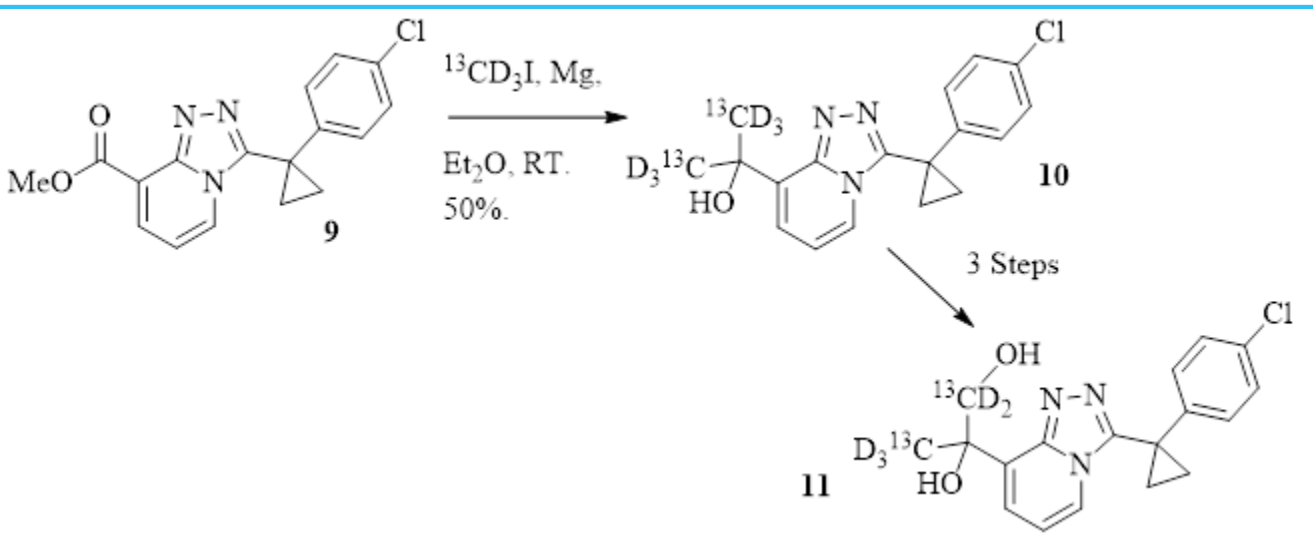
[8]L. A. Arnold *et coll*., *Eur. J. Med. Chem*. **2017**, *126*, 550-560



SCHEME 6

If methyl iodide has been extensively used as an alkylating agent, their corresponding organometallic derivatives such as Grignard reagents, methyllithium and lithium dimethylcuprate [$(CH_3)_2CuLi$] are excellent nucleophilic species, which can be engaged in various types of transformations, such as substitution or addition reactions with appropriate substrates.

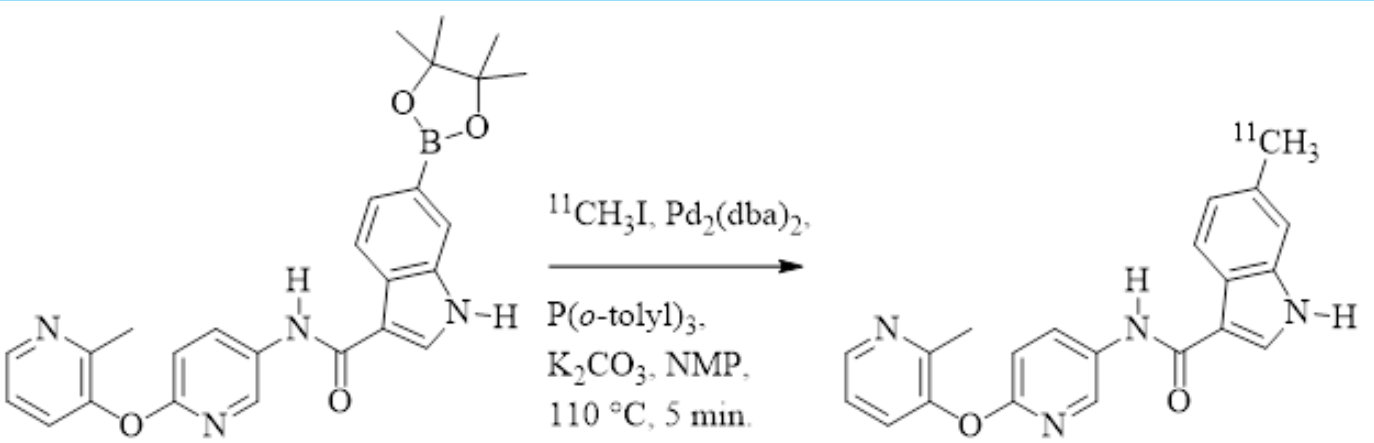
During human ADME clinical study of BMS-823778 (inhibitor of 11β-hydroxysteroid dehydrogenase type 1 to treat type 2 diabetes) the stable-isotope labeled metabolite standard **11** has been prepared as presented in Scheme 7.[9] The methyl ester intermediate **9** was treated with $^{13}CD_3MgI$ to afford the labeled tertiary alcohol **10** in 50% yield.



SCHEME 7

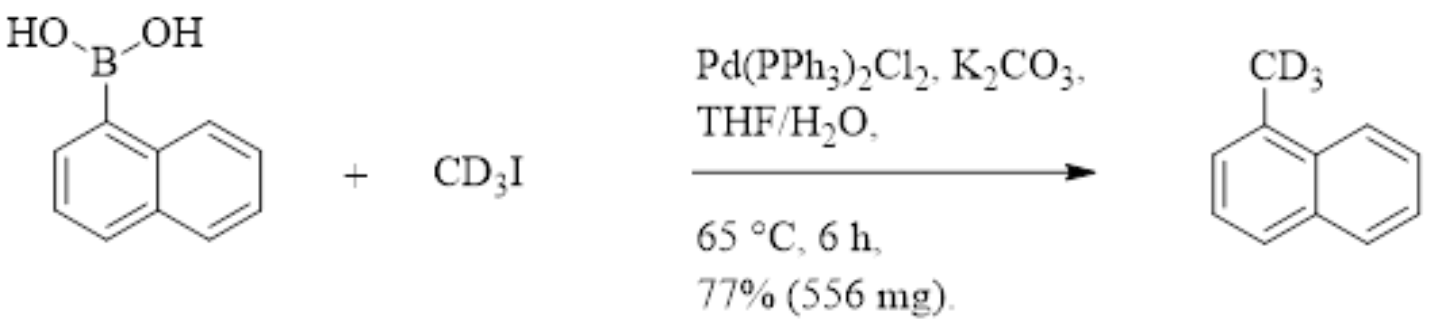
[9]B. D. Maxwell, S. B. Tran, M. Lago, J. Li, S. J. Bonacorsi, *J. Label. Compd. Radiopharm.* **2016**, *59*, 255-259.

In the last decade, the development of palladium-mediated cross-coupling reactions using methyl iodide has significantly extended the scope of this reagent for the preparation of labeled compounds. This methodology is currently very popular for ^{11}C-labeling with [^{11}C]-methyl iodide in the context of positron emission tomography (PET) as shown in Scheme 8.[10]



SCHEME 8

This efficient palladium-mediated C-C bond formation could expand the arsenal of methodologies to prepare labeled compounds either with $^{13}CH_3I$, $^{13}CD_3I$ or CD_3I. A recent example is presented in Scheme 9.[11]

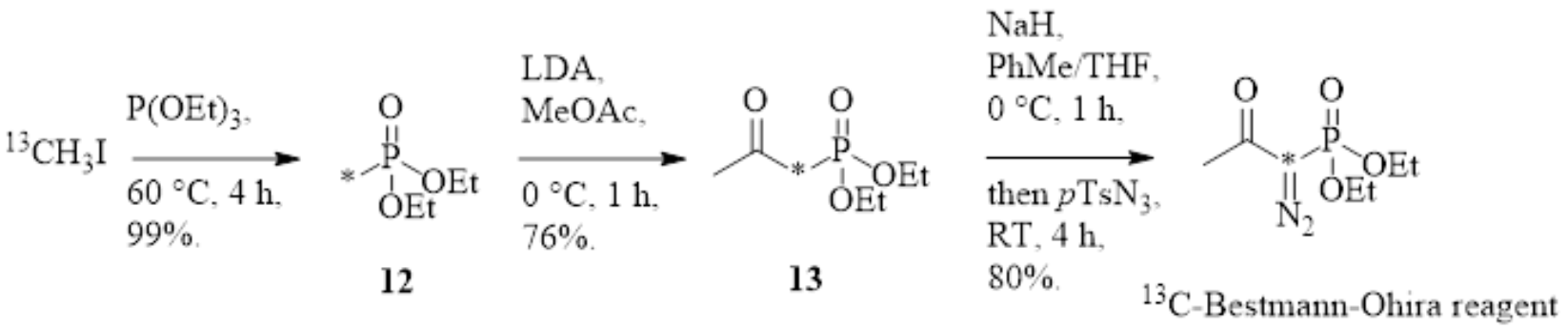


SCHEME 9

[10]F. Zeng, J. A. Nye, R. J. Voll, L. Howell, M. M. Goodman, *ACS Med. Chem. Lett*. **2018**, *9*, 188-192.
[11]G. Qin, Y. Wang, H. Huang, *Org. Lett*. **2017**, *19*, 6352-6335.

Labeled methyl iodide has also served as starting material in the preparation of various important labeled reagents.

The well-known Bestmann-Ohira reagent (diethyl(1-diazo-2-oxopropyl)phosphonate) is a unique and powerful reagent which allows the conversion of aldehydes into the corresponding terminal alkynes in good yields following a one-pot sequence under mildly basic conditions (K_2CO_3, MeOH, RT).[12] The ^{13}C-labeled Bestmann-Ohira reagent has been efficiently prepared from $^{13}CH_3I$, as reported in Scheme 10.[13] Treatment of $^{13}CH_3I$ with triethyl phosphite without solvent afforded the corresponding labeled diethyl methylphosphonate ester **12** through a Michaelis-Arbuzov reaction. Then acylation of the α-lithiated phosphonate anion of **12** led to the formation of β-oxophosphonate **13** which was deprotonated with sodium hydride in toluene, followed by treatment with tosyl azide to afford the ^{13}C-labeled Bestmann-Ohira reagent *via* a diazo-transfer.



SCHEME 10

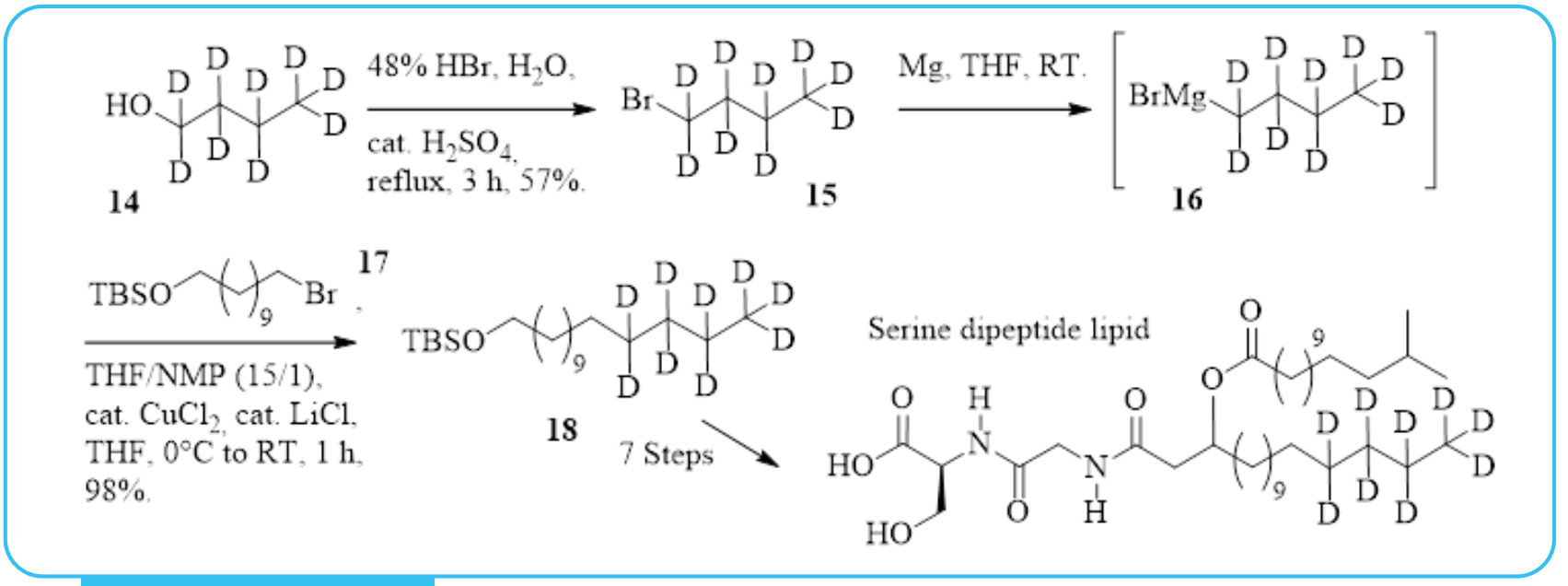
[12]For a recent review on this reagent, see: M. Dhameja, J. Pandey, *Asian J. Org. Chem*. **2018**, *7*, 1502-1523.

[13]A. Brunner, L. Hintermann, *Chem. Eur. J.* **2016**, *22*, 2787-2792.

From commercially available labeled starting materials

The commercial availability of suitable isotopically enriched starting materials is one of the limiting factors in many cases. Nevertheless, more and more labeled compounds are available from suppliers and allow to prepare the labeled molecules according to previous route developed in non-labeled series.

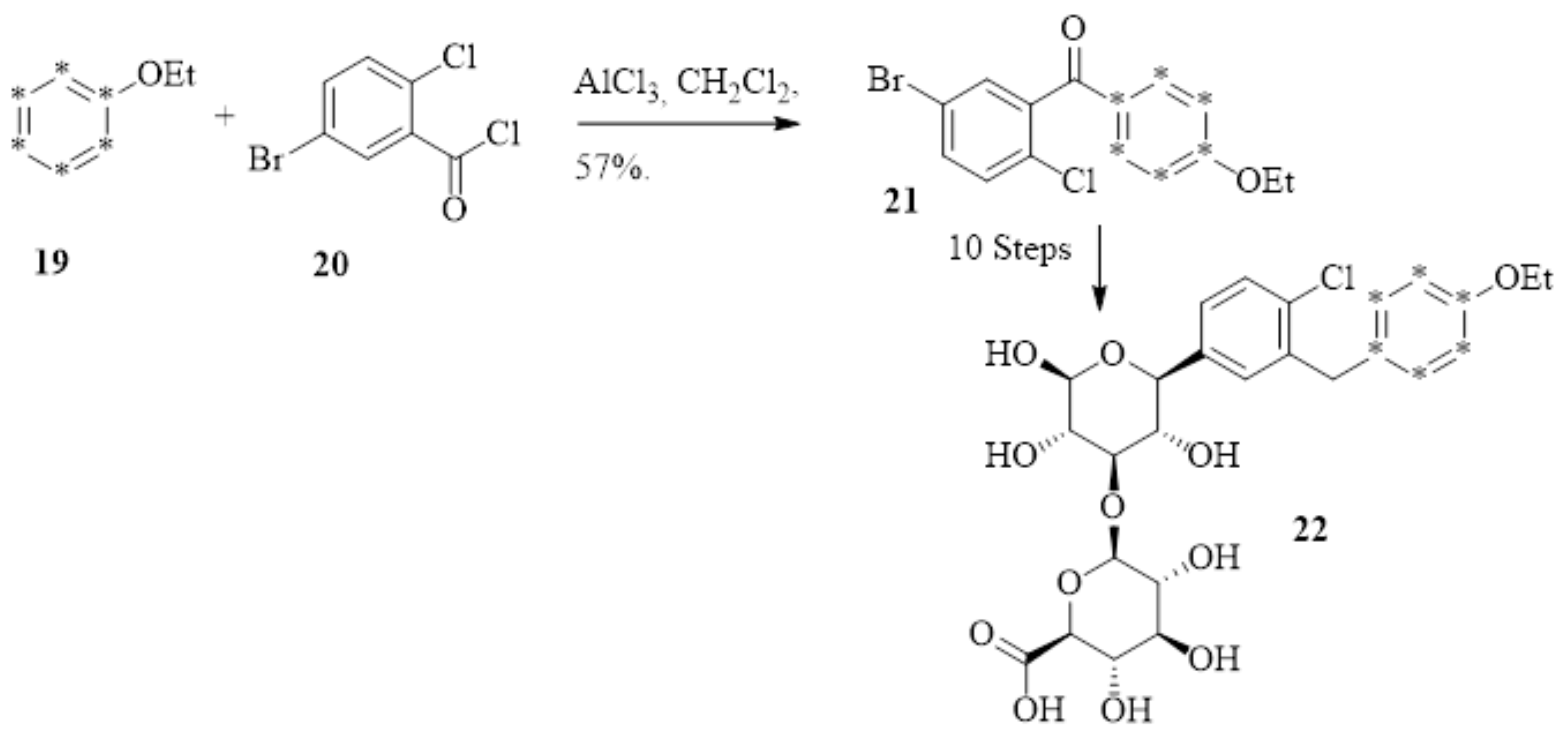
For example, commercially available [D_9]-butan-1-ol **14** was used as deuterated starting material for the preparation of deuterium-labeled internal standard Serine dipeptide lipid for accurate quantification of bacterial serine lipid in biological samples (Scheme 11).[14] Bromination of the deuterated alcohol **14** with 48% aqueous solution of HBr containing catalytic amount of H_2SO_4 gave the corresponding bromo derivative **15** in 57% yield. Then, Li_2CuCl_4-mediated C-C coupling reaction was carried out by addition of the Grignard reagent **16**, formed *in situ* from the previous bromo derivative **15**, to a solution of the protected 11-bromoundecanol **17** providing very efficiently the deuterated silyl ether **18** in 98% yield. The deuterium-labeled Serine dipeptide lipid was obtained in 9 steps from [D_9]-butan-1-ol **14**.



SCHEME 11

[14]C Dietz, R. B. Clark, F. C. Nichols, M. B. Smith, *J. Label. Compd. Radiopharm*. **2017**, *60*, 274-285.

In numerous synthesis of labeled pharmaceuticals and their metabolites containing at least one aromatic ring, commercially available uniformly labeled-[$^{13}C_6$] aromatic starting materials are often used to prepare labeled targeted standards according to the original synthetic route of the unlabeled reference, as outlined in Scheme 12.[15] The synthesis of glucuronide metabolite of Dapagliflozin **22** (used to treat type 2 diabetes) started with a Friedel-Crafts acylation of the [$^{13}C_6$]-phenetole **19** with 5-bromo-2-chlorobenzoyl chloride **20** followed by separation of the two regioisomers by crystallization to furnish the desired labeled *para*-benzophenone **21**.[16] This latter intermediate **21** was used to obtain the [$^{13}C_6$]-glucuronide metabolite of Dapagliflozin **22**, an internal standard for the urine analysis of patients in clinical studies.



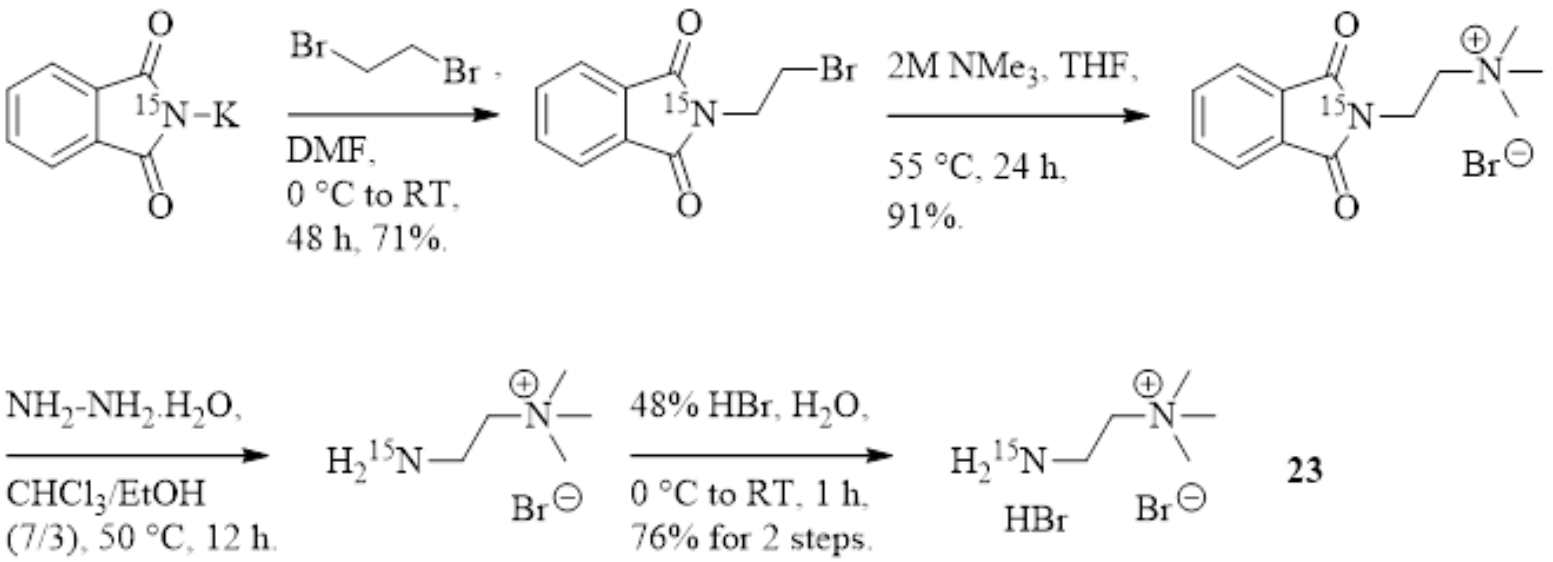
SCHEME 12

[15]K. Cao, J. A. Brailsford, M. Yao, J. Caceres-Cortes, R. Espina, S. J. Bonacorsi, *J. Label. Compd. Radiopharm*. **2016**, *59*, 150-159.

[16]W. N. Washburn *and coll*., *J. Med. Chem*. **2008**, *51*, 1145-1149.

Preparation of ^{15}N-enriched compounds

Concerning the preparation of ^{15}N-enriched compounds, the choice of ^{15}N-labeled reagents is considerably limited, $^{15}NH_4Cl$, $KC^{15}N$ and ^{15}N-phthalimide potassium salt are the most popular. As an illustration, ^{15}N-cholamine bromide hydrobromide **23** was prepared from ^{15}N-phthalimide potassium salt (see Scheme 13).[17] In this large scale synthesis of ^{15}N-cholamine bromide hydrobromide **23**, the final cleavage of the phthalimido group was more efficient using hydrazine compared to alkaline hydrolysis[18] with the formation of phthalic acid byproduct which was difficult to remove.



SCHEME 13

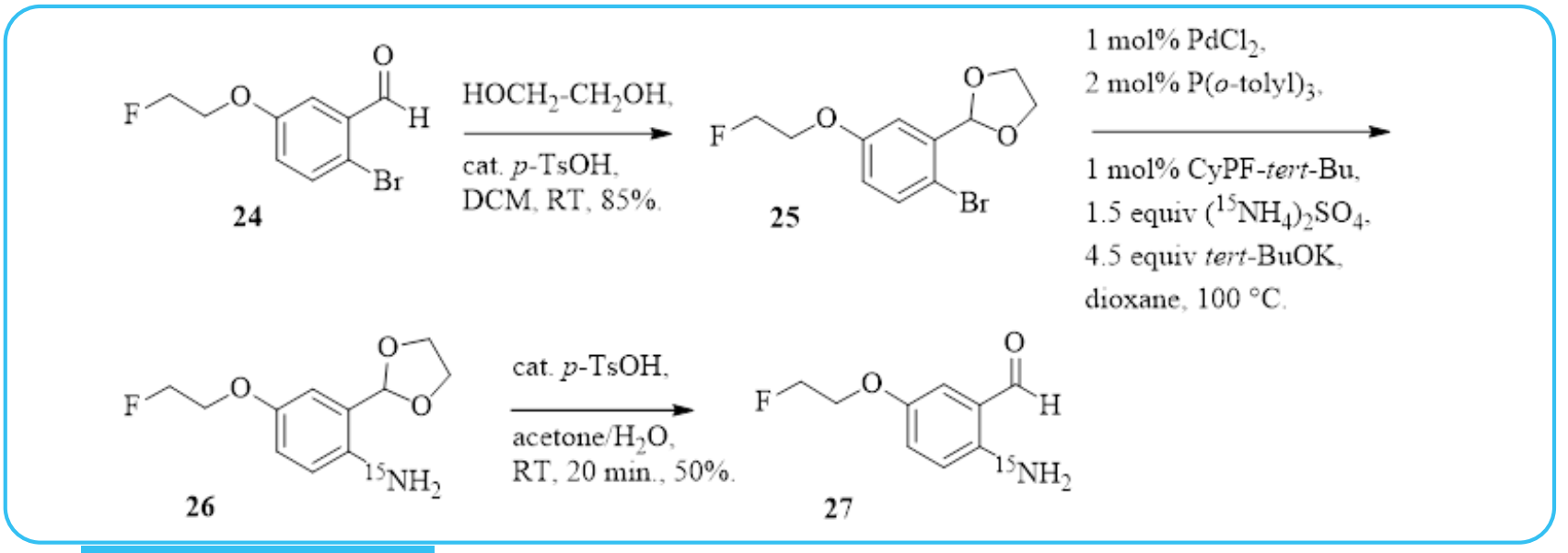
An efficient preparation of ^{15}N-THK-523, a Tau protein binder, using a palladium-catalyzed amination of aryl bromide intermediate was published in 2017 by Skrydstrup, as outlined in Scheme 14.[19] The application of the Hartwig protocol[20] on protected aryl bromide **25** using ^{15}N-ammonium sulfate as a source of ammonia provided the desired aniline **26** which was directly treated in acidic conditions leading to the 2-aminobenzaldehyde **27** in 50% overall yield. It should be pointed out that the protection of the aldehyde function of **24** as acetal is crucial for the palladium-catalyzed amination step. It is noteworthy that methodology avoided the nitration with an excess of expensive ^{15}N-labeled nitric acid followed by reduction into aniline derivatives to provide the desired ^{15}N-labeled aromatic compounds.

[17] J. Szeto, R. Lemoine, R. Nguyen, L. L. Olson, M. J. Tanga, *J. Label. Compd. Radiopharm.* **2018**, *61*, 391-394.

[18] F. Tayyari, G. A. N. Gowda, H. Gu, D. Raftery, *Anal. Chem.* **2013**, *85*, 8715-8721.

[19] K. T. Neumann, A. T. Lindhardt, B. Bang-Andersen, T. Skrydstrup, *J. Label. Compd. Radiopharm.* **2017**, *60*, 30-35.

[20] R. A. Green, J. F. Hartwig, *Org. Lett.*, **2014**, *16*, 4388-4391.



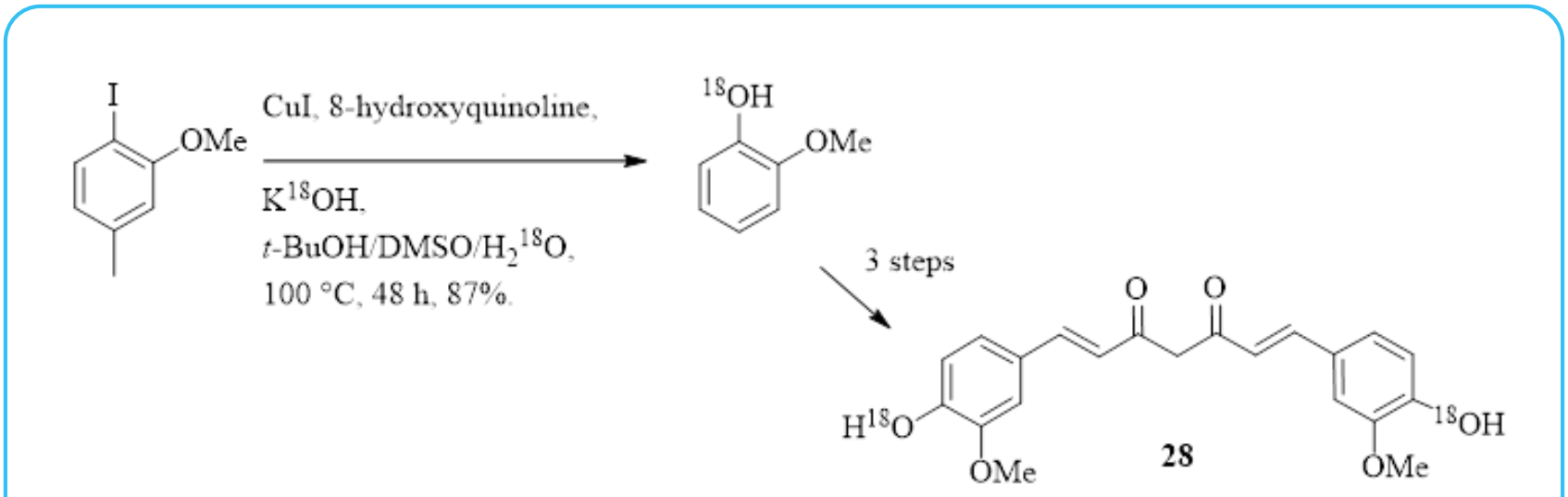
24 **25** **26** **27**

SCHEME 14

Preparation of ^{18}O-enriched compounds

Oxygen has two stable isotopes ^{17}O and ^{18}O which are naturally abundant in 0.037% and 0.204%, respectively. Synthesis of selectively ^{18}O-enriched compounds use in general ^{18}O-water as labeled reagent.[21]

^{18}O labeled curcumin **28** was successfully prepared based on a known copper-8-hydroxyquinoline-catalyzed direct hydroxylation of aryl iodides with K^{18}OH[22] as outlined in scheme 15.[23] Interestingly, K^{18}OH was obtained by the reaction of $H_2{}^{18}O$ with *tert*-BuOK in dry tetrahydrofuran and dried under vacuum before use.



28

SCHEME 15

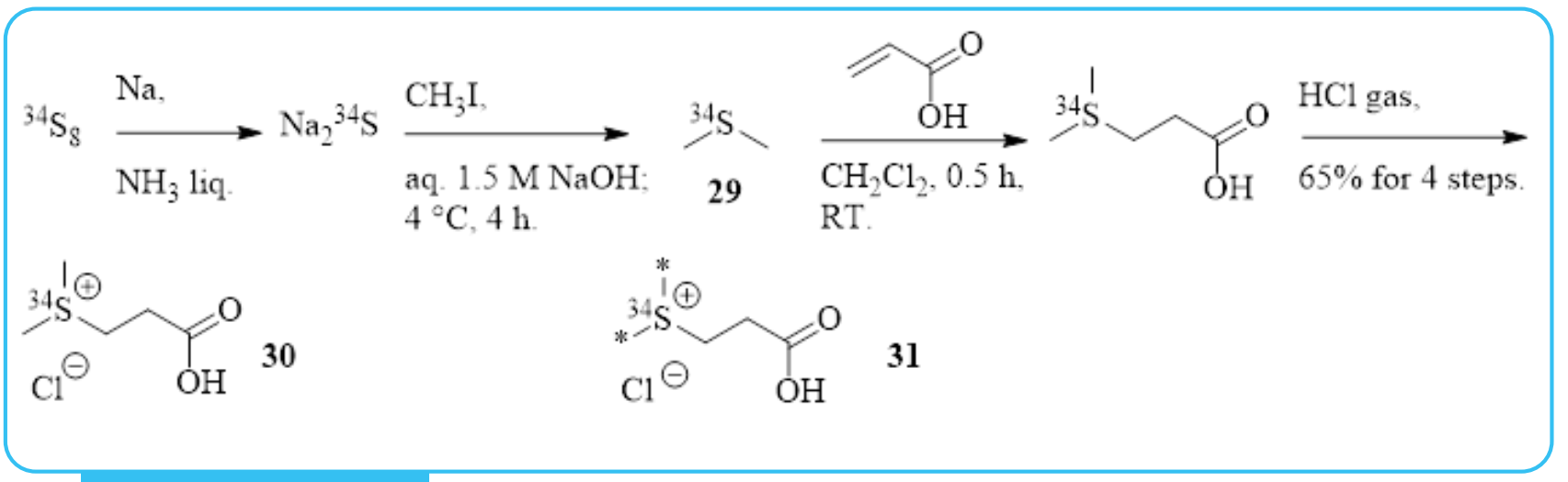
[21]For an excellent review on synthetic methodologies to introduce oxygen 17 and 18 atoms, see: V. Theodorou, K. Skobridis, D. Alivertis, I. P. Gerothanassisa, *J. Label. Compd. Radiopharm*. **2014**, *57*, 481-508.

[22]S. Maurer, W. Liu, X. Zhang, Y. Jiang, D. Ma, *Synlett* **2010**, 976-978.

[23]D. Gao, X. Chen, X. Yang, Q. Wu, F. Jin, H. Wen, Y. Jiang, H. Liu, *J. Am. Chem. Soc Mass Spectrom*. **2015**, *26*, 686-694.

Preparation of ^{34}S-enriched compounds

Sulfur 34 (^{34}S) is naturally abundant around 4% and is commercially available as elemental $^{34}S_8$ which is unfortunately quite expensive.[24] Nevertheless, due to the great importance of sulfur-containing natural products, various investigation of biosynthetic pathways have been studied using isotopically ^{34}S labeled compounds.[25] For example, the fate of dimethylsulfoniopropionate (DMSP) which is the main zwitterionic sulfur metabolite produced by many marine micro- and macro-algae in high intracellular concentrations, and the main source of marine organic sulfur, is not fully understood yet. In this context, a synthesis of (2-carboxyethyl)di(methyl)sulfonium-^{34}S hydrochloride **30**, as well as its isotopologue (2-carboxyethyl)di(methyl-^{13}C)sulfonium-^{34}S hydrochloride **31** from $^{34}S_8$ has been published (see Scheme 16).[26] Elemental $^{34}S_8$ was treated under Birch conditions and removal of ammonia provided the anhydrous $Na_2{}^{34}S$. Reaction of this latter salt in aqueous solution of NaOH under nitrogen with an excess of MeI gave the ^{34}S-dimethylsulfur **29** which after purification by distillation was directly engaged in a Michael addition with acrylic acid and finally exposed to dry HCl gas. The (2-carboxyethyl)di(methyl)sulfonium-^{34}S hydrochloride **30** was isolated as white crystals in 65% yield from elemental $^{34}S_8$. Following the same strategy, (2-carboxyethyl)di(methyl-^{13}C)sulfonium-^{34}S hydrochloride **31** was efficiently obtained using [^{13}C]-CH_3I.



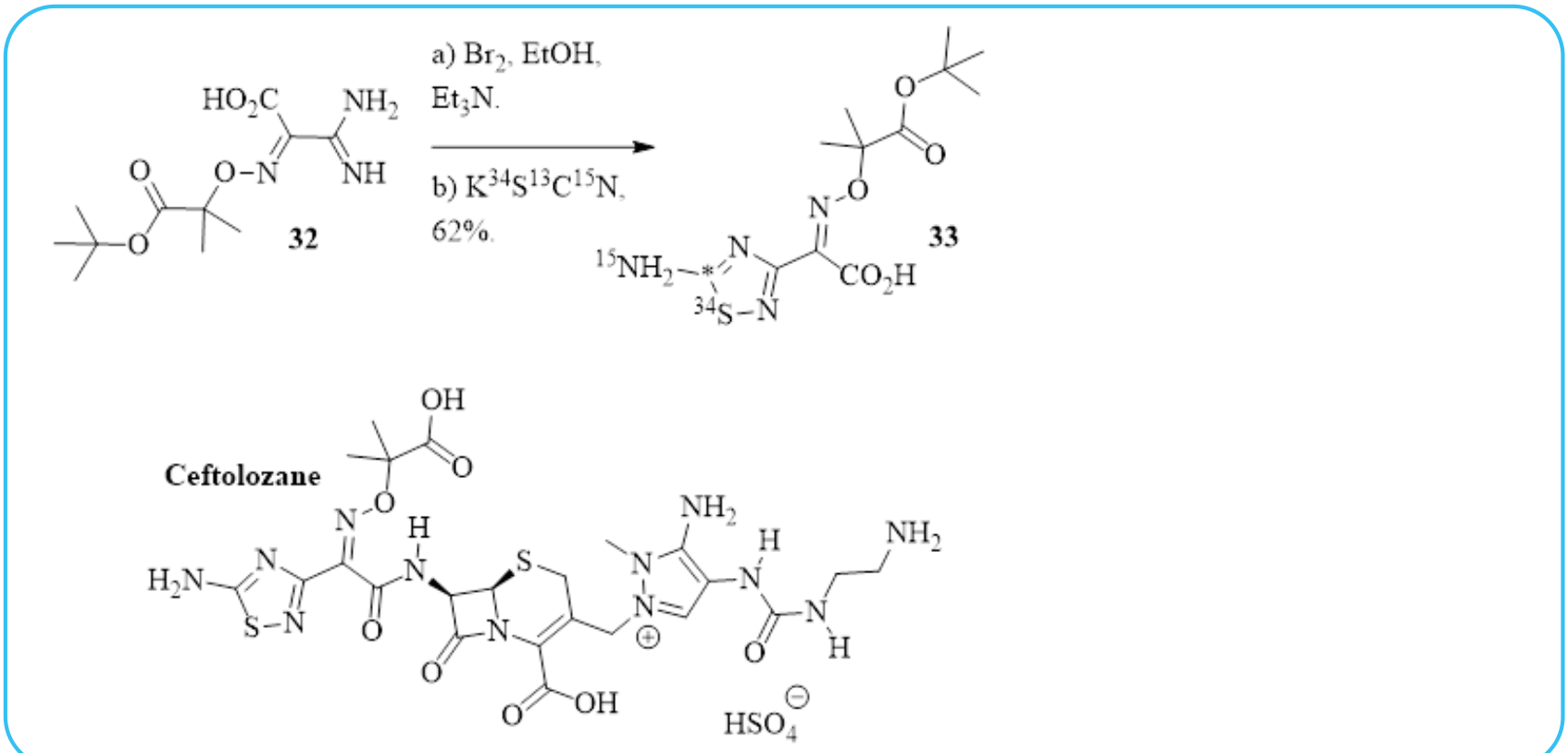
SCHEME 16

[24]For a recent review on synthesis of ^{34}S labeled compounds, see: S. Ren, P. S. Fier, H. Ren, A. J. Hoover, D. Hesk, R. Marques, I. Mergelsberg, *Chem. Eur. J.* **2018**, *24*, 7133-7136.

[25](a) J. Rinkel, J. S. Dickschat, *Beilstein J. Org. Chem*. **2015**, *11*, 2493-2508. (b) A. Spielmeyer, B. Gebser, G. Pohnert, *ChemBioChem* **2011**, *12*, 2276-2279.

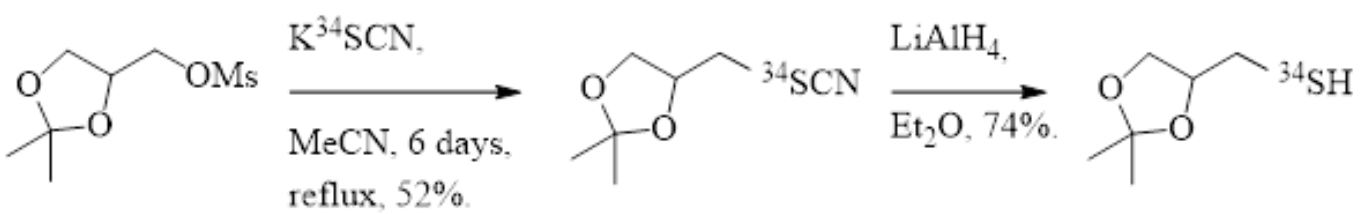
[26]J. S. Wirth, W. B. Whitman, *J. Label. Compd. Radiopharm*. **2019**, *62*, 52-58.

In the pharmaceutical industry, only one example of ^{34}S isotope labeled compound has been reported so far. At Merck, a synthesis of [^{13}C,^{15}N,^{34}S]-Ceftolozane, a fifth-generation Cephalosporin antibiotic developed for the treatment of infections with gram-negative bacteria, has been reported as outlined in Scheme 17.[24] The key labeled heterocyclic moiety **33** was obtained in 62% yield from the amidine **32** by treatment with bromine in ethanol followed by addition of $K^{34}S^{13}C^{15}N$. It should be noted that $K^{34}S^{13}C^{15}N$ was simply prepared by addition of $^{34}S_8$ into an aqueous solution of $K^{13}C^{15}N$ followed by two days at reflux, cooled, concentrated and then filtered.



SCHEME 17

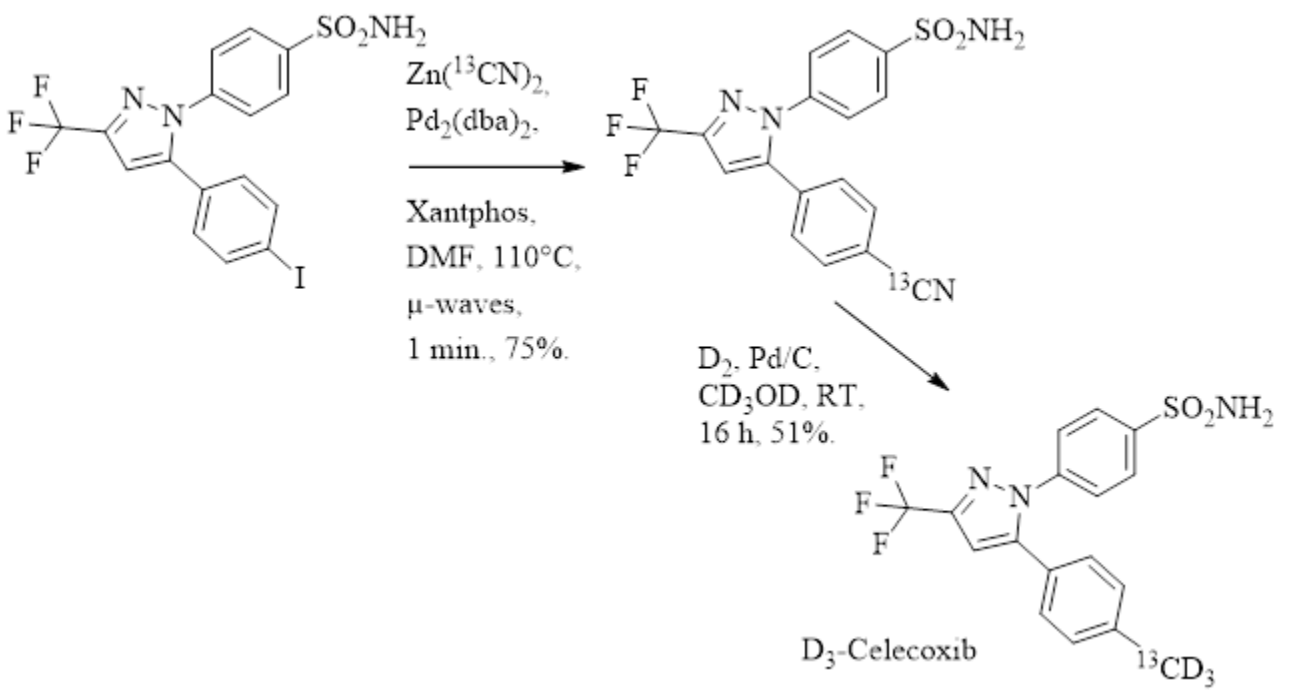
More generally, $K^{34}SCN$ is an excellent nucleophile to prepare labeled thiocyanate derivatives which could be reduced to the corresponding ^{34}S labeled thiols with $LiAlH_4$, as presented in the Scheme 18.[27]



SCHEME 18

An interesting sequence to end this *AtlanChimPharma Scientific Letter*

Palladium-catalyzed cyanation of aryl halides followed by hydrogenation of the resulting nitrile into the corresponding benzyl amine intermediates which by *in situ* hydrogenolysis led to the methyl derivatives. This elegant sequence was applied to the preparation of labeled Celecoxib (a nonsteroidal anti-inflammatory drug), as reported in Scheme 19.[28]



SCHEME 19

[27]E. Celik, M. Maczka, N. Bergen, T. Brinkhoff, S. Schulz, J. S. Dickschat, *Org. Biomol. Chem.* **2017**, *15*, 2919.

[28]K. Ellis-Sawyer, R. A. Bragg, N. Bushby, C. S. Elmore, M. J. Hickey, *J. Label. Compd. Radiopharm.* **2017**, *60*, 213-220.

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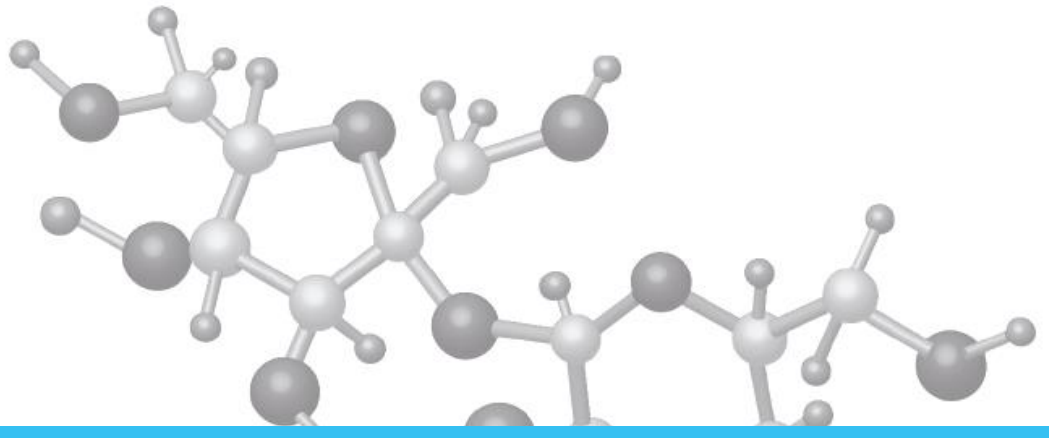

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