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Research Article

Synthesis of [3,5-dichlorobenzenesulfonamide-U-^{14}C)] labeled VLA-4 antagonists

Nathan X. Yu[1,*], Conrad E. Raab[1], Dennis C. Dean[1], Linus S. Lin[2] and David G. Melillo[1]

[1] *Department of Drug Metabolism, Merck Research Laboratories, RY80R-104, PO Box 2000, Rahway, NJ 07065, USA*

[2] *Department of Basic Chemistry, Merck Research Laboratories, RY80R-104, PO Box 2000, Rahway, NJ 07065, USA*

Summary

Radiolabeled tracers were required for the development of a series of VLA-4 antagonists. A method to synthesize [U-^{14}C]3,5-dichlorobenzenesulfonyl chloride was developed. From this key intermediate, various tracers were prepared in high yield. Copyright © 2004 John Wiley & Sons, Ltd.

Key Words: VLA-4 antagonist; asthma; [U-^{14}C]3,5-dichlorobenzenesulfonyl chloride

Introduction

VLA-4 (α4β1 protein), a key regulator of inflammation and auto-immune disease response, has been a target for the treatment of a number of inflammatory conditions including asthma.[1] The series of VLA-4 antagonists, **1**–**5**, were investigated as potential drug candidates.[2,3] Carbon-14 radiolabeled tracers of these compounds were required for pharmacokinetic and metabolism studies (Scheme 1).

We report here a synthetic route to [U-^{14}C]3,5-dichlorobenzenesulfonyl chloride (**6**), which can allow the rapid preparation of tracers of **1**–**5**, as well as other 3,5-dichlorobenzenesulfonamides. [^{14}C]Aniline hydrochloride was chosen as the starting material because it is readily available and reasonably priced. The amino group, very important in directing aromatic substitution, was easily removed at the end of the synthesis by a diazotization/dediazotization sequence.

*Correspondence to: N.X. Yu, Merck Research Laboratories, Department of Drug Metabolism, RY80R-104, Rahway, NJ 07065, USA. E-mail: xiao_yu@merck.com

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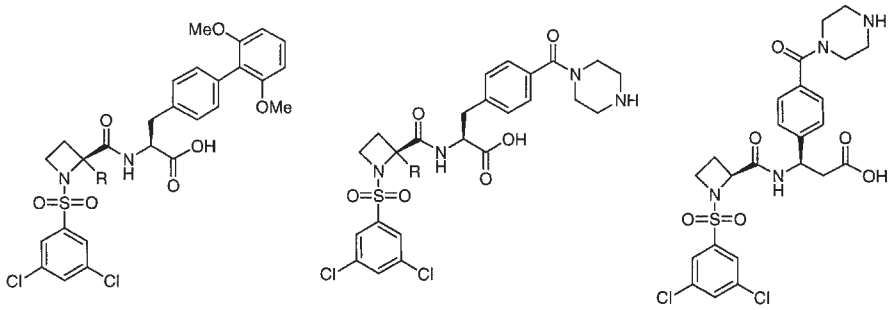


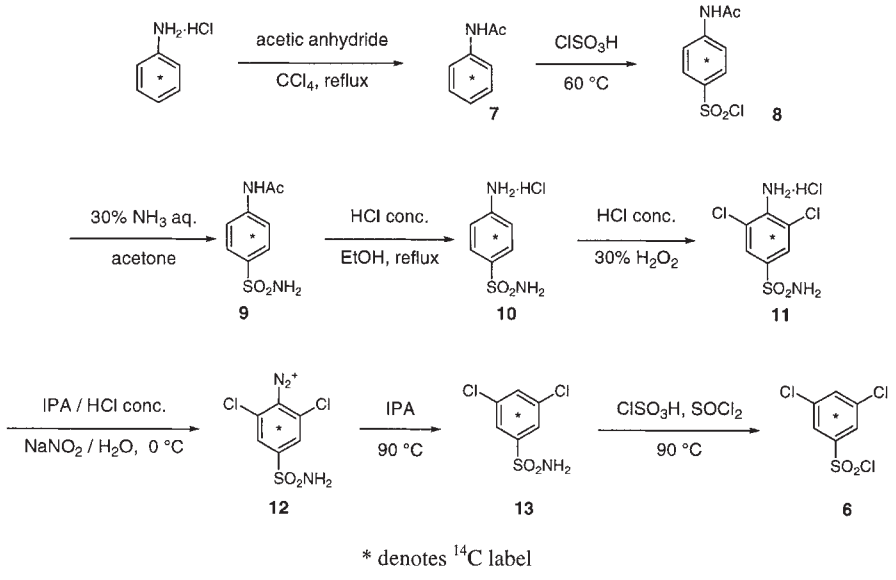
Scheme 1. VLA-4 antagonists

Results and discussion

To prevent possible *N*-oxidation or *bis*-sulfonation, aniline was acetylated with acetic anhydride.[4] Acetamide **7** was then sulfonated with chlorosulfonic acid to give sulfonyl chloride **8**,[5] which was stirred in an ammonium hydroxide/acetone solution to provide the corresponding sulfonamide **9**.[6] Subsequent ethanolysis under acidic conditions removed the acetyl protecting group.[7] Even though unlabeled and small-scale probe reactions proceeded well (50 and 60% yields of **11**, respectively), the main batch of chlorination of **10** provided **11** in only 13% radiochemical yield from **9**. This reaction provides better yields of the desired product if the reaction temperature is limited. The lower yield in the main batch reaction was probably due to a local elevation of the reaction temperature, resulting in the formation of azo- or azoxybenzenes.[8] Deamination of **11** was performed through initial formation of the diazonium salt **12**, which was heated at 90°C in 2-propanol to promote nitrogen loss leading to sulfonamide **13**.[9] The reaction of **13** proceeded smoothly with chlorosulfonic acid and thionyl chloride to give 42 mCi of the target sulfonyl chloride **6** (8.4% radiochemical yield from aniline).[10] Storage of this labeled intermediate in anhydrous benzene at −27°C resulted in less than 5% decomposition over a 3-year period (Scheme 2).

The appropriately protected amines (**15**–**19**) were prepared using procedures similar to those described in Lin *et al.*[3], allowing for late stage carbon-14 incorporation. Thus, sulfonylation of the amines with **6** gave, in high yield, esters **20**–**24**, which were saponified to provide the corresponding acids, **1**, **2**, **25**–**27**. The protecting groups on **25**–**27** were removed by acidic hydrolysis or hydrogenation. All five final tracers were purified by preparative HPLC (Scheme 3).

In summary, we have developed a synthetic route to [U-^{14}C]3,5-dichlorobenzenesulfonyl chloride (**6**), from which the sulfonamide tracers (**1**–**5**) were rapidly prepared. The [^{14}C]sulfonyl chloride (**6**) is stable in storage

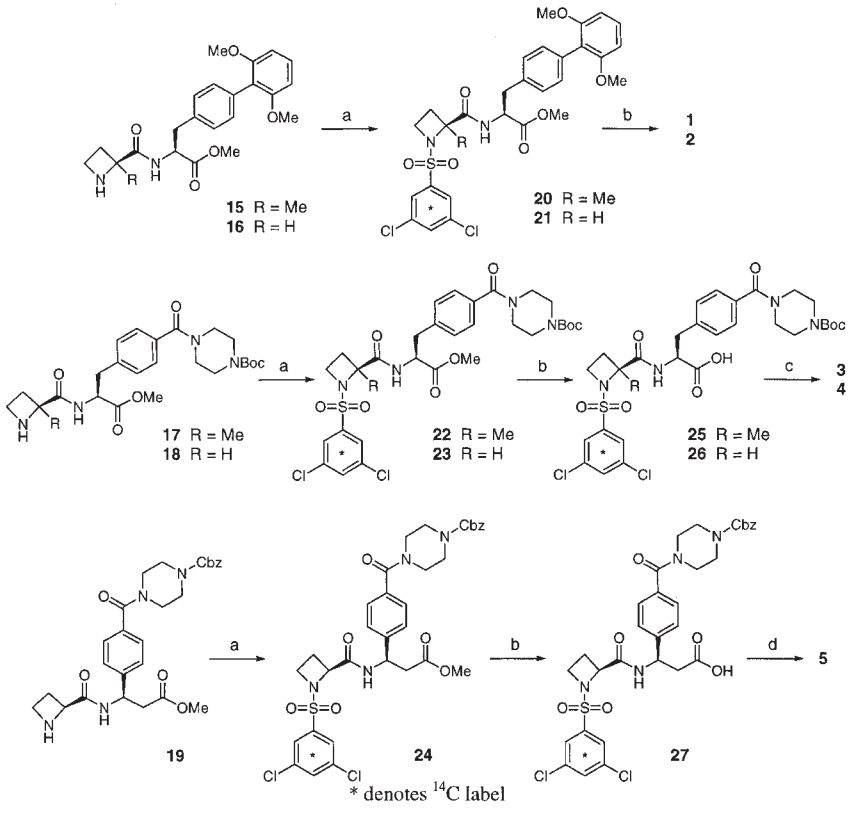


Scheme 2. Synthesis of sulfonyl chloride 6

over a long period of time and can be used as an off-the-shelf radiolabeling reagent.

Experimental

Radioactivity measurements were carried out using a Bioscan Lumi-Scint liquid scintillation counter with Packard Ultima Gold™ scintillant. Analytical HPLC measurements were performed on a system consisting of Shimadzu LC-10ADVP pumps, SPD-10AVP UV detector (215/254 nm), CTO-10ASVP column oven heated at 30°C, SIL-10ADVP auto-injector, SCL-10AVP system controller and Packard Radiomatic™ 150TR flow monitor controlled by a Shimadzu Class-VP software. All HPLC analyses were conducted on 4.6×250 mm columns at 1.0 ml/min, eluting with acetonitrile (A) and either 0.1% aqueous trifluoroacetic acid (B) or 0.1% aqueous perchloric acid (C), and concluded with a 10 min wash of 100% acetonitrile. All retention times (t_R) refer to the radioactive channel. Preparative HPLC was performed on a system consisting of Gilson 322 pump, UV/VIS-155 detector, and 215 liquid handler controlled by UniPoint™ software. Silica gel chromatography was performed on a Biotage FlashElute™ system, using FlashElute™ cartridges. Mass spectra were recorded on an HP-1100 LCMSD instrument in API-ES positive ionization mode. The MS data reported are the masses of the most abundant molecular ions. ^{1}H NMR spectra were measured at 400 MHz on a Varian Unity-400 spectrometer.



(a) **14**, DIPEA, DMAP, CH_2Cl_2, THF; (b) LiOH· H_2O, MeOH, THF, H_2O; (c) HCl, EtOAc; (d) PtO_2, H_2, IPA, 1 M HCl aq.

Scheme 3. Synthesis of the VLA-4 tracers (1–5)

[U-^{14}C]Aniline hydrochloride was obtained from American Radiolabeled Chemicals. Amines **15–19** were obtained from Merck Basic Chemistry, Rahway. Anhydrous solvents were obtained from Aldrich Chemical Co. and dried over 4 Å molecular sieves for at least 24 h prior to use. All other reagents were obtained from Aldrich and used as is. All reactions were carried out under a nitrogen atmosphere. All radioactive compounds were stored at −27°C.

The identities of all intermediates and the final products were established by ^{1}H NMR and/or by co-elution on HPLC with authentic standards obtained from Aldrich, TCI America or Merck Basic Chemistry.

*[phenyl-U-^{14}C]N-Acetylaniline (**7**)*

Acetic anhydride (6.5 ml, 68.9 mmol) was added to a solution of [^{14}C]aniline (500 mCi, 60 mCi/mmol, 8.33 mmol) in carbon tetrachloride (12 ml), with stirring. The reaction mixture was refluxed at 85°C for 1 h, then cooled to

room temperature. The solvent was removed *in vacuo*. Water (25 ml) was added. The mixture was refluxed at 100°C for 1 h, then cooled to room temperature. It was extracted with methylene chloride, dried with anhydrous sodium sulfate, filtered and counted (500 mCi, 97.1% radiochemical purity). The solvent was removed *in vacuo* to give **7** (97% radiochemical yield) as a white solid, which was analyzed by HPLC (Phenomenex Curosil PFP, 40A/60B isocratic, $t_R = 5.92$ min, 97.1% radiochemical purity). Crude **7** was used in the following reaction without purification. ^{1}H NMR ($CDCl_3$) δ 7.49 (d, 2H, $J = 7.9$ Hz), 7.31 (t, 2H, $J = 7.9$ Hz), 7.10 (t, 1H, $J = 7.9$ Hz), 2.15 (s, 3H). MS Calculated for **7** $[M + H^+]$: 136, Found: 136.

*[phenyl-U-^{14}C]4-(N-Acetylamino)benzenesulfonamide (**9**)*

Chlorosulfonic acid (10 ml, 150 mmol) was added to **7** (500 mCi, 97.1% radiochemical purity, 8.09 mmol) at 0°C with stirring. The reaction mixture was heated to 60°C for 1 h, then cooled to room temperature and poured onto crushed ice. A white solid formed, which was isolated by filtration, then dissolved in acetone (40 ml). At 0°C, ammonium hydroxide (10 ml, 28% NH_3 in water) was added. The mixture was stirred at room temperature for 1 h, then counted (400 mCi, 98.6% radiochemical purity) and analyzed by HPLC (Phenomenex Curosil PFP, 40A/60B isocratic, $t_R = 6.81$ min, 98.6% radiochemical purity). The solvent was removed *in vacuo* to give **9** (81% radiochemical yield) as a white solid, which was used in the following reaction without purification. ^{1}H NMR (DMSO-d_6) δ 10.66 (s, 1H), 7.74 (m, 4H), 7.22 (b, 2H), 2.08 (s, 3H). MS Calculated for **9** $[M + H^+]$: 215, Found: 215.

*[phenyl-U-^{14}C]4-Aminobenzenesulfonamide (**10**)*

Concentrated hydrochloric acid (8 ml) was added to a solution of **9** (400 mCi, 98.6% radiochemical purity, 6.57 mmol) in ethanol (50 ml) at room temperature, with stirring. The reaction mixture was refluxed at 90°C for 30 min, then counted (400 mCi, 96.9% radiochemical purity) and analyzed by HPLC (Phenomenex Curosil PFP, 20A/80B isocratic, $t_R = 4.12$ min, 96.9% radiochemical purity). The solvent was removed *in vacuo* to give **10** (98% radiochemical yield) as a light yellow solid, which was used in the following reaction without purification. MS Calculated for **10** $[M + Na^+]$: 195, Found: 195. The identity of **10** was established by co-elution with an authentic sample of unlabeled material obtained commercially.

*[phenyl-U-^{14}C]4-Amino-3,5-dichlorobenzenesulfonamide (**11**)*

The crude **10** (400 mCi, 96.9% radiochemical purity, 6.46 mmol) was dissolved in water (25 ml) and concentrated hydrochloric acid (25 ml). Aqueous hydrogen peroxide (30%, 2.5 ml) was added, and the mixture stirred at room

temperature for 1 h, then extracted with methylene chloride. The organic layers were dried with anhydrous sodium sulfate, filtered, counted (385 mCi, 20.6% radiochemical purity), and analyzed by HPLC (Phenomenex Curosil PFP, 40A/60B isocratic, $t_R = 8.02$ min, 20.6% radiochemical purity). Purification by silica gel chromatography (methylene chloride/ethyl acetate 4/1) gave 65 mCi of **11** (80% radiochemical purity, 13% radiochemical yield), which was stored in methanol. The identity of **11** was established by co-elution with an authentic sample of unlabeled material obtained commercially.

*[phenyl-U-^{14}C]3,5-Dichlorobenzenesulfonamide (**13**)*

A methanol solution of **11** (65 mCi, 80% radiochemical purity, 0.87 mmol) was dried *in vacuo*, and the residue dissolved in 2-propanol (5 ml). Concentrated hydrochloric acid (0.67 ml) was added at room temperature. After cooling to 0°C, a solution of sodium nitrite (190 mg, 2.75 mmol) in water (0.48 ml) was added. The mixture was stirred at 0°C for 30 min, then refluxed at 90°C for 2 h. The solution was counted (61.5 mCi), then concentrated *in vacuo*. The residue was dissolved in methanol and purified by preparative HPLC (Zorbax SB-C18, 21.2 × 250 mm, 215/254 nm, 27A/73B isocratic, flow rate = 20 ml/min) to give 42 mCi of **13** (100% radiochemical purity, 81% radiochemical yield) as a solution in methanol. HPLC analysis: Zorbax SB-C18, 35A/65B isocratic, $t_R = 12.03$ min, 100% radiochemical purity. The identity of **13** was established by co-elution with an authentic sample of unlabeled material obtained commercially.

*[phenyl-U-^{14}C]3,5-Dichlorobenzenesulfonyl chloride (**6**)*

A methanol solution of **13** (42 mCi, 0.70 mmol) was dried *in vacuo*. Chlorosulfonic acid (300 µl) was added to the residue, and the reaction mixture stirred at 90°C for 100 min. Thionyl chloride (50 µl) was then added, and the mixture stirred at 90°C for another 60 min. After cooling to room temperature, the mixture was poured onto ice and extracted with methylene chloride. The combined organic layers were quickly washed with saturated aqueous sodium bicarbonate solution and then brine at 0°C. The solution was dried with anhydrous sodium sulfate and filtered. The solvent was removed *in vacuo*, and the residue dissolved in anhydrous benzene (10 ml) to give 42 mCi of **6** (100% radiochemical purity, 100% radiochemical yield). HPLC analysis: Phenomenex Curosil PFP, 20A/80B isocratic, $t_R = 5.52$ min, 100% radiochemical purity. The identity of **11** was established by co-elution with an authentic sample of unlabeled material obtained commercially.

*[3,5-dichlorophenyl-U-^{14}C]Methyl(2S)-2-({(2S)-1-[(3,5-dichlorophenyl) sulfonyl]-2-methylazetidin-2-yl}carbonylamino)-3-[4-(2,6-dimethoxyphenyl) phenyl]propanoate (**20**)*

A benzene solution of **6** (10 mCi, 0.17 mmol) was dried *in vacuo*. A solution of **15** (75 mg, 0.18 mmol) in methylene chloride (2 ml), tetrahydrofuran (850 µl) and diisopropylethylamine (425 µl) was added to the residue at 0°C, with stirring. The reaction mixture was allowed to reach room temperature slowly and was then stirred overnight. The solvent was removed *in vacuo*, and the residue dissolved in methanol to give 10 mCi of **20** (95.7% radiochemical purity, 96% radiochemical yield). HPLC analysis: Phenomenex Curosil PFP, 80A/20B isocratic, $t_R = 5.41$ min, 95.7% radiochemical purity. The identity of **20** was established by co-elution with an authentic sample obtained from Merck Basic Chemistry.

*[3,5-dichlorophenyl-U-^{14}C]Methyl (2S)-2-({(2S)-1-[(3,5-dichlorophenyl) sulfonyl]-azetidin-2-yl}carbonylamino)-3-[4-(2,6-dimethoxyphenyl)phenyl] propanoate (**21**)*

A benzene solution of **6** (1.2 mCi, 0.02 mmol) was dried *in vacuo*. A solution of **16** (8.3 mg, 0.02 mmol) in methylene chloride (240 ml), tetrahydrofuran (100 µl) and diisopropylethylamine (50 µl) was added to the residue at 0°C, with stirring. A catalytic amount of 4-dimethylaminopyridine was also added. The reaction mixture was allowed to reach room temperature slowly and was then stirred overnight. The solvent was removed *in vacuo*, and the residue dissolved in methanol to give 1.2 mCi of **21** (100% radiochemical yield). HPLC analysis: Phenomenex Curosil PFP, 80A/20B isocratic, $t_R = 5.01$ min, 99.7% radiochemical purity. The identity of **21** was established by co-elution with an authentic sample obtained from Merck Basic Chemistry.

*[3,5-dichlorophenyl-U-^{14}C]Methyl (2S)-2-({(2S)-1-[(3,5-dichlorophenyl)sulfonyl]-2-methylazetidin-2-yl}carbonylamino)-3-[4-({4-[(tert-butyl)oxycarbonyl]piperazinyl}-carbonyl)phenyl]propanoate (**22**)*

The reaction of **6** (1.9 mCi, 0.032 mmol) with **17** (17 mg, 0.035 mmol), following the procedure described for the preparation of **21**, yielded 1.9 mCi of **22** (87.8% radiochemical purity, 88% radiochemical yield). HPLC analysis: Zorbax Rx-C18, 70A/30C isocratic, $t_R = 7.53$ min, 87.8% radiochemical purity. The identity of **22** was established by co-elution with an authentic sample obtained from Merck Basic Chemistry.

*[3,5-dichlorophenyl-U-^{14}C]Methyl (2S)-2-({(2S)-1-(3,5-dichlorophenyl) sulfonyl]-azetidin-2-yl}carbonylamino)-3-[4-({4-[(tert-butyl)oxycarbonyl] piperazinyl}carbonyl)-phenyl]propanoate (**23**)*

The reaction of **6** (2.0 mCi, 0.033 mmol) with **18** (17 mg, 0.036 mmol), following the procedure described for the preparation of **21**, yielded 2.0 mCi

of **23** (78.4% radiochemical purity, 78% radiochemical yield). HPLC analysis: Phenomenex Curosil PFP, 80A/20B isocratic, $t_R = 4.22$ min, 78.4% radiochemical purity. The identity of **23** was established by co-elution with an authentic sample obtained from Merck Basic Chemistry.

*[3,5-dichlorophenyl-U-^{14}C]Methyl (3R)-3-({(2S)-1-[(3,5-dichlorophenyl) sulfonyl]-azetidin-2-yl}carbonylamino)-3-[4-({4-[benzyloxycarbonyl]piperazinyl)carbonyl)-phenyl]propanoate (**24**)*

The reaction of **6** (2.7 mCi, 0.045 mmol) with **19** (25 mg, 0.050 mmol), following the procedure described for the preparation of **21**, yielded 2.7 mCi of **24** (94.0% radiochemical purity, 94% radiochemical yield). HPLC analysis: Zorbax Rx-C18, 60A/40B isocratic, $t_R = 8.52$ min, 94.0% radiochemical purity. The identity of **24** was established by co-elution with an authentic sample obtained from Merck Basic Chemistry.

*[3,5-dichlorophenyl-U-^{14}C](2S)-2-({(2S)-1-[(3,5-Dichlorophenyl)sulfonyl]-2-methyl-azetidin-2-yl}carbonylamino)-3-[4-(2,6-dimethoxyphenyl)phenyl]-propanoic acid (**1**)*

To a solution of methyl ester **20** (8.8 mCi, 95.7% radiochemical purity, 0.14 mmol) in methanol (1.9 ml) and tetrahydrofuran (1.9 ml) was added a solution of lithium hydroxide monohydrate (45 mg) in water (1.9 ml) at 0°C. After stirring at 0°C for 1 h, the solvent was removed *in vacuo*, and the residue extracted with ethyl acetate and water. The combined organic layers were dried with anhydrous sodium sulfate, filtered, counted (8.8 mCi) and analyzed by HPLC (Phenomenex Curosil PFP, 80A/20B isocratic, $t_R = 4.52$ min, 95.3% radiochemical purity). The tracer was purified by preparative HPLC (Zorbax Rx-C18, 21.2 × 250 mm, 215/254 nm, 49A/51B isocratic, flow rate = 20 ml/min) to give 5.3 mCi of **1** (63% radiochemical yield), which was isolated by solid phase extraction and stored in acetonitrile. HPLC analysis: Zorbax Rx-C18, 60A/40B isocratic, $t_R = 10.72$ min, 100% radiochemical purity. ^{1}H NMR (CD_3OD) δ 7.85 (d, 2H), 7.77 (t, 1H), 7.72 (br d, 1H), 7.23 (t, 1H), 7.16 (ABq, 4H), 6.67 (d, 2H), 4.75 (m, 1H), 3.91 (m, 1H), 3.62 (s, 6H), 3.51 (m, 1H), 3.38 (dd, 1H), 3.06 (dd, 1H), 2.18 (m, 1H), 2.03 (m, 1H), 1.62 (s, 3H). MS Calculated for **1** [$M + H^+$]: 607, Found: 607. The identity of **1** was also established by co-elution with an authentic sample obtained from Merck Basic Chemistry.

*[3,5-dichlorophenyl-U-^{14}C](2S)-2-({(2S)-1-[(3,5-Dichlorophenyl)sulfonyl] azetidin-2-yl}carbonylamino)-3-[4-(2,6-dimethoxyphenyl)phenyl]propanoic acid (**2**)*

The saponification of **21** (1.2 mCi, 99.7 radiochemical purity, 0.02 mol), following the procedure described for the preparation of **1**,

yielded 1.2 mCi of **2** (96.9% radiochemical purity, 97% radiochemical yield). HPLC analysis: Phenomenex Curosil PFP, 80A/20B isocratic, $t_R = 4.32$ min, 96.9% radiochemical purity. MS Calculated for **2** $[M + H^+]$: 593, Found: 593. The identity of **2** was established by co-elution with an authentic sample obtained from Merck Basic Chemistry.

*[3,5-dichlorophenyl-U-^{14}C](2S)-2-({(2S)-1-[(3,5-Dichlorophenyl)sulfonyl]-2-methyl-azetidin-2-yl}carbonylamino)-3-[4-({4-[(tert-butyl)oxycarbonyl] piperazinyl}carbonyl)-phenyl]propanoic acid (**25**)*

The saponification of **22** (1.9 mCi, 87.8% radiochemical purity, 0.028 mmol), following the procedure described for the preparation of **1**, yielded 1.9 mCi of **25** (86.7% radiochemical purity, 99% radiochemical yield), which was used in the next reaction without purification. HPLC analysis: Zorbax Rx-C18, 50A/50C isocratic, $t_R = 11.03$ min, 86.7% radiochemical purity. The identity of **25** was established by co-elution with an authentic sample obtained from Merck Basic Chemistry.

*[3,5-dichlorophenyl-U-^{14}C](2S)-2-({(2S)-1-[(3,5-Dichlorophenyl)sulfonyl] azetidin-2-yl}carbonylamino)-3-[4-({4-[(tert-butyl)oxycarbonyl]piperazinyl}-carbonyl)phenyl]propanoic acid (**26**)*

The saponification of **23** (2.0 mCi, 78.4% radiochemical purity, 0.026 mmol), following the procedure described for the preparation of **1**, yielded 2.0 mCi of **26** (78.0% radiochemical purity, 99% radiochemical yield), which was used in the next reaction without purification. HPLC analysis: Phenomenex Curosil PFP, 80A/20B isocratic, $t_R = 5.25$ min, 78.0% radiochemical purity. The identity of **26** was established by co-elution with an authentic sample obtained from Merck Basic Chemistry.

*[3,5-dichlorophenyl-U-^{14}C](3R)-3-({(2S)-1-[(3,5-Dichlorophenyl)sulfonyl] azetidin-2-yl}carbonylamino)-3-[4-({4-[benzyloxycarbonyl]piperazinyl}carbonyl)phenyl]propanoic acid (**27**)*

The saponification of **24** (2.7 mCi, 94.0% radiochemical purity, 0.042 mmol), following the procedure described for the preparation of **1**, yielded 2.1 mCi of **27** (97.2% radiochemical purity, 80% radiochemical yield), which was used in the next reaction without purification. HPLC analysis: Zorbax Rx-C18, 50A/50B isocratic, $t_R = 10.62$ min, 97.2% radiochemical purity. The identity of **27** was established by co-elution with an authentic sample obtained from Merck Basic Chemistry.

*[3,5-dichlorophenyl-U-^{14}C](2S)-2-({(2S)-1-[(3,5-Dichlorophenyl)sulfonyl]-2-methyl-azetidin-2-yl}carbonylamino)-3-[4-(piperazinylcarbonyl)phenyl] propanoic acid (**3**)*

A solution of **25** (1.9 mCi, 86.7% radiochemical purity, 0.027 mmol) in ethyl acetate (5 ml) was saturated with hydrogen chloride gas by bubbling. The reaction mixture was stirred at room temperature for 15 min, and then the solvent removed *in vacuo*. The residue was dissolved in acetonitrile, counted (0.9 mCi), and analyzed by HPLC (Zorbax Rx-C18, 50A/50C isocratic, $t_R = 3.21$, 97.5% radiochemical purity). The tracer was purified by preparative HPLC (Zorbax Rx-C18, 21.2 × 250 mm, 215/254 nm, 25A/75C isocratic, flow rate = 20 ml/min) to give 0.59 mCi of **3** (35% radiochemical yield), which was isolated as an acetonitrile solution after solid phase extraction. HPLC analysis: Zorbax Rx-C18, 29A/71C isocratic, $t_R = 14.82$ min, 99.8% radiochemical purity. ^{1}H NMR (CD_3OD) δ 7.85 (br d, 1H), 7.82–7.78 (m, 3H), 7.40 (ABq, 4H), 4.75 (m, 1H), 3.91 (m, 1H), 3,83 (br, 4H), 3.70 (m, 1H), 3.35 (dd, 1H), 3.25 (br, 4H), 3.19 (dd, 1H), 2.42 (m, 1H), 2.09 (m, 1H), 1.55 (s, 3H). MS Calculated for **3** [$M + H^+$]: 583, Found: 583. The identity of **3** was also established by co-elution with an authentic sample obtained from Merck Basic Chemistry.

*[3,5-dichlorophenyl-U-^{14}C](2S)-2-({(2S)-1-[(3,5-Dichlorophenyl)sulfonyl] azetidin-2-yl}carbonylamino)-3-[4-(piperazinylcarbonyl)phenyl]propanoic acid (**4**)*

A solution of **26** (2.0 mCi, 78.0% radiochemical purity, 0.026 mmol) in ethyl acetate (10 ml) was saturated with hydrogen chloride gas by bubbling. The reaction mixture was stirred at room temperature for 15 min, and then the solvent removed *in vacuo*. The residue was dissolved in acetonitrile, counted (2.0 mCi), and analyzed by HPLC (Phenomenex Curosil PFP, 20A/80B to 50A/50B gradient over 15 min, $t_R = 12.6$, 35.1% radiochemical purity). The tracer was purified by preparative HPLC (Zorbax Rx-C18, 21.2 × 250 mm, 215/254 nm, 23A/77B isocratic, flow rate = 20 ml/min) to give 0.34 mCi of **4** (22% radiochemical yield), which was isolated as an acetonitrile solution after solid phase extraction. HPLC analysis: Zorbax Rx-C18, 30A/70B isocratic, $t_R = 7.22$ min, 100% radiochemical purity. MS Calculated for **4** [$M + H^+$]: 569, Found: 569. The identity of **4** was established by co-elution with an authentic sample obtained from Merck Basic Chemistry.

*[3,5-dichlorophenyl-U-^{14}C](3R)-3-({(2S)-1-[(3,5-Dichlorophenyl)sulfonyl] azetidin-2-yl}carbonylamino)-3-[4-(piperazinylcarbonyl)phenyl]propanoic acid (**5**)*

Acid **27** (2.1 mCi, 97.2% radiochemical purity, 0.034 mmol) was dissolved in 2-propanol (8 ml) and 1 M hydrochloric acid (2 ml). Platinum(IV) oxide (8 mg)

was added, and the reaction mixture stirred at room temperature under hydrogen gas (1 atm) for 1 h. Then the mixture was filtered through celite, rinsed with 2-propanol, counted (2.0 mCi), and analyzed by HPLC analysis (Zorbax Rx-C8, 50A/50B isocratic, $t_R = 7.12$, 43.2% radiochemical purity). The tracer was purified by preparative HPLC (Waters XTerra RP-18, 19×300 mm, 215/254 nm, 25A/75C isocratic, flow rate = 20 ml/min) to give 0.6 mCi of **5** (29% radiochemical yield), which was isolated as an acetonitrile solution after solid phase extraction. HPLC analysis: Waters XTerra RP-18, 30A/70C isocratic, $t_R = 13.42$ min, 99.0% radiochemical purity. ^{1}H NMR (CD_3OD) δ 8.09 (s, 1H), 7.84 (s, 2H), 7.50 (ABq, 4H), 5.39 (m, 1H), 4.50 (m, 1H), 4.0–2.6 (m, 12H), 2.26 (m, 2H). MS Calculated for **3** [$M + H^+$]: 569, Found: 569. The identity of **5** was also established by co-elution with an authentic sample obtained from Merck Basic Chemistry.

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