Asian Journal of Chemistry; Vol. 36, No. 3 (2024), 740-742

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2024.31197

A Useful Method for *in situ* Indirect Radiolabeling of Carbonyl-Containing Biomolecules

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Received: 10 January 2024; *Accepted*: 21 February 2024; *Published online*: 28 February 2024; AJC-21569

[^{125}I] 2-(2,3-diiodo-4-hydroxyphenyl)-1,3,4-oxadiazole was synthesized (48% yield) and acid-hydrolyzed *in situ* with direct derivatization with *p*-nitro benzaldehyde to obtain the required ^{125}I hydrazone (89% yield). A blood clearance kinetic study was performed on the ^{125}I oxadiazole in a Sprague-Dawley rat and a biphasic behaviour was obtained. This study shows that ^{125}I oxadiazole has potential to be metabolically stable and enough to allow imaging and tracking for a minimum of 2 h, making it a viable option for *in situ* indirect labeling of carbonyl containing macromolecules and keto steroids.

Keywords: Oxadiazole, Radiolabeling, Hydrazone, Blood clearance, Biphasic behaviour.

INTRODUCTION

Electrophilic radioiodination of aromatic amines and hydrazine derivatives at no carrier added (^{127}I) is complicated by the presence of N-H bonds in the molecules being labeled. These undergo oxidative side reactions using up the electrophilic radioiodine. Earlier synthesis used esters as a protecting group, which were converted to carbohydrazide's after radioiodination [1]. Other methods used amide/phthalimide groups, which could mask N-H functionality and be subsequently cleaved after iodination [2]. Furthermore, electrophilic radioiodination does not occur on small organic molecules which lack activated aromatic rings. In addition, biologically important macromolecules can undergo massive structural change during attempted direct electrophilic radioiodination with various iodinating reagents, which are oxidizers and can damage sensitive tertiary structures.

There is definite need for a prosthetic radioiodination group, carrying a non-metabolized tracer, useful for carbonyl derivatization, which can be prepared at high specific activity. Many *in vivo* diagnostic radiopharmaceuticals, labeled proteins used in hormonal studies and some tagged antigens needed for radioimmunoassay, require high specific activity radionuclide incorporation. This is especially true for the radio iodinated monoclonal antibodies used in the new field of radioimmune imaging.

Oxadiazole motif is well-known due to its huge importance in medicinal chemistry [3-6]. A logical prosthetic group would be an acyl hydrazide directly iodinated at an aryl ring. The N-H functionalities of hydrazide could be masked or protected as a 1,3,4-oxadiazole. Not only can oxadiazoles usually be readily made from hydrazides but they can also be readily opened back to hydrazides with acid catalysis. Direct iodination of *p*-hydroxybenzoic acid hydrazide is exceedingly difficult to carry out and is impossible in high specific activity labeling. However, the masked form of hydrazide can be conveniently and cleanly iodinated and radio-iodinated to the corresponding 2-(3,5-diiodo-4-hydroxyphenyl)-1,3,4-oxadiazole, which can be acid-hydrolyzed *in situ* with direct derivatization of carbonyl containing small molecules present in the same medium (*e.g. p*-nitro benzaldehyde).

EXPERIMENTAL

The melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. The NMR spectra were taken in DMSO-d_6 as a solvent on a JEOL FX90-Q spectrometer using tetramethyl silane (TMS) as an internal standard. Infrared spectra were determined in KBr pellet on a Perkin-Elmer model 283 infrared spectrophotometer.

Blood clearance study of [^{125}I] 2-(2,3-diiodo-4-hydroxyphenyl)-1,3,4-oxadiazole in Sprague Dawley rat: A Sprague Dawley rat was anesthetized, weighed, an area on the neck was shaved and the spot rubbed with saline. An incision was made to delineate the jugular vein. It was freed of surrounding tissue and a small nick was made at the lower part of jugular vein. A piece of elastic tubing was inserted in the vein and tied with a thread. The tubing was connected to a canula and a syringe. To initiate the study, 50 µL of [^{125}I] 2-(2,3-diiodo-4-hydroxyphenyl)-1,3,4-oxadiazole in 95% ethanol was added to 1.0 mL of saline and then injected intravenous (IV) into the rat. At time intervals over a period of 2 h, 0.10 mL samples of blood were withdrawn from the rat, weighed and counted using a gamma counter. Percent injected dose per gram of blood was then calculated for each count.

[^{125}I] 2-(2,3-Diiodo-4-hydroxyphenyl)-1,3,4-oxadiazole (2): A solution of 0.50 g (3.37 mmol) of 2-(4-hydroxyphenyl)-1,3,4-oxadiazole, 0.50 g (3.00 mmol) of KI, 3 mL of 2 N aqueous acetic acid, 5 mL of 95% ethanol and mCi of ^{125}I sodium iodide was refluxed for 15 min and treated to the dropwise addition over 0.5 h of 0.30 g (1.65 mmol) of iodic acid dissolved in 2 mL of water. Following 1 h of reflux, the remaining amount of KI (0.50 g) in 3 mL of water was added with refluxing continued for additional 1 h. Evaporation *in vacuo*, chilling and filtration afforded 0.61 g (yield: 48%) of [^{125}I] 2-(2,3-diiodo-4-hydroxyphenyl)-1,3,4-oxadiazole of specific activity 51×10^{-3} µCi/mg. A single spot of R_f = 0.25 with ethylene dichloride eluant was coincident with authentic 2-(2,3-diiodo-4-hydroxyphenyl)-1,3,4-oxadiazole coeluted under similar conditions.

[^{125}I] N_1-(*p*-Nitro benzylidene)-N_2-(3,5-diiodo-4-hydroxybenzoyl)hydrazine (4): A solution of 0.30 g (0.73 mmol), 24 µCi of [^{125}I] 2-(2,3-diiodo-4-hydroxyphenyl)-1,3,4-oxadiazole, 0.4 mL of concentrated hydrochloric acid and 15 mL of THF refluxed for 3 h, evaporated to a low volume ($\simeq$ 5 mL), mixed with 0.11 g (0.73 mmol) of *p*-nitro benzaldehyde in 15 mL of THF and refluxed for 2 h. Evaporation and filtration of residue gave 0.35 g (89% yield) of hydrazone; TLC gave a single spot, R_f = 0.60, using THF as eluant.

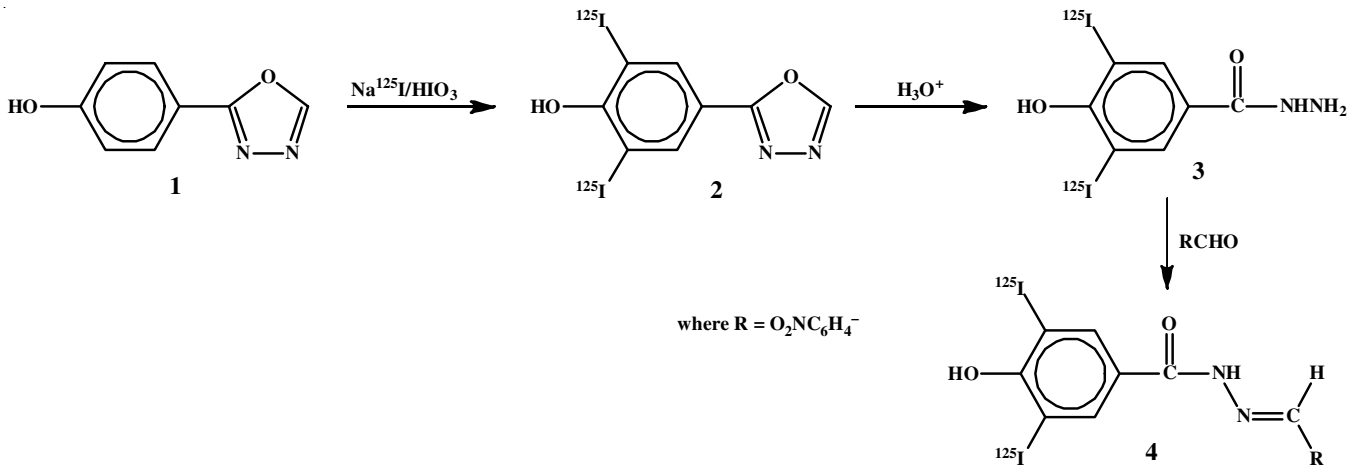
N_1-(*p*-Nitro benzylidene)-N_2-(3,5-diiodo-4-hydroxybenzoyl)hydrazine (authentic hydrazone): A solution of 0.30 g (0.72 mmol) 2-(2,3-diiodo-4-hydroxyphenyl)-1,3,4-oxadiazole, 0.20 mL of conc. HCl and 15 mL of THF was refluxed with constant stirring for 3 h. The reaction mixture was then evaporated to dryness *in vacuo* to remove excess acid. A solution of THF (15 mL) containing 0.11 g (0.72 mmol) of *p*-nitro benzaldehyde was added to the reaction flask and refluxed with stirring for 2 h. The residue obtained after evaporation to dryness was recrystallized from ethanol and then dried. Yield: 0.29 g (75%), m.p.: 265-270 ºC. IR (KBr, ν_{max}, cm^{-1}): 3320 (C=O); 1510 and 1350 (NO_2); ^{1}H NMR (DMSO-d_6) δ: 12.15 (s,1, NH); 10.23 (Br, s,1, OH); 8.55 (s,1, -CH=N-); 8.35 (d,4, H-2, H-3, H-5, H-6); 7.95 (s,2, H-2, H-6 of ArH). Anal. calcd. (found) % for $C_{14}H_9N_3O_4I_2$: C, 31.31 (31.58); H, 1.69 (1.84); N, 7.72 (7.61).

Animal ethics: The project was approved by the Institutional Ethics Committee of Jordan University of Science and Technology IRB (810-2020). All studies were conducted under pentobarbital sodium anesthesia to minimize any possible distress experienced by the animals involved in the study.

Animals: The Jordan University of Science and Technology (JUST) supplied healthy and four-week-old Sprague-Dawley rats (200-260 g in weight). The study's protocol was reviewed and approved by the Animals Care Unit Committee at JUST. Over the course of 28 days, the animals were given the test drug by swallowing. The weight of each rat was recorded at the beginning of the experiment and then every week until the necropsy was planned. Cage housing was provided for each rat. The animal room controls in the barrier system were designed to keep the temperature at 22 ± 2 ºC and the relative humidity between 40% and 70%.

RESULTS AND DISCUSSION

Scheme-I show that compound [^{125}I] **4** was synthesized in 89% yield from [^{125}I] **2** prepared in 46% radiochemical yield by electrophilic *in situ* iodination of **1** with [^{125}I] sodium iodide. The use of **2** as a readily obtained, stable precursor of an [^{125}I] acid hydrazide [^{125}I] **3** did constitute, a useful method for *in situ* indirect labeling of keto steroids and carbonyl containing biomolecules [7].



Scheme-I

Blood clearance study: To be of value as a prosthetic labeling agent for radioiodine attachment, the covalently bonded iodine would have to display a suitable resistance to enzymatic deiodination. Counsell & Ice [8] reported that some *ortho*-iodinated phenols undergo a very rapid scission of iodine from the aromatic ring with subsequent removal of tracer from blood into gastric mucosa and thyroid. The primary agent developed in this study is a radio iodinated phenyl-substituted oxadiazole. This species and a hydrazone derivative were prepared in radioactive (^{125}I) form by a method described in the experimental section. The chemical yield of ^{125}I oxadiazole was 48% and it conversion to the hydrazone was affected in 89% yield A blood clearance kinetic study was performed on the ^{125}I oxadiazole in Sprague-Dawley rat. A biphasic behaviour was obtained and the raw data is shown in Table-1.

TABLE-1
PERCENT INJECTED DOSE PER GRAM OF BLOOD OF [^{125}I] 2-(2,3-DIIODO-4-HYDROXYPHENYL)-1,3,4-OXADIAZOLE IN SPRAGUE-DAWLEY RAT AT VARIOUS TIMES

Time (min)	% ID/g	Time (min)	% ID/g
1	4.02	30	0.55
2	1.06	40	0.68
5	0.98	50	0.54
10	0.78	60	0.55
20	0.56	120	0.34

After the initial rapid loss of tracer from blood, the percent dose falls to about one-third of initial values from 1.06% at 2 min to 0.34% at 120 min over a 2 h period. Since free circulating iodide, if present, would be expected to be rapidly absorbed into the gastric mucosa and thyroid [9], these counts may be presumed to be from the iodinated oxadiazole. The kinetic plot of the data is shown in Fig. 1. A first pass clearance slope of -0.76% injected dose/g/min, which was followed by a whole body clearance slop of -0.0046% injected dose/g/min. In first order kinetic processes these slops are, of course, equal to rate constants. The best fit line for whole-body clearance was determined by a computer derived least square treatment of the data. The biological half-life for whole-body clearance of the tracer was calculated from that slop as $t_{½}$ = 136 min. Such biphasic behaviour is both common and expected in drug clearances [10].

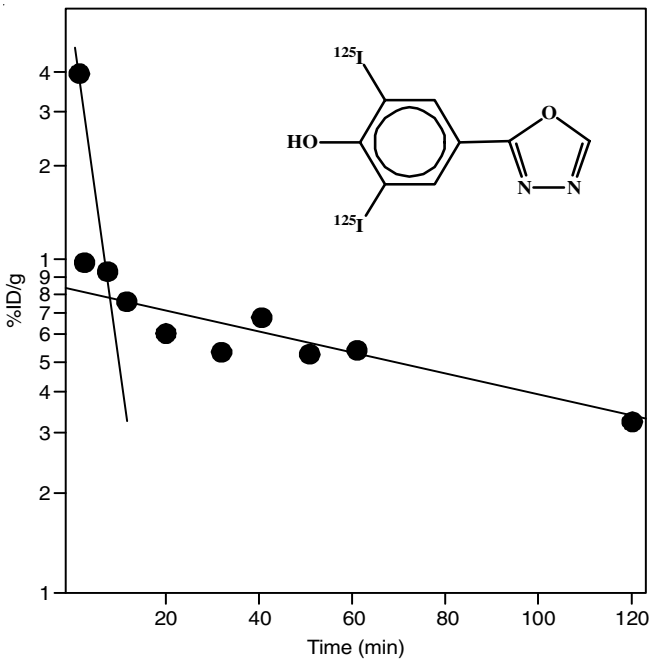


Fig. 1. Blood clearance curve for [^{125}I]2-(3,5-diiodo-4-hydroxyphenyl)-1,3,4-oxadiazole [Note: (%ID/g) is the percent injected dose per g]

Conclusion

In this study, a prosthetic and indirect radioiodination method was developed. The general method involves the use of radio iodinated aryl oxadiazole as a protective functionality for the final aryl acid hydrazide. The latter was employed to derivatize carbonyl-functionality in biomolecules. A blood clearance study was done on a Sprague Dawley rat after being injected with a dose of [^{125}I] 2-(2,3-diiodo-4-hydroxyphenyl)-1,3,4-oxadiazole. The results showed that this agent could have sufficient metabolic stability to permit tracking and imaging over at least a 2 h period.

ACKNOWLEDGEMENTS

The authors thank Lehigh University, Bethlehem, PA, USA, for funding this work. One of the authors (BMS) also thanks his daughter Ms. Nevein Shabsoug for technical assistance in typing this manuscript.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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