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Synthesis of [^{14}C]boceprevir, [$^{13}C_3$]boceprevir, and [D_9]boceprevir, a hepatitis C virus protease inhibitor

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Boceprevir is a hepatitis C virus (HCV) NS3 protease inhibitor for HCV treatment. [^{14}C]Boceprevir (SCH 503034, trade name Victrelis) was synthesized from K^{14}CN in 11 steps with an overall yield of 16.4%. [$^{13}C_3$]Boceprevir was synthesized in 16 steps with a 2.5% overall yield. The carbon-13 in the molecule was distributed along the peptide chain. [D_9]Boceprevir was synthesized from [D_9]-*t*-butylamine in four steps with an overall yield of 69%.

Keywords: boceprevir; Victrelis; SCH 503034; HCV protease inhibitor; carbon-13; carbon-14; synthesis

Introduction

Chronic hepatitis C virus (HCV) infection is a major global public health problem, affecting more than 170 million people worldwide. Boceprevir, a novel, potent and orally bioavailable NS3 protease inhibitor, has been approved recently by the FDA to be used in combination with Peginterferon and ribavirin to treat hepatitis C genotype 1 infection.[1–5]

[^{14}C]Boceprevir was synthesized to support ADME studies and environmental assessment study. [D_9]Boceprevir was initially requested as an internal standard in the development of bioanalytical LC/MS/MS methods. [$^{13}C_3$]Boceprevir was requested for the development of LC-MS methods for profiling and structural characterization of metabolites in various biological matrices. After oral administration of a mixture containing unlabeled boceprevir, [^{14}C]boceprevir, and [$^{13}C_3$] boceprevir to male rats, the drug-derived material obtained from various matrices was collected and analyzed. The isotopic pattern of [$^{13}C_3$]SCH 503034 facilitated the detection of drug-derived material in the presence of endogenous ions.

Results and discussion

Synthesis of [^{14}C]boceprevir

[^{14}C]Boceprevir was synthesized from K^{14}CN as shown in Schemes 1 and 2. (*S*)-*t*-[^{14}C]Leucine (**5**) was synthesized by a diastereoselective Strecker reaction using (*R*)-phenylglycine amide **1** as a chiral auxiliary, as shown in Scheme 1.[6] (*R*)-(−)-2-Phenylglycine amide **1**, synthesized from (*R*)-(−)-2-phenylglycine methyl ester hydrochloride by a modified literature procedure,[7] reacted with pivaldehyde to form the imine *in situ*, which was reacted with K^{14}CN in aqueous HOAc to give amino nitrile **2**. The mixture of (*R,S*) and (*R,R*) amino nitrile underwent a crystallization-induced asymmetric transformation, and (*R,S*) amino nitrile precipitated out in 79% yield. Hydrolysis of **2** with H_2SO_4 gave diamide **3** in 82% yield. Catalytic hydrogenolysis to remove the chiral auxiliary afforded (*S*)-*t*-[^{14}C]leucine amide **4** in 94% yield. Hydrolysis of amide **4** with HCl gave (*S*)-*t*-[^{14}C]leucine **5** in quantitative yield. Reaction of **5** with $(Boc)_2O$ in the presence of Et_3N gave Boc-(*S*)-*t*-[^{14}C]leucine **6** in 68% yield.

The rest of the synthesis of [^{14}C]boceprevir is as shown in Scheme 2.

(*S*)-*t*-[^{14}C]Leucine was coupled with amine **7** to give compound **8** in 74% yield. The Boc group was removed with HCl to generate compound **9** in quantitative yield. Treatment of amine **9** with *tert*-butyl isocyanate gave ester **10** in 84% yield. Hydrolysis of ester **10** gave acid **11** in quantitative yield. Coupling of acid **11** with amine **12** using EDCI/HOBt chemistry gave compound **13** as an approximately equal mixture of four diastereomers in 98% yield. Finally, oxidation of hydroxyamide **13** using Dess–Martin reagent gave [^{14}C] boceprevir as an approximately equal mixture of two diastereomers in 65% yield. The diastereomer ratio is same as that of the unlabeled boceprevir.

Synthesis of [$^{13}C_3$]boceprevir

Boceprevir is a linear, peptide-like molecule. It was required that the molecule was labeled in multiple sites along the tri-peptide

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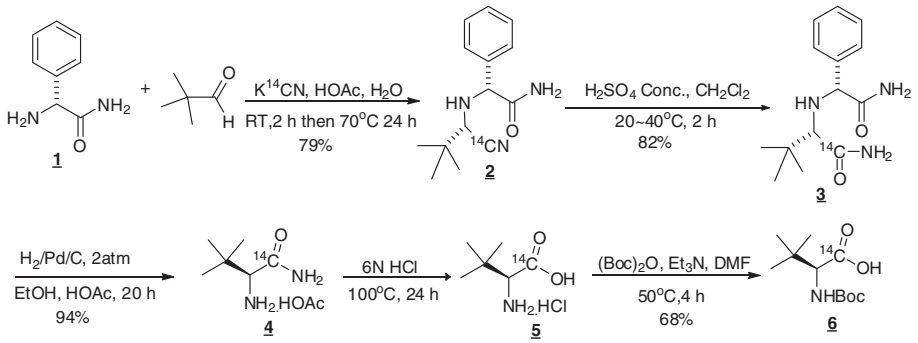
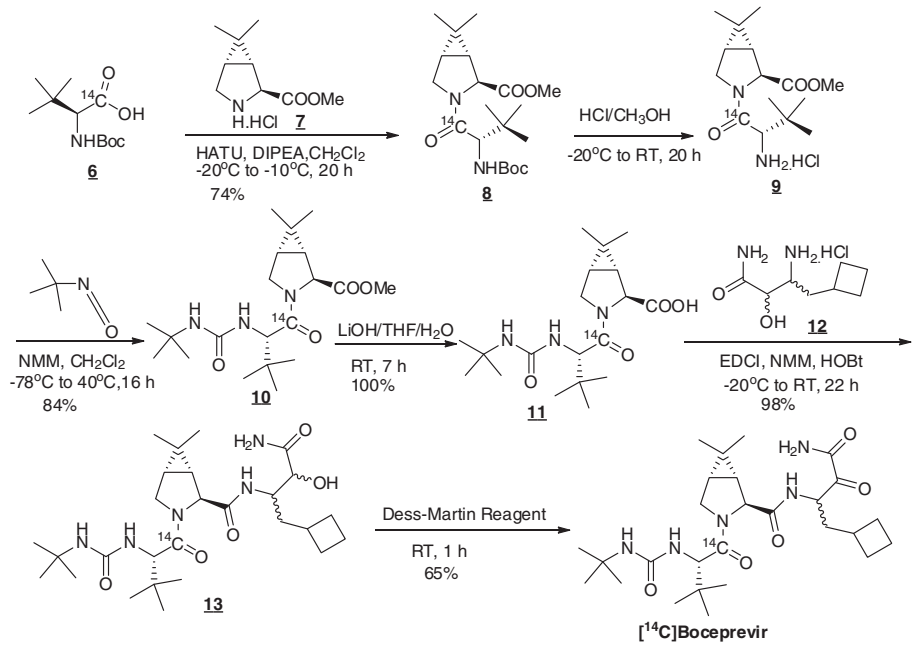


Scheme 1. Synthesis of Boc-(*S*)-*t*-[^{14}C]leucine.



Scheme 2. Synthesis of [^{14}C]boceprevir.

backbone. The retrosynthetic analysis is shown in Scheme 3. None of the labeled synthetic intermediates were commercially available. They were synthesized as shown in Schemes 4 and 5.

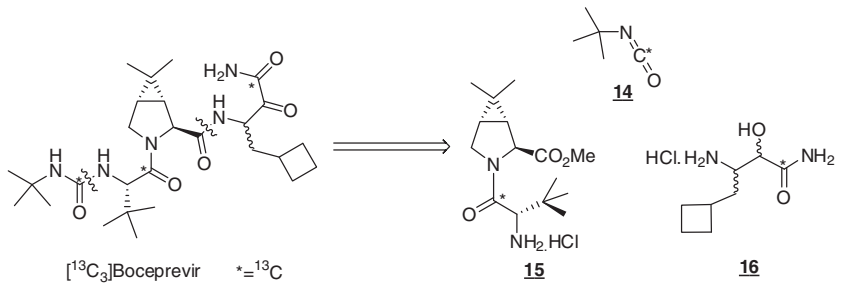
[^{13}C]Compound **15** was synthesized from K^{13}CN in seven steps with 35% overall yield in the same manner as that of [^{14}C]compound **9** (Schemes 1 and 2).

tert-Butyl [^{13}C]isocyanate (**14**; Scheme 4) was synthesized from *tert*-butyl amine and [^{13}C]CO_2 in the presence of Mitsunobu zwitterions according to the literature procedure[8] for the unlabeled compound, as shown in Scheme 4.

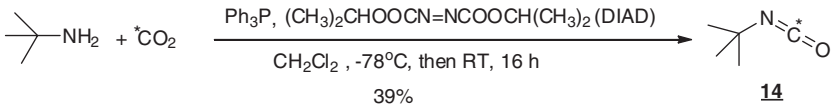
[^{13}C]CO_2 was reacted with *t*-butyl amine to give carbamic acid. The carbamic acid solution was treated with the pre-formed Mitsunobu zwitterion to give compound **14** in 39% yield after distillation.

Compound **16** was synthesized in four steps in 79% overall yield as outlined in Scheme 5.

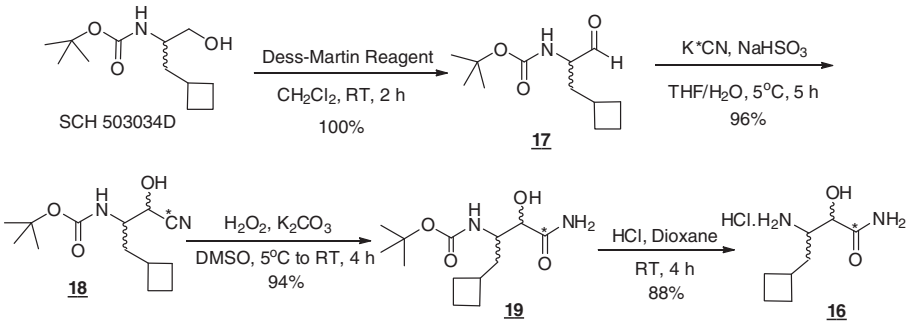
SCH 503034D was oxidized with Dess–Martin periodinane to generate aldehyde **17** in quantitative yield. Treatment of aldehyde **17** with K^{13}CN and $NaHSO_3$ gave **18** as a diastereoisomeric mixture in 96% yield. Cyanohydrin **18** was converted



Scheme 3. Retrosynthesis of [$^{13}C_3$]boceprevir.



Scheme 4. Synthesis of *tert*-butyl [^{13}C]isocyanate.



Scheme 5. Synthesis of [^{13}C]compound **16**.

to hydroxyamide **19** in 94% yield by reacting with H_2O_2/K_2CO_3/DMSO. Finally, removal of the Boc group with HCl gave compound **16** in 88% yield.

The final compound [$^{13}C_3$]boceprevir as an approximately equal mixture of two diastereomers was synthesized with these labeled intermediates in four steps in 23% overall yield (Scheme 6) in a similar manner to the synthesis of [^{14}C]boceprevir. In the last step of the synthesis, DMSO/dichloroacetic acid/EDCI was used as oxidizing reagent, which gave a comparable yield with that using Dess–Martin reagent.

Synthesis of [D_9]boceprevir

[D_9]Boceprevir was synthesized from [D_9]-*t*-butylamine in four steps with an overall yield of 69%. Compound **23** was synthesized from (S)-*t*-leucine and amine **7** in the same way as [^{14}C]compound **8**. Amine **23** was reacted with triphosgene to generate the isocyanate, which was reacted with [D_9]-*t*-butylamine to give urea **24** in 93% yield. The rest of the synthesis was completed in the same manner as that of [$^{13}C_3$]boceprevir (Scheme 7).

Experimental

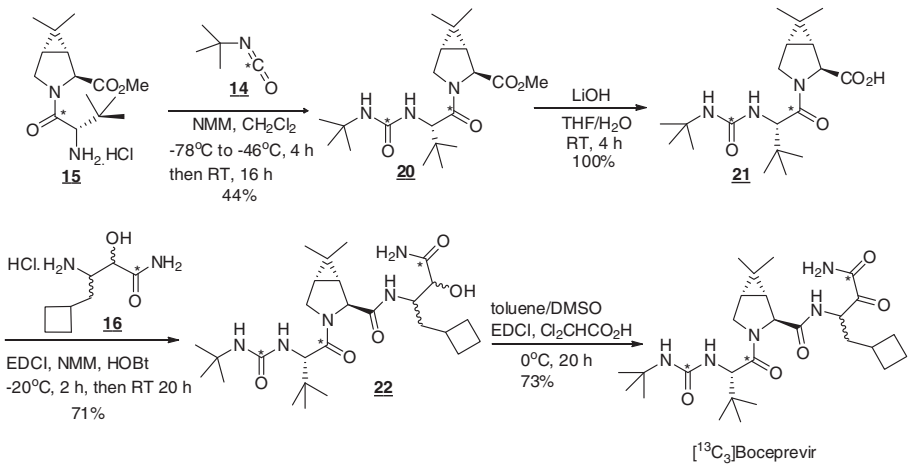
General

[^{13}C]CO_2 was purchased from Isotec. K^{13}CN was purchased from Cambridge Isotope Laboratories, Inc. [$^2H_9$]-*t*-Butylamine was purchased from C/D/N Isotopes, Inc. [^{14}C]Potassium cyanide was obtained from Amersham. Compounds **7, 12**, SCH 503034D and unlabeled boceprevir were from Merck Research Laboratories, Process Chemistry. All remaining reagents, authentic standards, and solvents were purchased from standard commercial sources (Aldrich, Fluka, Fisher, Acros) and were used without further purification.

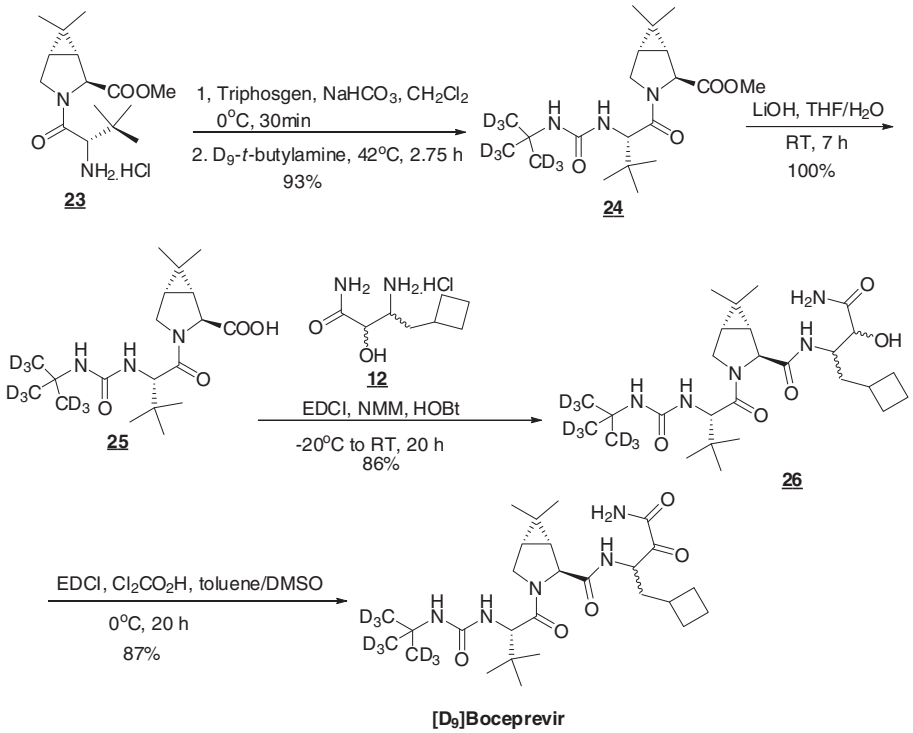
LC-MS: Waters Micromass with Waters 2695 Separation Module operating in the ES^+ ionization mode. YMC-Pack ProC18 column, 150 mm × 4.6 mm, 230 nm, isocratic, pH 9.5, 0.04 M NH_4OAc/H_2O:CH_3OH (35:65), 1.0 mL/min.

Analytical HPLC system 1: Cyclobond 2000I, 150 × 4.6 mm, 220 nm, CH_3CN/water 99:1, 1.0 mL/min, LS Cocktail 2.4 mL/min, isocratic.

Analytical HPLC system 2: Waters 600 Multisolvent Delivery System with Waters 2487 Absorbance Detector: YMC-Pack Pro



Scheme 6. Synthesis of [$^{13}C_3$]boceprevir.



Scheme 7. Synthesis of [D_9]boceprevir.

C18 column, 150 mm × 4.6 mm, 230 nm, 30°C, 0.05 M pH 7.5, K_2HPO_4/H_2O:CH_3OH (35:65), 1.0 mL/min, isocratic.

Analytical HPLC system 3: Restek Allure Biphenyl 150 mm 3.2 mm, 5 μm, 220 nm, 40°C. MeOH/0.05 M TEAP pH 6 (65:35) for 20 min followed by a step gradient to CH_3CN for 20 min, 0.5 mL/min.

Semi-preparative HPLC: Waters 600 Multisolvent Delivery System with Waters 2487 Absorbance Detector: Zorbox SB Phenyl C18, 250 mm × 9.4 mm, 220 nm, 0.01 M pH 7.5, K_2HPO_4/H_2O:CH_3OH (30:70), 4.0 mL/min, isocratic.

^{1}H NMR (400 or 600 MHz) spectra were obtained on a Varian spectrometer.

Synthesis of [^{14}C]boceprevir

Synthesis of [^{14}C]amino nitrile ***2***

To a stirred suspension of (*R*)-(−)-2-phenylglycine amide **1** (0.682 g, 4.54 mmol) in H_2O (3.0 mL) was added pivaldehyde (0.50 mL, 4.54 mmol) at room temperature (RT). K^{14}CN (250 mCi, 0.305 g, 4.54 mmol, 55.0 mCi/mmol) was added. The vial containing K^{14}CN was washed with H_2O (2 × 0.5 mL), and the mixture was added to the reaction flask. Finally, glacial acetic acid (0.28 mL, 4.77 mmol) was added over 10 min. The mixture was stirred at RT under N_2 for 2 h, warmed to 70°C and stirred for 24 h. A big chunk of white solid formed in the reaction and was broken to small pieces periodically. After being cooled to RT, the product was collected by filtration and washed with H_2O (2 × 3 mL). The solid, after being concentrated and dried, was dissolved in CH_2Cl_2 (12 mL) and counted to give 196.8 mCi (79%) of [^{14}C] amino nitrile **2** as a light yellow solid. TLC of the final product indicated 99% radiochemical purity (RCP) (SiO_2, ethyl acetate/hexanes 60:40, R_f = 0.54).

Synthesis of [^{14}C]diamide ***3***

To a solution of concentrated H_2SO_4 (5.4 mL) at 10°C, [^{14}C] amino nitrile **2** (196.8 mCi, 3.58 mmol) in CH_2Cl_2 (7.0 mL) was added dropwise over 30 min. The flask containing the [^{14}C] amino nitrile **2** was washed with CH_2Cl_2 (2 × 1 mL), and the mixture was added to the reaction over 10 min. The reaction was stirred at 20°C for 30 min, warmed to 40°C, and stirred for an additional 2 h. After being cooled to RT, the mixture was poured on ice, neutralized with 25% NH_4OH to pH ~ 9, and extracted with EtOAc (7 × 35 mL). The combined organic phase was washed with brine (50 mL), dried over Na_2SO_4, filtered, and concentrated to give 178 mCi of crude compound **3**. The crude product was purified on a silica column (elution with 0–10% CH_3OH/CH_2Cl_2) to give 162 mCi (82%) of (*S*),(*R*)- [^{14}C]diamide **3** with 95.0% RCP by analytical HPLC system 1. A small amount of (*R*),(*R*)- [^{14}C]diamide **3** diastereomer was removed during the purification.

Synthesis of (S)-[^{14}C]*tert*-leucine amide **4**

To a solution of [^{14}C]diamide **3** (162 mCi, 2.94 mmol) in EtOH (30 mL, 200 proof), Pd/C (10% wt on activated carbon, 150 mg) and glacial HOAc (3.0 mL) were added. The mixture was shaken under H_2 (55 psi) for 20 h and filtered through a Celite pad. The Celite was washed with EtOH (3 × 20 mL), and the combined organic phase was concentrated (this sometimes did not go to completion; in such case, the mixture was re-submitted to the hydrogenation condition to push the reaction to completion). The crude product was purified by flash chromatography on silica gel (elution with 0–10% CH_3OH/CH_2Cl_2) to give 152 mCi (94% yield) of (*S*)-[^{14}C]*tert*-leucine amide **4** with 98.8% RCP by radio-TLC (10% CH_3OH/CH_2Cl_2, R_f = 0.29).

Synthesis of (S)-[^{14}C]tert-leucine **5**

(*S*)-[^{14}C]*tert*-Leucine amide **4** (152 mCi, 2.76 mmol) was refluxed in aqueous HCl (60 mL, 6 N) for 24 h and then cooled to RT. The solvent was removed under reduced pressure to give the crude product **5** in 79.8% RCP by radio-TLC (10% CH_3OH/CH_2Cl_2, $R_f = 0.028$). The crude product was used directly in the next step.

Synthesis of (S)-N-Boc-[^{14}C]tert-leucine **6**

To a solution of **5** (152 mCi, 2.76 mmol) in anhydrous DMF (5.0 mL), di-*t*-butyl-dicarbonate (1.50 g, 6.87 mmol) and Et_3N (0.96 mL, 6.88 mmol) were added. The reaction was stirred at 50°C under N_2 for 2 h, and an additional Et_3N (0.5 mL) was added. The heavy suspension was stirred at 50°C for 2 h, then at RT over the weekend. After removing the solvent under vacuum at 50°C, the residue was dissolved in HCl (30 mL, 1 N, pre-cooled on ice) and extracted with EtOAc (4 × 40 mL). The combined organic phase was dried (Na_2SO_4), filtered, and concentrated. The crude product was purified by flash chromatography on silica gel (elution with 1% CH_3OH/0.5% $HOAc/CH_2Cl_2$) to give 104 mCi of **6** with 95.3% RCP by radio-TLC as a white solid in 68% yield. The product co-eluted with authentic *N*-Boc-*t*-leucine (Fluka) on TLC ($R_f = 0.18$, 10% CH_3OH/0.5% $HOAc/CH_2Cl_2$,).

Synthesis of [^{14}C]compound ***8***

The amine HCl salt **7** (204 mg, 0.993 mmol) and DIPEA (0.53 mL, 2.97 mmol) were dissolved in CH_2Cl_2 (anhydrous, 3.0 mL) at −20°C, and added to the flask containing [^{14}C] compound **6** (42.0 mCi, 0.764 mmol). The reaction mixture was cooled to −20°C under N_2, and HATU (377 mg, 0.993 mmol) was added. The reaction mixture was gradually warmed to −10 °C and stirred at −10 °C under N_2 for 20 h. The reaction was diluted with CH_2Cl_2 (50 mL) and washed with $NaHCO_3$ (saturated, 20 mL) and brine (20 mL). The organic phase was dried (Na_2SO_4), filtered, and concentrated. The crude product was purified by flash chromatography on silica gel (50 g silica, elution with EtOAc/hexanes) to give 31.0 mCi with 97.3% RCP by radio-TLC (20% EtOAc in hexanes, $R_f = 0.22$) of compound **8** as a light yellow solid in 74% yield.

Synthesis of [^{14}C]compound ***9***

[^{14}C]Compound **8** (31.0 mCi, 0.563 mmol) was dissolved in CH_3OH (2.0 mL) and cooled to −20 °C. To the solution, HCl in CH_3OH (2.0 mL, prepared fresh by bubbling HCl gas into cold CH_3OH for 20 min) was added. The reaction was gradually warmed to RT and stirred under N_2 for 20 h. The reaction mixture was concentrated in vacuo to give [^{14}C] amine hydrochloride salt with 96.7% RCP by radio-TLC (5% CH_3OH (7 N NH_3)/CH_2Cl_2, $R_f = 0.56$) in quantitative yield. The compound was used directly in the next step.

Synthesis of [^{14}C]compound ***10***

[^{14}C]Compound **9** (31.0 mCi, 0.563 mmol) was dissolved in CH_2Cl_2 (anhydrous, 6.0 mL) and cooled to −78 °C under N_2. To the solution, *N*-methylmorpholine (0.12 mL, 1.13 mmol) was added dropwise, followed by the addition of *t*-butyl isocyanate (0.13 mL, 1.13 mmol). The reaction was gradually warmed to 40°C and stirred at 40°C under N_2 16 h. The reaction was diluted with CH_2Cl_2 (40 mL) and washed with HCl (1 N, 30 mL). The aqueous phase was extracted with CH_2Cl_2 (3 × 30 mL). The combined organic phase was dried (Na_2SO_4), filtered, concentrated, and purified by silica gel chromatography (0–40% EtOAc/hexanes) to give 26.1 mCi of **10** (84% yield) with 100% RCP by radio-TLC (20% EtOAc in hexanes, $R_f = 0.13$).

Synthesis of [^{14}C]compound ***11***

[^{14}C]Compound **10** (26.1 mCi, 0.474 mmol) was dissolved in THF (3.0 mL) at RT, and LiOH (50.0 mg in 2.0 mL of H_2O) was added. The reaction was stirred at RT under N_2 for 7 h and concentrated. The residue was diluted with EtOAc (20 mL) and acidified with HCl (0.3 N, 20 mL), extracted with EtOAc (3 × 30 mL), washed with brine (30 mL), dried (Na_2SO_4), filtered, and concentrated to give 26.1 mCi of **11** with 100% RCP by radio-TLC (20% EtOAc in hexanes, R_f =0.07) in quantitative yield.

Synthesis of [^{14}C]compound ***13***

A suspension of [^{14}C]compound **11** (26.1 mCi, 0.474 mmol), compound **12** (119 mg, 0.569 mmol), HOBt (64 mg, 0.474 mmol), and EDCI (136 mg, 0.712 mmol) in anhydrous THF/DMF (4 mL/2.0 mL, anhydrous) was treated with *N*-methylmorpholine (0.24 mL, 2.14 mmol) at −20°C under N_2. The reaction was gradually warmed to RT during 2 h and stirred at RT for 20 h. The reaction was diluted with EtOAc (20 mL) and washed with HCl (1 N, 30 mL). The aqueous phase was extracted with EtOAc (3 × 40 mL), and the combined organic phase was washed with saturated $NaHCO_3$ (30 mL) and brine (30 mL), dried (Na_2SO_4), filtered, concentrated, and purified by silica gel chromatography (gradient, 10–50% acetone/hexanes) to give 25.5 mCi of [^{14}C]compound **13** as an approximately equal mixture of four diastereomers (98% yield) with 99.6% RCP by analytical HPLC system 2. All the four diastereomers were separated in this HPLC system; the retention times of the peaks were identical with the authentic unlabeled standard.

Synthesis of [^{14}C]boceprevir

To a solution of [^{14}C]compound **13** (2.0 mCi, 0.036 mmol) in CH_2Cl_2 (0.6 mL, anhydrous), Dess–Martin periodinane (15 mg, 0.036 mmol) was added. The reaction was stirred under N_2 at RT for 1 h. The reaction was diluted with diethyl ether (20 mL) and then washed with saturated $NaHCO_3$/brine (20 mL, 1:1) and water (20 mL). The aqueous phase was extracted with diethyl ether (2 × 10 mL). The combined organic phase was dried (Na_2SO_4), filtered, and concentrated. The crude product was initially purified by silica gel chromatography (gradient, 10–40% acetone/hexanes) and then by semi-preparative HPLC to give 1.3 mCi of [^{14}C]boceprevir as an approximately equal mixture of two diastereomers in 65% yield with 98.5% RCP by analytical HPLC system 3. The retention times of the peaks were identical with the authentic unlabeled standard. The specific activity of the compound was 55.7 mCi/mmol.

Synthesis of [$^{13}C_3$]boceprevir

Synthesis of tert-butyl [^{13}C]isocyanate **14**

[^{13}C]CO_2 (3 L) was bubbled through a solution of *t*-butylamine (1.85 mL, 17.6 mmol) in CH_2Cl_2 (40 mL) at −10°C for 10 min and then cooled to −78°C to form the carbamate salt solution. In a separate flask, diisopropyl azodicarboxylate

(4.2 mL, 21.3 mmol) was added dropwise to a solution of PPh_3 (5.54 g, 21.1 mmol) in CH_2Cl_2 (60 mL) under N_2 at −20°C. The resulting light yellow solution was cooled to −78°C, then transferred via cannula to the above carbamate salt solution. Additional [^{13}C]CO_2 (1 L) was bubbled into the reaction for 5 min. The reaction was warmed to RT and stirred in the sealed flask overnight. Most of the CH_2Cl_2 was removed by fractional distillation at 60°C. The rest of the CH_2Cl_2 and *tert*-butyl [^{13}C]isocyanate **1** was separated from the byproducts PPh_3O and $DIADH_2$ by vacuum distillation. A final fractional distillation to remove the small amount of CH_2Cl_2 gave 693 mg of *tert*-butyl [^{13}C]isocyanate (**14)** in 39% yield as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.37 (s, 9H). LC-MS (EI^+) *m/z* 133 $(M + CH_3OH + H)^+$.

Synthesis of compound ***17***

To a solution of SCH 503034D (1.0 g, 4.36 mmol) in CH_2Cl_2 (15 mL), Dess–Martin periodinane (15.0 g, 15 wt/wt% solution in CH_2Cl_2, 5.30 mmol) was added dropwise at RT. After being stirred for 2 h, the reaction mixture was diluted with Et_2O (50 mL) and then washed with NaOH (25 mL, 1 N), H_2O (25 mL), and brine (25 mL). The organic phase was dried (Na_2SO_4), filtered, and concentrated to give 0.99 g of crude product **17** in quantitative yield. Compound **17** was used directly in the next step without further purification. ^{1}H NMR (400 MHz, CD_3OD): δ 9.45 (s, 1H), 6.90 (s, 1H), 3.82–3.80 (m, 1H), 2.40–2.19 (m, 2H), 2.06–1.44 (m, 7H), 1.42 (s, 9H). LC-MS (EI^+) *m/z* 479 $(2M + Na + H)^+$.

Synthesis of [^{13}C]compound ***18***

To a solution of **17 (**2.0 g, 8.8 mmol) and K^{13}CN (684 mg, 10.4 mmol) in THF/H_2O (10 mL, 1:1) at 5°C, a saturated aqueous solution of sodium bisulfate (8.0 mL) was added slowly over 2 h, and the mixture was stirred for a further 5 h at 5°C. The reaction was diluted with H_2O (40 mL) and extracted with CH_2Cl_2 (5 × 100 mL). The combined organic phase was washed with brine, dried (Na_2SO_4), filtered, and concentrated. The crude product was purified by flash chromatography on silica gel (50 g silica, elution with 10–30% EtOAc/hexanes) to give 2.17 g of diastereomer mixture of **18** as a colorless liquid in 96% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.35 (bs, 0.5H, NH), 4.85–4.76 (m, 1H), 4.46 (bs, 1.5H, NH, OH), 3.81–3.48 (m, 1H), 2.41–1.57 (m, 9H), 1.45(s, 9H). LC-MS (EI^+) *m/z* 256 $(M + H)^+$, 200 $(M\text{-}CHCNOH + H)^+$.

Synthesis of [^{13}C]compound ***19***

To a solution of **18** (2.00 g, 7.84 mmol) in DMSO (13 mL) at 5°C, K_2CO_3 (886 mg, 6.41 mmol) was added. H_2O_2 (3.5 mL, 30.9 mmol) was then added dropwise during 30 min. The reaction mixture was warmed to RT, stirred for 4 h, diluted with H_2O (12 mL), and stirred for an additional 10 min. The resulting white precipitate was filtered, washed with H_2O (3 × 5 mL), and dried under vacuum to give 1.21 g of compound **19**. The filtrate was extracted with EtOAc (3 × 50 mL); the combined organic phase was washed with H_2O (2 × 50 mL), dried (Na_2SO_4), filtered, and concentrated to give an additional 0.80 g of compound **19** for a total 94% yield. Compound **19** was used directly in the next step. LC-MS (EI^+) *m/z* 296 $(M + Na)^+$, 274 $(M + H)^+$, 218 $(M\text{-}t\text{-}Bu + H)^+$, 174 $(M\text{-}Boc + H)^+$.

Synthesis of [^{13}C]compound ***16***

HCl (11 mL, 4.0 M in dioxane) was added dropwise to a solution of compound **19** (2.01 g, 7.35 mmol) in dioxane (11 mL). The reaction mixture was stirred at RT for 4 h, and the product was collected by filtration. The solid was washed with Et_2O (2 × 5 mL) and dried to give 1.36 g of **16** in 88% yield. The product was used directly in the next step. LC-MS (EI^+) *m/z* 196 $(M + Na)^+$, 174 $(M + H)^+$, 130 $(M\text{-}CONH_2 + H)^+$.

The rest of the synthesis of [$^{13}C_3$]boceprevir was conducted in the same manner as that of [^{14}C]boceprevir. In the last step of the synthesis, DMSO/dichloroacetic acid/EDCI was used as oxidizing reagent, which gave a comparable yield with that using Dess–Martin reagent. The detailed procedure is as follows:

Synthesis of [$^{13}C_3$]boceprevir

To a solution of compound **17** (455 mg, 0.867 mmol) in DMSO (2.5 mL) and toluene (5.0 mL) at 0°C, EDCI (1.60 g, 8.346 mmol) was added. To the resulting suspension, dichloroacetic acid (0.40 mL, 4.86 mmol) was added dropwise, and the mixture was stirred under N_2 at 0°C for 20 h. The reaction was diluted with EtOAc (40 mL) and then washed with HCl (1 N, 40 mL), saturated $NaHCO_3$ (40 mL) and brine (40 mL). The organic solution was dried (Na_2SO_4), filtered, concentrated, and purified by silica gel chromatography (gradient, 30–40% acetone/hexanes) to give 332 mg of [$^{13}C_3$]boceprevir as a white solid in 73% yield. This batch was combined with another batch of product (total, 830 mg) and repurified by silica gel chromatography (gradient, 30–40% acetone/hexanes) to give a final batch of 722 mg of [$^{13}C_3$]boceprevir as an approximately equal mixture of two diastereomers. The chemical purity was 95.2% by HPLC system 3. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.20 (dd, J = 41.70 Hz, J = 7.32, 1H, NH), 8.00–7.73 (m, 2H, NH_2), 5.95 (s, 1H, NH), 5.84 (t, J = 10.25, 1H, NH), 4.99–4.81 (m, 1H), 4.28 (s, 1H), 4.15–4.08 (m, 1H), 3.99–3.91 (m, 1H), 3.79–3.71 (m, 1H), 2.51–2.32 (m, 1H), 1.99–1.90 (m, 2H), 1.81–1.71 (m, 3H), 1.66–1.54 (m, 3H), 1.45–1.40 (m, 1H), 1.29–1.24 (m, 1H), 1.15 (s, 9H), 1.10–0.80 (m, 15H). ^{13}C NMR (DMSO-d_6, δ): 197.8, 170.9, 170.8, 162.8, 157.3, 59.1, 56.6, 51.7, 48.9, 47.4, 36.7, 34.0, 32.1, 30.6, 29.1, 27.7, 27.3, 27.0, 26.4, 26.1, 18.5, 17.6, 12.6. LC-MS (EI^+) *m/z* 523 $(M + H)^+$, 423 $(M\text{-}tBuNHCO + H)^+$, 309 $(M\text{-}tBuNHCONHCHtBuCO + H)^+$. HRMS-FAB (*m/z*): $[M + Na]^+$calcd for $^{12}C_{24}^{13}C_3H_{45}N_5O_5Na$, 545.34190; found 545.34430.

Synthesis of [D_9]boceprevir

Synthesis of [D_9]compound ***24***

Into a 250-mL round-bottomed flask containing compound **23** (which was prepared in the same manner as that of compound **8**, 1.05 g, 3.29 mmol) was added CH_2Cl_2 (14 mL) and saturated $NaHCO_3$ (14 mL). The mixture was stirred at 0 °C, and triphosgene (0.322 g, 1.09 mmol) was added. The reaction mixture was stirred for 30 min at 0 °C and then extracted with CH_2Cl_2 (3 × 30 mL). The combined organic extracts were dried (Na_2SO_4) and then concentrated by rotary evaporation to give a yellow oil (isocyanate) that was resuspended in anhydrous CH_2Cl_2 (32 mL), and the mixture was stirred under argon. [$^2H_9$]-*t*-Butylamine (0.431 mL, 3.62 mmol) was added, and the reaction was heated in a 42 °C oil bath for 2.75 h. The reaction was concentrated by rotary

evaporation to give a light yellow oil, which was purified on a silica gel column eluting with 2:8 EtOAc/hexanes to give [D_9] compound **24** as a white solid (1.19 g, 93%).

The rest of the synthesis of [D_9]boceprevir as an approximately equal mixture of two diastereomers was conducted in the same manner as that of [$^{13}C_3$]boceprevir.

In summary, [^{14}C]boceprevir, [$^{13}C_3$]boceprevir, and [D_9] boceprevir were synthesized to support the drug development at different stages.

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Conflict of Interest

The authors did not report any conflict of interest.

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