Supporting Information for:

One-pot radioiodination of aryl amines via stable diazonium salts: preparation of $^{125}\text{I}$-imaging agents

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1. General Experimental

All reagents and starting materials were obtained from commercial sources and used as received unless otherwise stated. Dry solvents (dichloromethane) were purified using a PureSolv 500 MD solvent purification system. All reactions were performed open to air unless otherwise stated. Brine is defined as a saturated aqueous solution of sodium chloride. Room temperature refers to 20–25 °C. Flash column chromatography was carried out using Fisher Matrix silica 60. Macherey-Nagel aluminium-backed plates, pre-coated with silica gel 60 (UV254) were used for thin layer chromatography and were visualised using UV light (254/365 nm) then potassium permanganate solution. $^\text{1}H$ and $^\text{13}C$ NMR spectra were recorded on a Bruker DPX 400 ($^\text{1}H$: 400 MHz; $^\text{13}C$: 101 MHz) spectrometer or a Bruker 500 ($^\text{1}H$: 500 MHz; $^\text{13}C$: 126 MHz) spectrometer with chemical shift values reported in ppm relative to a residual solvent peak and in the solvent stated. Assignment of $^\text{1}H$ NMR signals is based on COSY experiments. Assignment of $^\text{13}C$ NMR signals is based on HSQC and/or DEPT experiments. All coupling constants, $J$, are quoted in Hz. Infrared spectra were recorded using Golden Gate apparatus on a JASCO FTIR 410 spectrometer directly as either a solid or liquid. Mass spectra were obtained using a JEOL JMS-700 spectrometer. Melting points were determined on a Gallenkamp melting point apparatus. For use as a standard for HPLC analysis, 4-chloro-2-iodonitrobenzene was purchased from a commercial supplier. All other standards were prepared using the one-pot diazotisation-iodination procedure (see below).

Ethyl 7-nitro-5,6-dihydro-5-methyl-6-oxo-4H-imidazo[1,5-a][1,4]benzodiazepine-3-carboxylate (3c) was supplied from GE Healthcare Ltd. N-Methyl-N-(3-nitrophenyl)cyanamide, required for the preparation of CN1261, was prepared as previously reported.$^1$ Ethyl 4-(2'-nitrophenyl)quinoline-2-carboxylate, required for the preparation of the TSPO agent, was prepared as previously reported.$^2$ 4-[4'-Fluoro-3'-(piperazine-1''-carbonyl)benzyl]-2H-phthalazin-1-one, for the preparation of the PARP-1 imaging agent, was prepared as previously reported.$^3$

2. Experimental Procedures and Spectroscopic Data for all Compounds

General Procedure for Preparation of Polymer-Supported Nitrite$^4$

The polymer-supported nitrite reagent was prepared by the addition of Amberlyst$^{\text{®}}$ A26 hydroxide form resin (1.00 g, 4.00 mmol) to a solution of NaNO$_2$ (0.55 g, 8.00 mmol) in water (20 mL). The mixture was stirred at room temperature for 0.5 h. The
polymer-supported nitrite was filtered and washed with water until the pH of the filtrate became neutral. The content of the polymer-supported nitrite was 3.5 mmol of NO₂/g.⁴

**General Procedure for One-Pot Diazotisation and Iodination: 4-Iodonitrobenzene⁵**

To a solution of p-toluenesulfonic acid monohydrate (0.27 g, 1.1 mmol) in acetonitrile (2 mL) was added 4-nitroaniline (0.050 g, 0.36 mmol). The solution was cooled in a water bath to 10–15 °C. Polymer-supported nitrite (0.31 g, containing 1.1 mmol of NO₂) was added, followed by sodium iodide (0.11 g, 0.54 mmol) in water (0.2 mL). The reaction mixture was stirred for 1 h then warmed to room temperature and stirred for 2 h in total. The mixture was filtered from the resin and the resin was washed with diethyl ether (50 mL). The reaction mixture and diethyl ether washings were combined and washed with water (50 mL). The aqueous layer was then extracted with diethyl ether (3 × 50 mL). The combined organic layers were dried (MgSO₄) and concentrated in vacuo. The crude material was purified using flash column chromatography eluting with 20% ethyl acetate in petroleum ether (40–60) to give 4-iodonitrobenzene as a pale yellow solid (0.080 g, 86%). Mp 170–172 °C (lit.⁵ 169–171 °C); δ_H (400 MHz, CDCl₃) 7.91 (2H, d, J 8.0 Hz, 3-H and 5-H), 7.94 (2H, d, J 8.0 Hz, 2-H and 6-H); δ_C (101 MHz, CDCl₃) 102.8 (C), 125.0 (2 × CH), 138.8 (2 × CH), 148.0 (C); m/z (Cl) 250 (MH⁺, 60%), 209 (12), 193 (15), 124 (30), 113 (22), 85 (78), 69 (100).

**4-Iodobromobenzene⁶**

The reaction was carried out according to the previously described general procedure for 4-iodonitrobenzene using 4-bromoaniline (0.050 g, 0.28 mmol), p-toluenesulfonic acid monohydrate (0.17 g, 0.87 mmol), polymer-supported nitrite
(0.25 g, containing 0.87 mmol of NO₂) and sodium iodide (0.090 g, 0.58 mmol). Purification by flash column chromatography eluting with 5% diethyl ether in petroleum ether (40–60) gave 4-iodobromobenzene as a white solid (0.063 g, 77%). Mp 91–92 °C (lit.⁶ 90 °C); δ_H (400 MHz, CDCl₃) 7.23 (2H, d, J 8.6 Hz, 3-H and 5-H), 7.54 (2H, d, J 8.6 Hz, 2-H and 6-H); δ_C (101 MHz, CDCl₃) 92.2 (C), 122.4 (C), 133.6 (2 × CH), 139.2 (2 × CH); m/z (EI) 282 (M⁺, 48%), 155 (32), 84 (100), 49 (48).

4-Iodoanisole⁷

![4-Iodoanisole](image)

The reaction was carried out according to the previously described general procedure for 4-iodonitrobenzene using 4-methoxyaniline (0.050 g, 0.40 mmol), p-toluenesulfonic acid monohydrate (0.23 g, 1.2 mmol), polymer-supported nitrite (0.34 g, containing 1.2 mmol of NO₂) and sodium iodide (0.12 g, 0.80 mmol). The crude material was purified using flash column chromatography eluting with 5% ethyl acetate in petroleum ether (40–60) to give 4-iodoanisole as a white solid (0.070 g, 71%). Mp 47–48 °C (lit.⁷ 49–50 °C); δ_H (400 MHz, CDCl₃) 3.78 (1H, s, OCH₃), 6.68 (2H, d, J 9.0 Hz, 2-H and 6-H), 7.56 (2H, d, J 9.0 Hz, 3-H and 5-H); δ_C (101 MHz, CDCl₃) 55.5 (CH₃), 82.9 (C), 116.5 (2 × CH), 138.4 (2 × CH), 159.6 (C); m/z (EI) 234 (M⁺, 100%), 219 (22), 191 (11), 92 (14).

3,4,5-Trimethoxyiodobenzene⁸

![3,4,5-Trimethoxyiodobenzene](image)

The reaction was carried out according to the previously described general procedure for 4-iodonitrobenzene using 3,4,5-trimethoxyaniline (0.050 g, 0.29 mmol), p-toluenesulfonic acid monohydrate (0.16 g, 0.82 mmol), polymer-supported nitrite (0.23 g, containing 0.82 mmol of NO₂) and sodium iodide (0.080 g, 0.55 mmol). The crude material was purified using flash column chromatography eluting with 20%
diethyl ether in petroleum ether (40–60) to give 3,4,5-trimethoxyiodobenzene as a yellow solid (0.060 g, 73%). Mp 84–86 °C (lit.8 83–85 °C); δ_H (400 MHz, CDCl_3) 3.80 (3H, s, OCH_3), 3.82 (6H, s, 2 × OCH_3), 6.87 (2H, s, 2 × ArH); δ_C (101 MHz, CDCl_3) 56.5 (2 × CH_3), 61.0 (CH_3), 86.2 (C), 115.1 (2 × CH), 138.4 (C), 154.1 (2 × C); m/z (EI) 294 (M^+, 100%), 279 (58), 251 (19), 236 (18), 124 (21), 109 (12), 84 (70).

2-Iodobenzophenone^9

\[
\begin{align*}
\text{COPh} & \quad \text{I} \\
& \quad \text{Ar} \\
\end{align*}
\]

The reaction was carried out according to the previously described general procedure for 4-iodonitrobenzene using 2-aminobenzophenone (0.050 g, 0.25 mmol), p-toluenesulfonic acid monohydrate (0.14 g, 0.76 mmol), polymer-supported nitrite (0.22 g, containing 0.76 mmol of NO_2) and sodium iodide (0.080 g, 0.51 mmol). The crude material was purified using flash column chromatography eluting with 5% diethyl ether in petroleum ether (40–60) to give 2-iodobenzophenone as an orange solid (0.060 g, 79%). Mp 30–31 °C (lit.9 31–32 °C); δ_H (400 MHz, CDCl_3) 7.15–7.21 (1H, m, ArH), 7.30 (1H, dd, J 7.6, 1.5 Hz, ArH), 7.42–7.49 (3H, m, 3 × ArH), 7.58–7.63 (1H, m, ArH), 7.79–7.83 (2H, m, 2 × ArH), 7.93 (1H, dd, J 8.0, 0.8 Hz, ArH); δ_C (101 MHz, CDCl_3) 92.3 (C), 127.9 (CH), 128.6 (CH), 128.8 (CH), 130.6 (2 × CH), 131.2 (2 × CH), 133.8 (CH), 135.7 (C), 139.8 (CH), 144.5 (C), 197.3 (C); m/z (ESI) 331 (MNa^+, 100%).

2-Iodo-4-methylanisole^10

\[
\begin{align*}
\text{OMe} & \quad \text{I} \\
& \quad \text{Ar} \\
\end{align*}
\]

The reaction was carried out according to the previously described general procedure for 4-iodonitrobenzene using 2-methoxy-5-methylaniline (0.050 g, 0.36
mmol), p-toluenesulfonic acid monohydrate (0.21 g, 1.1 mmol), polymer-supported nitrite (0.31 g, containing 1.1 mmol of NO₂) and sodium iodide (0.11 g, 0.73 mmol). The crude material was purified using flash column chromatography eluting with 20% ethyl acetate in petroleum ether (40–60) to give 2-iodo-4-methylanisole as a white solid (0.070 g, 82%). Mp 28–29 °C (lit. 10 30 °C); δ_H (500 MHz, CDCl₃) 2.26 (3H, s, 4-CH₃), 3.85 (3H, s, OCH₃), 6.72 (1H, d, J 8.3 Hz, 6-H), 7.10 (1H, dd, J 8.3, 1.6 Hz, 5-H), 7.60 (1H, d, J 1.6 Hz, 3-H); δ_C (126 MHz, CDCl₃) 20.1 (CH₃), 56.6 (CH₃), 85.9 (C), 110.9 (CH), 130.1 (CH), 132.2 (C), 140.0 (CH), 156.2 (C); m/z (EI) 248 (M⁺. 100%), 233 (28), 121 (11), 84 (28), 78 (17).

### 4,5-Dimethoxy-2-iodobenzonitrile

![Structure of 4,5-Dimethoxy-2-iodobenzonitrile](image)

The reaction was carried out according to the previously described general procedure for 4-iodonitrobenzene using 2-amino-4,5-dimethoxybenzonitrile (0.050 g, 0.28 mmol), p-toluenesulfonic acid monohydrate (0.16 g, 0.84 mmol), polymer-supported nitrite (0.24 g, containing 0.84 mmol of NO₂) and sodium iodide (0.080 g, 0.56 mmol). Purification by flash column chromatography eluting with 20% ethyl acetate in petroleum ether (40–60) gave 4,5-dimethoxy-2-iodobenzonitrile as an orange solid (0.070 g, 86%). Mp 98–99 °C (lit. 7 103–104 °C); δ_H (400 MHz, CDCl₃) 3.87 (3H, s, OCH₃), 3.91 (3H, s, OCH₃), 7.02 (1H, s, 3-H), 7.24 (1H, s, 6-H); δ_C (101 MHz, CDCl₃) 56.4 (CH₃), 56.6 (CH₃), 88.8 (C), 112.3 (C), 115.9 (CH), 119.9 (C), 121.7 (CH), 149.5 (C), 153.0 (C); m/z (ESI) 312 (MNa⁺. 100%).
Ethyl 7-amino-5,6-dihydro-5-methyl-6-oxo-4H-benzo[f]imidazo[1,5-a][1,4]diazepine-3-carboxylate

Ethyl 7-nitro-5,6-dihydro-5-methyl-6-oxo-4H-imidazo[1,5-a][1,4]benzodiazepine-3-carboxylate (0.030 g, 0.090 mmol) was dissolved in ethanol (1 mL) before addition of tin(II) chloride dihydrate (0.10 g, 0.45 mmol). The reaction mixture was stirred under reflux for 16 h. After cooling to room temperature, the reaction was quenched by addition of a saturated aqueous solution of sodium hydrogen carbonate and the product was extracted with ethyl acetate (3 × 5 mL). The organic layers were dried (MgSO₄), filtered and concentrated in vacuo. The crude material was purified using flash column chromatography eluting with 1% methanol in dichloromethane to give ethyl 7-amino-5,6-dihydro-5-methyl-6-oxo-4H-benzo[f]imidazo[1,5-a][1,4]diazepine-3-carboxylate as a white solid (0.019 g, 71%). Mp 218–220 °C; νmax/cm⁻¹ (neat) 3460 (NH), 3313 (NH), 2926 (CH), 1696 (C=O), 1646 (C=O), 1614, 1582, 1493, 1377, 1317, 1284, 1222, 1195, 1142, 1096, 942, 800, 761, 704; δH (400 MHz, CDCl₃) 1.43 (3H, t, J 7.1 Hz, CO₂CH₂CH₃), 3.20 (3H, s, NCH₃), 4.34–4.50 (3H, m, CO₂CH₂CH₃ and 4-HH), 5.07 (1H, d, J 15.5 Hz, 4-HH), 5.29 (2H, br s, NH₂), 6.65 (1H, dd, J 8.0, 1.0 Hz, 8-H), 6.76 (1H, dd, J 8.0, 1.0 Hz, 10-H), 7.27 (1H, t, J 8.0 Hz, 9-H), 7.81 (1H, s, 1-H); δC (126 MHz, CDCl₃) 14.5 (CH₃), 35.3 (CH₃), 42.4 (CH₂), 61.0 (CH₂), 111.4 (CH), 112.2 (C), 116.6 (CH), 128.4 (C), 132.2 (CH), 133.7 (C), 135.5 (CH), 136.1 (C), 150.0 (C), 163.3 (C), 166.8 (C); m/z (ESI) 323.1101 (MNa⁺. C₁₅H₁₆N₄NaO₃ requires 323.1115).
Ethyl 7-iodo-5,6-dihydro-5-methyl-6-oxo-4H-benzo[f]imidazo[1,5-a][1,4]diazepine-3-carboxylate (Iomazenil)

The reaction was carried out according to the previously described general procedure for 4-iodonitrobenzene using ethyl 7-amino-5,6-dihydro-5-methyl-6-oxo-4H-benzo[f]imidazo[1,5-a][1,4]diazepine-3-carboxylate (0.020 g, 0.070 mmol), p-toluenesulfonic acid monohydrate (0.040 g, 0.21 mmol), polymer-supported nitrite (0.060 g, containing 0.21 mmol of NO₂) and sodium iodide (0.020 g, 0.14 mmol). The reaction mixture was heated to 60 °C and stirred for 16 h. The crude material was purified using flash column chromatography eluting with 5% methanol in dichloromethane to give ethyl 7-iodo-5,6-dihydro-5-methyl-6-oxo-4H-benzo[f]imidazo[1,5-a][1,4]diazepine-3-carboxylate as a white solid (0.020 g, 72%).

Mp 232–234 °C; νmax/cm⁻¹ (neat) 3091, 2924 (CH), 1694 (C=O), 1647(C=O), 1577, 1486, 1390, 1372, 1348, 1303, 1281, 1240, 1198, 1070, 1050, 938, 799, 772, 705, 664; δH (400 MHz, CDCl₃) 1.44 (3H, t, J 7.1 Hz, CO₂CH₂CH₃), 3.20 (3H, s, NCH₃), 4.33–4.51 (3H, m, CO₂CH₂CH₃ and 4-HH), 5.14 (1H, d, J 15.8 Hz, 4-HH), 7.25 (1H, t, J 8.0 Hz, 9-H), 7.37 (1H, dd, J 8.0, 0.7 Hz, 10-H), 7.89 (1H, s, 1-H), 8.06 (1H, dd, J 8.0, 0.7 Hz, 8-H); δC (126 MHz, CDCl₃) 14.5 (CH₃), 35.1 (CH₃), 42.7 (CH₂), 61.2 (CH₂), 97.4 (C), 122.4 (CH), 129.4 (C), 132.4 (CH), 132.5 (C), 134.0 (C), 135.1 (CH), 136.0 (C), 141.2 (CH), 163.0 (C), 166.1 (C); m/z (ESI) 433.9963 (MNa⁺. C₁₅H₁₄IN₃NaO₃ requires 433.9972).

N-(1-Naphthyl)-N'-[3-nitrophenyl]-N'-methylguanidine

1-Naphthylamine (0.13 g, 0.75 mmol) was dissolved in diethyl ether (1 mL) and converted to its hydrochloride salt by dropwise addition of aqueous hydrochloric acid
(1 M) in diethyl ether (1 mL) to give a pink precipitate. This was filtered and dried. This was added to N-methyl-N-(3-nitrophenyl)cyanamide (0.13 g, 0.75 mmol) under argon and the reaction mixture was stirred at 160 °C neat for 3 h. After cooling to room temperature, the reaction mixture was diluted with dichloromethane (10 mL) and washed with 0.1 M NaOH (10 mL). The aqueous layer was extracted with dichloromethane (3 × 10 mL). The combined organic layers were dried (MgSO$_4$), filtered and concentrated in vacuo. The crude material was purified using flash column chromatography eluting with 1% methanol and 1% triethylamine in dichloromethane to give N-(1-naphthyl)-N'- (3-nitrophenyl)-N'-methylguanidine as a viscous oil (0.20 g, 84%). $\nu_{\text{max}}$/cm$^{-1}$ (neat) 3485 (NH), 3386 (NH), 2924 (CH), 1631, 1567, 1522 (NO), 1482, 1379, 1345 (NO), 1234, 960, 854, 783, 771, 732, 684; $\delta_H$ (400 MHz, CDCl$_3$) 3.60 (3H, s, NCH$_3$), 7.02 (1H, dd, $J$ 7.2, 1.0 Hz, 2'-H), 7.39–7.58 (5H, m, 5 × ArH), 7.72 (1H, ddd, $J$ 8.1, 2.1, 1.0 Hz, 6-H), 7.79–7.86 (1H, m, ArH), 8.03 (1H, ddd, $J$ 8.1, 2.1, 1.0 Hz, 4-H), 8.07–8.13 (1H, m, ArH), 8.23 (1H, t, $J$ 2.1 Hz, 2-H); $\delta_C$ (101 MHz, CDCl$_3$) 39.0 (CH$_3$), 117.5 (CH), 120.1 (CH), 120.6 (CH), 122.8 (CH), 124.0 (CH), 125.4 (CH), 126.2 (CH), 126.5 (CH), 128.1 (CH), 128.6 (C), 130.2 (CH), 131.9 (CH), 134.9 (C), 145.9 (C), 146.5 (C), 149.1 (C), 150.1 (C); m/z (ESI) 321.1333 (MH$^+$). $C_{18}H_{17}N_4O_2$ requires 321.1346.

**N-(1-Naphthyl)-N’-(3-aminophenyl)-N’-methylguanidine**

![Structure](image)

The reaction was carried out according to the previously described procedure for ethyl 7-amino-5,6-dihydro-5-methyl-6-oxo-4H-benzo[f]imidazo[1,5-a][1,4]diazepine-3-carboxylate using N-(1-naphthyl)-N’-(3-nitrophenyl)-N’-methylguanidine (0.23 g, 0.73 mmol) and tin(II) chloride dihydrate (0.82 g, 3.6 mmol) in ethanol (10 mL). The reaction mixture was stirred under reflux for 16 h to give N-(1-naphthyl)-N’-(3-aminophenyl)-N’-methylguanidine as a brown solid (0.12 g, 95%). Mp 96–98 °C; $\nu_{\text{max}}$/cm$^{-1}$ (neat) 3322 (NH), 3200 (NH), 3051, 1618, 1599, 1580, 1560, 1491, 1383, 1275, 1227, 970, 781, 750; $\delta_H$ (400 MHz, CDCl$_3$) 3.50 (3H, s, NCH$_3$), 6.53 (1H, ddd,
J 7.9, 2.2, 0.9 Hz, 4-H), 6.63 (1H, t, J 2.2 Hz, 2-H), 6.70 (1H, ddd, J 7.9, 2.2, 0.9 Hz, 6-H), 7.05 (1H, dd, J 7.4, 1.1 Hz, 2'-H), 7.15 (1H, t, J 7.9 Hz, 5-H), 7.40 (1H, dd, J 8.4, 7.4 Hz, 3'-H), 7.43–7.49 (2H, m, 2 × ArH), 7.52 (1H, br d, J 8.4 Hz, 4'-H), 7.78–7.84 (1H, m, ArH); δC (101 MHz, CDCl₃) 39.0 (CH₃), 113.4 (CH), 113.5 (CH), 116.7 (CH), 118.4 (CH), 122.5 (CH), 124.1 (CH), 125.2 (CH), 126.0 (CH), 126.4 (CH), 128.0 (CH), 129.2 (C), 130.6 (CH), 134.9 (C), 145.5 (C), 145.7 (C), 148.0 (C), 151.2 (C); m/z (EI) 290.1528 (M⁺. C₁₈H₁₈N₄ requires 290.1531), 247 (11%), 234 (10), 168 (22), 122 (56), 93 (8).

N-(1-Naphthyl)-N'-{(3-iodophenyl)-N'-methylguanidine (CNS1261)

The reaction was carried out according to the previously described general procedure for 4-iodonitrobenzene using N-(1-naphthyl)-N'-{(3-aminophenyl)-N'-methylguanidine (0.061 g, 0.21 mmol), p-toluenesulfonic acid monohydrate (0.12 g, 0.62 mmol), polymer-supported nitrite (0.18 g, containing 0.62 mmol of NO₂) and sodium iodide (0.062 g, 0.41 mmol). The reaction mixture was stirred at room temperature for 16 h. Purification by flash column chromatography eluting with 60–100% ethyl acetate in petroleum ether gave N-(1-naphthyl)-N'-{(3-iodophenyl)-N'-methylguanidine as a viscous oil (0.049 g, 58%). νmax/cm⁻¹ (neat) 3482 (NH), 3385 (NH), 3053, 2924 (CH), 1624, 1557, 1474, 1376, 1266, 1233, 943, 782, 693; δH (400 MHz, CDCl₃) 3.53 (3H, s, NCH₃), 7.03 (1H, dd, J 7.2, 1.1 Hz, 2'-H), 7.13 (1H, t, J 8.0 Hz, 5-H), 7.35 (1H, ddd, J 8.0, 2.0, 0.9 Hz, 6-H), 7.41 (1H, dd, J 8.1, 7.2, Hz, 3'-H), 7.44–7.49 (2H, m, 2 × ArH), 7.52 (1H, br d, J 8.1 Hz, 4'-H), 7.58 (1H, ddd, J 8.0, 2.0, 0.9 Hz, 4-H), 7.73 (1H, t, J 2.0 Hz, 2-H), 7.79–7.85 (1H, m, ArH), 8.09–8.16 (1H, m, ArH); δC (101 MHz, CDCl₃) 39.2 (CH₃), 94.6 (C), 117.9 (CH), 122.6 (CH), 124.1 (CH), 125.3 (CH), 126.1 (CH), 126.2 (CH), 126.5 (CH), 128.1 (CH), 129.0 (C), 131.1 (CH), 134.9 (C), 135.5 (CH), 135.9 (CH), 145.9 (C), 146.2 (C), 150.5 (C); m/z (ESI) 402.0445 (MH⁺. C₁₈H₁₇N₃ requires 402.0462).
2-(4′-Dimethylaminophenyl)-6-nitrobenzoxazole

4-(Dimethylamino)benzoic acid (0.530 g, 3.25 mmol), 5-nitro-2-aminophenol (0.500 g, 3.25 mmol) and boric acid (0.200 g, 3.25 mmol) in p-xylene (50 mL) were stirred under Dean-Stark conditions for 72 h. After cooling to room temperature, the reaction mixture was filtered and washed with xylene before concentrating in vacuo. Water was added to the residue (20 mL) and the mixture made basic with 1 M sodium hydroxide solution. The aqueous layer was extracted with dichloromethane (3 × 20 mL) and the organic layers combined, dried (MgSO₄), filtered and concentrated in vacuo. The crude material was purified using flash column chromatography eluting with 40% ethyl acetate in petroleum ether (40–60) to give 2-(4′-dimethylaminophenyl)-6-nitrobenzoxazole as an orange solid (0.485 g, 54%). Mp 215–217 °C; νmax/cm⁻¹ (neat) 3422, 3315, 2924 (CH), 1615 (C=C), 1510, 1491, 1357, 822; δH (500 MHz, CDCl₃) 3.10 (6H, s, 2 × NCH₃), 6.75 (2H, d, J 9.1 Hz, 3′-H and 5′-H), 7.69 (1H, d, J 8.7 Hz, 4-H), 8.10 (2H, d, J 9.1 Hz, 2′-H and 6′-H), 8.26 (1H, dd, J 8.7, 2.1 Hz, 5-H), 8.37 (1H, d, J 2.1 Hz, 7-H); δC (126 MHz, CDCl₃) 40.2 (2 × CH₃), 106.7 (CH), 111.7 (2 × CH), 112.5 (C), 118.5 (CH), 120.9 (CH), 130.1 (2 × CH), 144.2 (C), 148.6 (C), 149.9 (C), 153.3 (C), 168.9 (C); m/z (EI) 283.0956 (M⁺. C₁₅H₁₃N₃O₃ requires 283.0957), 253 (31%), 209 (48), 145 (11).

2-(4′-Dimethylaminophenyl)-6-aminobenzoxazole

The reaction was carried out according to the previously described procedure for ethyl 7-amino-5,6-dihydro-5-methyl-6-oxo-4H-benzo[f]imidazo[1,5-a][1,4]diazepine-3-carboxylate using 2-(4′-dimethylaminophenyl)-6-nitrobenzoxazole (0.12 g, 0.42 mmol) and tin(II) chloride dihydrate (0.48 g, 2.1 mmol) in ethanol (10 mL). The reaction mixture was stirred under reflux for 16 h. The crude material was purified using flash column chromatography eluting with 1–5% methanol in dichloromethane to give 2-(4′-dimethylaminophenyl)-6-aminobenzoxazole as an orange solid (0.089 g, 83%). Mp 186–187 °C; νmax/cm⁻¹ (neat) 3430 (NH), 3314 (NH), 2916 (CH), 1613
(C=C), 1508, 1489, 1356, 1126, 949, 820; δH (500 MHz, CDCl₃) 3.05 (6H, s, 2 × NCH₃), 6.65 (1H, dd, J 8.4, 2.1 Hz, 5-H), 6.75 (2H, d, J 9.0 Hz, 3'-H and 5'-H), 6.84 (1H, d, J 2.1 Hz, 7-H), 7.45 (1H, d, J 8.4 Hz, 4-H), 8.03 (2H, d, J 9.0 Hz, 2'-H and 6'-H); δC (126 MHz, CDCl₃) 40.3 (2 × CH₃), 96.8 (CH), 111.8 (2 × CH), 112.8 (CH), 115.0 (C), 119.4 (CH), 128.6 (2 × CH), 135.3 (C), 144.1 (C), 151.9 (C), 152.1 (C), 162.6 (C); m/z (Cl) 254.1289 (MH⁺. C₁₅H₁₅N₃O requires 254.1293), 165 (3%), 113 (4), 69 (12).

2-(4'-Dimethylaminophenyl)-6-iodobenzoxazole (IBOX, 5e)¹¹

The reaction was carried out according to the previously described general procedure for 4-iodonitrobenzene using 2-(4'-dimethylaminophenyl)-6-aminobenzoxazole (0.050 g, 0.20 mmol), p-toluenesulfonic acid monohydrate (0.11 g, 0.60 mmol), polymer-supported nitrite (0.17 g, containing 0.60 mmol of NO₂) and sodium iodide (0.060 g, 0.40 mmol). The reaction mixture was heated to 60 °C and stirred for 16 h. Purification by flash column chromatography eluting with 20% ethyl acetate in petroleum ether (40–60) gave 2-(4'-dimethylaminophenyl)-6-iodobenzoxazole as a yellow solid (0.040 g, 59%). Mp 183−184 °C; δH (400 MHz, CDCl₃) 3.08 (6H, s, 2 × NCH₃), 6.77 (2H, d, J 9.0 Hz, 3'-H and 5'-H), 7.43 (1H, d, J 8.4 Hz, 4-H), 7.60 (1H, dd, J 8.4, 1.4 Hz, 5-H), 7.86 (1H, d, J 1.4 Hz, 7-H), 8.08 (2H, d, J 9.0 Hz, 2'-H and 6'-H); δC (101 MHz, CDCl₃) 40.3 (2 × CH₃), 86.5 (C), 111.9 (2 × CH), 113.8 (C), 119.5 (CH), 120.7 (CH), 129.5 (2 × CH), 133.5 (CH), 142.7 (C), 151.5 (C), 152.7 (C), 164.6 (C); m/z (ESI) 387 (MNa⁺. 100%). Spectroscopic data was in agreement with previously published data.¹¹
4-(2'-Nitrophenyl)-N,N-diethylquinoline-2-carboxamide

Ethyl 4-(2'-nitrophenyl)quinoline-2-carboxylate (0.380 g, 1.18 mmol) was dissolved in 50% aqueous ethanol (20 mL) before addition of ground sodium hydroxide (0.190 g, 4.72 mmol). The mixture was stirred under reflux for 16 h. After cooling to room temperature, the ethanol was removed \textit{in vacuo} and the water layer acidified (pH 4) with 1 M hydrochloric acid solution. The product was extracted with dichloromethane (3 × 50 mL), washed with water (2 × 50 mL), dried (MgSO$_4$), filtered and concentrated \textit{in vacuo} to give 4-(2'-nitrophenyl)quinoline-2-carboxylic acid as a brown solid (0.320 g, 92%), which was used without further purification. 4-(2'-Nitrophenyl)quinoline-2-carboxylic acid (0.090 g, 0.30 mmol) was dissolved in N,N'-dimethylformamide (10 mL), before addition of triethylamine (61 µL, 0.44 mmol) and N,N,N',N'-tetramethyl-O-(1H-benzotriazol-1-yl)uronium hexafluorophosphate (0.12 g, 0.32 mmol). The mixture was stirred at room temperature for 1 h and then heated to 50 °C, with the addition of diethylamine (30 µL, 0.44 mmol). The reaction mixture was stirred at 50 °C for 16 h. Water was added and the mixture stirred for an additional 1 h. After cooling to room temperature, the reaction mixture was diluted in ethyl acetate (20 mL) and washed with a 5% aqueous solution of lithium chloride (3 × 20 mL), followed by brine (20 mL). The organic layer was dried (MgSO$_4$), filtered and concentrated \textit{in vacuo}. The crude material was purified using flash column chromatography eluting with 60% ethyl acetate in petroleum ether (40–60) to give 4-(2'-nitrophenyl)-N,N-diethylquinoline-2-carboxamide as a colourless oil (0.055 g, 54%). $\nu_{\text{max}}$/cm$^{-1}$ (neat) 2974 (CH), 1630 (C=O), 1528, 1346, 1275, 1098, 766; $\delta$$_{4}$ (400 MHz, CDCl$_3$) 1.27 (3H, t, $J$ 7.2 Hz, NCH$_3$C$^n$H$_3$), 1.32 (3H, t, $J$ 7.2 Hz, NCH$_2$CH$_3$), 3.40–3.72 (4H, m, 2 × NCH$_2$CH$_3$), 7.42 (1H, dd, $J$ 8.4, 1.2 Hz, ArH), 7.47–7.53 (2H, m, 2 × ArH), 7.55 (1H, s, 3-H), 7.66–7.80 (3H, m, 3 × ArH), 8.16–8.24 (2H, m, 2 × ArH); $\delta$$_{c}$ (101 MHz, CDCl$_3$) 13.1 (CH$_3$), 14.6 (CH$_3$), 40.7 (CH$_2$), 43.7 (CH$_2$), 119.9 (CH), 124.6 (CH), 124.9 (CH), 126.4 (C), 128.1 (CH), 129.9 (CH), 130.2 (CH), 130.5
(CH), 132.5 (CH), 132.9 (C), 133.5 (CH), 145.9 (C), 146.9 (C), 148.8 (C), 154.4 (C), 168.5 (C); m/z (ESI) 372.1303 (MNa+). C20H19N3O3Na requires 372.1319.

4-(2'-Aminophenyl)-N,N-diethylquinoline-2-carboxamide

![Chemical Structure]

4-(2'-Nitrophenyl)-N,N-diethylquinoline-2-carboxamide (0.050 g, 0.15 mmol) was dissolved in ethanol (2 mL) before addition of tin(II) chloride dihydrate (0.18 g, 0.77 mmol). The solution was stirred under reflux for 16 h. After cooling to room temperature, the reaction was quenched by addition of a saturated aqueous solution of sodium hydrogen carbonate and the product was extracted with ethyl acetate (3 x 5 mL). The organic layers were dried (MgSO4), filtered and concentrated in vacuo. The crude material was purified using flash column chromatography eluting with 1% methanol in dichloromethane to give 4-(2'-aminophenyl)-N,N-diethylquinoline-2-carboxyamide as a colourless oil (0.045 g, 91%). \( \nu_{\text{max}}/\text{cm}^{-1} \) (neat) 3348 (NH), 2973 (CH), 1620 (C=O), 1486, 1451, 1275, 1099, 750; \( \delta_H \) (500 MHz, CDCl3) 1.27 (3H, t, J 7.2 Hz, NCH2CH3), 1.32 (3H, t, J 7.2 Hz, NCH2CH3), 3.42–3.67 (6H, m, 2 x NCH2CH3 and NH2), 6.83 (1H, dd, J 8.2, 1.2 Hz, ArH), 6.88 (1H, td, J 8.2, 1.2 Hz, ArH), 7.14 (1H, dd, J 8.2, 1.2 Hz, ArH), 7.28 (1H, td, J 8.2, 1.2 Hz, ArH), 7.53 (1H, ddd, J 8.2, 7.2, 1.2 Hz, ArH), 7.64 (1H, s, 3-H), 7.71–7.78 (2H, m, 2 x ArH), 8.17 (1H, d, J 8.2 Hz, ArH); \( \delta_C \) (126 MHz, CDCl3) 13.1 (CH3), 14.6 (CH3), 40.5 (CH2), 43.6 (CH2), 115.9 (CH), 118.6 (CH), 121.4 (CH), 122.7 (C), 126.2 (CH), 126.7 (C), 127.6 (CH), 129.9 (CH), 130.2 (CH), 130.4 (CH), 143.9 (C), 147.3 (C), 147.4 (C), 155.0 (C), 168.8 (C); m/z (ESI) 320.1743 (MH+). C20H22N3O requires 320.1757.
4-(2-Iodophenyl)-N,N-diethylquinoline-2-carboxamide

![Chemical Structure](image)

The reaction was carried out according to the previously described general procedure for 4-iodonitrobenzene using 4-(2-aminophenyl)-N,N-diethylquinoline-2-carboxamide (0.040 g, 0.12 mmol), p-toluenesulfonic acid monohydrate (0.070 g, 0.38 mmol), polymer-supported nitrite (0.11 g, containing 0.38 mmol of NO₂) and sodium iodide (0.038 g, 0.25 mmol). The reaction mixture was heated to 60 °C and stirred for 16 h. The crude material was purified using flash column chromatography eluting with 40% ethyl acetate in petroleum ether (40–60) to give 4-(2-iodophenyl)-N,N-diethylquinoline-2-carboxamide as a pale yellow solid (0.037 g, 67%). Mp 58–59 °C (lit.² 64–66 °C); δ_H (500 MHz, CDCl₃) 1.25 (3H, t, J 7.1 Hz, NCH₂CH₃), 1.33 (3H, t, J 7.1 Hz, NCH₂CH₃), 3.38–3.60 (2H, m, NCH₂CH₃), 3.63 (2H, q, J 7.1 Hz, NCH₂CH₃), 7.18 (1H, td, J 7.8, 1.6 Hz, 4'-H), 7.33 (1H, dd, J 7.8, 1.6 Hz, 5-H), 7.44–7.55 (4H, m, 3-H, 6-H, 5'-H and 6'-H), 7.75 (1H, ddd, J 8.4, 7.8, 1.6 Hz, 7-H), 8.01 (1H, dd, J 7.8, 1.6 Hz, 3'-H), 8.19 (1H, d, J 8.4 Hz, 8-H); δ_C (126 MHz, CDCl₃) 13.1 (CH₃), 14.7 (CH₃), 40.6 (CH₂), 43.6 (CH₂), 98.5 (C), 120.8 (CH), 125.9 (CH), 126.5 (C), 127.6 (CH), 128.3 (CH), 130.1 (CH), 130.2 (CH), 130.3 (CH), 139.5 (CH), 142.6 (C), 147.2 (C), 151.3 (C), 154.5 (C), 168.7 (C); m/z (EI) 430.0543 (M⁺. C₂₀H₁₉ΙN₂O requires 430.0542), 359 (84%), 331 (91), 294 (23), 203 (100), 176 (26), 149 (26), 72 (78), 69 (31).
4-[3’-[4’’-(4’’’-Nitrobenzoyl)piperazine-1’’-carbonyl]-4’-fluorobenzyl]-2H-phthalazin-1-one

To a solution of 4-nitrobenzoic acid (92.0 mg, 0.550 mmol) in N,N'-dimethylformamide (6 mL) was added triethylamine (119 µL, 0.880 mmol), followed by O-benzotriazole-N,N,N',N'-tetramethyluroniumhexafluorophosphate (231 mg, 0.610 mmol) and the mixture was stirred at room temperature for 2 h. 4-[4’-Fluoro-3’-(piperazine-1’’-carbonyl)benzyl]-2H-phthalazin-1-one (200 mg, 0.550 mmol) was added and the mixture was stirred at room temperature for a further 48 h. Water (12 mL) was then added, followed by 1 h of stirring, after which the mixture was cooled to 0 °C. The resulting precipitate was collected by vacuum filtration, washed with water (4 × 20 mL) and dried in vacuo to yield 4-[3’-[4’’-(4’’’-nitrobenzoyl)piperazine-1’’-carbonyl]-4’-fluorobenzyl]-2H-phthalazin-1-one (239.0 mg, 84%) as an orange foam. NMR spectra showed a 60:40 mixture of rotamers. Only data for the major rotamer were recorded. \( \nu_{\text{max}}/\text{cm}^{-1} \) (neat) 3167 (NH), 3012 (CH), 2905 (CH), 1638 (CO), 1599 (C=C), 1346, 1433, 1265, 766, 735; \( \delta_{\text{H}} \) (400 MHz, \( d_6 \)-DMSO) 3.22 (2H, br s, N-CH\(_2\)), 3.38 (2H, br s, NCH\(_2\)), 3.57–3.86 (4H, m, NCH\(_2\)), 4.39 (2H, d, \( J = 18.6 \text{ Hz} \), 7'-H/H and 7''-H/H), 7.21–7.53 (3H, m, 2'-H, 5'-H and 6'-H), 7.69–8.05 (5H, m, 5-H, 6-H, 7-H, 2'''-H and 6'''-H), 8.25–8.38 (3H, m, 8-H, 3'''-H and 5'''-H), 12.65 (1H, br s, NH); \( \delta_{\text{C}} \) (101 MHz, \( d_6 \)-DMSO) 37.5 (CH\(_2\)), 41.8 (2 × CH\(_2\)), 46.6 (2 × CH\(_2\)), 116.0 (d, \( J = 18.2 \text{ Hz} \), CH), 123.5 (d, \( J = 18.0 \text{ Hz} \), C), 123.9 (2 × CH), 125.5 (CH), 126.2 (CH), 127.9 (C), 128.5 (2 × CH), 129.0 (CH), 129.1 (C), 131.7 (CH), 131.9 (d, \( J = 8.8 \text{ Hz} \), CH), 133.6 (CH), 134.9 (d, \( J = 3.4 \text{ Hz} \), C), 141.9 (C), 145.0 (C), 148.0 (C), 156.5 (d, \( J = 245.7 \text{ Hz} \), C), 159.5 (C), 164.2 (C), 167.5 (C); \( m/z \) (ESI) 538.1488 (MNa\(^+\). C\(_{27}\)H\(_{22}\)FN\(_5\)NaO\(_5\) requires 538.1497).
4-[3’-[4’’-(4’’-Aminobenzoyl)piperazine-1’’-carbonyl]-4’-fluorobenzyl]-2H-phthalazin-1-one

The reaction was carried out according to the previously described procedure for ethyl 7-amino-5,6-dihydro-5-methyl-6-oxo-4'H-benzo[7][imidazo[1,5-a][1,4]diazepine-3-carboxylate using 4-[3’-[4’’-(4’’-nitrobenzoyl)piperazine-1’’-carbonyl]-4’-fluorobenzyl]-2H-phthalazin-1-one (55.0 mg, 0.107 mmol) and tin(II) chloride dihydrate (120 mg, 0.533 mmol) in ethanol (10 mL). The suspension was stirred under reflux for 16 h after which the mixture was cooled to ambient temperature and a saturated solution of sodium hydrogen carbonate (12 mL) was added. The product was extracted into ethyl acetate (3 × 10 mL), dried (MgSO₄), filtered and concentrated in vacuo to give 4-[3’-[4’’-(4’’-aminobenzoyl)piperazine-1’’-carbonyl]-4’-fluorobenzyl]-2H-phthalazin-1-one (45.0 mg, 87%) as an orange foam. ν_max/cm⁻¹ (neat) 3347 (NH), 2924 (CH), 1605 (CO), 1424, 1256, 1177, 1003, 748, 729; δ_H (400 MHz, CDCl₃) 3.30 (2H, br s, NCH₂), 3.49–4.16 (8H, m, 3 × NCH₂, and 4’’-NH₂), 4.27 (2H, s, 7’-H₂), 6.61 (2H, d, J 8.4 Hz, 3’’’-H and 5’’’-H), 7.01 (1H, t, J 8.7 Hz, 5’-H), 7.22 (2H, d, J 8.4 Hz, 2’’’-H and 6’’’-H), 7.28–7.36 (2H, m, 2’-H and 6’-H), 7.66–7.77 (3H, m, 5-H, 6-H and 7-H), 8.42–8.48 (1H, m, 8-H), 11.61 (1H, s, NH); δ_c (101 MHz, CDCl₃) 37.5 (CH₂), 42.1 (2 × CH₂), 47.0 (2 × CH₂), 114.0 (2 × CH), 116.0 (d, J 22.0 Hz, CH), 123.6 (d, J 17.9 Hz, C), 123.9 (C), 124.9 (CH), 127.0 (CH), 128.1 (C), 129.1 (d, J 3.6 Hz, CH), 129.3 (2 × CH), 129.4 (C), 131.5 (CH), 131.6 (d, J 8.1 Hz, CH), 133.5 (CH), 134.3 (d, J 3.5 Hz, C), 145.4 (C), 148.6 (C), 156.8 (d, J 247.6 Hz, C), 160.7 (C), 165.1 (C), 171.1 (C); m/z (ESI) 508.1912 (MNa⁺. C_{27}H_{24}FN_{5}NaO_{3} requires 508.1902).
4-[3’-[4’’-(4’’’-iodobenzoyl)piperazine-1’’-carbonyl]-4’-fluorobenzyl]-2H-phthalazin-1-one³

The reaction was carried out according to the previously described general procedure for 4-iodonitrobenzene using 4-[3’-[4’’-(4’’’-aminobenzoyl)piperazine-1’’-carbonyl]-4’-fluorobenzyl]-2H-phthalazin-1-one (30.0 mg, 0.035 mmol), p-toluenesulfonic acid monohydrate (20.0 mg, 0.105 mmol), polymer-supported nitrite (30.0 mg, containing 0.105 mmol of NO₂) and sodium iodide (10.5 mg, 0.070 mmol). The reaction mixture was stirred at room temperature for 1 h. Purification by flash column chromatography, using 4% methanol in dichloromethane gave 4-[3’-[4’’-(4’’’-iodobenzoyl)piperazine-1’’-carbonyl]-4’-fluorobenzyl]-2H-phthalazin-1-one (28.0 mg, 67%) as a white foam. νmax/cm⁻¹ (neat) 3198 (NH), 2899 (CH), 1628 (CO), 1587 (C=C), 1427, 1254, 1225, 1001, 747; δH (400 MHz, CDCl₃) 3.14–4.02 (8H, m, 4 × NCH₂), 4.29 (2H, s, 7’-H₂), 7.03 (1H, t, J 7.8 Hz, 5’-H), 7.14 (2H, d, J 8.0 Hz, 3’’’-H and 5’’’-H), 7.29–7.37 (2H, m, 2’-H and 6’-H), 7.67–7.84 (5H, m, ArH), 8.42–8.51 (1H, m, 8-H), 10.96 (1H, br s, NH); δc (101 MHz, CDCl₃) 37.8 (CH₂), 42.2 (2 × CH₂), 47.3 (2 × CH₂), 96.6 (C), 116.3 (d, J 21.7 Hz, CH), 123.6 (d, J 17.7 Hz, C), 125.1 (CH), 127.3 (CH), 128.4 (C), 128.9 (2 × CH), 129.4 (d, J 3.6 Hz, CH), 129.6 (C), 131.8 (CH), 131.9 (d, J 8.0 Hz, CH), 133.8 (CH), 134.5 (C), 134.6 (d, J 3.7 Hz, C), 138.0 (2 × CH), 145.6 (C), 157.1 (d, J 247.1 Hz, C), 160.7 (C), 165.3 (C), 169.8 (C); m/z (ESI) 619.0597 (MNa⁺. C₂₇H₂₂FIN₄NaO₃ requires 619.0613).
3. Radiochemistry Methodology

General Experimental for Radioiodination of Anilines with $[^{125}\text{I}]\text{NaI}$

Reductant free $[^{125}\text{I}]\text{NaI}$ was purchased from Perkin Elmer (product number NEZ033H005MC) with a concentration of 12.95 GBq/mL and specific radioactivity of 643.8 GBq/mg in 0.1 M NaOH (pH 12–14) aqueous solution. All radiochemical yields were determined by radio-HPLC analysis of the crude product.

Analytical Radio-HPLC Method for determination of Radioiodide incorporation

Analytical HPLC was performed with a Dionex Ultimate 3000 HPLC system equipped with a Flowstar LB 513 NaI scintillation detector and a DAD-3000 UV detector using a Synergi 4 µm Hydro-RP 80 Å column (150 × 4.6 mm) with 10 mm guard cartridge, UV 254 nm and flow 1 mL/min. The mobile phase for the analysis of substrates was water:acetonitrile. Analysis of the reaction mixture (to assess radioiodide incorporation) used a gradient profile of water and acetonitrile, as shown below.

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<th>Time (mins)</th>
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Co-elution with the UV signal from the $^{127}\text{I}$-compound was used to confirm identity of the $^{125}\text{I}$-product from each reaction described.

General Method for Radiodination: 4-$[^{125}\text{I}]$iodonitrobenzene (2a)

To a solution of $p$-toluenesulfonic acid monohydrate (2.1 mg, 11.0 µmol) in acetonitrile (0.2 mL) was added 4-nitroaniline (0.50 mg, 3.6 µmol). The solution was cooled in a water bath to 10–15 °C. Polymer-supported nitrite (3.2 mg, containing 11.0 µmol of NO$_2$) was added and the reaction mixture was stirred for 0.25 h. A 4–6
MBq solution of $[^{125}\text{I}]\text{NaI}$ in water (0.01 mL) was added and the reaction mixture warmed to 20 °C and stirred for 2 h. The mixture was removed from the resin by syringe and diluted by the addition of water (0.2 mL). Analysis of this solution by analytical radio-HPLC showed a radiochemical yield of 93%.

**4-$[^{125}\text{I}]$iodobromobenzene (2b)**

![Structure of 4-$[^{125}\text{I}]$iodobromobenzene (2b)](image)

The reaction was done using 4-bromoaniline (1.0 mg, 5.8 µmol) in acetonitrile (0.2 mL) as described in the general method except for the following: six equivalents of both polymer-supported nitrite and $p$-toluenesulfonic acid monohydrate were used. Analysis by radio-HPLC gave a radiochemical yield of 73%.

**4-$[^{125}\text{I}]$idoanisole (2c)**

![Structure of 4-$[^{125}\text{I}]$idoanisole (2c)](image)

The reaction was done using 4-methoxyaniline (0.50 mg, 4.1 µmol) as described in the general method except for the following: six equivalents of both polymer-supported nitrite and $p$-toluenesulfonic acid monohydrate were used and the reaction was performed at 40 °C. Analysis by radio-HPLC gave a radiochemical yield of 97%.

**3,4,5-Trimethoxy-$[^{125}\text{I}]$iodobenzene (2d)**

![Structure of 3,4,5-Trimethoxy-$[^{125}\text{I}]$iodobenzene (2d)](image)

The reaction was done using 3,4,5-trimethoxyaniline (1.0 mg, 5.5 µmol) in acetonitrile (0.2 mL) as described in the general method except for the following: six equivalents of both polymer-supported nitrite and $p$-toluenesulfonic acid monohydrate were used. Analysis by radio-HPLC gave a radiochemical yield of 94%.
2-[^125]Iodobenzophenone (2e)

The reaction was done using 2-aminobenzophenone (0.5 mg, 2.5 µmol) in acetonitrile (0.2 mL) as described in the general method except for the following: six equivalents of both polymer-supported nitrite and p-toluenesulfonic acid monohydrate were used and the reaction required 4 h. Analysis by radio-HPLC gave a radiochemical yield of 94%.

2-[^125]Iodo-4-methylanisole (2f)

The reaction was performed using 2-methoxy-5-methylaniline (0.50 mg, 3.6 µmol) in acetonitrile (0.2 mL) as described in the general method except for the following: one equivalent of both polymer-supported nitrite and p-toluenesulfonic acid monohydrate were used. Analysis by radio-HPLC gave a radiochemical yield of 83%.

4,5-Dimethoxy-2-[^125]iodobenzonitrile (2g)

The reaction was performed using 2-amino-4,5-dimethoxybenzonitrile (1.0 mg, 5.6 µmol) in acetonitrile (0.2 mL) as described in the general method except for the following: six equivalents of both polymer-supported nitrite and p-toluenesulfonic acid monohydrate were used. Analysis by radio-HPLC gave a radiochemical yield of 61%.
4-Chloro-2-[\textsuperscript{125}I]iodonitrobenzene (2h)

![Chemical structure of 4-Chloro-2-[\textsuperscript{125}I]iodonitrobenzene]

The reaction was done using 2-nitro-5-chloroaniline (0.50 mg, 2.9 µmol) in acetonitrile (0.2 mL) as described in the general method except for the following: one equivalent of both polymer-supported nitrite and \( p \)-toluenesulfonic acid monohydrate were used. Analysis by radio-HPLC gave a radiochemical yield of 64%.

Ethyl 7-[\textsuperscript{125}I]iodo-5,6-dihydro-5-methyl-6-oxo-4\( H \)-benzo[f]imidazo[1,5-a][1,4]diazepine-3-carboxylate (2i)

![Chemical structure of Ethyl 7-[\textsuperscript{125}I]iodo-5,6-dihydro-5-methyl-6-oxo-4\( H \)-benzo[f]imidazo[1,5-a][1,4]diazepine-3-carboxylate]

The reaction was performed using ethyl 7-amino-5,6-dihydro-5-methyl-6-oxo-4\( H \)-benzo[f]imidazo[1,5-a][1,4]diazepine-3-carboxylate (0.50 mg, 1.7 µmol) as described in the general method except for the following: six equivalents of both polymer-supported nitrite and \( p \)-toluenesulfonic acid monohydrate were used and the reaction was performed at 60 °C. Analysis by radio-HPLC gave a radiochemical yield of 92%.

\( N \)-(1-Naphthyl)-N’-(3-[\textsuperscript{125}I]iodophenyl)-N’-methylguanidine (2j)

![Chemical structure of \( N \)-(1-Naphthyl)-N’-(3-[\textsuperscript{125}I]iodophenyl)-N’-methylguanidine]

The reaction was performed using \( N \)-(1-naphthyl)-N’-(3-aminophenyl)-N’-methylguanidine (1.0 mg, 3.4 µmol) as described in the general method except for the following: six equivalents of both polymer-supported nitrite and \( p \)-toluenesulfonic acid monohydrate were used and the reaction was performed at 60 °C. Analysis by radio-HPLC gave a radiochemical yield of 64%.
2-(4’-Dimethylaminophenyl)-6-[125I]iodobenzoxazole (2k)

The reaction was performed using 2-(4’-dimethylaminophenyl)-6-aminobenzoxazole (1.0 mg, 4.0 µmol) as described in the general method except for the following: six equivalents of both polymer-supported nitrite and p-toluensulfonic acid monohydrate were used. Analysis by radio-HPLC gave a radiochemical yield of 47%.

4-(2-[125I]iodophenyl)-N,N-diethylquinoline-2-carboxamide (2l)

The reaction was performed using 4-(2-aminophenyl)-N,N-diethylquinoline-2-carboxamide (0.50 mg, 1.6 µmol) as described in the general method except for the following: six equivalents of both polymer-supported nitrite and p-toluensulfonic acid monohydrate were used and the reaction was performed at 60 °C. Analysis by radio-HPLC gave a radiochemical yield of 50%.

4-{3’-[4”-(4”-[125I]iodobenzoyl)piperazine-1”-carbonyl]-4’-fluorobenzyl}-2H-phthalazin-1-one (2m)

The reaction was performed using 4-{3’-[4”-(4”-aminobenzoyl)piperazine-1”-carbonyl]-4’-fluorobenzyl}-2H-phthalazin-1-one (0.50 mg, 1.0 µmol) in acetonitrile (0.2 mL) as described in the general method except for the following: six equivalents of both polymer-supported nitrite and p-toluensulfonic acid monohydrate were used. Analysis by radio-HPLC gave a radiochemical yield of 59%.
Semi-Preparative Radio-HPLC Method for Purification of Ethyl 7-[\(^{125}\)I]iodo-5,6-dihydro-5-methyl-6-oxo-4H-benzo[f]imidazo[1,5-a][1,4]diazepine-3-carboxylate (2i): Semi-preparative HPLC was performed with a Dionex Ultimate 3000 HPLC system equipped with a Knauer Advanced Scientific Instruments Smartline UV Detector 2500 and a photomultiplier tube (PMT) connected to a Lab Logic Flow-Count radiodetector and using a Synergi 4 μm Hydro-RP 80 Å column (150 × 10 mm) with 10 mm guard cartridge, UV 254 nm and flow 3 mL/min. A gradient profile of water and acetonitrile was used, as shown below.

<table>
<thead>
<tr>
<th>Time (mins)</th>
<th>%MeCN</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–20</td>
<td>10–95</td>
</tr>
<tr>
<td>20–24</td>
<td>95</td>
</tr>
<tr>
<td>24–25</td>
<td>95–10</td>
</tr>
<tr>
<td>25–30</td>
<td>10</td>
</tr>
</tbody>
</table>

Synthesis and Purification of Ethyl 7-[\(^{125}\)I]iodo-5,6-dihydro-5-methyl-6-oxo-4H-benzo[f]imidazo[1,5-a][1,4]diazepine-3-carboxylate (2i)

To a solution of \(\text{p-toluenesulfonic acid monohydrate (2.1 mg, 11.0 \, \mu\text{mol}) in acetonitrile (0.2 mL)}\) was added ethyl 7-amino-5,6-dihydro-5-methyl-6-oxo-4H-benzo[f]imidazo[1,5-a][1,4]diazepine-3-carboxylate (0.50 mg, 1.7 \, \mu\text{mol}). The solution was cooled in a water bath to 10–15 °C. Polymer-supported nitrite (3.2 mg, containing 11.0 \, \mu\text{mol of NO}_2) was added and the reaction mixture was stirred for 0.25 h. A 4–6 MBq solution of \(\text{[}^{125}\text{I]}\text{NaI in water (0.01 mL)}\) was added and the reaction mixture warmed to 60 °C and stirred for 2 h. The mixture was removed from the resin by syringe and diluted by the addition of water (0.2 mL). The crude product was purified by semi-preparative HPLC. The fraction containing ethyl 7-[\(^{125}\)I]iodo-5,6-dihydro-5-methyl-6-oxo-4H-benzo[f]imidazo[1,5-a][1,4]diazepine-3-carboxylate (2i) was evaporated to dryness, then reconstituted in 10.0% ethanol in 0.9% saline to afford ethyl 7-[\(^{125}\)I]iodo-5,6-dihydro-5-methyl-6-oxo-4H-benzo[f]imidazo[1,5-
a][1,4]diazepine-3-carboxylate (2i) in 75 ± 9.9% radioactivity yield (estimated using the measured radioactivity of the isolated product), with molar activity of 16.2 ± 1.22 GBq µmol⁻¹ (n=2). The radiochemical purity of the final product was determined by analytical HPLC and was >99% (n=2). The identity of the product was confirmed by comparing the retention time of the ethyl 7-[¹²⁵]Iodo-5,6-dihydro-5-methyl-6-oxo-4H-benzo[f]imidazo[1,5-a][1,4]diazepine-3-carboxylate (2i) against the retention time of ethyl 7-[¹²⁷]Iodo-5,6-dihydro-5-methyl-6-oxo-4H-benzo[f]imidazo[1,5-a][1,4]diazepine-3-carboxylate.
4. Radio-HPLC Chromatograms for all Compounds
For target compounds, an overlay of the UV-Vis HPLC trace (in black) is provided along with the radio-HPLC trace (in blue):
5. References


