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Synthesis of Iodine-123 Labelled Analogues of the Partial Agonist (S)- and (R)-Bretazenil for the Study of CNS Benzodiazepine Receptors using SPECT

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Summary

The (S) and (R)-[^{123}I]iodinated analogues of the benzodiazepine receptor partial agonist bretazenil have been synthesised for study of the central benzodiazepine receptor using SPECT. (S)- and (R)-[^{123}I]iodobretazenil were prepared from the appropriate tin precursors by electrophilic iododestannylation with Na[^{123}I] in the presence of Chloramine-T. The products were purified by semi-preparative reverse-phase HPLC with radiochemical yields of 80% in a total synthesis time of 50 minutes. The specific activity was determined to be greater than 2500 Ci/mmol. The radiochemical and chemical purity assessed by radio-TLC and HPLC were found to be 98%. The enantiomeric purity of the (S) and (R) isomers were greater than 97% as assessed by analytical chiral HPLC analysis.

Key Words: Bretazenil, Iodine-123, benzodiazepine receptor, partial agonist, SPECT.

Introduction

Bretazenil, *tert*-butyl (S)-8-bromo-11,12,13,13α-tetra-hydro-9-oxo-9H-imidazo[1,5α] pyrrolo[2,1-c][1,4]-benzodiazepine-1-carboxylate, (Ro 16-6028) **1** (Fig. 1) is a high affinity (Ki 1.1 nM) benzodiazepine receptor (BZR) partial agonist which has been shown to display selective physiological properties in experimental animals and humans.[1,2] It possesses powerful anxiolytic and anticonvulsant properties with markedly reduced sedative-hypnotic and alcohol potentiating effects, no disturbance of motor control and no development of tolerance or physical dependence. Furthermore, the anxiolytic effects are produced at much lower doses and over a much wider dose range than diazepam.[3] In clinical trials it exhibits strong anxiolytic and antipsychotic activity[4] while the corresponding (R) enantiomer **2** is devoid of any activity at CNS benzodiazepine receptor.[5]

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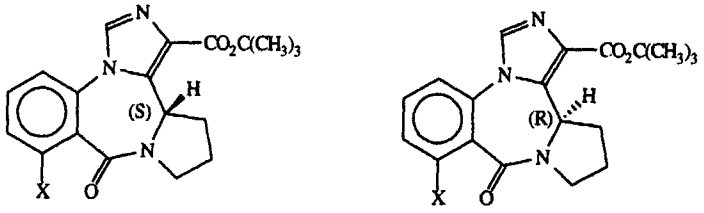
Although the detailed mechanism of BZR-ligand interactions is not fully understood, the selective pharmacological profile of some ligands have been explained on the basis of specific interactions with receptor subtypes.[6] Recent work suggested that the selective pharmacological response of bretazenil was due to its ability to activate specific receptor subtypes to various degrees as a consequence of specific interactions or intrinsic efficacy.[7,8] Furthermore, different functional states of the BZR corresponding to different conformations of the protein complexes with different interactions between agonists, antagonists and inverse agonists has also been proposed.[8]

Changes in the biochemical integrity and function of the benzodiazepine-GABA complex have been implicated in various neurological and psychiatric disorders, including epilepsy, Huntington's and Alzheimer's disease and hepatic encephalopathy. A large number of benzodiazepine ligands both agonists and antagonists have been labelled with a variety of radionuclides for use in biochemical, pharmacological and clinical studies of which the antagonists [^{11}C]flumazenil and [^{123}I]iomazenil being the most widely investigated using positron emission tomography (PET) and single photon emission computed tomography (SPECT) respectively.[9,10] Bretazenil contains a bromine atom in the 8-position which lends itself to the development of other halogenated derivatives with retention of biological and pharmacological activity. Therefore the incorporation of the radionuclide iodine-123 in the 8-position may allow the study of partial agonist BZR-ligand interactions using SPECT. In addition the preparation and labelling of the (R)- or inactive isomer may allow the estimation of non-specific binding. Herein we report the synthesis and radiolabelling of iodine-123 labelled analogues of (S)- and (R)-bretazenil by (i) electrophilic iododestannylation in the presence of a variety of oxidising agents and (ii) nucleophilic halogen exchange.



1. X= Br
3. X= I
5. X= ^{123}I
7. X= $SnBu_3$

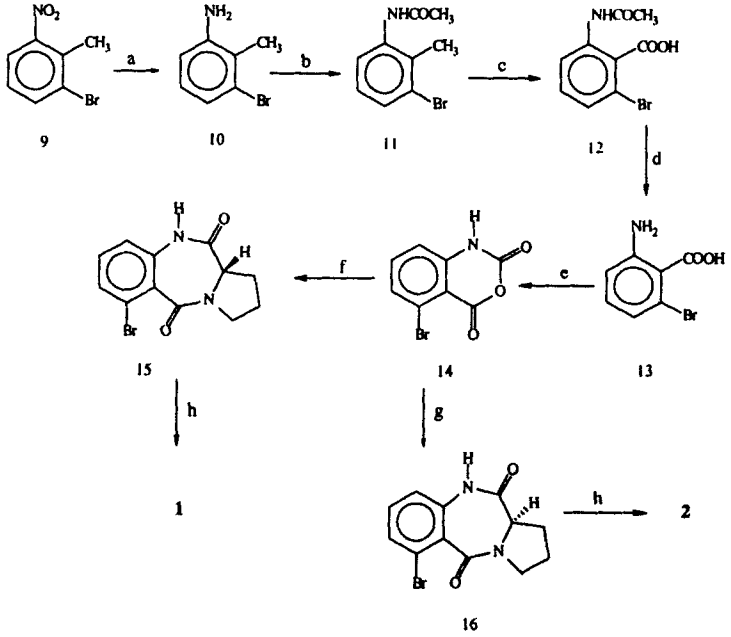
2. X= Br
4. X= I
6. X= ^{123}I
8. X= $SnBu_3$

Fig. 1

Results and Discussion

(S)- and (R)-bretazenil **1** and **2** and the iodinated analogues **3** and **4** were synthesised according to modified methods described in the patent literature.[5] For bretazenil (Scheme 1) the commercially available 6-bromo-2-nitrotoluene **9** was reduced with $SnCl_2$ in HCl to the corresponding amine **10**. Acetylation of the amino group followed by alkaline permanganate oxidation gave the carboxylic acid **12**. Hydrolysis of the acetate group with HCl (10M) at 50-60° gave **13** whereas excessive heating above 60° led to decarboxylation to give 3-bromoaniline.[11] Base hydrolysis using NaOH (3M) also yielded the desired product however, this was accompanied by

considerable debromination at higher temperatures. The addition of phosgene (20% solution in toluene) to a solution of **13** in NaOH (1M) gave anhydride **14**. Reaction of **14** with 1 equivalent of L-proline in refluxing DMF yielded the pyrrolobenzodiazepine **15** in 60% yield after crystallisation. Use of L-proline yielded the enantiomer with the (S)-configuration at C-11. Similarly reaction of **14** with the unnatural D-proline gave **16** with the (R)-configuration at C-11.

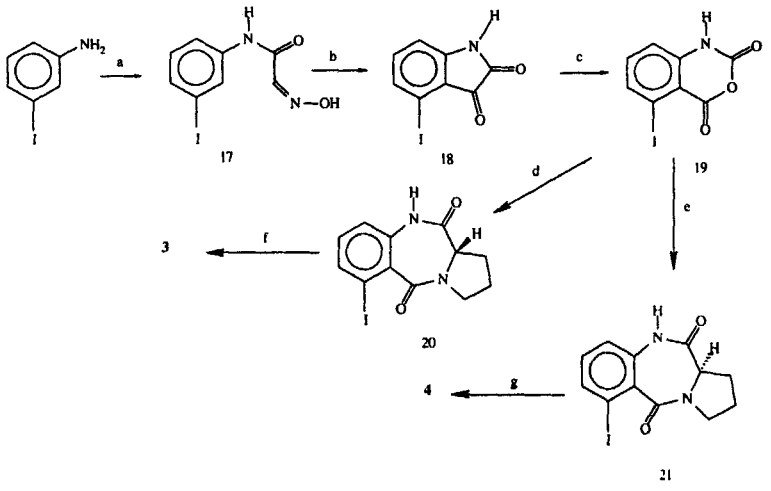


Scheme 1

Reagents. a: $SnCl_2$, HCl; b: $(CH_3CO)_2O$, CH_3COOH; c: $KMnO_4$, Na_2CO_3; d: HCl, 55°; e: $COCl_2$, NaOH; f: L-proline, DMF; g): D-proline, DMF; h: NaH, diethyl chlorophosphate, *tert*-butyl isocyanoacetate/NaH, -40°C.

The enantiomeric purity as determined by chiral HPLC was found to be greater than 97%. Addition of the sodium salt of *tert*-butylisocyanoacetate in THF at -20° to a solution of the iminophosphonate, prepared by the successive addition of NaH and diethyl chlorophosphate to **15** in THF at -40° under nitrogen gave bretazenil **1** with retention of the (S) stereochemistry at C-13. Similar treatment of the (R)-pyrrolobenzodiazepine **16** gave bretazenil **2** with the (R) configuration at C-13. The presence of excess base in this reaction mixture resulted in significant racemisation of the imidazo-pyrrolobenzodiazepines **1** and **2**. This was attributed to deprotonation of the chiral C-13 proton of the final product since no racemisation was detected in experiments where the diones **15** or **16** alone were treated with excess base and quenched with ammonium chloride at room temperature.

The corresponding 8-iodo imidazo-pyrrolobenzodiazepines **3** and **4** were prepared via the iodoisatin **18** (Scheme 2). Heating a mixture of 3-iodoaniline with trichloroacetaldehyde, Na_2SO_4, and hydroxylamine hydrochloride in HCl (5M) to reflux for 30-60 minutes gave the 3-iodo *iso*nitrosoacetanilide **17**.



Scheme 2

Reagents a: CCl_3CHO, NH_2OH-HCl, Na_2SO_4, HCl; b: H_2SO_4, 95°; c: CrO_3, $(CH_3CO)_2O$, CH_3COOH; d: L-proline, DMF; e: D-proline, DMF; f: NaH, diethyl chlorophosphate, *tert*-butylisocyanoacetate/NaH, -40°C.

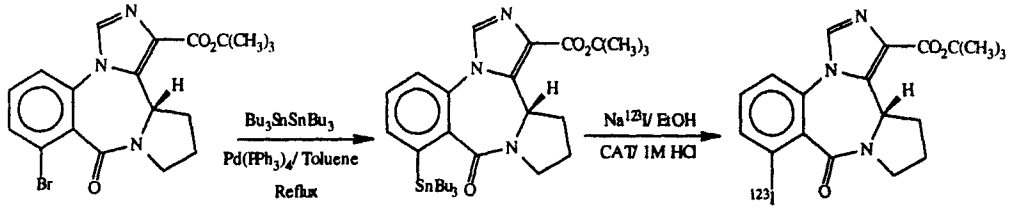
Addition of **17** to concentrated H_2SO_4 at 95° promoted ring closure to give a mixture of the 4 and 6-iodo isatins in the ratio of 3:1.[12] Fractional crystallisation from hot acetone several times enabled the more insoluble 4-isomer **18** to be isolated which after oxidation with CrO_3 in the presence of acetic anhydride and acetic acid at 90° gave the required 6-iodoisatoic anhydride **19**.[13] The 6-iodo-pyrrolobenzodiazepine **20** with the (S) configuration was obtained as above by refluxing the anhydride **19** with L-proline. Use of D-proline gave the corresponding (R) enantiomer **21**. (S) and (R) iodobretazenil **3** and **4** were prepared by reaction with *tert*-butylisocyanoacetate as described above and were found to be greater than 97% enantiomerically pure by chiral HPLC analysis.

The corresponding (S)- and (R)-tributylstannanes **7** and **8** were prepared in greater than 80% yield by heating to reflux a mixture of **1** or **2** with an excess of hexabutylditin, and a catalytic amount of palladium tetrakistriphenylphosphine in dry toluene for 6-24 h (Scheme 3).[14,15] The alternate preparation of iodobretazenil via the stannane required the addition of excess inactive iodine to a solution of the stannanes **7** or **8** in methanol containing dilute HCl or acetic acid and heating at reflux for several hours.

Synthesis and purification of (R)- and (S)-[^{123}I]Iodobretazenil

(S)- and (R)-[^{123}I]iodobretazenil were prepared by standard electrophilic iododestannylation reactions in ethanol or methanol using no carrier added Na^{123}I (Scheme 3). During the preparation of these radiotracers a number of reaction conditions and oxidising agents were investigated. Chloramine-T proved to be the optimum oxidising agent at a reaction concentration of approximately 10^{-3} M in 1 M HCl (100 µL) pH<1.5 and a reaction time of 5-10 minutes. The use of excess chloramine-T however, resulted in the formation of a non-active byproduct with a retention

time of 20 minutes. After the addition of sodium metabisulfite solution the reaction mixture was purified by reverse phase semipreparative HPLC using ethanol:water (43:57) as the eluent. The retention time of [^{123}I]iodobretazenil was 25 minutes at a flow rate of 2.5 ml/min and the product was recovered in greater than 80% radiochemical yield with radiochemical and chemical purity greater than 98% as assessed by analytical HPLC and radio-TLC. The enantiomeric purity of the labelled products as determined by chiral HPLC were found to be greater than 97% and co-elluted with the iodinated standards **3** and **4**. Total synthesis time was approximately 50 minutes and specific activity exceeded 2500 Ci/ mmol.[16] Pretreatment of the Na^{123}I (0.1-0.5 ml, 100-150 mCi, 0.1M NaOH) solution by passing it through a Biorad AG-W-X8 cation exchange membrane enabled large scale preparations of [^{123}I]Iodobretazenil.

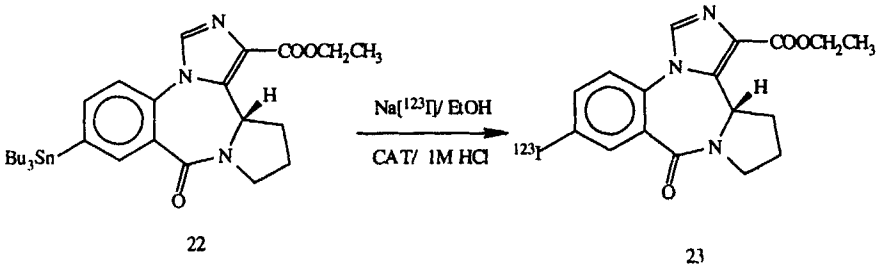


Scheme 3

The radiochemical yield of [^{123}I]iodobretazenil was found to be pH dependent with optimim conditions being pH < 1.[15] The use of phosphate buffers at pH's ranging from 3-7 as well as the use of solvents such as acetic acid reduced the radiochemical yield considerably. The use of peracetic acid as oxidising agent enhanced the formation of mixtures of active and non-active byproducts resulting in significantly lower yields of the desired product. Furthermore oxidation with peracetic acid in the presence of phosphoric or acetic acids or with chloramine-T in dilute HCl at pH> 1.5 led to the formation of radioactive volatile material (10-50%). This volatile material, previously identified as [^{123}I]butyl iodide[17] was not observed when chloramine-T in 1M HCl (pH<1.5) was used. Its formation also seemed to be pH dependent and was responsible for the low yields of product described above. The formation of [^{123}I]butyl iodide can be explained by competitive electrophilic reaction at the aliphatic butyl groups of the stannane compared to the aryl site enhanced by the neighbouring *ortho* electron-withdrawing amide group.[17,18] Presumably at the lower pH, the electron-withdrawing effect of the amide group is reduced by protonation effectively enhancing reaction at the aryl site. This was further supported by the need for acidic conditions during the inactive synthesis of iodobretazenil from the corresponding stannane in methanol. Conversely iodination of the 7-substituted tributylstannyl benzodiazepine **22** with the electron withdrawing group *meta* to the stannane with inactive iodine proceeded rapidly in neat methanol at room temperature to yield the corresponding iodinated analogue. Similarly reaction of **22** with Na[^{123}I] using chloramine-T or peracetic acid at various pH levels and solvents proceeded in high yields to give **23** without any significant loss of activity (Scheme 4).

The use of iodogen in phosphate buffers or KIO_3 in 1M HCl as oxidising agents at room temperature also yielded radiochemically and chemically pure products of [^{123}I]iodobretazenil in

radiochemical yields of 20-30%. Although no UV impurity at 20 minutes was detected for either reaction KIO_3 formed [^{123}I]iodobretazenil with a lower specific activity.



22 23

Scheme 4

In an attempt to avoid competing electrophilic reactions and the formation of byproducts, the synthesis of [^{123}I]iodobretazenil using activated nucleophilic bromine-iodine exchange was also investigated. Heating bretazenil in acetic acid in the presence of Cu(II) nitrate and ascorbic acid at 100° for 15 minutes[19] resulted in the formation of [^{123}I]iodobretazenil in 50-55% radiochemical yield. The remainder of the activity was associated with a more polar byproduct (Rt ≈ 12 min) and consistent with the corresponding carboxylic acid derivative formed after hydrolysis of the *tert*-butyl group. In contrast heating solutions of the activity and bromobretazenil in acetic acid at 110° for 15-20 minutes in the absence of copper salts did not yield any [^{123}I]iodobretazenil. Heating a mixture of dried Na^{123}I and bretazenil in 100-200 µl of acetic or pivalic acids with and without Cu(I) or Cu(II)/ascorbic acid, at 150-160° for 30-60 minutes, conditions used previously for the preparation of [^{123}I]Iomazenil,[20] resulted in very low yields (10-20%) of the desired product. The bulk of the activity being the polar byproduct. [^{123}I]Iodobretazenil was separated from the bromoprecursor by RP semipreparative HPLC using ethanol:0.1M ammonium acetate (40:60) and a flow rate of 3 ml/min.

Experimental

Materials and Methods

6-Bromotoluene, 3-Iodoaniline, hexabutylditin and tetrakistriphenylphosphine palladium were purchased from Aldrich. Ethyl isocyanoacetate, tert-butylisocyanoacetate, phosgene, potassium *tert*-butoxide and diethyl chlorophosphate were purchased from Fluka. ^{1}H-NMR spectra were obtained on a Joel FX400 NMR spectrometer. Mass spectra were performed on a VG Quattro Triple Quadrupole in Electrospray mode in acetonitrile. Melting points were carried out on a Gallenkamp melting point apparatus and are uncorrected. Chromatographic separations were carried out on an Alltech semipreparative RP C-18 column (10µ, 10mm x 250 mm) using a Waters 510 pump, a Spectrophysics-Linear UV detector set at 254 nm and a modified on line NaI -Berthold radioactivity detector. Chiral HPLC analysis was carried out on a Daicel CHIRACEL OD chiral column (10µ x 4.6mm x 250mm) using isopropanol:hexane as the eluent. No carrier added Na^{123}I was produced by the National Medical Cyclotron, Sydney Australia using the Xe(p,2n) reaction.

Chemical Synthesis

2-N-Acetyl-6-bromo toluene (11). A solution of **9** (25g, 0.116 mol) and $SnCl_2$ (80g, 0.35 mol) in HCl (10M, 230 mL) was heated at 60-70° for 6 hr. The cooled solution was basified with NH_4OH (20M) to pH 12 and extracted with ethyl acetate (4 x 120 mL). Drying (Na_2SO_4) and evaporation of the solvent yielded 2-amino-6-bromo toluene **10** as a pale yellow oil (20g, 93%). The crude amine **10** (20g, 0.108 mol) in glacial acetic acid (100 mL) and acetic anhydride (50 mL) was heated to reflux for 1 h, cooled and poured into water (400 mL). The white precipitate formed was filtered to give **11** (22.5g, 91%) as white needles m.p. 157-159°C. ^{1}H-NMR (DMSO) δ 2.05 (s, CH_3.), 2.24 (s, $COCH_3$), 7.11 (dd, J=8.2 Hz, 1H, H4), 7.33 (dd, J=8.2, J=1.2 Hz, 1H, Ar), 7.47 (dd, J=8.2, J=1.2 Hz, 1H, Ar), 9.56 (s, 1H, NH). MS(CI) m/e: 230(M^{+2}), 228(M^+), 187, 185, 149, 106.

2-N-Acetyl-6-bromo benzoic acid (12). To a suspension of **11** (12.5g, 54.8 mmol) in hot water (250 mL) containing Na_2CO_3 (6.5g, 52 mmol) was added portionwise $KMnO_4$ (20g, 0.12 mol). Reflux was continued for a further 6 h until the purple colour was discharged. The cool suspension was filtered and the filtrate acidified to pH 3-4 with 10 M HCl to give a precipitate of **12** as white needles m.p. 220-222°C (7.8g, 55%). ^{1}H-NMR (DMSO) δ 7.3-7.4 (m, 2H, Ar), 7.45-7.55 (m, 1H, Ar). MS (CI) 260(M^{+2}), 259, 258(M^+), 257, 242, 240, 215, 192.

2-Amino-6-bromo benzoic acid (13). A suspension of **12** (5g, 19.4 mmol) in HCl (10M, 45 mL) was heated at 50-60° for 24 h. After cooling the reaction mixture was concentrated and the resultant fine needles filtered to give **13** as a white solid m.p. 180-182°C (3.5g, 84%). ^{1}H NMR (DMSO) δ 7.33 (dd, J=8 Hz, 1H, H4), 7.49 (d, J=7.9 Hz, 1H, Ar), 7.48 (d, J=7.9 Hz, 1H, Ar). MS (Fab) 218(M^{+2}), 216(M^+), 198, 157, 133, 124.

6-Bromoisatoic anhydride (14). To a solution of **13** (6.5g, 0.03 mol) in NaOH (40 mL, 1M, 0.035 mol) was added dropwise a solution of phosgene (1.93 M, 23 mL, 0.045 mol) in toluene at 5°. Stirring for an additional 30-40 min gave **14** (6.5g, 89%) as a white solid m.p. 260-262°. ^{1}H-NMR (DMSO) δ 7.14 (dd, J=8, J=1.5 Hz, 1H, Ar), 7.51 (dd, J=8, J=1.5 Hz, 1H, Ar), 7.56 (dd, J=8 Hz, 1H, H4). MS (ES) m/z: 242(M^+), 241, 240, 198,197, 195, 127, 126.

(S)-6-Bromo-1,2,3,11a-tetrahydro-5H-pyrrolo-[2,1-c][1,4]benzodiazepine 5,11[10H]dione (15). A mixture of **14** (3.0g, 0.0124 mol) and L-proline (1.43g, 0.0124 mol) in DMF (40 mL) was heated to reflux for 2 h. The DMF was evaporated and the residue left to stand overnight. The deposited solid was triturated with ethanol, filtered and recrystallised from ethyl acetate to give **17** (1.6g, 45%) as white crystals m.p. 224-226° (lit.[5] 221-224°). ^{1}H-NMR ($CDCl_3$) δ 2.0–2.1 (m, 3H); 2.66-2.76 (m, 1H); 3.54-3.55 (m, 1H); 3.88-3.96 (m, 1H); 4.14 (d, J= 6.4 Hz, H11α), 6.97 (dd, J= 8.1, J= 1.0 Hz, 1H, Ar), 7.25 (dd, J=8.1 Hz, H8), 7.52 (dd, J=8.0, J=1.0 Hz, 1H, Ar), 8.1 (s, 1H, NH). MS (ES) m/z: 297(M^{+2}), 296, 295(M^+), 294, 216. R_t = 14 min., (CHIRALCEL OD) isopropanol: hexane (20:80) at 1 mL/min. Anal. calc'd for $C_{12}H_{11}BrN_2O_2$: C, 48.80; H, 3.73; N, 9.49. Found: C, 49.06; H, 3.73; N, 9.49.

(R)-6-Bromo-1,2,3,11a-tetrahydro-5H-pyrrolo-[2,1-c][1,4]benzodiazepine5,11[10H]dione (16). Heating a mixture of **14** (3.0g, 0.0124 mol) and D-proline (1.43g, 0.0124 mol) in DMF (40 mL) for 2h as above resulted in the formation of the (R)-enantiomer **16** m.p. 224-226°. Rt = 12.5 min,

(CHIRALCEL OD) isopropanol: hexane (20:80) at 1 mL/min. Anal. calc'd for $C_{12}H_{11}BrN_2O_2$: C, 48.84; H, 3.73; N, 9.49. Found: C, 48.29; H, 3.39; N, 9.05.

***tert*-Butyl (S)-8-bromo-11,12,13,13α-tetra-hydro-9-oxo-9H-imidazo[1,5α]pyrrolo[2,1-c][1,4] benzodiazepine-1-carboxylate (1).** To a solution of **15** (2.4g, 8.1 mmol) in dry THF 20 mL at room temperature under nitrogen was added NaH (95%) (0.22g, 8.9 mmol). After 40 min the brown suspension was cooled to -40° and treated dropwise with diethyl chlorophosphate (1.54g, 8.7 mmol). Meanwhile to a stirred solution of NaH (0.22g, 8.9 mmol) in dry THF (5 mL) under nitrogen at -20° was added tert-butyl isocyanoacetate (1.3g, 9.2 mmol). The red-orange solution was added dropwise via cannular to the solution of the iminophosphonate at -30° and stirred with warming to -10° for 90 min. The reaction mixture was quenched with NH_4Cl and extracted with chloroform (4 x 50 mL). Purification by flash chromatography using ethylacetate:petroleum spirit (60:40) followed by recrystallisation from ethyl acetate gave **1** (1.9g, 56%) as a white solid m.p. 199-200° (lit.[5] 206-208°). ^{1}H-NMR ($CDCl_3$) δ 1.64 (s, 9H C_4H_9), 2.15-2.30 (m, 3H), 3.48-3.61 (m, 2H), 2.82-3.88 (m, 1H), 4.76 (d, J=7.0 Hz, H13α), 7.28 (dd, J=6.2, J=0.9 Hz, 1H, Ar), 7.42 (dd, J=8.1 Hz, H6), 7.79, dd, J=8.1, J=0.9 Hz, 1H, Ar), 7.83 (s, H3). MS(EI) m/z: 420(M^{+2}), 418(M^+), 364, 362, 346, 344, 318, 289, 261, 236. Rt = 24 min, (CHIRALCEL OD) isopropanol: hexane (15:85) at 1 mL/min.

***tert*-Butyl (R)-8-bromo-11,12,13,13α-tetra-hydro-9-oxo-9H-imidazo[1,5α]pyrrolo[2,1-c][1,4] benzodiazepine-1-carboxylate (2).** The (R)-enantiomer was prepared from **16** as above. Rt = 18 min, (CHIRALCEL OD) isopropanol: hexane (15:85) at 1 mL/min.

***tert*-Butyl (S)-8-tributylstannyl-11,12,13,13α-tetra-hydro-9-oxo-9H-imidazo[1,5α]pyrrolo [2,1-c][1,4] benzodiazepine-1-carboxylate (9).** A mixture of bretazenil **1** (0.5g, 1.2 mmol), hexa-butylditin (1.5g, 2.6 mmol) and palladium tetrakistriphenylphosphine (100 mg) in dry toluene (15 mL) under nitrogen was heated to reflux for 12-18 h. The resultant black reaction mixture was diluted with dichloromethane and filtered. The filtrate was washed with 0.1M $AgNO_3$ and the dichloromethane evaporated to give a pale yellow oil. Purification by flash chromatography (ethyl acetate:petroleum spirit 60:40) yielded the tri-n-butylstannane **9** as a white amorphous solid (0.45, 60%) m.p. < 30° . ^{1}H-NMR ($CDCl_3$) δ 0.85-0.95 (m, 9H), 1.04-1.1 (m, 6H), 1.25-1.35 (m, 6H), 1.40-1.54 (m, 6H), 1.63 (s, 9H, C_4H_9), 2.07-2.35 (m, 3H), 3.45-3.58 (m, 2H), 3.73-3.85 (m, 1H), 4.73 (d, J=6.7, Hz, H13α), 7.25 (dd, J=8.0, J=1.1 Hz, 1H, Ar), 7.53 (dd, J=8.0 Hz, 1H, H6), 7.69 (dd, J=7.8, J=1.0 Hz, 1H, Ar), 7.83 (s, 1H, H3). MS (ES) m/z: 631, 630, 629, 628, 627, 626, 575, 574, 573, 572, 574.

***tert*-Butyl (R)-8-tributylstannyl-11,12,13,13α-tetra-hydro-9-oxo-9H-imidazo[1,5α]pyrrolo [2,1-c][1,4]-benzodiazepine-1-carboxylate (8)** was obtained as a colourless low melting solid according to the above procedure using (R)-bretazenil **2.**

Synthesis of (R)- and (S)-Iodo-Bretazenil

4-Iodoisatin (18): A mixture of 3-iodoaniline (75g, 0.30 mol), anhydrous trichloroacetaldehyde (60g, 0.361 mol), sodium sulfate (85g, 0.66 mol) hydroxylamine hydrochloride (73g, 1.1 mol) and HCl (10M, 150 mL) in water 250 mL was heated to reflux for 2.5 h. The reaction mixture was cooled in ice and the tan solid filtered to give the 3-iodo *iso*nitrosoacetanilide **17** (61g, 70%)

m.p. 162-164°. ^{1}H-NMR (DMSO) δ 7.13 (dd, J=8.1, J=1 1 Hz, H5), 7.45 (d, J=7.9 Hz, 1H, Ar), 7.66, (dd, J=8.1, J=1.2 Hz, 1H, Ar), 8.18, (dd, J=1.8 Hz, 1H, H2), 10.3 (s, 1H, vinylic H'). MS (CI) m/e 291(M^{+1}), 290(M^+), 272, 259, 245, 219,203, 146, 118. To sulfuric acid 100 mL at 95° was added portionwise **17** over 45 min while maintaining the temperature between 95-100° . The dark solution was heated for a further 1 h and then poured over ice. The orange precipitate was filtered and dried to give a mixture of the 4-iodo and 6-iodo- isatins in a ratio of 3:1 as determined by ^{1}H-NMR analysis. Recrystallisation from hot acetone gave 4-iodoisatin **18** as a red-orange solid m.p. 232-234° (30-40% yield). ^{1}H-NMR (DMSO) 6.90 (dd, J= 7.8, J= 1.1 Hz, 1H, Ar), 7.25 (dd, J=8.0, J=1.1 Hz, 1H, Ar), 7.48 (dd, J=7.8, J=1.1, Hz, 1H, Ar). MS (ES) m/z: 273(M^+). Anal. calc'd for $C_8H_4INO_2$: C, 35.19; H, 1.48; N, 5.21. Found: C, 35.52; H, 1.26; N, 5.16.

6-Iodoisatoic anhydride (19). To a solution of **18** (5g, 0.018 mol) in a 1:1 mixture of acetic acid and acetic anhydride (80 mL) was added CrO_3 (4.0g, 0.04 mol) portionwise over 30 min while maintaining the temperature of the reaction at 90°. After stirring for an additional 30 min the green suspension was poured into water 120 mL and the resultant yellow precipitate collected to give **19** (4 g, 75%) m.p. 270-272°. ^{1}H-NMR (DMSO) 7.14 (dd, J=8.0, J=1.1, Hz, 1H, Ar), 7.34 (dd, J=8.0, Hz, 1H, Ar), 7.84 (dd, J=7.8, J=1.1 Hz, 1H, Ar). MS (ES) 290 (M^{+1}), 289(M^+). Anal. calc'd for $C_8H_4INO_3$: C, 33.25; H, 1.39; N, 4.85. Found: C, 33.46; H, 1.37; N, 4.86.

(S)-6-Iodo-1,2,3,11a-tetrahydro-5H-pyrrolo-[2,1-c][1,4]benzodiazepine 5,11[10H]dione (20): A mixture of **19** (1.0g, 6.17 mmol) and L-proline (0.71g, 6.17 mmol) in DMF (20 mL) were heated to reflux for 4 h. The solvent was removed under reduced pressure and the residue purified by flash chromatography (ethylacetate:petroleum spirit 1:1). Recrystallisation from ethyl acetate gave **20** as a white solid m.p. 212-214° (lit.[5] 212-214°). ^{1}H NMR ($CDCl_3$) δ 2.0-2.1 (m, 3H), 2.69-2.76 (m, 1H), 3.55-3.65 (m, 1H), 3.87-3.97 (m 1H), 4.12 (d, J=7.2 Hz, H11α), 7.00 (dd, J=8.1, J=1 Hz, 1H, Ar), 7.07 (dd, J=7.9 Hz, H8), 7.82 (dd, J=7.6, J=1 Hz, 1H, Ar), 8.21 (s, 1H, NH). MS(ES) m/z: 344(M^{+2}), 343(M^{+1}),285, 284. Anal. calc'd for $C_{12}H_{11}IN_2O_2$: C, 42.13; H, 3.24; N, 8.19. Found: C, 42.28; H, 2.95; N, 8.19. Rt = 14 min (CHIRALCEL OD) isopropanol: hexane (20:80) at 1 mL/min.

(R)-6-Iodo-1,2,3,11a-tetrahydro-5H-pyrrolo-[2,1-c][1,4]benzodiazepine 5,11[10H]dione (21). Heating a mixture of **19** (3.0g, 0.0124 mol) and D-proline (1.43g, 0.0124 mol) in DMF (40 mL) for 4 h as above gave **21** m.p. 213-214°. Rt = 18.5 min (CHIRALCEL OD) isopropanol: hexane (20:80) at 1 mL/min.

***tert*-Butyl (S)-8-iodo-11,12,13,13α-tetra-hydro-9-oxo-9H-imidazo[1,5α]pyrrolo[2,1-c][1,4]-benzodiazepine-1-carboxylate (3):** Treatment of a solution of the dione **20** (260 mg) and diethyl chlorophosphate (170 mg, 0.98 mmol) followed by a solution of tert-butyl isocyanoacetate (140 mg, 0.98 mmol) and sodium hydride (95%) (22 mg, 0.92 mmol) in dry THF (5 mL) as above gave **3** (160 mg, 44%) as a white solid m.p. 223-225° from ethyl acetate. Alternatively heating a solution of the stannane **7** (200 mg, 0.32 mmol) and iodine (0.5 mg, 1.9 mmol) in methanol (20 mL) containing HCl (4mL, 3M) to reflux for 3 h gave **3** (70 mg, 45%) identical to the above material. ^{1}H NMR ($CDCl_3$) δ 1,65 (s, 9H C_4H_9), 2.13-2.31 (m, 3H), 3.48-3.54 (m, 2H), 3.8-3.9 (m, 1H), 4.76 (d, J=7.0 Hz, H13α), 7.24 (dd, J=8.0 Hz, 1H, H6), 7.34 (dd, J=8.0, J=0.9 Hz, 1H, Ar), 7.96 (s, 1H, H3), 8.11

(dd, J=8.0, J=0.9 Hz, 1H, Ar). MS (ES) m/z: 466(M^{+1}), 465(M^+), 410, 392. Anal. calc'd for $C_{19}H_{20}IN_3O_3$: C, 49.05; H, 4.33; N, 9.03. Found: C, 49.15; H, 3.97; N, 8.91. Rt = 20 min (CHIRALCEL OD) isopropanol: hexane (20:80) at 1 mL/min.

***tert*-Butyl (R)-8-bromo-11,12,13,13α-tetra-hydro-9-oxo-9H-imidazo[1,5α]pyrrolo[2,1-c][1,4]-benzodiazepine-1-carboxylate (4):** The (R)-enantiomer was prepared from **21** as above. Anal. calc'd for $C_{19}H_{20}IN_3O_3$: C, 49.05; H, 4.33; N, 9.03. Found: C, 48.84; H, 3.96; N, 8.79 Rt = 16 min (CHIRALCEL OD) isopropanol: hexane (20:80) at 1 mL/min.

Synthesis and purification of (R)- and (S)-[^{123}I]Iodobretazenil

a) Electrophilic iododestannylation. To a solution of **7** (0.3 mg, 0.48 μmol) in ethanol 300 μL was added Na[^{123}I] (1-10 mCi) in NaOH (0.1N, 10-50 μL) followed by a solution of chloramine-T (100 μg, 0.4 μmol) in HCl (1 M, 100 μL). After standing for 10 minutes with intermittent shaking the reaction mixture was quenched with $Na_2S_2O_5$ (100 mg/mL, 100 μL) and injected onto a semi-preparative RP HPLC column. The mixture was eluted at a flow rate of 2.5 mL/min using a mobile phase of 43:57 ethanol:water. The radioactivity peak corresponding to [^{123}I]Iodobretazenil (R_t = 25 min, k' = 6) was collected and evaporated to dryness. The residue was reconstituted in sterile saline and filtered through a sterile, 0.22 μm filter (Millex GS, Millipore) into a sterile, pyrogen-free evacuated vial, and the radioactivity measured. The total synthesis time was 50 minutes with radiochemical yields of 80%. Radiochemical and chemical purity assessed by both radio-TLC, and analytical HPLC were 98%. For the determination of specific activity aliquots of the final solution of known volume and radioactivity were injected onto a Goldpack C-18 (10μ x 5mm x 250mm) reverse phase HPLC column. A mobile phase of 50:50 ethanol:water with a flow rate of 1 mL/min was used to elute the radioligand (t_R =7.5 min). The radioactive product co-eluted with the iodinated standard and the area of the UV absorbance peak measured at 254 nm corresponding to carrier product was measured and compared to a standard curve relating mass to UV absorbance. The specific activities calculated at the end of synthesis using the above reaction conditions were greater than 2500 Ci/mmol. Treatment of a solution of **10** (0.3 mg, 0.48 μmol) in ethanol 300 μL as described above yielded (R)-[^{123}I]Iodobretazenil.

b) Nucleophilic Substitution: A solution of the activity (1-10 mCi) was evaporated to dryness under nitrogen and treated successively with a solution of $Cu(NO_3)_2 \cdot 3H_2O$ (50 μg) in glacial acetic acid (50 μl), ascorbic acid (25 mg) in water (250 μl) and bretazenil (1 mg) in acetic acid (100 μl). After heating the reaction mixture in a sealed vial at 100-110° for 15 minutes, it was quenched with $NaHCO_3$ (200mg/ml), diluted with mobile phase and injected onto a RP HPLC semiprep column (ethanol:0.1M ammonium acetate (40:60) at 3 ml/min to give [^{123}I]Iodobretazenil in 55-60% radiochemical yield.

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