Research Article

Journal of Labelled Compounds and Radiopharmaceuticals

Received 10 December 2013, Revised 16 January 2014, Accepted 17 January 2014 Published online 13 February 2014 in Wiley Online Library

(wileyonlinelibrary.com) DOI: 10.1002/jlcr.3187

Hepatitis C virus serine protease: synthesis of radioactive and stable isotope-labeled potent inhibitors

Bachir Latli,* Matt Hrapchak, Vida Gorys, Montse Llinàs-Brunet, Scot S. Campbell, Jinhua Song, and Chris H. Senanayake

Drug candidates labeled with radioactive and stable isotopes are required for absorption, distribution, metabolism, and excretion (ADME) studies, pharmacokinetics, autoradiography, bioanalytical, and other research activities. The findings from these studies are crucial in the development of a drug candidate and its approval for human use. Herein, we report the synthesis of potent and selective hepatitis C virus serine protease inhibitors related to BILN 2061 and BI 201335 labeled with radioactive and stable isotopes. Synthetic efforts were focused on the common substituted thiazole moiety, which is easily accessible via a Hantzsch's reaction of α-bromoketones and mono-substituted thioureas. In the radioactive synthesis, commercially available carbon-14 thiourea was utilized to prepare mono-substituted thioureas, which upon condensation with α-bromoketones in isopropanol followed by ester hydrolysis gave the desired carbon-14-labeled protease inhibitors. The same strategy was used to prepare these inhibitors labeled with stable isotopes. Mono-substituted thioureas were obtained from commercially available deuterium-labeled intermediates and then condensed with α-bromoketones followed by ester hydrolysis to give the deuterium-labeled inhibitors.

Keywords: HCV; NS3 protease inhibitors; BILN 2061; BI 201335; carbon-14; deuterium; radiosynthesis

Introduction

Since its identification in 1989, [1] hepatitis C virus (HCV) has been viewed as the major cause of chronic hepatitis in humans.[2] This virus can cause liver diseases ranging from mild illness lasting a few weeks to a serious condition that leads to liver cirrhosis or cancer. About 25% of liver cancer is attributed to HCV. In the USA, where the virus has overtaken HIV as a cause of death,[3] four million Americans are unknown carriers. The Centers of Disease Control and Prevention has recently urged baby boomers to test for HCV.[4] Each year, between three to four million people get infected, and more than 350,000 people die from HCV-related liver disease worldwide.[5,6] There are seven known genotypes of HCV, with genotype 1 being the hardest to treat and representing 60% of world infection.[7,8] The challenge to tailor treatments to these various genotypes represents a great opportunity and huge potential market for pharmaceuticals companies. Hepatitis C is a single-stranded RNA virus. Its genome encoded a single protein of about 3000 amino acids. This protein is cleaved by proteases into smaller structural and nonstructural (NS) proteins[9–11], with NS3/4A protease playing an important role in viral replication.[12,13] Drugs acting directly on the virus, such as those working as NS3/4A protease inhibitors, are just emerging. The first clinical proof of concept for this mechanism was achieved with BILN 2061.[14] Since then, two new drugs, telaprevir and boceprevir were approved by the Food and Drug Administration for HCV genotype 1.[15] Both drugs inhibit HCV's protease, but they must be taken with interferon and ribavirin.[16] The search for drugs that are not used in conjunction with interferon-α is an ongoing endeavor.[17–19] Since the disclosure of BILN 2061, several other series of C-terminus carboxylic acid tripeptides that are very potent and selective inhibitors of HCV NS3/4A protease have been reported.[20–22] Pharmacokinetics, excretion mass balance, isolation, identification, and quantification of metabolites, and determination of the partitioning of radioactivity between plasma and red blood cells of these peptide mimetic inhibitors are best performed when these inhibitors are co-administered with carbon-14-labeled analogs. Indeed, carbon-14 has been the radioisotope of choice for ADME studies.[23,24] The radioactive carbon is usually incorporated into the drug carbon skeleton in a site where it is not lost through metabolism. In one such study, BI201335 was mixed with carbon-14-labeled BI201335, 1% by weight of the total dose and administered to healthy human males.[25] Blood, urine, feces, and saliva samples were then collected at intervals through the study. The identification of the metabolites in this human study was greatly aided with carbon-14-labeled BI201335 because of the sensitivity of radio-detection. Analytical methods, such as HPLC and liquid chromatography–tandem mass spectrometry were used to identify the metabolites.[25] Along with radioactive isotopes, the use of stable isotopes in drug development is critical for developing validated, sensitive, and selective bioanalytical methods for the quantitative evaluation of drug candidates and their metabolites in biological matrixes (blood, plasma, serum, and urine).[26] In this manuscript;

Chemical Development, Boehringer Ingelheim Pharmaceuticals, Inc., 900 Ridgebury Road, PO Box 368, Ridgefield, CT 06877-0368, USA

**Correspondence to: Bachir Latli, Chemical Development, Boehringer Ingelheim Pharmaceuticals, Inc. 900 Ridgebury Road, PO Box 368, Ridgefield, CT 06877-0368, USA. E-mail: bachir.latli@boehringer-ingelheim.com*

we report the synthesis of five HCV protease inhibitors labeled with carbon-14 and with deuterium (Figure 1).

Results and discussion

The tripeptide HCV NS3/4A serine protease inhibitors **1–5** are complex molecules, wherein even the most practical and economical large-scale synthesis required 17 steps.[27] Each compound has five stereo centers, a cyclopropyl, quinoline, and thiazole moieties. Introducing a radioactive carbon at the thiazole moiety was envisioned as the simplest way to access carbon-14-labeled HCV inhibitors in a fast and efficient manner, requiring only α-bromoketones, which are available from in house syntheses,[20] and mono-substituted thioureas and using the classical Hantzsch's thiazole synthesis.[28] The mono-substituted thiourea derivatives were prepared with the carbon-14 in the thiourea ($^{14}C=S$) and not on the side chain, because of the fact that 2-substituted aminothiazoles may be prone to metabolic cleavage to aminothiazoles. Thus, carbon-14-labeled thiourea was condensed with the corresponding acylchloride derivatives in toluene to provide the labeled mono-substituted thioureas **[^{14}C]-6**, **[^{14}C]-7** (Scheme 1). The preparation of carbon-14 isopropylthiourea **[^{14}C]-8** was described previously.[29]

Simple condensations of the substituted thioureas [^{14}C]-**6**, [^{14}C]-**7**, and [^{14}C]-**8** with α-bromoketones **9**, **10**, **11**, and **12** in hot isopropanol, followed by ester hydrolysis using aqueous solution of lithium hydroxide in tetrahydrofuran and methanol at room temperature, provided the carbon-14-labeled compounds **2**, **3**, **4**, and **5** in overall yields ranging from 31% to 65% after purifications (Scheme 2). The low yields reported from the radiosynthesis is due to the fact that radioactive reagents are not very stable, and only fractions with more than 98% radiochemical purities of radiolabeled compounds were combined during the purifications.

Similarly, for the synthesis of the deuterium-labeled HCV inhibitors, the deuterium-labeled mono-substituted thiourea derivatives were prepared. It is desirable to have the drug candidate labeled with at least three amu higher to distinguish it from the parent drug candidate during liquid chromatography–tandem mass spectrometry analyses. Commercially available [D_7]-isobutyric acid was converted to the acetylchloride derivative using oxalyl chloride in methylene chloride and subsequently condensed with thiourea in toluene at 110 °C to give **[D_7]-6** in 40% yield over two steps. Deuterium-labeled *tert*-butyl acetic acid **[D_9]-7** was prepared from the reaction of [D_9]-*tert*-butyl alcohol and dichloroethylene in concentrated deuterated sulfuric acid and deuterated water.[30] The acylchloride of this acid was then condensed as previously described with thiourea to give **[D_9]-7** in 40% over all yield after crystallization (Scheme 3). The synthesis of **[D_7]-8**, which was described previously, also proceeded well.[29]

Condensation of the deuterium-labeled mono-substituted thioureas and α-bromoketones **9**, **10**, **11**, and **12** in isopropanol at 70 °C followed by acid hydrolysis gave **[D_7]-2**, **[D_7]-3**, **[D_9]-4**, and **[D_7]-5** in yields ranging from 66% to 93% (Scheme 4).

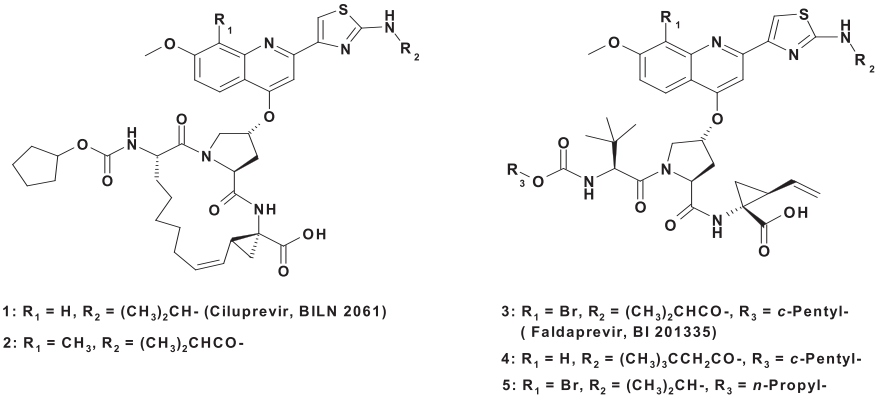
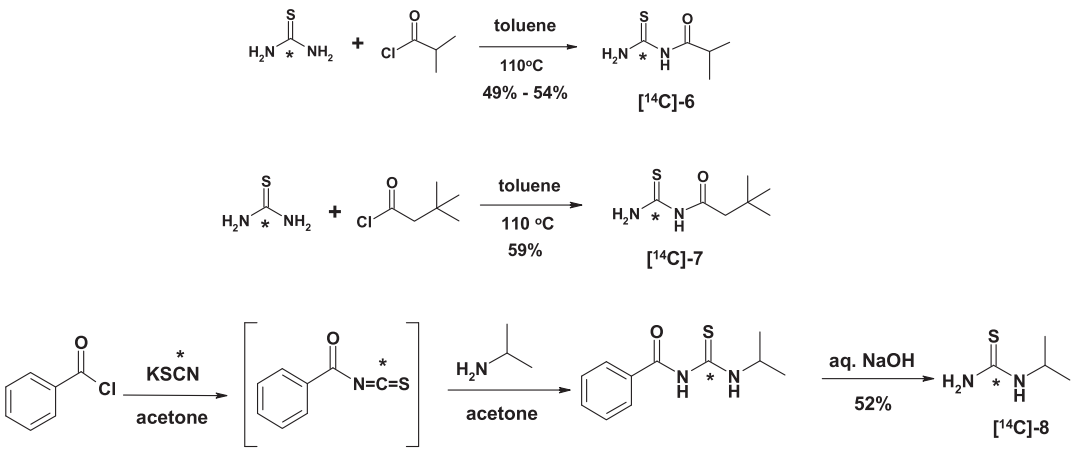
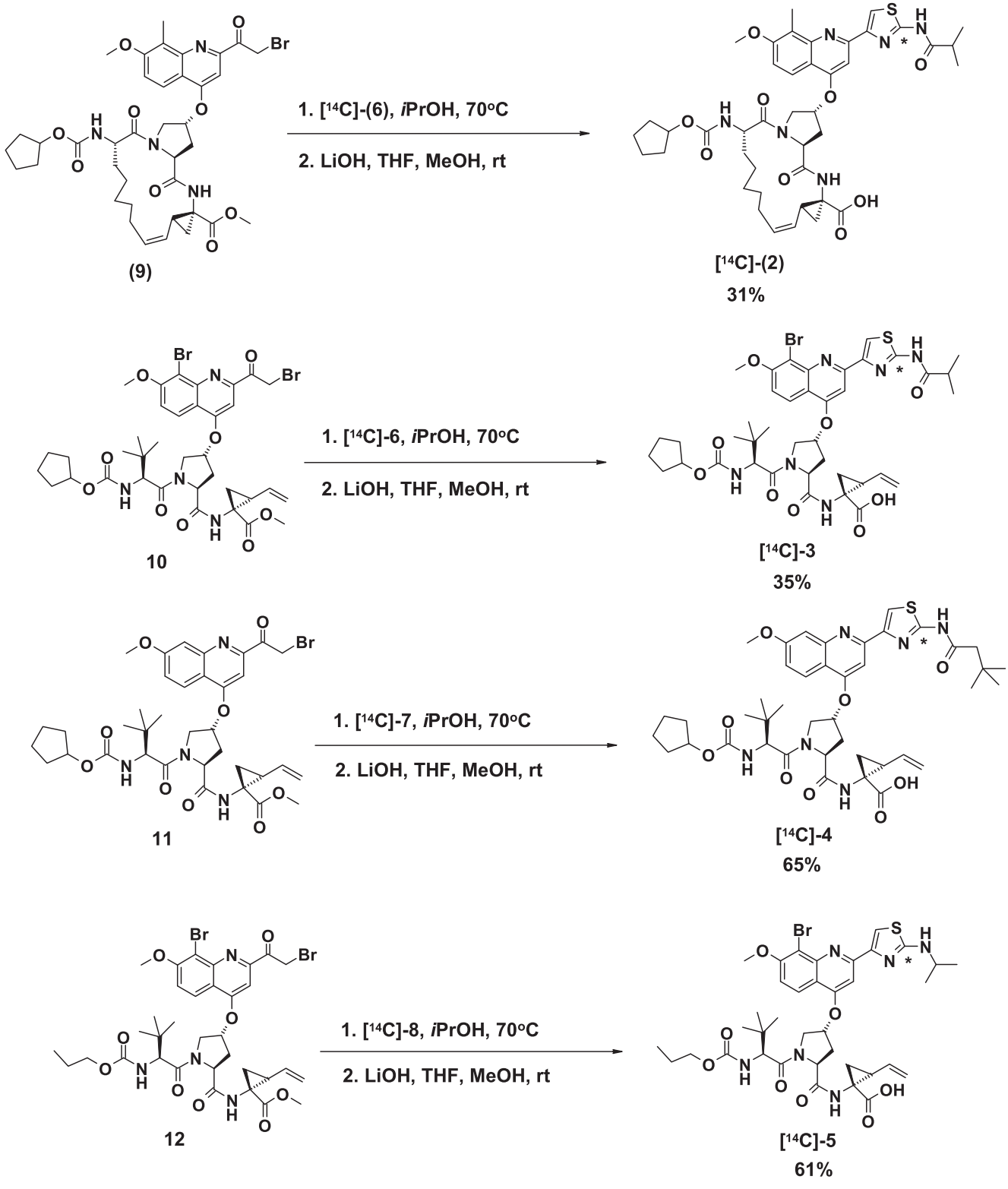


Figure 1. Some of Boehringer Ingelheim's hepatitis C virus protease inhibitors.



Scheme 1. Synthesis of carbon-14-labeled thiourea derivatives; asterisks indicate position of carbon-14.

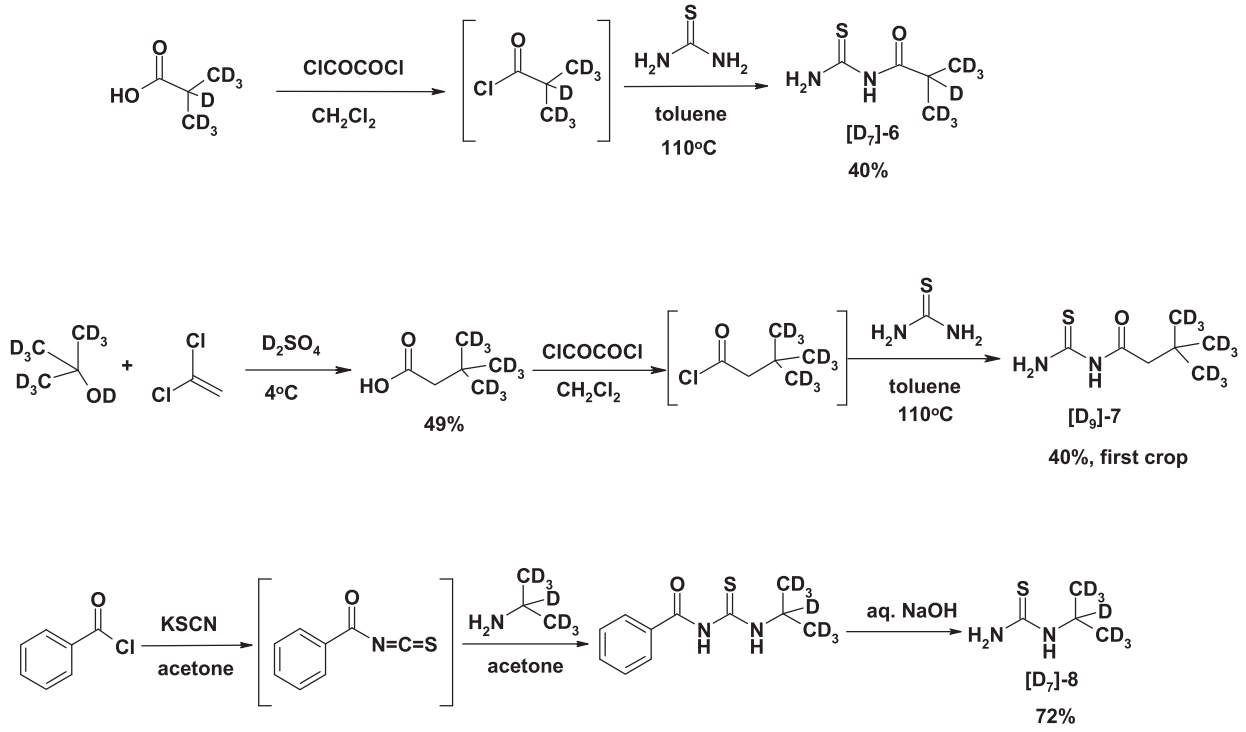


Scheme 2. Synthesis of carbon-14-labeled hepatitis C virus serine protease inhibitors.

Experimental

Materials and methods

Nuclear magnetic resonance (NMR) spectra of radioactive compounds were recorded on a Bruker 500 MHz spectrometer (Bruker-Biospin Corp., NMR Division, Billerica, MA) using double encapsulated NMR tubes in deuterated dimethyl sulfoxide. These compounds exist in two slowly interconverting conformational states. Only the major rotomer of each compound was reported and compared with unlabeled standards. NMR spectra of compounds labeled with stable isotopes were recorded on Bruker 400 MHz spectrometer in a suitable deuterated solvent. Liquid scintillation counting was accomplished using a Beckman LS6500TA and UltimaGold™ cocktail (PerkinElmer, MA). Pre-coated TLC sheets (silica gel 60 F_{254}) and silica



Scheme 3. Synthesis of deuterium-labeled mono-substituted thiourea derivatives.

gel 60-200 Mesh (Nominal, I.D., grade 62) for flash chromatography were obtained from EM Science (Gibbstown, NJ). HPLC analysis was performed on an Agilent 1200 instrument with a Zorbax Eclipse XDB-C18 (5 μm, 4.6 × 150 mm) column and a mobile phase gradient of 20% to 100% MeCN in H_2O (both 0.1% TFA) over 30 min, flow rate: 1 mL/min. The chemical purity of prepared compounds was greater than 98%. The radiochemical purity was measured using a radio-HPLC detector *β*-Ram model 4 or model 3 (LabLogic systems, Inc. Brandon, FL) connected to the Agilent instrument. The radiochemical purity of the final compounds was greater than 98%. [^{14}C]-thiourea was purchased from ViTrax (Plancentia, CA). [D_7]- 2-Methyl propionic acid (99.9 atom% D), [D_{10}]-*tert*-butanol (99.9 atom % D) and [D_7]-isopropylamine (98.9 atom % D) were purchased from CDN (Pointe Claire, Quebec, Canada). [D_2]-Sulfuric acid 98 wt.% solution in D_2O (99.8 atom% D), and the rest of the reagents were purchased from Sigma-Aldrich Company. Work up as usual refers to dissolving in ethyl acetate, washing with a saturated solution of $NaHCO_3$, drying over Na_2SO_4 or $MgSO_4$, filtration and concentration under reduced pressure. Intermediates **9**, **10**, **11**, and **12** were prepared by reported procedures.[20]

Carbon-14 synthesis

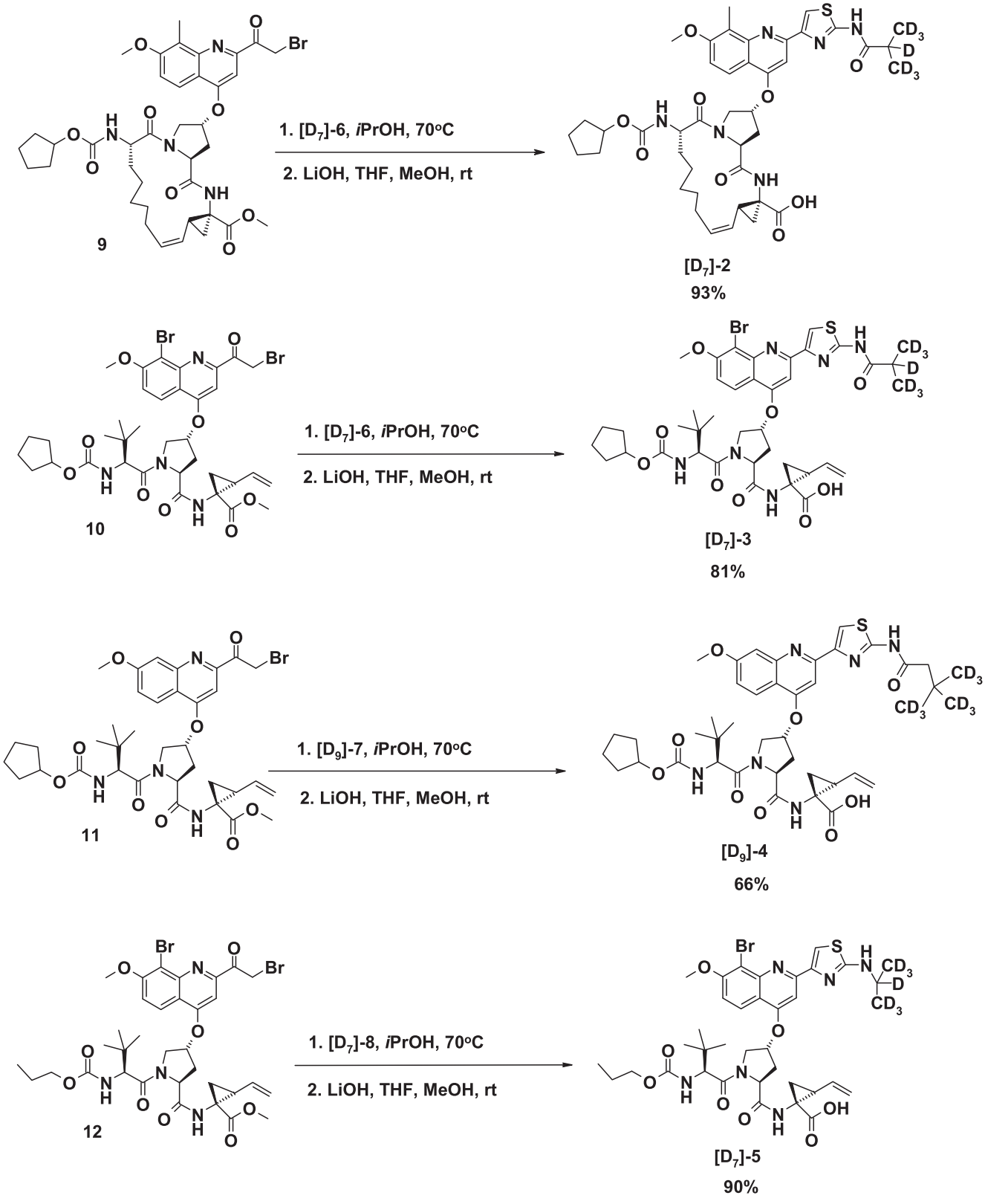
Synthesis of [^{14}C]-2

[^{14}C]-N-Carbamothioyl-2-methyl-propanamide, [^{14}C]-6. 2-Methylpropanoyl chloride (201 mg, 1.90 mmol) was added to [^{14}C]-thiourea (100 mCi at 52 mCi/mmol, 1.92 mmol) in toluene (3 mL). The reaction mixture was heated at 110 °C for 16 h. The mixture was cooled to room temperature and worked up as usual. The crude solid was purified by flash chromatography (0–3% MeOH/CH_2Cl_2) to give 152 mg of a light yellow solid in 54% yield, which eluted with unlabeled authentic material[20] on TLC and HPLC (98% radiochemical purity).

(Z)-(1-[2-(2-[2-^{14}C]isobutyramidothiazol-4-yl)-7-methoxy-8-methylquinolin-4-yloxy)-2,15-dioxo-3,16-diaza-tricyclo[14.3.0.0^{4,6}]nonadec-7-ene-4-carboxylic acid, [^{14}C]-2. *α*-Bomoketone **9** (815 mg, 1.04 mmol) was added to a solution of **[^{14}C]-6** (152 mg, 1.04 mmol) in isopropanol (15 mL) at room temperature. The mixture was heated to 70 °C and held at this temperature for 2 h. After cooling to room temperature, the mixture was worked up as usual. The crude solid was purified using silica gel flash chromatography (50% EtOAc/hexanes) to give 367 mg as a light yellow solid in 43% yield. The product co-eluted with unlabeled authentic material on TLC plate and on HPLC. A 3.2 M LiOH aqueous solution (1.2 mL, 3.8 mmol) was added to a solution of the aforementioned material (367 mg, 0.44 mmol) in THF (7 mL) and methanol (3.5 mL) at room temperature. After 6 h, a second portion of aqueous 3.2 M LiOH (0.5 mL, 1.6 mmol) was added to complete the reaction, and the mixture was stirred for 16 h. The pH was adjusted to ≈5 by addition of 5% HCl, and the mixture was worked up as usual to give yellow solid. The crude solid was purified using silica gel flash chromatography (10–50% EtOAc/CH_2Cl_2) to give 262 mg (16.7 mCi) of a white solid in 73%, yield with a specific activity of 52 mCi/mmol. Radiochemical purity of 99%, HPLC, t_R = 17.83 min. ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.28 (s, 1H, NH), 12.21(s, 1H, OH), 8.60(s, 1H, NH), 8.04(d, J = 9.1Hz, 1H), 8.02 (s, 1H), 7.43(s, 1H), 7.28(s, 1H), 7.21(d, J = 9.1 Hz, 1H), 5.52(m, 2H), 5.45(brs, 1H), 5.28(d, J = 16.5 Hz, 1H), 4.64(m, 1H), 4.53(d, J = 8.3 Hz, 2H), 4.47(t, J = 8.3, 1H), 4.13(m, 1H), 3.93(s, 3H), 2.82(m, 1H), 2.61(s, 3H), 2.53(m, 2H), 2.52(m, 2H), 2.20(q, J = 8.6, 1H), 1.74(m, 2H), 1.71–1.58(m, 4H), 1.64–1.35(m, 4H), 1.46 (m, 2H), 1.38(m, 2H), 1.36(m, 2H), 1.33(m, 2H), 1.16(d, J = 7.5 Hz, 6H).

Synthesis of [^{14}C]-BI 201335, [^{14}C]-3

(1R,2S)-1-{[(2S,4R)-4-[8-Bromo-2-(2-[^{14}C]-2-isobutyrylamino-thiazol-4-yl)-7-methoxy-quinolin-4-yloxy]-1-((S)-2-cyclopentyloxycarbonylamino-3,3-dimethyl-butyryl)-pyrrolidine-2-carbonyl]-amino}-2-vinyl-cyclopropane carboxylic acid, [^{14}C]-3. A mixture of *α*-bromoketone **10** (795 mg, 0.884 mmol) and **[^{14}C]-6** (139 mg, 0.938 mmol) in isopropanol (10 mL) was placed in a preheated oil bath at 70 °C. The resulting solution was stirred for 2 h. After cooling to room temperature, the solvent was evaporated under a stream of nitrogen. The solid residue was dissolved in methylene chloride (5 mL) and worked up as usual. Purification by silica gel chromatography using a 40 g RediSep™ (Lincoln, NE) disposable column and methylene chloride then 10% to 50% EtOAc : CH_2Cl_2 gave 336 mg of a cream colored solid in 43% chemical yield. Total activity = 19.85 mCis.



Scheme 4. Synthesis of deuterium-labeled hepatitis C virus serine protease inhibitors.

HPLC (98%) co-eluted with unlabeled authentic sample. To a solution of the aforementioned methyl ester in THF (3.5 mL) and methanol (2 mL) was added a solution of LiOH in water (0.525 mL, 3.2 M solution). The reaction was stirred at room temperature for 12 h. TLC analysis showed no starting material (100% EtOAc). The reaction was concentrated then treated with aqueous 1 M HCl (2 mL) and worked up as usual. The residue was purified by silica gel chromatography using a 12 g RediSep and methylene chloride then 50% EtOAc : CH_2Cl_2 to give 145 mg of a yellowish solid in 81% chemical yield. Total activity = 8.54 mCi and the specific activity = 51.35 mCi/mmol. HPLC co-elutes with unlabeled standard, t_R = 18.5 min,

radiochemical purity > 99%. ^{1}H NMR (500 MHz, DMSO-d$_6$): δ 12.47(s, 1H), 12.34(s, 1H), 8.56(s, 1H), 8.16(d, J = 9.1 Hz, 1H), 8.04(s, 1H), 7.46(s, 1H), 7.34(d, J = 9.1 Hz, 1H), 7.00(d, J = 7.5 Hz, 1H), 5.72(m, 1H), 5.41(s, 1H), 5.20(m, 1H), 5.06(m, 1H), 4.61(m, 1H), 4.45(m, 1H), 4.37(m, 1H), 4.01(d, J = 11.6 Hz, 1H), 4.00(s, 3H), 3.95(dd, J = 8.3 Hz, 1H), 2.82(m, 1H), 2.56(m, 1H), 2.26 (m, 1H), 2.02(q, J = 8.6 Hz, 1H), 1.69(m, 1H), 1.60(m, 1H), 1.56(m, 1H), 1.26–1.81(m, 4H), 1.45(m, 1H), 1.31(m, 1H), 1.28(m, 1H), 1.16(d, J = 7.5 Hz, 6H), 0.99(s, 9H).

Synthesis of [^{14}C]-4

[^{14}C]-N-Carbamothioyl-3,3-dimethyl-butanamide, [^{14}C]-7. 3,3-Dimethylbutanoyl chloride (158 mg, 1.92 mmol) was added via a syringe to a solution of [^{14}C]-thiourea (100 mCi, 1.92 mmol) in toluene (4.0 mL) at room temperature in a sealed tube. The mixture was heated at 110°C for 16 h before it was cooled to room temperature. The mixture was worked up as usual to give 200 mg of [^{14}C]-*N-tert*-butylacetylthiourea in 60% yield as a yellow solid. TLC (50% EtOAc : hexanes), R_f = 0.6, same as unlabeled authentic sample.[23]

(R)-1-[(1-(2S-Cyclopentyloxycarbonylamino-3,3-dimethyl-butyryl)-4-{2-[(^{14}*C)-[2-(3,3-dimethyl-butyrylamino)-thiazol-4-yl]]-7-methoxy-quinolin-4R-yloxy}-pyrrolidine-2S-carbonyl)-amino]-2-vinyl-cyclopropanecarboxylic acid, [^{14}C]-4.* α-bromoketone **11** (1.1 g, 1.45 mmol) was added to a solution of **[^{14}C]-7** (200 mg, 1.13 mmol) in isopropanol (25 mL) at room temperature and heated at 70 °C for 1.5 h. The mixture was cooled to room temperature and concentrated under reduced pressure to a solid residue. The residue was worked up as usual giving 1.3 g of a solid. The crude was purified by silica gel flash chromatography using 50% ethyl acetate/hexane to give 460 mg of light yellow foam (HPLC radiochemical purity 99%) and 337 mg (HPLC radiochemical purity 97%) as a yellow solid for a total of 797 mg in 66% yield. The total activity of the pure product was found to be 30.6 mCi, with a specific activity of 55.55 mCi/mmol. The activity of the 97% pure product was found to be 22.5 mCi. A solution of LiOH (40 mg, 1.74 mmol) in H_2O (2.5 mL) was added to a solution of the above 99% pure material (167 mg, 11.1 mCi, 0.2 mmol) in THF (5 mL) and MeOH (2.5 mL) at room temperature. The mixture was stirred at room temperature overnight. The reaction mixture was concentrated and then diluted with ethyl acetate (10 mL) and brine (10 mL). The pH was adjusted to ca. 5.0 by addition of 1 N aqueous HCl and worked up as usual giving 164 mg as a light yellow solid. The total activity of the pure product was found to be 11.02 mCi, with a specific activity of 55.1 mCi/mmol. HPLC, t_R = 16.5 min (99% radiochemical purity). ^{1}H NMR (500 MHz, DMSO-d$_6$): δ 12.47 (s, 1H, OH), 12.32(s, 1H, NH), 8.53(s, 1H, NH), 8.14(d, J = 8.6 Hz, 1H), 8.03 (s, 1H), 7.51(s, 1H), 7.32(s, 1H), 7.02(d, J = 8.6 Hz, 1H), 7.00(s, 1H, NH), 5.85(m, 1H), 5.44(s, 1H), 5.22(d, J = 16.5 Hz, 2H), 5.08(d, J = 11.8 Hz, 1H), 4.82(m, 1H), 4.47(t, J = 8.7 Hz, 1H), 4.35(d, J = 11.8 Hz, 1H), 4.21 (d, J = 8.7 Hz, 1H), 3.95(s, 3H), 3.93(m, 1H), 2.65(m, 1H), 2.34(s, 2H), 2.21 (m, 1H), 2.02(m, 1H), 1.21–1.85(m, 9H), 1.04(s, 9H), 0.99(s, 9H).

Synthesis of [^{14}C]-5

(R)-1-{[(2S,4R)-4-[8-Bromo-2-(2-[2-^{14}*C]-2-isopropylamino-thiazol-4-yl)-7-methoxy-quinolin-4-yloxy]-1-((S)-3,3-dimethyl-2-propoxycarbonylamino-butyryl)-pyrrolidine-2-carbonyl]-amino}-2-vinyl-cyclopropanecarboxylic acid, ([^{14}C]-5.* α-bromoketone **12** (1.43 g, 1.77 mmol) was added to a solution of **[^{14}C]-8** (172 mg, 1.46 mmol) in isopropanol (15 mL) at room temperature. The mixture was heated at 70 °C for 1 h. The reaction mixture was cooled to room temperature and concentrated to a solid residue. This residue was diluted with 5% Na_2CO_3 (10 mL), extracted with CH_2Cl_2 (10 mL ×3). The combined extracts were dried over Na_2SO_4 and concentrated. The crude residue was purified twice using CombiFlash Companion (40 g, 40–60% EtOAc/hexanes) to give 790 mg (48.1 mCi) as light yellow solid in 65% yield. HPLC: t_R = 12.94 min (99% radiochemical purity), co-eluted with unlabeled authentic material. A solution of LiOH (5.0 mmol) in water (5.0 mL) was added to a solution of the aforementioned material (263 mg, 0.32 mmol) in THF (10 mL) and MeOH (5.0 mL) at room temperature. The mixture was stirred for 3 h. The pH was adjusted to ≈5 by addition of 5% HCl. The mixture was worked up as usual giving an orange solid. The crude solid was crystallized from *n*-propanol (3 mL) to give 118 mg as light orange solid after drying under high vacuum in 45% yield. A total of 8.07 mCi at 55.8 mCi/mmol was obtained. HPLC: t_R = 15.51 min, radiochemical purity 99%. ^{1}H NMR (500 MHz, DMSO-d$_6$): δ 12.45(s, 1H, COOH), 8.54(s, 1H, NH), 8.12(d, J = 9.12 Hz, 1H), 7.71(d, J = 7.51 Hz, 1H, NH), 7.51(s, 1H), 7.45(s, 1H) 7.33(d, J = 9.12 Hz, 1H), 7.05(d, J = 8.58 Hz, 1H, NH), 5.75(m, 1H), 5.43(s, 1H), 5.21(d, J = 16.45 Hz, 1H), 5.06(d, J = 11.76 Hz, 1H), 4.46(t, J = 8.34 Hz, 1H), 4.32(d, J = 11.76 Hz, 1H), 4.15(d, J = 8.65 Hz 1H), 4.05(m, 2H), 4.01(S, 3H), 3.82(septet, J = 6.36 Hz, 1H), 3.74 (m, 2H), 2.52(m, 2H), 2.03(q, J = 8.64 Hz, 1H), 1.56(m, 2H), 1.41(m, 2H), 1.25(d, J = 6.36 Hz, 6H), 0.98(brs, 8H), 0.81(t, J = 7.32 Hz, 3H).

Synthesis of deuterium-labeled inhibitors

Synthesis of [D$_7$]-2

[D$_7$]-N-Carbamothioyl-2-methyl-propanamide, [D$_7$]-6. To a solution of [D$_7$]-2-methylpropionic acid (1.5 g, 15.78 mmol) in methylene chloride (10 mL) was added oxalyl chloride (7 mL, 78.64 mmol) drop wise at 0 °C. The resulting colorless solution was warmed to room temperature and stirred overnight. The solution was then concentrated *in vacuo* and then diluted with methylene chloride (10 mL ×2) and concentrated to remove excess oxalyl chloride. The remaining residue of deuterium-labeled 2-methylpropanoyl chloride and thiourea (1.22 g, 15.86 mmol) were heated in toluene (20 mL) at 110 °C for 12 h. After cooling to room temperature, the mixture was concentrated *in vacuo* to a yellow solid residue and dissolved in ethyl acetate (20 mL) and worked up as usual. Crystallization from (1:1) hexane: ethyl acetate gave 0.8 g of a pale yellow solid in 33% yield. ^{1}HNMR (400 MHz, $CDCl_3$): δ 10.01(s, 1H), 9.57(s, 1H), 7.72(s, 1H). ^{13}CNMR (90 MHz, $CDCl_3$): δ 182.44, 178.02, 39.2(m), 17.63(m). MS (ES+) calculated for $C_5H_3N_2OSD_7 + H^+$ 154.25, found 154.20.

(Z)-(1S,4R,6S,14S,18R-14-Cyclopentyloxycarbonylamino)-18-[2-(2-[D$_7$]-isobutyramidothiazol-4-yl)-7-methoxy-8-methylquinolin-4-yloxy)-2,15-dioxo-3,16-diaza-tricyclo[14.3.0.0^{4,6}]nonadec-7-ene-4-carboxylic acid, [D$_7$]-2. This material was prepared as described for **[^{14}C]-2** from α-bromoketone **9** (0.4 g, 0.487 mmol) and **[D$_7$]-6** (90 mg, 0.59 mmol) to give 390 mg of the pure methyl ester product as a white solid in 96% yield. MS (ES+) calculated for $C_{43}H_{47}N_6O_9SD_7 + H^+$ 839.05, found 839.27. The ester (390 mg, 0.465 mmol) was hydrolyzed similarly and Purified by silica gel flash chromatography using methylene chloride and 10 to 50% EtOAc:CH_2Cl_2. The product was dissolved in 2 mL of methylene chloride and precipitated by addition of hexanes to give 377 mg of a white solid after drying *in vacuo* in 98% yield. HPLC, t_R = 18.17 min (99% chemical purity). MS (ES+): calculated for $C_{42}H_{45}N_6O_9SD_7 + H^+$ 825.02, found 825.6 ^{1}H NMR (400 MHz, DMSO-d$_6$): δ 12.28 (s, 1H, NH), 12.21(s, 1H, OH), 8.6(s, 1H, NH), 8.04(d, J = 9.1 Hz, 1H), 8.02(s, 1H), 7.43(s, 1H), 7.28 (s, 1H), 7.21(d, J = 9.1 Hz, 1H), 5.51(m, 2H), 5.45(s, 1H), 5.28 (d, J = 16.5 Hz, 1H), 4.64(m, 1H), 4.53(d, J = 8.3 Hz, 2H), 4.47(t, J = 8.3 Hz, 1H), 4.13(m, 1H), 3.93(s, 3H), 2.61(s, 3H), 2.53(m, 2H), 2.52(m, 2H), 2.2 (q, J = 8.6 Hz, 1H), 1.74(m, 2H), 1.71-1.58(m, 4H), 1.64-1.35(m, 4H), 1.46 (m, 2H), 1.38(m, 2H), 1.36(m, 2H), 1.33(m, 2H).

Synthesis of [D$_7$]-BI 201335, [D$_7$]-3

(1R,2S)-1-{[(2S,4R)-4-[8-Bromo-2-(2-[D$_7$]-(isobutyrylamino-thiazol-4-yl)-7-methoxy-quinolin-4-yloxy]-1-((S)-2-cyclopentyloxycarbonylamino-3,3-dimethyl-butyryl)-pyrrolidine-2-carbonyl]-amino}-2-vinyl-cyclopropanecarboxylic acid, [D$_7$]-3. This material was prepared as previously described for **[^{14}C]-3** from **[D$_7$]-6** (0.16 g, 1.04 mmol) and α-bromoketone **10** (0.41 g, 0.46 mmol) to give after purification by flash chromatography using a 40 g RediSep disposable column and using CH_2Cl_2 then 10 to 50% EtOAc: CH_2Cl_2 as eluent, 382 mg of methyl ester derivative as yellowish solid (98% chemical purity) in 94% yield. This ester (0.32 g, 0.36 mmol) was hydrolyzed as in

2:1 THF: MeOH (10 mL) and solution of 3.2 M LiOH in water (1.0 mL) to give after the usual work up 400 mg of a yellow solid. Purification using a 40 g RediSep column and methylene chloride, then% 10 to 50% EtOAc:CH_2Cl_2 gave 270 mg of product in 86% yield as a white solid. HPLC: t_R = 17.5 min, 99.6% chemical purity, MS (ES+): calculated for $C_{40}H_{42}BrN_6O_9SD_7 + H^+$ 877.88, found 877.50. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.47(s, 1H), 12.34 (s, 1H), 8.56(s, 1H), 8.16(d, J = 9.1 Hz, 1H), 8.04(s, 1H), 7.46(s, 1H), 7.34 (d, J = 9.1 Hz, 1H), 7.00(d, J = 7.5 Hz, 1H), 5.72(m, 1H), 5.41(s, 1H), 5.20 (m, 2H), 5.06(m, 1H), 4.61(m, 1H), 4.45(m, 1H), 4.37(m, 1H), 4.01 (d, J = 11.6 Hz, 1H), 4.00(s, 3H), 3.95(dd, J =, 8.3 Hz, 1H), 2.56(m, 1H), 2.26 (m, 1H), 2.02(q, J = 8.6 Hz, 1H), 1.69(m, 1H), 1.60(m, 1H), 1.56(m, 1H), 1.26–1.81(m, 4H), 1.45(m, 1H), 1.31(m, 1H), 1.28(m, 1H), 0.99(s, 9H).

Synthesis of [D_9]-4

[D_9]-tert-Butylacetic acid. In a three-neck round bottom flask fitted with a thermometer, a dropping funnel and a condenser, was placed concentrated [D_2]-sulfuric acid (30 mL). A solution of [D_9]-*tert*-butanol (4.6 g, 62.1 mmol) and vinylidene chloride (6.2 mL, 76.8 mmol) is added dropwise at temperature of 4–6 °C in a 2-h period. The resulting yellowish solution was further stirred at this temperature for 90 min. The solution was then poured into 150 mL of crushed ice and extracted with hexane (150 mL ×3). The combined extracts were washed with 300 mL of 2 N aqueous KOH. The basic solution was then treated with 80 mL of concentrated HCl and extracted with methylene chloride, dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give 3.53 g of colorless oil in 49% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.2 (s).

[D_9]-N-Carbamothioyl-3,3-dimethyl-butanamide, [D_9]-7. To a solution of the aforementioned acid (2.0 g, 17.22 mmol) in methylene chloride (12 mL) was added oxalyl chloride (10 mL, 113.5 mmol) at room temperature. The solution was then stirred overnight. The yellow solution was then concentrated *in vacuo* to give 2.5 g of material. Thiourea (1.32 g, 17.2 mmol) was added to this acetyl chloride (2.5 g, 17.22 mmol) in toluene (25 mL) and refluxed for 12 h. The yellow solution was cooled to room temperature, and the mixture was concentrated *in vacuo*. The solid residue was dissolved in ethyl acetate (20 mL) and worked up as usual to give 1.95 g of a yellow solid. Crystallization from ethyl acetate (5 mL) and hexanes (10 mL) gave white needles, which were filtered, washed with hexane and dried under pressure to give 1.2 g of material in 40% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.92(s, 1H), 9.61(s, 1H), 9.34(s, 1H), 2.22(s, 2H). MS (ES+): calculated for $C_7H_5N_2OSD_9 + H^+$ 184.18, found 184.10.

(R)-1-[(1-(2-Cyclopentyloxycarbonylamino-3,3-dimethyl-butyryl)-4-{2-[2-[D_9]-(3,3-dimethyl-butyrylamino)-thiazol-4-yl]-7-methoxy-quinolin-4R-yloxy)-pyrrolidine-2S-carbonyl)-amino]-2-vinyl-cyclopropanecarboxylic acid, [D_9]-4. This compound was prepared similar to **[^{14}C]-4** from α-bromoketone **11** (0.76 g, 1.0 mmol) and [D_9]-*t*-butylacetyl thiourea (0.21 g, 1.1 mmol) to obtain 0.88 g of a yellowish solid. TLC (5% MeOH/$CHCl_3$): *t*-butylacetyl thiourea, R_f = 0.6; starting material, R_f = 0.7. Purification by flash chromatography using $CHCl_3$ then 5 to 50% EtOAc:$CHCl_3$ gave 728 mg of cream colored solid. A second purification using silica gel packed in hexanes and 30% to 40% EtOAc in hexane provided 707 mg of material in 84% yield and more than 98% chemical purity by HPLC. MS (ES+): calculated for $C_{43}H_{47}N_6O_9SD_9 + H^+$ 843.1, found 843.0. TLC (10% MeOH/$CHCl_3$): R_f = 0.58. co-eluted with unlabeled sample. The methyl ester derivative (0.7 g, 0.84 mmol) was hydrolyzed and worked up as before to give 537 mg of a bright yellow solid. The solid was dissolved in isopropanol and left to crystallize at -4 °C overnight. The solid was filtered and dried under reduced pressure to give 256 mg of a bright yellow solid in 78% yield. TLC (5% MeOH/CH_2Cl_2): starting material, R_f = 0.36; product, R_f = 0.25. HPLC: t_R = 16.8 min, 99% chemical purity, MS (ES+): calculated for $C_{42}H_{45}N_6O_9SD_9 + H^+$ 829.05, found 829.70. MS (ES-) calculated $C_{42}H_{45}N_6O_9SD_9$-H^+ 827.03, found 827.70. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.47(s, 1H), 12.32(s, 1H), 8.53(s, 1H), 8.14(d, J = 8.6 Hz, 1H), 8.03(s, 1H), 7.51(s, 1H), 7.32(s, 1H), 7.02(d, J = 8.6 Hz, 1H), 7.00(s, 1H), 5.85(m, 1H), 5.44(s, 1H), 5.22(d, J = 16.5 Hz, 2H), 5.08 (d, J = 11.8 Hz, 1H), 4.82(m, 1H), 4.47(t, J = 8.7 Hz, 1H), 4.35(d, J = 11.8 Hz, 1H), 4.21(d, J = 8.7 Hz, 1H), 3.95(s, 3H), 3.92(m, 1H), 2.64(m, 1H), 2.35(s, 2H), 2.20(m, 1H), 2.02(m, 1H), 1.21–1.87(m, 9H), 0.99(s, 9H).

Synthesis of [D_7]-5

(R)-1-{[(2S,4R)-4-[8-Bromo-2-(2-[D_7]-isopropylamino-thiazol-4-yl)-7-methoxy-quinolin-4-yloxy]-1-((S)-3,3-dimethyl-2-propoxycarbonylamino-butyryl)-pyrrolidine-2-carbonyl]-amino}-2-vinyl-cyclopropanecarboxylic acid, [D_7]-5. α-bromoketone **12** (300 mg, 0.37 mmol) and **[D_7]-8** (46 mg, 0.37 mmol) were used as described for **[^{14}C]-5** giving 387 mg of an off white solid in 95% yield. HPLC: 13.9 min, 98% chemical purity. Ester hydrolysis of this product (387 mg, 0.37 mmol) gave 320 mg of an orange solid in 100% yield. Half of the crude solid was purified by CombiFlash Companion using a 12-g disposable silica gel column and 0–10% MeOH/CH_2Cl_2, to give 138 mg of an orange solid. MS (ES+): calculated for $C_{37}H_{40}BrN_6O_8SD_7 + H^+$ 823.83, found 823.50. HPLC (retention time): 12.8 min, 99% pure. ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.45(s, 1H, COOH), 8.54(s, 1H, NH), 8.12(d, J = 9.12 Hz, 1H), 7.71(d, J = 7.51 Hz, 1H, NH), 7.51 (s, 1H), 7.45(s, 1H) 7.33(d, J = 9.12 Hz, 1H), 7.05(d, J = 8.58 Hz, 1H, NH), 5.75(m, 1H), 5.43(s, 1H), 5.21(d, J = 16.45 Hz, 1H), 5.06(d, J = 11.76 Hz, 1H), 4.46(t, J = 8.34 Hz, 1H), 4.32(d, J = 11.76 Hz, 1H), 4.15(d, J = 8.65 Hz 1H), 4.05(m, 2H), 4.01(S, 3H), 3.82(s, 1H), 3.74 (m, 2H), 2.52(m, 2H), 2.03(q, J = 8.64 Hz, 1H), 1.56(m, 2H), 1.41(m, 2H), 0.98(brs, 8H), 0.81 (t, J = 7.32 Hz, 3H).

Conclusion

Simple and efficient syntheses of radioactive and stable isotopes of Boehringer Ingleheim's leading HCV NS3 protease inhibitors were developed to support drug metabolism, pharmacokinetics, mass balance, bioanalytical, and other studies. In the radioactive synthesis, commercially available carbon-14 thiourea was utilized to prepare mono-substituted thiourea derivatives, which were then condensed with α-bromoketones according to Hantzsch's reaction. Subsequent ester hydrolysis gave the desired radioactive compounds. For the preparation of stable isotopes labeled compounds, a similar strategy was employed by initially preparing the mono-substituted thiourea derivatives from commercially available deuterium-labeled intermediates, followed by condensation with α-bromoketones, and then, ester hydrolysis provided the desired deuterium-labeled compounds. These labeled compounds are indispensable in enabling clinical studies and other ADME studies.

Acknowledgement

We thank Dr. Carl Busacca for reading this manuscript.

Conflict of Interest

The authors did not report any conflict of interest.

References

[1] (a)Q.-L. Choo, G. Kuo, A. J. Weiner, L. R. Overby, D. W. Bradley, M. Houghton, *Science* **1989**, *244*, 359–362; (b)G. Kuo, Q.-L. Choo, H. G. Alter, G. L. Grrnick, A. G. Redeker, R. H. Purcell, T. Miyamura, J. L. Dienstag, M. J. Alter, C. E. Stevens, G. E. Tegtmeier, F. Bonino, M. Colombo, W.-S. Lee, C. Kuo, K. Berger, J. R. Shuster, L. R. Overby, D. W. Bradley, M. Houghton, *Science* **1989**, *244*, 362–364.
[2] B. D. Lindenbach, M. J. Evans, A. J. Syder, B. Wolk, T. L. Tellinghuisen, C. C. Liu, T. Maruyama, R. O. Hynes, D. R. Burton, J. A. Mckeating, C. M. Rice, *Science* **2005**, *309*, 623–626.
[3] K. N. Ly, J. Xing, M. Klevens, R. B. Jiles, J. W. Ward, S. D. Holmberg, *Ann. Intern. Med.* **2012**, *156*, 271–278.

[4] http://www.cdc.gov/hepatitis/HCV/HCVfaq.htm#Section3. [28 June 2014].
[5] World Health Organization, Hepatitis C, Fact sheet N° 164, July **2012**.
[6] M. G. Ghany, D. B. Strader, D. L. Thomas, L. B. Seeff, *Hepatology* **2009**, *49*, 1335–1374.
[7] L. Gravitz, *Nature* **2011**, *474*, S2–S4.
[8] T. Nakano, G. M. Lau, *Liver Int.* **2011**, *32*, 339–345.
[9] Q. M. Wang, M. X. Du, M. A. Hockman, R. B. Johnson, X.-L. Sun, *Drugs Fut.* **2000**, *25*, 933–944.
[10] A. A. Kolykhalov, K. Mihalik, S. M. Feinstone, C. M. Rice, *J. Virol.* **2000**, *74*, 2046–2051.
[11] T. L. Tellinghuisen, M. J. Evans, T. von Hahn, C. M. Rice, *J. Virol.* **2007**, *81*, 8853–8867.
[12] V. Rajagopal, M. Gurjar, M. K. Levin, S. S. Patel, *J. Biol. Chem.* **2010**, *285*, 17821–17832.
[13] K. J. Blight, A. A. Kolykhalov, C. M. Rice, *Science* **2000**, *290*, 1972–1974.
[14] D. Lamarre, P. C. Anderson, M. Baily, P. Beaulieu, G. Bolger, P. Bonneau, M. Bös, D. R. Cameron, M. Cartier, M. G. Cordingley, A.-M. Faucher, N. Goudreau, S. H. Kawal, G. Kukolj, L. Lagacé, S. R. LaPlante, H. Narjes, M.-A. Poupart, J. Rancourt, R. E. Sentjens, R. St. George, B. Simoneau, G. Steinmann, D. Thibeault, Y. S. Tsantrizos, S. M. Weldon, C.-L. Yong, M. Llinàs-Brunet, *Nature* **2003**, *426*, 186–189.
[15] D. M. Jensen, *N. Engl. J. Med.* **2011**, *364*, 1272–1274.
[16] J. Schlütter, *Nature* **2011**, *474*, S5–S7.
[17] K. Garber, *Nat. Biotechnol.* **2011**, *29*, 963–966.
[18] J. Cohen, *Science* **2012**, *337*, 1450–1451.
[19] N. Alkhouri, N. N. Zein, *Cleveland Clin. J. Med.* **2012**, *79*, 213–222.
[20] M. Llinàs-Brunet, M. D. Baily, N. Goudreau, P. K. Bhardwaj, J. Bordeleau, M. Bös, Y. Bousquet, M. G. Cordingley, J. Duan, P. Forgione, M. Garneau, E. Ghiro, V. Gorys, S. Goulet, T. Halmos, S. H. Kawai, J. Naud, M.-A. Poupart, P. W. White, *J. Med. Chem.* **2010**, *53*, 6466–6476.
[21] S. R. LaPlante, M. Llinàs-Brunet, *Curr. Med. Chem. – Anti-Infective Agents* **2005**, *4*, 111–132.
[22] M. Llinàs-Brunet, M. Baily, F. Gulrez, S. Goulet, T. Halmos, S. LaPlante, R. Maurice, M. Poirier, M.-A. Poupart, D. Thibeault, D. Wernic, D. Lamarre, *Biorg. Med. Chem. Lett.* **1998**, *8*, 1713–1718.
[23] N. Penner, L. Xu, C. Prakash, *Chem. Res. Toxicol.* **2012**, *25*, 513–531.
[24] E. M. Isin, C. S. Elmore, G. N. Nilsson, R. A. Thompson, L. Weidolf, *Chem. Res. Toxicol.* **2012**, *25*, 532–542.
[25] L. Chen, P. Rose, Y. Mao, C.-L. Yong, R. St. George, Y. Huang, B. Latli, D. Mandarino, Y. Li, *Antimicrob. Agents Chemother.* Accepted for publication February 02, 2014.
[26] A. E. Mutlib, *Chem Res. Toxicol.* **2008**, *21*, 1672–1689.
[27] C. A. Busacca, X. Wei, N. Haddad, S. Kapadia, J. C. Lorenz, A. K. Saha, R. J. Varsolona, T. Berkenbusch, S. C. Campbell, V. Farina, X. W. Feng, N. C. Gonnella, N. Grinberg, P.-J. Jones, H. Lee, Z. Li, O. Niemeier, W. Samstag, M. Sarvestani, J. Schroeder, J. Smoliga, E. M. Spinelli, J. Vitous, C. H. Senanayake, *Asian J. Org. Chem.* **2012**, *1*, 80–89.
[28] A. Hantzsch, J. H. Weber, *Chem. Ber.* **1887**, *20*, 3118–3132.
[29] B. Latli, M. Hrapchak, V. Gorys, C. A. Busacca, C. H. Senanayake, *J. Label. Compd. Radiopharm.* **2005**, *48*, 447–455.
[30] S. Randriamahefa, P. Deschamps, R. Gallo, *Synthesis* **1985**, *5*, 493–495.

