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Synthesis of [^{3}H]CP-93,129: A Selective Radioligand for the 5-HT$_{1B}$ Receptor

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SUMMARY

The synthesis of [^{3}H]-CP-93,129, a potent radioligand for the serotonin 5-HT$_{1B}$ receptor, is described. 5-Butoxypyrrolo[3,2-b]pyridine (**2**) is converted in 3 steps to 5-butoxy-1-phenylsulfonyl-2-tritiopyrrolo[3,2-b]pyridine (**3c**) via lithiation of C2, iodination of that anion, and tritium reduction. (**3c**) is converted to the title compound in two more steps analogous to methodology used in the synthesis of CP-93,129, a potent and selective 5-HT$_{1B}$ agonist.

Key Words: CP-93,129, 3-(1,2,5,6-tetrahydropyrid-4-yl)pyrrolo[3,2-b]pyrid-5-one, serotonin, 5-HT$_{1B}$ receptor, tritium, radioligand

INTRODUCTION

The study of serotonin (5-HT) receptors has been hampered by the lack of specific ligands for the individual receptors (1). Within the multitude of types (5-HT$_1$, 5-HT$_2$, and 5-HT$_3$) and subtypes (i.e: 5-HT$_{1A}$, 5-HT$_{1B}$, 5-HT$_{1C}$, and 5-HT$_{1D}$) of 5-HT receptors (2), only the 5-HT$_{1A}$ receptor has a specific agonist: 8-OH-DPAT [8-hydroxy-2-(N,N-dipropylamino)tetralin]. Recently, we reported the synthesis and pharmacology of a rationally designed, potent, and selective agonist for the 5-HT$_{1B}$ receptor: CP-93,129 (**1a**) (3, 4). 3-(1,2,5,6-Tetrahydropyrid-4-yl)pyrrolo[3,2-b]pyrid-5-one (**1a**) binds to the 5-HT$_{1B}$ receptor with an IC$_{50}$ of 15 nm versus [^{3}H]-5-HT and with the following selectivities vs. other 5-HT receptors: 200x vs. 5-HT$_{1A}$, 425x vs. 5-HT$_{1C}$, 150x vs. 5-HT$_{1D}$, and

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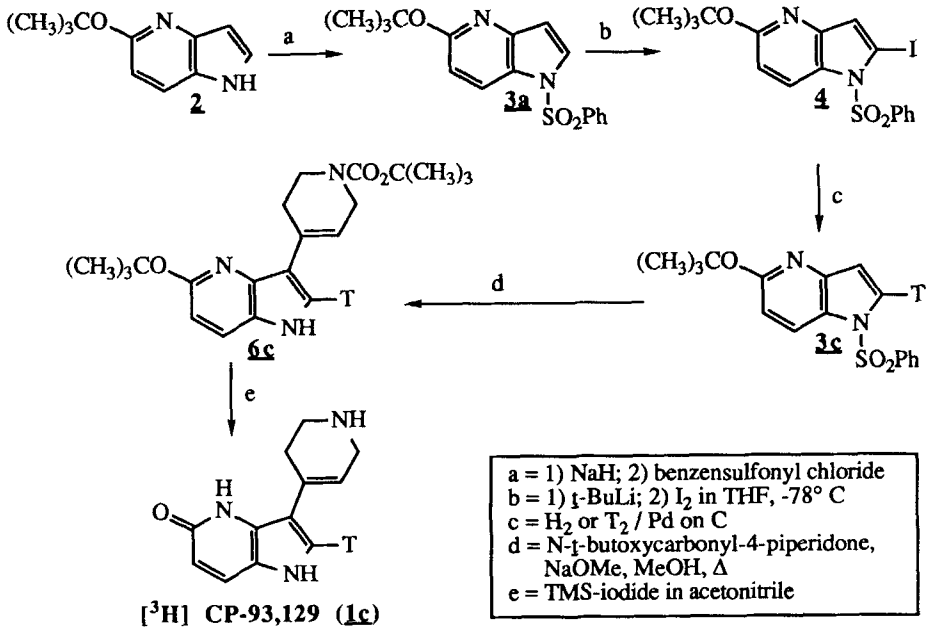
over 600x vs. 5-HT_2 (3). This potency and selectivity for the 5-HT_{1B} receptor is unprecedented, and therefore, CP-93,129 represents an important pharmacological tool in the study of the location and function of serotonin receptors, specifically 5-HT_{1B} receptors. The synthesis of a radiolabelled version of CP-93,129 was seen as a necessary extension of these findings, and in this report we will present such a compound.

RESULTS AND DISCUSSION

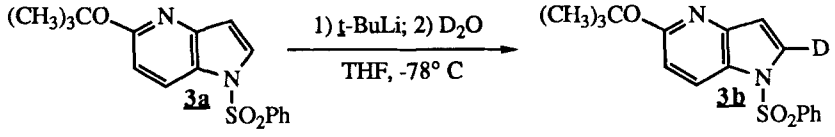
Scheme 1 outlines our successful approach to [^{3}H]CP-93,129 (**1c**). Palladium catalyzed tritium-halogen exchange was viewed as the most direct method of radiolabel incorporation into CP-93,129. We envisioned lithiation of C-2 of our pyrrolo[3,2-b]pyridine heterocycle as our means of introducing a halogen into an appropriate precursor of CP-93,129. Sundberg and Russell had previously used a N-1 phenylsulfonamide as the ortho-directing group in C-2 lithiation of indoles (5). We expected that pyrrolo[3,2-b]pyridines would be analogous to indoles in such a reaction.



Scheme 1 - Synthesis of [^{3}H] CP-93,129

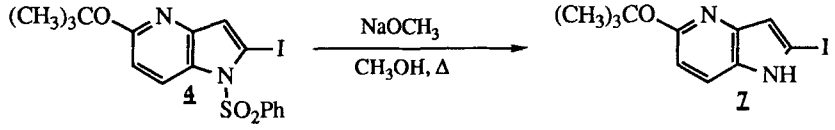
Therefore, 5-t-butoxypyrrolo[3,2-b]pyridine (**2**) (3) was deprotonated with sodium hydride and reacted with phenylsulfonyl chloride to form the required 1-phenylsulfonylpyrrolo[3,2-b]pyridine (**3**, 81%). To test the validity of our approach, **3a** was deprotonated using a slight excess of t-butyl

lithium in anhydrous tetrahydrofuran, and the resulting anion was quenched with D_2O (Scheme 2). This reaction consistently led to greater than 95% deuterium incorporation into C-2 of **3** (as determined by ^1H NMR) forming 2-deuterio-5-t-butoxy-1-phenylsulfonylpyrrolo[3,2-b]pyridine (**3b**).



Scheme 2

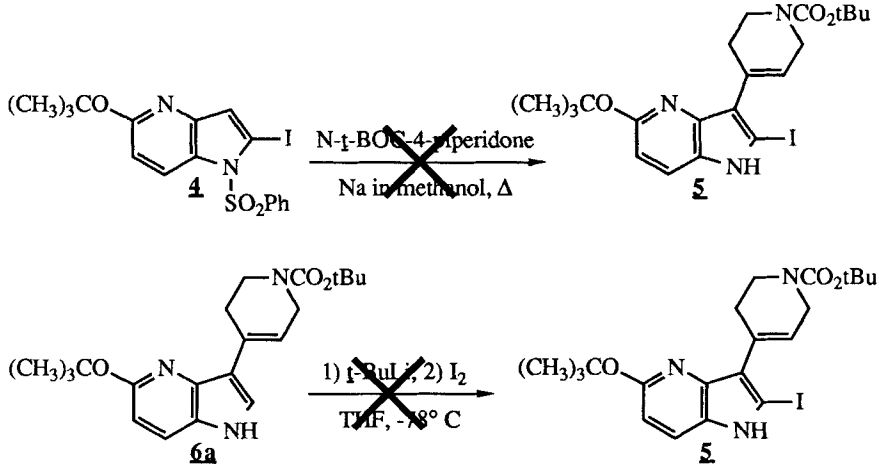
Therefore, upon treatment of the C-2 anion of **3a** with a solution of iodine in THF (Scheme 1), the 2-iodopyrrolo[3,2-b]pyridine (**4**) was synthesized (61%). The major by-product from this reaction was recovered starting material. To our knowledge, this represents the first example of this type of halogenation on C2 of an indole-like molecule. It should be noted that N1-deprotection of **4** was straightforward using sodium methoxide in refluxing methanol to yield 5-t-butoxy-2-iodopyrrolo[3,2-b]pyridine (**7**, 97%, Scheme 3) . Although the palladium catalyzed iodo-hydrogen exchange in **7** proceeded smoothly to yield **2**, iodo-tritium exchange in **7** was never attempted since tritiation of **4** had already been successful (discussed below).



Scheme 3

Attempts to cyclize **4** with 4-piperidone to form 5-t-butoxy-2-iodo-3-(1,2,5,6-tetrahydropyridyl)pyrrolo[3,2-b]pyridine (**5**, Scheme 4) under both standard (refluxing sodium methoxide in methanol) (6) and more vigorous conditions (i.e. more base, higher temperatures) failed completely, probably as a result of the steric hindrance of of C2 iodine atom. In this regard, it should also be noted that attempted iodination of 5-t-butoxy-3-(1,2,5,6-tetrahydropyridyl)pyrrolo[3,2-b]pyridine (**6a**, Scheme 4) (3) to form **5** using our previously described conditions failed also, presumably as a result of steric hindrance from the 3-tetrahydropyridyl group. Therefore, direct iodo-hydrogen or iodo-tritium exchange using 10% Pd/C as catalyst under atmospheric pressure on the

protected 2-iodopyrrolopyridine (4) was seen as the most desirable alternative (Scheme 1). This procedure was effective, and iodo-hydrogen exchange occurred quantitatively on 4 returning 3a; iodo-tritium exchange produced the 2-tritiopyrrolo[3,2-b]pyridine (3c) in high yield with 92% purity as determined by Radio-TLC analysis. It is important to note that an excess of triethylamine was needed as the acid scavenger in these reactions since the conversion of the tert-butoxy ether of 3 to the pyrrolo[3,2-b]pyrid-5-one was extremely facile in the presence of acid (produced by iodo-hydrogen [iodo-tritium] exchange).



Scheme 4

As mentioned above, removal of the N1-phenylsulfonyl protecting group was easily effected using sodium methoxide in refluxing methanol. Therefore, reaction of crude 3c with N-t-butoxycarbonyl-4-piperidone in refluxing sodium methoxide/methanol first removed the phenylsulfonamide, and then piperidone condensation proceeded as previously published (3) in the synthesis of CP-93,129 (1a) to yield the 3-(1,2,5,6-tetrahydropyridyl)-2-tritiopyrrolo[3,2-b]pyridine (6c) with 87% purity as determined by Radio-TLC (Scheme 1) (7). Crude 6c was then deprotected using iodotrimethylsilane in dry acetonitrile to afford our desired crude product (1c). Using reverse phase HPLC purification, [^{3}H]CP-93,129 (1c) was isolated in > 99% radiochemical purity (as determined by HPLC) with a specific activity of 19 Ci/mmol (as determined by UV spectrophotometry).

The utility of [^{3}H]CP-93,129 (1c) as a pharmacological tool for the study of 5-HT$_{1B}$ receptors is underway, and the results of this research will be disclosed in due course.

EXPERIMENTAL SECTION

Melting points were determined on a Thomas-Hoover open capillary melting point apparatus and are uncorrected. Infrared spectra were obtained from a Perkin Elmer IR-283B Infrared Spectrophotometer, and NMR spectra were recorded on either a Bruker AM-300 (300 MHz) or a Varian XL300 (300 MHz) spectrometer. NMR data are reported in parts per million (δ) and are referenced to the deuterium lock signal from the sample solvent. Low resolution mass spectra were obtained on a Finnigan 4310 instrument; high resolution mass spectra were obtained on a AEI MS-30 instrument. Elemental analyses were performed at Central Research Division, Pfizer, Inc., Groton, CT. and at Schwarzkopf Microanalytical Laboratory, Woodside, N.Y.

Commercial reagents (Aldrich Chemical Co.) were utilized without further purification, including Aldrich Gold Label tetrahydrofuran (THF). Room temperature (RT) refers to 20 - 25° C.

Radio-TLC scans were obtained with Bioscan Bid 100 and 200 radiochromatogram scanners. Scintillation counting was carried out with a Beckman LS 3801 liquid scintillation counter using Fisher ScintiVerse LC scintillation cocktail. Preparative and analytical high performance liquid chromatography (HPLC) was carried out on a Varian 5560 instrument with a Rheodyne 7125 injector (200 µL loop).

3-(1,2,5,6-Tetrahydropyridyl)-2-tritiopyrrolo[3,2-b]pyrid-5-one ([^{3}H]CP-93,129 (1c)). A solution of **6c** (1.6 Ci) and iodotrimethylsilane (75 µL, 2 eq) in dry acetonitrile (10 mL) was heated at 50 ° C for 2 h. TLC (LK6F silica gel, methanol:triethylamine, 9:1) showed complete conversion of **6c** (R_f=0.9) to **1c** (R_f=0.1). Solvent was removed under reduced pressure and the crude product was suspended in saturated sodium hydrogen carbonate (5 mL). The aqueous phase was extracted with methylene chloride (3 x 5 mL), and the combined organic phase was washed with a solution of saturated sodium thiosulfate and dried (Na_2SO_4). Solvent was removed under reduced pressure, and the crude product (1.51 Ci) was taken up in methanol:water (11:1) containing 1% ascorbic acid. Radio-TLC indicated a purity of 85%. Prior to purification by HPLC, the solvent was removed and replaced with ethyl acetate: methanol: acetic acid (75:24.75:0.25). Purification was carried out using a Dupont Zorbax CN column (4.6 x 250 mm) using methylene chloride: methanol: 10% aqueous acetic acid (ammonia added to pH=4) (80:18:2) as the mobile phase (isocratic, 1 mL/min) with UV detection at 334 nm. The product (average retention time = 15.5 min) was obtained with > 99% radiochemical purity (HPLC). The specific activity was determined to be 19 Ci/mmol by UV spectrophotometry. (**1c**) was identical to (**1a**) (3) by UV analysis.

5-*tert*-Butoxy-1-phenylsulfonylpyrrolo[3,2-b]pyridine (3a). To a stirred solution of 5-t-butoxypyrrolo[3,2-b]pyridine (2, 4.04 g, 21.2 mmol) in anhydrous tetrahydrofuran (THF, 35 mL) at 0° C under nitrogen was added 60% NaH (0.934 g, 23.4 mmol, 1.1 eq). The resultant mixture was stirred at RT for 30 min, and then re-cooled to 0° C. Benzenesulfonyl chloride (3.00 mL, 23.5 mmol, 1.1 eq) was added neat dropwise slowly, and the resulting mixture was stirred at RT under nitrogen for 12 h. Water (70 mL) was added, and this aqueous mixture was extracted with ethyl acetate (70 mL). This extract was then washed with water (50 mL), dried ($MgSO_4$), and evaporated under reduced pressure to yield a tan solid (7.86 g). This solid was placed in refluxing hexanes (100 mL), the cloudy solution was filtered hot through celite, the filtrate brought back to reflux, and then slowly cooled overnight to 0° C. The resulting precipitated solid (5.64 g) was filtered and redissolved in refluxing hexanes (75 mL).This cloudy solution was hot filtered through celite, and the filtrate was again brought to reflux and then slowly cooled. The resulting crystals were filtered to afford 3a (4.83 g, 14.6 mmol, 69%) as off-white crystals: mp 109.0-111.0° C; IR ($CDCl_3$) 1590, 1575, 1465, 1450, 1405, 1375, 1165, 1140 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.07 (d, J=8.9 Hz, 1H), 7.82 (br d, J=7.2 Hz, 2H), 7.61 (d, J=3.7 Hz, 1H), 7.53-7.49 (m, 1H), 7.45-7.39 (m, 2H), 6.63 (d, J=3.7 Hz, 1H), 6.59 (d, J=8.8 Hz, 1H), 1.55 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 161.5, 145.7, 138.2, 134.0, 129.4, 128.1, 126.7, 123.7, 123.6, 110.5, 110.3, 79.7, 28.7; LRMS (m/z, relative intensity) 331 (31), 330 (M+, 26), 276 (22), 274 (100), 141 (31), 134 (26), 133 (62), 105 (37), 77 (51), 57 (31). Anal. calcd for $C_{17}H_{18}N_2O_3S$: C, 61.80; H, 5.49; N, 8.48; found: C, 61.89; H, 5.40; N, 8.44.

5-*tert*-Butoxy-2-deuterio-1-phenylsulfonylpyrrolo[3,2-b]pyridine (3b). To a stirred solution of 3a (0.300 g, 0.91 mmol) in anhydrous THF (5 mL) at -78° C under nitrogen was added a solution of t-butyllithium (1.7 M in pentane, 0.80 mL, 1.36 mmol, 1.5 eq) slowly such that the temperature of the reaction solution was maintained at or below -60° C. The resultant solution was stirred at -60° C under nitrogen for 30 min. Deuterium oxide (D_2O, 3.0 mL, large excess) was then added slowly to quench the anion, and the reaction was allowed to warm to RT. Water (10 mL) and ether (25 mL) were added to the reaction mixture, and the organic layer was removed, dried ($MgSO_4$), and evaporated under reduced pressure to afford 3b (0.300 g, 0.91 mmol, 100%) as a white solid: mp, 112.0-113.0° C; IR ($CDCl_3$) 1590, 1575, 1445, 1405, 1375, 1165 cm^{-1}; ^1H NMR ($CDCl_3$) 8.07 (d, J=8.9 Hz, 1H), 7.82 (br d, 7.7 Hz, 2H), 7.55-7.50 (m, 1H), 7.45-7.40 (m, 2H), 6.62 (s, 1H), 6.59 (d, J=8.9 Hz, 1H), 1.55 (s, 9H); ^{13}C NMR ($CDCl_3$) 161.5, 145.7, 138.2, 133.9, 129.3, 129.2, 126.7, 123.7, 123.6, 110.5, 110.1, 79.7, 28.7; LRMS (m/z, relative intensity)

332 (17), 331 (M+, 10), 277 (29), 275 (100), 135 (26), 134 (59), 106 (37), 77 (43), 57 (32). Anal. calcd for $C_{17}H_{17}DN_2O_3S$: C, 61.61; H,D: 5.78; N, 8.45; found: C, 61.57; H,D 5.53; N, 8.38. Note that ^1H NMR indicated 100% deuterium incorporation; no **3a** detected in spectrum (resonance at δ 7.61 absent).

5-*tert*-Butoxy-2-tritio-1-phenylsulfonylpyrrolo[3,2-b]pyridine (3c). A mixture of **4** (78.6 mg, 0.172 mmol), 10% Pd/C (15 mg), and triethylamine (50 μL, 0.36 mmol) was taken up in acetonitrile (5 mL). The resultant mixture was degassed under reduced pressure and then stirred under 14 Ci (0.48 mmol) of tritium gas at room temperature for 2 h. TLC (Whatman LK6F silica gel; hexane:ethyl acetate, 11:1) indicated complete conversion of **4** (R_f=0.4) to **3c** (R_f=0.5). Catalyst was removed by filtration through celite, and solvent was removed under reduced pressure. Labile tritium was removed by co-distillation with methanol (3 x 2 mL). The resulting product (3.6 Ci, 92% pure by Radio-TLC) was used without further purification.

5-*tert*-Butoxy-2-iodo-1-phenylsulfonylpyrrolo[3,2-b]pyridine (4). To a stirred solution of **3a** (1.11 g, 3.36 mmol) in anhydrous THF (11 mL) at -78° C under nitrogen was added a solution of *t*-butyllithium (1.7 M, 2.4 mL, 4.1 mmol, 1.2 eq). Reaction temperature allowed to rise to -60° C, and upon completion of addition, reaction maintained at -60° C under nitrogen for 60 min. A solution of iodine (0.85 g, 3.35 mmol, 1.0 eq) in anhydrous THF (8 mL) was then added dropwise slowly maintaining temperature at -60° C, and reaction solution stirred at -78° C under nitrogen for 15 min. A solution of 10% sodium thiosulfate and saturated sodium hydrogen carbonate (1:1, 10 mL) was added to quench the reaction, and this mixture was allowed to warm to room temperature. Water (15 mL) was added to the mixture, and this aqueous mixture was extracted with ethyl acetate (2 x 25 mL). The organic extracts were combined, dried ($MgSO_4$), evaporated under reduced pressure to afford a black oil (1.42 g). Flash column chromatography of this oil using silica gel (32-64 μm, approx 100 g) and elution with methylene chloride/hexanes (7:5) afforded **4** (0.93 g, 2.04 mmol, 61%) as a pale grey, crystalline solid: mp 113.0-115.0° C; IR ($CHCl_3$) 1585, 1450, 1405, 1380, 1165 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.32 (d, *J*=9.0 Hz, 1H), 7.86 (br d, *J*=8.0 Hz, 2H), 7.59-7.54 (m, 1H), 7.47-7.42 (m, 2H), 6.98 (s, 1H), 6.55 (d, *J*=9.2 Hz, 1H), 1.56 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 161.6, 146.1, 138.0, 134.2, 129.3, 127.8, 127.3, 125.3, 124.9, 110.5, 80.0, 28.6; LRMS (m/z, realtive intensity) 456 (M+, 15), 400 (66), 274 (34), 260 (31), 259 (100), 231 (35), 141 (59), 133 (45), 77 (83), 57 (57); HRMS calcd for $C_{17}H_{17}IN_2O_3S$ 456.0005, found 456.0013. Anal. calcd for $C_{17}H_{17}IN_2O_3S$: C, 44.75; H, 3.76; N, 6.14; found: C, 44.74; H, 3.69; N, 6.20.

5-*tert*-Butoxy-3-(4-*tert*-butoxycarbonyl-1,2,5,6-tetrahydropyridyl)-2-tritiopyrrolo-[3,2-b]pyridine (6c). A mixture of 3c (3.6 Ci) and N-t-butoxycarbonyl-4-piperidone (0.72 mg, 0.36 mmol) was taken up in dry methanol (2.5 mL) and treated with 25% methanolic sodium methoxide (360 μL, 1.56 mmol). The resulting solution was heated at reflux under an argon atmosphere for 1 h after which TLC (silica gel; hexane:ethyl acetate, 1:1) indicated partial conversion of 3c (R_f=0.7) to 6c (R_f=0.5). A second addition of N-t-butoxycarbonyl-4-piperidone (0.48 mg, 0.24 mmol) was made, and the reaction solution was heated for an additional 4 h to complete the reaction. Solvent was removed under reduced pressure, and the crude product was dissolved in ethyl acetate (5 mL).The resulting solution was washed with a saturated solution of sodium hydrogen carbonate (5 mL),and the aqueous phase was extracted with ethyl acetate (5 mL). The combined organic extract was dried (Na_2SO_4), and the solvent was removed under reduced pressure to afford 6c. Radio-TLC indicated a purity of 87%, and this product was used without further purification.

5-t-Butoxy-2-iodopyrrolo[3,2-b]pyridine (7). To a stirred solution of sodium hydride (60% dispersion in mineral oil, 0.165 g, 4.12 mmol, 2.0 eq) in methanol (10 mL) at 0° C was added 4 (0.93 g, 2.04 mmol) as a solid rapidly. The resultant mixture was heated at reflux (to become a yellow solution) under nitrogen for 1 h. A saturated solution of sodium hydrogen carbonate (10 mL) was added to the reaction, and methanol was removed via evaporated under reduced pressure. The resultant aqueous mixture was extracted with ethyl acetate (2 x 20 mL), and the organic extracts were dried ($MgSO_4$) and evaporated under reduced pressure to yield a yellow solid. Recrystallization of this oil in methylene chloride/hexanes afforded 7 (0.62 g, 1.96 mmol, 97%) as a white, crystalline solid: mp, decomposes 191-195° C; IR (KBr) 1610, 1570, 1460, 1395, 1155 cm^{-1}; ^1H NMR (DMSO-d_6) δ 11.7 (br s, 1H), 7.55 (d, J=8.7 Hz, 1H), 6.58 (s, 1H), 6.38 (d, J=8.8 Hz, 1H), 1.52 (s, 9H); ^{13}C NMR (DMSO-d_6) δ 158.5, 143.4, 127.5, 120.9, 110.6, 107.7, 80.9, 78.1, 28.6; LRMS (m/z, relative intensity) 316 (M+, 23), 261 (30), 260 (100), 232 (48), 133 (17). Anal. calcd for $C_8H_7IN_2O$: C, 41.79; H, 4.14; N, 8.86; found: C, 41.63; H, 4.04; N, 8.56.

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4. Note that throughout this paper **Xa** refers to C2-hydrogen, **Xb** refers to C2-deuterium, and **Xc** refers to C2-tritium.
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7. (**6c**) and (**1c**) were identical by TLC and UV spectrophotometric analyses to (**6a**) and (**1a**), respectively, which were previously synthesized as described in reference 3.