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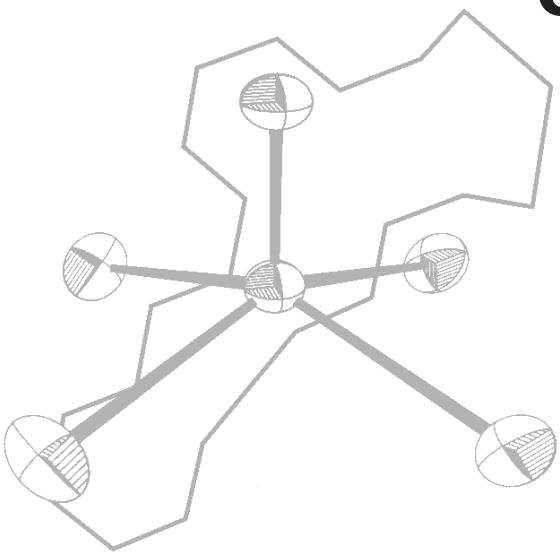

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Synthesis of ^{123}I-Labelled Analogues of Imidazobenzodiazepine Receptor Ligands*

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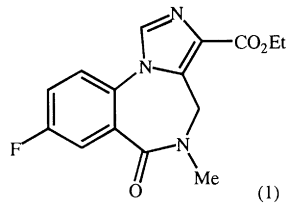
Reaction of bromo- or iodo-substituted isatoic anhydrides with *N*-methylglycine, L-proline or D-proline afforded bromo- or iodo-substituted 1,4-benzodiazepinediones which on condensation with ethyl or t-butyl isocyanoacetates gave ethyl or t-butyl bromo- or iodo-imidazobenzodiazepine carboxylates. These aryl halides were converted into the corresponding tributylstannanes with bis(tributyltin) in the presence of (triphenylphosphine)palladium(0), and the stannanes were treated with sodium (^{123}I)iodide in the presence of chloramine-T to give the required ^{123}I-labelled analogues of the imidazobenzodiazepine receptor ligands flumazenil and bretazenil.

The imidazobenzodiazepines, of which flumazenil (1), bretazenil (2), Ro 15-4513 (3) and iomazenil (4) are key examples, are among the most important classes of benzodiazepine receptor ligands.[1–3] However, these substances have some undesirable properties which have been detailed in many reports.[1,3,4–11] Although the properties of bretazenil (2) suggest that its ability to separate the desirable and undesirable effects is due to partial agonism, other ligands may separate these effects due to their ability to bind to particular receptor subtypes,[5] and the identification of receptor subtypes responsible for one or a few of the effects could be helpful in the design of improved pharmaceuticals. Ligands could be developed that were specific to a particular receptor subtype, thus providing drugs that only produce the effect mediated by that receptor subtype. Another consideration is that a ligand not only has different affinities for particular receptor subtypes, but it may also have different intrinsic efficacies at each receptor subtype.[12,13]



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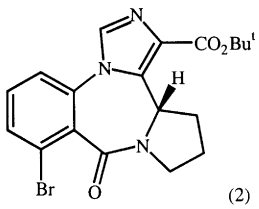

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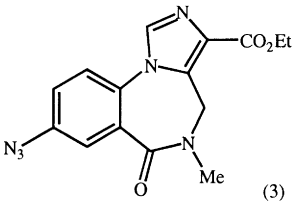

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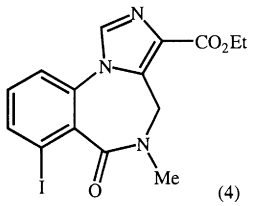

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Radioligands have been used to investigate receptor systems and the use of radiolabelled benzodiazepine receptor ligands in nuclear imaging provides information on the functioning of the benzodiazepine receptor in both diseased and healthy patients. The information obtained can be used in the development of better substances for the treatment of illness, and in the development of nuclear imaging procedures for non-invasive diagnosis.

One aim of our work involves the preparation of iodine-123 labelled 1,4-benzodiazepines as potential SPECT radiopharmaceuticals to investigate the involvement of benzodiazepine receptors in neurodegenerative diseases. We report here on the synthesis of radioiodinated derivatives of flumazenil (1) and bretazenil (2).

The radionuclide chosen for the preparation of the radioligands was iodine-123. This choice was based on the fact that the imidazobenzodiazepines (1), (2) and (4) all possess halogen atoms on the aromatic ring, and suggested that iodinated derivatives may retain biological and pharmacological activity. The longer half-life of iodine-123 would also allow the measurement of pharmacology consistent with the biological half-life of some of these substances.

The preparation of the receptor ligands essentially involved two parts. First, the appropriately substituted tributylstannyl derivatives, and the non-radioactive iodinated ligands were prepared. These iodinated ligands were required for the development of the h.p.l.c. protocol for the purification of the iodine-123 derivatives, and for binding studies such as saturation and blocking studies. Also, as the radioiodinated products are only formed in picomole amounts (due to the high specific activities of the radioligands) normal analyses cannot be used in their identification;

* This paper is dedicated to Professors W. R. Jackson, J. T. Pinhey, R. W. Rickards, S. Sternhell and W. C. Taylor.

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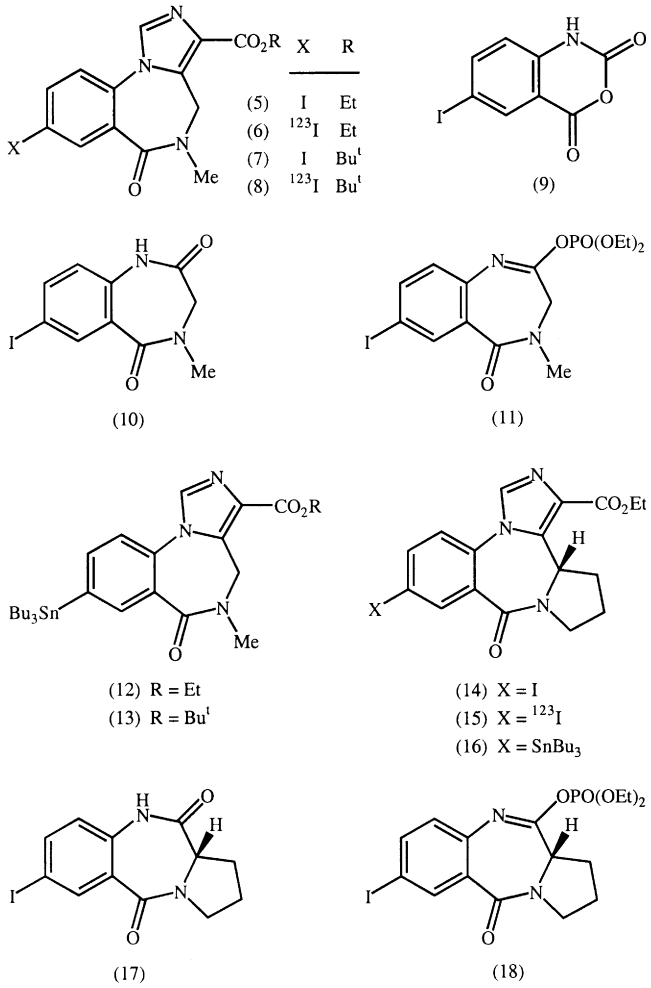


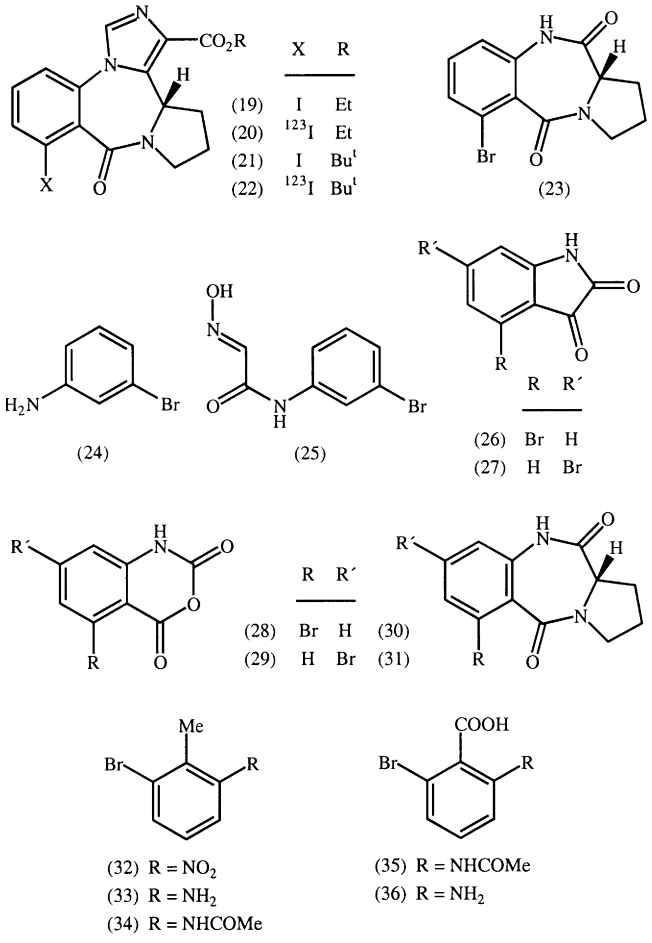
therefore, the comparison of h.p.l.c. retention times was the only means of ensuring that the radioiodination procedure was producing the desired products. The second part of the synthesis was the radioiodination of the tributylstannyl derivatives by iodo-de-stannylation, and the purification of the radioiodinated product.

The initial ligands chosen for investigation were the iodo ethyl ester (5) and its iodine-123 analogue (6), and the corresponding t-butyl esters (7) and (8). In the first step, cyclocondensation of 5-iodoisatoic anhydride (9) and *N*-methylglycine gave the benzodiazepinedione (10) in 32% yield. In order to incorporate the imidazo ring, activation of the N 1–C 2 amide of the *N*-methyl dilactam (10) as the imino phosphate (11) was required, and was achieved by treatment of the anion of the *N*-methyl dilactam with diethyl chlorophosphate. Subsequent treatment of the solution obtained with a solution of ethyl isocyanoacetate and potassium t-butoxide in dimethylformamide gave the *N*-methyl ethyl ester (5) in 19% yield. However, with a modification of a procedure reported by Gu *et al.*,[14] a one-pot reaction in which a cold solution of the *N*-methyl dilactam (10) in tetrahydrofuran was treated first with potassium t-butoxide, then with diethyl chlorophosphate and finally with ethyl isocyanoacetate/potassium t-butoxide afforded the *N*-methyl ethyl ester (5) in an improved yield of 42%.

The *N*-methyl ethyl ester (5) was then treated with bis(tributyltin) and tetrakis(triphenylphosphine)palladium(0) in toluene to afford the stannane (12) in 70% yield. It is important to note that the workup of this stannane, and in fact of all the stannanes reported here, involved washing a solution of the stannane with silver nitrate prior to purification. This was performed in an attempt to remove any residual iodide that could reduce the specific activity of the radioiodinated products formed during the radioiodo-de-stannylation reaction.

The corresponding t-butyl ester derivatives were prepared in a similar manner. Thus, a solution of the *N*-methyl dilactam (10) was treated with sodium hydride, diethyl chlorophosphate, and then with the anion of t-butyl isocyanoacetate, to give the *N*-methyl t-butyl ester (7) in 37% yield. This ester was converted into the crystalline *N*-methyl t-butyl ester stannane (13) in 71% yield according to the usual procedure. The preparation of this stannane has been previously reported by He *et al.*,[15] but their method afforded the *N*-methyl t-butyl ester stannane, as an oil, in only 16% yield.

A similar set of reactions was then carried out to obtain the tetracyclic derivatives (14) and (15) which contained the pyrrolo ring in a position similar to that in the partial agonist bretazenil (2). Thus, a solution of the *N*-unsubstituted iodo dilactam (17)[16] was treated with potassium t-butoxide, and diethyl chlorophosphate to generate the imino phosphate (18) which was treated immediately with the anion generated



from ethyl isocyanoacetate. This method afforded the (*S*) 7-iodo ethyl ester (14) which was then converted into the corresponding stannane (16) according to the usual procedure.

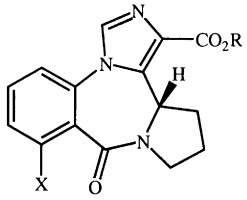
Attention was next turned to the preparation of isomeric iodo tetracycles in which the iodo substituent occurred in the position corresponding to the bromo substituent of bretazenil, and the ethyl ester (19) and t-butyl ester (21) were initial targets. The common starting material required was the bromo dilactam (23) and in the first approach *m*-bromoaniline (24) was converted into 3-bromo-α-(hydroxyimino)acetanilide (25) according to a modification of the procedure of Webber for related bromo anilines.[17] Ring closure of the bromo oxime (25) was effected with hot concentrated sulfuric acid to afford the desired 4-bromoisatin (26) and its isomer, 6-bromoisatin (27), in a combined yield of 83%.[18] ^{1}H n.m.r. analysis of the mixture indicated approximately 60% of the product was the desired 4-bromoisatin (26). The separation of the isomeric isatins was attempted by recrystallization and chromatography; however, only small quantities of 4-bromoisatin (26) could be isolated. Due to the time-consuming nature of the separation of the isatins it was decided to take the mixture of isatins through to the next step of the synthesis, and to attempt separation of the isomers at a later stage.

Oxidation of the bromoisatin mixture to the corresponding bromoisatoic anhydrides (28) and (29) was achieved according to a method reported for the formation of 5-fluoroisatoic anhydride.[19] Thus a solution of the mixture of isatins (26) and (27), acetic acid and acetic anhydride was slowly treated with chromium trioxide to ensure that the temperature remained between 80 and 90°. Higher temperatures resulted in the ignition of the reaction mixture, and the formation of a black tar. The product obtained was a mixture of the desired 6-bromoisatoic anhydride (28) and 4-bromoisatoic anhydride (29) in a combined yield of 84%. Again the purification of these isomers only allowed isolation of small amounts of the desired bromoisatoic anhydride (28) at a time. Therefore, the mixture of isatoic anhydrides was heated with L-proline to give a mixture containing the desired 6-bromo dilactam (30) and its 8-bromo isomer (31) in 35% yield. The separation of the isomers was achieved by using both recrystallization and chromatography, but again only small amounts of the desired bromo dilactam (30) could be isolated.

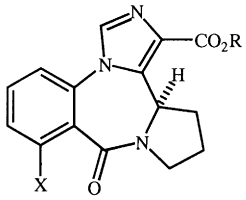
Due to the consistent problems encountered in the separation of the isomers in this synthetic route, an alternative method for the preparation of the bromo dilactam (23) was undertaken. Accordingly, 6-bromo-2-nitrotoluene (32) was reduced to 2-amino-6-bromotoluene (33) and the amino group was then protected as the acetamide (34) for the subsequent oxidation step. Oxidation of the aromatic methyl group to the corresponding benzoic acid (35) was achieved by treatment of the acetamide (34) with potassium permanganate in the presence of magnesium sulfate.

While attempted deprotection of the amide (35) with hot potassium hydroxide gave a mixture of products, treatment of the amide with warm HCl cleanly gave 2-amino-6-bromobenzoic acid (36) but only provided the temperature was maintained between 55 and 60°. Higher reaction temperatures led to products arising from decarboxylation.

Treatment of the amine (36) with phosgene afforded 6-bromoisatoic anhydride (28) which on reaction with L-proline gave the desired bromo dilactam (30). Incorporation of the imidazo ring was accomplished in the usual manner by using the two-step reaction in which a solution of the bromo dilactam (30) was treated sequentially with sodium hydride/diethyl chlorophosphate, followed by the addition of the anion generated from ethyl isocyanoacetate. The (*S*) 8-bromo ethyl ester (37) was obtained in 53% yield when the cyclocondensation was carried out between –40 and –20° and this product was found to be greater than 98% enantiomerically pure by chiral h.p.l.c. analysis. However, when the reaction was conducted at higher temperatures racemization occurred (as indicated by analysis of the product by chiral h.p.l.c.).



	X	R
(37)	Br	Et
(38)	$SnBu_3$	Et
(39)	Br	Bu^t
(40)	$SnBu_3$	Bu^t



	X	R
(41)	I	Et
(42)	^{123}I	Et
(43)	I	Bu^t
(44)	^{123}I	Bu^t
(45)	Br	Et
(46)	$SnBu_3$	Et
(47)	$SnBu_3$	Bu^t

The stannane (38) was next prepared from the (*S*) bromo derivative (37) according to the usual procedure in 81% yield but iodo-de-stannylation of this stannane with iodine in chloroform gave several products that could not be isolated. Zea-Ponce *et al.* have reported that the radioiodination of the stannane of iomazenil (4) was dependent on the pH of the reaction mixture, that is at pH within the range of 1.8–3.3 iodination occurred whereas at pH 5.8 reduced yields resulted, and at pH 7 there was no iodination.[20] Consistent with these results it was found that when the (*S*) 8-tributylstannyl ethyl ester (38) was heated with iodine in a chloroform solution which had been adjusted to pH 3 by the addition of glacial acetic acid the 8-iodo ethyl ester (19) was obtained in 74% yield.

Next, the t-butyl (13a*S*) 8-iodo tetracyclic ester (21) was obtained from the bromo derivative (30). Thus use of t-butyl isocyanoacetate for the incorporation of the imidazo ring gave the 8-bromo t-butyl ester (39) which was converted into the corresponding stannane (40). The iodo-de-stannylation reaction under acidic conditions gave the (*S*) 8-iodo t-butyl ester (21) which was found to be greater than 98% pure by chiral h.p.l.c. analysis.

In order to investigate the importance of the stereochemistry about C 13a the (*R*) 8-iodo ethyl ester (41) was prepared in an analogous manner except that D-proline instead of L-proline was used in the condensation with bromoisatoic anhydride (28). Details of the synthesis are given in the Experimental section. The (*R*) 8-iodo ethyl ester (41) was found to be greater than 97% pure by chiral h.p.l.c. analysis.

The incorporation of iodine-123 into all derivatives was achieved by using standard electrophilic iodo-de-stannylation reactions; however, there were several considerations in these radioiodination procedures. The first consideration was that the iodine-123 was obtained in the form of iodide ion; thus, an oxidizing agent was required in order to obtain an electrophilic source of iodine. Commonly used oxidizing agents include chloramine-T, iodogen, and peracetic acid.[21] The second consideration was that the iodine-123 was supplied in a hydroxide solution; therefore, appropriate buffers were required to obtain the desired pH. The final and perhaps most important consideration was the 13.3 h half-life of iodine-123, which required that the radioiodination times and purification times of the radioiodinated product were minimized in order to obtain the radioligand in high specific activity.

Before attempting the radioiodination reaction the h.p.l.c. conditions for the separation of the radioiodinated product from the reactants were optimized. This involved the use of the 'cold' iodo standards to obtain the retention times of the radioiodinated product, as well as 'cold' iodination reactions to ensure that the radioiodinated product would be well separated from other species in the reaction mixture.

The radiolabelled *N*-methyl ethyl ester (6) was prepared by treating a solution of *N*-methyl ethyl ester stannane (12); in ethanol, with 2–20 mCi* of sodium (^{123}I)iodide, followed by the addition of an acidic solution of the oxidizing agent chloramine-T. After a 5 min reaction time sodium metabisulfite was added and the mixture was purified by reverse-phase h.p.l.c. to give the *N*-methyl iodo ester (6) in 85% radiochemical yield and with a radiochemical purity of 98%. The specific activity was determined to be greater than 2500 Ci/mmol. Similar treatment of the *N*-methyl t-butyl ester stannane (13) gave the radiolabelled *N*-methyl t-butyl ester (8) (85% radiochemical yield, radiochemical purity of 98% and specific activity greater than 2500 Ci/mmol).

From the 7-tributylstannyl ethyl ester (16) was prepared the tetracyclic derivative (15) in a 72% radiochemical yield and a radiochemical purity of greater than 98%. This radioiodination was also investigated by using the oxidizing agent peracetic acid in Na_2HPO_4/NaH_2PO_4 buffer with a pH of 7.3 and a reaction time of 10 min. This method was found to give the radioiodinated product in a similar radiochemical yield of 70%.

The (*S*) 8-iodo ethyl ester (20) was prepared from the 8-tributylstannyl ethyl ester (38). The optimum reaction conditions required the use of chloramine-T in a concentration of approximately 10^{-3} M in 1 M hydrochloric acid so that the pH was less than 1.5. Under these optimum conditions the (*S*) 8-iodo ethyl ester (20) was obtained in greater than 80% radiochemical yield with radiochemical and chemical purity greater than 98% as assessed by analytical h.p.l.c. and radio-t.l.c.

The radiochemical yield of the 8-iodo ethyl ester (20) was found to be pH-dependent with optimum conditions being at a pH less than 1.5. The use of peracetic acid as the oxidizing agent enhanced the formation of mixtures of active and non-active by-products resulting in significantly lower yields of the desired product. Furthermore, oxidation with peracetic acid in the presence of phosphoric or acetic acids, or chloramine-T in dilute HCl at a pH greater than 1.5 led to the formation of a volatile radioactive material (10–50% radiochemical yield). This volatile material was previously identified by Zea-Ponce as butyl (^{123}I)iodide, arising from aliphatic iodo-de-stannylation.[20] The butyl (^{123}I)iodide was not observed when chloramine-T in 1 M HCl with a pH of less than 1.5 was used. These results are in agreement with the proposal of Zea-Ponce that the carbonyl *ortho* to the aryl carbon bearing the tin substituent reduces the reactivity of this carbon to electrophilic attack. The proposal of Zea-Ponce was further supported by the successful radioiodination of the 7-tributylstannyl ethyl ester (16) at a pH of 7.3 as described above. This stannane could be radioiodinated in good yield at high pH as it did not possess an *ortho* electron-withdrawing group which would reduce the reactivity of the aryl carbon bearing the tin substituent.

The corresponding (*R*) 8-iodo ethyl ester (42) was prepared from (*R*) 8-tributylstannyl ethyl ester (46). The product was analysed by chiral h.p.l.c. and was found to have a retention time of 32 min compared to the 44 min obtained for the (*S*) isomer.

Finally, the (*S*) 8-iodo t-butyl ester (22) was obtained from the 8-tributylstannyl t-butyl ester (40) in a radiochemical yield of 80% and with a radiochemical purity of greater than 98%. The specific activity was greater than 2500 Ci/mmol. The (*R*) 8-iodo t-butyl ester (44) was prepared from the (*R*) 8-tributylstannyl t-butyl ester (47) which was obtained from the ANSTO Biomedicine and Health Program. The two isomers were analysed by chiral h.p.l.c. and the retention times for the (*S*) 8-iodo t-butyl ester (22) and the (*R*) 8-iodo t-butyl ester (44) were found to be 20 min and 16 min respectively.

Experimental

General Procedures

General conditions have been reported elsewhere.[16]

Ethyl isocyanoacetate, t-butyl isocyanoacetate, and *m*-bromoaniline were distilled immediately prior to use. Preparative high-performance liquid chromatography (h.p.l.c.) separations were carried out on an Alltech semipreparative reverse-phase (RP) C18 column (10 μm, 10 mm by 250 mm), and analytical separations on a Goldpak Excil RP-C18 column (10 μm, 4.6 mm by 250 mm). The systems consisted of a Waters 510 pump, a Spectrophysics—linear ultraviolet detector set at 254 nm, and a modified online NaI—Berthold radioactivity detector. Chiral h.p.l.c. analysis was carried out on a Daicel CHIRACEL OD chiral column (10 μm, 4.6 mm by 250 mm) with 20% isopropyl alcohol/hexane as the eluent.

* 1 Ci = 3.7×10^{10} Bq = 3.7×10^{10} s^{-1}.

No-carrier-added (^{123}I)NaI was produced by the National Medical Cyclotron, Sydney, Australia, by using the Xe(p, 2n) reaction. The t-butyl ester* (47) was obtained from the ANSTO Biomedicine and Health Program.[22]

*7-Iodo-4-methyl-3,4-dihydro-2*H-*1,4-benzodiazepine-2,5(1*H)*-dione (10)*

A solution of 6-iodo-2*H*-3,1-benzoxazine-2,4(1*H*)-dione (9)[16] (2.0 g, 6.9 mmol), *N*-methylglycine (0.80 g, 9.0 mmol), and dimethylformamide (10 ml) was warmed gently until gas evolution ceased. The solution was heated at reflux for 1 h, cooled, and poured onto iced water (250 ml), and the cream precipitate was collected by filtration. The filtrate was extracted with ethyl acetate (3×50 ml), washed with water (3×100 ml), dried over anhydrous sodium sulfate, and the solvent was removed under reduced pressure to give a yellow solid. The solids were combined and recrystallized from ethyl acetate to afford the benzodiazepinedione (10) as a white crystalline solid (0.7 g, 32%), m.p. 257–259° (lit.[14] 249–250°). ^{1}H n.m.r. ($CDCl_3$): δ 8.27 (1H, d, *J* 2.1 Hz, H 6); 8.24 (1H, s, NH); 7.75 (1H, dd, *J* 2.1, 8.4 Hz, H 8); 6.74 (1H, d, *J* 8.4 Hz, H 9); 3.88 (2H, s, CH_2); 3.28 (3H, s, CH_3).

*Ethyl 8-Iodo-5-methyl-6-oxo-5,6-dihydro-4*H-*imidazo[1,5-*a*][1,4]benzodiazepine-3-carboxylate (5)*

Method 1. A solution of the benzodiazepinedione (10) (200 mg, 0.63 mmol) and dimethylformamide (10 ml) was stirred at room temperature under nitrogen. The solution was cooled to –20° and sodium hydride (60% dispersion in oil, 0.03 g, 0.75 mmol) was added, and the mixture was stirred at –20° for 45 min. Diethyl chlorophosphate (0.12 ml, 0.83 mmol) was added, and the mixture was stirred for a further 30 min at –20°.

Simultaneously, a solution of potassium t-butoxide (0.10 g, 0.89 mmol) and dimethylformamide (5 ml) was stirred under nitrogen and cooled to –40°. Ethyl isocyanoacetate (0.10 ml, 0.91 mmol) was added dropwise, and after the final addition the solution was stirred at –40° for 30 min. This solution was then added to the first solution via cannula. The resulting solution was stirred at –20° for 1.5 h, and glacial acetic acid (1 ml) and water (10 ml) were added. The solution was extracted with ethyl acetate (3×30 ml), washed with sodium carbonate (10%, 2×20 ml) and water (2×20 ml), dried over anhydrous sodium sulfate, and the solvent was removed under reduced pressure to give a yellow oil. Purification of the oil by flash chromatography (100% ethyl acetate) afforded a yellow oil which solidified upon standing. Recrystallization from ethyl acetate gave the *ethyl ester* (5) as a cream solid (50 mg, 19%), m.p. 223–224 (Found C, 43.6; H, 3.3; N, 10.0. $C_{15}H_{14}IN_3O_3$ requires C, 43.8; H, 3.4; N, 10.2%). ν_{max} ($CHCl_3$) 3003m, 1703m, 1642s, 1495s, 1378m, 1285m, 1251m, 1210s, 1119m, 1064m, 941m.† ^{1}H n.m.r. ($CDCl_3$): δ 8.39 (1H, d, *J* 2.0 Hz, H 7); 7.95 (1H, dd, *J* 8.5, 2.0 Hz, H 9); 7.87 (1H, s, H 1); 7.15 (1H, d, *J* 8.5 Hz, H 10); 5.10–5.35 (1H, br s); 4.25–4.49 (3H, m); 3.24 (3H, s, NCH_3); 1.45 (3H, t, *J* 7.1 Hz, $CH_2C\mathbf{H_3}$). Mass spectrum *m/z*: 411 (M^+, 58%), 365 (84), 337 (100), 42 (53).

Method 2. A solution of the dione (10) (200 mg, 0.63 mmol) and tetrahydrofuran (15 ml) was stirred under nitrogen and cooled to –15°. Potassium t-butoxide (0.08 g, 0.71 mmol) was added and the solution was stirred at –15° for 5 min. Diethyl chlorophosphate (0.10 ml, 0.69 mmol) was added and the resulting solution was stirred at room temperature until the temperature reached 10°. The solution was recooled to –15° and treated with ethyl isocyanoacetate (0.10 ml, 0.91 mmol), and potassium t-butoxide (0.08 g, 0.71 mmol). The solution was then stirred at room temperature for 1.5 h, and acetic acid (1 ml), and water (10 ml) were added. The mixture was worked up according to the procedure described above to afford a yellow oil. Purification by flash chromatography (100% ethyl acetate) gave a solid which was recrystallized from ethyl acetate to afford the ethyl ester (5) as a white solid (0.11 g, 42%), m.p. 222–223°.

*Ethyl 5-Methyl-6-oxo-8-tributylstannyl-5,5-dihydro-4*H-*imidazo[1,5-*a*][1,4]benzodiazepine-3-carboxylate (12)*

A solution of the 8-iodo ethyl ester (5) (40 mg, 0.10 mmol) and toluene (10 ml) was stirred under nitrogen. Bis(tributyltin) (0.12 ml, 0.24 mmol) and tetrakis(triphenylphosphine)palladium(0) (50 mg, 0.04 mmol) were added and the solution was heated to reflux. After 3 h a black precipitate formed and the mixture was filtered through Celite and the residue was washed with ethyl acetate. The filtrate and washings were combined and the solvent was removed under reduced pressure to give a yellow oil. The oil was dissolved in ethyl acetate (30 ml), washed with silver nitrate (0.1 M, 2×10 ml), and water (20 ml), dried over anhydrous sodium sulfate, and the solvent was removed under reduced pressure. Purification of the oil by flash chromatography (40% light petroleum/ethyl acetate) gave the *8-tributylstannyl ethyl ester* (12) as an oil which solidified upon standing (40 mg, 70%), m.p. 64–65° (Found: C, 56.0; H, 7.3. $C_{27}H_{41}N_3O_3Sn$ requires C, 56.5; H, 7.1%). ν_{max} ($CHCl_3$) 2950m, 2927m, 2872w, 2853w, 1701m(br), 1635s, 1578m, 1496s, 1464m, 1396m, 1377s, 1292m, 1253m. ^{1}H n.m.r. ($CDCl_3$): δ 8.13 (1H, d, *J* 0.9 Hz, H 7); 7.88 (1H, s, H 1); 7.70 (1H, dd, *J* 0.9, 7.8 Hz, H 8); 7.34 (1H, d, *J* 7.8 Hz, H 9); 5.05–5.35 (1H, br s); 4.30–4.52 (3H, m); 3.25 (3H, s, NCH_3); 1.05–1.82 (21H, m); 0.89 (9H, t, *J* 7.2 Hz, $Sn(CH_2CH_2CH_2C\mathbf{H}_3)_3$).

*t-Butyl 8-Iodo-5-methyl-6-oxo-5,6-dihydro-4*H-*imidazo[1,5-*a*][1,4]benzodiazepine-3-carboxylate (7)*

A solution of the benzodiazepinedione (10) (200 mg, 0.63 mmol), dimethylformamide (10 ml), sodium hydride (60% dispersion in oil, 0.03 g, 0.75 mmol) and diethyl chlorophosphate (0.10 ml, 0.69 mmol) was treated with a solution of potassium t-butoxide (0.10 g, 0.89 mmol), dimethylformamide (5 ml) and t-butyl isocyanoacetate (0.15 ml, 1.0 mmol) as described above. The brown oil obtained upon workup was purified by flash chromatography (50–60% ethyl acetate/light petroleum) to afford a yellow oil which solidified on standing. Recrystallization from ethyl acetate afforded the t-butyl ester (7) as a white crystalline solid (0.10 g, 37%), m.p. 212–213° (lit.[14] 212–214°). ^{1}H n.m.r. ($CDCl_3$): δ 8.39 (1H, d, *J* 2.1 Hz, H 7); 7.94 (1H, dd, *J* 2.1, 8.4 Hz, H 9); 7.85 (1H, s, H 1); 7.15 (1H, d, *J* 8.4 Hz, H 10); 5.05–5.30 (1H, br s, H 4); 4.16–4.40 (1H, br s, H 4); 3.24 (3H, s, CH_3); 1.65 (9H, s, $C(CH_3)_3$). Mass spectrum *m/z*: 439 (1.4%), 383 (89), 337 (79), 42 (100).

*t-Butyl 5-Methyl-6-oxo-8-tributylstannyl-5,6-dihydro-4*H-*imidazo[1,5-*a*][1,4]benzodiazepine-3-carboxylate (13)*

A solution of the 8-iodo t-butyl ester (7) (48 mg, 0.11 mmol), toluene (10 ml), bis(tributyltin) (0.12 ml, 0.24 mmol) and tetrakis(triphenylphosphine)palladium(0) (50 mg, 0.04 mmol) was heated at reflux for 2 h. The oil obtained upon workup was purified by flash chromatography (50% ethyl acetate/light petroleum) to give a white solid. Recrystallization from light petroleum afforded the 8-tributylstannyl t-butyl ester (13) as white needles (47 mg, 71%), m.p. 120–122° (previously reported as an oil[15]) (Found: C, 57.3; H, 7.8. Calc. for $C_{29}H_{45}N_3O_3Sn$: C, 57.8; H, 7.5%). ν_{max} ($CHCl_3$) 2951s, 2927s, 1724m, 1693s, 1635s, 1576w, 1496s, 1375s. ^{1}H n.m.r. ($CDCl_3$): δ 8.14 (1H, d, *J* 1.1 Hz, H 7); 7.88 (1H, s, H 1); 7.72 (1H, dd, *J* 1.1, 7.8 Hz, H 9); 7.35 (1H, d, *J* 7.8 Hz, H 10); 5.03–5.30 (1H, br s, H 4); 4.30–4.50 (1H, br s, H 4); 3.28 (3H, s, NCH_3); 1.67 (9H, s, $C(CH_3)_3$); 1.08–1.50 (18H, m); 0.91 (9H, t, *J* 7.1 Hz, $(C\mathbf{H}_3CH_2CH_2CH_2)_3Sn$). Mass spectrum *m/z*: 546 (M^+ – Bu, 100%), 544 (98), 490 (15), 439 (20), 434 (20), 376 (24), 57 (37), 41 (79%).

*Ethyl (13a*S*)-7-Iodo-9-oxo-11,12,13,13a-tetrahydro-9*H-*imidazo[1,5-*a*]pyrrolo[2,1-*c*][1,4]benzodiazepine-1-carboxylate (14)*

A solution of the (11a*S*) tricyclic dione (17)[16] (500 mg, 1.46 mmol), dimethylformamide (20 ml), potassium t-butoxide (200 mg, 1.78 mmol), and diethyl chlorophosphate (0.25 ml, 1.73 mmol), was treated

* t-Butyl 9-oxo-8-tributylstannyl-11,12,13,13a-tetrahydro-9*H*-imidazo[1,5-*a*]pyrrolo[2,1-*c*][1,4]benzodiazepine-1-carboxylate.
† ν_{max} are in cm^{-1} throughout.

with a solution of potassium t-butoxide (200 mg, 1.78 mmol), dimethylformamide (20 ml) and ethyl isocyanoacetate (0.20 ml, 1.8 mmol). The yellow oil obtained upon workup as described above was purified by flash chromatography (100% ethyl acetate) to afford a white solid. Recrystallization from ethyl acetate gave the *ethyl tetracyclic ester* (14) as colourless crystals (0.17 g, 27%), m.p. 234–235° (Found: C, 46.9; H, 3.6; N, 9.4. $C_{17}H_{16}IN_3O_3$ requires C, 46.7; H, 3.7; N, 9.6%). ν_{max} ($CHCl_3$) 1720s, 1633s, 1547w, 1495m, 1442s, 1370m, 1323m, 1301w, 1278w, 1253s, 1187m, 1120m, 961m. ^{1}H n.m.r. ($CDCl_3$): δ 8.45 (1H, d, *J* 2.1 Hz, H 8); 7.95 (1H, dd, *J* 2.1, 8.4 Hz, H 6); 7.83 (1H, s, H 3); 7.14 (1H, d, *J* 8.4 Hz, H 5); 4.75 (1H, m); 4.42 (2H, q, *J* 7.1 Hz, CH_2); 3.72–3.86 (1H, m); 3.40–3.65 (2H, m); 2.11–2.41 (3H, m); 1.44 (3H, t, *J* 7.1 Hz, CH_3). Mass spectrum *m/z*: 437 (M^+, 19%), 391 (68), 363 (100).

*Ethyl (13a*S*)-9-Oxo-7-tributylstannyl-11,12,13,13a-tetrahydro-9*H*-imidazo[1,5-*a*]pyrrolo[2,1-*c*][1,4]benzodiazepine-1-carboxylate (16)*

A solution of the 7-iodo ethyl ester (14) (50 mg, 0.11 mmol), toluene (10 ml), bis(tributyltin) (0.13 ml, 0.26 mmol) and tetrakis(triphenylphosphine)palladium(0) (10 mg, 0.01 mmol) was heated at reflux for 20 h. The oil obtained upon workup was purified by flash chromatography (40% ethyl acetate/light petroleum) and gave the *7-tributylstannyl ethyl ester* (16) as a colourless oil (46 mg, 70%) (Found: C, 58.2; H, 7.4. $C_{29}H_{43}N_3O_3Sn$ requires C, 58.1; H, 7.2%). ^{1}H n.m.r. ($CDCl_3$): δ 8.19 (1H, d, *J* 0.9 Hz, H 8); 7.85 (1H, s, H 3); 7.71 (1H, dd, *J* 0.9, 7.8 Hz, H 6); 7.33 (1H, d, *J* 7.8 Hz, H 5); 4.72–4.80 (1H, m); 4.41 (2H, q, 7.1 Hz, C**H**$_2$CH$_3$); 3.71–3.87 (1H, m); 3.43–3.67 (2H, m); 2.10–2.40 (3H, m); 1.04–1.80 (21H, m); 0.89 (9H, t, *J* 7.1 Hz, (C**H**$_3$CH$_2$CH$_2$CH$_2$)$_3$Sn). Mass spectrum *m/z*: 544(M^+ – Bu, 100%), 430 (M^+ – (3×Bu), 33), 356 (19), 57 (15).

3-Bromo-α-(hydroxyimino)acetanilide (25)

Anhydrous sodium sulfate (18 g, 0.13 mol) was added over 1 min to a stirred solution of water (150 ml) and chloral hydrate (11.6 g, 70 mmol). Separately, 3-bromoaniline (24) (10 g, 58 mmol) was added slowly to a stirred solution of hydrochloric acid (10 M, 6 ml) and water (50 ml). The 3-bromoaniline solution was then added to the first solution, and a precipitate formed. A solution of hydroxylamine hydrochloride (15 g, 0.22 mol) and water (70 ml) was then added. The mixture was heated slowly to reflux, resulting in dissolution of the precipitate, and almost immediately another precipitate formed. The mixture was heated at reflux for a further 10 min, cooled, filtered and the residue washed well with water, and dried under high vacuum. The bromo oxime (25) was obtained as a cream solid (11.76 g, 83 %), m.p. 161–162° (lit.[18] 165°). ^{1}H n.m.r. ($(CD_3)_2SO/CDCl_3$): δ 11.80 (1H, br s); 8.75 (1H, br s); 7.95 (1H, s); 7.45–7.60 (2H, m); 7.10–7.27 (2H, m).

4-Bromoisatin (26)

Sulfuric acid (18 M, 10 ml) was warmed to 60° and the bromo oxime (25) (2.0 g, 8.2 mmol) was added slowly to ensure that the temperature remained between 60 and 80°. After the final addition the mixture was stirred at 80° for 1 h. The mixture was cooled, and poured onto crushed ice (100 g). The precipitate was collected by filtration and washed with water, and dried to constant weight under high vacuum to give the crude product as a bright orange solid (1.54 g, 83%). T.l.c. analysis (40% ethyl acetate/light petroleum) indicated two products. A small sample was purified by flash chromatography (80% ethyl acetate/light petroleum) and the two products in order of elution were as follows.

(A) 6-Bromoisatin (27), orange crystals, m.p. 269–270° (lit.[18] 267°). ^{1}H n.m.r. ($(CD_3)_2SO/CDCl_3$): δ 10.71 (1H, s, NH); 7.40 (1H, d, *J* 8.0 Hz, H 4); 7.20 (1H, dd, *J* 1.4, 7.5 Hz, H 5); 7.12 (1H, d, *J* 1.4 Hz, H 7). Mass spectrum *m/z*: 227 (M^+, ^{81}Br, 64%), 225 (M^+, ^{79}Br, 64), 199 (100), 197 (99), 172 (24), 170 (41), 90 (62), 63 (43).

(B) 4-Bromoisatin (26), orange crystals, m.p. 272–273° (lit.[18] 270°). ^{1}H n.m.r. ($(CD_3)_2SO/CDCl_3$): δ 10.71 (1H, s, NH); 7.32 (1H, m); 7.17 (1H, m); 6.87 (1H, m). Mass spectrum *m/z*: 227 (M^+, ^{81}Br, 51%), 225 (M^+, ^{79}Br, 57), 199 (98), 197 (100), 172 (42), 170 (53), 63 (43).

^{1}H n.m.r. analysis of the original mixture indicated that 40% was the 6-bromoisatin (27) and 60% the desired 4-bromoisatin (26).

*5-Bromo-2*H*-3,1-benzoxazine-2,4(1*H*)-dione (28)*

The mixture of bromoisatins (26) and (27) (1.0 g, 4.4 mmol) was added to a solution of acetic acid (5 ml) and acetic anhydride (5 ml), and the mixture was warmed to 60°. Chromium trioxide (0.74 g, 7.4 mmol) was added slowly to ensure that the temperature remained between 80 and 90°. After the final addition the mixture was stirred for 10 min at 80°, cooled, and the precipitate collected by filtration. The precipitate was washed with water, and dried to a constant weight under high vacuum, to give the product as a pale yellow solid (0.90 g, 84%). A small sample was purified by flash chromatography (40% ethyl acetate/light petroleum); in order of elution the products were as follows.

(A) 7-Bromo-2*H*-3,1-benzoxazine-2,4(1*H*)-dione (29), m.p. 252–253° (lit.[23] 256°). ^{1}H n.m.r. ($(CD_3)_2SO$): δ 7.80 (1H, d, *J* 8.4 Hz, H 5); 7.39 (1H, dd, *J* 1.8, 8.4 Hz, H 6); 7.26 (1H, d, *J* 1.8 Hz, H 8).

(B) The 5-bromo dione (28), a white solid. Recrystallization from ethyl acetate gave colourless microcrystals, m.p. 260–261° (lit.[22] 260–262°). ^{1}H n.m.r. ($(CD_3)_2SO$): δ 11.82 (1H, s, NH); 7.46–7.59 (2H, m); 7.13 (1H, dd, *J* 1.9, 7.3 Hz). Mass spectrum (electrospray) *m/z*: 242 (M^+), 241, 240, 198, 197, 195, 127, 126.

*(11a*S*)-6-Bromo-1,2,3,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine-5,11(10*H*)-dione (30)*

The mixture of the bromoisatoic anhydrides (28) and (29) (0.73 g, 3.0 mmol), dimethylformamide (20 ml) and L-proline (0.50 g, 4.3 mmol) was heated gently with stirring until gas evolution ceased. The solution was then heated at reflux for 1 h, cooled, and poured onto water (150 ml). The mixture was extracted with chloroform (3×50 ml), washed with water (3×50 ml), dried over anhydrous magnesium sulfate, and the solvent was removed under reduced pressure to give a yellow oil. T.l.c. analysis indicated two products (0.31 g, 35%). A small sample was purified by flash chromatography (100% chloroform), and the two products, in order of elution, were as follows.

(A) The *8-bromo dione** (31), a white powder. Recrystallization from ethyl acetate gave white microcrystals, m.p. 293–295° (Found: C, 49.1; H, 3.7; N, 9.5. $C_{12}H_{11}BrN_2O_2$ requires C, 48.8; H, 3.7; N, 9.5%). ^{1}H n.m.r ($CDCl_3$): δ 8.39 (1H, s, NH); 7.88 (1H, d, *J* 8.4 Hz, H 9); 7.39 (1H, dd, *J* 1.7, 8.4 Hz, H 7); 7.20 (1H, d, *J* 1.7 Hz, H 6); 3.99–4.15 (1H, m); 3.71–3.87 (1H, m); 3.50–3.68 (1H, m); 2.64–2.88 (1H, m); 1.92–2.10 (3H, m). Mass spectrum *m/z*: 296 (M^+, ^{81}Br, 25%), 294 (M^+, ^{79}Br, 26), 238 (16), 70 (100).

(B) The 6-bromo dione (30), a white solid. Recrystallization from ethyl acetate gave white crystals, m.p. 231–232° (lit.[24] 221–224°). ^{1}H n.m.r. ($CDCl_3$): 8.49 (1H, s, NH); 7.51 (1H, dd, *J* 1.0, 8.0 Hz); 7.23 (1H, m, H 8); 6.99 (1H, dd, *J* 1.0, 8.3 Hz); 4.08–4.19 (1H, m); 3.86–3.98 (1H, m); 2.62–2.82 (1H, m); 1.93–2.15 (3H, m). Mass spectrum *m/z*: 296 (M^+, ^{81}Br, 12.5%), 294 (M^+, ^{79}Br, 14), 70 (100).

2-Acetamido-6-bromotoluene (34)

A solution of stannous chloride dihydrate (12.8 g, 57 mmol), 6-bromo-2-nitrotoluene (30) (4.0 g, 18.5 mmol) and ethanol (100 ml) was stirred at room temperature. After 24 h, t.l.c. analysis (20% ethyl acetate/light petroleum) indicated that some starting material remained. The solution was heated at reflux for 3 h, cooled, and concentrated to 10 ml under reduced pressure. The residue was made alkaline by the addition of sodium hydroxide (2 M), and extracted with ethyl acetate (3×50 ml). The organic phases were washed with water (2×40 ml), dried over anhydrous sodium sulfate, and the solvent was removed under reduced pressure to give the 2-amino-6-bromotoluene (33) as a yellow oil (3.18 g, 92%). ^{1}H n.m.r. ($CDCl_3$): δ 6.99 (1H, dd, *J* 1.1, 7.9 Hz, H 5); 6.85 (1H, m, H 4); 6.60 (1H, dd, *J* 1.1, 7.8 Hz, H 3); 3.74 (2H,

* (11a*S*)-8-Bromo-1,2,3,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine-5,11(10*H*)-dione.

s, NH_2); 2.26 (3H, s, CH_3). Mass spectrum *m/z*: 187 (M^+, ^{81}Br, 100%), 185 (M^+, ^{79}Br, 97), 106 (M^+ – Br, 83), 77 (26). Acetic anhydride (5 ml, 53 mmol) was added to 2-amino-6-bromotoluene (33) (3.0 g, 16 mmol) with stirring. A precipitate formed immediately and the mixture was stirred for a further 10 min. The solvent was removed by distillation under reduced pressure to give a cream precipitate. Recrystallization from ethanol gave the 2-acetamido-6-bromotoluene (34) as white needles (2.8 g, 77%), m.p. 166–167° (lit.[25] 164.5°). ^{1}H n.m.r. ($CDCl_3$): δ 7.59 (1H, d, *J* 8.2 Hz); 7.40 (1H, d, *J* 8.0 Hz); 6.96–7.19 (2H, m, H 4, NH); 2.34 (3H, s, CH_3CO); 2.20 (3H, s, ArC**H$_3$**).

2-Acetamido-6-bromobenzoic Acid (35)

A mixture of 2-acetamido-6-bromotoluene (34) (3.0 g, 13 mmol) magnesium sulfate heptahydrate (6.5 g, 26 mmol), and water (150 ml) was heated at reflux. A solution of potassium permanganate (6.5 g, 41 mmol) and water (70 ml) was added portionwise over 4 h. After the final addition the mixture was heated at reflux for 2 h, and the hot mixture was filtered. The filtrate was cooled and acidified with hydrochloric acid (3 M). The crude product precipitated as white crystals which were collected by filtration, and dried under high vacuum. Recrystallization from ethanol afforded the 2-acetamido-6-bromobenzoic acid (35) as a white crystalline solid (2.44 g, 73%), m.p. 225–226° (lit.[26] 224°). ^{1}H n.m.r. ($(CD_3)_2SO$): δ 9.63 (1H, s, NH); 7.27–7.51 (3H, m, aromatic); 1.99 (3H, s, CH_3).

2-Amino-6-bromobenzoic Acid (36)

2-Acetamido-6-bromobenzoic acid (35) (3.0 g, 12 mmol) was suspended in hydrochloric acid (10 M, 30 ml) and the mixture was stirred at 55–60° for 48 h. The mixture was filtered and the residue was washed with ether, and dried under high vacuum to give the 2-amino-6-bromobenzoic acid hydrochloride salt as a white solid (2.22 g, 73%). A small sample of the hydrochloride salt was neutralized with sodium carbonate (10%) and reacidified to pH 4 with hydrochloric acid (3 M). A white precipitate formed, and the mixture was extracted with ether (2×30 ml), washed with water (2×20 ml), dried over anhydrous sodium sulfate, and the solvent was removed under reduced pressure to give a white solid. Recrystallization from ethyl acetate gave 2-amino-6-bromobenzoic acid (36) as white crystals, m.p. 134–135° (lit.[26] 136°). ^{1}H n.m.r. ($CDCl_3$): δ 6.89–7.02 (2H, m, H 4,5); 6.63 (1H, dd, *J* 2.0, 7.3 Hz, H 3).

*5-Bromo-2*H*-3,1-benzoxazine-2,4(1*H*)-dione (28) via 2-Amino-6-bromobenzoic Acid (36)*

A solution of 2-amino-6-bromobenzoic acid (36) (3.0 g, 12 mmol), sodium hydroxide (1.00 g, 25 mmol) and water (20 ml) was cooled to 0°. To this solution was added phosgene (1.93 M in toluene, 10 ml, 19 mmol), the rate ensuring that the temperature remained between 0 and 5°. After the final addition the mixture was stirred for 15 min. The precipitate was collected by filtration to give a white solid which upon recrystallization from ethyl acetate gave the bromo dione (28) as colourless microcrystals (2.68 g, 92%), m.p. 246–248°. This product was found to be identical to the product described above.

*(11a*S*)-6-Bromo-1,2,3,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine-5,11(10*H*)-dione (30) via 2-Amino-6-bromobenzoic Acid (36)*

A solution of L-proline (1.3 g, 11 mmol), 5-bromo-2*H*-3,1-benzoxazine-2,4(1*H*)-dione (28) (2.5 g, 10 mmol) and dimethylformamide (20 ml) was warmed gently until gas evolution ceased. The solution was heated at reflux for 1 h, and the solvent was removed under reduced pressure to give a brown oil. The oil was dissolved in chloroform (50 ml), washed with water (4×50 ml), dried over anhydrous magnesium sulfate, and the solvent was removed under reduced pressure to give an oily foam. Recrystallization from ethyl acetate gave the pyrrolobenzodiazepinedione (30) as colourless crystals (1.18 g, 40%), m.p. 230° (lit.[24] 221–224°). This product was found to have identical properties to the product formed above.

*Ethyl (13a*S*)-8-Bromo-9-oxo-11,12,13,13a-tetrahydro-9*H*-imidazo[1,5-a]pyrrolo[2,1-c][1,4]benzodiazepine-1-carboxylate (37)*

A solution of the pyrrolobenzodiazepinedione (30) (1.0 g, 3.4 mmol), dimethylformamide (50 ml), sodium hydride (60% dispersion in oil, 0.15 g, 3.8 mmol) and diethyl chlorophosphate (0.60 ml, 4.2 mmol) was treated with a solution of potassium t-butoxide (0.45 g, 4.0 mmol), dimethylformamide (50 ml) and ethyl isocyanoacetate (0.50 ml, 4.6 mmol). The oil obtained upon work up was purified by flash chromatography (100% ethyl acetate) and gave a white solid. Recrystallization from ethyl acetate/light petroleum afforded the *8-bromo ethyl ester* (37) as a white crystalline solid (700 mg, 53%), m.p. 196–197 (Found: M^+ 389.0364. $C_{17}H_{16}BrN_3O_3$ requires M^+ 389.0375). ν_{max} ($CHCl_3$) 1718m, 1643s, 1591w, 1570w, 1546w, 1496m, 1455m, 1416w, 1370w, 1350w, 1320m, 1275w, 1253m, 1222s, 1214s, 1186m, 1104m, 1059w, 1043w. ^{1}H n.m.r ($CDCl_3$): δ 7.87 (1H, s); 7.81 (1H, dd, *J* 1.2, 8.0 Hz); 7.43 (1H, m); 7.31 (1H, dd, *J* 1.2, 8.0 Hz); 4.78 (1H, m); 4.42 (2H, q, *J* 7.1 Hz, C**H$_2$**CH_3); 3.77–3.92 (1H, m); 3.44–3.67 (2H, m); 2.13–2.39 (4H, m); 1.44 (3H, t, *J* 7.1 Hz, CH_3). Mass spectrum *m/z*: 391 (M^+, ^{81}Br, 19%), 389 (M^+, ^{79}Br, 17), 345 (31), 343 (28), 313 (81), 315 (100), 289 (33), 287 (30), 209 (44), 179 (31), 75 (46), 41 (41).

*Ethyl (13a*S*)-9-Oxo-8-tributylstannyl-11,12,13,13a-tetrahydro-9*H*-imidazo[1,5-a]pyrrolo[2,1-c][1,4]benzodiazepine-1-carboxylate (38)*

A solution of the 8-bromo ethyl ester (37) (100 mg, 0.26 mmol), toluene (20 ml), bis(tributyltin) (0.30 ml, 0.59 mmol) and tetrakis(triphenylphosphine)palladium(0) catalyst (10 mg, 0.01 mmol) was heated at reflux overnight. The oil obtained was purified by flash chromatography (40% ethyl acetate/light petroleum) to afford an oil which solidified upon standing. The *8-tributylstannyl ethyl ester* (38) was obtained as a white solid (126 mg, 81%), m.p. 60–62° (Found: C, 57.6, H, 7.4. $C_{27}H_{43}N_3O_3Sn$ requires C, 58.1; H, 7.2%). ν_{max} ($CHCl_3$) 2957m, 2923m, 1716m, 1624s, 1548w, 1494w, 1456m, 1416w, 1370w, 1321m, 1250m, 1323s, 1244s. ^{1}H n.m.r. ($CDCl_3$): δ 7.86 (1H, s, H 3); 7.73 (1H, dd, *J* 1.2, 7.1 Hz); 7.57 (1H, m); 7.30 (1H, m); 4.72–4.82 (1H, m); 4.42 (2H, q, *J* 7.1 Hz, C**H$_2$**CH_3); 3.72–3.88 (1H, m); 3.40–3.63 (2H, m); 2.09–2.41 (3H, m); 0.92–1.64 (21H, m); 0.87 (9H, t, *J* 7.12 Hz, (C**H$_3$**$CH_2CH_2CH_2)_3Sn$). Mass spectrum *m/z*: 544 (M^+ – Bu, 100%), 430 (M^+ – (3×Bu), 40), 356 (24), 238 (9), 57 (Bu, 17).

*Ethyl (13a*S*)-8-Iodo-9-oxo-11,12,13,13a-tetrahydro-9*H*-imidazo[1,5-a]pyrrolo[2,1-c][1,4]benzodiazepine-1-carboxylate (19)*

The 8-tributylstannyl ethyl ester (38) (100 mg, 0.17 mmol) was dissolved in chloroform (30 ml) acidified to pH 3 by the addition of glacial acetic acid. Iodine (0.24 g, 0.94 mmol) was added and the mixture was heated gently for 3 h. The mixture was cooled, washed with sodium thiosulfate solution (10%, 3×30 ml), sodium carbonate (10%, 3×30 ml), and water (2×30 ml). The solvent was removed under reduced pressure to give a yellow oil. Purification of the oil by flash chromatography (100% ethyl acetate) gave a white solid, which upon recrystallization from ethyl acetate gave the *8-iodo ethyl ester* (19) as colourless crystals (54 mg, 74%), m.p. 200–202° (Found: M^+ 437.0293. $C_{17}H_{16}IN_3O_3$ requires M^+ 437.0236). ν_{max} ($CHCl_3$) 1718m, 1641s, 1496m, 1455m, 1415w, 1370w, 1320m, 1251m. ^{1}H n.m.r ($CDCl_3$): δ 8.11 (1H, dd, *J* 1.3, 7.4 Hz); 7.85 (1H, s, H 3); 7.20–7.38 (2H, m); 4.77 (1H, br d, *J* 6.7 Hz, H 13a); 4.42 (2H, q, *J* 7.1 Hz, C**H$_2$**CH_3); 3.78–3.90 (1H, m); 3.47–3.68 (2H, m); 2.15–2.44 (3H, m); 1.44 (3H, t, *J* 7.1 Hz, CH_2C**H$_3$**). Mass spectrum *m/z*: 437 (M^+, 26%), 391 (92), 363 (100), 335 (27). (Chiral h.p.l.c. R_t 44 min.)

*t-Butyl (13a*S*)-8-Bromo-9-oxo-11,12,13,13a-tetrahydro-9*H*-imidazo[1,5-a]pyrrolo[2,1-c][1,4]benzodiazepine-1-carboxylate (39)*

A solution of the pyrrolobenzodiazepinedione (30) (200 mg, 0.68 mmol), dimethylformamide (20 ml), sodium hydride (60% dispersion in oil, 0.03 g, 0.75 mmol) and diethyl chlorophosphate (0.12 ml, 0.83 mmol) was treated with a solution of potassium t-butoxide (0.09 g, 0.80 mmol), dimethylformamide (30 ml) and t-butyl isocyanoacetate (0.16 ml, 1.1 mmol). The yellow oil obtained was purified by flash chromatography (100% ethyl acetate) to yield a white solid. Recrystallization from ethyl acetate/light petroleum gave the 8-bromo

t-butyl ester (39) as a colourless crystalline solid (122 mg, 43%), m.p. 200–203° (lit.[24] 206–208°). ^{1}H n.m.r. ($CDCl_3$): δ 7.83 (1H, s, H 3); 7.79 (1H, dd, *J* 0.9, 8.1 Hz); 7.42 (1H, m); 7.28 (1H, dd, *J* 0.9, 6.2 Hz); 4.74 (1H, br d, *J* 7.0 Hz, H 13a); 2.82–3.88 (1H, m); 3.48–3.61 (m, 2H); 2.15–2.30 (m, 3H); 1.64 (9H, s, Bu^t).

*t-Butyl (13a*S*)-9-Oxo-8-tributylstannyl-11,12,13,13a-tetrahydro-9*H-*imidazo[1,5-*a*]pyrrolo[2,1-*c*][1,4]benzodiazepine-1-carboxylate (40)*

A solution of the 8-bromo t-butyl ester (39) (100 mg, 0.24 mmol), toluene (20 ml), bis(tributyltin) (0.28 ml, 0.55 mmol) and tetrakis-(triphenylphosphine)palladium(0) (10 mg, 0.01 mmol) was heated at reflux for 3 h. The oil obtained upon workup was purified by flash chromatography (60% ethyl acetate/light petroleum) to give the *8-tributylstannyl t-butyl ester* (40) as a colourless oil (114 mg, 76%) (Found: C, 57.9, H, 7.3. $C_{29}H_{47}N_3O_3Sn$ requires C, 57.7; H, 7.8%). ν_{max} ($CHCl_3$) 2957s, 2923s, 2871m, 2853m, 1709s, 1624s, 1579w, 1545w, 1492m, 1456m, 1415m, 1151s. ^{1}H n.m.r. ($CDCl_3$): δ 7.82 (1H, s, H 3); 7.71 (1H, dd, *J* 1.03, 7.2 Hz); 7.54 (1H, dd, *J* 7.2, 7.8 Hz, H 6); 7.25 (1H, dd, *J* 1.03, 7.8 Hz); 4.68–4.79 (1H, m); 3.69–3.88 (1H, m); 3.40–3.64 (2H, m); 2.05–2.40 (1H, m); 1.64 (9H, s, Bu^t); 1.20–1.60 (14H, m); 1.08 (6H, t, *J* 8.3 Hz, $(CH_3CH_2CH_2C\mathbf{H_2})_3Sn$); 0.88 (9H, t, *J* 7.1 Hz, $(C\mathbf{H_3}CH_2CH_2CH_2)_3Sn$). Mass spectrum (electrospray) *m/z*: 631, 630, 629, 628, 627, 626, 575, 574, 573, 572.

*t-Butyl (13a*S*)-8-Iodo-9-oxo-11,12,13,13a-tetrahydro-9*H-*imidazo[1,5-*a*]pyrrolo[2,1-*c*][1,4]benzodiazepine-1-carboxylate (21)*

A solution of the 8-tributylstannyl t-butyl ester (40) (100 mg, 0.16 mmol), and chloroform (30 ml) acidified to pH 3 by the addition of glacial acetic acid, was treated with iodine (0.24 g, 0.94 mmol) for 2 h. The yellow solid obtained upon workup was purified by flash chromatography (100% ethyl acetate) and gave the *8-iodo t-butyl ester* (21) as a white solid (52 mg, 70%), m.p. 223–225° (Found: C, 49.2; H, 4.0; N, 8.9. $C_{19}H_{20}IN_3O_3$ requires C, 49.1; H, 4.3; N, 9.0%). ν_{max} ($CHCl_3$) 1712m, 1604s, 1492m, 1454m, 1415w, 1327m, 1254m, 1151s. ^{1}H n.m.r. ($CDCl_3$): δ 8.10 (1H, dd, *J* 1.6, 7.4 Hz); 7.82 (1H, s, H 3); 7.31 (1H, dd, *J* 1.6, 7.9 Hz); 7.19–7.27 (1H, m); 4.73–4.78 (1H, m); 3.78–3.92 (1H, m); 3.48–3.67 (2H, m); 2.12–2.40 (3H, m); 1.64 (9H, s, Bu^t). Mass spectrum (electrospray) *m/z*: 466 (M^++1), 465 (M^+), 410, 392.

*Ethyl (13a*R*)-8-Bromo-9-oxo-11,12,13,13a-tetrahydro-9*H-*imidazo[1,5-*a*]pyrrolo[2,1-*c*][1,4]benzodiazepine-1-carboxylate (45)*

5-Bromo-2*H*-3,1-benzoxazine-2,4(1*H*)-dione (28) (2.5 g, 10 mmol) was treated with D-proline (1.3 g, 11 mmol) according to the procedure described above for the preparation of the (*S*)-enantiomer (30) to afford the (11a*R*) compound* (1.03 g, 35%), m.p. 224–226° (lit.[24] 221–224°). (Chiral h.p.l.c. R_t 12.5 min.) Further treatment of the (11a*R*) product according to the procedure described above for the preparation of the (*S*)-enantiomer, afforded the *8-bromo ethyl ester* (45) in 41% yield, m.p. 196–197° (Found: M^+ 389.0348. $C_{17}H_{16}BrN_3O_3$ requires M^+ 389.0375). The ^{1}H n.m.r. spectrum of this (*R*)-enantiomer was identical to that of the (*S*)-enantiomer (37) reported above.

*Ethyl (13a*R*)-8-Iodo-9-oxo-11,12,13,13a-tetrahydro-9*H-*imidazo[1,5-*a*]pyrrolo[2,1-*c*][1,4]benzodiazepine-1-carboxylate (41)*

Treatment of the 8-bromo ethyl ester (45) according to the procedure described above for the (*S*)-enantiomer afforded the 8-tributylstannyl ethyl ester (46) in 87% yield, m.p. 60–61°. Subsequent treatment of the 8-tributylstannyl ethyl ester (46) according to the procedure described for the (*S*)-enantiomer afforded the 8-iodo ethyl ester (41) in 78% yield, m.p. 200–202°. (Chiral h.p.l.c. R_t 32 min.) The ^{1}H n.m.r. spectrum of this (*R*)-enantiomer was identical to that of the (*S*)-enantiomer (19) reported above.

Ethyl 8-(^{123}*I)Iodo-5-methyl-6-oxo-5,6-dihydro-4*H-*imidazo[1,5-*a*]*[1,4]benzodiazepine-3-carboxylate (6)*

To a solution of the 8-tributylstannyl ethyl ester (12) (200 μg, 0.42 μmol) and ethanol (200 μl) was added sodium (^{123}I)iodide (2–20 mCi) in NaOH (0.1 M, 20–300 μl). A solution of chloramine-T (100 μg, 0.4 μmol) in HCl (1 M, 100 μl) was added and the solution was allowed to stand for 5 min with intermittent shaking. The reaction mixture was quenched with $Na_2S_2O_5$ (100 mg/ml, 100 μl) and $NaHCO_3$ (100 mg/ml, 100 μl), and was injected onto a semipreparative RP h.p.l.c. column (42% ethanol/water, 2.5 ml/min). The fraction with activity corresponding to the ^{123}I-labelled ester (6) was collected (R_t 19 min) and the solution was evaporated to dryness. The residue was reconstituted into sterile saline, and filtered through a sterile 0.22 μm filter (Millex GS, Millipore) into a sterile evacuated vial and the radioactivity was measured. The product was obtained in a radiochemical yield of 85% with a radiochemical purity of 98% as assessed by radio-t.l.c. and analytical h.p.l.c.

Aliquots of known volume and activity were taken and injected onto an analytical h.p.l.c. column (45% ethanol/water, 1 ml/min). The radioactive product was co-eluted with the iodinated standard (R_t 6.5 min), and the u.v. absorbance peak was measured at 254 nm corresponding to the carrier product and compared to a standard curve relating mass to u.v. absorbance. The specific activity calculated at the end of the synthesis under the above reaction conditions was greater than 2500 Ci/mmol.

t-Butyl 8-(^{123}*I)Iodo-5-methyl-6-oxo-5,6-dihydro-4*H-*imidazo-[1,5-*a*]*[1,4]benzodiazepine-3-carboxylate (8)*

A solution of the 8-tributylstannyl t-butyl ester (13) (200 μg, 0.3 μmol) and ethanol (200 μl) was treated with sodium (^{123}I)iodide as described above for stannane (12). The fraction with activity corresponding to the *^{123}I-labelled ester* (8) was collected at R_t 39 min (semipreparative) and R_t 10.5 min (analytical). The product (8) was obtained in a radiochemical yield of 85% with a radiochemical purity of 98%. The specific activity was calculated to be greater than 2500 Ci/mmol.

*Ethyl (13a*S*)-7-(*^{123}*I)Iodo-9-oxo-11,12,13,13a-tetrahydro-9*H-*imidazo[1,5-*a*]pyrrolo[2,1-*c*][1,4]benzodiazepine-1-carboxylate (15)*

To a solution of the 7-tributylstannyl ethyl ester (16) (500 μg, 0.8 μmol) and ethanol (250 μl) was added a solution of sodium (^{123}I)iodide (1.95 mCi) and NaOH (0.1 M, 10 μl). A solution of chloramine-T (500 μg, 2 μmol) and HCl (1 M, 100 μl) was added and the mixture was allowed to stand for 6 min with intermittent shaking. The reaction was quenched with $Na_2S_2O_5$ (100 mg/ml, 100 μl) and $NaHCO_3$ (100 mg/ml, 100 μl). The mixture was injected onto a semipreparative RP h.p.l.c. column (40% ethanol/ammonium acetate (0.1 M), 2.5 ml/min) and the fraction with activity corresponding to the *^{123}I-labelled ester* (15) (R_t 19.5 min) was collected and the solution was evaporated to dryness. The residue was reconstituted into sterile saline, and filtered through a sterile 0.22 μm filter into a sterile evacuated vial, and the radioactivity measured. The radiochemical yield and radiochemical purity were determined as 72 and 98% respectively.

*Ethyl (13a*S*)-8-(*^{123}*I)Iodo-9-oxo-11,12,13,13a-tetrahydro-9*H-*imidazo[1,5-*a*]pyrrolo[2,1-*c*][1,4]benzodiazepine-1-carboxylate (20)*

To a solution of the 8-tributylstannyl ethyl ester (38) (300 μg, 0.69 μmol) and ethanol (300 μl) was added sodium (^{123}I)iodide (1–10 mCi) in NaOH (0.1 M, 100 μl). A solution of chloramine-T (100 μg, 0.4 μmol) and HCl (1 M, 100 μl) was added and the solution was allowed to stand, with intermittent shaking, for 10 min. The reaction mixture was quenched with $Na_2S_2O_5$ (100 mg/ml, 100 μl) and injected onto a semipreparative RP h.p.l.c. column (40% ethanol/water, 2.5 ml/min). The radioactivity peak corresponding to the *^{123}I-labelled ester* (20) (19.5 min) was collected and the solution was evaporated to dryness. The residue was reconstituted in sterile saline, and filtered through a

* (11a*R*)-6-Bromo-1,2,3,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine-5,11(10*H*)-dione.

sterile 0.22 μm filter (Millex GS, Millipore) into a sterile, pyrogen-free evacuated vial, and the radioactivity measured. The ethyl ester (20) was obtained in a radiochemical yield of 80%, and radiochemical and chemical purity assessed by both radio-t.l.c. and analytical h.p.l.c. were greater than 98%. The specific activity was determined as described above (R_t 6.5 min), and was found to be greater than 2500 Ci/mmol. The product was found to be greater than 97% enantiomerically pure by chiral h.p.l.c. (1 ml/min, R_t 44 min).

*Ethyl (13a*R*)-8-(*^{123}*I)Iodo-9-oxo-11,12,13,13a-tetrahydro-9*H-*imidazo[1,5-*a*]pyrrolo[2,1-*c*][1,4]benzodiazepine-1-carboxylate (42)*

A solution of the 8-tributylstannyl ethyl ester (46) was treated as described above for the (*S*)-enantiomer to give the *^{123}I-labelled ester* (42). (Chiral h.p.l.c., 1 ml/min, R_t 32 min.)

*t-Butyl (13a*S*)-8-(*^{123}*I)Iodo-9-oxo-11,12,13,13a-tetrahydro-9*H-*imidazo[1,5-*a*]pyrrolo[2,1-*c*][1,4]benzodiazepine-1-carboxylate (22)*

A solution of the 8-tributylstannyl t-butyl ester (40) (0.3 mg, 0.48 μmol) and ethanol (300 μl) was treated as described for the ethyl ester (38). The fraction with activity corresponding to the *^{123}I-labelled ester* (22) was collected at R_t 44 min (semipreparative) and R_t 11.5 min (analytical), and was obtained in a radiochemical yield of 80%. The radiochemical and chemical purity were greater than 98%, and the specific activity was calculated to be greater than 2500 Ci/mmol. The product was found to be greater than 97% enantiomerically pure by chiral h.p.l.c. (1 ml/min, R_t 20 min).

*t-Butyl (13a*R*)-8-(*^{123}*I)Iodo-9-oxo-11,12,13,13a-tetrahydro-9*H-*imidazo[1,5-*a*]pyrrolo[2,1-*c*][1,4]benzodiazepine-1-carboxylate (44)*

A solution of the 8-tributylstannyl t-butyl ester (47) (0.3 mg, 0.48 μmol) and ethanol (300 μl) was treated as described above for the (*S*)-enantiomer and gave the *^{123}I-labelled ester* (44). (Chiral h.p.l.c., 1 ml/min, R_t 16 min.)

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