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Synthesis of potent lymphocyte function-associated antigen-1 inhibitors labeled with carbon-14 and deuterium, part 1

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The lymphocyte function-associated antigen-1 (LFA-1) is an essential component in normal immune system function and is a target for drug discovery for its broad therapeutic potential in treating inflammatory diseases. Here, we report the synthesis of three potent antagonists of LFA-1 labeled with carbon-14 and deuterium to support drug metabolism and pharmacokinetics studies. Carbon-14 labeled (*R*)-1-acetyl-5-(4-bromobenzyl)-3-(3,5-dichlorophenyl)-5-methyl-imidazolidine-2,4-dione (1) was prepared in 27% radiochemical yield in two steps and with a specific activity of 2.1 GBq/mmol by using [^{14}C]-phosgene. Carbon-14 labeled 5-bromopyrimidine was used to prepare (*R*)-5-(1-piperazinylsulfonyl)-1-(3,5-dichlorophenyl)-3-[4-(5-pyrimidinyl) benzyl]-3-methyl-1-*H*-imidazo[1,2a]imidazol-2-one (2) and (*R*)-1-[7-(3,5-dichlorophenyl)-5-methyl-6-oxo-5-(4-pyrimidin-5-yl-benzyl)-6,7-dihydro-5*H*-imidazo[1,2-a]imidazole-3-sulfonyl]piperidin-4-carboxylic acid amide (3) via a Suzuki reaction with the corresponding boronic acid esters in 42% and 67% radiochemical yield and specific activities of 1.85 GBq/mmol and 1.95 GBq/mmol, respectively. Deuterium labeled piperazine was reacted with the sulfonyl chloride derivative (7), followed by a Suzuki coupling to the pyrimidine boronic ester to give deuterium labeled (2) in 47% yield. Deuterium labeled isonipecotamide was reacted in a similar way with the sulfonyl chloride derivative (14) to furnish deuterium labeled (3) in one step and in 94% yield.

Keywords: carbon-14; deuterium; LFA-1 antagonists; metabolism; synthesis

Introduction

Adhesion between T cells and antigen-presenting cells is necessary for the formation of the immunological synapse. This adhesion is mediated by T cell surface molecules including the lymphocyte function-associated antigen-1 (LFA-1) and the intracellular adhesion molecule (ICAM) ligands.[1–4] To appreciate the physiological importance of LFA-1, individuals with leukocyte adhesion deficiency lack the ability to clear pathogens from their bodies, suffer from recurrent infections, and die at a young age.[5] LFA-1 plays a role in organ transplantation and in the progression of diseases including rheumatoid arthritis, multiple sclerosis, Crohn's diseases, and psoriasis. Because of this broad therapeutic potential in treating these inflammatory diseases, LFA-1 is a target for drug discovery. Inhibitors of LFA-1 to its counter-receptors ICAM-1, ICAM-2, ICAM-3, and other receptors have attracted considerable attention in the pharmaceutical industry.[6–13] For example, the primary drug resistance is a major problem in the incurable disease of the bone marrow, multiple myeloma. Targeting LFA-1 by monoclonal antibodies or by small molecules was shown to reduce cell adhesion-mediated drug resistance significantly, which is responsible for this strong primary drug resistance.[14] The inhibition of ICAM-1/LFA-1 interactions was also shown to play a role in HIV-1 attachment to target cells. Once ICAM-1 is acquired by HIV-1, it promotes virus infectivity via ligation of LFA-1, which helps in HIV-1 adsorption into the host cells.[15,16]

BIRT377 (Figure 1) was the first small molecule reported to reversibly and specifically inhibit LFA-1 binding to ICAM-1.[6] The binding site of BIRT377 was also identified.[17] Although this molecule demonstrated good molecular and cellular potency, it was poorly soluble in aqueous solution and was rapidly metabolized *in vitro* by human liver microsomes by *N*-demethylation. Replacement of this methyl group with an acetyl, such as that in compound (**1**), led to improved metabolic stability. However, (**1**) was still prone to phase I metabolic *N*-deacetylation. The search for compounds with superior pharmacokinetic profiles led to the introduction of the five-membered ring at this nitrogen and the adjacent carbon in compounds (**2**) and (**3**).[18] Further, replacement of the bromide by a pyrimidine moiety enhanced the water solubility.[19]

[^{14}C]-(**1**) was prepared according to Scheme 1. Thus, (*R*)-2-amino-3-(4-bromophenyl)-*N*-(3,5-dichlorophenyl)-2-methyl-propionamide hydrochloride (**4**)[20,21] underwent cyclization to hydantoin by using carbon-14 labeled phosgene under Schotten–Baumann conditions.[22] The isocyanate was postulated as being formed first and subsequently cyclized to the hydantoin.[23] The product (*R*)-5-(4-bromobenzyl)-3-(3,5-dichlorophenyl)-5-methyl-imidazolidine-2,4-dione (**5**) was easily isolated in methylene chloride solution. Treatment of this compound in dry THF with

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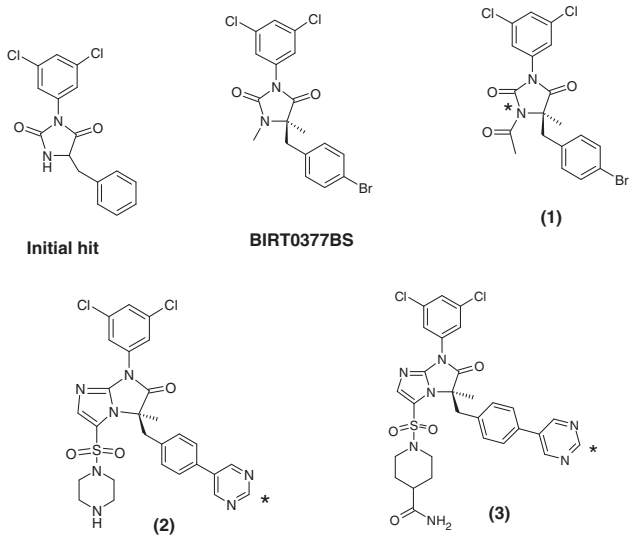
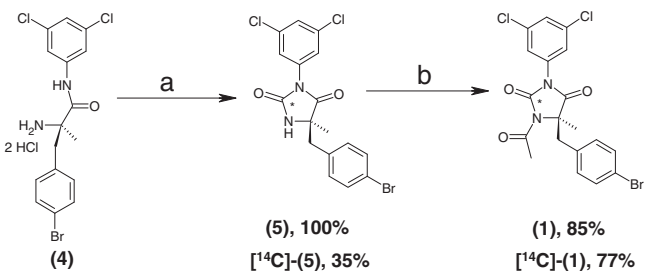


Figure 1. Structures of LFA-1 antagonists. Asterisks indicate position of the radioactive carbon.

KHMDS at −25 °C, followed by the addition of acetyl chloride, gave the desired product [^{14}C]-(**1**) in 27% overall radiochemical yield and a specific activity of 56.75 mCi/mmol (Scheme 1).

In the synthesis of [^{14}C]-(**2**) (Scheme 2), the iodo derivative (**6**)[24] was converted to the sulfinate salt by treatment with cyclopentyl magnesium bromide at −40 °C, and the Grignard's product was quenched with sulfur dioxide.[25] Reaction with *N*-chlorosuccinimide gave the sulfonyl chloride (**7**) that was reacted directly with 1-*tert*-butoxycarbonyl piperazine and triethylamine as a base in THF to give (**8**) in 81% overall yield. Heating compound (**8**) with *bis*(pinacolato)diboron in the presence of catalytic amounts of [1,1′-*bis*(diphenylphosphino)-ferrocene] dichloropalladium [$PdCl_2$(dppf)] and 4.5 equivalent of potassium acetate in DMF at 80 °C gave the boronate ester (**9**) in 77% yield after flash chromatography purification. The availability of carbon-14 labeled 5-bromopyrimidine from commercial suppliers[26] prompted us to prepare the boronic ester (**9**) and then couple it to [^{14}C]-5-bromopyrimidine in one radiosynthesis under Suzuki conditions to give (**10**). Removing the protecting group by using TFA gave the product [^{14}C]-(**2**) with a specific activity of 50 mCi/mmol and in 42% radiochemical yield.

For the deuterium labeled compounds, it was very important to have the molecular ion peak of these compounds at least five units higher than the unlabeled compounds to avoid overlapping with ion peaks resulting from the two chlorine atoms



Scheme 1. (a) ClCOCl or Cl^{14}COCl, sat'd $NaHCO_3$, CH_2Cl_2, 0 °C to rt; (b) KHMDS, THF, CH_3COCl, −25 °C to rt.

during analytical measurement by mass spectrometry. Deuterium labeled (**1**) was not synthesized because of no further interest in this compound due to its metabolism as mentioned previously. In the synthesis of [$^2H_8$]-(**2**), the sulfonyl chloride (**7**) was reacted with [$^2H_8$]-piperazine dihydrochloride monohydrate to give the bromide derivative (**11**) in 59% yield. At least two equivalents of labeled piperazine had to be used to minimize the double sulfonylation of piperazine. Higher yields were obtained when using protected piperazine. However, monoprotected labeled piperazine was not available commercially. The bromide derivative (**11**) was then reacted with 5-(4,4,5,5,-tetramethyl-[1,2,3]dioxaborolan-2-yl)-pyrimidine under Suzuki conditions by using sodium carbonate and toluene : ethanol as solvent to give the desired product in 80% yield (Scheme 2).

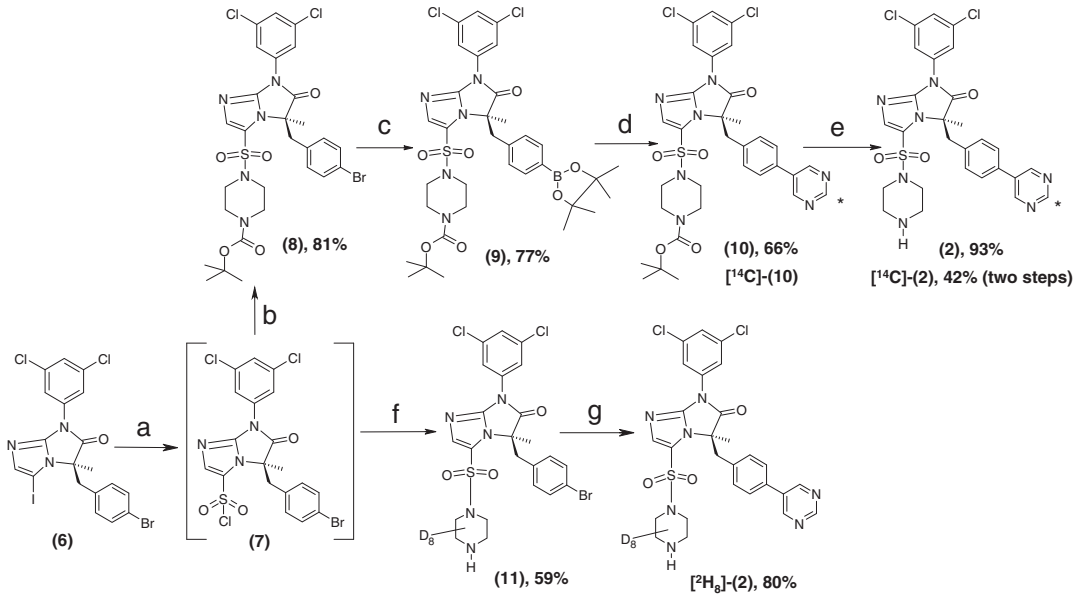
Compound [^{14}C]-(**3**), 1-[7-(3,5-dichlorophenyl)-5-methyl-6-oxo-5-(4-pyrimidin-5-yl-benzyl)-6,7-dihydro-5*H*-imidazo[1,2-a]imidazole-3-sulfonyl]-piperidine-4-carboxylic acid amide, labeled with carbon-14, was prepared with a specific activity of 52.8 mCi/mmol. The synthesis was simple and straightforward, starting from the in-house available (**12**) and the commercially available [^{14}C]-labeled 5-bromopyrimidine, as seen before (Scheme 3). The boronic ester (**13**) was prepared in yields ranging from 80% to 90% as described previously by using *bis*(pinacolato)diboron in the presence of potassium acetate and catalytic amounts of [$PdCl_2$(dppf)] in DMF. The same catalyst was used in the second step, the only radiosynthetic step with potassium carbonate and dimethoxyethane as solvent. Thus, the resulting boronic ester was reacted with 5-bromopyrimidine,[2-^{14}C]- to give labeled (**3**) in 67% radiochemical yield.

Deuterium labeled (**3**) was prepared from the in-house available sulfonyl chloride derivative (**14**) with the pyrimidine already attached and deuterated isonipecotamide to give deuterated (**3**) in one step and in 94% yield.

Experimental procedures

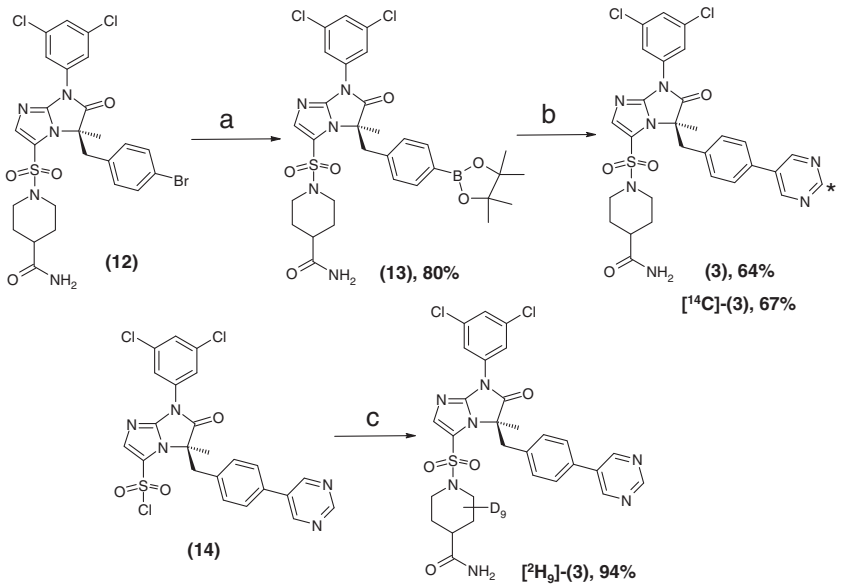
Materials and methods

Liquid scintillation counting was accomplished using a Beckman LS5000TA and ready safe™ cocktail (Beckman, Fullerton, CA, USA). Radio-TLC was carried out on a BIOSCAN System 200 imaging scanner by using an auto-changer 1000 and WinScan software version 2.1a (Bioscan, Inc., Washington, DC, USA). The quantification of the HPLC chromatograms was carried out using an HPLC system composed of a radiomatic A515 Flo-one/beta radioactivity flow detector (Packard Instrument Company, Meriden, CT, USA), two pumps (HITACHI L-6200A intelligent pump), a linear UVIS 200, Ultima Flo™ AP cocktail (Packard, Meriden, CT, USA), and radiomatic 500TR V 3.60 for data evaluation. The analytical HPLC purity verification for compound (**1**) was carried out on a 3MZ-18 column, particle size 3 μm, 4.6 × 150 mm (Cohesive Technologies, Inc., Franklin, MA, USA). Mobile phase: A (water), B (methanol), both solvents contain triethylamine (TEA, 10 mM) and use a gradient: 50% to 100%B in 30 min. For compound (**2**), the HPLC was carried out on an MV-C18 column, particle size 5 μm, 5 × 250 mm (Rainin Instrument Company, Inc., Woburn, MA, USA). Mobile phase: A (water), B (acetonitrile), both solvents contain TFA (10 mM) and use a gradient: 40% to 80%B in 20 min, hold at 80%B to 30 min. For compound (**3**), HPLC was carried out on a Zorbax Eclipse XDB-C8 column, particle size 5 μm, 4.6 × 150 mm (Agilent Technologies, Inc., Santa Clara, CA, USA). Mobile phase: A (water), (acetonitrile), both solvents



Scheme 2. (a) CyMgBr, SO_2, −40 °C, NCS, −20 °C, THF; (b) Boc-piperazine, TEA, CH_2Cl_2, rt; (c) bis(pinacolato)diboron, Pd_2Cl_2(dppf), CH_2Cl_2, KOAc, DMF, 80 °C; (d) [^{14}C]-5-bromopyrimidine, K_2CO_3, $PdCl_2$(dppf), CH_2Cl_2, DME, 85 °C; (e) TFA, CH_2Cl_2, rt; (f) [$^2H_8$]-piperazine, TEA, THF, −20 °C to rt; (g) 5-pyrimidine boronic acid pinacol ester, $PdCl_2$(dppf)·CH_2Cl_2, Na_2CO_3, toluene : EtOH, 85 °C.

contain TFA (10 mM) and use a gradient: 5% to 100%B in 30 min. UV detection for all compounds was carried out at 254 nm. Evaporation of solvents and non-radioactive volatile components was accomplished at reduced pressure by using Büchi rotary evaporator unless stated otherwise. Mass spectra for non-radioactive compounds were acquired by a Hewlett-Packard auto sampler Series 1100, connected to a Micromass Platform LCZ in the ES mode. NMR spectra were recorded with a Bruker 400 MHz DPX spectrometer by using deuterated chloroform as a solvent and tetramethyl silane as the internal standard unless stated otherwise. Melting points are uncorrected and were obtained using MEL-TEMP® 3.0 (Laboratory Devices, Inc., Cambridge, MA, USA). Pre-coated TLC sheets (silica gel 60 F_{254}) and silica gel 60–200 Mesh (Nominal, ID, grade 62) for flash chromatography were obtained from EM Science (Gibbstown, NJ, USA). Phosgene, 20% solution in toluene was obtained from Fluka (Milwaukee, WI, USA). [$^2H_8$]-Piperazine·2HCl·H_2O, 98 at.%^{2}H was purchased from Aldrich. [$^2H_9$]-Isonipecotamide, 99 at.%^{2}H was purchased from CDN (Pointe Claire, Quebec, Canada). [^{14}C]-Phosgene with a specific activity of 58 mCi/mmol was obtained from Vitrax (Placentia, CA, USA). 5-Bromopyrimidine,[2-^{14}C]- with a specific activity of 53 mCi/mmol was purchased from Moravek Biochemicals,



Scheme 3. (a) Bis(pinacolato)diboron, $PdCl_2$(dppf)·CH_2Cl_2, KOAc, DMF, 80 °C; (b) [^{14}C]-5-bromopyrimidine, K_2CO_3, $PdCl_2$(dppf)·CH_2Cl_2, DME, 80 °C; (c) [$^2H_9$]-isonipecotamide, THF : DMF (10:1), TEA, rt.

Inc. (Brea, CA, USA). The rest of the reagents were purchased from Aldrich Chemicals Company.

Synthesis of (*R*)-1-actyl-5-(4-bromobenzyl)-3-(3,5-dichlorophenyl)-5-methyl-imidazolidine-2,4-dione (**1**) and [^{14}C]-(**1**) (Scheme 1)

(*R*)-5-(4-Bromobenzyl)-3-(3,5-dichlorophenyl)-5-methyl-imidazolidine-2,4-dione (**5**): A mixture of (**4**) (633.4 mg, 1.33 mmol) in methylene chloride (30 mL) and a saturated solution of $NaHCO_3$ (40 mL) was stirred at room temperature until all the amine was dissolved. The clear two-phase solution was then cooled in an ice bath to 0 °C. Stirring was stopped, and a solution of phosgene in toluene (1.14 mL, 2 mmol) was added to the organic phase. Stirring was then resumed, and the reaction was warmed to room temperature and stirred for 4 h. The organic phase was then removed, and the aqueous was extracted with methylene chloride. The combined extracts were concentrated *in vacuo* to give 661 mg of a white solid, which was used in the next step without further purification. $R_f = 0.57$ in 10% MeOH/$CHCl_3$, $t_R = 13.25$ min. ^{1}H NMR ($CDCl_3$): δ 7.50(d, $J = 8.26$ Hz, 2H), 7.38(t, $J = 1.83$ Hz, 1H), 7.09(d, $J = 8.26$ Hz, 2H), 7.03(d, $J = 1.83$ Hz, 2H), 6.61(s, 1H, ex.), 3.1(dd, $J = 13.69$, 90.29 Hz, 2H), 1.64(s, 3H). MS-ES$^-$: $[M-1]^- = 427$.

(*R*)-1-Acetyl-5(4-bromobenzyl)-3-(3,5-dichlorophenyl)-5-methyl-imidazolidine-2,4-dione (**1**): To a solution of the aforementioned compound (621 mg, 1.45 mmol) in anhydrous THF (8 mL), stirred at −25 °C under nitrogen, was added a solution of potassium *bis* (trimethylsilyl)amide (3.25 mL, 1.624 mmol, 0.5 M in toluene) dropwise. The resulting yellow solution was stirred at this temperature for 10 min before acetyl chloride (114 μL, 1.6 mmol) was added dropwise. After stirring at −25 °C for 5 min, the cooling bath was removed, and stirring was continued for 90 min at room temperature. Most of the solvent was then evaporated, and the residue was dissolved in methylene chloride (30 mL). A solution of aqueous HCl (1.0 N, 10 mL) was added, and the mixture was stirred vigorously. The organic phase was removed, and the aqueous was extracted with methylene chloride. The combined extracts were dried over $MgSO_4$, filtered and concentrated *in vacuo* to give 973 mg of yellow oil. Purification by flash chromatography using 1% MeOH/$CHCl_3$ gave 636 mg of pure product as viscous colorless oil. $R_f = 0.75$ in 10% MeOH/$CHCl_3$. Crystallization from warm hexane gave 531 mg of white crystalline material in 85% yield. $t_R = 19.6$ min. ^{1}H NMR ($CDCl_3$): δ 7.42 (d, $J = 8.54$ Hz, 2H), 7.38(t, $J = 1.83$ Hz, 1H), 6.95(d, $J = 8.54$ Hz, 2H), 6.90(d, $J = 1.83$ Hz, 2H), 3.50(dd, $J = 14.05$, 237.40 Hz, 2H), 2.56(s, 3H), 1.90(s, 3H).

[^{14}C]-(**5**): As described previously, a solution of [^{14}C]-phosgene (100 mCi, SA = 58 mCi/mmol) in anhydrous toluene (2 mL) was added at 0 °C to the propionamide derivative (**4**) (634 mg, 1.33 mmol). After stirring for 10 h at room temperature, the methylene chloride phase was removed, and the aqueous was extracted with methylene chloride (2 × 30 mL). The combined extracts were concentrated *in vacuo*, and the residue was purified by flash chromatography to give 315 mg of a white solid in 35% radiochemical yield. $R_f = 0.16$ in 1% MeOH/$CHCl_3$.

[^{14}C]-(**1**): To a solution of the aforementioned compound (315 mg, 0.736 mmol) in anhydrous THF (6 mL) stirred under nitrogen atmosphere at −25 °C was added a solution of KHMDS (1.65 mmol, 0.824 mmol, 0.5 M in toluene) dropwise. Acetyl chloride (59 μL, 0.824 mmol) was added. The cooling bath was removed, and the reaction was warmed to room temperature and stirred for 12 h. An aqueous solution of HCl (10 mL, 1%) was added and stirred for 15 min. The aqueous phase was extracted with methylene chloride, and the combined extracts were concentrated under reduced pressure. The residue was purified by flash chromatography using 1% MeOH/$CHCl_3$ to give a white solid, which was crystallized from hot hexane to give 222 mg of material. A total activity of 27 mCi was obtained with a specific activity of 56.75 mCi/mmol. HPLC ($t_R = 17.4$ min) and radio-TLC (plate developed in 5% MeOH/$CHCl_3$) showed the product to be more than 99% pure. ^{1}H NMR in $CDCl_3$ was identical to unlabeled material.

Synthesis of (*R*)-5-(1-piperazinylsulfonyl)-1-(3,5-dichlorophenyl)-3-[4-(5-pyrimidinyl)benzyl]-3-methyl-1-H-imidazo[1,2-a]imidazol-2-one (**2**), [$^2H_8$]-(**2**), and [^{14}C]-(**2**) (Scheme 2)

4-[(*R*)-5-(4-Bromobenzyl)-7-(3,5-dichlorophenyl)-5-methyl-6-oxo-6,7-dihydro-5H-imidazo[1,2-a]imidazole-3-sulfonyl]-piperazine-1-carboxylic acid *tert*-butyl ester (**8**): To a solution of (**6**) (2.83 g, 4.9 mmol) in THF (50 mL), stirred at −40 °C under nitrogen atmosphere, was added dropwise a solution of cyclopentyl magnesium bromide (2.94 mL, 5.88 mmol, 2.0 M solution in ether). The resulting solution was further stirred at this temperature for 20 min, and then sulfur dioxide was slowly bubbled into the reaction flask for 5 min at −50 °C. The reaction flask was warmed gradually to room temperature and was stirred for 1 h. Solvents and excess sulfur dioxide were then removed under reduced pressure, and the residue was further dried to give a yellow-orange foam, which was dissolved in dry THF (30 mL) and added at −20 °C to a suspension of *N*-chlorosuccinimide (3 g, 22.05 mmol) in dry THF (30 mL). The resulting mixture was stirred at this temperature for 20 min to give (**7**). To this mixture, 1-Boc-piperazine (2 g, 10.78 mmol) and triethylamine (1.5 mL, 10.78 mmol) in dry THF (30 mL) were added. The reaction was warmed to room temperature and was stirred for 12 h then diluted with ethyl acetate (200 mL). The organic phase was washed with aqueous HCl (0.1 N, 200 mL), brine (200 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo* to give 5.5 g of brown foam. Purification by flash chromatography gave 3 g of pure product. ^{1}H NMR ($CDCl_3$): δ 7.36(m, 2H), 7.32(m, 2H), 7.26 (m, 2H), 6.81(d, $J = 8.29$ Hz, 2H), 3.81(d, $J = 13.91$ Hz, 1H), 3.57 (brs, 4H), 3.21(d, $J = 13.91$ Hz, 1H), 3.20(m, 4H), 1.97(s, 3H), 1.45 (s, 9H). $R_f = 0.31$ in 10% EtOAc : CH_2Cl_2. MS-ES$^+$: $[MH]^+ = 700.2$.

4-{(*R*)-7-(3,5-Dichlorophenyl)-5-methyl-6-oxo-5-[4-(4,4,5,5,-tetramethyl-[1,3,2]dioxaborolan-2-yl]6,7-dihydro-5H-imidazo[1,2-a]imidazole-3-sulfonyl}-piperazine-1-carboxylic acid *tert*-butyl ester (**9**): To a mixture of (**8**) (0.97 g, 1.4 mmol), potassium acetate (0.61 g, 6.23 mmol), *bis*(pinacolato)diboron (0.53 g, 2.1 mmol), $PdCl_2$ (dppf)·CH_2Cl_2 (0.17 g, 0.21 mmol), was added dry DMF (10 mL) under nitrogen atmosphere, and the resulting dark mixture was heated to 80 °C overnight. After cooling to room temperature, the mixture was filtered through a short pad of Celite®, and the filtrate was washed with water (250 mL), brine (100 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by flash chromatography using 30% EtOAc : Hexanes as eluant to give 795 mg of white foam in 77% yield. $R_f = 0.23$ in 10% EtOAc : CH_2Cl_2. ^{1}H NMR ($CDCl_3$): δ 7.55(d, $J = 7.57$ Hz, 2H), 7.36(s, 1H), 7.27(m, 1H), 7.21(m, 2H), 6.90(d, $J = 7.65$ Hz, 2H), 3.84(d, $J = 13.76$ Hz, 1H), 3.57(brs, 4H), 3.24(d, $J = 13.76$ Hz, 1H), 3.57(brs, 4H), 3.24(d, $J = 13.76$ Hz, 1H), 3.23(brs, 4H), 1.98(s, 3H), 1.45(s, 9H), 1.26(brs, 12H). MS-ES$^+$: $[MH]^+ = 746.5$.

4-[(*R*)-7-(3,5-Dichlorophenyl)-5-methyl-6-oxo-5(4-pyrimidin-5-yl-benzyl)6,7-dihydro-5-H-imidazo[1,2-a]imidazole-3-sulfonyl]-piperazine-1-carboxylic acid *tert*-butyl ester (**10**): A mixture of the aforementioned boronic acid ester (**9**) (0.34 g, 0.46 mmol), 5-bromopyrimidine (74 mg, 0.46 mmol), $PdCl_2$(dppf)·CH_2Cl_2 (40 mg,

0.04 mmol), and potassium carbonate (0.32 g, 2.3 mmol) in dimethoxyethane (8 mL) was heated at 80 °C under nitrogen atmosphere for 7 h. The resulting dark mixture was then cooled to room temperature, and water (50 mL) was added, and the mixture was extracted with ethyl acetate (2 × 150 mL). The combined extracts were dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification of the residue by flash chromatography using 5% to 10% EtOAc : Hexanes followed by crystallization from ethyl acetate : hexanes gave 210 mg of white solid in 66% yield. $R_f = 0.27$ in 50% EtOAc : Hexanes. ^{1}H NMR ($CDCl_3$): δ 9.17 (s, 1H), 8.84(s, 2H), 7.36(m, 3H), 7.31(m, 3H), 7.08(d, $J = 8.10$ Hz, 2H), 3.93(d, $J = 13.85$ Hz, 1H), 3.60(brs, 4H), 3.32(d, $J = 13.85$ Hz, 1H), 3.26(brs, 4H), 2.02(s, 3H), 1.62(s, 3H), 1.45(s, 9H), 1.26(s, 9H). MS-ES$^+$: $[MH]^+ = 698.4$.

(*R*)-5-(1-Piperazinylsulfonyl)-1-(3,5-dichlorophenyl)-3-[4-(5-pyrimidinyl)benzyl]-3-methyl-1-H-imidazo[1,2-a]imidazol-2-one (**2**): A solution of (**10**) (64 mg, 0.0916 mmol) in methylene chloride (5 mL) and TFA (0.5 mL) was stirred at room temperature for 90 min. It was then poured into aqueous NaOH (1.0 N, 10 mL) and extracted with methylene chloride. The combined extracts were dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by flash chromatography gave 51 mg of the desired product in 93% yield. ^{1}H NMR ($CDCl_3$): δ 9.16(s, 1H), 8.83(s, 2H), 7.35 (m, 3H), 7.31(m, 2H), 7.27(m, 1H), 7.08(d, $J = 8.14$ Hz, 2H), 3.93(d, $J = 13.81$ Hz, 1H), 3.46(s, 3H), 3.26(m, 4H), 3.28(d, $J = 13.80$ Hz, 1H), 3.01(m, 4H), 2.01(s, 3H). MS-ES: MH^+: 598.4 (100%). MS-ES$^-$: $[M + OAc]^- = 656$.

(*R*)-3-(4-Bromobenzyl)-1-(3,5-dichlorophenyl)-3-methyl-5-(piperazine-1-sulfonyl)-1H-imidazo[1,2-a]imidazol-2-one (**11**): To a solution of (**6**) (1.41 g, 2.45 mmol) in THF (30 mL), stirred at −40 °C under nitrogen atmosphere, was added dropwise a solution of cyclopentyl magnesium bromide (1.47 mL, 2.94 mmol, 2.0 M solution in ether). The resulting solution was further stirred at this temperature for 20 min. Then, sulfur dioxide was slowly bubbled into the reaction flask for 5 min at −50 °C. The reaction was then warmed gradually to room temperature and was stirred for another hour. Solvent and excess sulfur dioxide were removed *in vacuo*, and the residue was further dried for 1 h under reduced pressure to give a yellow-orange foam, which was used in the next step without further purification. The aforementioned sulfinate salt was dissolved in dry THF (15 mL) and was added at −20 °C to a suspension of *N*-chlorosuccinimide (0.5 g, 3.67 mmol) in dry THF (15 mL). The resulting mixture was stirred at this temperature for 20 min to give (**7**), which was then added via cannulation to a flask containing [$^2H_8$]-piperazine-hydrochloride monohydrate (1.0 g, 5.4 mmol), triethylamine (2.2 mL, 15.8 mmol), and ethanol (10 mL) at −30 °C. The resulting mixture was warmed to room temperature slowly and then concentrated *in vacuo*. The residue was dissolved in ethyl acetate (200 mL) and was washed with water (200 mL), brine (100 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo* to give 2.02 g of a yellow solid. Purification by flash chromatography using chloroform and then 10% methanol/chloroform gave 0.87 g of white foam in 59% yield. ^{1}H NMR ($CDCl_3$): δ 7.38(m, 2H), 7.40(s, 1H), 7.32(m, 1H), 7.25(m, 2H), 6.82(d, $J = 8.35$ Hz, 2H), 3.83(d, $J = 13.84$ Hz, 1H), 3.22(d, $J = 13.91$ Hz, 1H), 1.98(s, 3H). MS-ES$^+$: $[MH]^+ = 606.1$: 80%, 608.1: 100%, 610.1: 60%.

[$^2H_8$]-(**2**): A mixture of the aforementioned compound (0.7 g, 1.132 mmol), 5-pyrimidine boronic acid pinacol ester (0.546 g, 2.65 mmol), $PdCl_2(dppf)\cdot CH_2Cl_2$ (28 mg, 3 mol%), aqueous Na_2CO_3 (2.0 M solution, 2 mL) in toluene (8 mL) and ethanol (4 mL) was heated at 85 °C for 4 h. The mixture was then cooled to room temperature, and most of the solvents were removed under reduced pressure. Ethyl acetate (100 mL) and water (50 mL) were added, and the aqueous phase was extracted with ethyl acetate (2 × 100 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo* to give 1.0 g of a foamy residue. Purification by flash chromatography followed by preparative TLC gave 682 mg of pure product in 80% yield. ^{1}H NMR ($CDCl_3$): δ 9.17(s, 1H), 8.84(s, 2H), 7.36(m, 5H), 7.28(m, 1H), 7.09(d, $J = 8.15$ Hz, 2H), 3.95(d, $J = 13.81$ Hz, 1H), 3.32(d, $J = 13.77$ Hz, 1H), 2.02(s, 3H). MS-ES$^+$: $MH^+ = 606.2$: 100%, $[MH + CAN]^+ = 747.2$ (20%). MS-ES$^-$: $[M + OAc]^- = 664.0$ (100%). HPLC: $t_R = 9.8$ min, 99.9%.

[^{14}C]-(**2**): A mixture of the boronic acid ester (**9**) (239 mg, 0.32 mmol), 5-bromopyrimidine,[2-^{14}C]- (16 mCi, 48 mg, 0.3 mmol, SA = 53 mCi/mmol), $PdCl_2(dppf)\cdot CH_2Cl_2$ (40 mg, 0.05 mmol), potassium carbonate (221 mg, 1.5 mmol) in DME (10 mL) was refluxed for 8 h. After cooling to room temperature, water was added (40 mL), and the mixture was extracted with ethyl acetate. The combine organic extracts were dried over $MgSO_4$, filtered and concentrated *in vacuo*. The pure product was obtained after flash chromatography purification using 30% to 50% EtOAc : Hexanes, $R_f = 0.25$ in 50% EtOAc : Hexanes; HPLC and TLC similar to (**9**). This material was then dissolved in methylene chloride (10 mL), and TFA (2 mL) was added. The resulting solution was stirred at room temperature for 90 min. Then it was poured into an ice cold solution of NaOH (1.0 N, 40 mL) and extracted with methylene chloride. The combined extracts were dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by flash chromatography using 50% EtOAc : Hexanes to give 80 mg of pure material as a white solid. Total activity was 6.68 mCi and a specific activity of 50 mCi/mmol. ^{1}H NMR was identical to unlabeled (**2**).

Synthesis of (*R*)-1-[7-(3,5-dichlorophenyl)-5-methyl-6-oxo-5-[4-pyrimidin-5-yl-benzyl)-6,7-dihydro-5H-imidazo[1,2-a]imidazole-3-sulfonyl]-piperidine-4-carboxylic acid amide (**3**), [$^2H_9$]-(**3**), and [^{14}C]-(**3**) (Scheme 3)

1-{(*R*)-7-(3,5-Dichlorophenyl)-5-methyl-6-oxo-5-[4,4,5,5-tetramethyl-[1,3,2]dioxaborolan-2-yl)-benzyl]-6,7-dihydro-5H-imidazo[1,2-a] imidazole-3-sulfonyl}-piperidine-4-carboxylic acid amide (**13**): A mixture of (**12**) (1.28 g, 2.0 mmol), potassium acetate (0.881 g, 9.0 mmol), $PdCl_2(dppf)\cdot CH_2Cl_2$ (0.245 g, 0.3 mmol), and *bis*(pinacolato)diboron (0.9 g, 3.47 mmol) in DMF (20 mL) was heated at 80 °C under nitrogen atmosphere for 14 h. The dark mixture was cooled to room temperature and diluted with ethyl acetate (300 mL) then poured into water (200 mL). The organic phase was removed, washed twice with water (2 × 100 mL), brine (200 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo* to give 1.8 g of a dark solid residue, which was purified by flash chromatography using 10% ethanol in ethyl acetate to give 1.33 g of a foam contaminated with traces of the starting *bis* (pinacolato)diboron. A second purification using 100% ethyl acetate as eluant gave 1.1 g of the desired product in 80% yield. ^{1}H NMR ($CDCl_3$): δ 7.52(d, $J = 7.68$ Hz, 2H), 7.26(m, 4H), 7.27(m, 1H), 6.89(d, $J = 7.80$ Hz, 2H), 5.81(d, $J = 13.65$ Hz, 2H), 3.27(m, 3H), 3.21(d, $J = 13.69$ Hz, 1H), 2.85(m, 2H), 2.29(m, 1H), 1.98(s, 3H), 1.84(m, 2H), 1.27(brs, 12H).

1{(*R*)-[7-(3,5-Dichlorophenyl)-5-methyl-6-oxo-5-(4-pyrimidin-5-yl-benzyl)-6,7-dihydro-5H-imidazo[1,2-a]imidazole-3-sulfony}-piperidine-4-carboxylic acid amide (**3**). A mixture of the aforementioned boronic ester (344.3 mg, 0.5 mmol), $PdCl_2$ (dppf)·CH_2Cl_2 (45 mg, 0.05 mmol), 5-bromopyrimidine (72 mg,

0.44 mmol), and potassium carbonate (346 mg, 2.5 mmol) in DME (10 mL) was refluxed for 14 h under nitrogen atmosphere. After cooling to room temperature, water was added, and the organic phase was extracted with ethyl acetate (2 × 50 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo* to give 420 mg of a dark residue, $R_f = 0.40$ in 20% EtOH/EtOAc or 0.27 in 5% MeOH/$CHCl_3$. Purification by flash chromatography using 50% EtOAc : Hexanes then 100% EtOAc gave 205 mg of pure product as cream-colored solid in 64% yield. ^{1}H NMR ($CDCl_3$): δ 9.16(s, 1H), 8.83(s, 2H), 7.36(m, 3H), 7.31(m, 2H), 7.27(m, 1H), 7.09(d, $J = 8.07$ Hz, 2H), 5.53(d, $J = 15.65$ Hz, 2H), 3.93(m, 2H), 3.78(m, 1H), 3.28(d, $J = 13.78$ Hz, 1H), 2.86(m, 2H), 2.31(m, 1H), 2.06(m, 2H), 2.01(s, 3H), 1.90(m, 2H). MS-ES: $[MH]^+$ (640.1, 100%), (642.0, 80%), MS-ES$^-$: $[M + OAc]^- = 698.1$.

[$^2H_9$]-(**3**): To a solution of the sulfonyl chloride derivative (**14**) (549 mg, 1.0 mmol) in dry THF (22.5 mL) and DMF (2.5 mL) was added triethylamine (209 μL, 1.5 mmol) at 0 °C. Then, isonipecotamide-2,2,3,3,4,5,5,6,6-$^2H_9$ (205.5 mg, 1.5 mmol, 99 at.%^{2}H) was added in one portion, and the resulting mixture was warmed to room temperature and stirred overnight under nitrogen. The mixture was concentrated under reduced pressure, and the residue was diluted with ethyl acetate (150 mL) and washed with an aqueous solution of HCl (0.1 N, 50 mL) and brine (50 mL). The organic phase was dried over $MgSO_4$, filtered and concentrated *in vacuo* to give a white solid. Purification by flash chromatography using ethyl acetate as eluant and then 10% methanol : ethyl acetate gave 640 mg of a white solid. Crystallization from warm ethyl acetate and hexanes gave 610 mg of a white solid in 94% yield. $R_f = 0.5$ in 30 % EtOAc : Hexanes. ^{1}H NMR ($CDCl_3$): δ 9.17(s, 1H), 8.84(s, 2H), 7.37(s, 1H), 7.35(s, 2H), 7.32(m, 2H), 7.28(m, 2H), 7.09(d, $J = 8.5$ Hz, 2H), 5.44(d, $J = 12.62$ Hz, 2H), 3.94(d, $J = 13.77$ Hz, 1H), 3.30(d, $J = 13.77$ Hz, 1H), 2.04(s, 3H). MS-ES$^+$: $MH^+ = 649.1$. HPLC: $t_R = 16.83$, 99.13% co-elutes with unlabeled (**3**).

[^{14}C]-(**3**): To a 50 mL round-bottom flask containing 5-bromopyrimidine,[2-^{14}C]- (30 mCi, SA = 53 mCi/mmol, 0.566 mmol) was added a magnetic stirrer, the boronic ester (**13**) (470 mg, 0.682 mmol), potassium carbonate (470 mg, 3.4 mmol), and DME (15 mL). The mixture was stirred under nitrogen atmosphere for 10 min before $PdCl_2(dppf)\cdot CH_2Cl_2$ (70 mg, 0.079 mmol) was added in one portion, and the mixture was heated at reflux for 6 h. After the reaction was complete by TLC, it was cooled down to room temperature, and water (20 mL) was added. The aqueous phase was extracted with ethyl acetate, and the combined ethyl acetate extracts were dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by flash chromatography silica gel (packed in Hexanes) using ethyl acetate as eluant. The product was isolated in 67% radiochemical yield (245 mg, 20 mCi) with a specific activity of 52.86 mCi/mmol. HPLC: Rad-detection, $t_R = 17.30$ min (99%); UV detection, $t_R = 16.92$ min (99%). ^{1}H NMR was identical to unlabeled (**3**).

Conclusion

Three LFA-1 antagonists labeled with carbon-14 and deuterium were prepared to support DMPK studies. The radiosyntheses were straightforward, one or two steps, and used intermediates available in house. [^{14}C]-(**1**) was prepared in two steps in 27% radiochemical yield and a specific activity of 56.75 mCi/mmol. Both [^{14}C]-(**2**) and [^{14}C]-(**3**) were obtained using the commercially available labeled 5-bromopyrimidine in 42% and 67% radiochemical yield and specific activities of 50 mCi/mmol and 52.86 mCi/mmol, respectively. Deuterium labeled (**2**) and (**3**) were prepared using the commercially available deuterium labeled piperazine and deuterium labeled isonipecotamide.

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