Synthesis of 4-[2-(4-Azidophenyl)-5-(3-iodo-^{125}I-phenyl)-1H-imidazol-4-yl]pyridine (SB 206718-[^{125}I]), a Pyridinyl Imidazole Cytokine Inhibitor

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SUMMARY

The pyridinyl imidazole cytokine-suppressing anti-inflammatory drug (CSAID™), SB 206718 (**1**) was required in ^{125}I-labeled form for photoaffinity ligand studies. The target compound (SB 206718-[^{125}I], [^{125}I]**1**) was obtained via conversion of the highly functionalized **1** to a tributylstannyl derivative. Radioiododestannylation using Na^{125}I in the presence of chloramine-T gave good radiochemical yields of the title compound (42-69%, four radiosyntheses) at high radiochemical purity (>98%) after HPLC purification at specific activities of 1670-1736 Ci/mmol.

Key Words: Pyridinyl imidazole, CSAID™, iodine-125, photoaffinity labelling

Introduction

Cytokines are protein hormones which regulate immune and inflammatory responses. One such protein, interleukin 1 (IL-1), has been implicated in a wide range of human diseases such as hemodynamic shock, arthritis, inflammatory bowel disease and lethal sepsis (1). Along with tumor necrosis factor α (TNF-α), IL-1 also modulates bone cell proliferation and is thought to have a role in the development of postmenopausal osteoporosis (2,3). Modulators of cytokine function therefore make attractive targets for drug research. SB 206718 (**1**) belongs to a class of novel antiinflammatory agents with the potential to modify the course of chronic inflammatory disease by inhibition of IL-1 and TNF-α. An iodine-125 labeled analog of **1** was critical for pharmacologic studies aimed at identifying the cytokine-suppressing anti-inflammatory drug (CSAID™) binding protein. SB 206718-[^{125}I] has now been prepared in high yield and successfully used to identify, purify and characterize the CSAID binding protein (4).

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Results and Discussion

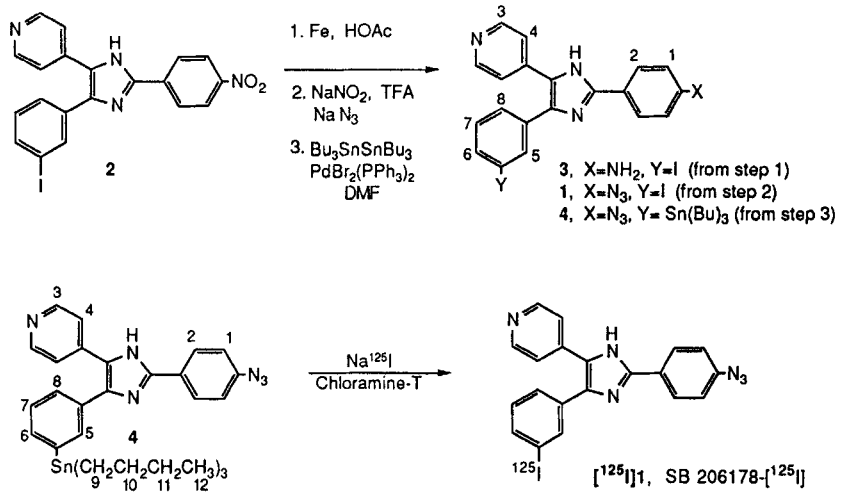
Halodestannylation has proven to be exremely useful method for introduction of a radiohalogen and is particularly useful for the regiospecific labeling at a meta position in an aromatic ring (5-10). For the preparation of **1** labeled with iodine-125, we chose to convert unlabeled **1** to its tributylstannyl derivative followed by radioiododestannylation.

The synthetic route used for the preparation of SB 206718-[^{125}I] is shown below. Reduction of 4-[5-(3-iodophenyl)-2-(4-nitrophenyl)-1**H**-imidazol-4-yl]pyridine (**2**) with iron powder in ethanol/water/acetic acid at reflux gave 4-[4-(3-iodophenyl)-5-(4-pyridinyl)-1**H**-imidazol-2-yl]benzeneamine (**3**) in 94% yield (11). Treatment of **3** with sodium nitrite in trifluoroacetic acid at room temperature, followed by addition of sodium azide gave the unlabeled target compound **1** in 79% yield.

The key intermediate in the synthesis, tributylstannane **4**, was obtained by palladium catalyzed coupling of **1** with bis(tributyltin). Several Pd(II) catalysts were examined, $PdBr_2(PPh_3)_2$, $PdCl_2(PPh_3)_2$ and $PdCl_2(C_6H_5CN)_2$, as well as one Pd(0) catalyst, $Pd(PPh_3)_4$. All gave the desired tributylstannane, though reaction with $PdBr_2(PPh_3)_2$ appeared to be slightly cleaner. Choice of solvent was critical to the successful stannylation of the highly functionalized starting material **1**. Reaction in toluene did not give any tributylstannane whereas reaction in DMF gave the desired compound in 55% yield 10-20 minutes after gentle heating.

Since the stannane **4** was prepared from the unlabeled form of the target compound, it was critical that any trace of **1** be removed in order to prevent dilution of the specific activity of [^{125}I]**1**. This was accomplished by rigorous purification by preparative TLC prior to destannylation. A long wave chromophore of **1** was easily visible so that contamination of **4** with **1** could be detected by UV.

Another concern was the possiblility of diluting the "effective" specific activity of [^{125}I]**1**with a non-labeled component which might be expected to compete for the CSAID binding protein. One such possible competitor, tributylstannane **4**, was easily removed by normal phase HPLC where the desired product **1** (R_T= 14.8 min) was well separated from the tributylstannane **4** (R_T= 6.6 min). Another possible competitor is the chlorinated analog of **1**. Possible production of such a chlorinated analog was checked in a control reaction using chloramine-T as the oxidant without any added sodium iodide. No product was observed to elute with the same retention time as **1**.



Radioiododestannylation of **4** (100 μg) by reaction with Na ^{125}I (2-5 mCi) in the presence of chloramine-T (1 μg, 0.03 equivalents) in 3% acetic acid in ethanol gave SB 206718-[^{125}I]). Four syntheses of [^{125}I]**1** have been completed and gave radiochemical yields of 59% ± 13% (mean ± SEM; n = 4) after HPLC purification. Specific activities of SB 206718-[^{125}I] were 1670-1736 Ci/mmol. The purified product [^{125}I]**1** exhibited high radiochemical purity (98.6-99.9%) by analytical HPLC. The final product was stored as an ethanol solution at 0.8-1.2 mCi/mL at -78°C in the dark. Solutions of SB 206718-[^{125}I] were stable for the time reqiured for photoaffinity studies under these storage conditions.

Conclusion

Reaction conditions for the preparation of an important photoaffinity label to study the CSAID binding protein have been developed. SB 206718-[^{125}I] has been prepared in good yield and at high specific activity via conversion of unlabeled SB 206718 to an intermediate suitable for regiospecific iodination. This intermediate, tributylstannane **4**, was easily coverted back to labeled SB 206718. This iodine-125 labeled photoaffinity label has played a key role in the characterization of the CSAID binding protein.[4]

Experimental

General: All organic reagents were obtained from Aldrich Chemical Company unless otherwise noted, and were used without further purification. Chloramine-T was obtained from Sigma Chemical. No-carrier added Na^{125}I was obtained from DuPont/NEN (2-5 mCi/ 50 μL

dilute aqueous NaOH, pH 10). HPLC equipment consisted of a Rheodyne 7125 injector, Beckman 110B Pump, Waters 481 UV absorbance detector (260 nm), a flow-through NaI(Tl) crystal scintillation detector comprised of Ludlum components, Gilson automatic fraction collector, HP 3380 integrator, and Houston Instruments strip chart recorders. Proton nuclear magnetic resonance spectra were obtained on a Bruker AM-400 instrument. Chemical shifts (δ) are reported downfield from tetramethylsilane. The HPLC response curve relating mass to UV peak area was generated using solutions of unlabeled SB 206718 standards in concentrations chosen to bracket the region of interest.

4-[4-(3-Iodophenyl)-5-(4-pyridinyl)-1H-imidazol-2-yl]benzeneamine (**3**) A 500 mg portion of 4-[5-(3-iodophenyl)-2-(4-nitrophenyl)-1H-imidazol-4-yl]pyridine (**2**, 1.07 mmol) was suspended in a mixture of 36 mL of ethanol and 25 mL of water. A 3.0 mL portion of acetic acid was added, followed by 298 mg of iron powder (5.34 mmol). The reaction mixture was heated at reflux with vigorous stirring for 90 minutes, at which point TLC (Merck silica, 9:1 v/v chloroform/methanol) showed no starting material (R_f 0.3) remaining. The reaction mixture was allowed to cool to ca. 30°C, filtered through Celite, and the filtrate was concentrated *in vacuo*. The residue was dissolved in 30 mL of 9:1:0.1 (v/v/v) chloroform/methanol/ammonium hydroxide and the resulting solution was vacuum filtered through silica gel. The product was eluted using the same solvent and was visible as a bright yellow band. The filtrate was concentrated *in vacuo*, resulting in 0.75 g of crude **3** as an orange glass. Purification by flash chromatography (100 g of Baker 40 μm flash silica gel, eluted with 9:1 v/v chloroform/methanol) provided 313 mg of **3** as a yellow solid (m.p. 261-262°C) after trituration with diethyl ether. An additional 125 mg was obtained from slightly less pure chromatography fractions, resulting in an overall chemical yield of 93.7%. ^{1}H-NMR (400 MHz, $CDCl_3$): δ8.40 (2H, d, J=6.2 Hz, H3), 7.87 (1H, s, H5), 7.69-7.73 (3H, m, H2 and H6), 7.50 (2H, d, J=6.2 Hz, H4), 7.44 (1H, d, J=7.8 Hz, H8), 7.14 (1H, t, J=7.8 Hz, H7), 6.77 (2H, d, J=8.5 Hz, H1)

4-[2-(4-Azidophenyl)-5-(3-iodophenyl)-1H-imidazol-4-yl]pyridine (**1**) A 313 mg portion (0.714 mmol) of **3** was dissolved in 12 mL of trifluoroacetic acid. To this, at room temperature, was added 148 mg (2.14 mmol, Aldrich) of sodium nitrite. The reaction was stirred 15 minutes and then 209 mg (3.22 mmol, Aldrich) of sodium azide was added all at once. Vigorous bubbling was observed. The solution was stirred 15 minutes and then poured into a saturated aqueous solution of sodium bicarbonate at 5°C. The resulting mixture was

made basic by addition of more saturated aqueous sodium bicarbonate. The solution was extracted with ethyl acetate (2 x 100 mL), dried over sodium sulfate, filtered, and concentrated to a yellow glassy solid *in vacuo*. The 360 mg of crude product thus obtained was purified by flash chromatography (100g of Merck 40 μm flash grade silica gel, eluted with 25:1 (v/v) chlroform/methanol). The product fractions were combined, concentrated to a yellow glassy solid *in vacuo* and then triturated with ether. This procedure gave 240 mg (73%) of SB 206718 (**1**) as a fine yellow powder . A second crop of 21 mg was also obtained from the mother liquors giving a total yield of 79%. **1**: ^{1}H-NMR (400 MHz, $CDCl_3$) δ8.46 (2H, d,J=4.4 Hz,H3), 8.03 (2H, d, J=8.6 Hz, H2), 7.91 (1H, s, H5), 7.76 (1H, d, J=6.7 Hz, H6), 7.54 (2H, s(broad), H4), 7.49 (1H, d,J=7.6 Hz, H8), 7.18-7.22 (3H, m, H1 and H7); CI-MS (NH_3) m/e (% intensity): 465 ($M+H^+$,62), 439 (8), 339 (61), 313 (100); Microanalysis Calcd. for $C_{20}H_{13}IN_6$: C, 51.74; H, 2.82; N, 18.10; Found: C, 51:55; H, 2.96; N, 17.42.

[3-[2-(4-Azidophenyl)-5-(4-pyridinyl)-1H-imidazol-4-yl]phenyl]-tributylstannane (**4**)

SB 206718 (**1**, 34 mg, 73 μmol) was dissolved in 1 mL of dry DMF. To this solution was added 92 μL of bis(tributyltin) (106 mg, 182 μmol, Aldrich) and ~4 mg of $PdBr_2(PPh_3)_2$ (Aldrich) in a nitrogen filled glove bag. The reaction was heated at 95-105°C in a closed vial. The vial was *immediately* removed from the oil bath as soon as the reaction turned dark and a precipitate formed (~10 minutes). The reaction mixture was partitioned between saturated aqueous potassium carbonate and ethyl acetate. The ethyl acetate layer was dried over sodium sulfate, filtered and concentrated to a yellow oil *in vacuo*. The oil was taken up in chloroform and eluted through a silica gel Sep-pak (Waters) with chloroform. The chloroform layer contained the product plus the excess bis(tributyltin). This chloroform layer was then evaporated and the residue taken up in hexane. This hexane solution was eluted through another silica gel Sep-pak (Waters) with hexane; the hexane eluate contained bis(tributyltin). The product was eluted from the Sep-pak with 10% methanol in chloroform. The solvent was removed *in vacuo* giving 25 mg (55%) of the tributylstannane derivative **4**. TLC analysis (95:5 chloroform/methanol) still showed a trace of starting iodide (R_f 0.26) contaminating the tributylstannane product (R_f 0.36). The 25 mg of product was then subjected to preparative TLC (Merck 2 mm thick 20 x 20 cm plate, eluted with 92.5:7.5 (v/v) chloroform/methanol). This procedure gave 17 mg of **4** which still showed a trace of starting iodide (readily evident under long wave UV light). A second preparative TLC using 95:5 (v/v) cloroform/methanol (two developments) gave 10 mg (22%) of pure **4**. ^{1}H-NMR (400 MHz, $CDCl_3$) δ8.32 (2H, d,

J=4.9 Hz, H3), 7.86 (2H, d, J=8.6 Hz, H2), 7.52 (2H, s(broad), H4), 7.49 (1H, s, H5), 7.43 (1H, d, J=6.6 Hz, H6), 7.29-7.34 (2H, m, H7 and H8), 7.03 (2H, d, J=8.6 Hz, H1), 1.38-1.46 (6H, m, H9), 1.21 (6H, m, H10), 0.90-1.00 (6H, m, H11), 0.78 (9H, t, J=7.3 Hz, H12).

4-[2-(4-Azidophenyl)-5-(3-iodo-^{125}I-phenyl)-1*H*-imidazol-4-yl]pyridine ([^{125}I]**1**) [3-[2-(4-Azidophenyl)-5-(4-pyridinyl)-1**H**-imidazol-4-yl]phenyl]-tributyl-stannane, **4** (100 μg, 0.159 μmol), was dissolved in 50 μL of 3% acetic acid in ethanol. To this solution was added 1.14 μg of chloramine-T hydrate (0.005 μmol) in 11.4 μL of water. To this solution was added 2.0 mCi of sodium [^{125}I]iodide in 15 μL of 0.1 N sodium hydroxide. Another 50 μL of 3% acetic acid in ethanol was added to make the reaction mixture homogeneous. The reaction was stirred 30 minutes at room temperature (in the dark). The reaction was then blown to dryness under a stream of dry nitrogen and the residue partitioned between chloroform (1 mL) and saturated aqueous sodium bicarbonate (1 mL). The aqueous layer was extracted with chloroform (2 x 1 mL), the organic layers were combined and dried by passing through a pipet filled with granular sodium sulfate. The solvent was removed under a stream of dry nitrogen and the residue from the organic layer was taken up in 50 μL of HPLC mobile phase and purified on a Baker SiO_2 column, 5 μm, 4.6mm ID x 250 mm, eluted at 1.0 mL/min with 90:10:1 (v/v/v) hexane/isopropanol/triethylamine, with UV monitoring at 260 nm. The product fractions were combined and blown to dryness under a stream of dry nitrogen. The product was taken up in 1.0 mL of absolute ethanol. This procedure gave 840 μCi (42%) of SB 206718-[^{125}I] (**1a**) at a radiochemical purity of 99.9% and a specific activity of 1718 Ci/mmol, based on mass and radioactivity concentrations.

The above procedure was repeated three times using 5.0 mCi of sodium [^{125}I]iodide, 250 μg of **4** (0.398 μmol), and 2.85 μg of chloramine-T in 100 μL of 3% acetic acid in ethanol. The mixture was kept for 55 minutes in the dark, with workup and purification as described above. This procedure gave radiochemical yields ranging from 55-69% of [^{125}I]**1** at radiochemical purities of 98.6% to 99.9% by HPLC (Baker, Silica, 5μm, 120 Å, 4.6 mm I.D. x 25 cm 90:10 (v/v)hexane/isopropanol, 1.0 mL/min, UV @ 260 nm) and specific activities of 1670-1736 Ci/mmol, calculated from radioactivity and mass concentrations of ethanol solutions of the final product.

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