The 1,3,4-Oxadiazole Moiety, A Protective Synthon for Indirect Radioiodination

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2-(4-Hydroxyphenyl)-1,3,4-oxadiazole can readily be iodinated/radioiodinated to 2-(3,5-diiodo-4-hydroxyphenyl)-1,3,4-oxadiazole which in a one-step hydrolysis-condensation serves as an indirect prosthetic moiety for iodination of carbonyl compounds.

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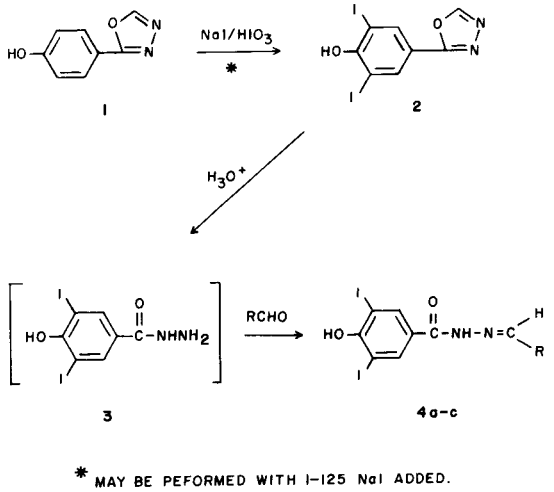
Procedures for indirect radioiodination of proteins and other biologically important molecules are finding increasing applicability in *in vivo* bioanalytical procedures and for production of I-125 labeled antigens for radioimmunoassays [1-3]. Such radioiodinated prosthetic groups are useful when the biological molecule to be labeled lacks activated aromatic functionality or possesses sensitive tertiary structure which might not survive the traditional direct radioiodination conditions. Most frequently such indirect labeling involves radioiodinated phenolic compounds which couple by azo, imidate, or activated ester groupings to amine nucleophiles in the protein or small molecule being labeled [4-7]. Several reviews have recently discussed indirect labeling methods [8,9,10].

Scheme I



* MAY BE PEFORMED WITH I-125 NaI ADDED.

Currently attention is being paid to the need to develop indirect radioiodination prosthetic groups for attachment at carbonyl centers. Ruliffson and coworkers pioneered a quantification of keto steroids by *in situ* derivatization with I-131 iodopyridinium hydrazonylacetate [11]. Carbohydrate moieties in nucleosides or glycoproteins have been oxidized to carbonyls by periodate or by galactose oxidase and subsequently derivatized by pre-radioiodinated tyramine or 4-hydroxyphenylpropionyl carbohydrazide [12,13]. Electrophilic radioiodination of aromatic amines and hydrazine derivatives is complicated by the presence of N-H bonds which undergo oxidative side reactions [14]. Earlier syntheses used esters which were converted to carbohydrazides after radioiodination [11,13] or used amide/phthalimide groups which could mask N-H functionality and be subsequently cleaved after iodination [15,16].

We wish to report an experimental method in which a 1,3,4-oxadiazole serves as a protective synthon for iodination (and radioiodination) of an acid hydrazide. Direct iodination of *p*-hydroxybenzoic acid hydrazide with either iodine monochloride or iodide/iodate combinations did not, in our hands, generate isolable quantities of **(3)** unless a vast excess of iodine were present. Such conditions, however, are obviously not possible in high specific activity labelings. In this alternative procedure 2-(4-hydroxyphenyl)-1,3,4-oxadiazole **(1)** (the masked form of *p*-hydroxybenzoic acid hydrazide), can be conveniently and cleanly iodinated/radioiodinated to 3-(3,5-diiodo-4-hydroxyphenyl)-1,3,4-oxadiazole **(2)**.

The latter can be acid-hydrolyzed *in situ* with direct derivatization of carbonyl-containing small molecules present in the same medium. By this process **4a-c** were prepared in 70-80% yield and (I-125 **4a**) was prepared in 80% chemical yield, from (I-125 **2**) prepared in 46% radiochemical yield by electrophilic *in situ* iodination of **(1)** with I-125 sodium iodide. The use of **(2)** as a readily obtained, stable precursor of a I-125 acid hydrazide (I-125 **3**) constitutes a useful method for *in situ* indirect-labeling of keto steroids, carbonyl-containing biomolecules, and oxidized nucleosides.

EXPERIMENTAL

The 2-(4-hydroxyphenyl)-1,3,4-oxadiazole **(1)** was prepared by the method of Maillard and Vincent [17]. Infrared spectra were obtained on a

Table 1

Preparation of Iodinated Benzoyl Hydrazones **4a-c**

Compound	R	Formula	Analysis % Calcd. C	Calcd. H	Found C	Found H	Mp °C	Yield %	Recrystallization Solvent	
4	*p*-Me_2N-C_6H_4-	$C_{16}H_{15}I_2N_3O_2$	35.91	2.83	35.67	2.90	237-239	81	Methanol:water	1:3
4	*p*-HOOC-C_6H_4	$C_{15}H_{10}I_2N_2O_4$	33.61	1.88	33.40	2.11	276-277	73	Methanol:water	1:3
4	$CH_3(CH_2)_4$-	$C_{12}H_{14}I_2N_2O_2$	30.53	2.99	30.61	3.14	184-185	70	Chloroform:hexane	1:3

Perkin Elmer Model 283 spectrophotometer. Proton nmr spectra were recorded on a JEOL FX90Q spectrometer with tetramethylsilane as an internal standard. Microanalyses were provided by George I. Robertson Microanalytical Laboratory, Florham Park, NJ. Thin layer chromatograms of both I-125 labeled and non-radioactive compounds were performed on Bakerflex silica sheets containing fluorescent indicator. The I-125 used in this study was supplied by NEN as a no-carrier added solution of (I-125) sodium iodide in a *p*H 8-10 aqueous solution (reductant free) at a specific activity of *ca* 350 mCi/ml.

2-(3,5-Diiodo-4-hydroxyphenyl)-1,3,4-oxadiazole (**2**).

A solution of 10.0 g (62.0 mmoles) of 2-(4-hydroxyphenyl)-1,3,4-oxadiazole in 75 ml of 95% ethanol was refluxed and treated to the addition of 40 ml of water containing 10.3 g (62.0 mmoles) of potassium iodide followed by 93 ml of 2*N* acetic acid (186 mmoles). To this solution was added in a dropwise fashion over 0.5 hour, 6.5 g (37.0 mmoles) of iodic acid in 50 ml of water. The resulting reaction mixture was heated at reflux for an additional hour after which a further 10.3 g (62.0 mmoles) of potassium iodide in 40 ml of water was added. After 1 hour of continued reflux the mixture was evaporated *in vacuo* to turbidity and chilled in an ice-water bath. The product precipitated, was filtered, and was recrystallized from 1:2 dioxane:water to afford 17.0 g, 66% yield of **2** as a cream-colored solid, mp 198-200°; ir (potassium bromide): 3140 (OH cm^{-1}; proton nmr (DMSO-d_6): δ 8.28 (s, 2, ArH), 9.29 (s, 1, oxadiazole H), and 10.28 ppm (br s, 1, H).

Anal. Calcd. for $C_8H_4I_2N_2O_2$: C, 23.21; H, 0.97; N, 6.76. Found: C, 23.40; H, 1.08; N, 6.57.

I-125 2-(3,5-Diiodo-4-hydroxyphenyl)-1,3,4-oxadiazole (I-125 **2**).

A solution of 2.0 g (12.3 mmoles) of **1**, 2.0 g (12.0 mmoles) of potassium iodide, 12 ml of 2*N* aqueous acetic acid, 20 ml of 95% ethanol, and 2.0 mCi of I-125 sodium iodide was refluxed for 15 minutes and treated to the dropwise addition over 0.5 hour of 1.2 g (6.6 mmoles) of iodic acid dissolved in 5 ml of water. Following 1 hour of reflux the remaining amount of potassium iodide, (2.0 g) in 8 ml of water was added with refluxing continuing for 1 additional hour. Evaporation *in vacuo*, chilling, filtration, and recrystallization (1:2 dioxane:water) afforded 3.2 g (65% chemical yield) of I-125 **2** of specific activity 120 μCi/mmole (46% radiochemical yield). A single spot of R_f = 0.20 with ethylene dichloride eluant was coincident in position with authentic **2** coeluted under identical conditions.

General Procedure for Acyl Hydrazones **4a-c**.

A solution of 0.30 g (0.72 mmole) of **2**, 0.20 ml (2.56 mmoles) of concentrated hydrochloric acid, and 15 ml of tetrahydrofuran was refluxed for 3.0 hours and evaporated to dryness *in vacuo*. A solution of 0.72 mmoles of the carbonyl-containing molecule in 15 ml of tetrahydrofuran was added and the resulting solution refluxed for 2 hours. Evaporation to dryness gave crude products which were recrystallized to analytical purity in the indicated solvents (see Table 1 for properties and yields).

Preparation of I-125 Acyl Hydrazone I-125 **4a**.

A solution of 0.50 g (1.2 mmoles, 140 μCi) of I-125 **2**, 0.4 ml of concentrated hydrochloric acid, and 25 ml of tetrahydrofuran was refluxed for 3 hours, evaporated to dryness *in vacuo*, mixed with 0.18 g (1.2 mmoles) of *p*-dimethylaminobenzaldehyde in 25 ml of tetrahydrofuran and refluxed for 2 hours. Evaporation and recrystallization of the residue from ethanol gave 0.51 g (80% yield) of I-125 **4a**, mp 236-238°; tlc gave a single spot, R_f 0.86, using ethanol as eluant. It was coincident with authentic **4a**.

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