Research article

Journal of Labelled Compounds and Radiopharmaceuticals

Received 24 July 2015, Revised 21 August 2015, Accepted 30 August 2015 Published online 22 September 2015 in Wiley Online Library

(wileyonlinelibrary.com) DOI: 10.1002/jlcr.3349

Synthesis of two potent glucocorticoid receptor agonists labeled with carbon-14 and stable isotopes

Bachir Latli,* Jonathan T. Reeves, Zhulin Tan, Matt Hrapchak, Jinhua J. Song, Carl B. Busacca, and Chris H. Senanayake

Two potent glucocorticoid receptor agonists were prepared labeled with carbon-14 and with stable isotopes to perform drug metabolism, pharmacokinetics, and bioanalytical studies. Carbon-14 labeled (1) was obtained from an enantiopure alkyne (5) via a Sonogashira coupling to a previously reported 5-amino-4-iodo-[2-^{14}C]pyrimidine [^{14}C]-(6), followed by a base-mediated cyclization (1) in 72% overall radiochemical yield. Carbon-14 labeled (2) was prepared in five steps employing a key benzoic acid intermediate [^{14}C]-(13), which was synthesized in one pot from enolization of trifluoromethylketone (12), followed by bromine–magnesium exchange and then electrophile trapping reaction with [^{14}C]-carbon dioxide. A chiral auxiliary (*S*)-1-(4-methoxyphenyl)ethylamine was then coupled to this acid to give [^{14}C]-(15). Propargylation and separation of diastereoisomers by crystallizations gave the desired diastereomer [^{14}C]-(17) in 34% yield. Sonogashira coupling to iodopyridine (10) followed by cyclization to the azaindole [^{14}C]-(18) and finally removal of the chiral auxiliary gave [^{14}C]-(2) in 7% overall yield. For stable isotope syntheses, [$^{13}C_6$]-(1) was obtained in three steps using [$^{13}C_4$]-(6) and trimethylsilylacetylene-[$^{13}C_2$] in 26% yield, while [$^{2}H_5$]-(2) was obtained by first preparing the iodopyridine [$^{2}H_5$]-(10) in five steps. Then, Sonogashira coupling to chiral alkyne (24) and cyclization gave [$^{2}H_5$]-(2) in 42% overall yield.

Keywords: glucocorticoid mimics; radiosynthesis; carbon-14; carbon-13; deuterium

Introduction

The glucocorticoid receptor (GR) plays significant roles in vertebrate physiology owing to that fact that it is expressed in most cell types and tissues.[1] Glucocorticoids (GCs) are small molecules that interact with this receptor.[2] The anti-inflammatory properties of GCs have been known for a long time. GCs are widely used in the treatment of several inflammatory diseases. For example, the development of inhaled steroids and combination of inhaled steroids and β2 agonists led to many formulations currently on the market for treating respiratory diseases. Fluticasone propionate and salmeterol (Figure 1) are among the top ten most prescribed and best-selling drugs in the world (www.medscape.com/viewarticle/829246).

However, these therapies are fraught with a number of serious side effects, like cross-reactivity with other steroid hormone receptors. These complications often hamper high dose and chronic administration. The search for non-steroidal drugs that would have the desired anti-inflammatory actions of GC but with significantly lower or no side effects remains an ongoing quest.[3–13] Herein, we report the synthesis of two potent GR agonists labeled with carbon-14 and with stable isotopes to aid in drug metabolism, pharmacokinetics, and bioanalytical and other studies (Figure 2).

As depicted in Figure 2, the quaternary center, trifluoromethylcarbinol, was reported to be an essential pharmacophore for the biological activity of these compounds. Its role in ligand–receptor interactions has been proposed on the basis of modeling studies to correspond to that of 11-β-OH of steroidal GC, namely, hydrogen-bonding interaction with N564 (Asn 564) in the active site, which has been recently confirmed by X-ray structure.[10] The trifluoromethyl moiety affects the functional activity. Analogs with this moiety behave as potential agonists.[13] Compounds (**1**) and (**2**) are structurally similar. Compound (**1**) was found to be at high risk of drug–drug interactions owing to its potent cytochrome P-450 inhibition across multiple isoforms.[14] Preliminary dose projections showed a potential for high clinical dose requirements of this compound owing to the overall high clearance and low bioavailability.[14] The added modifications on compound (**2**) were found to have a beneficial effect on cytochrome P-450 profile and better metabolic stability and aqueous solubility, and attenuate some of the other undesired properties of compound (**1**).[15] Both compounds are chiral, and the pure enantiomers were reported to have twofold improved potency against GR when compared with the racemic mixtures.[14,15]

Chemical Development, Boehringer Ingelheim Pharmaceuticals, Inc., 900 Ridgebury Road, PO Box 368, Ridgefield, CT, USA

**Correspondence to: Bachlir Latli, Chemical Development, Boehringer Ingelheim Pharmaceuticals, Inc., 900 Ridgebury Road, PO Box 368, Ridgefield, CT, USA. E-mail: bachir.latli@boehringer-ingelheim.com*

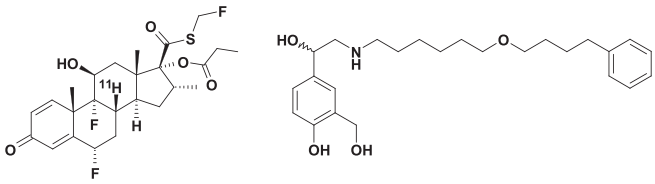


Figure 1. Structure of fluticasone (steroid) and salmeterol (β2 agonist).

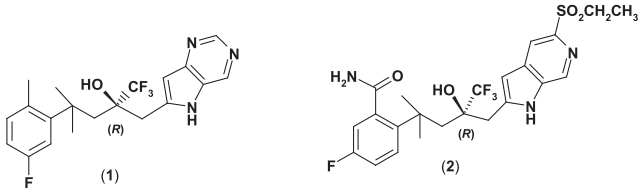
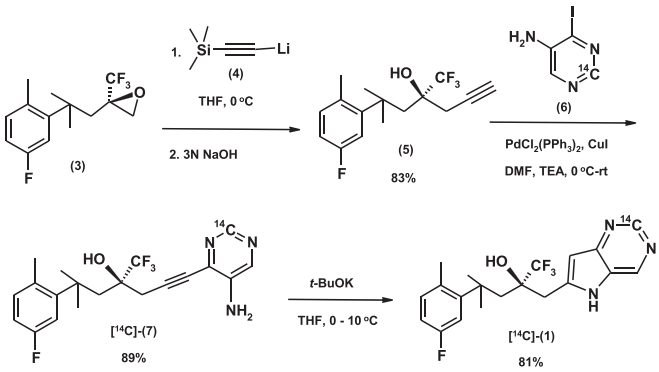


Figure 2. Structure of compounds (**1**) and (**2**).

Results and discussions

Synthesis of [^{14}C]-(1) and [$^{13}C_6$]-(1)

5-Amino-4-iodopyrimidine (**6**) is the most effective intermediate to access carbon-14 labeled (**1**) with fewer radioactive steps and more importantly avoid the synthesis of labeled chiral epoxide (**3**). This chiral epoxide (**3**)[16] was reacted with trimethylsilyl lithium acetylide (**4**), which was prepared *in situ* from trimethylsilyl acetylene and *n*-butyl lithium at 0 °C in THF, to give the alkyne (**5**) in 83% yield. Sonogashira coupling of this alkyne to 5-amino-4-iodo-[2-^{14}C]-pyrimidine [^{14}C]-(**6**)[17] gave [^{14}C]-(**7**) in 89% radiochemical yield.[12] Base-mediated cyclization using *t*-BuO$^-$K$^+$ in THF gave the diazaindole [^{14}C]-(**1**) in 81% radiochemical yield after two crystallizations from heptane-isopropyl acetate.[9] A total of 59.5 mCi of material was obtained with a specific activity of 49.6 mCi/mmol and radiochemical and chiral purities of more than 99% (Scheme 1).



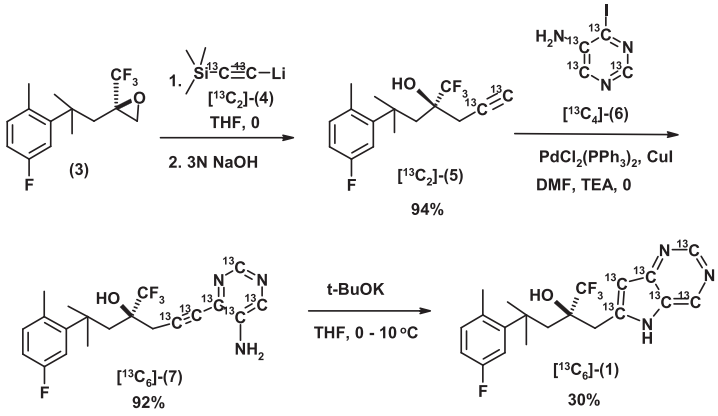
Scheme 1. Synthesis of [^{14}C]-(**1**).

The same approach was applied to the synthesis of [$^{13}C_6$]-(**1**). With the availability of [$^{13}C_2$]-trimethylsilylacetylene and 5-amino-6-iodo[$^{13}C_4$]-pyrimidine,[17] treatment of epoxide (**3**) with the lithium salt of trimethylsilylacetylene[1,2-$^{13}C_2$] gave alcohol [$^{13}C_2$]-(**5**) in 94% yield. Sonogashira coupling followed by cyclization gave [$^{13}C_6$]-(**1**) in 92% and 30% yields, respectively (Scheme 2).

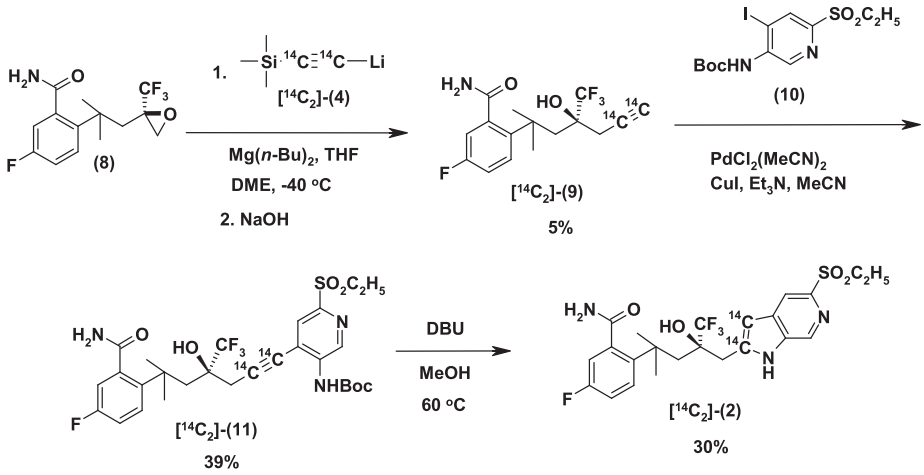
Synthesis of [$^{14}C_2$]-(2), [^{14}C]-(2), and [$^{2}H_5$]-(2)

Similar to the preceding syntheses, the epoxide (**8**)[16] was used to prepare [$^{14}C_2$]-(**2**) in three steps (Scheme 3). However, the first step gave very poor yield probably owing to the instability of trimethylsilylacetylene-[1,2-$^{14}C_2$]. About 10 mCi of [$^{14}C_2$]-(**9**) was obtained from 200 mCi of acetylene with specific activity of 110 mCi/mmol. This compound was then subjected as discussed before to the general Sonogashira coupling followed by base-mediated cyclization using 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in MeOH to give 2.9 mCi of the desired [$^{14}C_2$]-(**2**) after chromatographic purification (Scheme 3).[18]

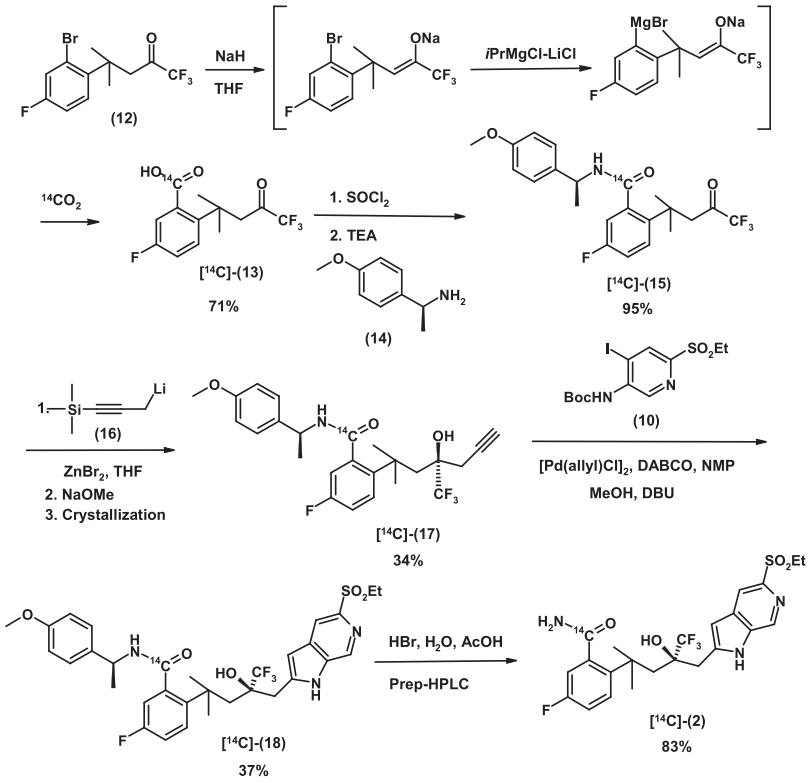
While the aforementioned synthesis was short, not enough material was obtained to cover the development studies. A second synthesis was devised to furnish [^{14}C]-(**2**) in five steps with a specific activity of 58 mCi/mmol and a radiochemical purity of 99.9% using carbon-14 labeled carbon dioxide (Scheme 4). The key intermediate [^{14}C]-(**15**) containing an *N*-(4-methoxyphenyl)ethyl amide was first synthesized from enolization of trifluoromethylketone (**12**)[19] followed by bromine–magnesium exchange, and then electrophile trapping reaction with [^{14}C]-carbon dioxide to [^{14}C]-(**13**) was accomplished in 71% radiochemical yield.[19] The acid [^{14}C]-(**13**) was then converted to the acyl chloride using thionyl chloride and reacted with (*S*)-1-(4-methoxyphenyl)ethylamine (**14**) to give [^{14}C]-(**15**) in 95% radiochemical yield. Addition of the lithium derivative (**16**) gave a mixture of diastereomers. Crystallization



Scheme 2. Synthesis of [$^{13}C_6$]-(**1**).



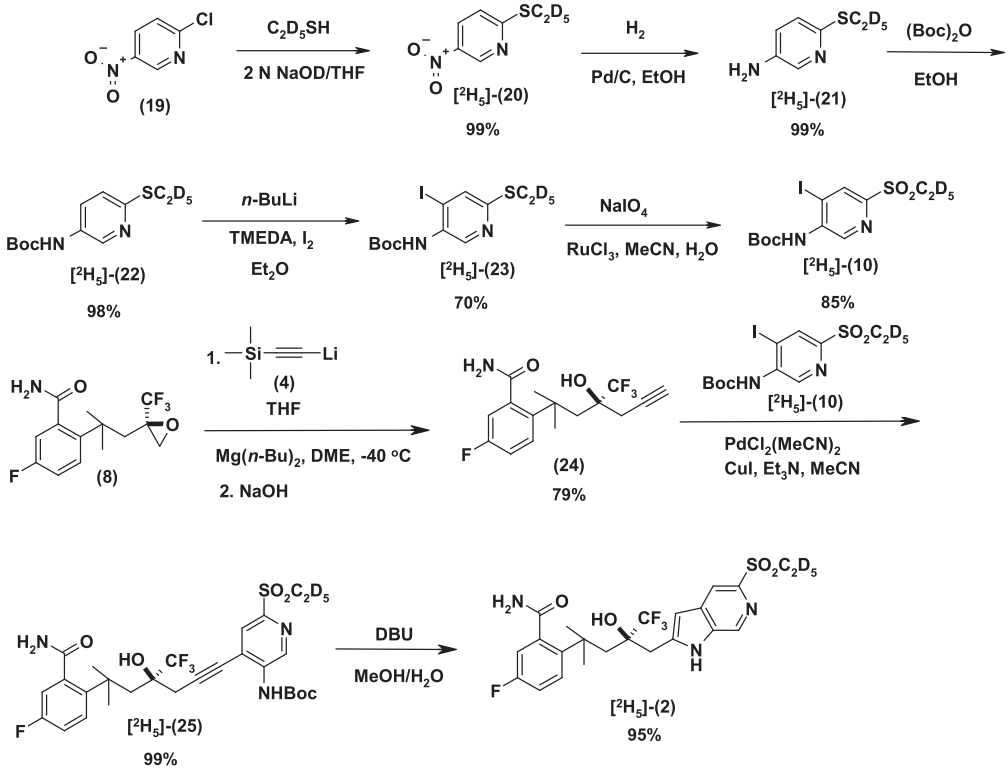
Scheme 3. Synthesis of [$^{14}C_2$]-(**2**).



Scheme 4. Second synthesis of [^{14}C]-(**2**).

from EtOAc acetate : heptane gave the desired diastereomer [^{14}C]-(**17**) in 34% radiochemical yield with more than 99% ee. Sonogashira coupling with iodopyridine (**10**) followed by cyclization gave [^{14}C]-(**18**) in 37% radiochemical yield. Removal of chiral *N*-phenethyl amide moiety using aqueous HBr and acetic acid gave [^{14}C]-(**2**) in 83% yield. Purification by preparative HPLC gave 37 mCi of the desired [^{14}C]-(**2**) in 7% overall radiochemical yield with 98% radiochemical purity, 99% chiral purity, and a specific activity of 58 mCi/mmol.

In the synthesis of [$^2H_5$]-(**2**) (Scheme 5), we first prepared [$^2H_5$]-(**10**) in five steps and in 57% overall chemical yield starting from the commercially available [$^2H_5$]-ethanethiol. Thus, coupling [$^2H_5$]-ethanethiol to 2-chloro-5-nitropyridine (**19**) using aqueous 2 N NaOD in deuterated water gave [$^2H_5$]-(**20**) in 99% yield. Reduction of the nitro group at 50 psi hydrogen for 20 h followed by reaction with di-*tert*-butyl dicarbonate to protect the amino-group gave [$^2H_5$]-(**22**) in 97% yield over two steps. Ortho metalation and subsequent iodination in anhydrous ethyl ether in the presence of tetramethylethylene diamine gave the iodo-derivative [$^2H_5$]-(**23**) in 70% yield. Oxidation with sodium periodate in the presence of ruthenium trichloride in acetonitrile and water gave [$^2H_5$]-(**10**) in 85% yield. General Sonogashira coupling reaction of this material with (**24**) gave [$^2H_5$]-(**25**) in 99% yield.[20] Finally, treatment with DBU in MeOH at 60 °C as seen before gave [$^2H_5$]-(**2**) in 95% yield after crystallization from EtOAc.



Scheme 5. Synthesis of $[^2H_5]$-(**2**).

Conclusion

Glucocorticoids play an important role in the regulation of the immune system and, therefore, are widely used in the treatment of inflammatory and immune diseases. However, because of their many undesirable side effects, the search for non-steroidal GC mimetics led to identification of compounds (**1**) and (**2**), with compound (**2**) identified as a clinical candidate. The detailed syntheses of these two potent GR agonists labeled with carbon-14 and with stable isotopes are described. Compound $[^{14}C]$-(**1**) was prepared in two radioactive steps in 72% overall yield and with a specific activity of 49.6 mCi/mmol and 99% radiochemical and chiral purities. Carbon-13 labeled (**1**) was prepared in three chemical steps in 26% overall yield and with 99% isotopic enrichment. Compound $[^{14}C]$-(**2**) was prepared in five radiochemical steps in 7% overall radiochemical yield and with a specific activity of 58 mCi/mmol and radiochemical purity of 99%. Deuterium labeled (**2**) was prepared in eight steps in 42% overall yield and with 99% isotopic enrichment. These labeled compounds were used in drug metabolism and pharmacokinetics and other studies.

Experimental procedures

Materials and methods

Liquid scintillation counting was accomplished using a Beckman LS6500 and ready safe™ cocktail (Beckman, Fullerton, CA, USA). NMR spectra were recorded with a Bruker 400 MHz DPX or Bruker 500 MHz spectrometers (Bruker, Billerica, MA, USA) using deuterated solvents and tetramethylsilane as the internal standard unless stated otherwise. Pre-coated thin-layer chromatography (TLC) sheets (silica gel $60F_{254}$) were obtained from EM Science (Gibbstown, NJ, USA). The developed plates were visualized using 254-nm UV illumination or by phosphomolybdic acid stain. HPLC, gradient 20% to 100% MeCN/H_2O (10 mM trifluoroacetic acid) over 20 min; column, Zorbax Eclipse XDB C8 (4.6 mm × 150 mm, 5 μm). These conditions are used throughout this synthesis unless stated otherwise. Disposable RediSep™ silica gel columns of different sizes were purchased from Isco, Inc. (Lincoln, NE, USA) and used for flash column chromatography using Isco Combiflash. Liquid chromatography–mass spectrometry (LCMS) data were obtained using UPLC on Waters Acquity, equipped with single quad detector, photodiode array detector, and evaporative light scattering detector in the $ESI^{+/-}$ mode; column, C18 BEH (2.1 mm × 50 mm, 1.7 μm); column temperature, 60 °C; gradient, 90% A to 100% B in 1.19 min hold at 100% B to 1.77 min, flow rate 0.8 mL/min; A, 95% water, 5% acetonitrile, 0.05% formic acid; B, acetonitrile, 0.05% formic acid. Chiral HPLC was performed on Chiralpak IA column, 235-nm detection, flow rate 2 mL/min, isocratic 70:30 v/v heptane/*i*-PrOH, 10 min run. Both $[^2H_5]$-ethanethiol (99.7 at.% ^2H) and $[^{13}C_2]$-trimethylsilylacetylene (99.9 at.% ^{13}C) were purchased from Isotec, an Aldrich company (Miamisburg, OH, USA). TMS-acetylene-$[^{14}C_2]$ was purchased from ViTrax (Placentia, CA, USA). Chiral epoxides (**3**) and (**8**) with chiral purity exceeding 99% were obtained from in-house synthesis. The rest of the reagents were purchased from Sigma-Aldrich Company (St. Louis, MO, USA).

Synthesis of $[^{14}C]$-(1)

*(4R)-6-(5-Fluoro-2-methyl-phenyl)-6-methyl-4-trifluoromethyl-hept-1-yn-4-ol (**5**)*

To a solution of trimethylsilylacetylene (400 mg, 4 mmol) in dry THF (4 mL) was added a solution of *n*-BuLi (2.5 M solution in hexanes, 2 mL) dropwise at 0 °C under nitrogen atmosphere. After stirring for 1 h, epoxide (**3**) (1.1 g, 4 mmol) in dry dimethyl sulfoxide (8 mL) was added dropwise, and the reaction was warmed gradually to room temperature and stirred for 14 h. A solution of aqueous NaOH (3 N, 3 mL) was then added and stirred for 1 h at ambient temperature. The mixture was cooled again to 0 °C, and aqueous HCl (2.75 N, 5 mL) was added. The aqueous solution was extracted with EtOAc (100 mL × 3), and the

combined extracts were washed with brine (100 mL), dried over $MgSO_4$, filtered, and concentrated under reduced pressure to give an oil that was purified by flash chromatography using 40 g RediSep™ column and 20% EtOAc : hexanes to give 0.74 g of a colorless oil in 83% yield. TLC: $R_f = 0.38$ in 20% EtOAc : hexanes. ^{1}H NMR ($CDCl_3$) δ: 7.16 (dd, $J = 2.73$, 12.0 Hz, 1H), 7.11 (dd, $J = 6.64$, 8.31 Hz, 1H), 6.87 (dt, $J = 2.73$, 7.71 Hz, 1H), 2.56 (s, 3H), 2.45–2.56 (m, 2H), 2.31–2.42 (m, 2H), 1.82 (m, 1H), 1.60 (s, 3H), 1.56 (s, 3H).

*1-(5-Amino[5-^{14}C]pyrimidin-4-yl)-6-(5-fluoro-2-methyl-phenyl)-6-methyl-4(R)-trifluoromethyl-hept-1-yn-4-ol, [^{14}C]-(**7**)*

A mixture of 5-amino-4-iodo[2-^{14}C]pyrimidine, [^{14}C]-(**6**)[17] (435 mg, 1.97 mmol, 95 mCi), $PdCl_2(Ph_3P)_2$ (14 mg, 0.02 mmol), and CuI (19 mg, 0.1 mmol) in degassed dimethylformamide (5 mL) was added under nitrogen atmosphere to alkyne (**5**) (595 mg, 1.97 mmol) in degassed triethylamine (TEA, 10 mL) dropwise at 0 °C. The reaction was warmed gradually to room temperature and stirred for 48 h. The dark mixture was treated with a saturated solution of NH_4Cl (25 mL), and the aqueous solution was extracted with EtOAc (100 mL × 3). The combined extracts were washed with brine (100 mL), dried over $MgSO_4$, filtered, and concentrated under reduced pressure to give a red-brown solid. Combiflash purification using 10% to 20% EtOAc : CH_2Cl_2 and 40 g RediSep™ gave 696 mg of orange foam (73.3 mCi) in 89% radiochemical yield, $R_f = 0.44$ in 10% MeOH/CH_2Cl_2.

*(R)-1,1,1-Trifluoro-4-(5-fluoro-2-methyl-phenyl)-4-methyl-2-(5H-pyrrolo[3,2-d][2-^{14}C]pyrimidin-6-ylmethyl)-pentan-2-ol, [^{14}C]-(**1**)*

To a solution of [^{14}C]-(**7**) (696 mg, 1.76 mmol, 73 mCi) in THF (5 mL) was added *t*-BuOK (434 mg, 3.87 mmol) in one portion at 0 °C under nitrogen. After stirring for 2 h, brine (5 mL) was added, and the mixture was warmed to room temperature and extracted with EtOAc (10 mL × 3). The combined extracts were washed with brine (20 mL), dried over $MgSO_4$, filtered, and concentrated under reduced pressure to give foam. Combiflash purification on 40 g RediSep™ and 0% to 100% EtOAc : CH_2Cl_2 gave 121 mg of not so pure product and 400 mg of the desired product, $R_f = 0.42$ in 10% MeOH/CH_2Cl_2. The pure material was further crystallized twice from isopropyl acetate : heptane (1.5 mL each) to give 325 mg of a white solid. HPLC: $R_t = 11.4$ min, radiopurity 99%, and chiral purity 99%. Total radioactivity 59.5 mCi, or 81.4% radiochemical yield. ^{1}H NMR (MeOH-d_4) δ: 8.71 (s, 1H), 8.67 (s, 1H), 7.16 (dd, $J = 12$, 2 Hz, 1H), 7.07 (m, 1H), 6.81 (m, 1H), 6.29 (s, 1H), 2.91 (d, $J = 15$ Hz, 1H), 2.75 (d, $J = 15$ Hz, 1H), 2.63 (d, $J = 15$ Hz, 1H), 2.52 (s, 3H), 2.12 (d, $J = 16$ Hz, 1H), 1.69 (s, 3H), 1.44 (s, 3H).

Synthesis of [$^{13}C_6$]-(1)

*6-(5-Fluoro-2-methyl-phenyl)-6-methyl-4-trifluoromethyl-hept-1-yn [1,2-$^{13}C_2$]-4-ol, [$^{13}C_2$]-(**5**)*

To a solution of trimethylsilylacetylene[1,2-$^{13}C_2$] (102 mg, 1 mmol) in dry THF (1 mL) was added a solution of *n*-BuLi (2.5 M solution in hexanes, 0.44 mL) dropwise at 0 °C under nitrogen atmosphere. After stirring for 1 h, epoxide (**3**) (280 mg, 1 mmol) in dry dimethyl sulfoxide (2 mL) was added dropwise, and the reaction was warmed gradually to room temperature overnight. A solution of NaOH (3.0 N, 0.6 mL) was then added and stirred for 1 h. The reaction was cooled again to 0 °C, and aqueous HCl (2.75 N, 1 mL) was added. The aqueous solution was extracted with EtOAc (50 mL × 3), and the combined extracts were washed with brine (50 mL), dried over $MgSO_4$, filtered, and concentrated under reduced pressure to give an oil that was purified by flash chromatography using 40 g RediSep™ and 20% EtOAc : hexanes to give 285 mg of a slight yellow oil in 94% yield, $R_f = 0.38$ in 20% EtOAc : hexanes. ^{1}H NMR ($CDCl_3$) δ: 7.16 (dd, $J = 2.73$, 12.0 Hz, 1H), 7.11 (dd, $J = 6.64$, 8.31 Hz, 1H), 6.87 (dt, $J = 2.73$, 7.71 Hz, 1H), 2.56 (s, 3H), 2.45–2.56 (m, 2H), 2.31–2.42 (m, 2H), 1.82 (m, 1H), 1.60 (s, 3H), 1.56 (s, 3H).^{13}C NMR ($CDCl_3$) δ: 162.54, 160.12, (146.99, 146.94), (134.65, 134.57), 131.66, 127.06, 124.20, (114.78, 114.56), (113.35, 113.15), (78.46, 76.73, 73.97, 71.83), 51.92, 40.27, 38.67, 31.36, 30.82, (25.74, 25.63, 25.09, 24.97), 22.66.

*1-(5-Amino-pyrimidin[$^{13}C_4$]-4-yl)-6-(5-fluoro-2-methyl-phenyl)-6-methyl-4(R)-trifluoromethyl-hept-1-yn-[1,2-$^{13}C_2$]-4-ol, [$^{13}C_6$]-(**7**)*

A mixture of [$^{13}C_4$]-(**6**)[17] (162 mg, 0.72 mmol), $PdCl_2(Ph_3P)_2$ (5 mg, 7 μmol), and CuI (7 mg, 35 μmol) was first degassed. Dimethylformamide (1 mL) was the added under nitrogen atmosphere, and the mixture was cooled in an ice bath. The alkyne [$^{13}C_2$]-(**5**) (213 mg, 0.7 mmol) in TEA (2 mL) was then added dropwise. The reaction was warmed gradually to room temperature and stirred for 12 h. The dark mixture was treated with a saturated solution of NH_4Cl (6 mL), and the aqueous solution was extracted with EtOAc (50 mL × 3). The combined extracts were washed with brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure to give 0.35 g of a red-brown solid. Combiflash purification using 10% to 20% EtOAc : CH_2Cl_2 gave 260 mg of red-brown foam in 92% yield, $R_f = 0.44$ in 10% MeOH/CH_2Cl_2. ^{1}H NMR ($CDCl_3$) δ: 8.56 (m, $J = 206$ Hz, 1H), 8.23 (m, 1H), 7.16 (dd, 1H), 7.09 (dd, 1H), 6.85 (dt, 1H), 4.23 (brs, 2H), 2.97 (s, 3H), 2.60–2.80 (m, 2H), 2.54 (s, 3H), 1.56 (s, 3H). ^{13}C NMR ($CDCl_3$) δ: 162.53, 160.27, 148.30 (m), 142.38 (m), 142.15 (m), 135.00–132.81 (m), 94.53 (dd), (79.77, 78.73, 77.97, 76.93), 40.72, 38.74, 36.55, 31.49, 30.87, (26.74, 26.64, 26.07, 25.97), 22.66. LCMS: MH^+ 402.27 (100%).

*(R)-1,1,1-Trifluoro-4-(5-fluoro-2-methyl-phenyl)-4-methyl-2-(5H-pyrrolo[3,2-d]pyrimidin-[$^{13}C_6$]-6-ylmethyl)-pentan-2-ol, [$^{13}C_6$]-(**1**)*

To the alkyne [$^{13}C_6$]-(**7**) (245 mg, 0.6 mmol) in THF (3 mL) was added *t*-BuOK (150 mg, 1.2 mol) in one portion at 0 °C under nitrogen. After 4 h, brine (3 mL) was added, and the mixture was extracted with EtOAc (10 mL × 3). The combined extracts were washed with brine (20 mL), dried over $MgSO_4$, filtered, and concentrated under reduced pressure to give 245 mg of foam. Combiflash purification on 40 g RediSep™ and 10% to 30% EtOAc : CH_2Cl_2 gave 70 mg of pure material in 30% yield, $R_f = 0.42$ in 10% MeOH/CH_2Cl_2. HPLC: $R_t = 11.327$ min. ^{1}H NMR ($CDCl_3$) δ: 8.90 (dt, $J = 202.46$, 9.84 Hz, 1H), 8.70 (dm, $J = 181.59$ Hz, 1H), 7.16 (dd, $J = 6.58$, 8.14 Hz, 1H), 7.07 (dd, $J = 6.58$, 8.41 Hz, 1H), 6.86 (dt, $J = 2.65$, 7.78 Hz, 1H), 6.43 (dm, $J = 176.32$ Hz, 1H), 2.94–3.12 (m, 2H), 2.45 (dd, $J = 15.72$, 75.03 Hz, 2H), 2.38 (s, 3H), 1.63 (s, 3H), 1.49 (s, 3H). ^{13}C NMR ($CDCl_3$) δ: 162.55, 160.13, 150.29 (t, $J = 53.71$ Hz), 146.31 (d, $J = 69.60$ Hz), 143.02 (d, $J = 69.6$ Hz), 138.081 (d, $J = 66.91$ Hz), 134.82, 134.74, 131.82, 127.55, 113.64 (m), 103.12 (m), 53.43, 42.56, 38.73, 34.20, 33.76, 31.66, 30.81, 22.41. LCMS: $MH^+ = 402.23$ (100%). High-resolution mass spectrometry: calc. 402.1894, found 402.1898.

Synthesis of [$^{14}C_2$]-(2)

*5-Fluoro-2-(R)-3-hydroxy-1,1-dimethyl-3-trifluoromethyl-hex-5-ynyl-[$^{14}C_2$])-benzamide, [$^{14}C_2$]-(**9**)*

To a solution of trimethylsilylacetylene[1,2-$^{14}C_2$] (200 mCi, 1.8 mmol) in anhydrous dimethoxyethylene (DME, 10 mL) was added *n*-BuLi (2.4 M in THF, 0.84 mL, 2 mmol) at −15 °C. The resulting yellow solution was warmed to room temperature and stirred for 1 h. A solution of epoxide (**8**) (0.52 g, 1.7 mmol) in DME (5 ML) was cooled to below −40 °C, and a solution of dibutyl magnesium (1 M in heptane, 1 mL) was added dropwise. After stirring for 1 h at this temperature, the resulting lithium acetylide was cooled to −20 °C and added via cannulation to the flask containing the epoxide (**8**). The reaction was left to warm gradually to room temperature and stirred for 2 h. After cooling to −10 °C, MeOH was added (10 mL), and the mixture was warmed to 0 °C in an ice bath. A solution of aqueous NaOH (1 N, 15 mL) was added dropwise, and the mixture was warmed to room temperature and stirred overnight. A saturated solution of NH_4Cl was added (50 mL) and extracted with EtOAc (20 mL × 3). The combined extracts were dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give brown oil. Purification by silica gel chromatography using 40 g disposable column wetted with CH_2Cl_2 and using up to 20% EtOAc : CH_2Cl_2 gave the desired product with some polar impurities. A second purification by HPLC gave 9.8 mCi of desired product with more than 98% radiochemical purity.

*{4-[(R)-6-(2-Carbamoyl-4-fluoro-phenyl)-4-hydroxy-6-methyl-4-trifluoromethyl-hept-1-ynyl-[1,2-$^{14}C_2$]-]-6-ethanesulfonyl-pyridin-3-yl}-carbamic acid tert-butyl ester, [$^{14}C_2$]-(**11**)*

A solution of TEA (64 μL, 0.46 mmol) in MeCN (3 mL) was degassed by bubbling argon through it for 20 min and then added to a mixture of the aforementioned acetylene derivative [$^{14}C_2$]-(**9**) (9.8 mCi, SA = 110 mCi/mmol), bis(acetonitrile)dichloropalladium (II) (2 mg, 8 μmol), CuI (5.2 mg, 27 μmol), and (**10**) (40 mg, 94 μmol) at room temperature. The resulting dark mixture was stirred for 12 h. The mixture was concentrated *in vacuo*, and the residue was dissolved in EtOAc and washed with water (5 mL) and brine (10 mL), dried over Na_2SO_4, filtered, and concentrated *in vacuo*. Purification by flash chromatography (12 g disposable silica gel column) using CH_2Cl_2 and then 20% EtOAc/CH_2Cl_2 gave 21 mg (3.8 mCi) of the desired product in 39% yield, which was used as it is in the next step.

*2-[(R)-3-(5-Ethanesulfonyl-1H-[2,3-^{14}C]pyrrolo[2,3-c]pyridin-2-ylmethyl)-4,4,4-trifluoro-3-hydroxy-1,1-dimethyl-butyl]-5-fluoro-benzamide, [$^{14}C_2$]-(**2**)*

To a solution of [$^{14}C_2$]-(**11**) (21 mg, 0.034 mmol) in MeOH (2 mL) was added DBU (40 μL, 0.27 mmol) in one portion at room temperature. The resulting solution was heated to 60 °C and stirred for 2.5 h. HPLC showed no starting material. The reaction was concentrated under a stream of nitrogen and then dissolved in EtOAc (5 mL) and washed with a saturated solution of NH_4Cl (3 mL), dried over Na_2SO_4, filtered, and concentrated overnight under a stream of nitrogen to give 21 mg of a residue. A sample of 14 mg of unlabeled (**2**) was added, and the mixture was dissolved in 10% MeOH/CH_2Cl_2 and then purified using a 12-g disposable silica gel column. An impurity remained that eluted just above the desired product (R_f = 0.65 for impurity; R_f = 0.52 for desired product in 50% EtOAc : CH_2Cl_2). A second purification using another 12 g silica gel column and 50% EtOAc : CH_2Cl_2 gave 32 mg of product as white solid, R_f = 0.27 in 10% MeOH/CH_2Cl_2. HPLC showed the product to be 92% pure (R_t = 10.31 min) with a major impurity (5%) at 12.74 min. The product was crystallized from absolute ethanol (1.0 mL) to give a grain-like white solid, which was washed with ethanol (0.5 mL × 2) to give 12 mg of the desired product and 15 mg of yellowish mother liquor. The mother liquor was concentrated, and the residue was dissolved in CH_2Cl_2 and purified by prep-TLC (0.5 mm) using 10% MeOH/$CHCl_3$. The layer corresponding to the product was scraped and extracted with 10% MeOH/$CHCl_3$, filtered, and concentrated *in vacuo*. The white solid was further dried under reduced pressure at 35 °C to give 13 mg of material with radiochemical purity of 98.8%. A total of 25 mg of combined product was obtained in 52% yield over two steps, and with a specific activity of 46 mCi/mmol.

Synthesis of [^{14}C]-(2)

*5-Fluoro-2-(4,4,4-trifluoro-1,1-dimethyl-3-oxo-butyl)-benzoic acid [carboxyl-^{14}C], [^{14}C]-(**13**)*

In a round-bottomed flask, sodium hydride (300 mg, 60 wt.% dispersion in mineral oil, 2.5 mmol) was added under N_2 atmosphere. Anhydrous THF (6 mL) was then added, and the mixture was stirred while cooling to internal temperature ~0–5 °C. Then, a solution of trifluoromethylketone (**12**) (2.21 g, 6.2 mmol) in THF (6 mL) was added at a rate that internal temperature does not exceed 10 °C. The mixture was stirred at 25 °C for 20 h. The mixture was cooled to 0–5 °C, and *iso*-propylmagnesium chloride lithium chloride complex (5.1 mL, 1.3 M in THF) was added at a rate that internal temperature does not exceed 20 °C in 5 min, and then 1,4-dioxane (1.7 mL) was added. The mixture was stirred at room temperature for 4 h. [^{14}C]-CO_2 generated from 500 mCi of $BaCO_3$ was then added to the reaction at −40 °C. The reaction was stirred below −20 °C for 2 h before aqueous 3 N HCl (12 mL) was added. The mixture was warmed to room temperature and extracted with toluene (20 mL × 3), filtered, and concentrated *in vacuo* to give 354 mCi (71% yield) of crude product, which was used as it is in the next step.

*5-Fluoro-N-[(S)-1-(4-methoxy-phenyl)-ethyl]-2-(4,4,4-trifluoro-1,1-dimethyl-3-oxo-butyl)-benzamide-[^{14}C], [^{14}C]-(**15**)*

The acid [^{14}C]-(**13**) (354 mCi, 6.1 mmol) in $SOCl_2$ (6 mL) was heated to 55 °C and stirred for 4 h. Excess thionyl chloride was removed *in vacuo*, and the residue was dissolved in THF (4 mL) and cooled to 0 °C. (*S*)-1-(4-Methoxyphenyl)ethylamine (**14**) (0.92 mL, 6.2 mmol) and TEA (2.9 mL, 21 mmol) were added at 0 °C. The reaction was warmed to room temperature and stirred for 2 h. The reaction was then concentrated *in vacuo*, and the residue was purified by silica gel chromatography using 35:1 CH_2Cl_2/MeOH to give 335 mCi of product in 95% yield.

*5-Fluoro-2-((R)-3-hydroxy-1,1-dimethyl-3-trifluoromethyl-hex-5-ynyl)-N-[(S)-1-(4-methoxy-phenyl)-ethyl]-benzamide-[^{14}C], [^{14}C]-(**17**)*

A solution of 1-trimethylsilylpropyne (1.3 mL, 8.8 mmol) in 50 mL of THF was treated dropwise over 15 min with *n*-BuLi (3.6 mL, 8.8 mmol, 2.5 M/hexanes) at −20 °C and stirred for 1 h and then treated with a solution of zinc bromide (1.45 g, 6.4 mmol) in THF (5 mL), keeping the temperature between −20 and −15 °C. The reaction mixture was stirred for 1 h at −20 °C. A solution of [^{14}C]-(**15**) (335 mCi, 5.78 mmol) in THF (5 mL) was added slowly, keeping the temperature between −20 and −15 °C. After 1 h, the reaction mixture was quenched with MeOH (7 mL) and concentrated to 1/3 volume. MeOH (7 mL) was added, followed by sodium methoxide (270 mg of Na and 1.5 mL of MeOH). The reaction mixture was stirred at room temperature for 1 h; 85% phosphoric acid (0.8 mL) in 50 mL of water was added. An additional 20 mL of water was added followed by 20 mL of EtOAc, and the slurry was stirred for 1 h and filtered. The solid (zinc oxide) was washed with 4 mL of water and then washed with EtOAc (10 mL). The filtrates were combined, and the layers were separated. The aqueous layer was extracted with EtOAc (30 mL × 3). The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated. Crystallization from EtOAc (8 mL) and heptane (1 mL) at room temperature overnight gave a white solid. The solid was filtered and dried to give 962 mg of product or 115.4 mCi of [^{14}C]-(**17**) in 34% radiochemical yield.

*2-[(R)-3-(5-Ethanesulfonyl-1H-pyrrolo[2,3-c]pyridin-2-ylmethyl)-4,4,4-trifluoro-3-hydroxy-1,1-dimethyl-butyl]-5-fluoro-N-[(S)-1-(4-methoxy-phenyl)-ethyl]-benzamide, [^{14}C]-(**18**)*

[^{14}C]-(**17**) (115.4 mCi, 1.99 mmol) and the iodopyridine derivative (**10**) (0.866 g, 2.04 mmol) in degassed MeOH (10 mL) were stirred under N_2 atmosphere. 1,4-Diazabicyclo(2,2,2)octane (430 μL, 3.87 mmol) and allylpalladium chloride dimmer (4 mg, 10.71 μmol) as a slurry in degassed MeOH (5 mL) were added. The reaction is heated to 50 °C. After 9 h, DBU (450 μL, 3.0 mmol) was added. All solid dissolved, and the yellow solution is heated up to 50 °C for 3 h. After cooling to room temperature, the reaction was concentrated *in vacuo*, and the residue was purified by silica gel chromatography using 30:1 CH_2Cl_2/MeOH. This residue was further purified using prep-TLC and 10:1 CH_2Cl_2/acetone to give 44.6 mCi in 37% radiochemical yield and with a specific activity of 58 mCi/mmol.

*2-[(4R)-4-{[5-(Ethylsulfonyl)-1H-pyrrolo[2,3-c]pyridin-2-yl]methyl}-5,5,5-trifluoro-4-hydroxy-2-methylpentan-2-yl]-5-fluoro-benzamide-[^{14}C], [^{14}C]-(**2**)*

[^{14}C]-(**18**) (44.6 mCi, 0.77 mmol) and acetic acid (2.5 mL) were stirred at room temperature. An off-white slurry is obtained. A solution of 48% aqueous HBr (1.3 mL) was added. The off-white slurry becomes red. The reaction was heated to 80 °C. It becomes homogeneous upon warming. After stirring at this temperature for 7 h, the reaction was cooled to room temperature overnight. After concentration *in vacuo*, the residue was dissolved in MeOH (20 mL) and treated with 1 N KOH (5 mL). The pH was adjust to 7, and the mixture was filtered, dried, and purified by flash chromatography using 15:1 CH_2Cl_2/MeOH to give 37 mCi of material in 83% radiochemical yield with a specific activity of 58 mCi/mmol and 99% radiochemical purity and 99% chiral purity. ^{1}H NMR (MeOH-d_4) δ 8.67 (s, 1H), 8.20 (s, 1H), 7.56 (dd, J = 9 Hz, J = 5 Hz, 1H), 7.10 (dd, J = 9 Hz, J = 3 Hz, 1H), 7.02 (ddd, J = 9 Hz, J = 9 Hz, J = 3 Hz, 1H), 6.47 (s,

1H), 3.34 (q, $J = 7$ Hz, 2H), 3.17 (d, $J = 15$ Hz, 1H), 3.02 (d, $J = 15$ Hz, 1H), 2.49 (d, $J = 15$ Hz, 1H), 2.42 (d, $J = 15$ Hz, 1H), 1.61 (s, 6H), 1.19 (t, $J = 7$ Hz, 3H).

Synthesis of [$^2H_5$]-(2)

*2-Ethyl-[$^2H_5$]-sulfanyl-5-nitropyridin, [$^2H_5$]-(**20**)*

To a mixture of [$^2H_5$]-ethanethiol (5 g, 74.5 mmol) in THF (115 mL) and aqueous NaO^2H (2 N, 46.5 mL) was added 2-chloro-5-nitropyridine (**19**) (9.84 g, 61.4 mmol) in one batch at 0 °C. After 2 h, the starting material was consumed. TLC (10% EtOAc : hexanes), $R_f = 0.24$ (starting material) and $R_f = 0.36$ (product). Water was added (150 mL) and extracted with EtOAc (100 mL × 3). The organic layer was washed with brine (100 mL), dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give 11.71 g of an off-white solid in 99% yield. LCMS (ESI^+): 95% to 5% water/MeCN gradient (0.1 formic acid): $MH^+ = 190.66$ (100%), $R_t = 1.29$ min. ^{1}H NMR ($CDCl_3$) δ: 9.23 (d, $J = 2.56$ Hz, 1H), 8.21 (dd, $J = 2.56$, 9.48 Hz, 1H), 7.26 (d, $J = 9.48$ Hz, 1H). ^{13}C NMR ($CDCl_3$) δ: 168.17, 145.05, 140.95, 130.22, 121.41, 24.21 (pent, $J = 21.13$ Hz), 13.22 (sept, $J = 19.12$ Hz).

*6-Ethyl-[$^2H_5$]-sulfanyl-pyridin-3-ylamine, [$^2H_5$]-(**21**)*

A mixture of [$^2H_5$]-(**20**) (11.56 g, 61.1 mmol) and 10% Pd/C (2 g) in absolute ethanol (200 mL) was stirred under 50 psi of hydrogen for 20 h in a Parr apparatus. TLC (10% EtOAc : hexanes) showed no starting material. The reaction was filtered through a short pad of Celite® and concentrated *in vacuo* to half its volume and then used as an ethanolic solution in the next step. LCMS (ESI^+): $MH^+ = 160.38$ (100%), $R_t = 0.77$ min. ^{1}H NMR ($CDCl_3$) δ: 8.21 (d, $J = 2.61$ Hz, 1H), 7.68 (dd, $J = 2.61$, 8.65 Hz, 1H), 7.14 (d, $J = 8.65$ Hz, 1H), 6.35 (s, 2H). ^{13}C NMR ($CDCl_3$) δ: 151.3, 145.10, 138.7, 127.3, 24.2 (pent. $J = 22.13$ Hz), 14.31 (sept, $J = 20.12$ Hz).

*(6-Ethyl-[$^2H_5$]-sulfanyl-pyridin-3-yl)carbamic acid tert-butyl ester, [$^2H_5$]-(**22**)*

To a solution of [$^2H_5$]-(**21**) from last reaction in ethanol (100 mL) was added di-*tert*-butyl dicarbonate (17 g, 63 mmol) in one portion at room temperature. The resulting solution was stirred overnight. The reaction was then concentrated under reduced pressure, and the residue was dissolved in EtOAc (300 mL) and washed with a saturated solution of $NaHCO_3$ (100 mL) and brine (50 mL), dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give 16.75 g of a viscous oil. Purification by Combiflash using 120 g disposable column and a gradient 0% to 20% EtOAc : CH_2Cl_2 gave 15.6 g of a viscous oil in 98% yield. The product solidified upon standing at room temperature. TLC in 10 MeOH/$CHCl_3$: starting amine, $R_f = 0.33$ (turned brown in the air); product, $R_f = 0.60$. LCMS (ESI^+): $MH^+ = 260.48$ (100%), $R_t = 1.70$ min. ^{1}H NMR ($CDCl_3$) δ: 8.31 (d, $J = 2.61$ Hz, 1H), 7.78 (s, 1H), 7.12 (d, $J = 8.67$ Hz, 1H), 6.74 (s, 1H), 1.54 (br s, 9H). ^{13}C NMR ($CDCl_3$) δ: 152.85, 140.30, 131.90, 127.06, 122.51, 81.09, 28.28, 24.28 (pent. $J = 22.13$ Hz), 13.57 (sept, $J = 20.12$ Hz).

*(6-Ethyl-[$^2H_5$]-sulfanyl-4-iodopyridin-3-yl)carbamic acid tert-butyl ester, [$^2H_5$]-(**23**)*

To a solution of [$^2H_5$]-(**22**) (15.5 g, 61 mmol) in anhydrous ether (150 mL) was added a solution of *n*-BuLi in hexanes (2.4 M, 60 mL) at −78 °C under nitrogen dropwise. The resulting solution was warmed to 0 °C in an ice bath and stirred for 3 h. The dark reaction was cooled again to −78 °C, and iodine (34 g, 134 mmol) dissolved in anhydrous ether (150 mL) was added dropwise with stirring. The reaction was warmed gradually to room temperature and stirred overnight. A solution of saturated ammonium chloride (350 mL) was added, and the ether layer was separated; washed with a solution of Na_2SO_3, water (50 mL), and brine (50 mL); and dried over $MgSO_4$. Filtration and concentration *in vacuo* gave 20.45 g of dark viscous oil. A portion of this crude product (10 g) was purified by Combiflash to give 1.6 g of the desired product in 70% yield based on reacted starting material. A batch of 4 g of unreacted starting material was recovered. LCMS: $MH^+ = 386.45$ (100%), $R_t = 1.93$ min. ^{1}H NMR ($CDCl_3$) δ: 9.21 (s, 1H), 8.41 (s, 1H), 7.32 (s, 1H), 1.53 (s, 9H). ^{13}C NMR ($CDCl_3$) δ: 152.41, 150.30, 140.13, 138.81, 131.85, 98.51, 81.98, 28.43, 24.15 (pent. $J = 22.13$ Hz), 13.85 (sept, $J = 20.12$ Hz).

*(6-Ethyl-[$^2H_5$]-sulfonyl-4-iodopyridin-3-yl)carbamic acid tert-butyl ester, [$^2H_5$]-(**10**)*

To a solution of [$^2H_5$]-(**23**) (1.6 g, 4.2 mmol) in acetonitrile (26 mL) and water (12 mL) was added sodium (*meta*)periodate (1.94 g, 9 mmol) and $RuCl_3$ (50 mg, 0.24 mmol) at room temperature. The resulting mixture (dark) was stirred overnight. LCMS shows only one product: $MH^+ = 418.57$ (100%), $R_t = 1.61$ min. Ether (300 mL) was added followed by aqueous NaCl (200 mL). The organic phase was removed, and the aqueous solution was extracted with another 300 mL of ether. The combined extracts were dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give 2.22 g of a solid material. Purification by Combiflash using a 120-g disposable silica gel column and a gradient 0% to 20% EtOAc in CH_2Cl_2 gave 1.4 g of a white solid in 85% yield. ^{1}H NMR ($CDCl_3$) δ: 9.34 (s, 1H), 8.42 (s, 1H), 7.03 (s, 1H), 1.56 (s, 9H). ^{13}C NMR ($CDCl_3$) δ: 151.37, 149.76, 140.13, 139.87, 132.67, 98.30, 82.97, 46.03 (pent, $J = 21.13$ Hz), 28.41, 5.99 (sept, $J = 20.13$ Hz).

*5-Fluoro-2-((R)-3-hydroxy-1,1-dimethyl-3-trifluoromethyl-hex-5-ynyl)-benzamide, (**24**)*

To a solution of trimethylsilylacetylene (1.2 g, 11.7 mmol) in anhydrous DME (10 mL) was added *n*-BuLi (2.4 M in THF, 5 mL, 12 mmol) at −15 °C. The resulting yellow solution was warmed to room temperature and stirred for 1 h. A solution of epoxide (**8**) (0.52 g, 1.7 mmol) in DME (5 mL) was cooled to below −40 °C, and a solution of dibutyl magnesium (1 M in heptane, 1 mL) was added dropwise. After stirring for 1 h at this temperature, the lithium acetylide was cooled to −20 °C and added via cannulation to the epoxide flask. The reaction was left to warm gradually to room temperature and stirred for 2 h. After cooling to −10 °C, MeOH was added (10 mL), and the mixture was warmed to 0 °C in an ice bath. A solution of NaOH in water (15 mL, 1 N) was added dropwise, and the mixture was warmed to room temperature and stirred overnight. A solution of NH_4Cl was added (50 mL) and extracted with EtOAc (50 mL × 3). The combined extracts were dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give 700 mg of brown oil, which solidified upon standing at room temperature. Purification by silica gel chromatography using 40 g disposable column wetted with CH_2Cl_2 and using up to 20% EtOAc : CH_2Cl_2 gave 440 mg of orange solid with $R_f = 0.5$ in 10% MeOH/$CHCl_3$. ^{1}H NMR ($CDCl_3$) δ: 7.51 (m, 1H), 7.10 (m, 1H), 7.02 (m, 1H), 6.33 (s, 1H), 6.06 (s, 1H), 5.76 (s, 1H), 5.30 (s, 1H), 2.51 (m, 2H), 2.42 (AB quart, $J = 15.01$, 50.77 Hz, 2H), 2.08 (s, 1H), 1.66 (s, 3H), 1.44 (s, 3H). LCMS: $R_t = 1.61$ min, 95%, $MH^+ = 332.72$.

*{4-[(R)-6-(2-Carbamoyl-4-fluoro-phenyl)-4-hydroxy-6-methyl-4-trifluoromethyl-hept-1-ynyl]-6-ethane-[$^2H_5$]-sulfonyl-pyridin-3-yl}-carbamic acid tert-butyl ester, [$^2H_5$]-(**25**)*

A solution of TEA (0.85 mL, 6.1 mmol) in acetonitrile (5 mL) was degassed by bubbling argon through it for 15 min and added via cannulation to a mixture of the alkyne derivative (**24**) (394 mg, 1.19 mmol), the labeled iodopyridine [$^2H_5$]-(**10**) (0.5 g, 1.2 mmol), bis(acetonitrile)dichloropalladium (II) (5 mg, 0.02 mmol), and cuprous iodide (69 mg, 0.355 mmol). The resulting dark solution was stirred for 3 h at room temperature. HPLC, in 22 min run, shows that the reaction was over. A new product at 14.27 min was detected, with the starting material at 10.79 min. The reaction was concentrated *in vacuo*, and the residue was dissolved in EtOAc (20 mL) and washed with water (20 mL). The organic phase was dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give 0.91 g of an orange residue. Purification using 40 g disposable column and up to 20% EtOAc : CH_2Cl_2 gave 691 mg of yellow foam. TLC (12% MeOH/$CHCl_3$): product, $R_f = 0.43$; starting material, $R_f = 0.37$. ^{1}H NMR ($CDCl_3$) δ: 9.49 (s, 1H), 8.29 (s, 1H), 7.56 (s, 1H), 7.48 (m, 1H), 7.04 (m, 2H), 6.51–6.70 (m, 2H), 6.20 (s, 1H), 3.48 (m, 1H), 2.76 (AB quart, $J = 17.51$, 42.6 Hz, 2H), 2.40–2.61 (m, 2H), 1.65 (s, 6H), 1.54 (s, 9H). ^{13}C NMR ($CDCl_3$) δ: 175.64, 161.72, 159.24, 151.65, 148.68, 140.18, 139.75, 139.04, 136.29, 130.17, 125.02, 119.14, 116.94, 116.34, 115.51, 115.28,

98.81, 82.47, 75.42 (q, $J = 27.17$ Hz), 45.13, 36.96, 33.92, 33.15, 28.12, 27.13. LCMS: $R_t = 1.83$ min (99%), $MH^+ = 621.85$. HPLC: $R_t = 14.36$ min, 99%.

2-[(S)-3-(5-Ethane-[$^2H_5$]-sulfonyl-1*H*-pyrrolo[2,3-*c*]pyridin-2-ylmethyl)-4,4,4-trifluoro-3-hydroxy-1,1-dimethyl-butyl]-5-fluoro-benzamide, [$^2H_5$]-(**2**)

To a solution of [$^2H_5$]-(**25**) (690 mg, 1.11 mmol) in MeOH (10 mL) was added DBU (0.5 mL, 3.34 mmol) in one portion at room temperature. The resulting solution was heated to 60 °C and stirred for 2 h. HPLC showed no starting material. The reaction was concentrated under reduced pressure and then dissolved in EtOAc (50 mL) and washed with a saturated solution of NH_4Cl (30 mL), dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give 0.58 g of a yellow solid. TLC (10% MeOH/$CHCl_3$): starting material, $R_f = 0.40$; product, $R_f = 0.27$. The solid was dissolved in boiling EtOAc (6 mL) and left to cool to room temperature to give a white solid. Filtration and drying gave 550 mg of material. HPLC: $R_t = 9.79$ min, 99%. LCMS: $MH^+ = 521.81$ as the only ion peak, $R_t = 1.51$ min. ^{1}H NMR (MeOH-d_4) δ: 8.68 (s, 1H), 8.21 (s, 1H), 7.57 (dd, $J = 5.42$, 8.94 Hz, 1H), 7.11 (dd, $J = 2.91$, 8.76 Hz, 1H), 7.03 (dt, $J = 2.91$, 12.70 Hz, 1H), 6.48 (s, 1H), 4.11 (q, $J = 7.15$ Hz, 2H, EtOAc), 3.12 (Abq, $J = 14.97$, 60.27 Hz, 2H), 2.46 (d, $J = 15.41$ Hz, 2H), 2.02 (s, 3H, EtOAc), 1.62 (s, 6H), 1.25 (t, $J = 7.15$ Hz, 3H, EtOAc). ^{13}C NMR (MeOH-d_4) δ: 176.15, 171.50, 161.65, 159.20, 143.70, 142.17, 139.94, 113.88, 134.45, 133.53, 132.54, 130.43, 130.36, 127.38, 124.51, 115.40, 115.16, 102.91, 76.06 (q, $J = 80.50$ Hz), 60.05, 45.02, 37.25, 32.67, 31.06, 19.38, 12.98, contains one equivalent of EtOAc. High-resolution mass spectrometry: calc. 521.18885, found 521.19016.

Conflict of interest

The authors did not report any conflict of interest.

References

[1] E. Charmandari, C. Tsigos, G. Chrousos, *Annu. Rev. Physiol.* **2005**, *67*, 259–284.
[2] H. Schäcke, M. Berger, T. G. Hansson, D. McKerrecher, H. Rehwinkel, *Expert Opin. Ther. Pat.* **2008**, *18*, 339–352.
[3] H. Razavi, D. Riether, C. Harcken, J. Bentzien, R. M. Dinallo, D. Souza, R. M. Nelson, A. J. Kukulka, T. Fadra-Khan, E. J. Pack, Jr., L. Zuvela-Jelaska, J. Pelletier, M. Panzenbeck, C. Torcellini, J. R. Proudfoot, G. Nabozny, D. S. Thomson, *Bioorg. Med. Chem. Lett.* **2014**, *24*, 1934–1940.
[4] D. DiSalvo, D. Kuzmich, J. Bentzien, J. Regan, A. Kukulka, A. Kukulka, R. Nelson, C. Harcken, D. S. Thomson, G. Nabozny, *Bioorg. Med. Chem. Lett.* **2013**, *23*, 6645–6649.
[5] D. Kuzmich, J. Bentzien, R. Betageri, D. DiSalvo, T. Fadra-Khan, C. Harcken, A. Kukulka, G. Nabozny, R. Nelson, E. Pack, *Bioorg. Med. Chem. Lett.* **2013**, *23*, 6640–6644.
[6] P. Norman, *Expert Opin. Ther. Pat.* **2011**, *21*, 1137–1142.
[7] R. Betargeri, T. Gilmore, D. Kuzmich, T. M. Kirrane, J. Bentzien, D. Wieddenmayer, Y. Bekkali, J. Regan, A. Berry, B. Latli, A. J. Kukulka, T. N. Fadra, R. Nelson, S. Goldrick, L. Zuvela-Jelaska, D. Souza, J. Pelletier, R. Dinallo, M. Panzenbeck, C. Torcellini, H. Lee, E. Pack, C. Harcken, G. Nabozny, D. S. Thomson, *Bioorg. Med. Chem. Lett.* **2011**, *21*, 6842–6851.
[8] H. Diallo, D. C. Angell, H. A. Barnett, K. Biggadike, D. M. Coe, T. W. J. Cooper, A. Craven, J. R. Gray, D. House, T. I. Jack, S. P. Keeling, S. J. F. Macdonald, I. M. McLay, S. Oliver, S. J. Taylor, I. J. Uings, N. Wellaway, *Bioorg. Med. Chem. Lett.* **2011**, *21*, 1126–1133.
[9] D. Riether, C. Harcken, H. Razavi, D. Kuzmich, T. Gilmore, J. Bentzien, E. J. Pack, D. Souza, R. M. Nelson, A. J. Kukulka, T. N. Fadra, L. Zuvela-Jelaska, J. Pelletier, R. Dinallo, M. Panzenbeck, C. Torcellini, G. Nabozny, D. S. Thomson, *J. Med. Chem.* **2010**, *53*, 6681–6698.
[10] M. Berlin, *Expert Opin. Ther. Pat.* **2010**, *20*, 855–873.
[11] D. Kuzmich, T. Kirrane, J. Proudfoot, Y. Bekkali, R. Zindell, L. Beck, R. Nelson, C.-K. Shih, A. J. Kukulka, Z. Paw, P. Reilly, R. Deleon, M. Cardozo, G. Nabozny, D. S. Thomson, *Bioorg. Med. Chem. Lett.* **2007**, *17*, 5025–5031.
[12] J. Regan, T. W. Lee, R. M. Zindell, Y. Bekkali, J. Bentzien, T. Gilmore, A. Hammach, T. M. Kirrane, A. J. Kukulka, D. Kuzmich, R. M. Nelson, J. R. Proudfoot, M. Ralph, J. Pelletier, D. Souza, L. Zuvela-Jelaska, G. Nabozny, D. S. Thomson, *J. Med. Chem.* **2006**, *49*, 7887–7896.
[13] R. Betargeri, Y. Zhang, R. M. Zindell, D. Kuzmich, T. M. Kirrane, J. Bentzien, M. Cardozo, A. J. Capolino, T. N. Fadra, R. Nelson, Z. Paw, D.-T. Shih, C.-K. Shih, L. Zuvela-Jelaska, G. Nabozny, D. S. Thomson, *Bioorg. Med. Chem. Lett.* **2005**, *15*, 4761–4769.
[14] C. Harcken, D. Riether, D. Kuzmich, P. Liu, R. Betageri, M. Ralph, M. Emmanuel, J. T. Reeves, A. Berry, D. Souza, R. M. Nelson, A. Kukulka, T. N. Fadra, L. Zuvela-Jelaska, R. Dinallo, G. Nabozny, D. S. Thomson, *J. Med. Chem.* **2014**, *57*, 1583–1598.
[15] C. Harcken, D. Riether, P. Liu, H. Razavi, U. Patel, T. Lee, T. Bosanac, Y. Ward, M. Ralph, Z. Chen, D. Souza, R. M. Nelson, A. Kukulka, T. N. Fadra-Khan, L. Zuvela-Jelaska, M. Patel, D. S. Thomson, G. H. Nabozny, *ACS Med. Chem. Lett.* **2014**, *5*, 1318–1323.
[16] (a)T. W. Lee, J. R. Proudfoot, D. S. Thomson, *Bioorg. Med. Chem. Lett.* **2006**, *16*, 654–657; (b)J. J. Song, Z. Tan, J.. Xu, J. T. Reeves, N. K. Yee, R. Ramdas, F. Gallou, K. Kuzmich, L. DeLattre, H. Lee, X. Feng, C. H. Senanayake, *J. Org. Chem.* **2007**, *72*, 292–294; (c)Z. Han, J. J. Song, N. K. Yee, Y. Xu, W. Tang, J. T. Reeves, Z. Tan, X.-J. Wang, B. Lu, D. Krishnamurthy, C. H. Senanayake, *Org. Proc. Res. Dev.* **2007**, *11*, 605–608; (d)J. J. Song, J. Xu, Z. Tan, J. Xu, J. T. Reeves, N. Grinberg, H. Lee, K. Kuzmich, X. Feng, N. K. Yee, C. H. Senanayake, *Org. Proc. Res. Dev.* **2007**, *11*, 534–538.
[17] B. Latli, P.-J. Jones, D. Krishnamurthy, C. H. Senanayake, *J. Labelled Compd. Rad.* **2008**, *51*, 54–58.
[18] C. Harken, Y. Ward, D. Thomson, D. Riether, *Syn lett* **2005**, *20*, 3121–3125.
[19] J. T. Reeves, D. R. Fandrick, Z. Tan, J. J. Song, S. Rodriguez, B. Qu, S. Kim, O. Niemeier, Z. Li, D. Byrne, S. Campbell, A. Chitroda, P. DeCroos, T. Fachinger, V. Fuchs, N. C. Gonnella, N. Grinberg, N. Haddad, B. Jäger, H. Lee, J. C. Lorenz, S. Ma, B. A. Narayanan, L. J. Nummy, A. Premasiri, F. Roschangar, M. Sarvestani, S. Shen, E. Spinelli, X. Sun, R. J. Varsolona, N. Yee, M. Brenner, C. H. Senanayake, *J. Org. Chem.* **2013**, *78*, 3616–3635.
[20] R. Luque, D. J. Macquarrie, D. J. *Org. Biomol. Chem.* **2009**, *7*, 1627–1632.