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NON-PEPTIDE FIBRINOGEN RECEPTOR ANTAGONISTS. 4[1]. THE SYNTHESIS OF [^{3}H]L-756,568.

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Summary

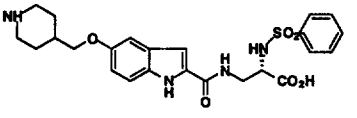
The synthesis of [^{3}H]L-756,568, an orally active fibrinogen receptor antagonist, is described. Two synthetic pathways were developed using either bromoindoles **2a/2b** or bromoaryl sulfonamide **11** as the precursor. Use of the bromoaryl sulfonamide precursor led to [^{3}H]L-756,568 with higher radiochemical purity, higher radiochemical yield, and slightly higher specific activity.

Keywords: Fibrinogen receptor antagonist, L-756,568, catalytic tritiation.

Introduction

As a result of cellular injury and stimulated by various agonists, the platelet surface receptor glycoprotein (GP) IIb/IIIa becomes activated and binds fibrinogen, leading to thrombus formation (2). Thrombus formation has been implicated in various coronary diseases such as stroke, myocardial infarction and unstable angina (3). Compounds that can disrupt the binding of fibrinogen to its platelet receptor and thereby prevent thrombus formation could be used to treat various cardiovascular diseases (4). Chronic treatment of these disorders would be possible with orally active compounds. One of the orally active, potent, nonpeptide GP IIb/IIIa inhibitors discovered at Merck is L-756,568, **1** (5), shown in Figure 1. As part of its development, radiolabelled L-756,568 was needed for absorption, distribution, metabolism and excretion (ADME) studies. This compound was labelled with tritium and its synthesis is described here.

Figure 1



1, L-756,568

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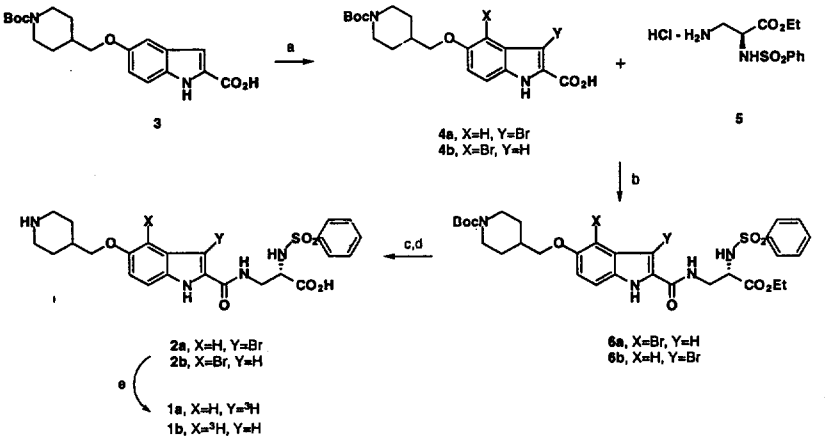
Results and Discussion

Scheme 1 shows the synthesis of bromoindoles **2a** and **2b** that were used as the precursor for [^{3}H]L-756,568. Bromination of indole **3** (5) with NBS in acetonitrile (6) gave, under unoptimized conditions, a mixture of the 3-bromo and 4-bromoindole products **4a/4b** in a 3:7 ratio. Rather than separating these isomers, this mixture was coupled with amine **5** (7). After deprotection and hydrolysis, a mixture of bromoindoles **2a** and **2b** resulted. In most cases this mixture was used for the subsequent hydrogenation and tritiation reactions. A small amount of the 3-bromoindole isomer, **2a**, was separated from this mixture to see if there was any advantage to using a pure isomer.

Scheme 1[a]. Synthesis of [^{3}H]L-756,568, 1a/1b, via bromoindole 2.



[a]Key (a) NBS, MeCN, 66% (b) BOP, NMM, MeCN, 47% (c) HCl, EtOAc, quantitative (d) 1N NaOH, MeOH, THF, 35% (e) T_2, 10% Pd/C, MeOH, Et_3N.

Initial model reduction reactions were carried out using a mixture of **2a/2b**. When using 10% Pd/C in methanol or DMF containing triethylamine, the reaction was approximately 70% complete in the first hour, with longer reaction times giving an unknown impurity that eventually became the major product at the expense of the desired product. When L-756,568 was submitted to these reaction conditions, it was converted to this byproduct, presumably from reduction of the indole ring. If the reaction was run using methanol or DMF without triethylamine, this byproduct did not form but longer reaction times were needed to give L-756,568. When this reduction was repeated using methanol and deuterium gas, ^{1}H NMR analysis of the crude product showed good levels of deuterium incorporation at the 3- and 4-position of the indole. No advantage was found to using a sample of the pure 3-bromoindole isomer **2a**.

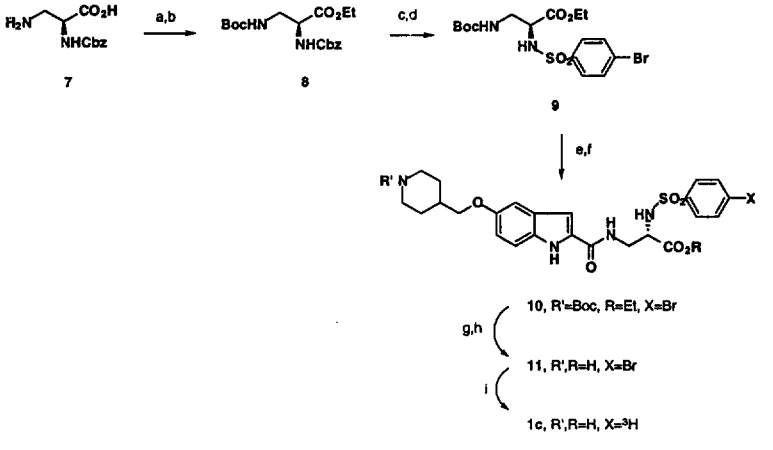
When the tritiation reaction was carried out using **2a/2b** with 10 curies of tritium gas and 10% Pd/C in methanol/triethylamine, rapid uptake of tritium gas was observed for the first 45 minutes with no uptake observed over the next 45 minutes of the reaction. After 1.5 hours, the reaction was terminated to give only 28 mCi of crude product with a

radiochemical purity of 70%. Analysis showed there was not unreacted starting material. This [^{3}H]L-756,568, **1a/1b**, was difficult to purify to 99% radiochemical purity and only had a specific activity of 2 Ci/mmol. Though deuterium model reactions indicated good levels of deuterium incorporation, a contributing factor to the low specific activity may have been the use of methanol as the solvent (8). Although this synthesis did lead to [^{3}H]L-756,568, the purification was quite tedious and it proved difficult to routinely provide this ligand, necessitating an alternate route to synthesize [^{3}H]L-756,568.

Based on previous work (9) labelling a compound containing the arylsulfonamide moiety, the bromophenyl sulfonamide **11** (Scheme 2) was synthesized to determine whether this type of precursor would behave better in the tritiation reaction. The protected diaminopropionic acid **7** (Fluka) was converted to bromosulfonamide **9** (7). Removal of the Boc protecting group and completion of similar chemistry as shown with the synthesis of **2a/2b** gave the desired precursor **11.**

With precursor **11** in hand, model reduction reactions were carried out as before. This reaction worked best when DMF/triethylamine was used in place of methanol/triethylamine with 10% Pd/C as the catalyst. A reaction time of about 2.5 hours was sufficient to cleanly convert **11** to L-756,568, **1.** The formation of side products was suppressed when DMF was the solvent. Deuterium model reactions showed good incorporation of deuterium into the phenyl ring as determined by ^{1}H NMR analysis. Repeating this same procedure with 10 curies of tritium gas required 3.5 hours and gave 85 mCi of crude [^{3}H]L-756,568, **1c**, with a radiochemical purity of 85%. The purification of this material proved to be much simpler than before. The final purified [^{3}H]L-756,568 had a radiochemical purity of >98% and a specific activity of 6 Ci/mmol.

Scheme 2[a]. Synthesis of [^{3}H]L-756,568, 1c, via bromoaryl sulfonamide, 11.



[a]Key: (a) HCl, EtOH, 94% (b) BOC_2O, Et_3N, CH_2Cl_2, quantitative (c) H_2, 10% Pd/C, EtOH, 98% (d) $ClSO_2PhBr$, Et_3N, CH_2Cl_2, 38% (e) HCl, EtOAc, quantitative (f) 3, BOP, NMM, MeCN, 92% (g) HCl, EtOAc, 84% (h) 1N NaOH, MeOH, THF, 65% (i) T_2, 10% Pd/C, Et_3N, DMF.

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 Zorbax RX-C8, 4.6 x 250 mm, column (MAC-MOD Analytical, Chadds Ford, PA) was used for analytical and preparative HPLC. 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. Nα-Z-L-2,3-diaminopropionate, **7**, was purchased from Fluka. The synthesis of **2a/2c** outlined in scheme 1 and the synthesis of **11** outlined in scheme 2 follows the procedure for the synthesis of L-756,568, **1**, outlined in reference 5.

[3- or 4-^{3}H]5-[(4-Piperidinyl)methoxy]-2-indolecarbonyl-2(S)-phenylsulfonylamino-β-alanine, 1a/1b: A solution of 5-[(4-Piperidinyl)methoxy]-3-bromo-2-indolecarbonyl-2(S)-phenylsulfonylamino-β-alanine, **2b**, and 5-[(4-Piperidinyl)methoxy]-4-bromo-2-indolecarbonyl-2(S)-phenylsulfonylamino-β-alanine, **2a**, (10 mg, 0.0173 mmol) in methanol (2.5 mL) was transferred to a 3 mL reaction vessel containing 10% Pd/C (8 mg). To this was added triethylamine (0.025 mL), the reaction vessel and its contents were degassed by three freeze-thaw evacuation cycles and then exposed to 10 curies of tritium gas. The reaction was terminated after a total of 1.5 hours at room temperature and atmospheric pressure. The reaction mixture was passed through a short column of celite which was then rinsed with methanol (2 x 1 mL). The solvents were removed *in vacuo* and any labile tritium was washed out by evaporation with methanol (3 x 2 mL). The residue was dissolved in methanol (20 mL) to give 28 mCi of activity. HPLC analysis (Zorbax RX-C8, 4.6 x250 mm, 25% MeCN:H_2O (10mM NH_4OAc with 0.1% TFA), 1 mL/min, 215 nm) showed the crude material (9.5 minute retention time for 7a/7b) had a radiochemical purity of ~70%. An aliquot was concentrated to dryness, diluted with 0.1N NaOH (0.05 mL) and H_2O (0.1 mL) and purified by HPLC (Zorbax RX-C8, 4.6 x 250 mm, 20% MeCN:H_2O (10mM NH_4OAc with 0.1% TFA), 1 mL/min, 215 nm). The [^{3}H]**1a,1b** had a retention time of 17 minutes. Fractions containing the desired product were pooled and concentrated and diluted in methanol to give [^{3}H]L-756,568, **1a/1b**, with a specific activity of 1.8 Ci/mmol and radiochemical and chemical purities >98%.

5-[(4-Piperidinyl)methoxy]-2-indolecarbonyl-2(S)-(4-^{3}H-phenyl)sulfonylamino-β-alanine, 1c: A solution of 5-[(4-Piperidinyl)methoxy]-2-indolecarbonyl-2(S)-(4-bromophenyl)sulfonylamino-β-alanine, **11**, (4.3 mg, 0.0074 mmol) in DMF (2.5 mL) was transferred to a 2 mL reaction vessel containing 10% Pd/C (3.2 mg). To this was added triethylamine (0.012 mL), the reaction vessel and its contents were degassed by three freeze-thaw evacuation cycles and then exposed to 10 curies of tritium gas. The reaction was terminated after a total of 3.5 hours at room temperature and atmospheric pressure. The reaction mixture was passed through a short column of celite which was then rinsed with methanol (3 mL). The solvents were removed *in vacuo* and any labile tritium was washed out by evaporation with methanol (3x1mL). The residue was dissolved in methanol (20 mL) to give 85 mCi of activity. HPLC analysis as for [^{3}H]**1a/1b** showed a radiochemical purity of 85%. Purification as for [^{3}H]**1a/1b** gave [^{3}H]L-756,568, **1c**, with a specific activity of 5.9 Ci/mmol and radiochemical and chemical purities >98%.

Acknowledgment

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References and Notes

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