SYNTHESIS OF A RADIOIODINATED ANALOGUE OF THE SEROTONIN 5-HT_{2A} RECEPTOR LIGAND RP 62203

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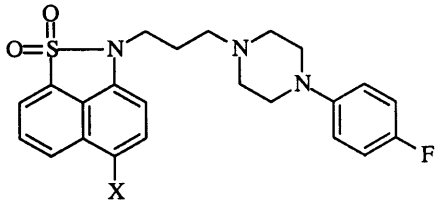
Summary

An analogue of RP 62203 having radioiodine in the 4-position of the 1,8-naphthosultam ring system has been prepared for evaluation as a ligand for the serotonin 5-HT_{2A} receptor. Non-radioactive 4-iodo-RP 62203 was synthesized in three steps (20% overall yield) by iodination of 1,8-naphthosultam with iodine monochloride followed by *N*-alkylation with 1-bromo-3-chloropropane and coupling with 1-(4-fluorophenyl)piperazine. The precursor for radiolabeling was prepared in 83% yield from 4-iodo-RP 62203 and hexamethylditin by palladium-mediated trimethylstannylation. Electrophilic radioiododestannylation gave 4-[^{125}I]-RP 62203 in good isolated yield (80 - 86%), with high purity (≥ 99.6%) and specific radioactivity (1200 - 2066 mCi/μmol).

Key Words: RP 62203, radioiodine, serotonin receptor

Introduction

RP 62203 (Figure 1) is a potent and selective serotonin 5-HT_{2A} receptor antagonist (1). The apparent affinity of RP 62203 for 5-HT_{2A} sites (K_i = 0.26 nM) is at least 60- to 100-fold higher than the most notable secondary binding interactions with histaminergic (H_1), adrenergic (α_1) or other serotonergic (5-HT_{2C}, 5-HT_{1A}) sites (1, 2). Tritiated RP 62203 displays high 5-HT_{2A} receptor affinity (K_d = 0.13 nM), is useful for quantitative autoradiography *in vitro*, and labels 5-HT_{2A} sites *in vivo* in rat brain (3, 4). RP 62203 also has been isotopically radiolabeled



X = H RP 62203

X = I 4-Iodo-RP 62203

X = ^{125}I 4-[^{125}I]-RP 62203

Figure 1. Structures of RP 62203 and iodinated RP 62203 analogues.

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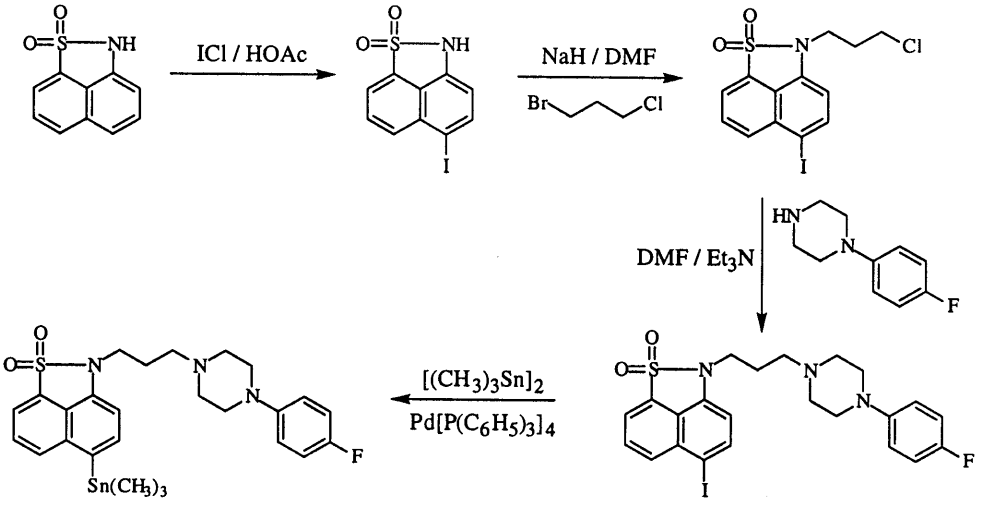
with F-18 for positron emission tomography (PET) studies (5 - 7). Complementary radioiodinated analogues of RP 62203 could prove useful for basic science studies (I-125), and for single photon emission computed tomography (SPECT) studies (I-123). Here we report the synthesis of 4-iodo- and 4-[^{125}I]-RP 62203 (Figure 1).

Results and Discussion

Qualitative structure-activity relationships for serotonergic 1,8-napthosultams have been reported by Malleron *et al.* (1). Incorporation of bromine or chlorine in the phenylpiperazine ring led to a loss of 5-HT_{2A} receptor affinity, while a three carbon chain length between the piperazine and the napthosultam proved optimal for good 5-HT_{2A} receptor selectivity. The effects of substitution on the napthosultam ring system, however, were not investigated. Therefore, we targeted a novel analogue of RP 62203 that would incorporate iodine at the synthetically accessible and pharmacologically interesting 4-position of the napthosultam moiety.

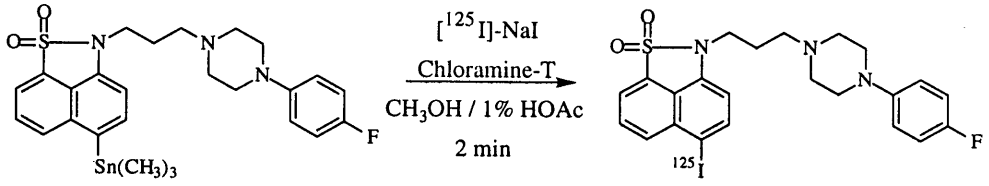
Scheme 1 shows the pathway taken for the preparation of 4-iodo-RP 62203 and the corresponding trimethylstannyl derivative chosen as the precursor for radioiodination. Commercially available 1,8-napthosultam was treated with iodine monochloride in acetic acid to give 4-iodo-1,8-naphthosultam in 45% yield as described previously by Mustafa *et al.* (8). Alkylation with 1-bromo-3-chloropropane in dimethylformamide (DMF) using sodium hydride as the base then gave a mixture of the *N*-3-chloropropyl and *N*-3-bromopropyl derivatives. A combination of spectroscopic and chromatographic techniques showed that *N*-(3-chloropropyl)-4-iodo-1,8-naphthosultam was the main component (90%). Treatment of the halopropyl naphthosultams with 1-(4-fluorophenyl)piperazine in DMF containing triethylamine at 120 °C for 3.5 hours provided 4-iodo-RP 62203 as a pale yellow solid (77%). Palladium-catalyzed stannylation in toluene at reflux for 4 hours occurred in good yield (83%). To ensure that carrier would not be contributed at the radiolabeling step, the trimethylstannyl derivative was purified



Scheme 1. Synthesis of 4-iodo- and 4-trimethylstannyl-RP 62203.

rigorously by preparative reverse-phase (C-18 Nova-Pak, 10 x 250 mm) high performance liquid chromatography (HPLC) followed by normal-phase column chromatography. Residual iodinated material was not observed in the colorless solid by mass spectroscopy, or by analytical HPLC at a detection limit of 0.005%.

Mild conditions were used for labeling that are known to give regiospecific radioiodination in high yield accompanied by minimal chloro- and protodestannylation (9, 10). As shown in Scheme 2, radioiododestannylation was done at ambient temperature on 0.2 mg of the precursor in methanolic acetic acid using chloramine-T as the oxidant for no-carrier-added [^{125}I]-NaI on a 2 mCi scale. After 2 min, aqueous sodium metabisulfite was added as a quenching agent.



Scheme 2. Radiosynthesis of 4-[^{125}I]-RP 62203.

Reaction mixtures were examined by reverse-phase HPLC (C-18 Nova-Pak, 8 x 100 mm) using a ternary mobile phase (Figure 2). Radioiodine incorporation was > 99% to give one product with a chromatographic profile (t_R = 32.3 min, k'= 41.5) identical to that of non-radioactive 4-iodo-RP 62203. The trimethylstannyl precursor was retained for more than 75 min under these HPLC conditions (not

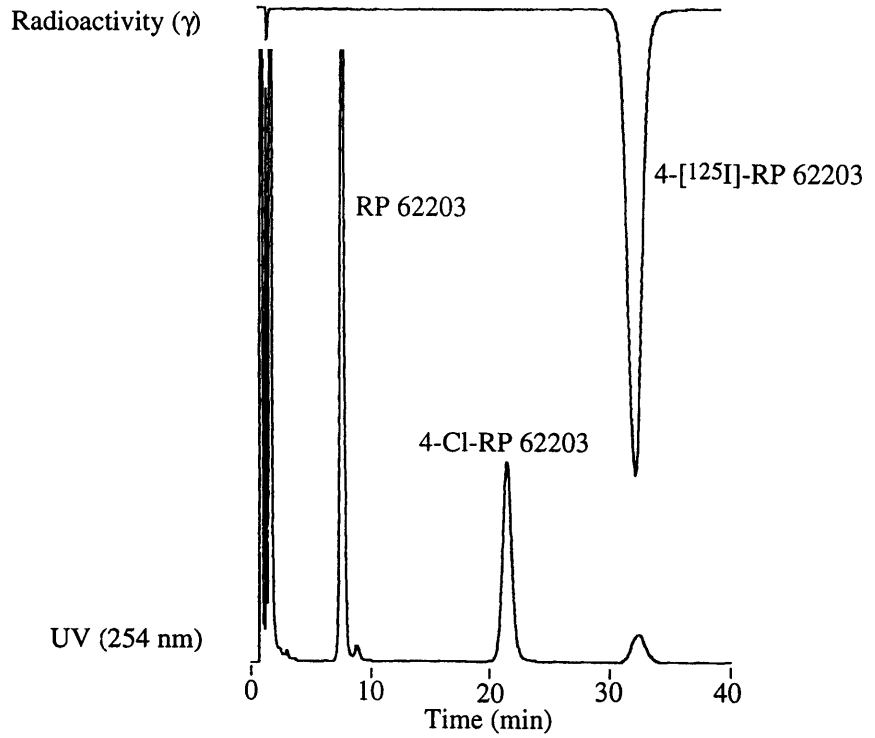


Figure 2. Preparative HPLC chromatograms for isolation of 4-[^{125}I]-RP 62203.

shown), and the radioligand was readily resolved from two primary side products (t_R = 7.6 min, k'= 8.9; t_R = 21.4 min, k'= 27.1) observed by UV detection (254 nm).

These two side products also were observed in model reactions performed in the absence of radioiodide. The relative proportion of the more lipophilic material increased as the concentration of chloramine-T was raised, while the less lipophilic material grew with higher percentages of acetic acid. These data are consistent with assignment of the compounds as 4-chloro-RP 62203 and RP 62203. With the assumption of equal HPLC response factors, approximately 2% of the precursor is consumed by oxidant-promoted chlorodestannylation and 3% suffers protodestannylation under the radioiodination conditions (*cf.* Figure 2).

Good isolated yields (80 - 86%) of 4-[^{125}I]-RP 62203 were obtained as concentrated solutions in ethanol after HPLC purification and solid-phase extraction. By analytical HPLC (C-18 Nova-Pak, 8 x 100 mm), final formulations of the radioligand (t_R = 5.8 min, k'= 6.6) matched standards of 4-iodo-RP 62203, and had ≥ 99.6% radiochemical purity with no detectable chemical impurities (Figure 3).

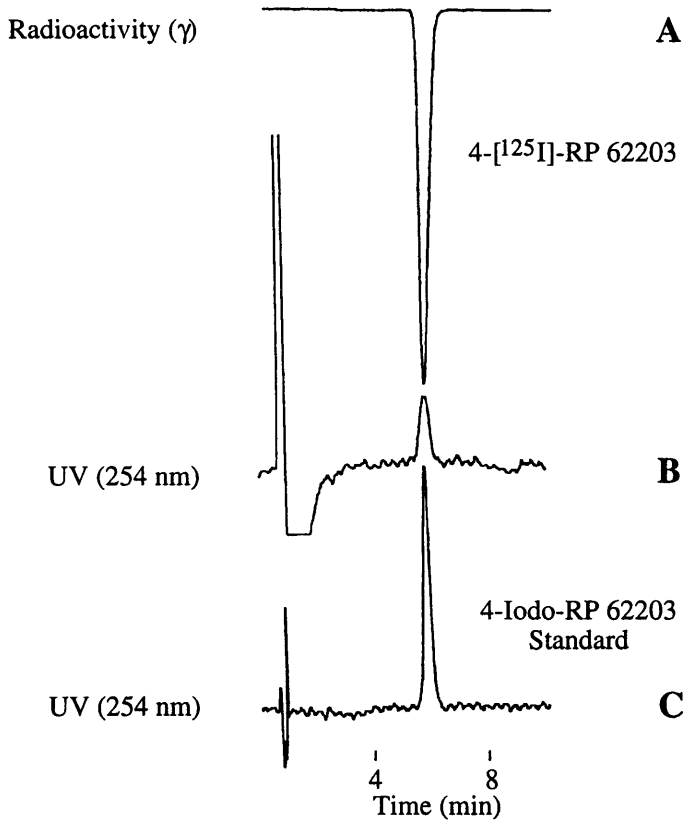


Figure 3. Analytical HPLC chromatograms of purified 4-[^{125}I]-RP 62203 used for a specific radioactivity determination. Top traces **A** (radioactivity) and **B** (UV) are of the final formulation of radioligand in ethanol. Bottom trace **C** (UV) is of one of the standard samples of 4-iodo-RP 62203 used to generate the HPLC response curve.

For specific radioactivity calculations, the mass of carrier in samples of 4-[^{125}I]-RP 62203 having known radioactivity was determined by HPLC. The UV absorbance (254 nm) peak height of the carrier was related to the equation for a linear (r^2 = 1.0), eight-point standard curve established with 4-iodo-RP 62203 over a concentration range (18 - 270 pmol) chosen to bracket the region of interest. For the sample shown in Figure 3, 76 pmol of carrier was associated with 157 μCi of the radioligand. The calculated values (1200 - 2066 mCi/μmol) for 4-[^{125}I]-RP 62203 were in good agreement (± 5%) with the manufacturer's data for the distinct batches of [^{125}I]-NaI employed.

Conclusions

We have prepared a novel analogue of RP 62203 that has iodine at the 4-position of the 1,8-naphthosultam ring system. The radioiodinated version, 4-[^{125}I]-RP 62203, was synthesized by electrophilic radioiododestannylation in good yield, with high specific radioactivity, and with a high degree of chemical and radiochemical purity. Initial studies indicate that high affinity for the serotonin 5-HT_{2A} receptor is maintained upon iodination of the naphthosultam moiety. The apparent affinity (K_i) of 4-iodo-RP 62203 for 5-HT_{2A} sites is 0.46 ± 0.05 nM measured against [^{3}H]ketanserin in rat frontal cortex membrane homogenates (Lever *et al.*, in preparation). Preliminary *in vivo* studies suggest that 4-[^{125}I]-RP 62203 selectively labels 5-HT_{2A} sites in mouse brain (Lever *et al.*, in preparation). However, the degree of specific *in vivo* binding appears to be substantially less than that shown by [^{3}H]RP 62203 *in vivo* in rat brain (4) or by the structurally unrelated ergot, D-(+)-*N*1-ethyl-2-[^{125}I]-LSD, *in vivo* in mouse brain (11). Thus, 4-[^{125}I]-RP 62203 is readily obtainable, and may prove useful as a serotonergic radioligand for certain *in vitro* and *in vivo* studies.

Experimental

Melting points (uncorrected) were determined using a Thomas-Hoover capillary apparatus. ^{1}H NMR spectra were obtained on a Bruker WM-300 (300.13 MHz) instrument. Chemical shifts are reported in ppm (δ) relative to internal Me_4Si. Low resolution electron impact mass spectroscopy (MS) was done at the University of Minnesota Mass Spectrometry Service Laboratory using a Finnigan MAT 90 instrument (source 200 °C, accelerating voltage 5000, solid probe). Data are given as observed *m/z* (M^+, relative intensity). Elemental analyses were determined by Atlantic Microlab, Inc. (Norcross, GA). Short-path column chromatography used E. Merck 7729 (< 230 mesh) silica gel under N_2 pressure. Dimethylformamide (DMF) was distilled under reduced pressure from CaH_2. Other chemicals and solvents were reagent grade, and were used as received from commercial sources. No-carrier-added [^{125}I]-NaI was obtained from Amersham Corp. (1 mCi / 10 μL dilute aqueous NaOH, pH 7-11). The HPLC equipment included a Rheodyne 7125 injector, Waters 510EF pumps, Waters 490 UV absorbance detector set at 254 nm, a flow-through NaI(Tl) crystal scintillation detector comprised of EE&G Ortec components, and Shimadzu CR-3A integrating recorders. Waters C-18 Nova-Pak (6 μm) columns for radial compression modules (RCM) were used for analytical (8 x

100 mm) and preparative (10 x 250 mm) HPLC. Ternary HPLC mobile phases consisted of an organic phase (MeOH / CH_3CN; 50:50, *v/v*) mixed in varying proportions with an aqueous solution of Et_3N (2.1% *v/v*) and HOAc (2.8% *v/v*). Waters SEP-PAK Light *t*-C-18 cartridges, activated prior to use by sequential elution with ethanol and distilled water, were used for solid-phase extraction.

4-Iodo-1,8-naphthosultam was synthesized as described by Mustafa *et al.* (8). In brief, a solution of ICl (4.03 g, 25.0 mmol) in glacial HOAc (30 mL) was mixed with a solution of 1,8-naphthosultam (5.00 g, 24.0 mmol) in glacial HOAc (40 mL) and allowed to stand at ambient temperature in the dark for 15 h. The mixture was filtered, and the solid was recrystallized from glacial HOAc, washed with water, and dried *in vacuo* to give 4-iodo-1,8-naphthosultam (3.67 g, 11.1 mmol) in 45% yield as a black solid (mp 198 - 210 °C; lit. 211 °C). ^{1}H NMR (DMSO-D_6) δ 6.78 (d, J = 14.8 Hz, 1H), 7.99 (t, J = 16.1 Hz, 1H), 8.12 (d, J = 16.1 Hz, 2H), 8.24 (d, J = 14.8 Hz, 1H). MS ($C_{10}H_6INO_2S$): *m/z* calcd 330.9; found 330.8 (M^+, 100%).

***N*-3-Chloropropyl-4-iodo-1,8-naphthosultam.** A solution of 4-iodo-1,8-naphthosultam (2.85 g, 8.59 mmol) in DMF (25 mL) containing NaH (0.120 g, 8.59 mmol) was stirred for 1 h under N_2. 1-Bromo-3-chloropropane (1.49 g, 9.45 mmol) was added, and the mixture was stirred 40 h. DMF was removed by Kugelrohr distillation under reduced pressure to give a brown residue. Short-path column chromatography (100 g silica gel; hexane:CH_2Cl_2:NEt_3, 90:8:2) followed by recrystallization from glacial HOAc gave 2.0 g (*ca.* 57%) of a yellow solid (mp 99 - 103 °C) determined to be a mixture of *N*-(3-chloropropyl)-4-iodo-1,8-naphthosultam and *N*-(3-bromopropyl)-4-iodo-1,8-naphthosultam in a 90:10 ratio by ^{1}H NMR ($CDCl_3$): δ 2.38 (m, 1.8 H; NC-CH_2-C-Cl), 2.47 (m, 0.2 H; NC-CH_2-C-Br), 3.61 (t, J = 5.8 Hz, 0.2 H; C-CH_2-Br), 3.76 (t, J = 5.8 Hz, 1.8 H; C-CH_2-Cl), 4.05 (t, J = 6.9 Hz, 2.0 H; SO_2NCH_2), 6.64 (d, J = 8.9 Hz, 1.0 H; Ar-H), 7.85 (d,d, J = 8.9, 8.9 Hz, 1.0 H; Ar-H), 8.02 (d, J = 7.9 Hz, 1.0 H; Ar-H), 8.04 (d, J = 7.9 Hz, 1.0 H; Ar-H) 8.14 (d, J = 8.9 Hz, 1.0 H). The mass spectral data were consistent with these assignments: MS ($C_{13}H_{11}ICINO_2S$) *m/z* calcd 406.9; found 406.2 (M^+, 100%); MS ($C_{13}H_{11}IBrNO_2S$) *m/z* calcd 450.9, 452.9; found 450.0, 452.0 (M^+, 8.4, 8.1%). Analytical HPLC (60% organic / 40% aqueous; 8 x 100 mm RCM) at 3.5 mL/min showed peaks for the chloro- (90%; t_R = 11.0 min; k' = 10.0) and bromo- (10%; t_R = 13.3 min; k' = 12.4) derivatives without evidence of other components, and the mixture was carried on without further separation.

2-[3-[4-(4-Fluorophenyl)-1-piperazinyl]propyl]-4-iodo-2*H*-naphth[1,8-*cd*]isothiazole 1,1-dioxide (4-Iodo-RP 62203). A mixture (90:10) of *N*-(3-chloropropyl)- and *N*-(3-bromopropyl)-4-iodo-1,8-naphthosultam (1.17 g, *ca.* 3.0 mmol), 1-(4-fluorophenyl)piperazine (1.00 g, 5.56 mmol) and triethylamine (0.83 g, 8.2 mmol) in DMF (12 mL) was heated at 120 °C for 3.5 h. The mixture was cooled, diluted with water (50 mL) and extracted with ethyl acetate. The organic extracts were dried (Na_2SO_4), filtered and concentrated under reduced pressure. Short-path column chromatography (50 g silica gel; hexane:EtOAc:Et_3N, 74:24:2) gave 1.26 g (2.28 mmol, 77%) of 4-iodo-RP 62203 as a pale yellow solid (mp 136 - 139 °C). ^{1}H NMR ($CDCl_3$): δ 2.08 (m, 2H), 2.54 (t, J = 6.6 Hz, 2H), 2.60 (m, 4H), 3.12 (m, 4H), 3.91 (t, J = 6.6 Hz, 2H), 6.65 (d, J = 8.7 Hz, 1H), 6.91 (m, 4H), 7.82 (t, J = 7.8 Hz, 1H), 7.97 (d,d, J = 8.9, 3.3 Hz, 2H), 8.7 (d, J = 8.9 Hz, 1H). MS: *m/z*

calcd 551.0; found 551.0 (M^+, 23%). Anal. calculated for $C_{23}H_{23}FIN_3O_2S$: C, 50.10; H, 4.20; N, 7.62; I, 23.01; S, 5.81. Found: C, 50.14; H, 4.24; N, 7.57; I, 23.01; S, 5.93.

2-[3-[4-(4-Fluorophenyl)-1-piperazinyl]propyl]-4-trimethylstannyl-2*H*-naphth[1,8-*cd*]isothiazole 1,1-dioxide (4-Trimethylstannyl-RP 62203). To a stirred solution of 4-iodo-RP 62203 (0.100 g, 0.181 mmol) in freshly distilled toluene (5.0 mL) was added tetrakis(triphenylphosphine)palladium(0) (5.0 mg, 0.043 μmol) followed by a solution of hexamethylditin (0.205 g, 0.630 mmol) in toluene (0.4 mL). The mixture was refluxed under N_2 for 4 h, cooled, and then filtered through Celite. The Celite was washed with toluene, and the filtrate and washings were concentrated under reduced pressure to give a brown residue. Short path column chromatography (12 g silica gel; hexane:CH_2Cl_2:NEt_3, 74:24:2) gave the stannylated material (0.091 g, 0.15 mmol) as a white solid in 83% yield. Further purification by preparative HPLC using the ternary mobile phase (70% organic / 30% aqueous; 10 x 250 mm RCM) at 14.0 mL/min gave the product with t_R = 19.5 min (k' = 15.3). The HPLC solvent was concentrated under reduced pressure, and then extracted with CH_2Cl_2. The organic extract was concentrated, and the material purified again by short-path column chromatography to give homogeneous product as a colorless powder (0.060 g) with mp 120 - 121 °C. Fine needles were obtained by slow evaporation of a methanol solution. ^{1}H NMR ($CDCl_3$): δ 0.43 (s, 9H), 2.10 (m, 2H), 2.58 (t, J = 6.0 Hz, 2H), 2.61 (m, 4H), 3.12 (m, 4H), 3.92 (t, J = 6.0 Hz, 2H), 6.80 (d, J = 7.5 Hz, 1H), 6.90 (m, 4H), 7.59 (d, J = 7.5 Hz, 1H), 7.72 (t, J = 8.6 Hz, 1H), 7.94 (m, 2H). MS: *m/z* calcd 589.1; found 589.0 (M^+, 11%). Anal. calculated for $C_{26}H_{32}FN_3O_2SSn$: C, 53.08; H, 5.48; N, 7.14; S, 5.45. Found: C, 53.37; H, 5.53; N, 7.15; S, 5.53.

2-[3-[4-(4-Fluorophenyl)-1-piperazinyl]propyl]-4-[^{125}I]-iodo-2*H*-naphth[1,8-*cd*]isothiazole 1,1-dioxide (4-[^{125}I]-RP 62203). A solution of [^{125}I]-NaI (20 μL, *ca.* 1.0 nmol; 2.05 mCi) was added to a glass vial sealed with a Teflon-faced septum. In rapid succession, a solution of the trimethylstannyl precursor (0.20 mg, 0.34 μmol) in CH_3OH (50 μL), CH_3OH containing glacial HOAc (50 μL; 98:2 *v/v*), and aqueous chloramine-T trihydrate (20 μL, 3.5 mM; 70 nmol) were added. After 2 min at ambient temperature, the reaction was quenched with $Na_2S_2O_5$ (20 μL, 50 mM; 1.0 μmol). The mixture was taken up in a syringe, and combined with a rinse of the vial with 0.15 mL of the ternary mobile phase used for HPLC (43% organic / 57% aqueous). Using a flow rate of 4 mL/min (8 x 100 mm RCM), the radioligand (t_R = 32.3 min, k'= 41.5) was collected in a 12.5 mL volume that was diluted to 50 mL with distilled water. This solution was passed through a solid-phase extraction cartridge that was then flushed with water (2.5 mL) to remove salts, and then with air. Elution with ethanol (1.0 mL) provided 1.76 mCi (86%) of 4-[^{125}I]-RP 62203. An aliquot assayed for radioactivity with a radioisotope dose calibrator (Capintec CRC-7) was checked by HPLC (65% organic / 35% aqueous; 8 x 100 mm RCM; 4 mL/min). The radioactive product (t_R = 5.8 min, k'= 6.6) had ≥ 99.6% chemical and radiochemical purity. The mass of carrier (76 pmol) associated with this sample (157 μCi) was determined using HPLC to relate the UV absorbance peak height to a linear (r^2 = 1.0), eight-point standard curve bracketing the region of interest (18 - 270 pmol). The specific radioactivity was calculated as 2066 mCi/μmol.

Acknowledgments

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