Synthesis of highly potent Lymphocyte Function-Associated Antigen-1 Antagonists Labeled with Carbon-14 and with Stable Isotopes, Part 3

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Abstract:

The drug candidates (**2**) and (**3**) are highly potent LFA-1 inhibitors. They were efficiently prepared labeled with carbon-14 using a palladium-catalyzed carboxylation of an iodo-precursor (**5**) and sodium formate-^{14}C to afford acid [^{14}C]-(**6**), which was coupled via an amide bond to chiral amines (**7**) and (**8**) in 52% and 48% overall yield respectively and with specific activities higher than 56 mCi/mmol and radiochemical purities of 99%. For stable isotopes synthesis, the amine [$^{2}H_8$]-(**7**) was synthesized in three steps from 2-cyanopyridine-$^{2}H_4$ using Kulinkovich-Szymonik aminocyclopropanation, followed by coupling to L-alanine-2,3,3,3-$^{2}H_4$-*N*-*t*-BOC, and then removal of the BOC-protecting group. Amide bond formation with acid (**6**) gave [$^{2}H_8$]-(**2**) in 36% overall yield. The amine [$^{13}C_4$,^{15}N]-(**8**) was obtained in two steps using L-threonine-$^{14}C_4$,^{15}N and then coupled to acid [^{13}C]-(**6**) to give [$^{13}C_5$,^{15}N]-(**3**) in 56% overall yield.

Keywords: LFA-1, carbon-14, carbon-13, nitrogen-15, deuterium, radiosynthesis

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Introduction:

The lymphocyte function associated antigen-1 (LFA-1) is an essential component in normal immune system function and a target for drug discovery due to its broad therapeutic potential in treating inflammatory diseases. We have previously described the synthesis of some potent LFA-1 antagonists, including compound (**1**) (Figure 1).[1] The continuous search for compounds with higher potency and better pharmacokinetics led to the discovery of compounds (**2**) and (**3**), in which the sulfonamide was replaced with an amide linkage, a fluorine atom was introduced on the dichlorophenyl moiety to suppress oxidative metabolism, and the trifluoromethoxy group was replaced with a nitrile group.[2] Further modifications on compound (**2**) included replacing the methyl group with a chiral 2-propanol moiety, and the pyridine ring with (1,3-dimethylpyrazole-4-yl)pyrimidine in compound (**3**),[2] Figure 1. Herein, we report the synthesis of compounds (**2**) and (**3**) labeled with carbon-14 and with stable isotopes.

Results and discussion:

Carbon-14 labeled (**2**) and (**3**), Scheme 1, were prepared in two radioactive steps in 52% and 48% radiochemical overall yield respectively. The chiral bicyclic imidazole (**4**) accessible from 3,5-dichloro-4-fluoroaniline according to published procedures,[3] was iodinated using one equivalent of *N*-iodosuccinimide and pyridinium *p*-toluenesulfonate (PPTS, 0.1 equivalent) in methylene chloride. The iodination gave about 10% of a diiodo-byproduct which was removed by silica gel chromatography using 100% toluene as the eluting solvent. The direct and selective palladium-catalyzed carboxylation of iodide (**5**) using sodium formate-^{14}C gave carbon-14 labeled carboxylic acid derivative [^{14}C]-(**6**) in 70% yield. This selective carboxylation, which presumably involves palladium catalyzed hydroxycarbonylation of *in-situ* generated carbon monoxide was suitable for this substrate which contains two chlorine atoms and a nitrile group susceptible to strong acids and bases.[2,4-7] An outstanding review on recent developments in carbonylation chemistry should be consulted as well.[8] Finally, amide bond formation between the acid [^{14}C]-(**6**) and the chiral amines (**7**) and (**8**) gave the desired [^{14}C]-(**2**) and [^{14}C]-(**3**) respectively with specific activities higher than 56 mCi/mmol and radiochemical purities of 99%. Several conditions for the amide bond formation were tried.[2] in our hands higher yields were obtained when using acylchloride derivatives.

As shown in Figure 1, both compounds contain two chlorine atoms; it was necessary that the stable isotope labeled compounds should be least 6 atomic mass units higher to easily distinguish them from the unlabeled drug candidates by mass spectroscopy. To prepare deuterium labeled (**2**), (*S*)-2-amino-*N*-(1-pyridin-$^{2}H_4$-2-yl-cyclopropyl)-propionamide [$^{2}H_8$]-(**7**) was prepared in three steps and in 50% overall yield starting from the commercially available 2-cyanopyridine-$^{2}H_4$, [$^{2}H_4$]-(**9**). The cyanopyridine was first converted to 1-pyridin-$^{2}H_4$ cyclopropylamine [$^{2}H_4$]-(**10**) using ethyl magnesium bromide and titanium tetraisopropoxide via Kulinkovich-Szymonik aminocyclopropanation.[9,10] Coupling to BOC-L-alanine-$^{2}H_4$, [$^{2}H_4$]-(**11**) using EDC and HOBT in THF gave [$^{2}H_8$]-(**12**). Finally, removal of the BOC-protecting group and coupling to the acid (**6**) gave deuterium labeled (**2**) with very high isotopic enrichment , see Figure 2, (Scheme 2).

The availability of L-threonine-$^{13}C_4$,^{15}N commercially, allowed the preparation of the chiral amine [$^{13}C_4$,^{15}N]-(**8**) in two steps with 80% overall yield. L-Threonine-$^{13}C_4$,^{15}N was first BOC-protected using aqueous sodium bicarbonate and methanol in quantitative yield.[11] Coupling to the amine (**14**)[12] using EDC and HOBT in methylene chloride as seen before afforded [$^{13}C_4$,^{15}N]-(**15**) in good yield. Removal of the BOC-protecting group gave [$^{13}C_4$,^{15}N]-(**8**) in quantitative yield. The acid [^{13}C]-(**6**), which was prepared during the cold trials of carbon-14 synthesis was used in the final amide bond formation with the amine [$^{13}C_4$,^{15}N]-(**8**) to give [$^{13}C_5$,^{15}N]-(**3**) in very good yield, (Scheme 3).

Experimental

Materials and methods

Liquid scintillation counting was accomplished using a Beckman LS6500TA and Ready Safe™ cocktail (Beckman, Fullerton, CA, USA). NMR spectra were recorded with a Bruker 400 and 500 MHz spectrometers using either deuterated chloroform or deuterated DMSO as a solvent and tetramethyl silane as the internal standard unless stated otherwise. Disposable silica gel columns were obtained from TELEDYNE-ISCO (Lincoln, NE, USA). HPLC was performed using Zorbax XDB C8 and 20% to 100% MeCN/water (both solvents contain 10 mM TFA) in 22 min runs, unless indicated otherwise. The chemicals 2-cyanopyridine-$^{2}H_4$ (99.6 atom % ^{2}H) and L-alanine-2,3,3,3-$^{2}H_4$-N-*t*-BOC (99.2 atom % ^{2}H) were purchased from CDN (Pointe Claire Quebec, Canada). L-Threonine-$^{13}C_4$,^{15}N (99 atom % ^{13}C and 98.5 atom % ^{15}N) and sodium formate-^{13}C (99.5 atom % ^{13}C) were obtained from ISOTEC (Sigma-Aldrich, Miamisburg, OH, USA). Carbon-14 sodium formate was obtained from

American Radiolabeled Compounds (St. Louis, MO, USA) and from ViTrax (Placentia, CA, USA). The rest of the reagents were purchased from Sigma-Aldrich Company (Milwaukee, WI, USA).

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

(*R*)-4-((1-(3,5-dichloro-4-fluorophenyl)-5-iodo-3-methyl-2-oxo-2,3-dihydro-1*H*-imidazo[1,2-a]imidazol-3-yl)methyl)benzonitrile, (5): To a solution of (**4**) (5.0 g, 12 mmol) in anhydrous CH_2Cl_2 (100 mL), was added PPTS (0.31 g, 1.2 mmol) and the solution was cooled in an ice-bath under nitrogen atmosphere. *N*-Iodosuccinimide (2.9 g, 12.2 mmol) was added in four portions in a 30 minute period at 0 ^oC. The mixture was stirred at 0 ^oC for 2 h in the dark. LCMS showed 29% remaining of starting material. The ice bath was removed and the dark mixture was further stirred for another 14 h at ambient temperature. A solution of 5% aqueous $Na_2S_2O_3$ (200 mL) was added and stirred for 15 minutes to give a light yellow mixture, which was extracted with CH_2Cl_2 (100 mL ×2). The combined extracts were washed with water (200 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo* to give 7.0 g of yellow foam. Purification by flash chromatography using a silica gel column (10" and 2.5" diameter silica gel) packed in hexanes and eluting with 100% toluene gave few fractions of the product contaminated with a diiodo-byproduct, which were combined and concentrated *in vacuo* to give 0.72 g of material. The fractions containing the desired product were combined and concentrated *in vacuo* at 45 ^oC to give 4.8 g of an off-white solid in 74% yield. TLC (100% toluene), R_f of the desired product = 0.13, R_f of the diiodo-byproduct = 0.2, the starting material remains on the base line. LCMS: R_t = 2.03 min, MH^+ = 541.4 (100%). HPLC: R_t= 7.22 min, 99%. ^{1}HNMR (400 MHz, $CDCl_3$) δ: 7.67(d, *J* = 5.8 Hz, 2H), 7.45(d, *J* = 8.3 Hz, 2H), 7.05(d, *J* = 8.3 Hz, 2H), 6.97(s, 1H), 3.66(d, *J* = 13.84 Hz, 1H), 3.34(d, *J* = 13.84 Hz, 1H), 1.95(s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 173.94, 154.09, 151.6, 148.6, 138.6, 136.0, 132.4, 130.3, 123.0(m), 122.3, 118.1, 112.1, 75.7(m), 68.2, 56.7, 42.4, 22.4.

(*R*)-5-(4-Cyano-benzyl)-7-(3,5-dichloro-4-fluoro-phenyl)-5-methyl-6-oxo-6,7-dihydro-5*H*-imidazo[1,2-*a*]imidazole-3-carboxylic-^{14}*C* acid, [^{14}C]-(6): Anhydrous DMF (2.0 mL) was added to a thick walled screw cap tube containing carbon-14 sodium formate (200 mCi), followed by Hünig's base (diisopropylethyl amine, DIPEA) (0.55 mL, 3.12 mmol) and acetic anhydride (306 μL, 3.18 mmol). After stirring for 30 min at room temperature, the mixture became thick. In a separate flask, LiCl (208 mg, 4.90 mmol) was dissolved in anhydrous DMF (4 mL) using sonication. To this, were added the iodide (**5**) (857 mg, 1.58 mmol) and $Pd(OAc)_2$ (74 mg, 0.33 mmol). The resulting solution was added to the tube containing

carbon-14 sodium formate mixture and the tube was heated to 80 °C and stirred for 14 h. An HPLC sample showed the acid [^{14}C]-(**6**) as the only peak in the chromatogram and co-eluted with the unlabeled (**6**) at 13.3 min. The dark mixture was transferred to a 100 mL Erlenmeyer flask using EtOAc (40 mL) and treated with 2.75 N of aqueous HCl (30 mL) with stirring. The organic phase was removed and the aqueous was extracted twice with EtAOc (40 mL ×2). The combined extracts were dried over Na_2SO_4, filtered and concentrated *in vacuo* to give 1.3 g of viscous oil. Purification by chromatography using 40 g disposable silica gel column and eluting with 10% to 20% EtOAc:CH_2Cl_2, then with 10% MeOH/CH_2Cl_2 gave 511 mg (70% based on the iodide) of the pure product. A total of 69 mCi was obtained. HPLC: t_R = 13.29 min. 96% chemical purity and 97% radiochemical purity.

(*R*)-3-(4-Cyanobenzyl)-1-(3,5-dichloro-4-fluorophenyl)-3-methyl-2-oxo-*N*-((*S*)-1-oxo-1-((1-(pyridin-2-yl)cyclopropyl)amino)propan-2-yl)-2,3-dihydro-1*H*-imidazo[1,2-a]imidazole-5-carboxamide-^{14}*C*, [^{14}C]-(2): To a solution of **[^{14}C]-(6)** (511 mg, 1.1 mmol, 69 mCi) and (**7**) (106 mg, 1.1 mmol) in anhydrous DMF (10 mL) was added HATU (418 mg, 1.2 mmol) in one portion. To this solution, Hünig's base (1 mL, 5.5 mmol) was added dropwise and the reaction was stirred at room temperature for 14 h. The reaction was concentrated *in vacuo* at 35 °C to remove most of the DMF, then diluted with $CHCl_3$ (100 mL) and washed with water (100 mL). The organic phase was removed and dried over Na_2SO_4, then filtered and concentrated *in vacuo* to give 0.85 g of foam. Purification by flash chromatography using a 40 g disposable silica gel column and 0→10% MeOH/CH_2Cl_2 gave 531 mg of white foam in 74% yield (48 mCi). HPLC: [^{14}C]-(**2**) eluted at 13.5 min and co-eluted with unlabeled (**2**) with 99.56 % chemical purity and 99.93% radiochemical purity. Specific activity of 58.6 mCi/mmol. ^{1}HNMR (500 MHz, $CDCl_3$) δ: 8.88 (exch, 1H), 8.47(s, 1H), 8.24 (exch, 1H), 7.64(d, *J* = 5.4 Hz, 2H), 7.57(m, 1H), 7.41(d, *J* = 8.3 Hz, 2H), 7.31(m, 2H), 7.14(d, 1H), 7.08(m, 1H), 7.01(d, *J* = 8.2 Hz, 2H), 6.94(s, 1H), 4.46(m, 1H), 4.14(d, *J* = 13.6 Hz, 1H), 3.28(d, *J* = 13.6 Hz, 1H), 1.93(s, 3H), 1.65(m, 2H), 1.59(d, *J* = 7.2 Hz, 3H), 1.26(m, 2H).

(*R*)-3-(4-Cyanobenzyl)-1-(3,5-dichloro-4-fluorophenyl)-*N*-((2*S*,3*R*)-1-((1-(5-(1,3-dimethyl-1*H*-pyrazol-4-yl)pyrimidin-2-yl)cyclopropyl)amino)-3-hydroxy-1-oxobutan-2-yl)-3-methyl-2-oxo-2,3-dihydro-1*H*-imidazo[1,2-a]imidazole-5-carboxamide-^{14}*C*, [^{14}C]-(3): [^{14}C]-(6) (458 mg, 0.99 mmol, 56 mCi) was dissolved in anhydrous THF (10 mL). Two drops of DMF were added and the solution was cooled in an ice-bath. Oxalyl chloride (152 mg, 1.2 mmol) was added dropwise and the resulting was warmed gradually to room

temperature and stirred for 4 h. A sample was taken and quenched with morpholine. HPLC indicated only a morpholine derivative was observed. The reaction was concentrated *in vacuo* to foam. The amine (**8**) (367 mg, 1.0 mmol) was suspended in anhydrous THF (8 mL). Hünig's base (0.34 mL, 2 mmol) was added dropwise at room temperature to give a colorless solution with some white precipitate at the bottom of the flask. The solution was cooled in an ice bath. The acylchloride derivative of [^{14}C]-(**6**) was dissolved in THF (5 mL) and then added dropwise to the flask containing the amine (**8**). After stirring for 1 h at 0 ^{o}C, the ice-bath was removed and the mixture was stirred for 14 h. Water (40 mL) was added and the organic was extracted with MTBE (50 mL ×2). The combined organic layers were washed with 2% aqueous $NaHCO_3$ (50 mL ×2), dried over Na_2SO_4, filtered and concentrated *in vacuo* to give 0.91 g of yellow solid. HPLC showed about 87% pure product by UV (230 nm) or 90% at 254 nm. Purification using a 40 g silica gel column and up to 10% MeOH/CH_2Cl_2 gave 532 mg of an off-white solid with a specific activity 56.02 mCi/mmol and total activity of 38.53 mCi in 69 % yield. HPLC (Halo C18, 2.1 x 50 mm, 2.7 μM), A: water (0.1% TFA), B: MeCN, 20 to 100% B in 9 min, injection volume 2 μL, UV 254 nm, R_t = 6.32 (99.5%) and 99.7% radiochemical purity. ^{1}HNMR(500 MHz, DMSO-d_6) δ: 8.85(exch, 1H), 8.71(s, 2H), 8.25(exch, 1H), 8.05(s, 1H), 7.85(s, 1H), 7.81(d, *J* = 5.1 Hz, 2H), 7.61(d, *J* = 8.1 Hz, 2H), 7.02(d, *J* = 8.1 Hz, 2H), 5.12(exch, 1H), 4.55(m, 1H), 4.20(m,1H), 4.0(d, *J* = 13.8 Hz, 1H), 3.78(s, 3H), 3.48(d, *J* = 13.8 Hz, 1H), 2.29(s, 3H), 1.91(s, 3H), 1.56(m, 2H), 1.30(d, *J* = 7 Hz, 3H), 1.25(m, 2H).

Synthesis of [$^2H_8$]-(2):

1-Pyridin-$^2H_4$-2-yl-cyclopropylamine, [^{2}H$_4$]-(10): To a 100 mL round bottom flask containing bis[2-(*N*,*N*-dimethylamino)ethyl]ether (4.0 mL, 20.36 mmol) and anhydrous THF (6 mL) at -15 ^{o}C, was added a solution of EtMgBr (21 mL, 1 M in THF) over a 90 minute-period. After the addition was completed, the white mixture was warmed to 0 ^{o}C for 15 min. In a separate 250 mL round bottom flask, a solution of 2-cyanopyridine-$^2H_4$ [^{2}H$_4$]-(**9**) (1 g, 9.25 mmol) in THF (20 mL) was heated to 50 ^{o}C in an oil bath. A solution of Ti(OiPr)$_4$ (3.25 mL, 11.1 mmol) was added to this heated solution under a nitrogen atmosphere followed by addition of the EtMgBr suspension via cannula. A 10 mL THF rinse was used to transfer the remaining EtMgBr mixture to the reaction flask. The reaction turned yellow and quickly darkened with reflux. The mixture thickened. After 2 h, HPLC showed most of the starting material was consumed. The reaction was cooled to room temperature and treated with a solution of 1N NaOH (30 mL) to give a yellow solution and a white precipitate. Me-THF (50

mL) was added and the organic phase was decanted, the solids were extracted with Me-THF (30 mL ×2) and the combined extracts were concentrated *in vacuo* to about 30 mL and used as it is in the next step. LCMS: one single peak, MH^+ = 161.13.

[(*S*)-1-(1-Pyridin-^{2}*H*$_4$-2-yl-cyclopropylcarbamoyl)-ethyl-^{2}*H*$_4$]-carbamic acid *tert*-butyl ester, [^{2}H$_8$]-(12): To a solution of crude [^{2}**H$_4$**]-(**10**) in Me-THF (30 mL) was added L-alanine-2,3,3,3,-^{2}*H*$_4$-*N*-*t*-Boc (1.55 g, 8 mmol), 1-hydroxybenzotriazole hydrate (HOBt, 1.1 g, 8.14 mmol), triethylamine (TEA, 1.12 mL, 8 mmol), and *N*-(3-diamethylaminopropyl)-*N*'-ethylcarbodiimide hydrochloride (EDC, 1.55 g, 8.1 mmol). The reaction was stirred for 14 h at room temperature. Water (50 mL) was added and the aqueous was extracted with CH_2Cl_2 (50 mL ×3). The combined extracts were washed with a saturated solution of $NaHCO_3$ (50 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo* to give 2.82 g of yellow foam. Purification by flash chromatography using a 150 g disposable silica gel column and 0→80% EtOAc in CH_2Cl_2, gave the pure material (1.24 g) as an off-white solid. R_f = 0.23 in 50% EtOAc:CH_2Cl_2. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.51(S, 1H), 5.54(s, 1H), 1.57(m, 2H), 1.44(br s, 9H), 1.18(m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 174.0, 160.5, 155.8, 155.3, 148.5(t, *J* = 26.77 Hz), 135.69(t, *J* = 24.31 Hz), 120.1(t, *J* = 23.57 Hz), 119.0(t, J = 23.57 Hz), 80.0, 50.8, 49.7(m), 35.6, 28.2, 19.1, 17.8(m).

(*S*)-2-Amino-N-(1-pyridin-^{2}*H*$_4$-2-yl-cyclopropyl)-propionamide-^{2}*H*$_4$, [^{2}H$_8$]-(7): To a solution of **[^{2}H$_8$]-(12)** (0.86 g, 2.75 mmol) in CH_2Cl_2 (10 mL) was added a solution of 4M HCl in 1,4-dioxane (7 mL) dropwise. The resulting mixture was stirred at room temperature for 4 h. TLC (10% MeOH/CH_2Cl_2) showed no starting material and the presence of polar material at the baseline. The mixture was concentrated *in vacuo* to give an off-white solid (0.8 g crude material). LCMS, one single peak (MH^+ = 214.49). ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.3(s, 1H), 5.54(brs, 4H), 1.61(m, 2H), 1.20(m, 2H).

***(R)*-3-(4-Cyanobenzyl)-1-(3,5-dichloro-4-fluorophenyl)-3-methyl-2-oxo-*N*-((S)-1-oxo-1-((1-(pyridin-^{2}*H*$_4$-2-yl)cyclopropyl)amino)propan-2-yl)-2,3-dihydro-1H-imidazo[1,2-a]imidazole-5-carboxamide ethyl-^{2}*H*$_4$, [^{2}H$_8$]-(2):** To a solution of acid (**6**) (1.26 g, 2.75 mmol) and amine **[^{2}H$_8$]-(7)** (0.8 g, 2.75 mmol) in anhydrous DMF (25 mL) was added HATU (1.25 g, 3.3 mmol) in one portion. To this solution, Hünig's base (2.4 mL, 13.6 mmol) was added dropwise and the reaction was stirred at room temperature for 14 h. The reaction was concentrated at 40 °C to remove most of the DMF, then diluted with $CHCl_3$ (200 mL) and washed with water (200 mL). The organic phase was dried over $MgSO_4$, filtered and concentrated *in vacuo* to give 3 g of viscous oil which contained some DMF. Purification by

flash chromatography using a 150 g disposable silica gel column and 0→10% MeOH/CH_2Cl_2 gave 1.32 g (72%) of product. HPLC, t_R = 10.47 min (99%) co-eluted with unlabeled standard,. TLC: 10% MeOH/CH_2Cl_2: R_f = 0.5. ^{1}HNMR (400 MHz, $CDCl_3$) δ: 7.70(d, *J* = 5.74 Hz, 2H). 7.50(s, 1H), 7.38(d, *J*= 8.06 Hz, 2H), 7.30(s, 1H), 7.15(s, 1H), 6.98(d, *J*= 8.11 Hz, 2H), 4.13(d, *J*= 13.48 Hz, 1H), 3.34(d, *J* = 13.48 Hz, 1H), 1.94(brs, 3H), 1.65(m, 2H), 1.31(m, 2H). ^{13}CNMR (101 MHz, $CDCl_3$) δ: 174.59, 173.49, 160.14, 159.15, 154.05, 151.55, 148.77(t, *J* = 26.77 Hz), 147.94, 139.77, 135.87 (t, *J* = 24.31 Hz), 132.25, 131.92, 130.08, 128.90, 128.86, 123.66, 122.95, 122.77, 122.01, 120.55(t, *J* = 23.57 Hz), 118.67(t, *J* = 23.57 Hz), 118.28, 111.57, 69.18, 53.47, 50.69, 48.92(m), 42.45, 36.54, 22.42, 18.96, 16.96(m). HRMS, calculated for $C_{32}H_{19}{}^2H_8O_3N_7Cl_2F$: 654.20331, found 654.20325.

***Synthesis of [$^{13}C_5$,^{15}N]-(3)*:**

(*tert*-Butoxycarbonyl)-L-threonine-$^{13}C_4$,^{15}N, [$^{13}C_4$,^{15}N]-(13): L-Threonine-$^{13}C_4$,^{15}N (1 g, 8.1 mmol) was mixed with methanol (12 mL) and a solution of sodium bicarbonate (1.04 g, 12.37 mmol) in water (12 mL) at room temperature. Di-*tert*-butyl-dicarbonate (2.57 g, 11.8 mmol) was added in one portion and the mixture was stirred for 30 h. LCMS showed all the starting material was consumed. The mixture was concentrated i*n vacuo* to remove most of the solvents and the residue was diluted with water (50 mL) and extracted with ether (50 ml ×2). The aqueous layer was made acidic, pH = 3 by adding 1.0 N aqueous HCl, and extracted with Me-THF (50 ml ×2). The combined extracts were dried over Na_2SO_4, filtered and concentrated *in vacuo* to give 2.1 g of colorless oil in quantitative yield. LCMS, R_t = 1.34 min, MH^+ = 225.0 (100%), EI$^-$, M^- = 223.1 (100%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 6.1(brs 2H), 5.73(m, 1H), 4.15-4.52(m, 2H), 1.42(brs, 9H), 1.16(m, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 174.42(d), 156.5, 80.2, 67.92(m), 60.2(m), 58.64(m), 28.2, 19.47(d).

***tert*-Butyl ((2*S*,3*R*)-1-((1-(5-(1,3-dimethyl-1H-pyrazol-4-yl)pyrimidin-2-yl)cyclopropyl)amino)-3-hydroxy-1-oxobutan-$^{13}C_4$-2-yl)carbamate-^{15}N, [$^{13}C_4$,^{15}N]-(15)**: To a mixture of (**14**) (2.4 g, 7.94 mmol) and [$^{13}C_4$,^{15}N]-(**13**) (1.77 g, 7.9 mmol) in anhydrous CH_2Cl_2 (80 mL) was added Hünig's base (8.3 mL, 47.65 mmol) slowly to give a colorless solution. HOBT (1.2 g, 8.88 mmol) and EDC (1.7 g, 8.87 mmol) were added and the solution was stirred at room temperature for 14 h. Most of the solvent was removed *in vacuo* and the residue was diluted with EtOAc (200 mL), and washed with 1.0 N aq. HCl (100 mL). The aqueous yellow solution was removed and the organic was washed with a saturated aqueous $NaHCO_3$ (100 mL), brine (100 mL), dried over Na_2SO_4, and concentrated. Purification on Combi-Flash using 120 g silica gel column and up to 20% MeOH/CH_2Cl_2 gave 2.2 g of an

off-white solid in 80% yield. LCMS: t_R = 0.69 min, MH^+ = 436.0 (100%), M^- = 434.1 (100%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.56(s, 2H), 7.45(s, 1H), 7.06(brs, 1H), 5.2-5.8(m, 1H), 3.8-4.6(m, 1H), 3.49(m, 1H), 2.34(m, 2H), 1.87(m, 1H), 1.4-1.7(m, 2H), 1.47(brs, 9H), 1.13(m, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 173.2(d), 166.7, 154.5, 145.9, 129.3, 128.9, 125.3, 124.5, 120.3, 113.5, 109.3, 80.1, 67.2(m), 62.8(m), 60.2(m), 58.6(m), 38.8, 36.7, 28.3(m), 18.4(d).

(2*S*,3*R*)-1-((1-(5-(1,3-dimethyl-1*H*-pyrazol-4-yl)pyrimidin-2-yl)cyclopropyl)amino)-3-hydroxy-1-oxobutan-^{13}*C*$_4$-2-amine-^{15}*N*, [^{13}C$_4$,^{15}N]-(8): To a solution of **[^{13}C$_4$,^{15}N]-(15)** (1.3 g, 3.0 mmol) in anhydrous CH_2Cl_2 (30 mL), was added a solution of 4N HCl in 1,4-dioxane (3.0 mL, 12 mmol) slowly at room temperature. A precipitate was formed instantly. The mixture was stirred at room temperature for 5 h, and then concentrated *in vacuo.* The white solid residue was treated with anhydrous ether (30 mL) and filtered. The solid was washed with anhydrous ether (10 mL ×2), collected, transferred to a round bottom flask, and dried at 35 ^{o}C for 4 h and then under reduced pressure overnight to give 1.2 g of a white solid in quantitative yield. LCMS, t_R = 0.19 min, MH^+ = 336.0 (100%). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.4(d, *J* = 4 Hz, 1H), 8.74(brs, 2H), 8.30(br, 2H), 8.12(br, 2H), 3.44(3, 3H), 2.51(m, 2H), 2.3(s, 3H), 1.25-160(m, 4H), 1.2(m, 3H). ^{13}CNMR (101 MHz, DMSO-d_6) δ: 168.58(d, *J* = 50 Hz), 154.12, 144.59, 130.39, 124.35, 112.73, 66.3(t, *J* = 37 Hz), 58.34(ddd, *J* = 37, 36, 52 Hz), 38.36, 36.10, 19.40, 12.80. **(*R*)-5-(4-Cyano-benzyl)-7-(3,5-dichloro-4-fluoro-phenyl)-5-methyl-6-oxo-6,7-dihydro-5*H*-imidazo[1,2-*a*]imidazole-3-carboxylic-^{13}*C* acid, [^{13}C]-(6):** Anhydrous DMF (2 mL) was added to a vial containing carbon-13 sodium formate (352 mg, 5.05 mmol) followed by Hünig's base (0.7 mL, 4.0 mmol) and acetic anhydride (0.4 mL, 4.0 mmol) and stirred at rt. Stirring was continued for 30 min at room temperature, the mixture become thick Gas bubbles were observed. LiCl (263 mg, 6.2 mmol) was dissolved in anhydrous DMF (4 mL) by sonication in a separate vial to give a clear solution. To this vial, were added the iodide (**5**) (1.1 g, 2.0 mmol) and $Pd(OAc)_2$ (90 mg, 0.4 mmol). The resulting solution was added to the sodium formate vial and the mixture was heated to 80 ^{o}C and stirred for 14 h. LCMS showed all the starting material was consumed and the presence of one major product co-elutes with the unlabeled acid standard (**6**). The dark mixture was diluted with EtOAc (50 mL) and treated with 2.0 N of aqueous HCl (40 mL). The organic phase was removed and washed with brine (40 mL ×2), dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give 1.2 g of foam. Purification by silica gel chromatography using 40 g disposable column and 10% to 30% EtOAc:CH_2Cl_2 gave 0.75 g of pure product as an

off-white solid in 81% yield. LCMS, R_t = 1.76 min, MH^+ = 459.48 (100%), 460.48(80%). ^{1}HNMR (400 MHz, $CDCl_3$) δ: 10.2(s, 1H), 8.1(s, 1H), 7.64(d, *J* = 5.8 Hz, 2H), 7.44(d, *J* = 8.21 Hz, 2H), 7.01(d, *J* = 8.21 Hz, 2H), 3.48(d, *J* = 13.64 Hz, 1H), 3.38(d, *J* = 13.64 Hz, 1H), 1.98(s, 3H).

(*R*)-3-(4-Cyanobenzyl)-1-(3,5-dichloro-4-fluorophenyl)-*N*-((2*S*,3*R*)-1-((1-(5-(1,3-dimethyl-1*H*-pyrazol-4-yl)pyrimidin-2-yl)cyclopropyl)amino)-3-hydroxy-1-oxobutan-^{13}*C*$_4$-2-yl)-3-methyl-2-oxo-2,3-dihydro-1*H*-imidazo[1,2-a]imidazole-5-carboxamide-^{13}*C*,^{15}*N*, [^{13}C$_5$,^{15}N]-(3): The acid **[^{13}C]-(6)** (461 mg, 1.0 mmol) was dissolved in anhydrous THF (10 mL). Two drops of DMF were added and the solution was cooled in an ice-bath. Oxalyl chloride (152 mg, 1.2 mmol) was added dropwise and the resulting solution was warmed gradually to room temperature and stirred for 14 h. HPLC of a sample quenched with morpholine showed only the morpholine derivative. The reaction was concentrated *in vacuo* to foam. The amine **[^{13}C$_4$,^{15}N]-(8)** (372 mg, 1.0 mmol) was suspended in anhydrous THF (10 mL). Hünig's base (260 mg, 2.0 mmol) was added dropwise at room temperature to give a colorless solution with some white precipitate. The mixture was stirred for 1 h. The above acylchloride was dissolved in THF (6 mL) and added dropwise to the reaction flask containing the amine **[^{13}C$_4$,^{15}N]-(8)**. The flask was washed with THF (4 mL) and added to the reaction. After stirring for 1 h at 0 ^{o}C, the ice-bath was removed and the mixture was stirred for 14 h at ambient temperature. The reaction was quenched with water (30 mL) and MTBE (50 mL) was added. The aqueous (bottom) phase was removed and the organic was washed with 2 % aqueous $NaHCO_3$ (50 ml ×2), dried over Na_2SO_4, filtered and concentrated *in vacuo* to give 0.9 g of a yellow solid. Purification using 40 g disposable silica gel column and up to 5% MeOH/CH_2Cl_2 gave 669 mg of pure material as an off-white solid in 86% yield. LCMS: t_R = 1.06 min, MH^+ = 775.6 (30%), 776.8 (100%), 779.8 (30%). EI^-, M^- = 774.1 (30%), 775.0(100%), 777.9(30%). HPLC: Halo-C18 (2.1 x 50 mm, 2.7 μm), 20% to 100% B in 9 min, A = H_2O (0.1% TFA), B= MeCN, t_R = 5.83 min, 99.25% at 230 nm. HRMS: MH^+ m/z 777.2263 observed, calculated m/z 777.2258; HPLC area % purity 99.1%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.59(s, 2H), 7.71(d, *J* = 4 Hz, 2H), 7.60(d, *J* = 28 Hz, 2H), 7.55(s, 1H), 7.48(s, 1H), 7.43(m, 1H), 7.41(d, *J* = 8 Hz, 2H), 7.15(s, 1H), 7.02(d, *J* = 8.0 Hz, 2H), 5.55(q, *J* = 7 Hz, 0.5 H), 5.30(m, 0.5 H), 4.95(m, 0.5 H), 4.79(m, 0.5 H), 4.60(m, 0.5 H), 4.42(m, 0.5 H), 4.16(d, *J* = 12Hz, 1H), 3.90(s, 3H), 3.58(q, *J* = 5 Hz, 1H), 3.37(d, *J* = 12Hz, 1H), 2.35(s, 3H), 1.94(s, 3H), 1.78(m, 2H), 1.56(m, 2H), 1.47(d, *J* = 4 Hz, 1H), 1.44(t, *J* = 6 Hz, 2H, 1.28(m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 172.01(d, *J* = 51 Hz), 159.25(d, *J* = 50 Hz),

154.79, 148.19, 145.97, 139.90, 131.91, 130.88, 129.06, 128.86, 124.85, 123.08, 122.81, 118.24, 113.54, 111.63, 69.30, 68.14(t, *J* = 38 Hz), 58.54(ddd, *J* = 11.06, 35.22, 41.30 Hz), 52.87, 42.31, 38.94, 36.94, 22.67, 18.20(d, *J* = 40.25 Hz), 14.12, 12.99.

Conclusion:

A two-step radiosynthesis route was used to prepare two highly potent LFA-1 antagonists labeled with carbon-14 with specific activities higher than 56 mCi/mmol and radiochemical purities of 99%. The synthesis included a palladium catalyzed [^{14}C]carbonylation of an iodide derivative using carbon-14 sodium formate and amide bond formation between the carbon-14 labeled carboxylic acid and the corresponding chiral primary amine derivatives (**7**) and (**8**). Deuterium labeled (**2**) was prepared in four steps using commercially available deuterium labeled 2-cyanopyridine which was subjected Kulinkovich-Szymoniak aminocyclopropanation followed by amide bond formation with deuterium labeled BOC-L-alanine, then removal of the BOC-protecting group and coupling to acid (**6**). The availability of L-threonine-$^{13}C_4$,^{15}N allowed the preparation of the amine [$^{13}C_4$,^{15}N]-(**8**) in only three steps with 80% overall yield. Coupling this amine to [^{13}C]-(**6**) gave [$^{13}C_5$,^{15}N]-(**3**) in very good yield. In conclusion, we report our approach to the preparation of these chiral compounds with rather complicated structures in fewer steps and provide valid tools for drug metabolism, pharmacokinetics, and other studies.

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[12] The synthesis of this intermediate will be published elsewhere.

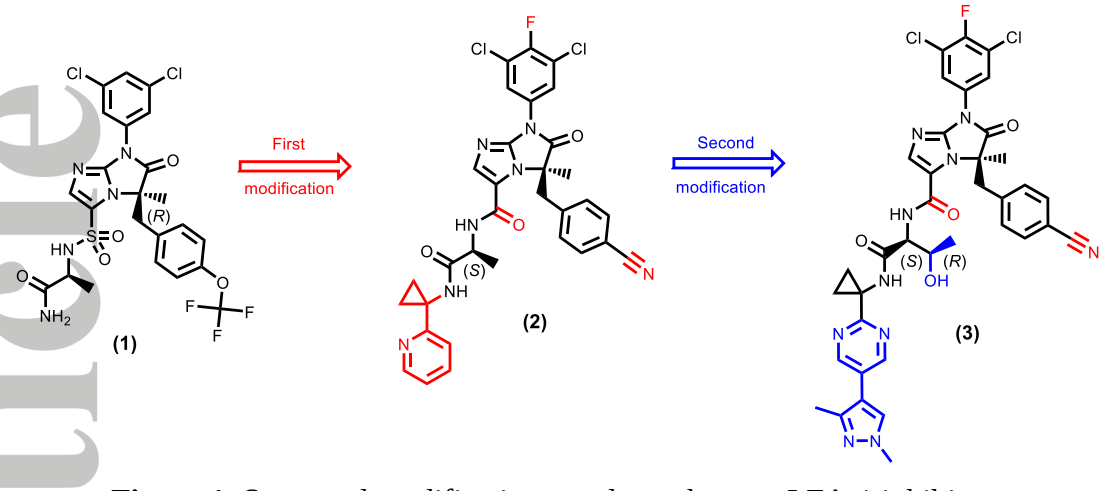


Figure 1: Structural modifications made on the new LFA-1 inhibitors

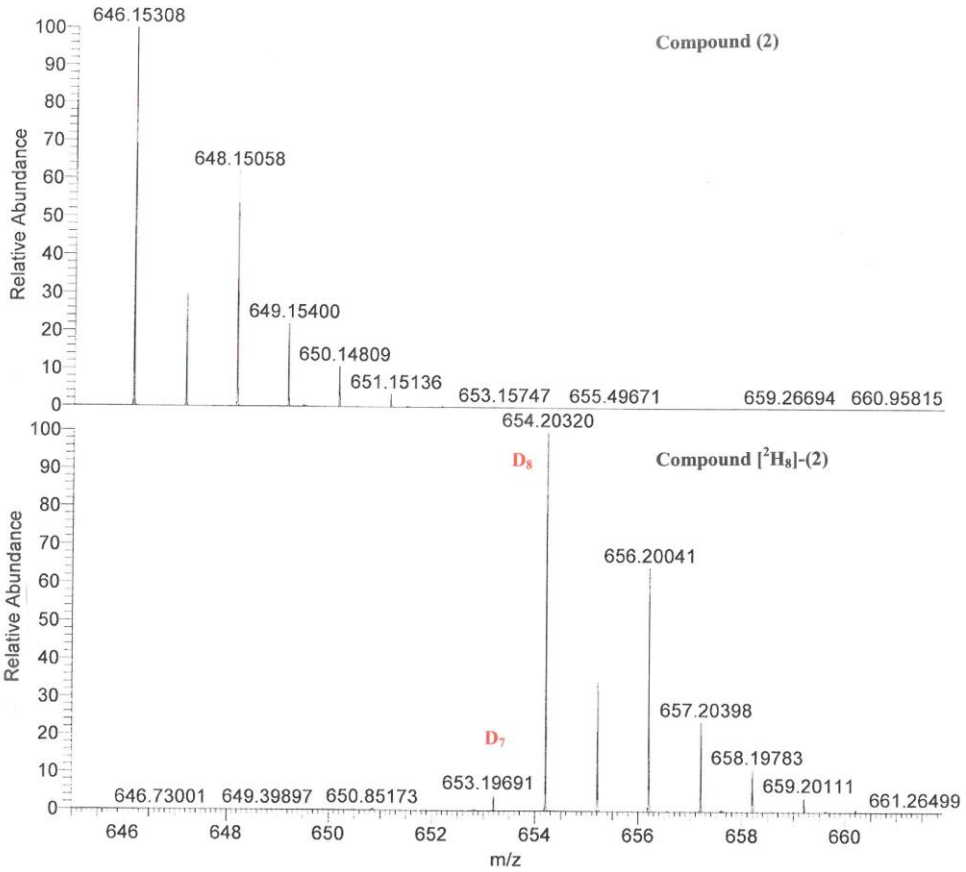
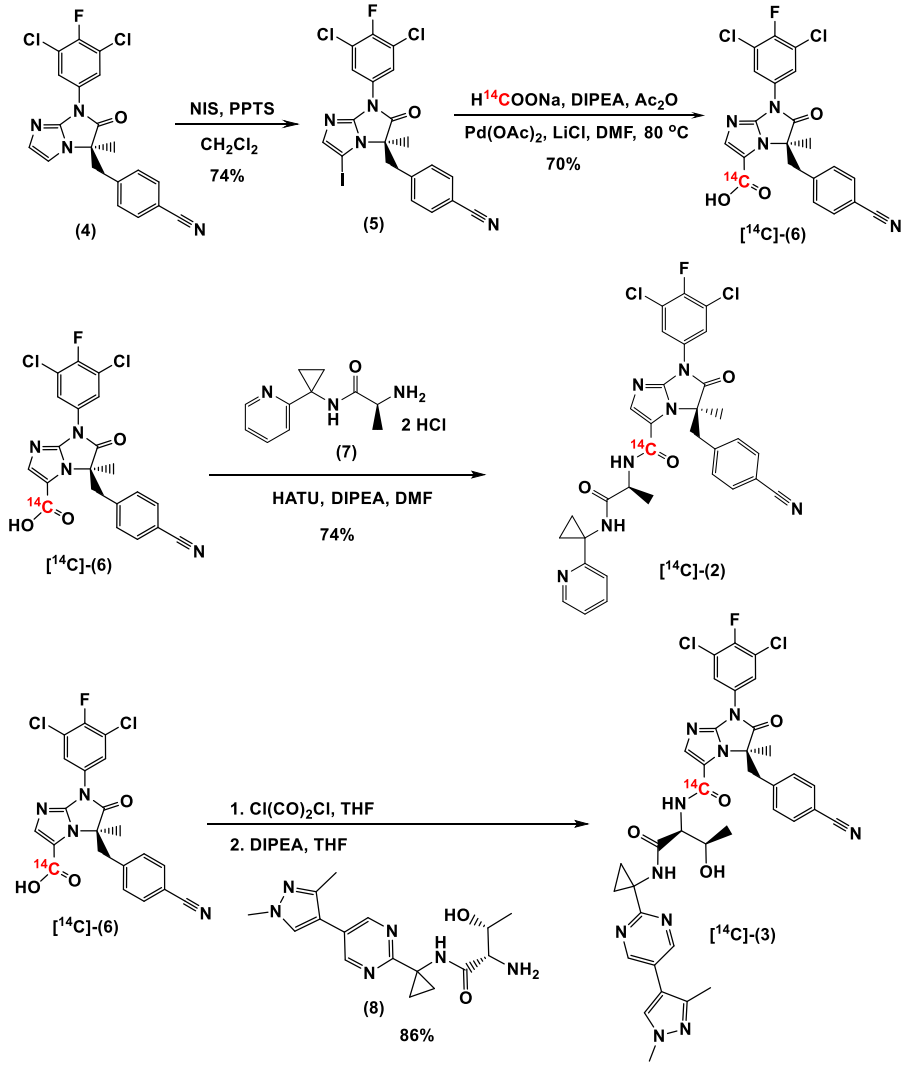
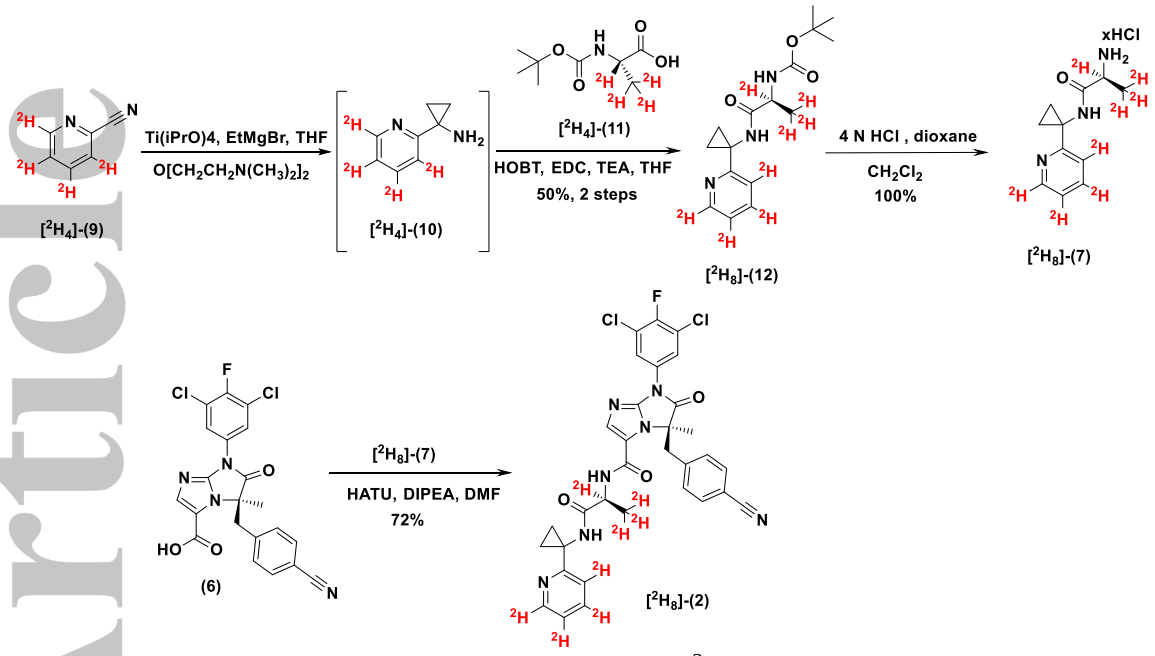


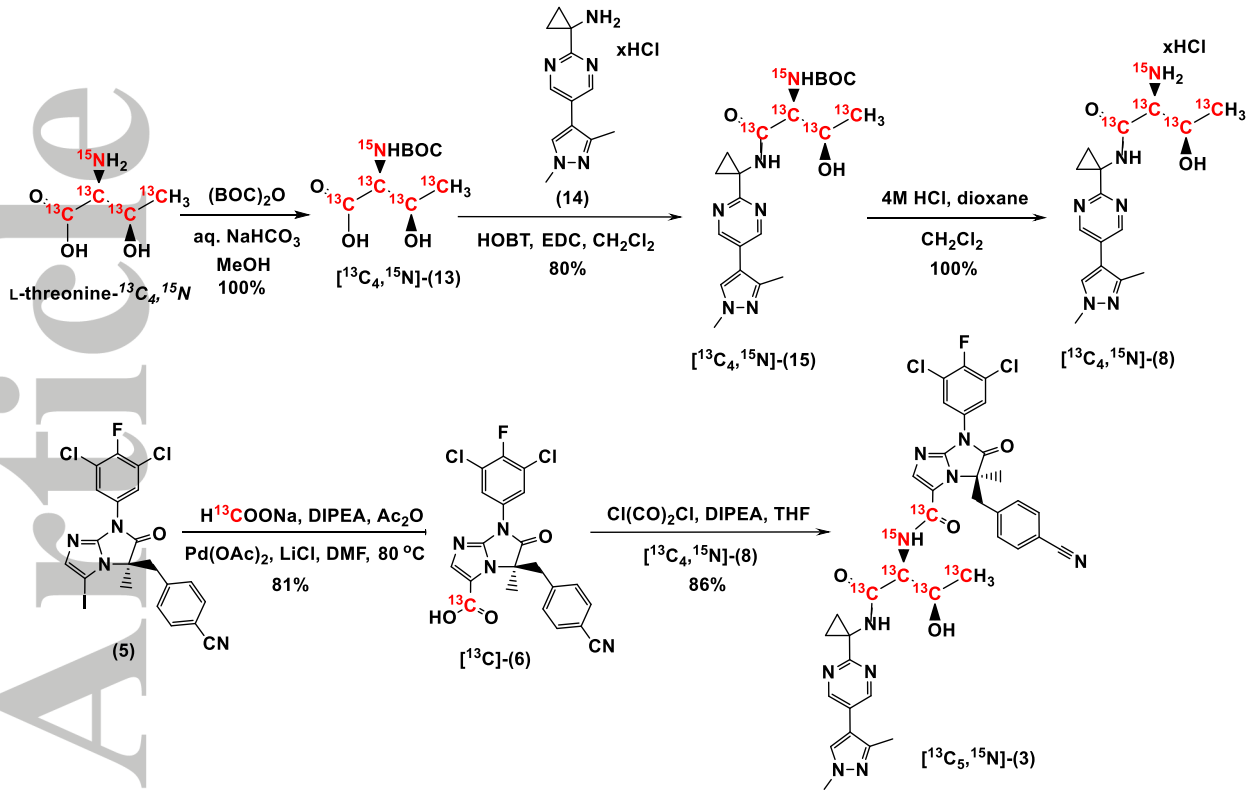
Figure 2: HRMS of unlabeled (**2**) and **[$^2H_8$]-(2)**



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



Scheme 2: Synthesis of [$^2H_8$]-(**2**)



Scheme 3: Synthesis of [$^{13}C_5$,^{15}N]-(**3**)

TOC:

Two highly potent LFA-1 inhibitors (**2**) and (**3**) were efficiently prepared labeled with carbon-14 using a palladium-catalyzed carboxylation of an iodo-precurssor (**5**) and sodium formate-^{14}C followed by amide bond formation with chiral amines (**7**) and (**8**). These two drug candidates were also labeled with stable isotopes by first preparing the chiral amines [$^{2}H_{8}$]-(**7**) and [$^{13}C_{4}$,^{15}N]-(**8**) and then coupling them to (**6**) and [^{13}C]-(**6**).