ChemComm

ROYAL SOCIETY OF CHEMISTRY

COMMUNICATION

View Article Online
View Journal | View Issue

Cite this: *Chem. Commun.*, 2021, **57**, 6680

Received 21st May 2021,
Accepted 26th May 2021

DOI: 10.1039/d1cc02665h

rsc.li/chemcomm

A general procedure for carbon isotope labeling of linear urea derivatives with carbon dioxide†

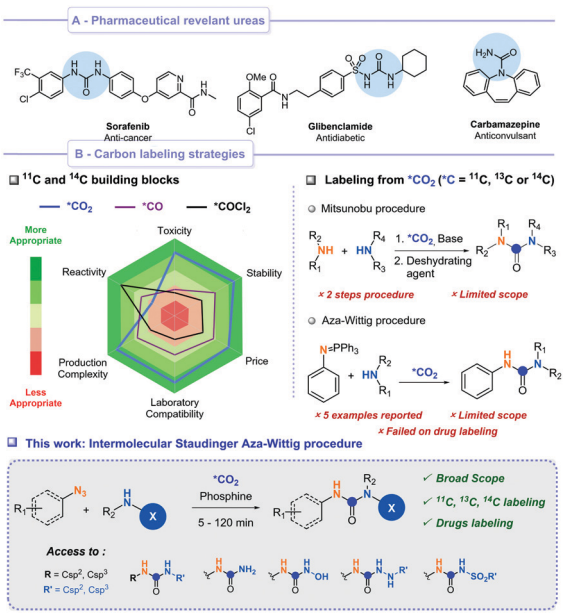
Victor Babin,[a] Antoine Sallustrau,[a] Olivier Loreau,[a] Fabien Caillé,[b] Amélie Goudet,[a] Héloïse Cahuzac,[c] Antonio Del Vecchio,[a] Frédéric Taran[a] and Davide Audisio*[a]

Carbon isotope labeling is a traceless technology, which allows tracking the fate of organic compounds either in the environment or in living organisms. This article reports on a general approach to label urea derivatives with all carbon isotopes, including ^{14}C and ^{11}C, based on a Staudinger aza-Wittig sequence. It provides access to all aliphatic/aromatic urea combinations.

Radioisotope labeling has multiple implications in drug design and development.[1–3] Two radioisotopes of carbon are pharmaceutically relevant: carbon-11 (^{11}C), a positon (β^+) emitter with a remarkably short half-life ($t_{1/2}$ = 20.4 minutes), and carbon-14 (^{14}C), a long-lived electron (β^-) emitter ($t_{1/2}$ = 5730 years).[4]

The urea substructure is found in numerous FDA approved drugs for a variety of human diseases and pharmaceutically relevant compounds (Scheme 1A).[5] This functional group represents an attractive target for isotope labeling. Three main reagents are known for the radiocarbonylation of ureas.[6] [$^{11/14}C$]Phosgene was used due to its high reactivity, but its complex chemical production requiring hazardous reactants, such as C-labeled CO and Cl_2, and the need for specialized equipment have reduced its utilization.[7,8] [$^{11/14}C$]Carbon monoxide is suitable for installing the carbonyl group of ureas, through metal-catalyzed procedures or selenium mediated synthesis.[9] Nevertheless, its utilization is not trivial due to the toxicity and the time-consuming production.[10] In addition, [^{14}C]CO was described to undergo radiolysis and its limited stability prevents storage.[11] [$^{11/14}C$]Carbon dioxide fulfills the handiest criteria: it is the primary radioactive source from which all ^{11}C- and ^{14}C-labeled compounds derive, and it has a remarkable stability to radiolysis and a suitable safety profile.[4]

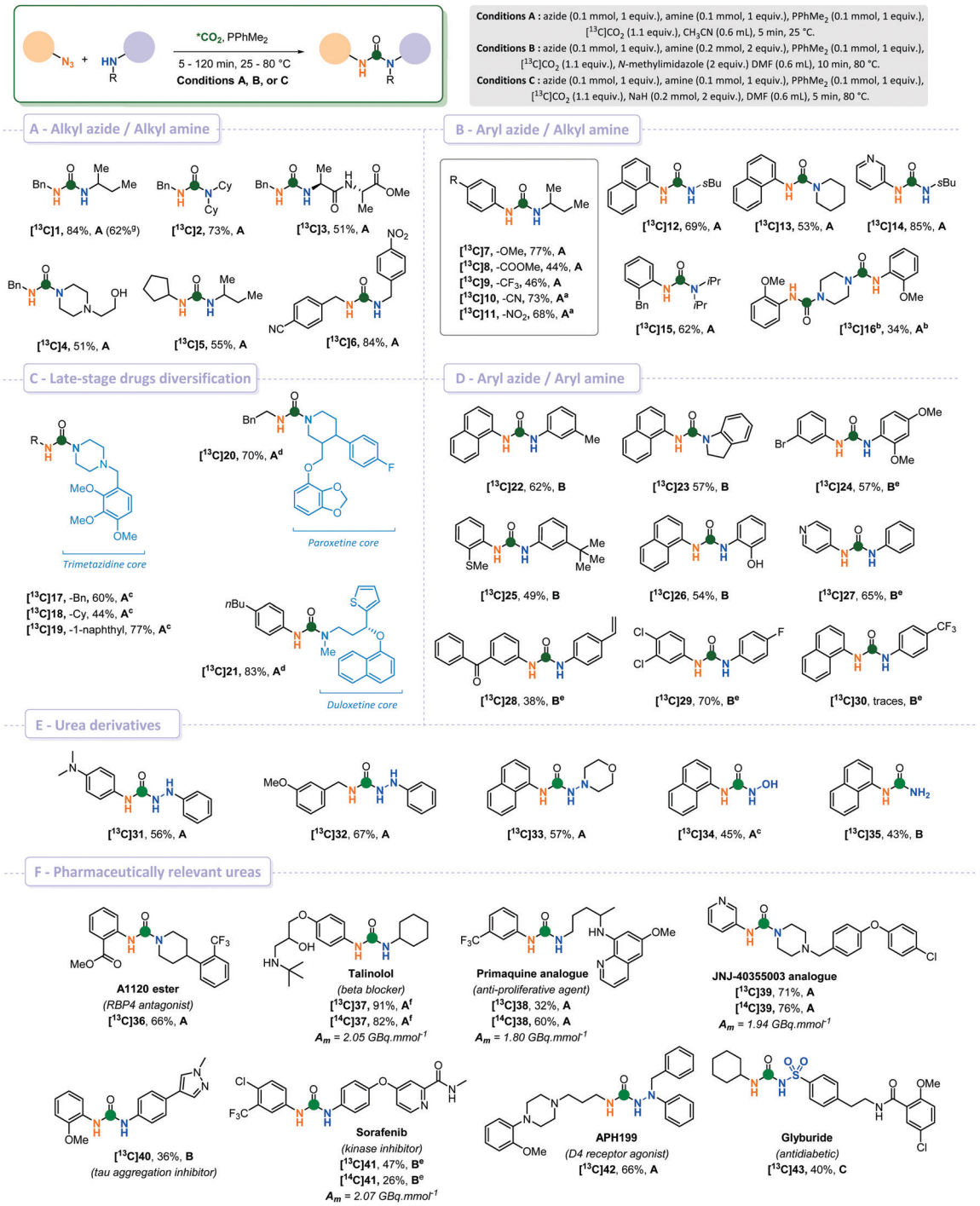
Two main procedures have been established for the labeling of linear ureas directly from [$^{11/14}$]CO_2. The first is the Mitsunobu approach using a base (BEMP or DBU) to trap CO_2 and a dehydrating agent ($POCl_3$) to afford unsymmetrical ureas.[12] This two-step procedure has been utilized both for ^{11}C and ^{14}C labeling, but shows moderate functional group compatibility. In 2006, van Tilburg published an aza-Wittig approach starting from commercial triphenylphosphine phenylimide and amines affording unsymmetrical ureas with moderate yields.[13] This methodology was applied only to four amine substrates. Importantly, it was unsuccessful for drug radiolabeling despite different attempts to modify and reverse the nature of the triphenylphosphinimine/amine partners.[14] It is worth noting that all previous procedures are limited to ureas



Scheme 1 (A) Examples of pharmaceutically relevant ureas; (B) synthetic strategies to access ureas from radiolabeled CO_2; bottom: Staudinger Aza-Wittig procedure.

[a] *Université Paris Saclay, CEA Service de Chimie Bio-organique et Marquage, DMTS, Gif-sur-Yvette, F-91191, France. E-mail: Davide.Audisio@cea.fr*
[b] *Université Paris-Saclay, Inserm, CNRS, CEA, Laboratoire d'Imagerie Biomédicale Multimodale Paris-Saclay, 91401, Orsay, France*
[c] *Université Paris-Saclay, Département Médicaments et Technologies pour la santé (DMTS), CEA, INRAE, SIMoS, Gif-sur-Yvette 91191, France*

† Electronic supplementary information (ESI) available. See DOI: 10.1039/d1cc02665h



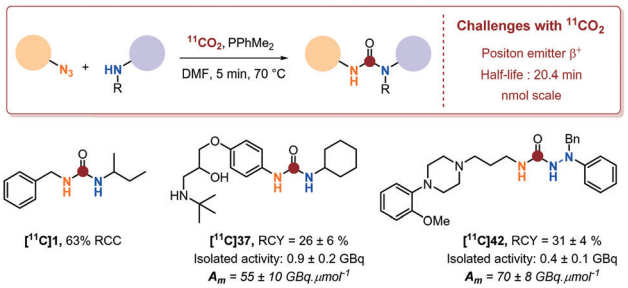
Scheme 2 Reaction scope. Green colored circles denote the position of the carbon atoms labeled. Reaction conditions: [a]70 °C instead of 25 °C; [b]azide (0.1 mmol, 2 equiv.), amine (0.05 mmol, 1 equiv.), $PPhMe_2$ (0.1 mmol, 2 equiv.), [^{13}C]CO_2 (2.2 equiv.); [c]DIPEA (2 equiv.) was added; [d]DIPEA (1 equiv.) was added. [e]2 hours instead of 10 min; [f]5 equivalents of cyclohexylamine; [g]0.5 mmol scale. A_m: molar activity. For all products, isolated yields are indicated.

bearing carbon-substituents on positions 1 and 3. Sulfonylureas, hydroxyl ureas, semicarbazides or terminal ureas still constitute a challenge for radiolabeling.[15]

The current state-of-the-art highlights the lack of a robust and general method to efficiently label unsymmetrical ureas and their derivatives. We reported an intramolecular Staudinger Aza-Wittig (SAW) approach to label cyclic ureas and carbamates from stoichiometric [$^{11/14}$]CO_2.[16] The close proximity of the nucleophile to the azide was fundamental for a facile intramolecular attack onto the isocyanate generated *in situ*. We now look to develop an intermolecular SAW procedure to offer straightforward access to unsymmetrical carbon labeled ureas including hydroxyl ureas, terminal ureas, semicarbazides and sulfonylureas.

At first, we explored the SAW procedure with a combination of aliphatic azides and aliphatic amines (Scheme 2A). The reaction was performed under previous optimized experimental conditions[16] with benzylazide and *s*-butylamine in acetonitrile, in the presence of one equivalent of $PPhMe_2$. [^{13}C]CO_2 was utilized as ^{14}C-surrogate, and its precise addition was monitored utilizing an RC Tritec carboxylation manifold. The introduction of [^{13}C]CO_2 resulted in rapid formation of expected urea **[^{13}C]1** at room temperature in less than 5 minutes in 84% isolated yield. On a 0.5 mmol scale, **[^{13}C]1** was isolated in 62% yield. The procedure is tolerant towards sterically bulky dicyclohexylamine (**[^{13}C]2**) and chemoselective for aliphatic amine in respect to alcohols (**[^{13}C]4**). The use of a dipeptide and varying the nature of the azide did not affect the reaction. Secondary cycloalkyl azide provided **[^{13}C]5** in 55% yield. In all the reactions performed, no parasite formation of symmetrical urea was observed in ^{13}C-NMR of the crude mixture. We subsequently explored the procedure with aromatic azides (Scheme 2B). The presence of electron donating groups on the azide partner successfully leads to the unsymmetrical ureas **[^{13}C]7**, **12–13**, and **15** in 53% to 77% yield. The presence of electron withdrawing groups (EWG) on the aromatic azide negatively impacted the outcome of the reaction and 4-trifluoromethyl and 4-methylbenzoate ureas **[^{13}C]8–9** were isolated in moderate yields. The anilines corresponding to the hydrolysis of the iminophosphorane (IP) intermediates were observed as major by-products. In the presence of stronger EWG such as cyano or nitro, only the aniline by-products were recovered and no traces of the desired ureas were observed. To prevent hydrolysis of the IP, the reactions were performed under strict anhydrous conditions in deuterated solvent. By ^{13}C-NMR, the formation of the expected urea was observed, but the major product detected was the IP intermediate (^{31}P-NMR δ = 11.9 ppm), suggesting that the isocyanate formation is the rate determining step (see the ESI† for details). To favor the aza-Wittig step, the procedure was reproduced in the glove box and the reaction heated at 70 °C for 10 minutes. Gratifyingly, it was possible to isolate the corresponding linear ureas **[^{13}C]10** and **[^{13}C]11** in 73% and 68% yield, respectively (see the ESI† for more details). In the presence of piperazine, a double SAW took place in the presence of two equivalents of phosphine, azide and [^{13}C]CO_2 and bis-urea **[^{13}C]16** was isolated in 34% yield. Though this result was not further optimized, it paves the way for the development of high molar activity ^{14}C-derivatives. Finally, the direct diversification of drugs was performed. The three commercially available pharmaceuticals trimetazidine, paroxetine and duloxetine were easily functionalized (Scheme 2C). We next explored the SAW in the presence of less nucleophilic aniline derivatives (Scheme 2D). As expected in the presence of *m*-toluidine, the corresponding urea **[^{13}C]22** was obtained in poor yield (see the ESI†). After careful optimization, *N*-methylimidazole was identified as the most suitable additive, affording the expected compound in ten minutes at 80 °C in 62% isolated yield (see the ESI† for details). Electron-donating anilines reacted smoothly affording the labeled unsymmetrical urea **[^{13}C]22–25** in the presence of both primary and secondary amines. Interestingly, the SAW exhibited high chemoselectivity toward aniline in respect to phenol: **[^{13}C]26** was isolated in 54% yield and no traces of carbamate were detected. Linear urea labeling was also possible with poor electron withdrawing aniline leading to aromatic ureas **[^{13}C]28** and **[^{13}C]29**. However, in the presence of strong electron-withdrawing anilines, the reaction did not occur and **[^{13}C]30** was not observed.

We next turned our attention to the labeling of urea derivatives (Scheme 2E). Semicarbazides, a precursor to semicarbazones, was labeled through this procedure with the use of both aromatic and aliphatic hydrazines **[^{13}C]31–33**. To the best of our knowledge, no radiochemical procedure has been described for radiocarbonylation of this family of derivatives. To access 1-hydroxy-3-(naphthalen-1-yl)urea **[^{13}C]34**, hydroxylamine hydrochloride was utilized in the presence of DIPEA. Pleasingly, enrichment was observed in ^{13}C-NMR corresponding to the expected hydroxyurea, which was next isolated in 45% yield. The utilization of a commercially available solution of ammonia in methanol, assisted by *N*-methylimidazole at 80 °C, allowed isolating the desired terminal urea in 43% yield. Though not optimized, this procedure allows access to terminal ureas in one single operation from CO_2 with no need of protecting groups. Next, a series of representative pharmaceuticals were labeled starting from appropriate combinations of azides and amines (Scheme 2F). **[^{13}C]36**, the methyl ester of A1120, an inhibitor of Retinol Binding Protein 4 (RBP4),[17] was isolated in 66% yield. The structure of beta-blocker talinolol **37** was particularly interesting as its labeling would provide a challenge using phosgene or the Mitsunobu/BEMP strategies. Pleasingly, the SAW enabled **[^{13}C]37** to be obtained in a fully chemo-selective and high yield. **[^{13}C]38**, a primaquine analogue,[18] and **[^{13}C]39**, a positional isomer of JNJ-40355003, an inhibitor of the Fatty Acid Amide Hydrolase (FAAH)[19] were synthesized in 32% to 71% yield, respectively. Aromatic urea **[^{13}C]40**, a tau aggregation inhibitor,[20] was obtained in 36% yield, while kinase inhibitor sorafenib **[^{13}C]41** was obtained in 47% yield in the presence of *N*-methylimidazole. Semicarbazide **[^{13}C]42**, recently described as a full agonist of the D_4 receptor,[21] was labeled with 66% yield. Finally, antidiabetic sulfonylurea glyburide **[^{13}C]43** was labeled for the first time on the carbonyl position. After deprotonation of the corresponding sulfonamide with sodium hydride, the sequence successfully provided the labeled urea with 40% yield using cyclohexylazide as a partner. Finally, we applied this protocol to ^{14}C-radiolabeling using high molar activity (A_m) [^{14}C]CO_2 (A_m: 2.17 GBq mmol^{-1}). In agreement with the results obtained with ^{13}C, four drugs were labeled with carbon 14 in high molar activities. Talinolol **[^{14}C]37** was labeled with a A_m of 2.05 GBq mmol^{-1} and 82% yield. **[^{14}C]38** and FAAH inhibitor **[^{14}C]39** were respectively labeled with A_m of 1.80 GBq mmol^{-1} and 1.94 GBq mmol^{-1} in 60% and 76% yields. Sorafenib **[^{14}C]41** was obtained with A_m of 2.07 GBq mmol^{-1}. Based on the short reaction time associated with the SAW procedure, application to ^{11}C-labeling seemed promising. Nonetheless, a series of challenges have to be faced: the high energy β^+ emission that requires the use of specialized automated systems, the minute concentrations of [^{11}C]CO_2 produced in the cyclotron and its short half-life (20.4 min).[22] Despite the challenges, **[^{11}C]1**



Scheme 3 Late-stage ^{11}C-radiolabeling of pharmaceutically relevant linear ureas. RCY: decay-corrected radiochemical yield; A_m: decay-corrected.

could be labeled in 63% RCC, by simply adapting the procedure (Scheme 3). With the SAW approach, the non-purified **[^{11}C]1** could be synthesized in only 10 minutes. We next decided to label talinolol. Under standard conditions, **[^{11}C]37** was isolated in 26 ± 6% radiochemical yield (RCY) and high A_m (55 ± 10 GBq μmol^{-1}).

To the best of our knowledge, carbonyl ^{11}C-labeling of semicarbazides is unprecedented. Pleasingly, **[^{11}C]42** could be labeled in 31 ± 4% RCY with a A_m of 70 ± 8 GBq μmol^{-1}. These preliminary examples showcase that the SAW approach will broaden the scope of original ^{11}C-labeled PET tracers available for imaging applications.

To conclude, a general methodology for the radiolabeling of linear ureas and derivatives such as semicarbazides, sulfonylureas, hydroxyl ureas, or simple terminal ureas has been developed. The reaction takes place with controlled amounts of CO_2, the first available building block for ^{14}C and ^{11}C radioisotopes, resulting in late-stage carbon isotope labeling of urea-containing drugs and analogues.[23]

We thank the European Union's Horizon 2020 research and innovation program under the Marie Sklodowska-Curie grant agreement no. 675071 and the CEA. The authors thank D.-A. Buisson and S. Lebrequier for the excellent analytical support.

Conflicts of interest

There are no conflicts to declare.

Notes and references

1 J. Atzrodt, V. Derdau, W. J. Kerr and M. Reid, *Angew. Chem., Int. Ed.*, 2018, **57**, 1758–1784.
2 (*a*) E. M. Isin, C. S. Elmore, G. N. Nilsson, R. A. Thompson and L. Weidolf, *Chem. Res. Toxicol.*, 2009, **25**, 532–542; (*b*) C. S. Elmore and R. A. Bragg, *Bioorg. Med. Chem. Lett.*, 2015, **25**, 167–171.
3 (*a*) P. M. Matthews, E. A. Rabiner, J. Passchier and R. N. Gunn, *Br. J. Clin. Pharmacol.*, 2012, **73**, 175–186; (*b*) N. Penner, L. Xu and C. Prakash, *Chem. Res. Toxicol.*, 2012, **25**, 513–531; (*c*) A. Del Vecchio, G. Destro, F. Taran and D. Audisio, *J. Labelled Compd. Radiopharm.*, 2018, **61**, 988–1007.
4 (*a*) R. Voges, J. R. Heys and T. Moenius, *Preparation of Compounds Labeled with Tritium and Carbon-14*, John Wiley & Sons, Ltd, 2009; (*b*) P. J. H. Scott, *Angew. Chem., Int. Ed.*, 2009, **48**, 6001–6004.
5 (*a*) P. Sikka, J. K. Sahu, A. K. Mishra and S. R. Hashim, *Med. Chem.*, 2015, **5**, 479–483; (*b*) A. K. Ghosh and M. Brindisi, *J. Med. Chem.*, 2020, **63**, 2751–2788.
6 (*a*) K. Dahl, C. Halldin and M. Schou, *Clin. Transl. Imaging*, 2017, **5**, 275–289; (*b*) X. Deng, J. Rong, L. Wang, N. Vasdev, L. Zhang, L. Josephson and S. H. Liang, *Angew. Chem., Int. Ed.*, 2019, **58**, 2580–2605.
7 (*a*) K. Kumata, J. Yui, Y. Zhang, Y. Kurihara, M. Ogawa, W. Mori, M. Fujinaga and M. R. Zhang, *Bioorg. Med. Chem. Lett.*, 2017, **27**, 4521–4524; (*b*) T. Yamasaki, M. Fujinaga, Y. Shimoda, W. Mori, Y. Zhang, H. Wakizaka, M. Ogawa and M. R. Zhang, *Bioorg. Med. Chem. Lett.*, 2017, **27**, 4114–4117.
8 For a recent highlight on labeled phosgene-free carbonyl insertion, see: J.-P. J. Bow and P. J. Riss, *Chem. Methods*, 2021, **1**, 139–141.
9 For recent labeling method using [^{11}C]CO see: (*a*) T. Kihlberg, K. Farhad Karimi and B. Långström, *J. Org. Chem.*, 2002, **67**, 3687–3692; (*b*) H. Doi, J. Barletta, M. Suzuki, R. Noyori, Y. Watanabe and B. Långström, *Org. Biomol. Chem.*, 2004, **2**, 3063–3066; (*c*) S. Kealey, S. M. Husbands, I. Bennacef, A. D. Gee and J. Passchier, *J. Labelled Compd. Radiopharm.*, 2014, **57**, 202–208.
10 J. Eriksson, G. Antoni, B. Långström and O. Itsenko, *Nucl. Med. Biol.*, 2021, **92**, 115–137.
11 (*a*) E. Hargraves, A. Lashford, A. Rees and B. Roughley, *J. Labelled Compd. Radiopharm.*, 2007, **50**, 435–436; (*b*) R. J. Hardy, J. C. Sheppard and M. J. Campbell, *Int. J. Appl. Radiat. Isot.*, 1984, **35**, 1071–1072.
12 (*a*) A. Schirbel, M. Holschbach and H. Coenen, *J. Labelled Compd. Radiopharm.*, 1999, **42**, 537–551; (*b*) A. A. Wilson, A. Garcia, S. Houle, O. Sadovski and N. Vasdev, *Chem. – Eur. J.*, 2011, **17**, 259–264; (*c*) A. K. Haji Dheere, N. Yusuf and A. Gee, *Chem. Commun.*, 2013, **49**, 8193–8195; (*d*) K. Horkka, K. Dahl, J. Bergare, C. S. Elmore, C. Halldin and M. Schou, *ChemistrySelect*, 2019, **4**, 1846–1849; (*e*) K. Dahl, T. L. Collier, R. Cheng, X. Zhang, O. Sadovski, S. H. Liang and N. Vasdev, *J. Labelled Compd. Radiopharm.*, 2018, **61**, 252–262; (*f*) J. Downey, S. Bongarzone, S. Hader and A. D. Gee, *J. Labelled Compd. Radiopharm.*, 2018, **61**, 263–271.
13 E. W. van Tilburg, A. D. Windhorst, M. van der Mey and J. D. M. Herscheid, *J. Labelled Compd. Radiopharm.*, 2006, **49**, 321–330.
14 A. J. Poot, B. van der Wildt, M. Stigter-van Walsum, M. Rongen, R. C. Schuit, N. H. Hendrikse, J. Eriksson, G. A. M. S. van Dongen and A. D. Windhorst, *Nucl. Med. Biol.*, 2013, **40**, 488–497.
15 For example of sulfonylurea labeling with [^{11}C]CO_2, see: J. R. Hill, X. Shao, N. L. Massey, J. Stauff, P. S. Sherman, A. A. B. Robertson and P. J. H. Scott, *Bioorg. Med. Chem. Lett.*, 2020, **30**, 127186.
16 (*a*) A. Del Vecchio, F. Caillé, A. Chevalier, O. Loreau, K. Horkka, H. Halldin, M. Schou, N. Camus, P. Kessler, B. Kuhnast, F. Taran and D. Audisio, *Angew. Chem., Int. Ed.*, 2018, **57**, 9744–9748; (*b*) A. Del Vecchio, A. Talbot, F. Caillé, A. Chevalier, A. Sallustrau, O. Loreau, G. Destro, F. Taran and D. Audisio, *Chem. Commun.*, 2020, **56**, 11677–11680.
17 (*a*) A. Motani, Z. Wang, M. Conn, K. Siegler, Y. Zhang, Q. Liu, S. Johnstone, H. Xu, S. Thibault, Y. Wang, P. Fan, R. Connors, H. Le, G. Xu, N. Walker, B. Shan and P. Coward, *J. Biol. Chem.*, 2009, **284**, 7673–7680; (*b*) C. Cioffi, N. Dobri, E. Freeman, M. Conlon, P. Chen, D. Stafford, D. Schwarz, K. Golden, L. Zhu, D. Kitchen, K. Barnes, B. Racz, Q. Qin, E. Michelotti, C. Cywin, W. Martin, P. Pearson, G. Johnson and K. Petrukhin, *J. Med. Chem.*, 2014, **57**, 7731–7757.
18 I. Perkovic, M. Antunovic, I. Marijanovic, K. Pavic, K. Ester, M. Kralj, J. Vlainic, I. Kosalec, D. Schols, D. Hadjipavlou-Litina, E. Pontiki and B. Zorc, *Eur. J. Med. Chem.*, 2016, **124**, 622–636.
19 J. Keith, R. Apodaca, M. Tichenor, W. Xiao, W. Jones, J. Pierce, M. Seierstad, J. Palmer, M. Webb, M. Karbarz, B. Scott, S. Wilson, L. Luo, M. Wennerholm, L. Chang, S. Brown, M. Rizzolio, R. Rynberg, S. Chaplan and J. Breitenbucher, *ACS Med. Chem. Lett.*, 2012, **3**, 823–827.
20 M. Altman, C. Fischer, J. D. Katz, T. M. Williams, X. F. Zhang and H. Zhou, WO 2013/181075 Al, 2013.
21 A. Pirzer, R. Lasch, H. Friedrich, H. Hubner, P. Gmeiner and M. Heinrich, *J. Med. Chem.*, 2019, **62**, 9658–9679.
22 (*a*) B. H. Rotstein, S. H. Liang, J. P. Holland, T. L. Collier, J. M. Hooker, A. A. Wilson and N. Vasdev, *Chem. Commun.*, 2013, **49**, 5621–5629; (*b*) B. H. Rotstein, S. H. Liang, M. S. Placzek, J. M. Hooker, A. D. Gee, F. Dollé, A. A. Wilson and N. Vasdev, *Chem. Soc. Rev.*, 2016, **45**, 4708–4726; (*c*) X. Deng, J. Rong, L. Wang, N. Vasdev, L. Zhang, L. Josephson and S. H. Liang, *Angew. Chem., Int. Ed.*, 2019, **58**, 2580–2605; (*d*) C. Taddei and V. W. Pike, *EJNMMI Radiopharmacy and Chemistry*, 2019, **4**, 25.
23 A similar work recently appeared: U. S. Ismailani, M. Munch, B. A. Mair and B. H. Rotstein, *Chem. Commun.*, 2021, **57**, 5266–5269.