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A One-Pot Radiosynthesis of [^{125}I]Iodoazido Photoaffinity Labels

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Summary

A useful method for preparing radioiodinated photoaffinity labels from alkyl anilines which offers significant advantages over present methods is described. The one-pot synthesis gives good radiochemical yields (40-64%) of pure, high specific activity (350 - 1500 mCi/μmol) ^{125}I labelled iodoaryl azides while minimising manipulation of radioactive materials. Purification of the [^{125}I]iodoazido photoaffinity labels is achieved by high performance liquid chromatography.

Key Words: photoaffinity label, ^{125}I, radioiodination, dopamine, receptors.

Introduction

Radioactive photoaffinity labels (RPLs) are very useful tools for the characterisation and isolation of large biomolecules such as receptor proteins and glycoproteins (1). Although ^{3}H has been used most often as the radionuclide in RPLs, ^{125}I labelled RPLs have enjoyed increasing popularity recently (2,3). The advantages of [^{125}I]-RPLs, which include high specific activities and short autoradiographic exposure times, have made them very valuable in attempts to isolate and characterise neurotransmitter receptors. We report here a convenient one-pot method for preparing iodoazido [^{125}I]-RPLs in high radiochemical yields

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and at high specific activities. This radiosynthetic method has been applied to the preparation of simple [^{125}I]iodoazido molecules and a series of [^{125}I]-RPLs designed as high affinity selective ligands for the dopamine transporter (4, 5).

Results and Discussion

Previous methods. A typical two-stage approach for preparing [^{125}I]-RPLs is depicted in Figure 1. A site of bulk tolerance in the ligand is first substituted with a para substituted alkylaniline **1**. The aniline ring is then radioiodinated under electrophilic substitution conditions in the position ortho to the amino group and the product **2** is isolated and purified by extraction and chromatography. The radioiodoaniline **2** is diazotized and deaminated with azide anion to give the radioiodinated arylazide **3**. Purification and isolation is again accomplished by a chromatographic procedure (2,3).

Such extensive manipulations and purifications are time consuming and potentially result in high radiation exposure to the chemist. Additionally, significant mechanical losses of radiolabelled material are, presumably, incurred during the handling, transfers, and extraction procedures* – ensuring lower isolated radiochemical yields.

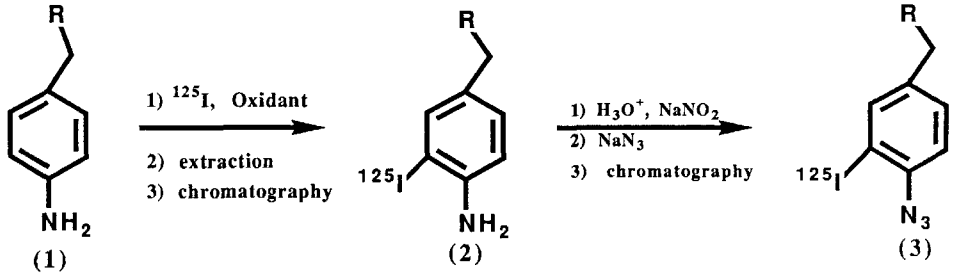


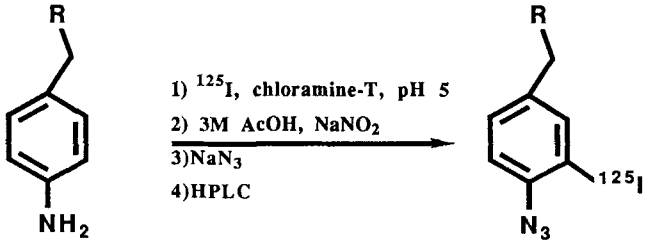
Figure 1. Common two-stage method for preparing ^{125}I labelled iodoazidophotoaffinity labels

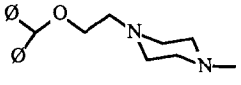
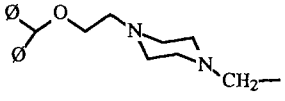
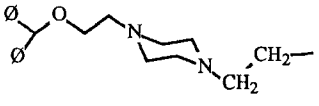
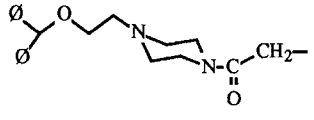
One-Pot Synthesis. As it was unclear why it was necessary to obtain pure radioiodinated aniline **2** before proceeding with the next step in the reaction sequence, reaction parameters were sought which would forego this step. A judicious choice of reaction conditions outlined in Table 1 allows conversion of the

* Although many such radiosyntheses of this type have been reported, scant information on radiochemical yields or specific activities obtained is available. For an exception see ref. # 3.

aniline **1** directly to the [^{125}I]-RPL **3** in a one-pot procedure; thus eliminating intermediate extraction, purification, and isolation of the radioiodinated aniline **2**.

Table 1. One-Pot Radiosynthesis of [^{125}I]Iodoazidophotoaffinity Labels (RPLs)



Cpd. #	R	% isolated radiochemical yield	Specific activity (mCi/μmol)
4	CH_3-	64	730
5	CH_3CO-	53	ND
6		44	495
7		45-50[a], 20-25[b,c]	400-1500[d]
8		40	610
9		48	ND

ND – not determined; a - range of seven runs; b - range of three runs; c - using the conventional method of preparation with chromatographic isolation of the intermediate radioiodoaniline **2**; d - specific activities from both methods of synthesis were in this range.

The method is applicable to simple alkyl anilines, e.g. 4-ethyl aniline (R = CH_3, **4**), as well as more complicated structures. The 1,4-substituted piperazines **6** - **9** in Table 1 are analogues of dopamine transporter ligands (4,5,7) from which the [^{125}I]-RPLs are readily prepared. A comparison of the one-pot method with the two-stage synthesis involving isolation of the intermediate radioiodinated aniline **2** was carried out with the 1,4-substituted piperazine **7** (Table 1). Isolated radiochemical yields doubled using the one-pot synthesis.

Purification of [^{125}I]-RPLs As is the case for most radioiodinated tracer preparations, stringent chromatography conditions are required to effect complete separation of the [^{125}I]-RPLs from the reaction by-products (8). The major mass peak (Figure 2, Peak A) is the non-iodinated aryl azide with a small amount of a more lipophilic product (Figure 2, Peak B) which is probably the chloroazide produced by action of the chloramine-T (9). Separation of these impurities from the product is critical, otherwise the effective specific activity of the [^{125}I]-RPL would be dramatically reduced (10). The use of the non-halogenated oxidising agent, peracetic acid (11), suppresses the formation of Peak B but also results in a substantial reduction of the radiochemical yields.

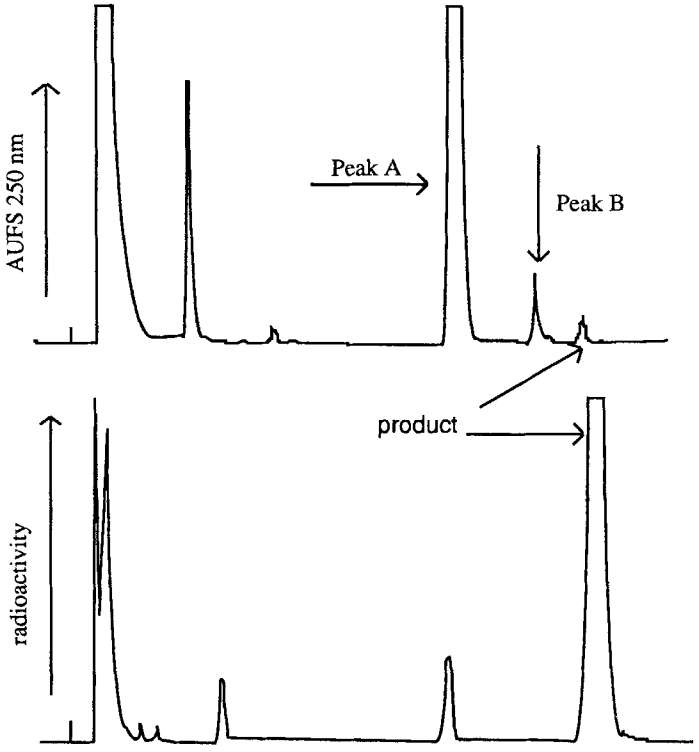


Fig. 2. HPLC chromatograms of the reaction mixture from the one-pot radiosynthesis of the iodoazido photoaffinity label derived from compound 7. The upper trace corresponds to mass (uv) detection; the lower to radioactivity.

Reaction Parameters. A pH range of 4 to 6 was found to be optimal for the radioiodination of the aniline ring. More acidic conditions gave a slower and lower yielding reaction while more radioactive impurities were observed at higher pH's,

also resulting in reduced radiochemical yields. The oxidising agent chloramine-T gave higher yields than N-chlorosuccinimide. Two μL of a 4.4 mM solution of chloramine-T were ideal as larger quantities increased the size of Peak B (Figure 2) whilst lower concentrations gave erratic results. The conditions described for the diazotization and azide deamination on the radioiodinated aniline **2** gave almost quantitative conversion (>95%) and for this reason were not manipulated further.

Conclusions

A method is described for the radiosynthesis of ^{125}I labelled RPL's which offers significant advantages over current techniques. The one-pot procedure offers improved radiochemical yields, high specific activities, short preparation times, and reduced radiation exposure. This method should be compatible with any compounds prepared by previous routes (2,3) as the reaction conditions used are similar to those in the conventional syntheses (oxidising agent, nitrous acid, azide anion, etc.) of iodoazido RPL's. The examples in Table 1 demonstrate that ether, non-activated aromatic, amino, ketone, and amide functionalities are stable to the reaction conditions employed.

Experimental

Purifications and analyses of ^{125}I-containing radioactive mixtures were performed on an HPLC system composed of a Rheodyne 7125 injector, two Waters 510 EF pumps, a UV detector (Waters 481) and an Ortec flow radioactivity detector. The HPLC columns used were either: A - Waters C18 Novapak (100 mm x 8 mm) or B - Waters C18 Novapak (150 mm x 3.9 mm). Peak areas were measured using Hewlett-Packard 3390A recording integrators. Isolated radiochemical yields were determined with a dose-calibrator (Capintec CRC-7). Sodium [^{125}I]iodide was obtained from Amersham Corporation (IMS·30 or IMS·300). Authentic samples of the "cold" iodoazides were prepared from the corresponding anilines **4 - 9** (Table 1) by standard literature procedures (4,5) and gave appropriate elemental and spectroscopic analyses.

Radioiodinations. Typically, a solution of the appropriate aniline (0.15 μmol)

in aqueous buffer (50 µL, 0.1 M AcOH, 0.22 M NaOAc, pH 5.0) was mixed with the sodium [^{125}I]iodide solution (10 µL, 1.0 mCi). This was followed by the addition of an aqueous solution of chloramine-T (2 µL, 4.4 mM) at 20° C. After 30 min, aqueous AcOH (50 µL, 3 M) was added and the solution was cooled to 0° C. An aqueous solution of $NaNO_2$ (10 µL, 0.5 M) was then added followed 15 min later by an aqueous solution of NaN_3 (10 µL, 0.5 M). The mixture was warmed to 20° C and, after 10 min, quenched with aqueous $Na_2S_2O_3$ (1 µL, sat.) and EtOH (50 µL). The total mixture was applied to HPLC column A and eluted with a mobile phase consisting of a mixture of CH_3CN/H_2O + 0.1 M NH_4HCO_2 + 1% AcOH at 2.5 mL/min. The composition of the HPLC mobile phase varied from compound to compound. The appropriate fraction (the typical $k'_{product}$ was between 18 to 22) was collected in a total volume of 3 - 4 mL and an aliquot analysed by analytical HPLC (column B) and TLC to establish (i) the identity of the product, (ii) the chemical and radiochemical purities, and (iii) the specific activities (6). In all cases radiochemical purities were >99% and specific activities of 400 - 1500 mCi/µmol were achieved.

Acknowledgements

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