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Journal Name

COMMUNICATION

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

Nikki L. Sloan,^a Sajinder K. Luthra,^b Graeme McRobbie,^b Sally L. Pimlott^c and Andrew Sutherland^{*a}

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An operationally simple, one-pot, two-step tandem procedure that allows the incorporation of radioactive iodine into aryl amines via stable diazonium salts is described. The mild conditions are tolerant of various functional groups and substitution patterns, allowing late-stage, rapid access to a wide range of ^{125}I -labelled aryl compounds and SPECT radiotracers.

The combination of radionuclide labelled molecules and nuclear imaging techniques has become important for the study of dynamic biochemical processes at the molecular and cellular level.¹ The application of these technologies has also extended into many aspects of medicine and healthcare and include the diagnosis and prognosis of disease, as well as utilisation in the drug discovery process, such as understanding drug action and establishing treatment strategies.² A key technique within this field is single photon emission computed tomography (SPECT) which, in combination with radioactive tracers is used for both preclinical in vitro measurements (^{123}I / ^{125}I) and in vivo diagnostic imaging (^{123}I).³

Despite the importance of SPECT imaging, one of the limitations preventing more widespread application of this technique is the dearth of methods for incorporating radioiodine into small molecules. In general, SPECT tracers bearing an iodoarene unit are produced by a substitution reaction using an electrophilic source of radioactive iodine, prepared under oxidising conditions.^{3a} The most commonly used approach involves the conversion of aryl halides into the corresponding organostannane via palladium(0)-catalysis, followed by

an oxidative iodo-destannylation reaction (Fig. 1a).^{2,3a} While this method generates SPECT imaging agents with high specific activity and radiochemical purity,⁴ there are obvious toxicity risks associated with generation of clinical tracers from organotin compounds. In addition, the unstable nature of organotin compounds prevents long-term storage and can result in unreliable radioiodinations.⁵ Although there are significant issues associated with this approach, there have been relatively few reports of new methods for the radioiodination of aryl compounds.⁶ In 2013, we reported a nickel(0)-catalysed radioiodination of aryl and heteroaryl bromides (Fig. 1b).⁷ While fast and efficient, this method requires elevated temperatures (180 °C) for complete conversion. More recently, the groups of Gouverneur and Zhang described the copper-mediated radioiodination of aryl boronic acids and esters (Fig. 1c).^{8–11} As well as showing broad substrate scope, these methods were used for the preparation of various SPECT imaging agents.^{8,9}

We were interested in developing a new general method for the radioiodination of aryl compounds with the criteria of using widely available starting materials and mild reaction conditions. The preparation of diazonium salts from aryl amines followed by a Sandmeyer-type reaction is a common approach for the preparation of aryl iodides.¹² However, previous attempts to use this two-step transformation for radioiodination were largely abandoned.¹³ The harsh acidic conditions required for the formation of diazonium salts and their unstable, potentially explosive nature led to unwanted by-products and low radiochemical yields. Here we now report an operationally simple, one-pot radioiodination of aryl amines that overcomes these previous limitations. Formation of the diazonium salts under mild conditions, using a polymer-supported nitrite reagent and *p*-toluenesulfonic acid (*p*-TsOH) as the proton source, followed by direct radioiodination, has produced labelled products in high radiochemical yield (Fig. 1d). Application of this new strategy for the preparation of various SPECT imaging agents is also described.

^a WestCHEM, School of Chemistry, The Joseph Black Building, University of Glasgow, Glasgow G12 8QQ, UK; Email: Andrew.Sutherland@glasgow.ac.uk; Telephone: +44 (0)141 330 5936; Fax: +44 (0)141 330 4888

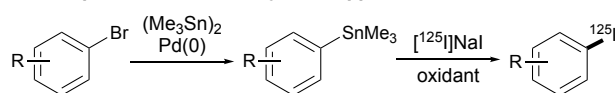
^b GE Healthcare, The Grove Centre, White Lion Road, Amersham, HP7 9LL, UK

^c West of Scotland PET Centre, Greater Glasgow and Clyde NHS Trust, Glasgow G12 0YN, UK

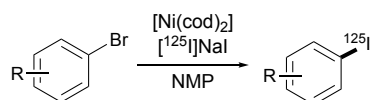
† Electronic Supplementary Information (ESI) available: Experimental procedures, spectroscopic data, radio-HPLC traces and NMR spectra for all compounds. See DOI: 10.1039/x0xx00000x



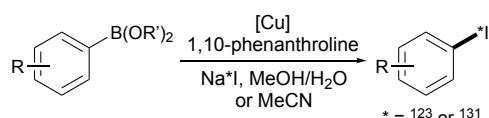
a) Two-step radioiodo-destannylation approach



b) Nickel(0)-mediated radioiodination of aryl bromides



c) Copper-mediated radioiodination of aryl boronic acids and esters



d) One-pot radioiodination of anilines- This work

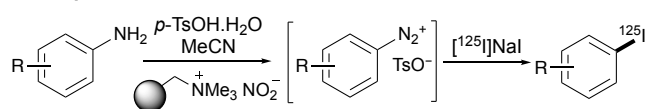


Fig. 1 Methods for radioiodination of arenes.

A number of groups have shown that the key limitations associated with the standard conditions of diazonium salt formation, such as the use of strong acids and sodium nitrite, which can lead to the release of nitrogen oxides, can be avoided using a polymer-supported nitrite reagent.¹⁴ These are simply prepared by the ion exchange of tetraalkylammonium functionalised resins, such as Amberlyst A-26, using an aqueous solution of sodium nitrite. Filimonov and co-workers have combined the use of a polymer-supported nitrite with mild acidic conditions (*p*-TsOH) and showed that the resulting tosylate diazonium salts were thermally stable and could be used for the stepwise preparation of aryl iodides under aqueous conditions.^{14c,d} These advances served as a starting point for the development of our rapid, one-pot tandem radioiodination of aryl amines.

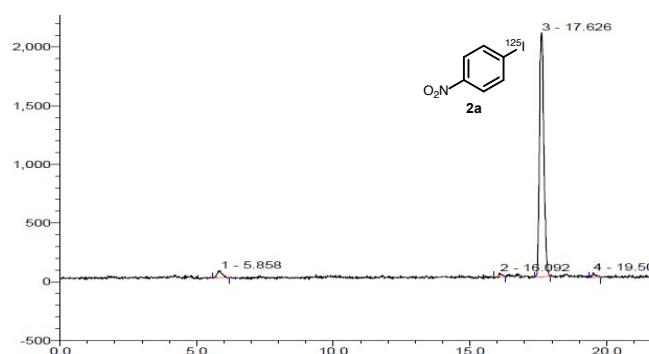
Our initial studies focused on the development of a mild, one-pot diazotisation-iodination of aryl amines that would also be amenable for radioiodination. Preliminary optimisation studies with 4-nitroaniline (**1a**) showed that when using acetonitrile as the solvent, all the reagents (polymer-supported nitrite, *p*-TsOH and sodium iodide) could be added together, resulting in fast and efficient tandem conversion to the corresponding iodide, **2a** (86% yield). These conditions were then investigated for the radioiodination of 4-nitroaniline (**1a**), where radiochemical yields (RCY) were determined by radio-HPLC analysis of the crude product.¹⁵ Using [¹²⁵I]NaI (4–6 MBq solution in water) as the limiting reagent and three equivalents of both the polymer-supported nitrite reagent and *p*-TsOH gave a low RCY (Table 1, entry 1). The concentration of **1a** in the reaction mixture was then varied and using more dilute conditions, which allowed better mixing of reagents, resulted in a dramatic increase in the RCY of

2a. In particular, a concentration of 0.018 mol/L of **1a** (0.5 mg of **1a** in 200 μL of solvent) gave an excellent RCY of 93% (entry 3). The corresponding radio-HPLC chromatogram for this reaction showed a particularly clean transformation with no other radiolabelled side-products (Fig. 2). Other variables of the one-pot tandem process were also investigated such as reaction temperature and the number of equivalents of reagents (entries 4–8). Higher temperatures led to a drop in RCY due to the formation of 4-nitrophenol as a side-product, while higher quantities of reagents again led to inefficient mixing of the small scale reaction and lower RCY. Although high RCY was achieved using one equivalent of both reagents (entry 7), the conditions used in entry 3 were deemed optimal for radioiodination.

Table 1 Optimisation of the one-pot diazotisation-radioiodination of 4-nitroaniline (**1a**).^a

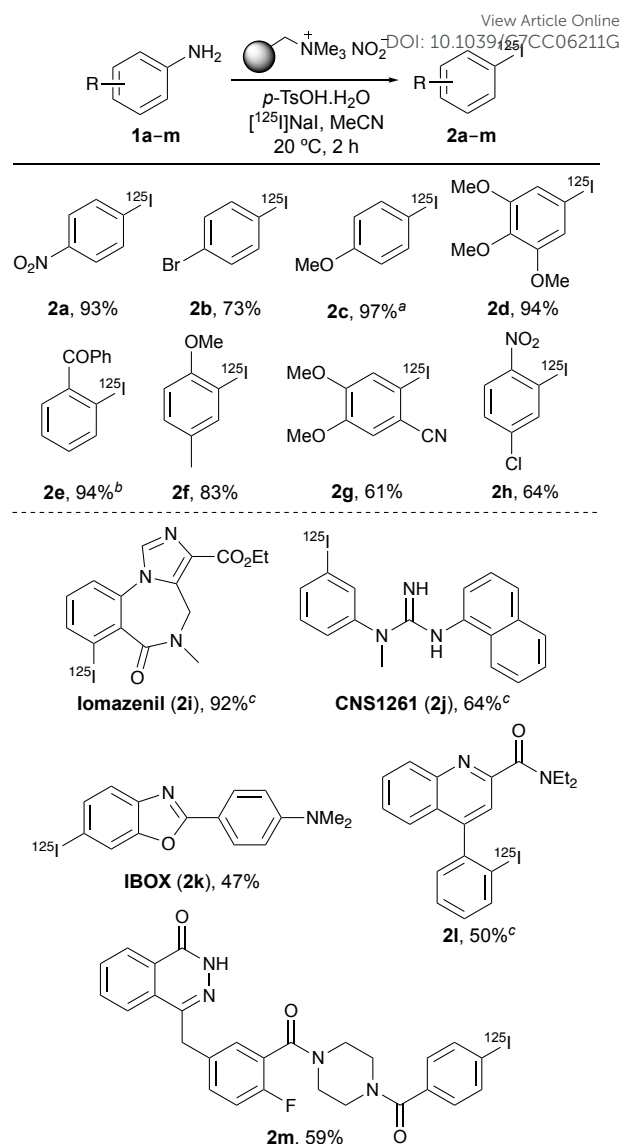
Entry	Conc. of 1a (mol/L)	Eq. of reagents ^b	Temp (°C)	RCY (%)
1	0.18	3	20	4
2	0.07	3	20	44
3	0.018	3	20	93
4	0.009	3	20	80
5	0.018	3	40	65
6	0.018	3	60	54
7	0.018	1	20	82
8	0.018	6	20	43

^a All reactions were performed using a 4–6 MBq solution of [¹²⁵I]NaI in water (0.01 mL). ^b Polymer-supported nitrite and *p*-TsOH.

Fig. 2 Chromatogram obtained by analytical radio-HPLC of the reaction mixture from the radioiodination of 4-nitroaniline (**1a**), showing a 93% yield of radioiodide incorporation.

The optimised radioiodination conditions were evaluated for the preparation of a range of ^{125}I -labelled aryl compounds (Scheme 1). Under these standard conditions, electron-deficient and electron-rich compounds (**1a–1h**) with various substitution patterns were found to be compatible and converted to the ^{125}I -iodinated compounds in excellent RCYs. Only two substrates required further optimisation. The highest RCY of 97% for 4- ^{125}I -iodoanisole (**2c**) was achieved when the one-pot tandem process was conducted at 40 °C, while a four-hour reaction gave the best RCY (94%) for 2- ^{125}I -iodobenzophenone (**2e**).

Having demonstrated the general scope of the one-pot tandem diazotisation-radioiodination process on simple arenes, the next stage of this work was to show that it could also be applied to the synthesis of more complex biologically active aryl iodides and SPECT radiotracers (**2i–2m**, Scheme 1).¹⁶ Again, using ^{125}I -NaI as the limiting reagent in dilute conditions, it was found that an increase in the amount of both the polymer-supported nitrite and *p*-TsOH (to six equivalents) was required to achieve good yields for a two hour reaction. Further optimisation showed that a higher reaction temperature (60 °C) gave improved RCYs for some of the substrates. Overall, the one-pot transformation was effective for all the targets, leading to a new radiosynthesis of the well-established imaging agents iomazenil (**2i**),¹⁷ a SPECT tracer of central-type benzodiazepine receptors in brain tissue, CNS1261 (**2j**)¹⁸ a radioligand of the *N*-methyl-D-aspartate (NMDA) receptor and IBOX (**2k**), a SPECT imaging agent of amyloid plaques.¹⁹ The best result was achieved for ^{125}I -iomazenil (**2i**), with a particularly clean reaction and a RCY of 92%. This is higher than the reported oxidative iodo-destannylation of a tri-*n*-butylstannane precursor (70%), which was complicated by the formation of non-radioactive reduced and halogenated by-products.²⁰ The synthesis of ^{125}I -iomazenil (**2i**) was also found to be highly reproducible, with a RCY of $89 \pm 8\%$ ($n = 9$). This study has also allowed the first preparation of a radioiodinated version of quinoline-2-carboxamide **2l**, a high affinity agent (K_i 5 nM) for the translocator protein (TSPO), a membrane protein that is overexpressed during chronic neurodegeneration or acute brain injury.²¹ As a radiosynthesis of this compound is now developed, the full potential of this tracer can be explored as a potential SPECT imaging agent of neuroinflammation processes associated with TSPO. The one-pot diazotisation-radioiodination was also effective for the preparation of phthalazinone **2m**, which is a nanomolar inhibitor (IC_{50} 7 nM) and SPECT imaging agent of poly(ADP-ribose) polymerase-1 (PARP-1), a therapeutic target for the treatment of cancer.²² The previous ^{125}I -labelled synthesis of **2m** was achieved by solid-state halogen exchange using a bromide precursor. Although this did give a 90% RCY, a reaction temperature of 210 °C was required.

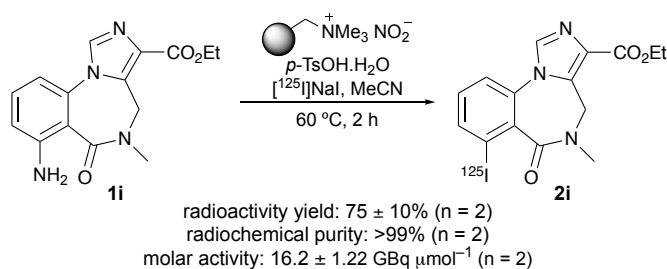


Scheme 1 Scope of one-pot diazotisation-radioiodination. ^a Reaction was performed at 40 °C. ^b Reaction required 4 h. ^c Reaction was performed at 60 °C

As well as using widely available starting materials, avoiding highly toxic reagents and harsh conditions, it should be emphasised that other advantages of this one-pot diazotisation-radioiodination process are a very simple work-up procedure and isolation protocol. Following completion of the reaction, the radioactive mixture is filtered to remove the polymer-supported nitrite, diluted and purified directly by HPLC to yield the ^{125}I -labelled compounds. These advantages were exemplified with validation of the one-pot method for the synthesis and purification of ^{125}I -iomazenil (**2i**). The amine precursor **1i** was treated with ^{125}I -NaI (4–6 MBq), polymer-supported nitrite and *p*-TsOH at 60 °C for 2 hours (Scheme 2). After HPLC purification, ^{125}I -iomazenil (**2i**) was isolated in $75 \pm 10\%$ radioactivity yield. The radiochemical purity of **2i** was measured as >99%, with a



molar activity of 16.2 ± 1.22 GBq μmol^{-1} . As with all reactions, identification of the product was confirmed using HPLC, demonstrating co-elution with a sample of unlabelled iomazenil.



Scheme 2 Radiosynthesis of [^{125}I]iomazenil (**2i**).

In summary, an operationally simple, one-pot two-step tandem process for the preparation of radioiodine containing arenes from readily available anilines has been developed. The use of mild conditions for diazonium salt formation resulted in a process with broad scope that is tolerant of a wide range of functional groups and substitution patterns. In particular, this process allowed the preparation of a range of important SPECT imaging agents, including [^{125}I]iomazenil, [^{125}I]CNS1261 and [^{125}I]IBOX, as well as the first radiosynthesis of a high affinity agent of TSPO, that can now be used to study neuroinflammation. Current studies are investigating the application of this method for the development of novel SPECT imaging agents and the use of solid-phase extraction for more simple and rapid purification.

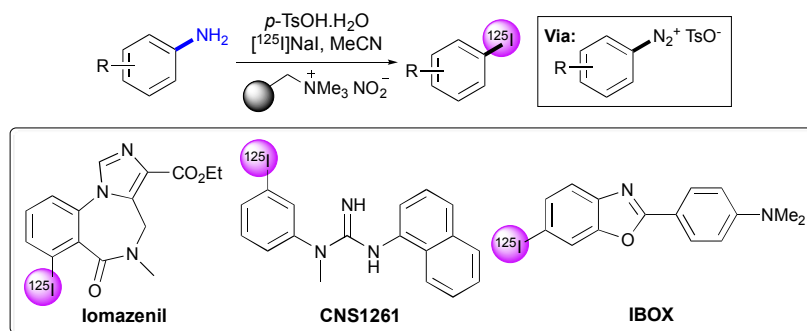
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Graphical Abstract Graphic:



Graphical Abstract Text:

A mild, general and efficient one-pot tandem process that produces radioiodinated SPECT imaging agents from readily available aryl amines via stable diazonium salts has been developed.

