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Synthesis of deleobuvir, a potent hepatitis C virus polymerase inhibitor, and its major metabolites labeled with carbon-13 and carbon-14

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Deleobuvir, (2*E*)-3-(2-{1-[2-(5-bromopyrimidin-2-yl)-3-cyclopentyl-1-methyl-1*H*-indole-6-carboxamido]cyclobutyl}-1-methyl-1*H*-benzimidazol-6-yl)prop-2-enoic acid (1), is a non-nucleoside, potent, and selective inhibitor of hepatitis C virus NS5B polymerase. Herein, we describe the detailed synthesis of this compound labeled with carbon-13 and carbon-14. The synthesis of its three major metabolites, namely, the reduced double bond metabolite (2) and the acyl glucuronide derivatives of (1) and (2), is also reported. Aniline-$^{13}C_6$ was the starting material to prepare butyl (*E*)-3-(3-methylamino-4-nitrophenyl-$^{13}C_6$)acrylate [$^{13}C_6$]-(11) in six steps. This intermediate was then used to obtain [$^{13}C_6$]-(1) and [$^{13}C_6$]-(2) in five and four more steps, respectively. For the radioactive synthesis, potassium cyanide-^{14}C was used to prepare 1-cylobutylaminoacid [^{14}C]-(23) via Buchrer–Bergs reaction. The carbonyl chloride of this acid was then used to access both [^{14}C]-(1) and [^{14}C]-(2) in four steps. The acyl glucuronide derivatives [$^{13}C_6$]-(3), [$^{13}C_6$]-(4) and [^{14}C]-(3) were synthesized in three steps from the acids [$^{13}C_6$]-(1), [$^{13}C_6$]-(2) and [^{14}C]-(1) using known procedures.

Keywords: deleobuvir; metabolites; NS5B polymerase; acyl glucoronide; carbon-14; carbon-13

Introduction

Hepatitis C virus (HCV) infection is a serious public health issue in the world. Each year between three and four million people become infected.[1,2] According to the Centers for Disease Control (CDC), most at risk are injection drug users, dialysis patients, people who acquire tattoos or body piercings with non-sterile instruments, some healthcare workers, people with HIV, and children born to mothers with hepatitis C. The highest prevalence of HCV worldwide is in North Africa and South Asia. In Egypt, for example, one in seven people is infected with the virus.[3,4] The main methods of transmission in this country are blood transfusions and unsafe medical procedures.[5] HCV is highly heterogeneous, which can be an obstacle for the development of a universal treatment and a preventive vaccine. Seven major HCV genotypes and several subtypes have been identified throughout the world. Subtypes 1a/b account for approximately 70% of all infections in the USA, Europe, China, and Japan.[6] These subtypes are the hardest to treat.[7,8] The remainders are generally genotypes 2, 3, and 4.[9] Hepatitis C is a single-stranded RNA virus. Its genome encoded a single protein of about three thousands amino acids.[10,11] This protein is cleaved into smaller structural and nonstructural (NS) proteins by proteases like NS3/4A. Polymerases play a major role in viral replication.[12] The polymerase NS5B catalyzes the synthesis of a complementary negative-stranded RNA using the positive-stranded RNA genome as a template.[13–15] Direct acting antiviral agents that target specific steps in the virus replication cycle have been pursued by pharmaceutical companies to eradicate this virus.[16,17] Deleobuvir is a non-nucleoside, potent, and selective HCV NS5B polymerase inhibitor developed for the treatment of HCV infection.[18–21]

Results and discussion

Boehringer Ingelheim has been in the forefront in tackling the problem of HCV infections by inventing drug candidates that specifically inhibit certain proteases and polymerases involved in the replication of this virus. Previously, we reported the synthesis of several NS3/4A protease inhibitors labeled with radioactive and stable isotopes.[22] In this paper, we describe the synthesis of a potent inhibitor of NS5B polymerase labeled with carbon-13 and carbon-14 isotopes as well as its major metabolites (Figure 1).

Two major metabolites of deleobuvir were identified in plasma; a metabolite with a reduced double bond formed in the gastro-intestinal tract by gut bacteria (**2**), and an acyl glucuronide (**3**). The reduced double bond metabolite (**2**) was further metabolized to glucuronide (**4**).[23] The glucuronation is common for drug candidates containing carboxylic acid, hydroxyl, amino,

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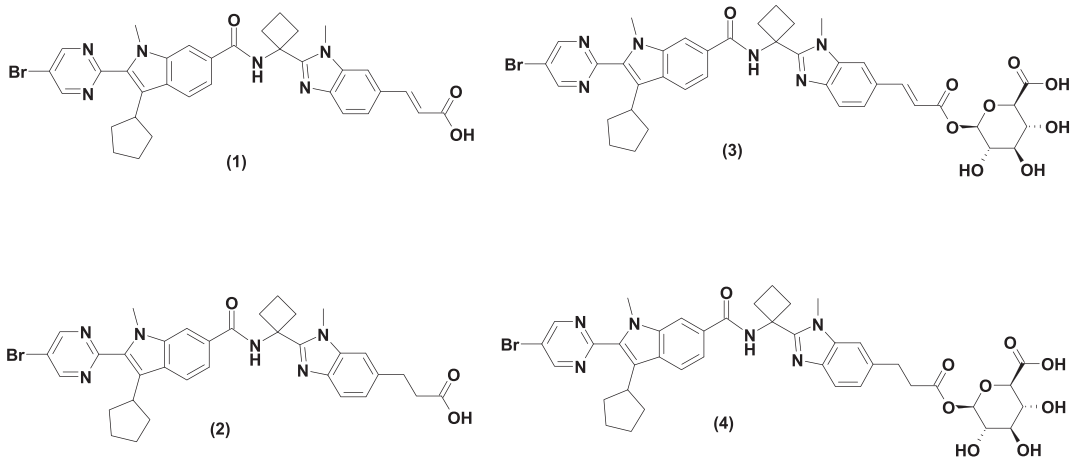


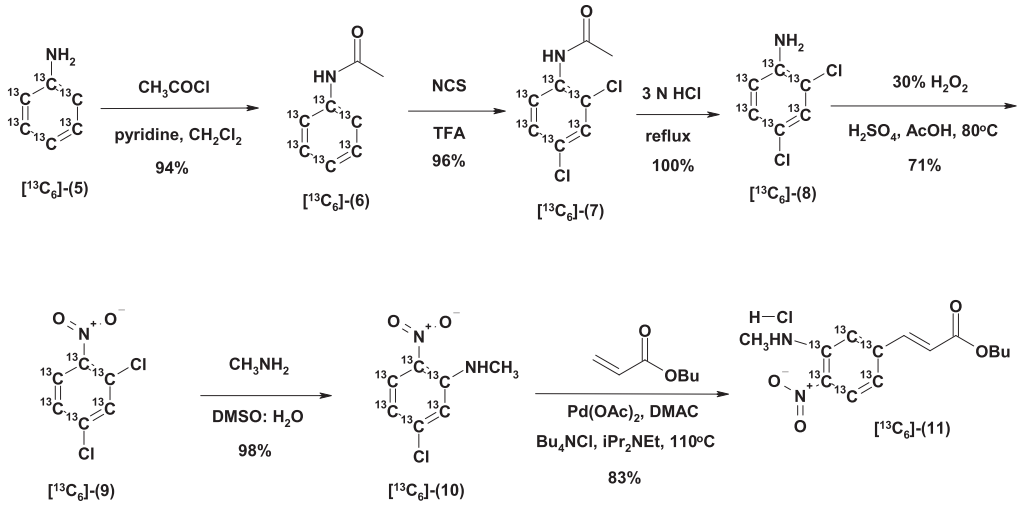
Figure 1. Structures of deleobuvir (**1**) and its metabolites (**2**), (**3**), and (**4**).

and sulfuryl moieties, and is usually considered a phase two metabolism process.[24] α,β-Unsaturated acids and ketones undergo enzymatic reduction of the carbon–carbon double bonds, and for certain compounds, for reasons that remain unclear, the reduction of the double bond is often a prerequisite for further metabolism.[25–27]

Synthesis of [$^{13}C_6$]-(1) and [$^{13}C_6$]-(2)

The synthesis of deleobuvir has been reported.[28] The commercially available 2,4-dichloronitrobenzene was used in the synthesis of the benzimidazole right-hand side of (**1**). This is a suitable region for introducing six ^{13}C atoms. However, carbon-13 labeled 2,4-dicholoronitrobenzene is not commercially available. From earlier work, we reported the preparation of 3,5-dichloroaniline-$^{13}C_6$ from aniline-$^{13}C_6$.[29] Similarly, we envisioned if we could oxidize the amino-moiety to a nitro group, we will have our starting material (Scheme 1). Direct chlorination of aniline with *N*-chlorosuccinimide (NCS) in chloroform gave 2,4-dichloroaniline in yields ranging from 40% to 50%.[30] Thus, aniline-$^{13}C_6$ was first protected as the acetanilide **[$^{13}C_6$]-(6)** in 94% using acetyl chloride and pyridine in methylene chloride.[31] Treatment of this acetanilide with NCS in trifluoroacetic acid (TFA) at room temperature gave 2,4-dicholoacetanilide **[$^{13}C_6$]-(7)** in 96% yield despite the fact that the reaction was slow and required four equivalents of NCS. Shorter reaction times were observed when we used freshly crystallized NCS. Removal of the acetyl group was accomplished in quantitative yield by refluxing in 2.5 N aqueous HCl. The 2,4-dichloroaniline **[$^{13}C_6$]-(8)** was isolated as a white solid, and the amino group was then oxidized to a nitro by heating with 30% hydrogen peroxide in acetic acid with traces of concentrated sulfuric acid at 80 °C for 14 h in 71% yield.[32] The oxidation step was necessary for the selective SN_{Ar} reaction with methylamine. The product 2,4-dichloro-nitrobenzene **[$^{13}C_6$]-(9)** was treated in DMSO with an aqueous 40% solution of methylamine to give 4-chloro-2-methylamino-nitrobenzene **[$^{13}C_6$]-(10)** in 98% yield. Ligandless Heck reaction with butyl acrylate gave (*E*)-(3-methylamino-4-nitrophenyl)acrylic acid butyl ester **[$^{13}C_6$]-(11)** in 83% yield,[28] (Scheme 1).

Reduction of the nitro group using tin dichloride gave the diamine derivative **[$^{13}C_6$]-(12)** in 62% yield. Condensation of this compound with 1-amino-cyclobutanecarbonyl chloride (**13**) in toluene and methanol at 90 °C gave the benzimidazole derivative **[$^{13}C_6$]-(14)** in 67% yield. The acid fragment (**15**), which is available from previous syntheses,[28] was treated with thionyl chloride at 0 °C in methylene chloride in the presence of triethylamine. The acyl-chloride (**16**) generated *in situ* was then



Scheme 1. Synthesis of [$^{13}C_6$]-(11).

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reacted with the amine fragment **[$^{13}C_6$]-(14)** and the butyl ester **[$^{13}C_6$]-(17)** was isolated in 84% yield. Hydrolysis in aqueous sodium hydroxide and *n*-propanol followed by flash chromatography purification, then crystallization from methanol-methylene chloride gave **[$^{13}C_6$]-(1)** as a white solid in quantitative yield (Scheme 2).

Our first attempts to prepare the metabolite **[$^{13}C_6$]-(2)** focused on using **[$^{13}C_6$]-(1)**. We devised a simple procedure that was tested successfully on the unlabeled butyl ester (**17**). The procedure used diimide generated *in situ* from potassium azodicarboxylic acid (PADA) and acetic acid in DMSO at ambient temperature.[33] The method is selective, and only the targeted double bond is reduced. However, direct reduction of acid (**1**) with PADA for extended periods of time resulted in no reduction of the double bond. Other direct reductions of the cinnamic double bond using nickel dichloride and sodium borohydride or samarium iodide in water failed to give (**2**).[34] With limited amounts of compound **[$^{13}C_6$]-(14)**, we turned our attention to **[$^{13}C_6$]-(11)**. The route described in Scheme 2 to prepare **[$^{13}C_6$]-(1)** was then followed to prepare the metabolite **[$^{13}C_6$]-(2)**. The only difference is the tandem reduction of the nitro group and the double bond with hydrogen in the presence of palladium on carbon. Compound **[$^{13}C_6$]-(2)** was synthesized in seven steps and in 21% overall yield from **[$^{13}C_6$]-(11)** (Scheme 3).

Synthesis of the acyl glucuronide derivatives [$^{13}C_6$]-(3) and [$^{13}C_6$]-(4)

To prepare the acyl glucuronide derivatives, the three-step procedure shown in Scheme 4 was used. The acid was first activated using HATU (1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate) in the presence of *N*-methylmorpholine (NMM), then reacted with allyl-D-glucuronate ((2*S*,3*S*,4*S*,5*R*,6*R*/*S*)-allyl-3,4,5,6-tetrahydroxytetrahydro-2*H*-pyran-2-carboxylate). Finally, removal of the allyl protecting group using palladium tetrakis triphenylphosphine gave the desired acyl glucuronide derivatives.[35–39] The products were isolated in most cases by reverse phase chromatography with yields ranging from 44% to 63%. Allyl-D-glucuronate is commercially available or can be easily prepared from glucuronic acid and allylbromide according to the literature.[40]

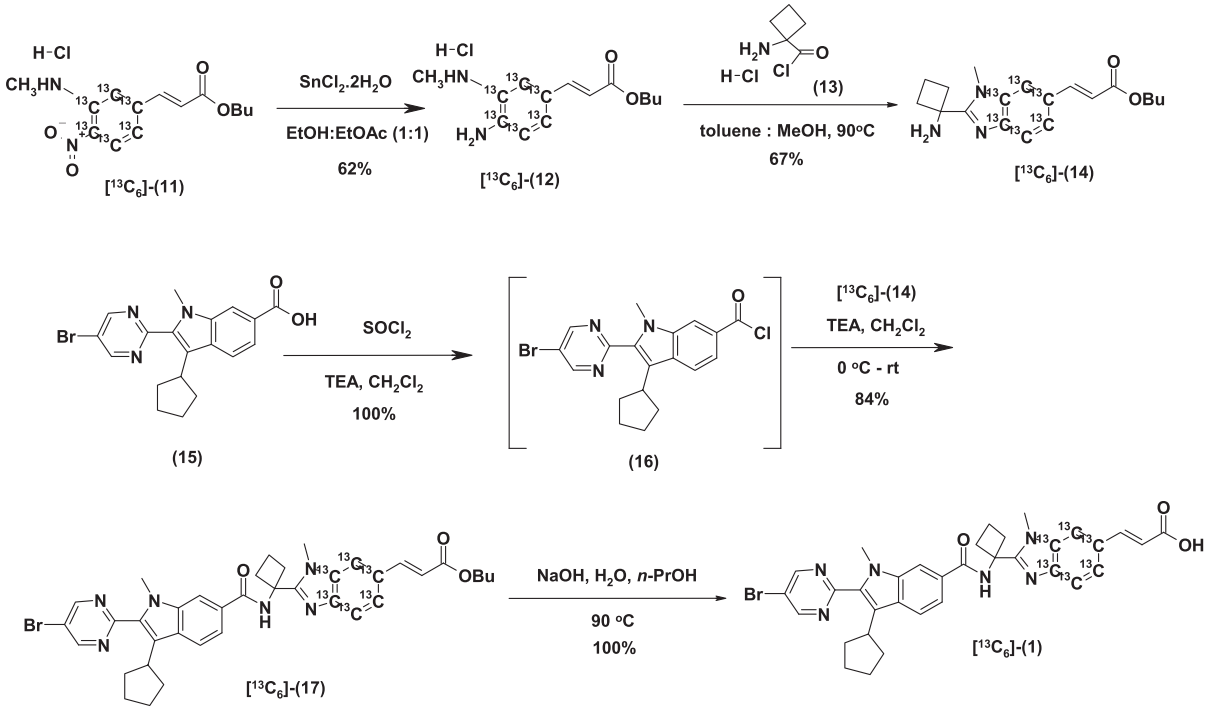
Synthesis of [^{14}C]-(1), [^{14}C]-(2), and [^{14}C]-(3)

The compound 1-amiocyclybutycarboxylic acid (**23**) seems to be a convenient intermediate that can be accessed in two steps using carbon-14 labeled potassium cyanide via the Bucherer–Bergs reaction.[41] Conversion of this amino acid to 1-amino-cyclobutane carbonyl chloride was accomplished using PCl_5 and 2-oxazolidone in acetonitrile,[28] (Scheme 5).

Carbon-14 labeled deleobuvir was then prepared in three steps as depicted in Scheme 6. The diamine derivative (**12**) which was obtained from the reduction of butyl ester (**11**) was condensed with the acyl chloride derivative **[^{14}C]-(13)** to give the amine **[^{14}C]-(14)** in 52% yield. Coupling to the acyl chloride (**16**), as seen before, gave the butyl ester **[^{14}C]-(17)**. Ester hydrolysis using aqueous NaOH in *n*-propanol gave **[^{14}C]-(1)** in 25% radiochemical overall yield and with a specific activity of 54.5 mCi/mmol. The chemical purity was 98.5% and a radiochemical purity of 98.7%. The sodium salt of this compound was easily prepared by a simple treatment with a solution of aqueous NaOH in THF and then crystallized from acetonitrile/THF.

The intermediate **[^{14}C]-(13)** was also used to prepare **[^{14}C]-(2)** as described in Scheme 7. This metabolite was obtained in 59% overall yield with a specific activity of 54.5 mCi/mmol and radiochemical purity of 98.8%.

The synthesis of the glucuronide **[^{14}C]-(3)** was accomplished as described before using **[^{14}C]-(1)** and allyl-D-glucuronate. The pure material was obtained after preparative HPLC purification with a specific activity of 52.3 mCi/mmol and radiochemical purity of 97.5%.



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

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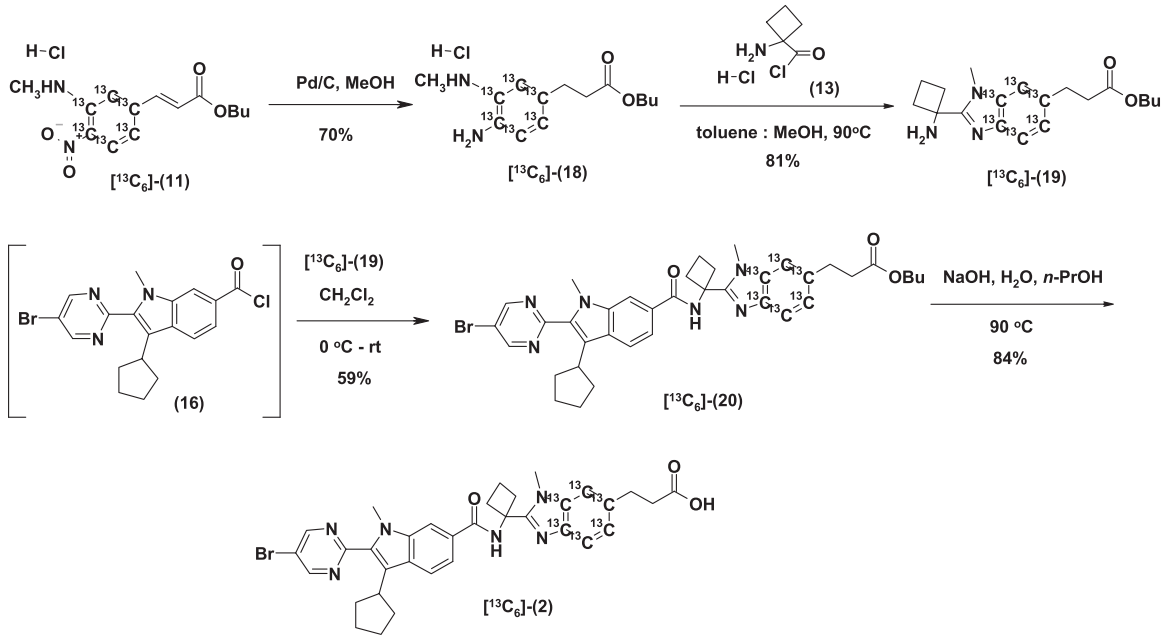
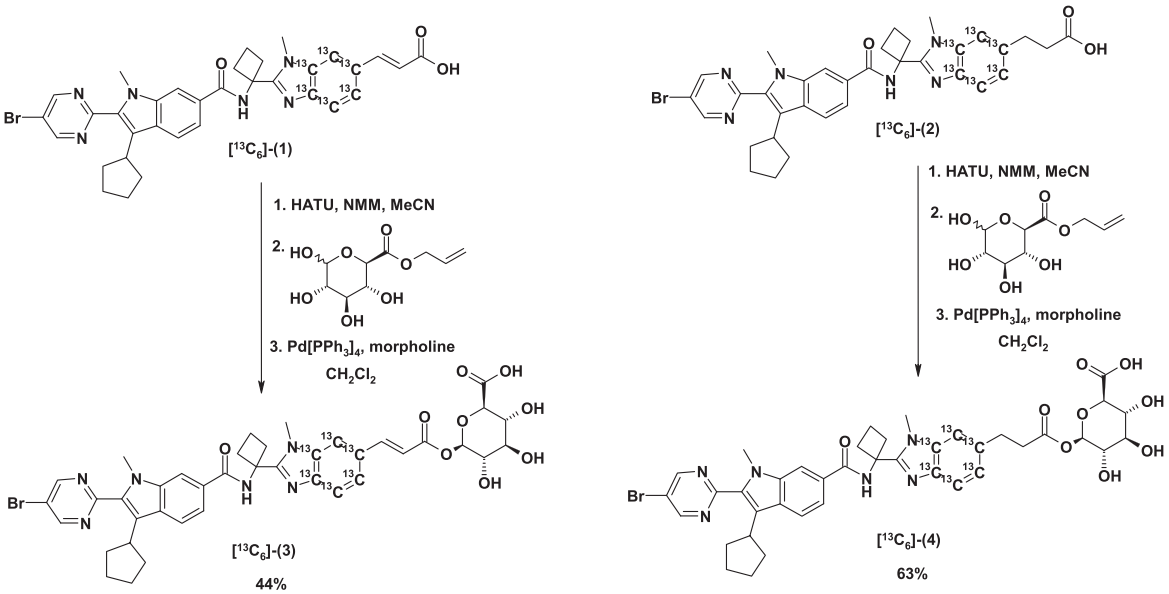
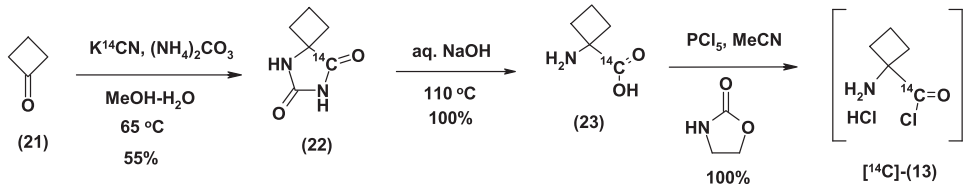


Scheme 3. Synthesis of [$^{13}C_6$]-(2).



Scheme 4. Synthesis of glucuronides [$^{13}C_6$]-(3) and [$^{13}C_6$]-(4).



Scheme 5. Synthesis of 1-aminocyclobutane-1-carbonyl chloride-^{14}C.

Conclusion

The carbon-13 labeled deleobuvir and its major metabolites were prepared starting from aniline-$^{13}C_6$. The preparation of the intermediate **[$^{13}C_6$]-(11)** in six steps and 52% overall yield allowed access to **[$^{13}C_6$]-(1)** and **[$^{13}C_6$]-(2)** in another five and four steps, respectively. The other metabolites **[$^{13}C_6$]-(3)** and **[$^{13}C_6$]-(4)** were then prepared in three steps from **[$^{13}C_6$]-(1)** and **[$^{13}C_6$]-(2)**. For carbon-14 synthesis, 1-amiocyclybutycarboxylic acid **[^{14}C]-(23)** was prepared in two

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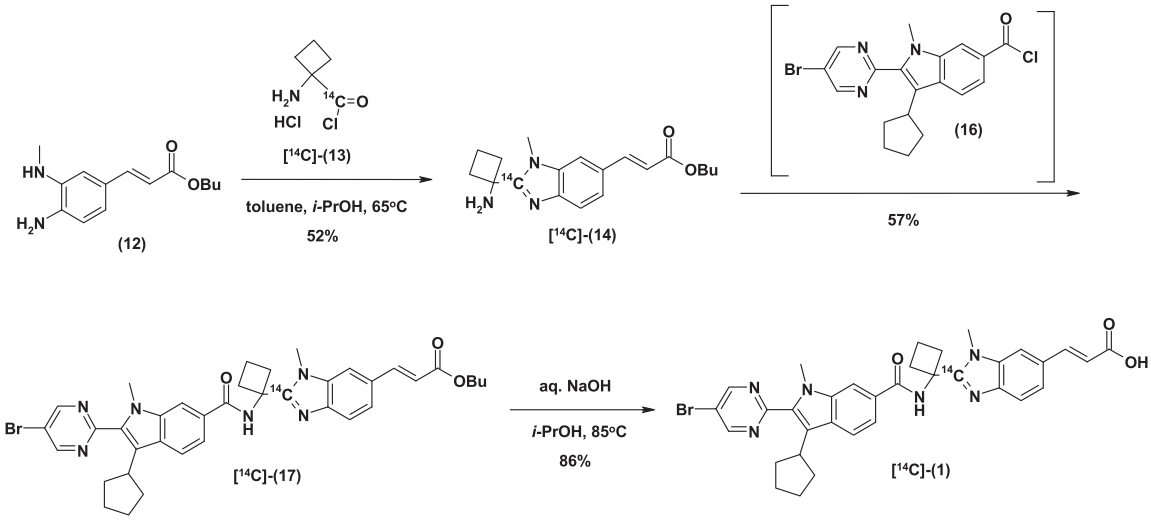
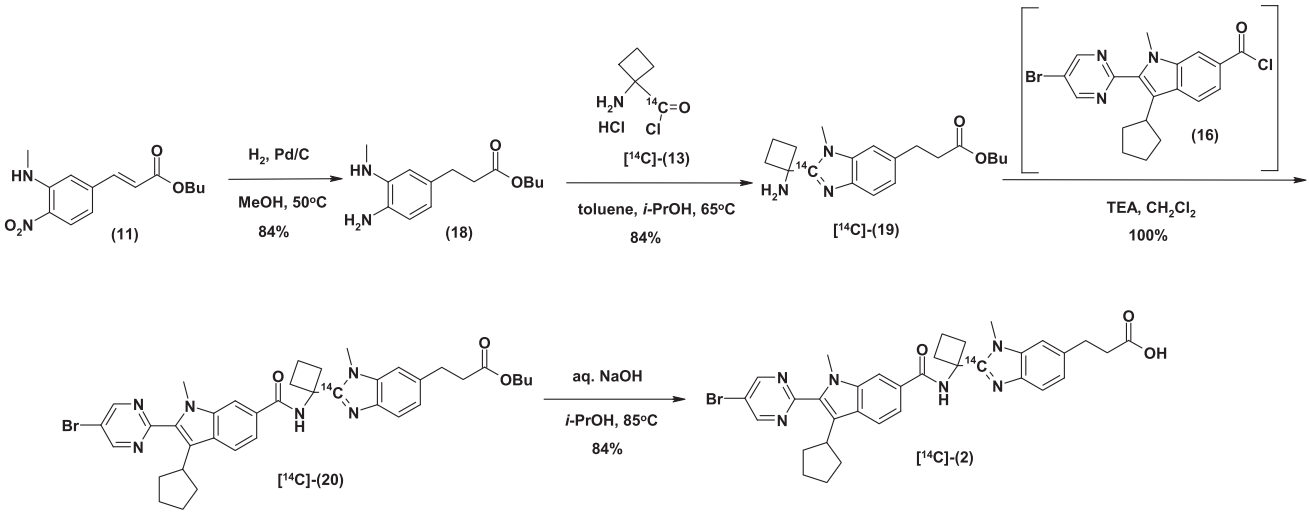


Scheme 6. Synthesis of [^{14}C]-(1).



Scheme 7. Synthesis of [^{14}C]-(2).

steps via Bucherer–Bergs reaction. Conversion of this amino acid to 1-amino-cyclobutane carbonyl chloride gave intermediate **[^{14}C]-(13)** which was used to prepare **[^{14}C]-(1)**, **[^{14}C]-(2)** in three radioactive steps. The metabolite **[^{14}C]-(3)** was prepared in a three-step synthesis starting from **[^{14}C]-(1)** and allyl-D-glucuronate.

Experimental

Materials and methods

The nuclear magnetic resonance (NMR) spectra of radioactive compounds were recorded on a Bruker 500 MHz spectrometer using double encapsulated NMR tubes in deuterated dimethyl sulfoxide. ^{1}H NMRs and ^{13}C NMR spectra of non-radioactive compounds were acquired in deuterated solvent using Bruker 400 MHz or Bruker Avance 600B spectrometers at 400.33 MHz and 600.04 MHz for ^{1}H NMR and 100.66 MHz and 150.88 MHz for ^{13}C NMR, respectively. Liquid scintillation counting was accomplished using a Beckman LS6500TA and UltimaGold™ cocktail (PerkinElmer, Boston, MA, USA). HPLC analysis was performed on an Agilent 1200 instrument. HPLC conditions: (a) mobile phase gradient 20% to 100% (MeCN/H_2O both 0.1% TFA) over 20 min, column: Eclipse XDB C18 (150 mm × 4.5 mm, 5 μm), injection volume 10 μL; (b) mobile phase gradient 75%A to 25%A in 25 min, A: 0.1% TFA in 95:5 water/methanol; B: 0.1% TFA in MeCN/methanol, column: Unison UK-C8 (150 mm × 4.6 mm, 3 μm). Liquid chromatography-mass spectrometry (LCMS) were acquired using either a fast medium polar method: run time 2.0 min, gradient 95% water (0.1% TFA) and 5% MeCN (0.1%TFA) to 5% water in 1.7 min, hold to 2 min at 5% water, flow 2.5 mL/min; column: Agilent Zorbax C18 SB (3.5 μm, 4.6 mm × 30 mm), or a long medium polar method, run time 9.0 min, gradient 95% water (0.1% TFA) and 5% MeCN (0.1% TFA) to 5% water in 7 min, hold to 9 min at 5% water, flow 1.5 mL/min; column: Agilent Zorbax Eclipse XDB-C8 (5 μm, 4.6 mm × 150 mm). The data were acquired on Waters Acquity™ Ultra Performance LC (Milford, MA, USA). The radiochemical purity was measured using a radio-HPLC detector β-Ram model 4 or model 3 (LabLogic systems, Inc. Brandon, FL, USA) connected to the Agilent instrument using IN-FLOW™ 2:1 liquid scintillation (LabLogic systems, Inc. Brandon, FL, USA). Carbon-14 potassium cyanide was obtained from Quotient Bioresearch (Cardiff, UK). Aniline-$^{13}C_6$ (99.4 atom % ^{13}C) was purchased from Isotec-Sigma-Aldrich (Miamisburg, OH, USA). The rest of the reagents were purchased from Sigma-Aldrich Company.

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

Acetanilide-$^{13}C_6$, [$^{13}C_6$]-(6): To a solution of aniline-$^{13}C_6$ (4.0 g, 40 mmol) in methylene chloride (50 mL), was added pyridine

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(6 mL). Acetyl chloride (3.13 mL, 44 mmol) was then added dropwise at 0 °C under nitrogen atmosphere. The reaction was warmed slowly to room temperature and stirred for 14 h. Methylene chloride (50 mL) was added and the reaction was washed with aqueous 2 N HCl (100 mL), water (100 mL), brine (100 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo* to give 5.4 g of an orange solid in 94% yield. $R_f = 0.14$ in 50% EtOAc : Hexane. ^{1}H NMR ($CDCl_3$) δ: 7.49 (m, $J_{C-H} = 167.2$ Hz, 2H), 7.30 (m, $J_{C-H} = 335.1$ Hz, 1H), 7.29 (m, $J_{C-H} = 159.2$ Hz, 2H), 2.15 (s, 3H). ^{13}C NMR ($CDCl_3$) δ: 168.68, 137.98 (dt, $J = 9.7$, 63.4 Hz), 128.94 (dt, $J = 6.8$, 56.2 Hz), 124.22 (dt, $J = 9.7$, 55.6 Hz), 119.97 (dt, $J = 6.2$, 55.9 Hz), 24.51. LCMS: ES$^+$ *m/z*: $MH^+ = 142.11$, 100%.

2,4-Dichloroacetanilide-$^{13}C_6$, [$^{13}C_6$]-(7): A solution of the aforementioned acetanilide (5.35 g, 38 mmol) in TFA (120 mL) was treated at room temperature with NCS (22 g, 165 mmol). The resulting solution was stirred until completion of the reaction in 5 days. The solution was poured into an aqueous cold solution of NaOH. The pH was adjusted to 9 by the addition of aqueous 4 N NaOH. The resulting precipitate was filtered off and washed with water. Purification by flash chromatography using 20% EtOAc : CH_2Cl_2 as eluent gave 7.7 g of an off-white solid in 96% yield. $R_f = 0.4$ in 50% EtOAc : Hexanes. ^{1}H NMR ($CDCl_3$) δ: 8.25 (m, $J_{C-H} = 168.6$ Hz, 1H), 7.66 (brs, 1H), 7.34 (m, $J_{C-H} = 164.7$ Hz, 1H), 7.20 (m, $J_{C-H} = 164.5$ Hz, 1H), 2.2 (s, 3H). ^{13}C NMR ($CDCl_3$) δ: 168.40, 133.68 (m), 127.13–129.84 (m), 121.97–125.22 (m), 124.75. LCMS: ES$^+$ *m/z*: $MH^+ = 209.96$, 100%.

2,4-Dichloroaniline-$^{13}C_6$, [$^{13}C_6$]-(8): A mixture of **[$^{13}C_6$]-(7)** (2.1 g, 10 mmol) in 2.5 N aqueous HCl (80 mL) was heated to reflux for 3 h. After cooling to room temperature, the solution was poured into a saturated aqueous solution of $NaHCO_3$ (100 mL). The solution was made basic to pH = 8 by adding more $NaHCO_3$ solution, and the resulting white solid was filtered off, washed with water, and dried *in vacuo* to give 1.93 g of material in quantitative yield. $R_f = 0.22$ in 20% EtOAc : Hexanes. ^{1}H NMR ($CDCl_3$) δ: 7.24 (m, $J_{C-H} = 167.6$ Hz,1H), 7.02 (m, $J_{C-H} = 164.3$ Hz, 1H), 6.69 (m, $J_{C-H} = 160.6$ Hz, 1H), 4.01 (brs, 2H). ^{13}C NMR ($CDCl_3$) δ: 141.0 (m), 128.32 (m), 127.07 (m), 122.77 (m), 118.91 (m), 115.73 (m). LCMS: ES$^+$ *m/z*: $MH^+ = 168.11$, 50%; $(MH + MeCN)^+ = 208.91$, 100%.

2,4-Dichloro-1-nitrobenzene-$^{13}C_6$, [$^{13}C_6$]-(9): To a solution of **[$^{13}C_6$]-(8)** (1.4 g, 8.2 mmol) in acetic acid (33 mL), was added a solution of 30% hydrogen peroxide (11 mL), followed by a concentrated solution of sulfuric acid (0.7 mL). The resulting solution was heated at 80 °C and stirred for 14 h. After cooling to room temperature, the reaction was poured into water (150 mL) and the resulting precipitate was extracted with ethyl acetate (100 mL ×3). The combined extracts were washed with a saturated solution of $NaHCO_3$ (50 mL), water (50 mL), and brine (50 mL), dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give 1.15 g of orange oil in 71% yield, which solidified upon standing at room temperature; $R_f = 0.55$ in 30% EtOAc : Hexanes. ^{1}H NMR ($CDCl_3$) δ: 7.86 (m, $J_{C-H} = 167.6$ Hz, 1H), 7.57 (m, $J_{C-H} = 164.3$ Hz, 1H), 7.39 (m, $J_{C-H} = 160.6$ Hz, 1H). ^{13}C NMR ($CDCl_3$) δ: 145.41 (m), 138.50 (m), 130.90 (m), 128.13 (m), 127.11 (m), 125.76 (m).

4-Chloro-2-methylamino-nitrobenzene-$^{13}C_6$, [$^{13}C_6$]-(10): To a solution of **[$^{13}C_6$]-(9)** (1.1 g, 5.7 mmol) in DMSO (10 mL), was added a solution of methylamine (2.5 mL, 40% solution in water). The resulting solution was stirred for 3 h at room temperature to give a precipitate. Water (30 mL) was added and the mixture was extracted with ethyl acetate (50 mL ×3). The combined extracts were washed with water (30 mL), brine (30 mL), dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The residue was purified by silica gel chromatography using a gradient 5–50% EtOAc in hexanes to give an orange solid, mp = 101–102 °C in 98% yield. $R_f = 0.54$ in 30% EtOAc : Hexanes or $R_f = 0.53$ in 100% CH_2Cl_2. ^{1}H NMR ($CDCl_3$) δ: 8.30 (brs, 1H, NH), 8.10 (m, $J_{C-H} = 165.7$ Hz, 1H), 6.83 (m, $J_{C-H} = 165.8$ Hz, 1H), 6.62 (m, $J_{C-H} = 170.1$ Hz, 1H), 3.02 (t, $J = 4.3$ Hz, 3H). ^{13}C NMR ($CDCl_3$) δ: 146.71 (t, $J = 66.1$ Hz), 142.92 (m), 130.63 (m), 128.20 (m), 115.77 (t, $J = 60.4$ Hz), 112.84 (m), 29.81. LCMS, ES$^+$ *m/z*: $MH^+ = 192.99$, 100%.

Butyl (*E*)-3-(3-methylamino-4-nitrophenyl-$^{13}C_6$)acrylate, [$^{13}C_6$]-(11): Argon was bubbled in a mixture of **[$^{13}C_6$]-(10)** (0.94 g, 4.9 mmol), tetrabutyl ammonium chloride (2.7 g, 10 mmol), Hünig's base (1.7 mL, 10 mmol) and butyl acrylate (1.4 mL, 10 mmol) in dimethyl acetamide (15 mL) for 10 min. Palladium acetate (110 mg, 0.5 mmol) was then added, and the mixture was heated to 110 °C for 8 h. The dark mixture was cooled to 50 °C, and 1-methylimidazole (0.78 mL, 10 mmol) was added, and the reaction was stirred for 1 h at this temperature. After cooling to room temperature, the mixture was filtered through a short pad of Celite® and washed with ethyl acetate (50 mL). The filtrate was poured into water (20 mL). The organic phase was removed and washed again with water (15 mL), brine (15 mL), dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The residue was purified by silica gel chromatography using 1–25% EtOAc : Hexanes to give 166 mg of starting material and 950 mg of product in 83% yield based on reacted chloride. $R_f = 0.3$ in 20 EtOAc : Hexanes as an orange solid, mp = 85–86 °C. ^{1}H NMR ($CDCl_3$) δ: 8.17 (dq, $J_{H-H} = 7.4$, $J_{13C-H} = 165.1$ Hz, 1H), 8.09 (brs, 1H, NH), 7.61 (m, 1H), 6.90 (dt, $J = 7.4$, 160.2 Hz, 1H), 6.84 (dq, $J = 7.4$, 165.4 Hz, 1H), 6.52 (dd, $J = 5.4$, 16.0 Hz, 1H), 4.23 (t, $J = 6.7$ Hz, 2H), 3.06 (t, $J = 4.3$ Hz, 3H), 1.70 (dt, $J = 6.8$, 7.8 Hz, 2H), 1.44 (dt, $J = 7.4$, 7.8 Hz, 2H), 0.97 (t, $J = 7.4$ Hz, 3H). ^{13}C NMR ($CDCl_3$) δ: 164.50, 144.40 (m), 139.89 (m), 130.20 (m), 125.50 (m), 123.94 (m), 114.87 (m), 11.81 (m), 111.2 (m), 62.97, 28.83, 27.90, 17.30, 11.86.

Butyl (*E*)-3-(4-amino-3-methylaminophenyl-$^{13}C_6$)acrylate, [$^{13}C_6$]-(12): A mixture of **[$^{13}C_6$]-(11)** (0.92 g, 3.6 mmol) and tin chloride dihydrate (3.7 g, 16.4 mmol) in ethyl acetate (20 mL) and ethanol (20 mL) was heated to reflux for 4 h. After cooling to room temperature, the solution was poured into an aqueous solution of NaOH (1.0 N, 100 mL) and extracted with ethyl acetate (100 mL ×2). The combined extracts were dried over $MgSO_4$, filtered and concentrated *in vacuo* to give 0.85 g of dark residue. Purification by silica gel chromatography using 1–20% EtOAc in methylene chloride gave 0.5 g of product in 62% yield; $R_f = 0.29$ in 20% EtOAc : CH_2Cl_2. ^{1}H NMR ($CDCl_3$) δ: 7.54 (dtd, $J = 1.5$, 5.5, 15.9 Hz, 1H), 6.79 (ddd, $J = 7.9$, 13.9, 158.6 Hz, 1H), 6.73 (dt, $J = 6.5$, 154.7 Hz, 1H), 6.57 (ddd, $J = 7.6$, 14.6, 154.7 Hz, 1H), 6.25 (dd, $J = 5.1$, 15.9 Hz, 1H), 4.11 (t, $J = 6.7$ Hz, 2H), 3.50 (brs, 2H), 2.78 (d, $J = 3.4$ Hz, 3H), 1.60 (dt, $J = 6.9$, 13.9 Hz, 2H), 1.35 (dt, $J = 7.4$, 15.1 Hz, 2H), 0.89 (t, $J = 7.4$ Hz, 3H). ^{13}C NMR ($CDCl_3$) δ: 166.36 (d, $J = 7.01$ Hz), 144.24 (d, $J = 56.3$ Hz), 142.91 (m), 125.14 (m), 119.17 (m), 113.84 (m), 108.70 (m), 62.57, 51.91, 29.32, 17.69, 12.23. LCMS, ES$^+$ *m/z*: $MH^+ = 255.61$ (100%).

Butyl (*E*)-3-(2-(1-Aminocyclobutyl)-1-methyl-1*H*-benzo[*d*]imidazole-6-yl-3a,4,5,6,7,7a-$^{13}C_6$)acrylate, [$^{13}C_6$]-(14): A mixture of **[$^{13}C_6$]-(12)** (408 mg, 1.6 mmol) and 1-amino-cyclobutane

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carbonyl chloride hydrochloride salt (**13**) (0.5 g, 2 mmol, 70 wt.%) in toluene (10 mL) was stirred at room temperature for 30 min to give a clear solution. The reaction was then heated to 90 °C and stirred for 2 h. A slurry was obtained to which methanol (4 mL) was added, and the heating was continued for 8 h. After cooling to room temperature, the reaction mixture was concentrated under reduced pressure, and the resulting solid was treated with ethyl acetate and a saturated solution of aqueous $NaHCO_3$ (100 mL). The organic phase was removed and then washed with water (50 mL), brine (30 mL), dried ($MgSO_4$), filtered, and concentrated *in vacuo* to give 0.5 g of product. Purification using a 40 g silica gel column and a gradient 10–50% EtOAc : CH_2Cl_2 gave 0.36 g of product in 67% yield; $R_f = 0.31$ in 15% MeOH/CH_2Cl_2. ^{1}H NMR ($CDCl_3$) δ: 7.82 (m, 1H), 7.75 (m, $J_{C-H} = 155.5$ Hz, 1H), 7.65 (m, 1H), 7.28 (m, 1H), 6.47 (dd, $J = 5.2$, 15.9 Hz, 1H), 4.22 (t, $J = 6.7$ Hz, 2H), 3.89 (d, $J = 2.8$ Hz, 3H), 3.01 (m, 2H), 2.27 (m, 2H), 2.04 (m, 2H), 1.85 (brs, 2H), 1.70 (dt, $J = 6.7$, 13.9 Hz, 2H), 1.45 (dt, $J = 7.5$, 15.0 Hz, 2H), 0.97 (t, $J = 7.4$ Hz, 3H). ^{13}C NMR ($CDCl_3$) δ: 167.43, 143.60 (m), 137.28 (m), 129.22 (m), 125.14 (m), 122.08 (m), 120.47 (m), 110.98 (m), 109.24 (m), 64.36, 55.95, 36.44. 31.27, 30.83, 19.24, 14.15, 13.78. LCMS: ES$^+$ *m/z*: $MH^+ = 334.65$ (100%).

Butyl (*E*)-3-(2-(1-(2-(5-bromo-pyrimidin-2-yl)-3-cyclopentyl-1-methyl-1*H*-indole-6-carboxamido)cyclobutyl)-1-methyl-1*H*-benzo[*d*]imidazol-6-yl-3a,4,5,6,7,7a-$^{13}C_6$)acrylate, [$^{13}C_6$]-(17): To a suspension of (**15**) (410 mg, 1 mmol) in methylene chloride (15 mL) was added thionyl chloride (0.1 mL, 1.4 mmol) at 0 °C under nitrogen atmosphere. After stirring for 15 min, triethylamine (0.43 mL, 3 mmol) was added dropwise to give a dark solution, which was stirred at 0 °C for 2 h and 30 min. The amine **[$^{13}C_6$]-(14)** (340 mg, 1 mmol) in methylene chloride (8 mL) was then added. After stirring at 0 °C for 1 h, the ice bath was removed, and the reaction was warmed to room temperature and stirred for 16 h. The reaction was poured into a saturated solution of Na_2CO_3 (50 mL) and extracted with ethyl acetate (50 mL ×3). The combined extracts were dried ($MgSO_4$), filtered, and concentrated *in vacuo* to give 0.8 g of a solid. Purification by silica gel chromatography using 10–50% EtOAc in CH_2Cl_2 gave 614 mg of product in 84% yield as a white solid with 98% chemical purity. $R_f = 0.17$ in 40% EtOAc : CH_2Cl_2. ^{1}H NMR ($CDCl_3$) δ: 8.93 (brs, 2H), 7.95 (s, 1H), 7.93 (m, 0.5 H), 7.80 (m, 1H), 7.7 (d, $J = 8.5$ Hz, 1H), 7.65 (m, 1H), 7.53 (m, 0.5H), 7.42 (dd, $J = 1.3$, 8.5 Hz, 1H), 7.32 (m, 1H), 7.01 (m, 1H), 6.47 (dd, $J = 5.2$, 15.9 Hz, 1H), 4.21 (t, $J = 6.7$ Hz, 2H), 3.97 (d, $J = 2.8$ Hz, 3H), 3.83 (s, 3H), 3.72 (m, 1H), 3.11 (m, 2H), 2.92 (m, 2H), 2.30 (m, 1H), 1.85–2.15 (m, 8H), 1.62 (m, 4H), 1.62 (brs, NH), 1.44 (m, 2H), 0.97 (t, $J = 7.4$ Hz, 3H). ^{13}C NMR ($CDCl_3$) δ:167.45, 157.70, 143.79 (m), 136.80 (m), 129.17 (m), 123.38, 122.21 (m), 121.28, 119.89 (t), 116.63, 110.60, 109.61 (m), 64.35, 56.33, 36.80, 33.63, 33.34, 31.91, 31.34, 30.83, 26.70, 19.24, 16.25, 13.78. LCMS ES$^+$ *m/z*: $MH^+ = 717.11$ (100%).

(*E*)-3-(2-(1-(2-(5-Bromopyrimidin-2-yl)-3-cyclopentyl-1-methyl-1*H*-indole-6-carboxamido)-cyclobutyl)-1-methyl-1*H*-benzo[*d*]imidazol-6-yl-3a,4,5,6,7,7a-$^{13}C_6$)acrylic acid, [$^{13}C_6$]-(1): A mixture of **[$^{13}C_6$]-(17)** (400 mg, 0.6 mmol) in *n*-propanol (11 mL), water (11 mL), and a 50% solution of NaOH (1 mL) was heated at 90 °C for 4 h. The reaction was concentrated *in vacuo* and the residue was treated with ethyl acetate (50 mL) and water (50 mL). The aqueous phase was extracted with ethyl acetate (50 mL), and the combined extracts were dried ($MgSO_4$), filtered, and concentrated *in vacuo* to give 430 mg of an off-white solid. Purification by silica gel chromatography and using up to 10% MeOH in methylene chloride gave 359 mg of a pale yellow solid in quantitative yield. The solid (200 mg) was further crystallized from 2 mL of 10% methanol/methylene chloride to give 160 mg of a white solid; $R_f = 0.1$ in 10% MeOH/CH_2Cl_2, mp 232 ° C decomposed. ^{1}H NMR (MeOH-d_4) δ: 9.07 (s, 2H), 8.04 (d, $J = 1.2$ Hz, 1H), 7.91 (m, 1H), 7.81 (d, $J = 8.43$ Hz, 1H), 7.73–7.85 (m, 1.5H), 7.59 (dd, $J = 1.5$, 8.4 Hz, 1H), 7.51 (m, 1H), 7.36 (m, 0.5H), 6.53 (dd, $J = 5.2$, 15.9 Hz, 1H), 3.88 (s, 3H), 3.87 (d, $J = 2.9$ Hz, 3H), 3.77 (quin, $J = 8.9$ Hz, 1H), 3.11 (m, 2H), 2.83 (m, 2H), 2.02–2.28 (m, 2H), 1.9–2.02 (m, 6H), 1.73 (m, 2H). ^{13}C NMR (DMSO-d_6) δ: 167.86, 165.98, 157.98, 145.44, 144.91, 143.34 (m), 137.29 (m), 135.05, 128.31, 127.46 (m), 127.03, 123.9 (t, $J = 55.1$ Hz), 121.93 (m), 120.22, 118.29 (m), 117.02, 111.97, 11.31, 110.39, 109.93 (m), 55.24, 54.88, 36.27, 32.77, 31.73, 31.03, 26.02, 14.98. LCMS, fast medium polar, run time 1.75 min, $R_t = 0.96$ min, 659.21:661.20 (1:1). HRMS: calculated 659.20716 (100%), 660.21051 (30%), 661.20511 (100%), 662.20847 (30%), 663.21182 (5%); found 659.20750 (100%), 660.21121 (30%), 661.20547 (100%), 662.20919 (30%), 663.21307 (5%). HPLC[a]: $R_t = 11.52$ min (99.74%).

Synthesis of [$^{13}C_6$]-(2)

Butyl 3-(4-amino-3-(methylamino)phenyl-$^{13}C_6$)-propanoate, [$^{13}C_6$]-(18): The cinnamic acid derivative **[$^{13}C_6$]-(11)** (0.37 g, 1.3 mmol) was divided into two tubes each containing 185 mg of Pd/C and 6 mL of methanol and stirred under 200 PSI of hydrogen at 50 °C for 20 h. The mixtures were filtered, combined and concentrated *in vacuo* to give 290 mg of viscous oil. Purification using a 12 g disposable column and 20–30% EtOAc : CH_2Cl_2 gave 236 mg in 70% yield. LCMS: ES$^+$ *m/z*: $MH^+ = 257.68$ (100%). ^{1}H NMR ($CDCl_3$) δ: 6.62 (dm, $J = 152.1$ Hz, 1H), 6.60 (dm, $J = 153.9$ Hz, 1H), 6.46 (dd, $J = 6.2$, 152.9 Hz, 1H), 4.06 (t, $J = 6.7$ Hz, 2H), 3.42 (s, 3H), 2.85 (m, 2H), 2.82 (d, $J = 7.9$ Hz, 3H), 2.59 (m, 2H), 1.58 (m, 2H), 1.36 (m, 2H), 0.91 (t, $J = 7.4$ Hz, 3H). ^{13}CNMR ($CDCl_3$) δ: 173.44, 139.18 (t, $J = 65.6$ Hz), 133.11 (t, $J = 56.5$ Hz), 132.11 (t, $J = 64.6$ Hz), 117.71 (t, $J = 58.8$ Hz), 116.32 (t, $J = 59.3$ Hz), 110.82 (t, $J = 66.4$ Hz), 64.49, 38.58, 32.22, 31.72, 30.12, 19.17, 14.28.

Butyl 3-(2-(1-aminocyclobutyl)-3-methyl-1*H*-benzo[*d*]]imidazol-6-yl-3a,4,5,6,7,7a-$^{13}C_6$)-propanoate, [$^{13}C_6$]-(19): To a mixture of (**23**) (182 mg, 1.2 mmol) in anhydrous MeCN (5 mL) were added 2-oxazolidone (46 mg, 0.5 mmol) followed by phosphorus pentachloride (250 mg, 1.2 mmol) and stirred for 2 h. The aforementioned diamino-derivative **[$^{13}C_6$]-(18)** (234 mg, 0.9 mmol) was added in MeCN (4 mL) and the resulting mixture was stirred for 5 h at room temperature. Toluene (2 mL) was added, and the mixture was heated to 60 °C and stirred overnight to give a homogeneous solution. Isopropanol (4 mL) was added, and the solution was stirred at 60 °C for another 4 h. After cooling to room temperature, the solution was concentrated *in vacuo* to half its volume then diluted with EtOAc (20 mL) and washed with a saturated solution of $NaHCO_3$ (20 mL), brine (15 mL), dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give 380 mg of residue. Purification using a 40 g disposable silica gel column and up to 10% MeOH in methylene chloride gave 188 mg of product in 81% yield. LCMS, ESI$^+$, *m/z*: $MH^+ = 336.79$ (100%). ^{1}H NMR ($CDCl_3$) δ: 7.67 (dm, $J = 159.5$ Hz, 1H), 7.15 (dm, $J = 157.9$ Hz, 1H),

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7.10 (dm, J = 155.4 Hz, 1H), 4.07 (t, J = 6.7 Hz, 2H), 3.84 (d, J = 2.8 Hz, 2H), 3.48 (brs, 3H), 3.09 (m, 2H), 2.97 (m, 2H), 2.69 (m, 2H), 2.25 (m, 2H), 1.85 (m, 2H), 1.58 (m, 2H), 1.33 (m, 2H), 1.30 (m, 2H), 1.21 (d, J = 6.27 Hz, 2H), 0.89 (t, J = 7.4 Hz, 3H).

Butyl 3-(2-(1-(2-(5-bromopyrimidin-2-yl)-3-cyclopentyl-1-methyl-1*H*-indole-6-carboxamido)cyclobutyl)-1-methyl-1*H*-benzo[*d*]imidazol-6-yl-3a,4,5,6,7,7a-$^{13}C_6$)-propanoate, [$^{13}C_6$]-(20): To a mixture of the acid (**15**) (358 mg, 0.9 mmol) in CH_2Cl_2 (7 mL) in an ice bath under nitrogen, was added thionyl chloride (90 μL, 1.2 mmol). After stirring for 15 min, triethylamine (446 μL, 3.2 mmol) was added, and the resulting dark mixture was stirred for 2 h. The aforementioned amine derivative **[$^{13}C_6$]-(19)** (0.3 g, 0.9 mmol) was then added in CH_2Cl_2 (4 mL). The flask was rinsed with another 4 mL of CH_2Cl_2 and added to the reaction and stirred for 2 h at 0 °C, and then, the ice bath was removed and the reaction was stirred at room temperature for 14 h. LCMS showed no starting material. Most of the solvent was removed *in vacuo* and the residue was dissolved in EtOAc (20 mL) and washed with a saturated solution of Na_2CO_3 (15 mL), brine (15 mL), dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give 0.65 g of an off-white foam. Purification on a 40 g disposable silica gel column and using 100% CH_2Cl_2 to 50% EtOAc : CH_2Cl_2 gave 378 mg in 59% yield. ^{1}H NMR ($CDCl_3$) δ: 8.90 (s, 1H), 8.06 (s, 1H), 7.92 (s, 1H), 7.73 (d, J = 8.5 Hz, 1H), 7.62 (m, 1H), 7.54 (d, J = 9.6 Hz, 1H), 7.10 (m, 1H), 4.06 (t, J = 6.7 Hz, 2H), 3.92 (d, J = 2.7 Hz, 3H), 3.71 (brs, 4H), 3.45 (s, 3H), 3.05 (m, H), 2.98 (t, J = 7.6 Hz, 2H), 2.65 (m, 2H), 2.24 (brs, 1H), 2.22 (m, 2H), 1.81–1.99 (m, 8H), 1.70 (m, 2H), 1.57 (m, 2H), 1.33 (m, 2H), 0.88 (t, J = 7.4 Hz, 3H). ^{13}C NMR ($CDCl_3$) δ: 173.11, 167.73, 158.75, 157.06, 140.25 (t, J = 62.01 Hz), 136.51 (t. J = 58.72 Hz), 135.34 (t, J = 56.33), 122.77 (t, J = 58.36 Hz), 119.07 (t, J = 63.9 Hz), 108.85 (t, J = 64.32 Hz), 64.38, 56.83, 50.50, 36.92, 36.67, 33.80, 32.37, 31.73, 30.65, 29.70, 26.67, 22.85, 19.11, 16.39, 13.70. LCMS long medium polar method: R_t = 9.38 min, m/z: MH^+ = 720.96 (100%).

3-(2-(1-(2-(5-Bromopyrimidin-2-yl)-3-cyclopentyl-1-methyl-1*H*-indole-6-carboxamido)cyclobutyl)-1-methyl-1*H*-benzo[*d*]imidazol-6-yl-3a,4,5,6,7,7a-$^{13}C_6$)propanoic acid, [$^{13}C_6$]-(2): To a solution of **[$^{13}C_6$]-(20)** (157 mg, 0.2 mmol) in methanol (5 mL), was added a solution of 2 N NaOH (1 mL) dropwise. The resulting mixture was stirred at room temperature for 14 h. The reaction was treated with acetic acid (0.2 mL) and concentrated *in vacuo* to remove most of the methanol. The residue was dissolved in water (10 mL) and extracted with ethyl acetate (20 mL ×2). The combined ethyl acetate extracts were dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue of 160 mg was purified using a 12 g disposable silica gel column and up to 10% MeOH/CH_2Cl_2. The fractions containing the pure material were combined and concentrated, then crystallized from hot methanol to give 130 mg of an off-white product in 84% yield. R_f = 0.6 in 20% MeOH/CH_2Cl_2. HPLC: R_t = 12.14 min (99%). ^{1}H NMR (DMSO-d_6) δ: 9.25 (s, 2H), 9.20 (s, 1H), 8.18 (s, 1H), 7.78 (d, J = 8.4 Hz, 1H), 7.65 (m, 1H), 7.55 (m, 1H), 7.30 (m, 1H), 7.15 (m, 1H), 3.92 (s, 3H), 5.75 (m, 4H), 3.21 (s, 2H), 2.90–3.10 (m, 4H), 2.75 (t, J = 8.1 Hz, 2H), 2.61 (m, 2H), 2.09 (m, 1H), 1.90 (m, 7H). ^{13}C NMR (DMSO-d_6) δ: 173.82, 165.88, 157.97, 141.55 (m), 136.95 (m), 134.95 (m), 128.50, 126.96, 121.72 (t, J = 55.3 Hz), 118.98, 118.39 (m), 110.32, 108.85 (m), 55.15, 48.56, 38.84, 36.25, 32.77, 31.72, 31.06, 30.81, 26.02, and 14.99. LCMS, long medium polar method: R_t = 8.35 min; m/z: 661.53:663.54 (1:1). LCMS fast medium polar method: R_t = 0.87 min. HRMS: calculated 661.22281 (100%), 662.22616 (30%), 663.22076 (100%), 664.22412 (30%), 665.22747 (5%); found 661.22283 (100%), 662.22655 (30%), 663.22087 (100%), 664.22458 (30%), 665.22807 (5%).

Synthesis of acyl glucuronides [$^{13}C_6$]-(3) and [$^{13}C_6$]-(4), Scheme 5

(2*R*,3*R*,4*R*,5*S*,6*R*)-6-{(*E*)-3-[2-(1-{[2-(5-Bromo-pyrimidin-2-yl)-3-cyclopentyl-1-methyl-1*H*-indole-6-carboxamido]}-cyclobutyl)-1-methyl-1*H*-benzo[*d*]imidazol-6-yl-3a,4,5,6,7,7a-$^{13}C_6$]-acryloyloxy}-3,4,5-trihydroxy-tetrahydro-pyran-2-carboxylic acid, [$^{13}C_6$]-(3): To a solution of **[$^{13}C_6$]-(1)** (145 mg, 0.22 mmol) and HATU (100 mg, 0.26 mmol) in anhydrous DMF (2 mL), was added NMM (30 μL, 0.27 mmol) at room temperature. The solution was stirred for 14 h. HPLC showed the consumption of the starting acid (R_t = 12.01 min) and the presence of a new product (R_t = 14.66 min); LCMS: MH^+ = 778.74 (100%). Allyl-D-glucuronate (103 mg, 0.427 mmol) was then added followed by NMM (60 μL, 0.545 mmol), and the solution was stirred for 14 h. The solution was diluted with EtOAc (20 mL) and washed with water (20 mL ×2), brine (20 mL), dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give 160 mg of a residue. The residue was dissolved in THF (3.0 mL). Tetrakis (triphenylphosphine)-palladium, polymer bound (200–400 mesh, 0.5–0.9 mmol/g, 150 mg) was added followed by morpholine (100 μL). The mixture was stirred at room temperature for 3 h. HPLC showed one single product, R_t = 10.6 min. Amberlite IR-120 (H+) (300 mg) was added and the mixture was stirred for 30 min. Filtration and washing with THF, then concentration *in vacuo* gave 140 mg of a solid. Purification by HPLC using YMC-Pack Pro C18 (30 × 30 mm ×150 mm, 5 μm), mobile phase A: water with 0.1% TFA, B: MeCN, flow rate: 32 mL/min and a gradient: 20% B to 95% B in 19 min, hold at 95% for 1 min, post run 3 min, run time 20 min, and collecting using ultraviolet 254 nm gave 80 mg of product in 44% yield and with a purity of 95.4%. ^{1}H NMR (600.3 MHz, d_6-DMSO) δ: 9.23 (s, 1H, NH), 9.18 (s, 2H), 8.25 (s, 1H), 8.01 (m, 0.5H), 7.72–7.85 (m, 1.5H), 7.80 (d, J = 15.8 Hz, 1H), 7.75 (d, J = 8.5 Hz, 1H), 7.68 (d, J = 8.3 Hz, 1H), 7.58 (m, 2H), 6.70 (d, J = 15.8 Hz, 1H), 5.52 (d, J = 7.9 Hz, 1H), 5.45 (m, 1H), 5.30 (m, 1H), 3.86 (s, 3H), 3.79 (t, J = 9.5 Hz, 1H), 3.78 (s, 3H), 3.71 (t, J = 9.0 Hz, 1H), 3.38 (t, J = 9.1 Hz, 1H), 3.34 (t, J = 8.8 Hz, 1H), 3.29 (m, 1H), 3.01 (m, 2H), 2.71 (q, J = 9.4 Hz, 2H), 2.06 (m, 1H), 1.94 (m, 7H), 1.68 (m, 2H). ^{13}C NMR (150.92 MHz, DMSO-d_6) δ: 171.10, 167.20, 165.8, 158.2, 157.3, 147.5, 143.6 (m), 137.4 (m), 137.3, 135.1, 128.3, 127.6 (m), 127.1, 122.3 (m), 121.4 (m), 120.2, 119.1 (m), 118.4, 118.2, 114.9, 110.6, 110.2, 94.3, 76.6, 75.1, 72.1, 71.0, 55.1, 36.2, 33.2, 33.0, 31.1, 27.1, 13.8.13. LCMS: m/z 1:1 835:837.

(2*R*,3*R*,4*R*,5*S*,6*R*)-6-{3-[2-(1-{[2-(5-Bromo-pyrimidin-2-yl)-3-cyclopentyl-1-methyl-1*H*-indole-6-carboxamido]}-cyclobutyl)-1-methyl-1*H*-benzo[*d*]imidazol-6-yl-3a,4,5,6,7,7a-$^{13}C_6$]-propyloxy}-3,4,5-trihydroxy-tetrahydro-pyran-2-carboxylic acid, [$^{13}C_6$]-(4): To a suspension of **[$^{13}C_6$]-(2)** (366 mg, 0.6 mmol), HATU (325 mg, 0.8 mmol) in anhydrous acetonitrile (10 mL), was added NMM (0.2 mL, 1.8 mmol) at room temperature and stirred for 14 h. HPLC showed no starting material. To this white mixture, was added anhydrous CH_2Cl_2 (10 mL) to give a clear yellow solution. Allyl-D- glucuronate (518 mg, 2.21 mmol) was added followed by NMM (0.8 mL, 7.2 mmol), and the resulting solution was stirred for 90 min. HPLC showed a new single product. LCMS: m/z (879.31:881.35, 1:1, 100%). The reaction was cooled in an ice

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bath under nitrogen and tetrakis triphenyl phosphine Pd(0) (64 mg, 0.055 mmol) was added followed by morpholine (0.145 mL, 1.7 mmol). The resulting solution was stirred for 2 h at 0 °C. HPLC and LCMS showed one single product corresponding to the acid *m/z* 1:1, 839:841. A solution of 2 N HCl (5 mL) was added followed by water (12 mL) at 0 °C. The resulting mixture was stirred at room temperature for 2 h then concentrated *in vacuo* to remove most of the organic solvents. The mixture was then decanted to remove the water (HPLC of aqueous showed no product). The solid residue showed the desired product. Purification using a 43-g C18 column, which was first preconditioned with 50% water/MeCN and 0–50% MeCN. Water (0.1% formic acid). The crude product was first loaded on C18 cartridge using methanol, and then most of the methanol was dried overnight. The fractions containing the pure material were combined, and most of the organic solvent was removed *in vacuo*, and the resulting white mixture (product + water) was freeze dried to give 293 mg of product in 63% yield. ^{1}HNMR (400.33 MHz, DMSO-d_6) δ: 9.11 (s, 2H), 9.07 (s, 1H, NH), 8.05 (s, 1H), 7.68 (d, J = 8.5 Hz, 1H), 7.75 (d, J = 8.5 Hz, 1H), 7.53 (m, 1H), 7.32 (m, 1H), 7.08 (m, 1H), 5.41 (d, J = 8.1 Hz, 1H), 5.35 (s, OH), 3.86 (s, 3H), 3.75 (t, J = 9.5 Hz, 1H), 3.71 (s, 3H), 3.35 (d, J = 9.3 Hz, 1H), 3.29 (t, J = 8.9 Hz, 1H), 3.20 (t, J = 8.6 Hz, 1H), 3.18 (s, OH), 2.01 (m, 4H), 2.73 (m, 4H), 2.53 (m, 2H), 1.90 (m, 6H), 1.64 (m, 2H). ^{13}C NMR (100.66 MHz, DMSO-d_6) δ: 171.2, 169.7, 166.0, 157.9, 156.0, 139.9 (m), 136.9 (m), 134.1 (m), 128.5, 127.0, 122.0, 121.9 (m), 120.2, 118.4 (m), 110.3, 108.9 (m), 109.1, 94.1, 76.1, 75.6, 72.1, 71.3, 55.2, 36.3, 35.9, 32.8, 31.7, 31.6, 30.8, 26.0, 14.9. HPLC: R_t = 10.35 min, 96.55%. LCMS (fast medium polar method) R_t = 0.76 min, 839.26 (MH^+): 837.47 (M^-). HRMS: calculated 837.25355 (100%), 838.25691 (30%), 839.25151 (100%), 840.25486 (30%), 841.25822 (5%); found 837.25429 (100%), 838.25846 (30%), 839.25185 (100%), 840.25589 (30%), 84125913 (5%).

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

5,7-Diazaspiro[3,4]octane-6,8-dione-8-^{14}*C*, [^{14}C]-(22): In a 24 mL screw–cap vial, were placed ammonium carbonate (730 mg, 7.6 mmol), potassium cynanide-^{14}C (224 mCi, SA = 59 mCi/mmol, 3.8 mmol). Methanol (3.5 mL), water (3.5 mL), and cyclobutanone (290 mg, 3.83 mmol) were then added, and the resulting solution was heated at 65 °C for 24 h. Another aliquot of cyclobutanone (300 μL) was added, and heating was continued for another 3 h. After cooling the reaction in an ice bath, aqueous HCl (4.75 mL, 6 N) was added slowly to give a white precipitate, which was filtered and dried under reduced pressure overnight. The product was obtained as an off-white solid in 55% radiochemical yield (300 mg, 124 mCi).

1-Amino-cyclobutane-1-carboxylic-^{14}*C* acid, [^{14}C]-(23): The hydantoin derivative **[^{14}C]-(22)** (300 mg, 2 mmol) in an aqueous solution of NaOH (5.0 N, 6 mL) was heated in a screw–cap vial at 110 °C for 24 h. After cooling in an ice bath, the mixture was transferred to a 100 mL round bottomed flask, and a concentrated solution of HCl (5 mL, 12 N) was added dropwise in a 30 min period. The resulting mixture was concentrated *in vacuo* to dryness. *n*-Propanol (60 mL) was added, and the mixture was stirred for 12 h to dissolve the amino acid. The mixture was filtered through a sintered funnel and washed with *n*-propanol. The solvent was removed under reduced pressure, and the solid residue was further dried at 40 °C for 12 h. The product was obtained as white solid (0.51 g, crude) and was used as it is in the next step without further characterization.

Butyl (*E*)-3-(4-amino-3-(methylamino)phenyl)acrylate (12): To a solution of (**11**) (2.8 g, 10 mmol) in ethyl acetate (60 mL) and ethanol (60 mL), was added tin chloride dihydrate (11.3 g, 50 mmol), and the resulting mixture was heated to reflux for 14 h. After cooling to room temperature, the reaction was poured into a saturated aqueous solution of Na_2CO_3 (250 mL) and extracted with EtOAc (250 mL ×2). The combined extracts were washed with brine (300 mL ×2), dried over $MgSO_4$, filtered, and concentrated *in vacu*o to give 2.76 g of a viscous honey-colored oil. Purification on 150 g disposable silica gel column using up to 20% EtOAc in CH_2Cl_2 gave 1.86 g of a bright yellow material in 75% yield. This solidified upon standing at room temperature. LCMS (fast medium polar method): R_t = 0.86 min, MH^+ = 249.24 (100%). TLC, R_f = 0.31 in 20% EtOAc:CH_2Cl_2. ^{1}H NMR ($CDCl_3$) δ: 7.62 (d, J = 16.0 Hz, 1H), 6.91 (dd, J = 1.0, 8.1 Hz, 1H), 6.83 (d, J = 1.0 Hz, 1H), 6.68 (d, J = 8.1 Hz, 1H), 6.29 (d, J = 16.0 Hz, 1H), 4.19 (t, J = 6.2 Hz, 2H), 3.56 (s, 3H), 2.89 (m, 3H), 1.70 (m, 2H), 1.45 (m, 2H), 0.96 (t, J = 6.2 Hz, 3H). ^{13}C NMR ($CDCl_3$) δ: 167.87, 145.77, 138.37, 137.46, 126.78, 120.78, 115.43, 114.02, 109.69, 64.13, 30.88, 19.25, 13.79.

Butyl (*E*)-3-(2-(1-aminocyclobutyl)-1-methyl-1*H*-benzo[*d*]imidazol-6-yl-2-^{14}*C*)acrylate, [^{14}C]-(14): A solution of phosphorous pentachloride (300 mg, 1.4 mmol) and 2-oxazolidone (250 mg, 2.9 mmol) in anhydrous acetonitrile (10 mL) was stirred at room temperature for 14 h. The acid **[^{14}C]-(23)** (154 mg, 1 mmol) was added in one portion, and the resulting mixture was stirred for 6 h. The diamine (**12**) (240 mg, 0.97 mmol) was then added and stirring was continued for 12 h. Toluene (1 mL) was added, and the mixture was stirred at 65 °C for 4 h. Isopropanol (3 mL) was added, and stirring was continued for another 3 h. TLC (10% MeOH/CH_2Cl_2) showed no starting material. The mixture was cooled to room temperature and concentrated to remove most of the solvents. Then, a saturated solution of $NaHCO_3$ (50 mL) was added, and the aqueous layer was extracted with ethyl acetate (50 mL ×2). The combined extracts were dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified on a 40-g RediSep™ column and up to 10% MeOH in methylene chloride to give 180 mg of a solid, which was used as is in the next step.

Butyl (*E*)-3-(2-(1-(2-(5-bromopyrimidin-2-yl)-3-cyclopentyl-1-methyl-1*H*-indole-6-carboxamido)cyclobutyl)-1-methyl-1*H*-benzo[*d*]imidazol-6-yl-2-^{14}*C*)acrylate, [^{14}C]-(17): To a suspension of (**15**) (262 mg, 0.7 mmol) in anhydrous methylene chloride (8 mL), was added thionyl chloride (66 μL, 0.9 mmol) at 0 °C under nitrogen atmosphere. After stirring for 15 min, triethylamine (0.32 mL, 2.3 mmol) was added dropwise to give a dark solution in few minutes. The stirring was continued for 2.5 h. The amine **[^{14}C]-(14)** (180 mg, 0.55 mmol) was added in methylene chloride (6 mL). The flask containing this amine was rinsed one more time with methylene chloride (6 mL) and added to the reaction flask. After stirring at 0 °C for 1 h, the ice bath was removed, and the reaction was warmed slowly to room temperature and stirred for 14 h. TLC (30% EtOAc : CH_2Cl_2) showed all the starting material was consumed. Most of the solvent was removed under reduced pressure, and the residue was dissolved in ethyl acetate. A saturated solution of Na_2CO_3 (30 mL) was added and extracted with methylene chloride (25 mL ×3). The combined extracts were dried (Na_2SO_4), filtered, and concentrated *in vacuo* to give 0.49 g of a foam. Purification using a 40 g disposable silica gel column

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and 10–45% ethyl acetate in methylene chloride as eluent gave 280 mg of an off-white solid or 22 mCi in 57% yield and a specific activity of 55.3 mCi/mmol. Crystallization from acetonitrile gave 264 mg of a fluffy white solid with radiochemical purity of 97%.

(*E*)-3-(2-(1-(2-(5-Bromopyrimidin-2-yl)-3-cyclopentyl-1-methyl-1*H*-indole-6-carboxamido)cyclobutyl)-1-methyl-1*H*-benzo[*d*]imidazol-6-yl-2-^{14}*C*)acrylic acid, [^{14}C]-(1): To a mixture of the butyl ester **[^{14}C]-(17)** (264 mg, 0.35 mmol) in *n*-propanol (6 mL), was added a solution of NaOH (6 mL, 0.2 N), and the mixture was heated at 90 °C for 6 h. After cooling to room temperature, the reaction mixture was treated with acetic acid (0.25 mL) to give a fluffy white solid. The mixture was treated with ethyl acetate (30 mL) and water (20 mL). The aqueous phase was extracted with ethyl acetate (30 mL ×2), and the combined extracts were dried (Na_2SO_4), filtered, and concentrated *in vacuo* to give 300 mg of an off-white solid. Purification using a 40 g disposable silica gel column and up to 10% MeOH/CH_2Cl_2 gave 160 mg of a white solid. The solid was crystallized form hot methanol to give 117 mg of a white solid or 9.3 mCi with a specific activity of 54.5 mCi/mmol with radio purity of 99% and chemical purity of 98%. The mother liquor was concentrated and recrystallized from hot methanol to give 85 mg of an off-white solid or 6.8 mCi with 97% radiochemical purity.

Sodium (E)-3-(2-(1-(2-(5-bromopyrimidin-2-yl)-3-cyclopentyl-1-methyl-1*H*-indol-6-carboxamido)cyclobutyl]-1-methyl-1*H*-benzo[*d*]imidazol-6-yl-2-^{14}*C*)acrylate, [^{14}C]-(1-NA): An aqueous solution of NaOH (2 N, 75 μL, 0.15 mmol) was added to a suspension of [^{14}C]-(**1**) (95 mg, 0.15 mmol, 8 mCi) in THF (1.0 mL). The mixture was stirred for 15 min to give a solution, which was warmed in an oil bath to 65 °C. Acetonitrile (0.5 mL) was added to give a cloudy solution. Few crystals of (**1**), ca; 1 mg in 24:1 MeCN : water (0.5 mL) were added. The mixture was stirred at this temperature for 1 h then acetonitrile (3 mL) was added, and the mixture was stirred for 30 min then allowed to cool to room temperature. The solid was collected by vacuum filtration, washed with 24:1 MeCN : water (3 mL), and then dried under reduced pressure for 14 h at room temperature then at 40 °C for 2.5 h to give 87 mg of a white solid in 88% yield. A total of 7 mCi was obtained with a specific activity of 54.5 mCi/mmol and with 98.7% radiochemical purity. HPLC[b], R_t = 17.3 min. ^{1}H NMR (DMSO-d_6) δ: 9.39 (s, 1H), 9.17 (s, 2H), 8.20 (s, 1H), 7.74 (d, *J* = 8.5 Hz, 1H), 7.62 (dd, *J* = 1.8, 8.3 Hz, 1H), 7.57 (d, *J* = 8.3 Hz, 1H), 7.56 (s, 1H), 7.34 (dd, *J* = 1.8, 8.3 Hz, 1H), 7.28 (d, *J* = 15.8 Hz, 1H), 6.44 (d, *J* = 15.8 Hz, 1H), 3.86 (s, 3H), 3.76 (s, 3H), 3.71 (m, 1H), 2.99 (m, 2H), 2.75 (m, 2H), 2.04 (m, 1H), 1.96 (m, 2H), 1.94 (m, 1H), 1.85 (m, 4H), 1.64 (m, 2H).

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

Butyl 3-(4-amino-3-(methylamino)phenyl)propanoate (18): A mixture of the cinnamic acid derivative (**11**) (3 g, 10.8 mmol) and Pd/C (10%, 3 g) in methanol (75 mL) was stirred under 200 PSI of hydrogen at 50 °C for 20 h in Parr apparatus. After cooling to room temperature, the mixture was filtered through a short pad of Celite® and concentrated *in vacuo* to give 2.8 g of a dark residue. Purification using a 150 g disposable silica gel column and up to 30% EtOAc : CH_2Cl_2 gave 2.25 g of material in 84% yield. LCMS (fast medium polar method): R_t at 0.64 min (MH^+ = 250.52, 100%). TLC, R_f = 0.7 in 20% EtOAc : CH_2Cl_2. ^{1}H NMR ($CDCl_3$) δ: 6.63 (d, *J* = 8.1 Hz, 1H), 6.52 (d, *J* = 2.1 Hz, 1H), 6.49 (dd, *J* = 2.1, 8.1 Hz, 1H), 4.09 (t, *J* = 6.5 Hz, 2H), 3.28 (s, 3H), 2.87 (t, *J* = 8.1 Hz, 2H), 2.85 (s, 3H), 2.60 (t, *J* = 8.1 Hz, 2H), 1.60 (m, 2H), 1.36 (m, 2H), 0.92 (t, *J* = 6.5 Hz, 3H). ^{13}C NMR ($CDCl_3$) δ: 173.40, 139.22, 133.28, 132.05, 117.67, 116.49, 110.68, 64.27, 36.55, 30.99, 30.93, 30.71, 19.15, 13.73.

Butyl 3-(2-(1-aminocyclobutyl)-1-methyl-1*H*-benzo[*d*]imidazol-6-yl-2-^{14}*C*)propanoate, [^{14}C]-(19): To (**13**) (172 mg, 1 mmol) in anhydrous acetonitrile (10 mL), was added the diamine **(18)** (226 mg, 1 mmol) in one portion, and the mixture was stirred for 14 h. Toluene (1 mL) was added, and the mixture was heated at 65 °C and stirred for 6 h. Isopropanol (3 mL) was added, and the mixture was heated for another 3 h at 65 °C. The mixture was concentrated to remove most of the solvents, and the residue was dissolved in a saturated aqueous solution of $NaHCO_3$ (40 mL) and EtOAc (50 mL). The aqueous layer was extracted one more time with EtOAc (50 mL), and the combined organic extracts were dried over Na_2SO_4, filtered through a column separator, and concentrated *in vacuo* to give 0.44 g of a residue. Purification using a 40 g disposable silica gel column, and up to 4% methanol in methylene chloride gave 260 mg of a pale yellow solid in 84% yield and in 98% chemical purity.

Butyl 3-(2-(1-(2-(5-bromopyrimidin-2-yl)-3-cyclopentyl-1-methyl-1*H*-indole-6-carboxamido)cyclobutyl)-1-methyl-1*H*-benzo[*d*]imidazol-6-yl-2-^{14}*C*)propanoate, [^{14}C]-(20): To a suspension of (**15**) (314 mg, 0.8 mmol) in anhydrous methylene chloride (8 mL), was added thionyl chloride (76 μL, 1 mmol) at 0 °C under nitrogen. After stirring for few minutes, triethylamine (385 μL, 2.8 mmol) was added slowly to give a clear solution. The solution was stirred for 2 h at 0 °C. The aforementioned amine (260 mg, 0.8 mmol) was dissolved in CH_2Cl_2 (8 mL) and added at 0 °C to the reaction flask, and the solution was stirred for 1 h at this temperature, then warmed to room temperature and stirred for 14 h. TLC (10% MeOH/CH_2Cl_2) showed that the starting amine was completely consumed. A saturated solution of Na_2CO_3 (35 mL) was added to the reaction flask, and the mixture was stirred vigorously. The methylene chloride layer was removed, and the aqueous was extracted (30 mL ×2) with methylene chloride. The combined organic extracts were dried over Na_2SO_4, filtered through a phase separator column, and concentrated *in vacuo* to give 0.7 g of a honey-colored residue. Purification by silica gel chromatography using a 40 g disposable silica gel column and using 10–50% EtOAc : CH_2Cl_2 as eluent gave 220 mg of a yellow solid in 48% yield and in 98% chemical purity.

3-(2-(1-(2-(5-Bromopyrimidin-2-yl)-3-cyclopentyl-1-methyl-1*H*-indole-6-carboxamido)cyclobutyl)-1-methyl-1*H*-benzo[*d*]imidazol-6-yl-2-^{14}*C*)propanoic acid, [^{14}C]-(2): A mixture of **[^{14}C]-(20)** (220 mg, 0.23 mmol) was treated with 0.2 N aqueous NaOH (4 mL) and *n*-propanol (4 mL). The cloudy mixture was heated at 90 °C and stirred for 6 h. HPLC showed the reaction to be complete, and only one major peak corresponding to **[^{14}C]-(2)** was observed. The reaction solution was cooled to room temperature and treated with acetic acid (0.17 mL). The solution was then concentrated *in vacuo* to remove most of the solvents and to give a white precipitate. The mixture was treated with water (20 mL) and extracted with methylene chloride. The emulsion was broken by adding methanol (2 mL). Then, the organic phase was removed and concentrated *in vacuo* to give 0.22 g of beige solid. The residue was further dried

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under vacuum at 32 °C to give 193 mg of beige solid. HPLC showed the product to be 82% pure. Purification of this material using 40 g disposable silica gel column and 2 to 20% methanol in methylene chloride gave 113 mg of a white solid. The specific activity was calculated to be 54.5 mCi/mmol, and a total of 9.53 mCi was obtained. HPLC[b], R_t = 17.2 min with radiochemical purity of 98.8%. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 9.41 (s, 1H), 9.20 (s, 2H), 8.21 (s, 1H), 7.74 (d, *J* = 8.5 Hz, 1H), 7.62 (d, *J* = 8.4 Hz, 1H), 7.48 (d, *J* = 8.2 Hz, 1H), 7.22 (d, *J* = 0.8 Hz, 1H), 7.02 (d, *J* = 8.3 Hz, 1H), 3.86(s, 3H), 3.75–3.65 (m, 1H), 3.71 (s, 3H), 3.33 (brs, 2H), 3.03 (m, 2H), 2.76 (t, *J* = 7.7 Hz, 2H), 2.76 (t, *J* = 9.1 Hz, 2H), 2.25 (t, J = 8.2 Hz, 2H), 2.01 (m, 2H), 1.90 (m, 6H), 1.70(m, 2H).

Synthesis of [^{14}C]-(3)

(2*S*,3*S*,4*S*,5*R*,6*S*)-6-(((*E*)-3-(2-(1-(2-(5-Bromopyrimidin-2-yl)-3-cyclopentyl-1-methyl-1*H*-indole-6-carboxamido)cyclobutyl)-1-methyl-1*H*-benzo[*d*]imidazole-6-yl-2-^{14}C)acryloyl)oxy)-3,4,5-trihydroxytetrahydro-2*H*-puran-2-carboxylic acid, [^{14}C]-(3): NMM (19.5 mg, 0.2 mmol) was added to a suspension of **[^{14}C]-(1)** (42 mg, 0.06 mmol, 3.1 mCi) and HATU (36.7 mg, 0.1 mmol) in MeCN (2 mL) at room temperature. The mixture was stirred for 14 h. HPLC showed complete consumption of the starting acid. The mixture was diluted with CH_2Cl_2 (3 mL) and D-allyl glucuronate (43 mg, 0.2 mmol) and NMM (19 mg, 0.2 mmol) were added, and the mixture was stirred overnight. A second portion of D-allylglucuronate (43 mg, 0.2 mmol) and NMM (19 mg, 0.2 mmol) were added and the mixture was stirred for 48 h. HPLC revealed consumption of the active ester. $Pd(PPh_3)_4$ (6.7 mg, 0.01 mmol) and morpholine (10 mg, 0.12 mmol) were added. The reaction mixture was stirred for 2 h, then HCl (2.0 M, 2 mL) was added, and the mixture was stirred overnight. The mixture was filtered and concentrated to remove most of the volatiles. The crude product was purified by HPLC, and the desired fractions were combined and lyophilized to give 482 μCi (7.7 mg) with a specific activity of 52.3 mCi/mmol and radiochemical purity of 97.5%, which co-eluted with unlabeled sample on HPLC[b], R_t = 10.37 min.

Conflict of Interest

The authors did not report any conflict of interest.

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