Journal of Labelled Compounds and Radiopharmaceuticals
J. Labelled Cpd. Radiopharm. **44**, 55-59 (2001)

The Synthesis of the $\alpha_v\beta_3$ Integrin Receptor Ligand [^{125}I]L-775,219.

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Summary

The $\alpha_v\beta_3$ integrin receptor ligand [^{125}I]L-775,219, **1c**, was synthesized for use in an *in vitro* receptor binding assay with high specific activity (>1500 Ci/mmol) and a radiochemical yield of 15-20%. Conversion of iodide **1b** to the corresponding aryl trimethylstannane **2**, followed by radioiodination using Na^{125}I/iodobead gave **1c**.

Keywords: Iodine-125, vitronectin, $\alpha_v\beta_3$ integrin, radioiodination.

Introduction

Integrins are heterodimeric transmembrane receptors comprised of α and β subunits that are involved in cell-cell and cell-matrix adhesion (1). The $\alpha_v\beta_3$ integrin receptor is found on osteoclasts and mediates the adhesion and migration of osteoclasts to bone during bone resorption (2). When the rate of bone resorption by osteoclasts exceeds the rate of bone formation, bone loss and, ultimately, bone fractures can result. Like many integrins, $\alpha_v\beta_3$ binds arginine-glycine-aspartic acid (RGD) containing proteins such as the adhesive protein, vitronectin, and the snake venom protein, echistatin. It has been shown that antibodies to $\alpha_v\beta_3$ (3), as well as nonpeptide RGD mimetics (4), inhibit bone resorption in vitro and in vivo. This suggests that orally active, nonpeptide $\alpha_v\beta_3$ receptor antagonists could be useful in the treatment of osteoporosis.

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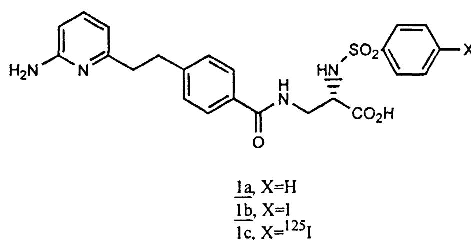
Received 4 May 2000
Revised 21 September 2000
Accepted 21 September 2000

In order to develop such compounds, an initial binding assay was developed to identify potent inhibitors of the binding of [^{125}I]echistatin (2,5) to $\alpha_v\beta_3$. However, this assay lacked the sensitivity required to differentiate high affinity compounds. For this reason, a new high affinity ligand with better binding characteristics than echistatin was required. Because high specific activity was desired, an iodine-125 labelled ligand was preferred. Compound **1a** and its 4-iodo isomer, **1b** (as shown in Figure 1), were found to be high affinity compounds (6) for the $\alpha_v\beta_3$ integrin receptor. Compound **1b** was chosen to radiolabel and evaluate as an $\alpha_v\beta_3$ radioligand. This paper describes the synthesis of [^{125}I]L-775,219, **1c**.

Figure 1.



1a, X=H
1b, X=I
1c, X=^{125}I

Discussion

Compound **1b** (6) is a high affinity $\alpha_v\beta_3$ receptor antagonist (IC_{50}=0.1nM)[7] that contains an aromatic iodine, and so was a candidate for labelling with iodine-125. As shown in scheme 1, heating iodide **1b** with Me_6Sn_2 and a catalytic amount of $Pd(PPh_3)_4$ in dioxane, followed by reverse phase preparative HPLC, yielded stannane **2**. Because the HPLC mobile phase contained trifluoroacetic acid, stanne **2** was isolated as its TFA salt, and lyophilization, without addition of ammonium hydroxide to the pooled fractions to neutralize the trifluoroacetic acid present, resulted in substantial loss of the trimethylstannyl group. With the addition of ammonium hydroxide however, stannane **2** could be isolated. With stannane **2** in hand,

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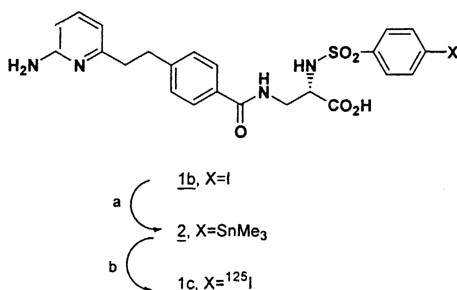
conditions for the iododestannylation were investigated. The oxidizing agent chosen was iodobead and the solvent used was H_2SO_4/methanol. It was found that 10% H_2SO_4/methanol for two minutes at room temperature reproducibly gave 15-20% radiochemical yields of **1c**, with high specific activity (>1500 Ci/mmol). The specific activity of this radioligand was determined using UV absorbance at 246 nm and had a range of 1580-1930 Ci/mmol.

Scheme 1. The Synthesis of 1c.



[a]Key: (a) Me_6Sn_2, cat $Pd(PPh_3)_4$, dioxane (b) $Na^{125}I$, iodobead, 10% H_2SO_4/MeOH, 2 min, RT.

This radioiodinated ligand has been used successfully in the primary *in vitro* binding assay for this program. The details of this binding assay as well as the characterization of this radioligand will be reported in the future.

Experimental

^1H NMR were recorded using a Varian Infinity-400 spectrometer operating at 400 MHz. Mass spectral analysis was carried out using a Waters ZMD LC/MS using electrospray in the positive ion mode. Analytical and preparative HPLC was carried out using a Waters 600E Powerline Multi Solvent Delivery System with 0.1 mL heads with a Rheodyne 7125 injector and a Waters 990 Photodiode Array Detector with a Gilson FC203 Microfraction collector. For analytical and preparative HPLC of **1c**, a

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Vydac peptide-protein C-18 column, 4.6 x 250 mm was used with a C-18 Brownlee modular guard column. For preparative HPLC of **2**, a C-18 Delta Pak, 40 x 100 mm column was used. The acetonitrile used for the HPLC analyses was Fisher Optima grade. The HPLC radiodetector used was a Beckman 170 Radioisotope detector. Solutions of radioactivity were concentrated using a Speedvac 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 a Packard A5530 gamma counter. Chemical reagents were purchased from Aldrich, Iodobeads were purchased from Pierce and the $Na^{125}I$ (IMS 300) was purchased from Amersham Pharmacia Biotech.

3-{4-[2-(2-Aminopyridin-6-yl)ethyl]benzoylamino}-2(S)-4-trimethylstannyl-benzenesulfonylamino-propionic acid, 2: A solution of iodide **1b** (70 mg, 0.12 mmol), Me_6Sn_2 (49 µl, 0.24 mmol), $Pd(PPh_3)_4$ (5 mg) and dioxane (7 ml) was heated to 90°C. After 2 h, the reaction was concentrated and then purified by preparative HPLC [Delta-Pak C_{18} 15 µM 100A, 40 x 100 mm; 40 mL/min, 50 minute linear gradient, 95:5 to 5:95 H_2O (0.1% TFA):MeCN (0.1% TFA), 215 nm, retention time 46.5 minutes] to provide the trifluoroacetate salt. The salt was suspended in H_2O (10 ml), treated with ammonium hydroxide (5 drops) and then lyophilized to provide 19.2 mg (26%) of **2** as a white solid: ^1H NMR (δ, d_6 DMSO) 8.40 (1H, m), 8.18 (1H, d, J=8Hz), 7.67 (5H, m), 7.56 (2H, d, J=8Hz), 7.29 (2H, d, J=8Hz), 6.95-7.52 (2H, m), 6.45 (2H, br s), 4.00 (1H, m), 3.50 (1H, m), 3.33 (1H, m), 2.97 (2H, m), 2.86 (2H, m), 0.25 (9H, t, J=27.4 Hz); MS m/z for $C_{26}H_{32}N_4O_5SSn$: 633 (M+1).

[^{125}I]3-{4-[2-(2-Aminopyridin-6-yl)ethyl]benzoylamino}-2(S)-4-iodobenzenesulfonylamino-propionic acid, 1c: An iodobead was added to a shipping vial of 10 mCi of $Na^{125}I$ and stirred for five minutes at room temperature. A solution of ~0.1 mg of **2** in methanol (45 µL) was treated with H_2SO_4 (5 µL) and immediately added to the $Na^{125}I$/iodobead vial.

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After stirring for two minutes at room temperature, approximately 0.04-0.05 mL of ammonium hydroxide was added so the reaction mixture was at pH 6-7. The entire reaction mixture was purified by HPLC [Vydac peptide-protein C-18 column, 4.6 x 250 mm, linear gradient of 10% MeCN:H_2O (0.1% TFA) to 90% MeCN:H_2O (0.1% TFA) over 30 minutes @ 1 mL/min]. The retention time of **1c** is approximately 17 minutes under these conditions. Fractions from the center cut were pooled, lyophilized and diluted with ethanol to give 1.7 mCi of **1c**, which coeluted on HPLC with an authentic sample of **1b**. The specific activity was determined using UV absorbance (246 nm) and was measured to be 1932 Ci/mmol.

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7. Displacement of [^{125}I]echistatin from human $\alpha_v\beta_3$.

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