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Design and synthesis of deuterated boceprevir analogs with enhanced pharmacokinetic properties

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As part of an ongoing effort to apply the Deuterated Chemical Entity Platform (DCE Platform™) to clinically validated drugs, the synthesis of deuterated analogs of the hepatitis C virus protease inhibitor boceprevir was carried out. The devised synthetic routes allowed for site-selective deuterium incorporation with high levels of isotopic purity. Application of the DCE Platform™ to boceprevir enabled the identification of several deuterated analogs that display marked levels of *in vitro* metabolic stabilization. Most notably, analog 1g exhibits a near doubling of *in vitro* half-life in human liver microsomal assays. The details of the convergent synthetic route to the boceprevir isotopologs and the results of the metabolic stability assays are described herein.

Keywords: deuteration; DCE Platform; boceprevir; HCV NS3 protease; KIE

Introduction

The deuterium kinetic isotope effect (KIE) has the potential to alter the biological fate of many drug molecules that are metabolized through pathways involving carbon–hydrogen bond cleavage.[1] Within biological systems, numerous competing effects can mask the deuterium KIE such that the observed magnitude and even direction of the KIEs are unpredictable and depend on a compound's molecular skeleton, substituents, specific substitution pattern, and the enzymes responsible for metabolism.[2] As a consequence of the KIE, site-selective deuteration of compounds can result in altered metabolism patterns including reduced metabolic rates and metabolic shunting effects.[3] Our research has shown that careful application of deuterium medicinal chemistry as part of the Deuterated Chemical Entity Platform (DCE Platform™) has the unique benefit, in select cases, of allowing for metabolic optimization of a compound while preserving the biochemical potency and selectivity of the agent.

Some important pharmacological effects of deuteration, observed in certain cases, can be classified as illustrated in Figure 1. The first panel illustrates the case where the major effect of deuteration is to reduce the rate of systemic clearance. This results in an increase of the biological half-life of the compound. Potential drug benefits could include a reduction in dosage and the ability to maintain similar systemic exposures with decreased peak levels (C_{max}) and enhance trough levels. This could result in a lower incidence of C_{max}-associated side effects and enhanced efficacy, depending on the particular drug's pharmacokinetic–pharmacodynanic relationship. The second panel in Figure 1 illustrates a largely pre-systemic (or first-pass) effect of deuteration. In such a case, reduced rates of oxidative metabolism in the gut wall and/or liver result in a larger percentage of unmetabolized drug reaching the systemic circulation, whereas the overall rate of systemic clearance remains unchanged. Deuterated drugs showing this effect may have reduced dosing requirements and produce lower metabolite loads. Because, in many instances, gastrointestinal irritation has been shown to be a dose-dependent rather than a plasma concentration-dependent phenomenon, this effect could allow for enhanced tolerability and/or the ability to achieve a higher maximum tolerated dose. The third panel in Figure 1 illustrates metabolic shunting effects in cases where a drug is metabolized to form both active and toxic metabolite species. In such a case, the deuterium KIE can result in the reduced formation of a toxic or reactive metabolite as well as the increased formation of desirable active metabolites. Each of the above cases highlights precision deuterium incorporation as a powerful medicinal chemistry tool capable of generating deuterated chemical entities (DCEs) with superior therapeutic properties.

Hepatitis C virus (HCV) infection is the major cause of chronic liver disease, leading to cirrhosis, hepatocellular carcinoma, or liver failure.[4] It is estimated that at least 170 million people worldwide are infected by this virus.[4] Currently, the most effective therapeutic regimen involves treatment with pegylated α-interferon in combination with the antiviral agent ribavarin.[5] However, even this combination treatment is effective in only 40% of patients with genotype 1 HCV, the most prevalent genotype in the United States, Japan, and Europe.[6]

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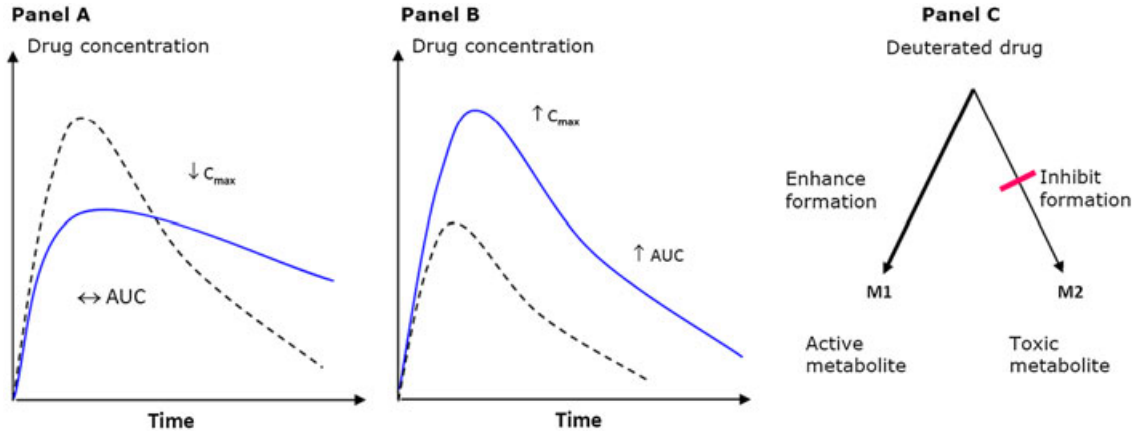


Figure 1. (A) Reduced systemic clearance resulting in increased half-life. (B) Decreased pre-systemic metabolism resulting in higher bioavailability of unmetabolized drug. (C) Metabolic shunting resulting in reduced exposure to undesirable metabolites or increased exposure to desired active metabolites.

Boceprevir (**1**) contains an α-ketoamide electrophilic warhead that reversibly binds to the NS3 serine protease, which plays a vital role in the replication of the HCV virus.[7] Boceprevir has shown dramatic viral responses in the clinic when administered with pegylated α-interferon and ribavarin, and is currently under expedited review in the United States and European Union as a new treatment option for patients living with chronic hepatitis C.[8] The present work is aimed at producing a DCE with enhanced pharmacokinetic properties that may improve the antiviral efficacy of boceprevir by raising the trough levels of the drug. Clinical studies have demonstrated that viral load reductions are positively correlated with boceprevir trough concentrations.[9] An enhanced pharmacokinetic profile may also allow for a more convenient dosing regimen over boceprevir's three-times-daily commercial dosing schedule.[10]

Results and discussion

Chemistry

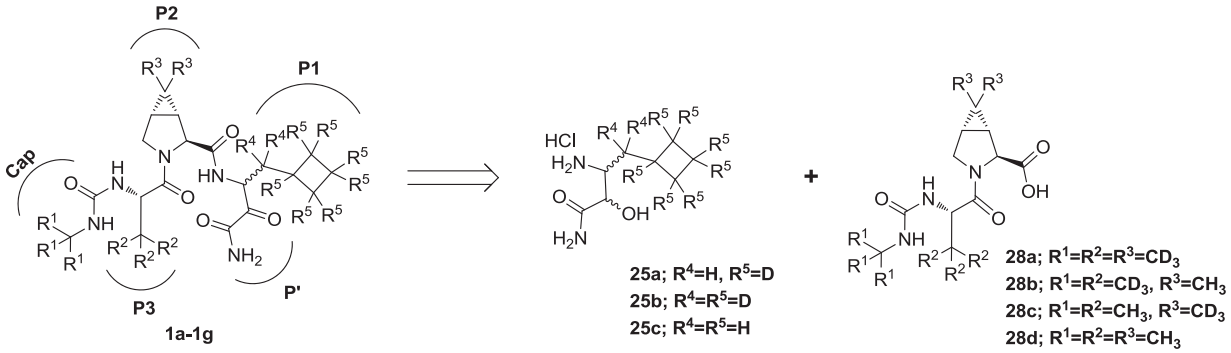
The retrosynthetic analysis, outlined in Scheme 1, depicts the key bond-forming reactions and required synthons for the preparation of deuterated analogs **1a–1g**.[11] As illustrated, individual target compounds arise from a series of peptide coupling reactions involving selectively deuterated intermediates. The syntheses culminate with the coupling of hydroxyamide synthons **25a–25c** with appropriately deuterated *tert*-butylurea intermediates **28a–28d** followed by subsequent oxidation to afford ketoamides **1a–1g**.

As shown in Scheme 1, the proposed route relies heavily upon the use of site-selectively deuterated synthons, each requiring individual preparation. The synthesis of the deuterated P3 subunit **7a** (**7b** purchased from Chem Impex Intl.), is outlined in Scheme 2, and begins with the formation of trimethylacetaldehyde-d9 (**2**) from *tert*-butyl chloride-d9.[12] An asymmetric Strecker with aldehyde **2** using an (*R*)-phenylglycine amide auxiliary in the presence of sodium cyanide and acetic acid afforded aminonitrile **3** as a single diastereomer.[13] Subsequent exposure of aminonitrile **3** to sulfuric acid afforded bisamide **4**, which was then converted to α-aminoamide **5** under palladium-catalyzed hydrogenolysis conditions. Exposure of **5** to 6 N HCl provided amino acid **6** as its hydrochloric acid salt, which was subsequently protected with di-*tert*-butyl dicarbonate, to afford *N*-Boc-*tert*-leucine-d9 (**7a**) in six overall steps.

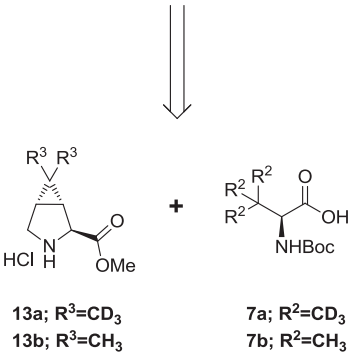
Preparation of deuterated P2 synthon **13a**[14] is outlined in Scheme 3. Diphenylethylsulfonium tetrafluoroborate-d3 (**8**) was obtained via treatment of diphenylsulfide with 2,2,2-iodoethane-d3 in the presence of silver tetrafluoroborate.[15] Deprotonation of **8** with LDA followed by the addition of iodomethane-d3 allowed for the *in situ* generation of the diphenylisopropylsulfonium-d6 salt. An additional equivalent of LDA served to generate the sulfonium ylide, which added to the less hindered *exo* face of enamide **9** to provide dimethylcyclopropane-d6 **10** as a single diastereomer.[16,17] It is important to note that when diphenylethylsulfonium tetrafluoroborate-d5 (prepared from iodoethane-d5) was employed in the cyclopropanation reaction, significantly lower yields were observed. This is believed to result from a dramatic KIE during one or both of the LDA deprotonation events.

With dimethylcyclopropane-d6 **10** in hand, removal of the benzylidene *N,O*-acetal was accomplished with $LiAlH_4$, affording *N*-benzyl aminoalcohol-d6 **11** in high yield.[18] Palladium-catalyzed transfer hydrogenolysis followed by treatment with di-*tert*-butyl dicarbonate provided the *N*-Boc-aminoalcohol-d6 **12**. Oxidation of the primary alcohol directly to the carboxylic acid under Sharpless conditions[19] followed by esterification and subsequent *N*-Boc deprotection ultimately afforded the deuterated P2 synthon **13a**.

Scheme 4 illustrates the preparation of the *N*-Boc-cyclobutylmethyl hydroxyamide synthons **25a** and **25b** from 1,3-dibromopropane-d6.[14] Cyclobutane dicarboxylic acid-d6 (**14**) was obtained in a two-step sequence involving alkylation and intramolecular cyclization[20] of diethyl malonate with 1,3-dibromopropane-d6 followed by saponification of the resulting diester. Decarboxylation in D_2O at 160 °C in a sealed tube generated cyclobutane carboxylic acid-d7 (**15**), which was subsequently reduced with $LiAlH_4$, affording cyclobutylmethanol-d7 (**16a**), or with $LiAlD_4$, affording cyclobutylmethanol-d9 (**16b**) (The remainder of the synthesis was completed in both the d7(**a**) and d9(**b**) series to obtain hydroxyamides **25a** and **25b**, respectively.) Conversion to tosylate **17** followed by subsequent treatment with lithium bromide provided (bromomethyl)cyclobutane **18**. Alkylation of **19** with bromide **18** afforded *rac*-cyclobutylalanine derivative **20**. Subsequent imine hydrolysis[21] and Boc protection delivered *rac*-*N*-Boc-cyclobutylalanine *t*-butyl ester **21**. Reduction of *t*-butyl ester **21** with diisobutylaluminum hydride[22] afforded aldehyde **22**, which, upon treatment with acetone cyanohydrin, was converted to cyanohydrin **23**. The cyclobutylmethyl hydroxyamide synthon



	R1	R2	R3	R4	R5
Boceprevir (1)	CH_3	CH_3	CH_3	H	H
1a	CH_3	CH_3	CH_3	H	D
1b	CH_3	CH_3	CH_3	D	D
1c	CH_3	CH_3	CD_3	D	D
1d	CH_3	CH_3	CD_3	H	H
1e	CD_3	CD_3	CH_3	H	H
1f	CD_3	CD_3	CD_3	H	H
1g	CD_3	CD_3	CD_3	D	D



Scheme 1. General retrosynthetic strategy to deuterated chemical entities.

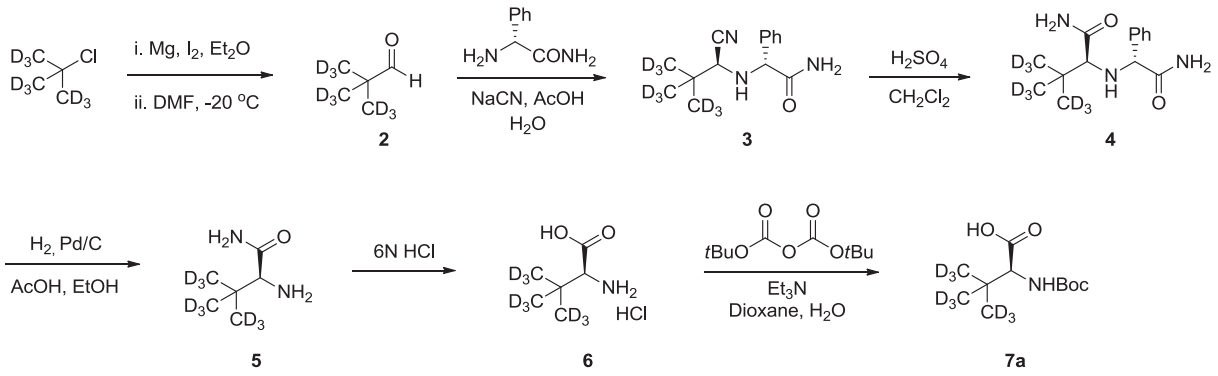
25 was then obtained via treatment of **23** with lithium hydroxide and hydrogen peroxide, affording *N*-Boc-amide **24**, followed by HCl-mediated Boc deprotection.

Final compounds **1a–1g** were ultimately assembled following the general route depicted in Scheme 5, employing the appropriately deuterated synthons as needed.[11] Coupling of *N*-Boc-*tert*-leucine **7a** or **7b** with dimethylcyclopropyl proline **13a** or **13b** was accomplished with BOP reagent to afford Boc-protected dipeptides **26a–26d**.[14] Deprotection of the *tert*-butyl carbamate followed by exposure to *tert*-butyl isocyanate **27a**[23] or **27b** provided *tert*-butyl ureas **28a–28d**.[14] Ester hydrolysis followed by EDC-mediated coupling with hydroxyamide **25a–25c** and subsequent oxidation under modified Moffatt conditions[24] afforded the deuterated boceprevir analogs **1a–1g**.

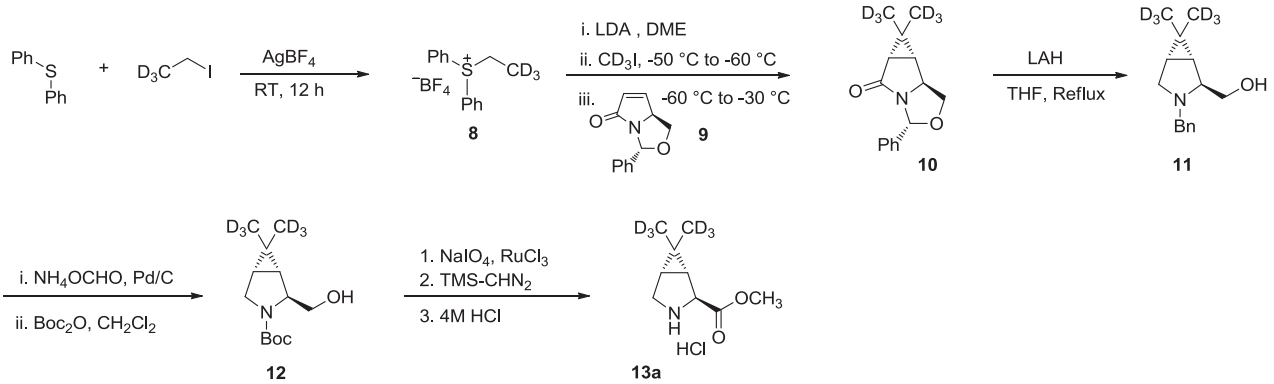
An alternative strategy for incorporation of the keto-amide subunit into the final compounds is shown in Scheme 6.[25] This approach involved oxidation and subsequent Boc deprotection of hydroxyamide intermediate **24b** to afford keto-amide **29**. Subsequent coupling with carboxylic acid fragments **28a** and **28d** afforded final compounds **1b** and **1g**, respectively. Similar yields have been observed for both strategies, and both methodologies provide final compounds **1** in high chemical and isotopic purities.

In vitro metabolic stability

In an effort to compare the *in vitro* metabolic stability of **1a–1g** with that of boceprevir, the compounds were incubated in human liver microsomes for a period of 30 min. Monitoring the percent parent remaining for each compound at various time points allows for the determination of *in vitro* $t_{1/2}$ and, ultimately, the average percent change in $t_{1/2}$ (%$\Delta t_{1/2}$) relative to boceprevir (Figure 2). As illustrated in Figure 2 and Table 1, deuteration of the P2 subunit alone seems to have little effect on *in vitro* metabolic stability, as compound **1d** exhibits a $t_{1/2}$ similar to that of



Scheme 2. Synthesis of P3 fragment (**7a**).



Scheme 3. Synthesis of P2 fragment (**13a**).

boceprevir. This may result from a masking of the KIE as a result of metabolic switching away from the P2 site on the molecule.[2,3] However, deuteration elsewhere within the molecule seems to play a more crucial role, and certain metabolic "hot spots" can be theorized. For instance, the analogs containing deuterium solely within the P1 subunit (**1a** and **1b**) displayed significant stability enhancement, with $\%\Delta t_{1/2} = 40\%$ and 51%, respectively. Additional metabolic stabilization was achieved by site-selective deuteration of multiple subunits as highlighted by analog **1g** exhibiting a $t_{1/2}$ nearly double that of boceprevir. These results may be indicative of diffuse metabolism at multiple sites within the parent scaffold. In such cases, the full potential of deuterium-mediated stabilization may only be realized through iterative labeling of several moieties within the compound of interest.

Experimental

General

Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Avance 400 spectrometer, operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C and with DMSO-d6 as solvent unless otherwise stated. Chemical shifts were reported in parts per million relative to tetramethylsilane, and *J* values were reported in hertz. Multiplicities were reported using the following abbreviations: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad. Low-resolution mass spectra were collected on an Agilent 1100 Series LC/MSD (column: 20 mm C18-RP 5–95% acetonitrile (ACN) + 0.1% HCO_2H/H_2O + 0.1% HCO_2H in 7 min with a 2-min hold at 95% acetonitrile (ACN) + 0.1% HCO_2H/H_2O + 0.1% HCO_2H; MSD: single quadrupole LC-MS (Agilent 6120) mass spectrometer using ESI in positive or negative mode). Optical rotation data were collected on a Perkin Elmer model 343 polarimeter at normal aperture and ambient temperature with a 100 mm path length cell. All reagents were purchased from commercial sources (Aldrich, Acros, Chem Impex Intl., Enamine Ltd., Alfa Aesar, CDN Isotopes, and Cambridge Isotope Labs) unless otherwise specified. The isotopic purity of commercial deuterated reagents has been noted in each experimental procedure. Maintenance of isotopic integrity was monitored using ^{1}H NMR and MS techniques throughout the synthetic sequences to the final compounds (**1a–1g**). Column chromatography was carried out on an ISCO CombiFlash® R_f system employing ISCO silica gel column cartridges. Human liver microsomes (20 mg/mL) were obtained from Xenotech, LLC (Lenexa, KS). β-Nicotinamide adenine dinucleotide phosphate, reduced form (NADPH), magnesium chloride ($MgCl_2$), and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich.

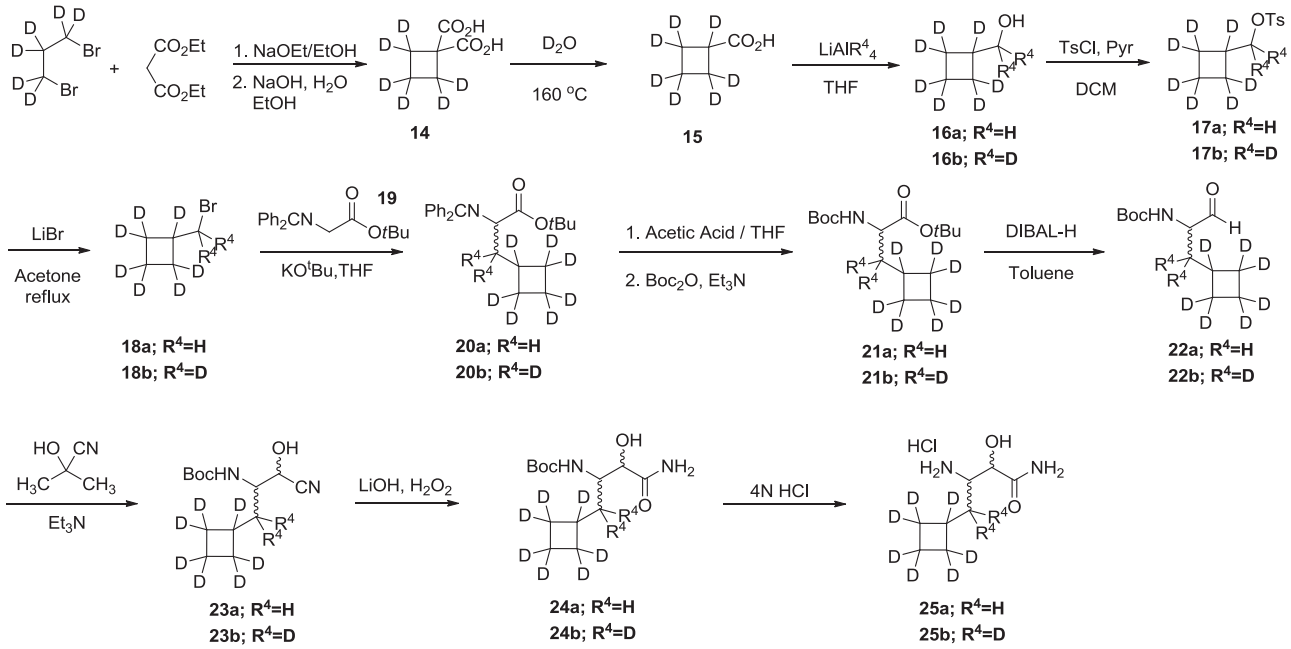
Labeling chemistry

Trimethylacetaldehyde-d9 ***(2)***

In a 3-L 4-necked round bottom flask fitted with mechanical stirrer, reflux condenser, dropping funnel, and thermometer was placed a few small crystals of iodine and then magnesium turnings (24.7 g, 1.03 mol). The bottom of the flask was heated with a heat gun until the iodine began to vaporize and was then allowed to cool, and a solution of *t*-butyl chloride-d9 (100.0 g, 1.03 mol, Cambridge Isotope Labs, 98 atom %D) in anhydrous ether was placed in the dropping funnel. A small amount of the solution of *t*-butyl chloride-d9 in ether (3–5 mL) was added directly to the dry magnesium. A large amount of anhydrous ether (1 L) and a few small crystals of iodine were added, and the resulting mixture was heated for 0.5 h to initiate the reaction. The rest of the solution of *t*-butyl chloride-d9 in ether was added with stirring at a rate not faster than one drop per second. The mixture was allowed to reflux during the addition, and no external cooling was applied. The reaction mixture was then heated at reflux for several hours until almost all of the magnesium disappeared. The mixture was then cooled to −20 °C, and a solution of anhydrous DMF (73.0 g, 1.00 mol) in ether (100 mL) was added over a 35-min period at such a rate that the temperature of the reaction did not exceed −15 °C. A second solution of anhydrous DMF (73.0 g, 1.00 mol) was then quickly added at −8 °C. After an additional 5 min, hydroquinone (0.5 g) was added, stirring was stopped, the cooling bath was removed, and the mixture was left standing overnight at ambient temperature under nitrogen. The mixture was then cooled to 5 °C, and aqueous 4 M HCl solution (600 mL) was added in portions to quench the reaction. The mixture was diluted with water (400 mL), and the layers were separated. The aqueous layer was extracted with ether (3 × 200 mL), and the combined organic layers were dried with $MgSO_4$ and filtered. The filtrate was subjected to fractional distillation under an atmosphere of nitrogen to remove most of the ether. The residue was transferred to a small flask, and fractional distillation was continued to collect aldehyde **2** at 65–75 °C (39.5 g, 40% yield) as a colorless oil. Compound **2** was stored under nitrogen in the freezer and used without further purification.

(R)-2-(((S)-1-Cyano-2,2-dimethylpropyl)amino)-2-phenylacetamide-d9 ***(3)***

To a stirred suspension of (*R*)-phenylglycine amide (60.7 g, 400 mmol) in water (400 mL) was added compound **2** (39.5 g, 415 mmol) at room temperature. Simultaneously, 30% aqueous NaCN solution (68.8 g, 420 mmol) and glacial acetic acid (25.4 g,



Scheme 4. Synthesis of P1 fragment (**25**).

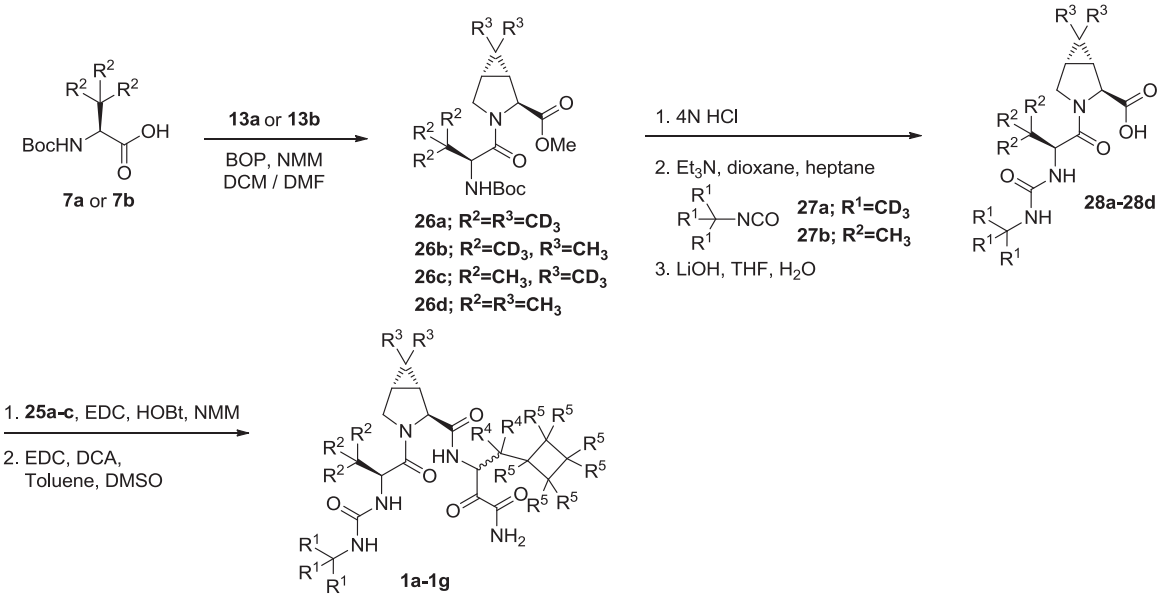
423 mmol) were added in 30 min, whereby the temperature of the reaction increased to 34 °C. The mixture was stirred for 2 h at 30 °C, followed by stirring at 70 °C for 20 h. After cooling to 30 °C, the product was isolated by filtration. The solid was washed with water (500 mL) and dried *in vacuo* at 50 °C to afford aminonitrile **3** (90.0 g, 88% yield) as a tan solid with $[\alpha]_D = -298°$ (c = 1.0, $CHCl_3$). MS (ESI) 255.3 $[(M+H)^+]$.

*(S)-2-(((R)-2-Amino-2-oxo-1-phenylethyl)amino)-3, 3-dimethylbutanamide-d9 (**4**)*

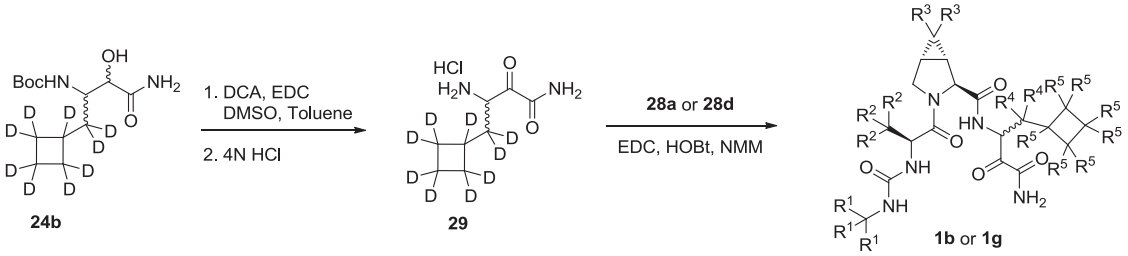
A solution of compound **3** (64.2 g, 252 mmol) in dichloromethane (500 mL) at 0 °C was added to conc. sulfuric acid (96%, 350 mL) at 15–20 °C through an addition funnel. The resulting mixture was stirred at room temperature for 1 h. The mixture was then poured on ice and carefully neutralized by NH_4OH solution to pH 9. The mixture was extracted with dichloromethane, and the combined organic layers were washed with water, dried with Na_2SO_4, filtered, and concentrated *in vacuo* to afford bisamide **4** (55.0 g, 80% yield) as a yellow foam with $[\alpha]_D = -140°$ (c = 1.0, $CHCl_3$). MS (ESI) 273.3 $[(M+H)^+]$.

*(S)-2-Amino-3,3-dimethylbutanamide-d9 (**5**)*

A mixture of compound **4** (77.0 g, 283 mmol), 10% Pd/C (~50% water, 20 g), and acetic acid (50 mL) in ethanol (1.2 L) was



Scheme 5. Fragment assembly to afford isotopologs **1a–1g**.



Scheme 6. Alternative fragment coupling approach.

subjected to hydrogenation at 30 psi at room temperature for several days until LC-MS showed that the reaction was complete. The mixture was filtered through Celite® and washed with EtOAc. After the filtrate was concentrated *in vacuo*, the residue was diluted with water (1 L) and basified with 1 M NaOH solution to pH 9. The mixture was extracted with dichloromethane, and the aqueous layer was concentrated *in vacuo* to half volume, saturated with solid NaCl, and extracted with THF. The combined extracts were dried with Na_2SO_4, filtered, and concentrated *in vacuo*. The resulting residue was diluted with toluene and subsequently evaporated to remove the remaining water, followed by trituration with dichloromethane to afford the aminoamide **5** (38.0 g, 96% yield) as a white solid, which was used without further purification.

*(S)-2-Amino-3,3-dimethylbutanoic acid-d9 hydrochloride (**6**)*

A mixture of compound **5** (31.0 g, 223 mmol) in 6 M aqueous HCl solution (1.5 L) was heated at reflux for 24 h. The mixture was concentrated *in vacuo* to give the crude product. The solid was redissolved in water (500 mL) and washed with EtOAc (2 × 200 mL) to remove impurities from previous steps. The aqueous layer was then concentrated *in vacuo*, diluted with toluene, evaporated, and dried *in vacuo* at 50 °C to afford the HCl salt of the desired compound (*S*)-2-amino-3,3-dimethylbutanoic acid-d9 hydrochloride (**6**; 33.6 g, 85% yield) as a white solid, which was used without further purification.

*N-Boc-tert-leucine-d9 (**7a**)*

To a solution of compound **6** (1.00 g, 5.66 mmol) in a mixture of dioxane (10 mL) and water (10 mL) was added triethylamine (3.16 mL, 22.6 mmol) followed by di-*tert*-butyl dicarbonate (1.48 g, 6.79 mmol). The resulting mixture, stirred at room temperature for 6 h, was then washed with heptane (2 × 20 mL). The aqueous fraction was cooled, with the pH adjusted to 2 with 1 M HCl, and extracted with ethyl acetate (3 × 50 mL). The combined extracts were dried with Na_2SO_4, filtered, and concentrated *in vacuo* to afford Boc-*tert*-leucine-d9 (**7a**, 1.10 g, 81% yield) as a yellow oil. MS (ESI) 239.2 $[(M-H)^-]$.

*Diphenyl(2,2,2-trideuteroethyl)sulfonium tetrafluoroborate (**8**)*

To a flask containing 2,2,2-iodoethane-d3 (10.0 g, 62.9 mmol, CDN Isotopes, 99 atom %D) was added diphenyl sulfide (2.32 mL, 13.8 mmol) and CH_2Cl_2 (15 mL). The mixture was cooled to 0 °C and stirred vigorously. To the cooled mixture was added silver tetrafluoroborate (2.45 g, 12.6 mmol) in one portion, and the mixture was warmed to ambient temperature over a period of 24 h. The reaction mixture was then diluted with CH_2Cl_2 (100 mL) and passed through a pad of Celite® to remove the precipitated silver iodide. The filtrate was concentrated *in vacuo*, and the crude oil triturated with Et_2O to afford a white solid. Recrystallization of the crude material from EtOH afforded **8** as a white powder (3.30 g, 86% yield). MS (ESI) 218.2 $[(M)^+]$.

*(3R,5aS,6aS,6βS)-3-Phenyl-6,6-bis(trideuteromethyl)tetrahydro-1H-cyclopropa[3,4]pyrrolo[1,2-c]oxazol-5(3H)-one (**10**)*

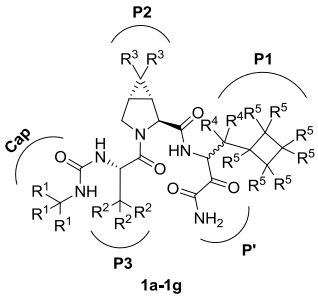
To a solution of freshly prepared LDA (0.94 M) in THF (13.0 mL, 12.4 mmol) at −70 °C was added a solution of **8** (3.64 g, 11.9 mmol) in DME (24 mL). The solution was stirred at −60 °C for a period of 30 min, followed by the addition of neat iodomethane-d3 (0.740 mL, 11.9 mmol, Aldrich, 99.5 atom %D). The mixture was then warmed slowly to −50 °C and stirred at −50 °C for a period of 2 h. The reaction mixture was then re-cooled to −70 °C, and a solution of LDA (0.94 M) in THF (13.8 mL, 13.1 mmol) was added; the mixture was stirred at −70 °C for a period of 1 h. To the stirred solution at −70 °C was then added a solution of commercially available (3*R*,7a*S*)-3-phenyl-1,7a-dihydropyrrolo[1,2-c]oxazol-5(3*H*)-one (**9**; 0.960 g, 4.77 mmol, Enamine Ltd.) in DME (9 mL). The reaction mixture was stirred for 1 h at −70 °C, followed by warming to −30 °C and stirring for an additional 2 h, at which point the mixture was diluted with saturated aqueous $NaHCO_3$ (30 mL) and warmed to ambient temperature. The mixture was extracted with Et_2O (3 × 25 mL) and the organic layers were dried with Na_2SO_4, filtered, and concentrated *in vacuo*. Purification by column chromatography (SiO_2, 20–40% EtOAc/heptane) afforded **10** as a white solid (980 mg, 82% yield, 33% with respect to CD_3I). MS (ESI) 250.1 $[(M+H)^+]$.

*((1R,2S,5S)-3-Benzyl-6,6-bis(trideuteromethyl)-3-azabicyclo[3.1.0]hexan-2-yl)methanol (**11**)*

To a solution of lactam-d6 **10** (376 mg, 1.51 mmol) in THF (3 mL) at 0 °C was added $LiAlH_4$ (2 M in THF, 1.51 mL, 3.02 mmol). The reaction was heated to reflux for 3 h, then cooled to 0 °C, and quenched by dropwise addition of 10% aqueous $KHSO_4$. The resulting slurry was diluted with ethyl acetate and filtered (washing the filter cake with ethyl acetate 2 × 10 mL). The resulting solution was diluted with water (20 mL) and extracted with ethyl acetate (3 × 30 mL). The combined organic layers were washed with brine, dried with $MgSO_4$, and concentrated *in vacuo* to afford alcohol-d6 **11** (350 mg, 98% yield). (ESI) 238.30 $[(M+H)^+]$.

*(1R,2S,5S)-tert-Butyl-2-(hydroxymethyl)-6,6-bis(trideuteromethyl)-3-azabicyclo[3.1.0]-hexane-3-carboxylate (**12**)*

To a solution of alcohol-d6 **11** (350 mg, 1.48 mmol) in methanol (15 mL) was added ammonium formate (571 mg, 9.06 mmol) followed by 10% palladium on carbon (70 mg, 20 wt.%). The reaction was then heated to reflux, taking precautions to limit ammonium formate sublimation inside the condenser. After stirring at reflux for 2 h, the reaction was cooled to room



	R^1	R^2	R^3	R^4	R^5
Boceprevir (1)	CH_3	CH_3	CH_3	H	H
1a	CH_3	CH_3	CH_3	H	D
1b	CH_3	CH_3	CH_3	D	D
1c	CH_3	CH_3	CD_3	D	D
1d	CH_3	CH_3	CD_3	H	H
1e	CD_3	CD_3	CH_3	H	H
1f	CD_3	CD_3	CD_3	H	H
1g	CD_3	CD_3	CD_3	D	D

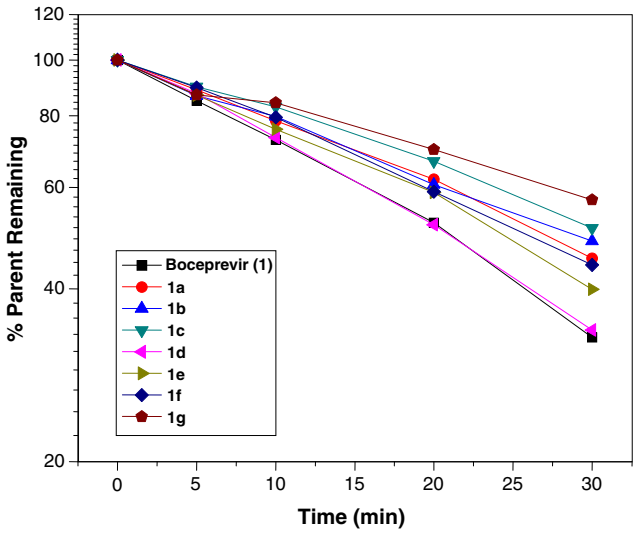


Figure 2. Metabolic stability of boceprevir and **1a–1g** in human liver microsomes.

temperature and filtered through Celite®. The Celite® pad was washed with methanol (2 × 10 mL) and then with dichloromethane (2 × 20 mL). The resulting solution was then concentrated *in vacuo* to afford the desired amino alcohol-d6. This material (~1.48 mmol) was dissolved in dichloromethane (5 mL), and triethylamine (273 µL, 1.96 mmol) was added followed by di-*tert*-butyl dicarbonate (428 mg, 1.96 mmol). The reaction was stirred at room temperature for 15 h, diluted with 1 M HCl (15 mL), and then extracted with dichloromethane (3 × 30 mL). The combined organic layers were washed with brine, dried with Na_2SO_4, filtered, and concentrated. The resulting residue was purified by column chromatography (SiO_2, 0–20% EtOAc/heptane) to afford alcohol-d6 **12** (311 mg, 83%, 2 step yield). (ESI) 270.2 $[(M + Na)^+]$.

*(1R,2S,5S)-Methyl-6,6-bis(trideuteromethyl)-3-azabicyclo[3.1.0] hexane-2-carboxylate hydrochloride (**13a**)*

To a solution of alcohol-d6 **12** (311 mg, 1.26 mmol) in ethyl acetate (10 mL) and ACN (10 mL) was added a solution of ruthenium trichloride monohydrate (5.50 mg, 0.0252 mmol) and sodium periodate (2.16 g, 10.0 mmol) in water (15 mL). After stirring at room temperature for 1 h, the reaction was filtered through Celite®. The Celite® pad was then washed with ethyl acetate (3 × 5 mL), and the resulting solution was concentrated to dryness. The resulting residue was diluted with 1 M HCl (10 mL) and extracted with ethyl acetate (3 × 20 mL). The organic layers were combined, washed with 1 M HCl, dried with Na_2SO_4, filtered, and concentrated to afford a dark tan solid. The crude acid-d6 (313 mg, 1.20 mmol) was dissolved in a mixture of benzene (5.0 mL) and methanol (0.50 mL), and a 2 M solution of trimethylsilyl diazomethane in hexanes (780 µL, 1.56 mmol) was added dropwise. The yellow solution was stirred at room temperature for 15 h and was subsequently quenched by dropwise addition of acetic acid until effervescence terminated. The reaction was then concentrated *in vacuo* with several repeated heptane dilutions/concentrations to remove excess acetic acid. The resulting residue was then purified by column chromatography (SiO_2, 0–30% EtOAc/heptane) to afford pure methyl ester-d6 (154 mg). To this material was added a 4 M solution of HCl in dioxane (5.0 mL), and the resulting solution was stirred at room temperature for 2 h. The reaction was then concentrated *in vacuo* to afford pure amine hydrochloride-d6 **13a** (128 mg, 41%, 3 step yield) as a colorless solid. (ESI) 176.2 $[(M + H)^+]$.

Table 1. Metabolic stability of DCEs in human liver microsomes

Compound	Ave $t_{1/2}$ (min)[a]	Ave $\%\Delta t_{1/2}$[b]
Boceprevir (**1**)	19.0 ± 0.87	—
1a	26.6 ± 1.70	40
1b	28.7 ± 2.19	51
1c	31.4 ± 2.36	65
1d	19.1 ± 1.19	<10
1e	23.1 ± 1.31	22
1f	25.0 ± 0.68	32
1g	37.3 ± 3.12	97

[a]Values are average of four experiments performed on separate days.
[b]$\%\Delta t_{1/2}$ was calculated according to the following equation: $\%\Delta t_{1/2} = [(t_{1/2}$ DCE) − ($t_{1/2}$ boceprevir)/($t_{1/2}$ boceprevir)] × 100%.

*2,2,3,3,4,4-Hexadeuterocyclobutane-1,1-dicarboxylic acid (**14**)*

A 21 wt% solution of sodium ethoxide in ethanol (24.1 mL, 64.2 mmol) was added dropwise to a stirred solution of 1,3-dibromopropane-d6 (3.54 mL, 33.7 mmol, CDN Isotopes, 99 atom %D) and diethylmalonate (4.87 mL, 32.1 mmol) in ethanol (35 mL), maintaining a temperature of 60–65 °C. Upon completion of the addition, the reaction was cooled to ~50 °C and was subsequently stirred at 100 °C until an aliquot added to water was neutral on pH paper. The reaction was then cooled to room temperature, diluted with water, and concentrated *in vacuo* to remove ethanol. The resulting aqueous solution was extracted with EtOAc (3 × 100 mL), dried with Na_2SO_4, filtered, and concentrated. The resulting residue was purified by column

chromatography (SiO_2, 0–20% EtOAc/heptane) to afford the diethyl ester derivative of **14** (4.00 g, 60% yield). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.20 (q, 2 H, J = 7.1 Hz), 1.25 (t, 3 H, J = 7.1 Hz). To a solution of this diester (4.00 g, 19.4 mmol) in ethanol (10 mL) was added 5 M aqueous sodium hydroxide (9.00 mL, 45.0 mmol). The reaction was then heated to reflux and stirred for 2 h. Upon cooling to room temperature, the reaction was concentrated *in vacuo* and diluted with 6 N HCl (100 mL). The resulting aqueous solution was extracted with diethyl ether (3 × 100 mL), and the combined organic layers were dried with Na_2SO_4, filtered, and concentrated. The resulting residue was then recrystallized from EtOAc/heptane to afford dicarboxylic acid **14** as white crystals (2.21 g, 76%). This material was carried forward into the next step without further purification.

*1,2,2,3,3,4,4-Heptadeuterocyclobutanecarboxylic acid (**15**)*

A solution of dicarboxylic acid **14** (2.21 g, 14.7 mmol) in D_2O (30 mL, Cambridge Isotope Labs, 99.8 atom %D) in a sealed pressure tube was heated to 160 °C for 15 h. After cooling to room temperature, the reaction was diluted with excess 1 M HCl and extracted with EtOAc (3 × 100 mL). The combined organic layers were dried with Na_2SO_4, filtered, and concentrated to afford carboxylic acid **15** (1.57 g, 100%) as a clear oil, which formed colorless crystals upon cooling in a −20 °C freezer. This material was carried forward crude into the next step.

*(Perdeuterocyclobutyl)methyl 4-methylbenzenesulfonate (**17a**)*

To a solution of **15** (1.00 g, 9.35 mmol) in THF (50 mL) at 0 °C was added a 2 M solution of $LiAlH_4$ in THF (5.61 mL, 11.2 mmol). The reaction was stirred at room temperature for 4 h and then cooled to 0 °C. Upon reaching 0 °C, the reaction was quenched by dropwise addition of 10% $KHSO_4$ until a solid white cake formed and further addition of $KHSO_4$ was unreactive. The cake was then broken up, filtered through Celite®, and washed with diethyl ether repeatedly. The resulting filtrate was concentrated *in vacuo*, dissolved in dichloromethane (100 mL), dried with Na_2SO_4, filtered, and concentrated. The resulting alcohol **(16a)** was dissolved in DCM (10 mL) and cooled to 0 °C, and pyridine (2.27 mL, 2.81 mmol) was added followed by *p*-toluenesulfonyl chloride (1.78 g, 9.35 mmol). The reaction was stirred at room temperature for 15 h and then diluted with diethyl ether (200 mL). The resulting solution was then washed with water (100 mL), 1 M HCl (3 × 50 mL), water (100 mL), and brine (100 mL). The organic layer was then dried with $MgSO_4$, filtered, and concentrated to afford tosylate **17a** as a clear oil (1.84 g, 80%), which was used without further purification. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.79 (d, J = 8.3 Hz, 2H), 7.34 (d, J = 8.1 Hz, 2H), 3.97 (s, 2H), 2.45 (s, 3H).

*1-(Bromomethyl)-1,2,2,3,3,4,4-heptadeuterocyclobutane (**18a**)*

A solution of tosylate **17a** (3.21 g, 13.0 mmol) and lithium bromide (1.81 g, 20.8 mmol) in acetone (50 mL) was heated to reflux and stirred for 3 h. The reaction was then cooled to room temperature, diluted with diethyl ether, and filtered through Celite® to remove lithium tosylate. The acetone and ether were then removed via careful distillation (bromomethylcyclobutane, bp = 123 °C). The resulting residue was then diluted with ether (50 mL), washed with water (2 × 50 mL), dried with $MgSO_4$, and filtered. Diethylether was then removed via careful distillation to afford bromide **18a** as a clear oil containing diethyl ether (2.02 g total, ~ 1.29 g of **11**, ~64% by ^{1}H NMR). This material was carried forward without further purification into the next step.

*tert-Butyl-2-((diphenylmethylene)amino)-3-(perdeuterocyclobutyl) propanoate (**20a**)*

To a solution of **19** (2.93 g, 9.92 mmol, purchased from Alfa Aesar) in THF (30 mL) at −78 °C was added a 1 M solution of potassium *tert*-butoxide in THF (9.92 mL, 9.92 mmol). The resulting solution was stirred at 0 °C for 1 h, at which time a solution of bromide **18a** (1.29 g, 8.27 mmol) in THF (2 mL) was added. The reaction was then stirred at room temperature for 15 h. The THF was then removed *in vacuo*, and the resulting residue was diluted with water (100 mL). This solution was then extracted with CH_2Cl_2 (3 × 50 mL), and the aqueous layer was acidified with 1 M HCl and re-extracted with CH_2Cl_2 (3 × 50 mL). All organic layers were combined, dried with Na_2SO_4, filtered, and concentrated to afford a red oil, which was purified by column chromatography (SiO_2, 0–20% EtOAc/heptane) to afford imine **20a** (1.93 g, 63%). MS (ESI) 371.3 $[(M + H)^+]$.

*tert-Butyl-2-((tert-butoxycarbonyl)amino)-3-(perdeuterocyclobutyl) propanoate (**21a**)*

To a solution of **20a** (1.93 g, 5.21 mmol) in a mixture of THF and water (1:1, 24 mL) was added acetic acid (12 mL). The reaction then stirred at room temperature for 6 h and then quenched with sat. $NaHCO_3$. The resulting solution was then extracted with EtOAc (3 × 50 mL). The organic layers were then combined, dried with Na_2SO_4, filtered, and concentrated to afford a 1:1 mixture of cyclobutylalanine *t*-butyl ester-d7 and benzophenone as observed by ^{1}H NMR. This mixture was then dissolved in CH_2Cl_2 (20 mL), and triethylamine (1.09 mL, 7.82 mmol) and di-*tert*-butyl dicarbonate (1.36 g, 6.25 mmol) were added. The reaction was stirred at room temperature for 15 h, diluted with 1 M HCl (15 mL), and then extracted with CH_2Cl_2 (3 × 30 mL). The combined organic layers were washed with brine, dried with Na_2SO_4, filtered, and concentrated. The resulting residue was purified by column chromatography (SiO_2, 0–20% EtOAc/heptane) to afford **21a** (1.26 g, 80%) as a clear oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.92 (br d, 1H), 4.08 (q, J = 5.6 Hz, 1H), 1.83 (dd, J = 5.3, 13.6 Hz, 1H), 1.65 (dd, J = 7.6, 13.6 Hz, 1H), 1.46 (s, 9H), 1.43 (s, 9H).

*tert-Butyl-(1-oxo-3-(perdeuterocyclobutyl)propan-2-yl)carbamate (**22a**)*

To a solution of **14** (1.26 g, 4.11 mmol) in toluene (20 mL) cooled to −78 °C was added a 1 M solution of diisobutylaluminum hydride in CH_2Cl_2 (2.12 mL, 2.12 mmol). The reaction was stirred at −78 °C for 1 h and then quenched with methanol (20 mL). After stirring at −78 °C for an additional 15 min, the reaction was poured into a flask containing saturated Rochelle's salt (40 mL) and subsequently stirred at room temperature for 3.5 h. The organic layer was separated, and the remaining aqueous layer was extracted with EtOAc (3 × 100 mL). The combined organic layers were dried with Na_2SO_4, filtered, concentrated *in vacuo*, and purified by column chromatography (SiO_2, 0–15% EtOAc/heptane) to afford aldehyde **22a** (583 mg, 61%). MS (ESI) 135.2 $[(M\text{-Boc} + H)^+]$.

*tert-Butyl-(1-cyano-1-hydroxy-3-(perdeuterocyclobutyl)propan-2-yl)carbamate (**23a**)*

Acetone cyanohydrin (471 µL, 5.15 mmol) was added to a solution of aldehyde **22a** (583 mg, 2.49 mmol) and triethylamine (420 µL, 3.01 mmol) in CH_2Cl_2 (4 mL). The reaction was stirred at room temperature for 15 h and then concentrated *in vacuo*. The resulting residue was diluted with 1 M HCl (10 mL) and extracted with CH_2Cl_2 (3 × 100 mL). The organic layers were combined, washed with water and then brine, dried with $MgSO_4$, filtered, and concentrated *in vacuo* to afford cyanohydrin **23a** (651 mg, 99%). MS (ESI) 284.2 $[(M + Na)^+]$.

*tert-Butyl-(4-amino-3-hydroxy-4-oxo-1-(perdeuterocyclobutyl) butan-2-yl)carbamate (**24a**)*

To a solution of cyanohydrin **23a** (651 mg, 2.49 mmol) in methanol (10 mL) at 0 °C was added 30% H_2O_2 (1.40 mL, 13.7 mmol) followed by lithium hydroxide (72.0 mg, 2.99 mmol). The reaction was stirred at 0 °C for 3 h and subsequently quenched at 0 °C via careful addition of excess sat. $NaHSO_3$. The resulting solution was then diluted with water (30 mL) and extracted with CH_2Cl_2 (3 × 100 mL). The combined organic layers were dried with Na_2SO_4, filtered, and concentrated *in vacuo* to afford hydroxyamide **24a** (469 mg, 67%) as a white solid. MS (ESI) 302.3 $[(M + Na)^+]$.

*3-Amino-2-hydroxy-4-(perdeuterocyclobutyl)butanamide (**25a**)*

A solution of hydroxyamide **24a** (469 mg, 1.68 mmol) was stirred in 4 M HCl in dioxane (5 mL) for 3 h. The reaction was then concentrated *in vacuo* to afford hydroxyamide hydrochloride salt-d7 **25a** (337 mg, 93%). MS (ESI) 180.2 $[(M + H)^+]$.

*Dideutero(perdeuterocyclobutyl)methyl 4-methylbenzenesulfonate (**17b**)*

The procedure was the same as that for **17a**, employing **15** (3.75 g, 35.0 mmol) and $LiAlD_4$ (Cambridge Isotope Laboratories, 98 atom %D) to provide **17b** (5.88 g, 67%). MS (ESI) 272.1 $[(M + Na)^+]$.

*1-(Bromodideuteromethyl)-1,2,2,3,3,4,4-heptadeuterocyclobutane (**18b**)*

The procedure was the same as that for **18a**, employing **17b** (5.88 g, 23.6 mmol) to provide **18b** (2.86 g, 76%), which was carried forward without further purification.

*tert-Butyl-3,3-dideutero-2-((diphenylmethylene)amino)-3-(perdeuterocyclobutyl)-propanoate (**20b**)*

The procedure was the same as that for **20a**, employing **18b** (2.86 g, 18.1 mmol) to provide **20b** (4.47 g, 66%). MS (ESI) 373.3 $[(M + H)^+]$.

*tert-Butyl-2-((tert-butoxycarbonyl)amino)-3,3-dideutero-3-(perdeuterocyclobutyl)-propanoate (**21b**)*

The procedure was the same as that for **21a**, employing **20b** (1.49 g, 3.99 mmol) to provide **21b** (973 mg, 79%). ^1H NMR ($CDCl_3$, 400 MHz) δ 4.92 (br d, 1H), 4.06 (d, $J = 8.3$ Hz, 1H), 1.46 (s, 9H), 1.43 (s, 9H).

*tert-Butyl-(1,1-dideutero-3-oxo-1-(perdeuterocyclobutyl)propan-2-yl)carbamate (**22b**)*

The procedure was the same as that for **22a**, employing **21b** (715 mg, 2.32 mmol) to provide **22b** (372 mg, 68%). MS (ESI) 137.3 $[(M\text{-Boc} + H)^+]$.

*tert-Butyl-(1,1-dideutero-3-cyano-3-hydroxy-1-(perdeuterocyclobutyl) propan-2-yl)carbamate (**23b**)*

The procedure was the same as that for **23a**, employing **22b** (372 mg, 1.57 mmol) to provide **23b** (414 mg, 100%). MS (ESI) 286.2 $[(M + Na)^+]$.

*tert-Butyl-(4-amino-1,1-dideutero-3-hydroxy-4-oxo-1-(perdeuterocyclobutyl)butan-2-yl)carbamate (**24b**)*

The procedure was the same as that for **24a**, employing **23b** (414 mg, 1.57 mmol) to provide **24b** (291 mg, 66%). MS (ESI) 304.3 $[(M + Na)^+]$.

*3-Amino-4,4-dideutero-2-hydroxy-4-(perdeuterocyclobutyl) butanamide (**25b**)*

The procedure was the same as that for **25a**, employing **24b** (272 mg, 0.984 mmol) to provide **25b** (208 mg, 97%). MS (ESI) 182.2 $[(M + H)^+]$.

*3-Amino-4-cyclobutyl-2-oxobutanamide-d9 (**29**)*

To a solution of amide **24b** (141 mg, 0.501 mmol) in 1:1 toluene/DMSO (10 mL) at 0 °C was added EDC (960 mg, 5.01 mmol) followed by dichloroacetic acid (207 µL, 2.50 mmol). The reaction was stirred at room temperature for 4 h, diluted with 1 M HCl (50 mL), and then extracted with DCM (3 × 50 mL). The combined organic layers were washed with 1 M HCl (50 mL), sat. $NaHCO_3$ (50 mL), and brine (50 mL); dried with Na_2SO_4; filtered; and concentrated *in vacuo*. The resulting residue was purified by column chromatography (SiO_2, 0–40% EtOAc/heptane) to afford the Boc-protected ketoamide (117 mg, 84%) as an off-white solid. MS (ESI) 302.2 $[(M + Na)^+]$, 180.2 $[M - Boc + H]$. The resulting ketoamide (117 mg, 0.419 mmol) was stirred in 4 M HCl in dioxane (7 mL) for 3 h. The reaction was then concentrated *in vacuo* to afford amine hydrochloride **29** (90 mg, 99%). This material was carried forward without purification.

*(1R,2S,5S)-Methyl-3-((S)-2-((tert-butoxycarbonyl)amino)-4,4,4-trideutero-3,3-bis(trideuteromethyl)butanoyl)-6,6-bis(trideuteromethyl)-3-azabicyclo[3.1.0]hexane-2-carboxylate (**26a**)*

To a solution of **7a** (121 mg, 0.506 mmol) and **13a** (128 mg, 0.607 mmol) in DCM/DMF (1:1, 3 mL) at 0 °C was added 4-methyl morpholine (167 µL, 1.52 mmol) and BOP reagent (269 mg, 0.607 mmol). The reaction was stirred at room temperature for 15 h, diluted with 1 M HCl, and extracted with DCM (3 × 30 mL). The combined organic layers were washed with 1 M HCl, sat. $NaHCO_3$, and brine; dried with $MgSO_4$; filtered; and concentrated *in vacuo*. The resulting residue was purified by column chromatography (SiO_2, 0–20% acetone/heptane) to afford methyl ester **26a** (112 mg, 56% yield). MS (ESI) 398.3 $[(M + H)^+]$.

*(1R,2S,5S)-Methyl-3-((S)-2-((tert-butoxycarbonyl)amino)-4,4,4-trideutero-3,3-bis(trideuteromethyl)butanoyl)-6,6-dimethyl-3-azabicyclo[3.1.0]hexane-2-carboxylate (**26b**)*

The procedure was the same as that for **26a**, employing amine **13b** (693 mg, 3.37 mmol) to afford **26b** (465 mg, 35%). MS (ESI) 392.4 $[(M + H)^+]$.

*(1R,2S,5S)-Methyl-3-((S)-2-((tert-butoxycarbonyl)amino)-3,3-dimethylbutanoyl)-6,6-bis(trideuteromethyl)-3-azabicyclo[3.1.0] hexane-2-carboxylate (**26c**)*

The procedure was the same as that for **26a**, employing acid **7a** (305 mg, 1.44 mmol) to afford **26c** (358 mg, 77%). MS (ESI) 389.4 $[(M+H)^+]$.

*(1R,2S,5S)-3-((S)-4,4,4-Trideutero-2-(3-(1,1,1,3,3,3-hexadeutero-2-(trideuteromethyl)propan-2-yl)ureido)-3,3-bis(trideuteromethyl) butanoyl)-6,6-bis(trideuteromethyl)-3-azabicyclo[3.1.0]hexane-2-carboxylic acid (**28a**)*

A solution of **26a** (545 mg, 1.37 mmol) in 4 M HCl in dioxane (15 mL) was stirred at room temperature for 3 h and then concentrated *in vacuo* to afford the amine hydrochloride salt. This material was dissolved in dioxane (3.00 mL), and triethylamine (1.15 mL, 8.22 mmol) was added. The mixture was subsequently cooled to −78 °C, and *tert*-butyl isocyanate-d9 (**27a**; 740 mg, 6.85 mmol) was added. The reaction was stirred at room temperature for 15 h, concentrated *in vacuo*, diluted with 1 M HCl, and extracted with DCM (3 × 50 mL). The combined organic layers were washed with 1 M HCl, sat. $NaHCO_3$, and brine; dried with $MgSO_4$; filtered; and concentrated *in vacuo*. The resulting residue was purified by column chromatography (SiO_2, 10–20% acetone/heptane) to afford d24-urea methyl ester as a white crunchy foam. MS (ESI) 428.5 $[(M+Na)^+]$. The resulting urea methyl ester was dissolved in a mixture of THF/H_2O (1:1, 10 mL), and lithium hydroxide (35.0 mg, 1.45 mmol) was added. The reaction was stirred for 3 h, quenched with 1 M HCl, and concentrated to remove THF. The resulting aqueous solution was extracted with EtOAc (3 × 10 mL), and the combined organic layers were dried with Na_2SO_4, filtered, and concentrated to afford carboxylic acid **28a** (360 mg, 67%), which was used without further purification.

*(1R,2S,5S)-6,6-Dimethyl-3-((S)-4,4,4-trideutero-2-(3-(1,1,1,3,3, 3-hexadeutero-2-(trideuteromethyl)propan-2-yl)ureido)-3,3-bis (trideuteromethyl)butanoyl)-3-azabicyclo[3.1.0]hexane-2-carboxylic acid (**28b**)*

The procedure was the same as that for **28a**, employing methyl ester **26b** (465 mg, 1.19 mmol) to afford **28b** (158 mg, 34%). Urea methyl ester intermediate: MS (ESI) 422.4 $[(M+Na)^+]$.

*(1R,2S,5S)-3-((S)-2-(3-(tert-Butyl)ureido)-3,3-dimethylbutanoyl)-6,6-bis(trideuteromethyl)-3-azabicyclo[3.1.0]hexane-2-carboxylic acid (**28c**)*

The procedure was the same as that for **28a**, employing methyl ester **26c** (358 mg, 0.921 mmol) and *tert*-butyl isocyanate (**27b**, 216 µL, 1.84 mmol) to afford **28c** (340 mg, 99%). Urea methyl ester intermediate: MS (ESI) 388.3 $[(M+H)^+]$.

*Boceprevir-d7 (**1a**) (method 1: hydroxyamide coupling strategy)*

To a solution of **28d** (71.0 mg, 0.193 mmol) dissolved in a mixture of CH_2Cl_2/DMF (1:1, 2.5 mL) at 0 °C was added amine hydrochloride salt **25a** (50.0 mg, 0.232 mmol), EDC (56.0 mg, 0.290 mmol), HOBt (39.0 mg, 0.290 mmol), and 4-methyl morpholine (85.0 µL, 0.772 mmol). The reaction was stirred at room temperature for 15 h and then concentrated *in vacuo*. The resulting residue was diluted with 1 M HCl and extracted with EtOAc (3 × 30 mL). The combined organic layers were washed successively with 1 M HCl, sat. $NaHCO_3$, and brine; dried with $MgSO_4$; filtered; and concentrated. To a solution of this material in a mixture of toluene/DMSO (1:1, 4 mL) at 0 °C was added EDC (362 mg, 1.89 mmol) and dichloroacetic acid (78.0 µL, 0.946 mmol). The reaction was stirred at room temperature for 4 h, diluted with sat. $NaHCO_3$, and then extracted with CH_2Cl_2 (3 × 30 mL). The combined organic layers were washed successively with sat. $NaHCO_3$, 1 M HCl, and brine; dried with $MgSO_4$; filtered; and concentrated *in vacuo*. The resulting residue was purified by column chromatography (SiO_2, 0–30% acetone/heptane) to afford **1a** (66.0 mg, 66%) as a white solid and as a mixture of diastereomers. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.28 (d, J = 7.3 Hz, 0.6H), 8.18 (d, J = 7.8 Hz, 0.4H), 8.03 (s, 0.6H), 7.99 (s, 0.4H), 7.77 (br s, 1H), 5.97 (s, 1H), 5.87–5.79 (m, 1H), 4.94 (d, J = 7.3 Hz, 0.6H), 4.82 (d, J = 7.3 Hz, 0.4H), 4.28 (br s, 1H), 4.16–4.06 (m, 1H), 4.00–3.90 (m, 1H), 3.80–3.69 (m, 1H), 1.78 (d, J = 3.8 Hz, 0.4H), 1.74 (d, J = 3.5 Hz, 0.6H), 1.65–1.50 (m, 1H), 1.46–1.39 (m, 1H), 1.27 (d, J = 4.0 Hz, 0.6H), 1.25 (d, J = 4.0 Hz, 0.4H), 1.17 (br s, 9H), 1.03–0.97 (m, 3H), 0.92–0.86 (m, 9H), 0.86–0.79 (m, 3H); MS (ESI) 527.5 $[(M+H)^+]$.

*Boceprevir-d9 (**1b**) (method 2: keto-amide coupling strategy)*

To a solution of carboxylic acid **28d** (59.0 mg, 0.160 mmol) and amine hydrochloride **29** (45.0 mg, 0.209 mmol) in ACN (1.5 mL) at 0 °C was added EDC (46.0 mg, 0.240 mmol), HOBt (6.00 mg, 0.0480 mmol), and *N*-methyl morpholine (19.0 µL, 0.176 mmol). The reaction was stirred at room temperature for 15 h, concentrated *in vacuo*, diluted with 1 M HCl, and extracted with EtOAc (3 × 20 mL). The combined organic layers were washed with 1 M HCl, sat. $NaHCO_3$, and brine; dried with $MgSO_4$; filtered; and concentrated *in vacuo*. The resulting residue was purified by column chromatography (SiO_2, 10–40% acetone/heptane) to afford **1b** (21 mg, 25%) as a white solid and as a mixture of diastereomers. ^{1}H NMR (CD_3OD, 400 MHz) δ 7.55 (br s, 0.3H), 6.07 (br s, 0.6H), 5.95–5.76 (m, 1.3H), 4.38 (s, 0.6H), 4.35–4.20 (m, 2H), 4.13–3.86 (m, 3H), 3.25–3.20 (m, 1H), 1.62–1.27 (m, 2H), 1.29–1.22 (m, 9H), 1.08–1.03 (m, 3H), 1.03–0.96 (m, 9H), 0.95–0.87 (m, 3H). MS (ESI) 529.5 $[(M+H)^+]$.

*Boceprevir-d15 (**1c**)*

The procedure was the same as that for **1a**, employing carboxylic acid **28c** to afford **1c** in 47% yield and as a mixture of diastereomers. NMR (DMSO-d_6, 400 MHz) δ 8.28 (d, J = 7.3 Hz, 0.6H), 8.18 (d, J = 7.8 Hz, 0.4H), 8.03 (s, 0.6H), 7.99 (s, 0.4H), 7.78 (br s, 1H), 5.94 (s, 1H), 5.87–5.79 (m, 1H), 4.94 (d, J = 7.3 Hz, 0.6H), 4.82 (d, J = 7.3 Hz, 0.4H), 4.28 (br s, 1H), 4.16–4.06 (m, 1H), 4.00–3.90 (m, 1H), 3.80–3.69 (m, 1H), 1.45–1.39 (m, 1H), 1.31–1.18 (m, 1H), 1.17 (br s, 9H), 0.89 (br s, 9H); MS (ESI) 535.5 $[(M+H)^+]$.

*Boceprevir-d6 (**1d**)*

The procedure was the same as that for **1a**, employing carboxylic acid **28c** and hydroxyamide **25c** to afford **1d** in 41% yield and as a mixture of diastereomers. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.28 (d, J = 7.3 Hz, 0.7H), 8.21 (d, J = 7.8 Hz, 0.3H), 8.03 (s, 0.7H), 7.99 (s, 0.3H), 7.76 (br s, 1H), 5.94 (s, 1H), 5.87–5.79 (m, 1H), 5.00–4.90 (m, 0.7H), 4.90–4.81 (m, 0.3H), 4.28 (s, 1H), 4.16–4.06 (m, 1H), 4.00–3.90 (m, 1H), 3.80–3.69 (m, 1H), 2.57–2.42 (m, 0.7H), 2.40–2.29 (m, 0.3H), 2.04–1.89 (m, 2H), 1.82–1.68 (m, 3H), 1.68–1.52 (m, 3H), 1.45–1.39 (m, 1H), 1.31–1.18 (m, 1H), 1.17 (br s, 9H), 0.89 (br s, 9H); MS (ESI) 526.5 $[(M+H)^+]$.

*Boceprevir-d18 (**1e**)*

The procedure was the same as that for **1a**, employing carboxylic acid **28b** and hydroxyamide **25c** to afford **1e** in 37% yield and as a mixture of diastereomers. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.27 (d, 0.8 H, J = 7.3 Hz), 8.17 (d, 0.2 H, J = 7.8 Hz),

8.02 (s, 0.8 H), 7.97 (s, 0.2 H), 7.76 (br s, 1.0 H), 7.34 (s, 0.5 H), 6.90 (s, 0.4 H), 5.94 (s, 0.8H), 5.93 (s, 0.2H), 5.87–5.79 (m, 1H), 5.00–4.90 (m, 0.8H), 4.90–4.81 (m, 0.2H), 4.28 (s, 0.8H), 4.27 (s, 0.2H), 4.16–4.06 (m, 1H), 4.00–3.90 (m, 1H), 3.80–3.69 (m, 1H), 2.57–2.42 (m, 0.8H), 2.40–2.29 (m, 0.2H), 2.04–1.89 (m, 2H), 1.82–1.68 (m, 3H), 1.68–1.52 (m, 3H), 1.45–1.39 (m, 1H), 1.31–1.18(m, 1H), 1.03–0.97 (m, 3H), 0.89–0.80 (m, 3H); MS (ESI) 538.5 [$(M+H)^+$].

*Boceprevir-d24 (**1f**)*

The procedure was the same as that for **1a**, employing carboxylic acid **28a** and hydroxyamide **25c** to afford **1f** in 23% yield and as a mixture of diastereomers. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.27 (d, 0.7 H, J = 7.3 Hz), 8.17 (d, 0.3 H, J = 7.8 Hz), 8.02 (s, 0.7 H), 7.97 (s, 0.3 H), 7.76 (br s, 1.0 H), 7.34 (s, 0.6 H), 6.90 (s, 0.6 H), 5.94 (s, 0.7H), 5.93 (s, 0.3H), 5.87–5.79 (m, 1H), 5.00–4.90 (m, 0.7H), 4.90–4.81 (m, 0.3H), 4.28 (s, 0.7H), 4.27 (s, 0.3H), 4.16–4.06 (m, 1H), 4.00–3.90 (m, 1H), 3.80–3.69 (m, 1H), 2.57–2.42 (m, 0.7H), 2.40–2.29 (m, 0.3H), 2.04–1.89 (m, 2H), 1.82–1.68 (m, 3H), 1.68–1.52 (m, 3H), 1.45–1.39 (m, 1H), 1.31–1.18(m, 1H); MS (ESI) 544.5 [$(M+H)^+$].

*Boceprevir-d33 (**1g**)*

The procedure was the same as that for **1b**, employing carboxylic acid **28a** to afford **1g** in 23% yield and as a mixture of diastereomers (28 mg, 36%) as a white solid. ^{1}H NMR (CD_3OD, 400 MHz) δ 7.55 (br s, 0.3H), 6.07 (br s, 1H), 5.95–5.76 (m, 1H), 4.35–4.20 (m, 2H), 4.20–3.87 (m, 3H), 3.22 (s, 1H), 1.62–1.27 (m, 2H). MS (ESI) 553.5 [$(M+H)^+$].

Determination of metabolic stability

Stock solutions (7.5 mM) of test compounds were prepared in DMSO. The 7.5 mM stock solutions were diluted to 12.5 µM in ACN. The 20 mg/mL human liver microsomes were diluted to 0.625 mg/mL in 0.1 M potassium phosphate buffer (pH 7.4) containing 3 mM $MgCl_2$. The diluted microsomes were added to wells of a 96-well deep-well polypropylene plate in triplicate. Ten microliters of the 12.5 µM test compound was added to the microsomes, and the mixture was pre-warmed for 10 min. Reactions were initiated by the addition of pre-warmed NADPH solution. The final reaction volume was 0.5 mL and contained 0.5 mg/mL human liver microsomes, 0.25 µM test compound, and 2 mM NADPH in 0.1 M potassium phosphate buffer (pH 7.4) and 3 mM $MgCl_2$. The reaction mixtures were incubated at 37 °C, and 50-µL aliquots were removed at 0, 5, 10, 20, and 30 min and then added to shallow 96-well plates, which contained 50 µL of ice-cold ACN with internal standard to stop the reactions. The plates were stored at 4 °C for 20 min, after which 100 µL of water was added to the wells of the plate before centrifugation to pellet the precipitated proteins. Supernatants were transferred to another 96-well plate and analyzed for amounts of parent remaining by LC-MS/MS using an Applied Bio-systems API 4000 mass spectrometer. Quantitative analysis by LC-MS/MS was performed using an Applied Bio-systems API 4000 mass spectrometer and utilized an APCI (Atmospheric Pressure Chemical Ionization) source operated in positive ion MRM (multiple reaction monitoring) mode. 7-Ethoxycoumarin (1 µM) was used as a positive control.

Data analysis

The *in vitro* $t_{1/2}$ values for test compounds were calculated from the slopes of the linear regression of the % parent remaining (ln) versus incubation time relationship. Data analysis was performed using Microsoft Excel Software; *in vitro* $t_{1/2} = 0.693/k$; $k = -$[slope of linear regression of % parent remaining(ln) vs incubation time].

Conclusions

In summary, several DCEs derived from the boceprevir scaffold have been prepared via synthetic routes that allow for highly site-selective deuterium incorporation with high levels of isotopic purity. Our studies demonstrate that judicious application of deuterium medicinal chemistry to the boceprevir scaffold can result in the identification of DCEs that display marked levels of *in vitro* metabolic stabilization in human liver microsomes. The highest degree of metabolic stabilization was observed with DCE **1g**, which exhibited a half-life approximately double that of boceprevir. On the basis of previous work in our laboratories, we anticipate that these metabolically stabilized DCEs will retain the biochemical potency and selectivity of boceprevir. Further studies aimed at evaluating the *in vivo* pharmacokinetics as well as confirming the preserved pharmacological activity of these DCEs will be reported in due course. This work highlights the utility of the DCE Platform™ as a medicinal chemistry tool capable of modifying the metabolic fate of a drug to potentially alter its therapeutic profile.

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