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NON-PEPTIDE FIBRINOGEN RECEPTOR ANTAGONISTS. 3[1]. THE SYNTHESIS OF [^{3}H]L-767,685 and [^{3}H]L-767,679.

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Summary

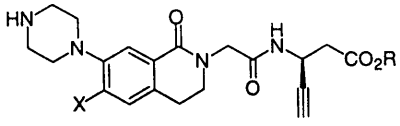
The synthesis of the fibrinogen receptor antagonist [^{3}H]L-767,679 and its ethyl ester prodrug [^{3}H]L-767,685 is described. Bromination of an appropriate benzolactam followed by catalytic tritiation with tritium gas gave a labelled benzolactam that was converted to [^{3}H]L-767,685 via a coupling/deprotection sequence. Hydrolysis of [^{3}H]L-767,685 then gave the acid [^{3}H]L-767,679. These two compounds were obtained with a specific activity of 10-16 Ci/mmol.

Keywords: Fibrinogen receptor antagonist, L-767,679, L-767,685, catalytic tritiation.

Introduction

Platelet aggregation and thrombus formation can be triggered by various activation mechanisms as a result of cellular injury (2). Though the activation mechanism may vary, the common final step in thrombus formation is the binding of the protein fibrinogen to the activated platelet glycoprotein IIb/IIIa (GP IIb/IIIa) receptor (3). Thrombus formation caused by the binding of fibrinogen to GP IIb/IIIa has been associated with various vascular diseases so antithrombotic agents that prevent this interaction would be important for the prevention of unstable angina and other vascular disorders (4). Because this type of therapy requires chronic treatment, orally active compounds are highly desirable.

Figure 1



1a, X=H, R=Et, L-767,685
1b, X=T, R=Et, [^{3}H]L-767,685
2a, X=H, R=H, L-767,679
2b, X=T, R=H, [^{3}H]L-767,679

One of the orally active, potent, nonpeptide GP IIb/IIIa inhibitors identified at Merck is L-767,679, **2a** (Figure 1) (5). As part of its development, radiolabelled L-767,679 and its ethyl ester prodrug L-767,685, **1a**, were needed for absorption, distribution, metabolism and excretion (ADME) studies. This pair of compounds was labelled with tritium and their synthesis is described here.

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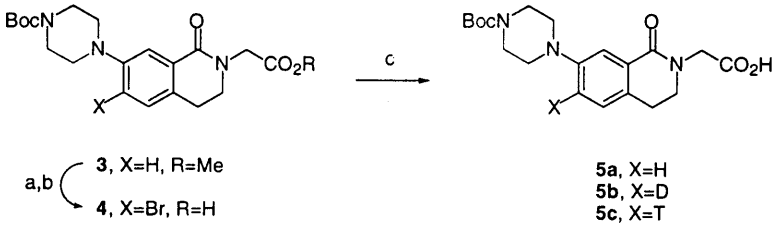
Results and Discussion

To synthesize L-767,769 and L-767,685 radiolabelled with tritium, we envisioned performing a catalytic reductive dehalogenation reaction using tritium gas (6). To avoid reduction of the alkyne moiety present in these compounds, catalytic tritiation would have to be carried out before the alkyne was introduced. This required the labelling of an intermediate that could be converted to the final products. By examining the synthetic sequence for the unlabelled compounds (5), and to minimize the number of synthetic steps after incorporation of the label, benzolactam **3** was chosen as the starting material. The resultant radiolabelled benzolactam could then rapidly be converted to the final products.

As shown in scheme 1, treatment of piperazinylbenzolactam **3** with bromine in acetic acid gave one monobrominated isomer which was hydrolyzed to give benzolactam **4**. A model hydrogenation reaction (10% Pd/C, Et_3N, EtOH) of **4** run overnight cleanly gave **5a**. This reaction was repeated using deuterium gas to check for isotope incorporation and to examine shorter reaction times. When this reaction was repeated using deuterium gas, HPLC analysis showed the reaction was complete within one hour and ^1H NMR analysis of crude **5b** showed the aromatic region contained approximately 2.5 protons. When this reaction was carried out using 15 curies of tritium gas, 490 mCi of crude **5c** was formed with a radiochemical purity of approximately 95%.

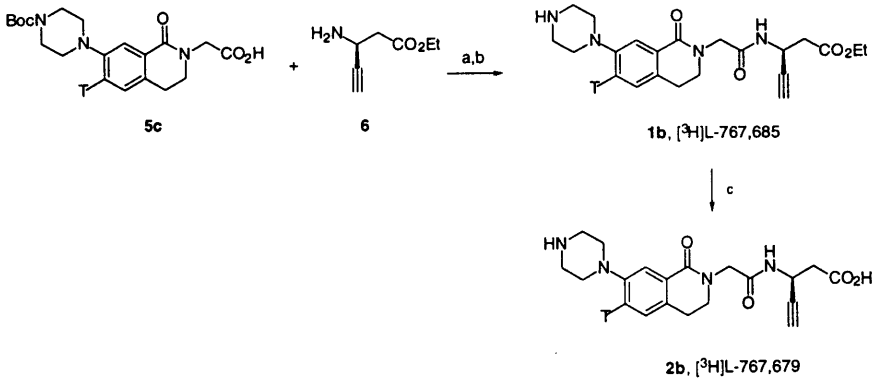
Scheme 1[a]. Synthesis of the [^3H]L-767,679/L-767,685 precursor.



[a]Key: a) Br_2, HOAc b) LiOH c) H_2, D_2 or T_2, 10% Pd/C, Et_3N, EtOH.

The conversion of **5c** to the final products is shown in scheme 2. To carry out the coupling reaction between **5c** and amine **6**, a specific activity of 20 Ci/mmol for **5c** was assumed for the stoichiometry. To simplify the purification of the final products, a minimum amount of unlabelled reagents was added. A portion of crude **5c** was treated with amine **6** (5) and EDC/HOBt in DMF. HPLC analysis showed clean conversion of the radioactive peak to a later eluting peak. Treatment with HCl saturated ethanol to remove the Boc protecting group cleanly gave [^3H]L-767,685, **1b**, which co-eluted on HPLC with an authentic standard. A portion of crude **1b** was then hydrolyzed using LiOH/MeOH to give [^3H]L-767,679, **2b**. Each of these compounds was then purified by HPLC to give [^3H]L-767,685 with a specific activity of 13-16 Ci/mmol and [^3H]L-767,679 with a specific activity of 10-13 Ci/mmol.

Scheme 2[a]. Synthesis of [^{3}H]L-767,679 and [^{3}H]L-767,685.



[a]Key: a) EDC, HOBt, Et_3N, DMF b) HCl(g), EtOH c) 1N LiOH, MeOH.

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, 3.9×300 mm, and a Zorbax RX-C8, 4.6 x 250 mm was used for analytical and preparative HPLC of the tritiated products. 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 co-elution with authentic standards. Reagents were purchased from Aldrich Chemical Co.

6-Bromo-7-(4-N-Boc-piperazin-1-yl)-3,4-dihydro-1(1H)-isoquinolinone-2-acetate, **4**: A solution of **3** (396 mg, 0.98 mmol) in acetic acid (2 mL) was treated with bromine (56 µL, 1.08 mmol) and the mixture was stirred at room temperature for 30 minutes. Additional bromine (20 µL, 0.39 mmol) was added and, after stirring an additional 15 minutes at room temperature, the reaction mixture was poured into water and extracted with EtOAc. The organic layer was washed with water, saturated $NaHCO_3$ solution and brine. The organic layer was dried ($MgSO_4$), filtered and the solvent was evaporated to give a yellow oil. Purification by column chromatography (silica gel; 1:1 hexane:EtOAc) afforded 91 mg (19%) of the methyl ester of **4**: ^{1}H NMR (δ, $CDCl_3$): 7.73 (1H, s), 7.45 (1H, s), 4.33 (2H, s), 3.76 (3H, s), 3.6 (6H, m), 3.0 (6H, m), 1.47 (9H, s). This ester (91 mg, 0.19 mmol) was stirred in a mixture of methanol (2 mL), THF (1 mL) and 1N LiOH solution (0.57 mL; 0.57 mmol) for 3 hours at room temperature. The reaction mixture was poured into 1N HCl (5 mL) and extracted with EtOAc and washed with water and brine. The combined organic layers were dried ($MgSO_4$), filtered and concentrated to give an oil. Trituration with ether then afforded **4** as a white crystalline solid: ^{1}H NMR (δ, d_4 MeOH): 7.66 (1H, s), 7.57 (1H, s), 4.30 (2H, s), 3.66 (2H, t), 3.6 (4H, m), 3.0 (6H, m), 1.48 (9H, s).

[6-^{2}H]-7-(4-N-Boc-piperazin-1-yl)-3,4-dihydro-1(1H)-isoquinolinone-2-acetate, **5b**: A solution of **4** (3 mg, 0.0064 mmol) in ethanol (0.3 mL) was treated with 10% Pd/C (1 mg), triethylamine (1.8 µL, 0.0128 mmol), degassed and stirred under a balloon of deuterium gas for 1 hour at ambient temperature. HPLC analysis (Waters C18 µBondapak, 3.9 x 300 mm, 10% AcCN:H_2O (0.1% TFA) to 90% AcCN:H_2O (0.1% TFA), linear gradient for 30 minutes, 1 mL/min, 254 nm) showed the disappearance of **4** (24 minute retention time) with formation of **5b** (19 minute retention time) as shown by coinjection with an authentic standard. The reaction mixture was filtered through celite, rinsed several times with ethanol and concentrated to give 4.8 mg of **5b** as a white solid: ^{1}H NMR (δ, d4 MeOH): 7.53 (1.5H, s), 7.17 (1H, s), 4.24 (2H, s), 3.67-2.95 (12H, m).

[6-^{3}H]-7-(4-N-Boc-piperazin-1-yl)-3,4-dihydro-1(1H)-isoquinolinone-2-acetate, **5c**: A solution of **4** (10 mg, 0.021 mmol) in ethanol (1 mL) was transferred to a 1 mL reaction vessel containing 10% Pd/C (3.5 mg). To this was added triethylamine (6 µL) and the reaction vessel and its contents were degassed by three freeze-thaw evacuation cycles and then exposed to 15 curies of tritium gas. The reaction was

terminated after 1 hour by passing through a short column of celite and washing with 3 mL of ethanol. The solvents were removed *in vacuo* and labile tritium was washed out by evaporation with 3 x 1mL of methanol. The residue was dissolved in ethanol (20 mL) to give 490 mCi of activity. HPLC analysis (Waters C18 μBondapak, 3.9 x 300 mm, 10% AcCN:H_2O (0.1% TFA) to 90% AcCN:H_2O (0.1% TFA), linear gradient for 30 minutes, 1 mL/min) showed 95% radiochemical purity and coelution with the unlabelled standard. For the following coupling reaction a specific activity of 20 Ci/mmol was assumed.

[6-^{3}H]N-[[7-Piperazin-1-yl)-3,4-dihydro-1(1H)-oxoisoquinolin-2-yl]acetyl]-3(S)-ethynyl-β-alanine ethyl ester, **1b**: An ampule containing 50 mCi of **5b** in 2 mL of ethanol was transferred to a small test tube and concentrated under an argon stream. The residue was treated with solutions of EDC (0.75 mg, 0.044 μL of 3.4 mg EDC/0.2 mL DMF), HOBt (0.41 mg, 0.01 μL of 3.9 mg HOBt/0.1 mL DMF), amine **6** (0.02 mL of 0.55 mg amine/0.02 mL DMF) and triethylamine (0.54 mL). The clear colorless solution was stirred at room temperature overnight. HPLC analysis (Waters C18 μBondapak, 3.9 x 300 mm, 10% AcCN:H_2O (0.1% TFA) to 90% AcCN:H_2O (0.1% TFA), linear gradient for 30 minutes, 1 mL/min) showed the radioactivity eluting at 23.5 minutes. The reaction mixture was concentrated to dryness and treated with HCl saturated ethanol (0.6 mL) and stirred at room temperature for forty minutes. HPLC analysis (Waters C18 μBondapak, 3.9 x 300 mm, 10% AcCN:H_2O (0.1% TFA) to 90% AcCN:H_2O (0.1% TFA), linear gradient for 30 minutes, 1 mL/min) showed the radioactivity eluting at 16 minutes and co-eluting with an authentic standard of L-767,685. An aliquot of the reaction mixture (0.1 mL, 10 mCi) was purified by HPLC (Waters C18 μBondapak, 3.9 x 300 mm, 10% AcCN:H_2O (0.1% TFA) to 90% AcCN:H_2O (0.1% TFA), linear gradient for 30 minutes, 1 mL/min, 254 nm, retention time 16 minutes) to give 3 mCi of [^{3}H]L-767,685 with a radiochemical purity of 99% and a specific activity of 16.4 Ci/mmol.

[6-^{3}H]N-[[7-Piperazin-1-yl)-3,4-dihydro-1(1H)-oxoisoquinolin-2-yl]acetyl]-3(S)-ethynyl-β-alanine, **2b**: An aliquot (0.2 mL, 20 mCi) of crude [^{3}H]L-767,685, **1b**, described above was concentrated almost to dryness and treated with methanol (0.2 mL) and 1N LiOH (0.1 mL) until basic to pH paper. After stirring for 2.5 hours at room temperature HPLC analysis (Waters C18 μBondapak, 3.9 x 300 mm, 10% AcCN:H_2O (0.1% TFA) to 90% AcCN:H_2O (0.1% TFA), linear gradient for 30 minutes, 1 mL/min) showed the radioactivity eluting at 12.5 minutes. Approximately half of this material was concentrated to dryness and treated with 0.2 mL of 0.02N NaH_2PO_4, pH 2.8. This was still slightly basic so 2 μl of conc. HCl was added to achieve neutral pH. HPLC purification (Zorbax RX-C8, 4.6 x 250 mm, 10% AcCN:H_2O (0.02N NaH_2PO_4, pH 2.8). 1 mL/min, 254 nm) gave 2 mCi of [^{3}H]L-767,679, **2b**, (14 minute retention time) with a radiochemical purity of 99% and a specific activity of 12 Ci/mmol.

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References and Notes

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