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NON-PEPTIDE FIBRINOGEN RECEPTOR ANTAGONISTS. 2[1]. THE SYNTHESIS OF [^{3}H]L-738,167.

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Summary

The synthesis of [^{3}H]L-738,167, an orally active fibrinogen receptor antagonist, is described. A precursor containing the 2-bromotolyl moiety was synthesized, and a reductive debromination reaction using tritium gas and Pearlman's catalyst gave the desired product with a final specific activity of 21 Ci/mmol.

Keywords: Fibrinogen receptor antagonist, L-738,167, catalytic tritiation.

Introduction

Platelets and fibrinogen play a central role in thrombus formation, which has been implicated in various occlusive vascular diseases such as unstable angina, myocardial infarction and stroke (2). When cellular injury occurs, various factors, such as collagen and von Willebrand factor, are released causing platelet activation and adherence (3). Regardless of the activating mechanism, the final step in thrombus formation is the cross linking of platelets caused by the binding of the protein fibrinogen to the glycoprotein IIb/IIIa (GP IIb/IIIa) receptor located on the platelet membrane (4).

Agents that inhibit thrombus formation by preventing the binding of fibrinogen to GP IIb/IIIa would be extremely useful in treating various thrombolytic disorders (5). For the chronic treatment of these disorders, an orally active fibrinogen receptor antagonist is highly desirable. One of the orally active, potent, nonpeptide GP IIb/IIIa inhibitors selected for development at Merck is L-738,167, **1a** (Figure 1) (6). As part of its development, radiolabelled L-738,167 was needed for absorption, distribution, metabolism and excretion (ADME) studies (7). This compound was labelled with tritium and its synthesis is described here.

Results and Discussion

Figure 1 shows the structure of L-738,167. As the precursor to [^{3}H]L-738,167, we chose to use bromide **2**, which could be synthesized by coupling 2-bromotoluenesulfonyl chloride (8) with the required amine. Catalytic reductive debromination of **2** using tritium gas (9) would provide [^{3}H]L-738,167.

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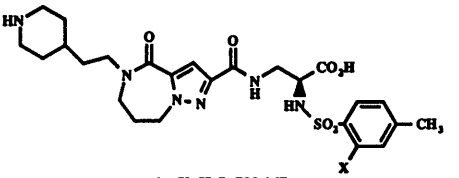
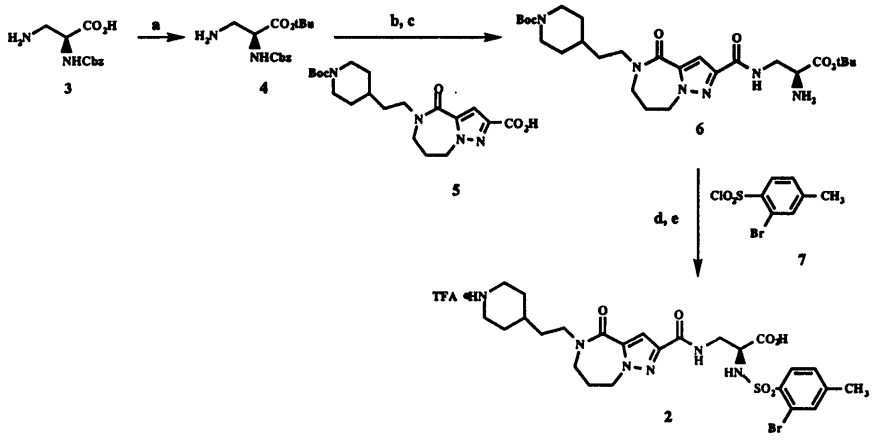
Figure 1



1a, X=H, L-738,167
1b, X=T
2, X=Br

The synthesis of bromide **2** is shown in Scheme 1. The commercially available amino acid **3** (10) was esterified and coupled with carboxylic acid **5** (11). After removal of the Cbz protecting group, amine **6** resulted. Coupling of this amine with 2-bromotoluenesulfonyl chloride **7** (8), followed by acid treatment gave bromide **2**. Conditions for the reductive debromination were then investigated (Scheme 2).

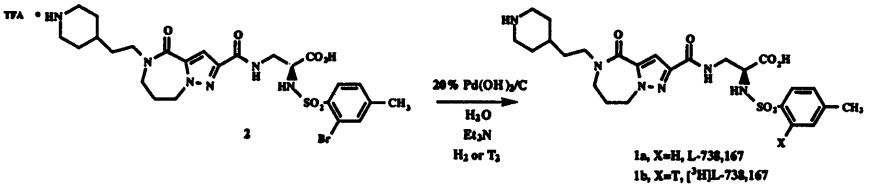
Scheme 1[a]. Synthesis of [^{3}H]L-738,167 precursor.



[a]Key (a) isobutylene, H_2SO_4, dioxane; (b) **5**, EDC, HOBT, CH_2Cl_2; (c) H_2, 10% Pd/C, EtOH; (d) **7**, Pyr, EtOAc; (e) TFA, CH_2Cl_2.

Initially high pressure conditions were used with 20% $Pd(OH)_2$ on carbon (Pearlman's catalyst) as the catalyst. An aqueous solution of **2** was combined with the catalyst and stirred for 18 hours in a Parr shaker under 50 psi of hydrogen to give a 95% yield of crude L-738,167 with 95% purity as measured by HPLC. In order to avoid high pressure and long reaction times in the gas tritiation reaction, this reaction was repeated at atmospheric pressure with triethylamine added. Under these conditions, after 2 hours HPLC analysis showed the disappearance of **2** and the presence of L-738,167. When this reaction was repeated using deuterium gas to check for deuterium incorporation as well as any isotope effects on reaction time, HPLC analysis at 2.5 hours showed the reaction was complete and ^{1}H NMR analysis showed good incorporation of deuterium. If a catalyst such as 10% Pd/C was used in place of Pearlman's catalyst, no improvement was seen.

Scheme 2. Reductive debromination of 2.



Initially the tritiation reaction was carried out under both high pressure and atmospheric pressure but with aqueous NaOH as the solvent rather than Et_3N/H_2O. The high pressure reaction using **2** and 10 Ci of tritium gas gave only 7 mCi of crude product with a radiochemical purity of 35%, and the atmospheric pressure reaction gave none of the desired product. Repeating the reaction at atmospheric pressure using Et_3N/H_2O with 10 Ci of tritium gas and a four hour reaction time gave 230 mCi of the desired product with a radiochemical purity of 47%. Further HPLC purification gave [^{3}H]L-738,167 with a radiochemical purity of 99% and a specific activity of 21 Ci/mmol.

Experimental

^{1}H NMR were recorded using a Varian Unity-300 spectrometer operating at 300 MHz. Analytical and preparative HPLC was carried out using a Waters 600E Powerline Multi Solvent Delivery System with 100 µL heads with a Rheodyne 7125 injector and a Waters 990 Photodiode Array Detector with a Gilson FC203 Microfraction collector. The acetonitrile was Fisher Optima grade. The HPLC radiodetector was a Beckman 171 Radioisotope detector with a Beckman 110B solvent delivery system and Beckman Ready Flow III liquid scintillation cocktail. A Waters C-18 µBondapak column, 3.9×150 mm, was used for analytical HPLC and a Waters C-18 µBondapak column, 3.9×300 mm, was used for preparative HPLC of the tritiated product. Solutions of radioactivity were concentrated using a Jouan vacuum centrifuge. Calibration curves and chemical concentrations were determined using a Hewlett Packard Model 8452A UV/Vis Diode Array Spectrophotometer. Sample radioactivities were determined in an LKB Wallac 1410 liquid scintillation counter. The identity of labelled compounds were determined by HPLC coelution with authentic compounds. Reagents were purchased from Aldrich Chemical Co. unless otherwise noted.

t-Butyl-2-(S)-Cbz-2,3-diaminopropionic acid, 4: Liquid isobutylene (60 mL) was slowly added to the commercially available amino acid **3**[9] (1.0 g, 4.20 mmol) in a mixture of dioxane (60 mL) and conc. H_2SO_4 (0.78 mL, 14.7 mmol) in a 500 mL pressure bottle. The securely stoppered bottle was stirred at room temperature for 60 h, then the contents were poured in to an ice cold mixture of sat. $NaHCO_3$ (200 mL) and ethyl acetate (200 mL). The aqueous layer was removed, washed with ethyl acetate (100 mL) and the combined organic layers washed with brine, dried over Na_2SO_4, filtered, and evaporated to afford **4** as a colorless crystalline solid (0.93 g, 75%): ^{1}H NMR (δ, $CDCl_3$): 7.38 (5H, m); 5.62 (1H, d, J = 7.2 Hz); 5.13 (2H, s); 4.23 (1H, m); 3.08 (2H, m); 1.51 (9H, s).

t-Butyl-2-(S)-[amino]-3-[[[5,6,7,8-tetrahydro-4-oxo-5-[2-(N-Boc-piperidin-4-yl) ethyl]- 4-H-pyrazolo[1,5-a][1,4]diazepin-2-yl]carbonyl]amino]-propionic acid, 6: A solution of **4** (445.5 mg, 1.51 mmol), **5**[10] (433 mg, 1.51 mmol), EDC (289.5 mg, 1.51 mmol), and HOBT (204 mg, 1.51 mmol) in 15 mL CH_2Cl_2 was stirred at room temperature for 8.5 h, then diluted with 100 mL CH_2Cl_2, washed successively with 10% $KHSO_4$, H_2O, sat. $NaHCO_3$, and brine (100 mL each), dried over Na_2SO_4, filtered and evaporated to afford a colorless glass (803 mg, 95%): ^{1}H NMR (δ, $CDCl_3$): 7.38 (5H, m); 7.19 (1H, s); 7.18 (1H, t); 5.83 (1H, d); 5.13 (2H, s); 4.40 (1H, m); 4.38 (2H, t); 4.05 (2H, br d); 3.85 (1H, m); 3.78 (1H, m); 3.57 (2H, m); 3.32 (2H, t); 2.63 (2H, t); 2.22 (2H, m); 1.71 (2H, d); 1.53 (1H, m); 1.45 (18H, s); 1.13 (2H, m). A solution of the above product (720 mg, 1.05 mmol) in methanol (15 mL) was treated with 10% Pd/C (50 mg) and the resulting mixture stirred under a H_2-filled balloon for 12.5 h. The catalyst was removed by filtration through Celite and the filtrate concentrated giving **6** in quantitative yield as a colorless glass. ^{1}H NMR (δ, $CDCl_3$): 7.38 (5H, m); 7.18 (1H, s); 7.06 (1H, t, J = 7.1 Hz); 4.38 (2H, t, J = 6.8 Hz); 4.32 (1H, m); 4.05 (2H, br d, J = 12.5 Hz); 3.82 (1H, m); 3.72 (1H, m); 3.57 (2H, m); 3.32 (2H, t, J = 6.8 Hz); 2.63 (2H, t, J = 7.1 Hz); 2.22 (2H, m); 1.71 (2H, d, J = 12.8 Hz); 1.53 (1H, m); 1.45 (18H, s); 1.13 (2H, m).

2-(S)-[2-Bromo-4-methyl-benzenesulfonylamino]-3-[[[5,6,7,8-tetrahydro-4-oxo-5-[2-(N-Bocpiperidin-4-yl)ethyl]-4-H-pyrazolo[1,5-a][1,4]diazepin-2-yl]carbonyl] amino]-propionic acid, 2: A solution of 6 (300 mg, 0.51 mmol) in ethyl acetate (25 mL) was treated with 7[7] (249 mg, 0.92 mmol) and pyridine (0.11 mL, 1.3 mmol) and stirred at reflux for 12.5 h. After cooling to room temperature, the mixture was diluted with ethyl acetate (100 mL) and washed successively with 10% $KHSO_4$, H_2O, sat. $NaHCO_3$, and brine (100 mL each), dried over Na_2SO_4, filtered and evaporated. The resulting brown residue was chromatographed on silica gel using ethyl acetate as eluent to afford a colorless solid (338 mg, 77%). ^1H NMR (δ, $CDCl_3$): 7.96 (1H, d); 7.53 (1H, s); 7.23 (1H, d); 7.20 (1H, t); 7.18 (1H, s); 6.18 (2H, d); 4.4 (2H, t); 4.08 (2H, d); 3.92 (1H, m); 3.87 (1H, m); 3.6 (2H, t); 3.41 (2H, t); 2.68 (2H, t); 2.40 (3H, s); 2.35 (2H, m); 1.78 (2H, d); 1.61 (1H, m); 1.43 (9H, s); 1.27 (9H, s); 1.21 (2H, m). A solution of the above product (530 mg, 0.68 mmol) in CH_2Cl_2 (10 mL) was treated with TFA (3.0 mL) and the mixture stirred at 0°C for 3.5 h and was then evaporated. The resulting oily residue was dissolved in H_2O (5 mL) then lyophilized to give 2 as an off-white powder. ^1H NMR (δ, CD_3OD): 8.03 (1H, d, J = 6.8 Hz); 7.58 (1H, s); 7.23 (1H, d, J = 6.8 Hz); 7.18 (1H, s); 4.4 (2H, t, J = 6.9 Hz); 3.92 (1H, m); 3.87 (1H, m); 3.6 (2H, t, J = 6.9 Hz); 3.51 (2H, d, J = 12.3 Hz); 3.41 (2H, t, J = 6.8 Hz); 2.68 (2H, t, J = 6.8 Hz); 2.42 (3H, s); 2.35 (2H, m); 1.78 (2H, d, J = 12.5 Hz); 1.61 (1H, m); 1.15 (2H, m).

2-(S)-[4-Methyl-benzenesulfonylamino]-3-[[[5,6,7,8-tetrahydro-4-oxo-5-[2-(N-Bocpiperidin-4-yl)ethyl]-4-H-pyrazolo[1,5-a][1,4]diazepin-2-yl]carbonyl] amino]-propionic acid, 1a: A mixture of 1.1 mg (0.0018 mmol) of 2 in 0.5 mL of H_2O was treated with 1 drop of triethylamine, 1.8 mg of Pearlman's catalyst, degassed and stirred under a hydrogen or deuterium atmosphere. After 2.5 h at room temperature HPLC analysis (Waters C-18 μBondapak column, 3.9 × 300 mm, 2% acetonitrile:H_2O (0.1% H_3PO_4) to 90% acetonitrile, 20 min linear gradient, 2 mL/min, 220nm) showed 1a (9.5 min retention time), as determined by coelution with an authentic standard, without 2 (10.2 min retention time).

[^{3}H]2-(S)-[4-Methyl-benzenesulfonylamino]-3-[[[5,6,7,8-tetrahydro-4-oxo-5-[2-(N-Bocpiperidin-4-yl)ethyl]-4-H-pyrazolo[1,5-a][1,4]diazepin-2-yl]carbonyl] amino]-propionic acid, 1b: A solution of 10 mg (0.016 mmol) of 2 in 2 mL of H_2O was transferred to a 2 mL reaction vessel containing 14 mg of Pearlman's catalyst. To this was added 0.1 mL (0.72 mmol) of triethylamine. The reaction vessel and it's contents were degassed by freeze-thaw evacuation cycle and then exposed to 10 Ci of tritium gas. The reaction was terminated after 4 h at room temperature and atmospheric pressure by removal of the catalyst through a short column of celite. This was washed with 1 mL of H_2O and 3 mL of methanol. The solvents were removed *in vacuo* and any labile tritium was washed out by evaporation with 3 x 1mL of methanol. The residue was dissolved in 10 mL of H_2O to give 230 mCi of activity. A 20 mCi aliquot (1 mL) was concentrated to about 200 μL and purified by HPLC [Waters C-18 μBondapak column, 3.9 × 300 mm, 1 mL/min, 0% AcCN:H_2O (0.06% TFA, pH 5.3) to 20 % AcCN, 30 min linear gradient then 20% AcCN:H_2O (0.06% TFA, pH 5.3) for 15 min, 220 nm]. The retention time for L-738,167 under these conditions is 36 min. The cleanest fractions were pooled to give 3 mCi of [^{3}H]L-738,167 with a specific activity of 21 Ci/mmol and a radiochemical purity of 99%.

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References and Notes

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