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Radioiodination of MIBG for Neuroendocrine Tumor Imaging

by

Khaled Dostrada

THESIS

Submitted in partial satisfaction of the requirements for the degree of

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of the

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by

Khaled Dostzada

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Radioiodination of MIBG for Neuroendocrine Tumor Imaging

Khaled Dostzada

Abstract

The aims of this project are to develop a new general method of nucleophilic radioiodination for aromatic tracers and to apply this method for the synthesis of radiolabeled metaiodobenzylguanidine (MIBG). MIBG is a radiolabeled pharmaceutical similar in structure to noradrenaline. The compound localizes to adrenergic tissues and can be used to image and treat neuroendocrine tumors overexpressing the human norepinephrine transporter (hNET). When labeled with I-131, MIBG can be used therapeutically to eradicate tumor cells that take up and metabolize norepinephrine. Preliminary studies with stable I-127 have shown that asymmetrical diaryliodonium salts can be iodinated with high yields. With this approach, it is possible to produce radiolabeled MIBG by using a protected diaryliodonium precursor. The effects of reaction pH, labeling solution, deprotection time, and HPLC solvent were examined for this reaction. It was found that acidic conditions gave the best labeling yields. Reaction for 7 minutes was found to be sufficient to fully deprotect the product and a labeling solution containing both CH₃CN and toluene was determined to give the best total yield. HPLC was used for separation of MIBG from side-products iodoanisole and metachlorobenzylguanidine (MCBG). Removal of precursor before deprotection was also found to increase specific activity 8-fold. These results show that iodonium chemistry is a novel and effective method for radioiodinating aromatic tracers and that this method may be applied to label other radioiodine compounds.

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Introduction

Nature of the Problem:

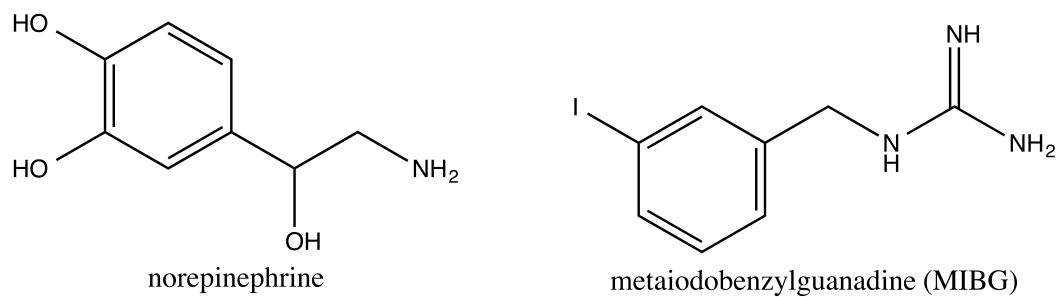


Figure 1. Comparison of norepinephrine and MIBG

Metaiodobenzylguanidine (MIBG) is a radiolabeled pharmaceutical similar in structure to noradrenaline. (Figure 1) MIBG was originally developed in the 1970s at the University of Michigan to image the adrenal medulla.¹ MIBG localizes to adrenergic tissues and can be used to image neuroendocrine tumors such as neuroblastoma and pheochromocytoma.⁶ When labeled with I-131, MIBG can also be used therapeutically to eradicate tumor cells that take up and metabolize norepinephrine. ¹³¹I-MIBG was approved by the Food and Drug Administration in 1994 and ¹²³I-MIBG was later approved for tumor imaging in 2008.²

Mechanism of Action:

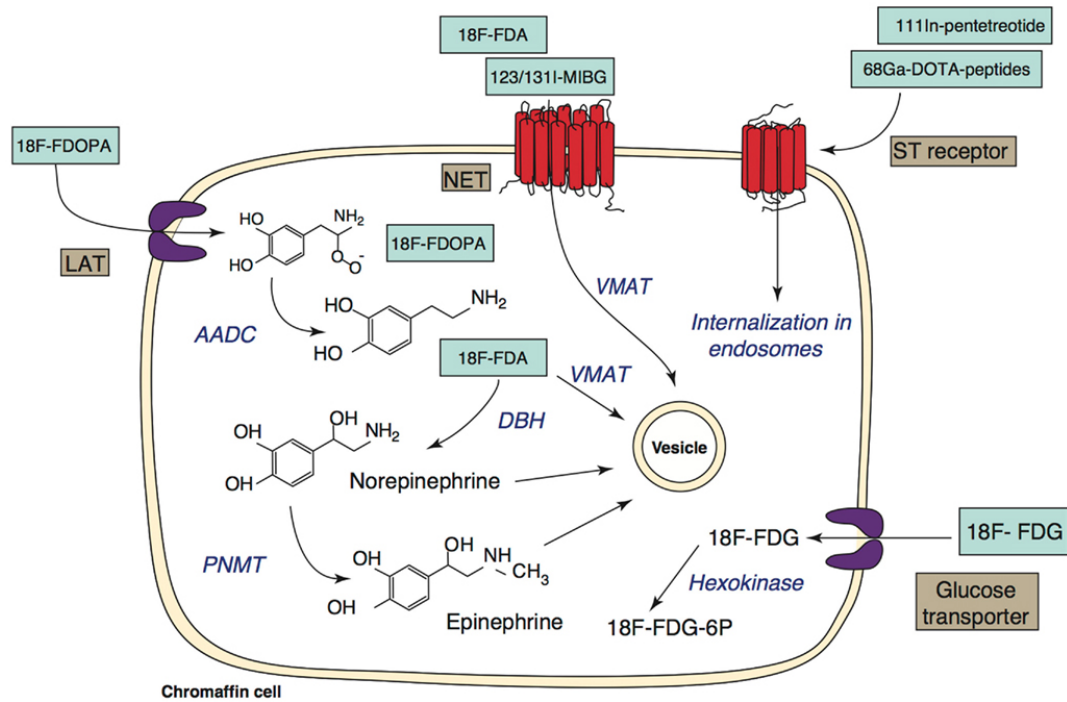


Figure 2. Mechanism of Action for MIBG

MIBG enters the intracellular environment via the human norepinephrine transporter (hNET). (Figure 2) Inside the cytoplasm, it is transported into synaptic vesicles via the action of the vesicular monoamine transporter (VMAT).³ MIBG remains trapped within the synaptic vesicles and can then be imaged or used for therapeutic purposes. hNET is overexpressed in the majority of neuroendocrine tumors such as neuroblastoma, a common extracranial solid cancer found primarily in children under the age of two.⁴ MIBG's specificity for hNET makes it a good candidate for imaging and treatment, given that hNET is overexpressed in these cell lines.

Current Methodology:

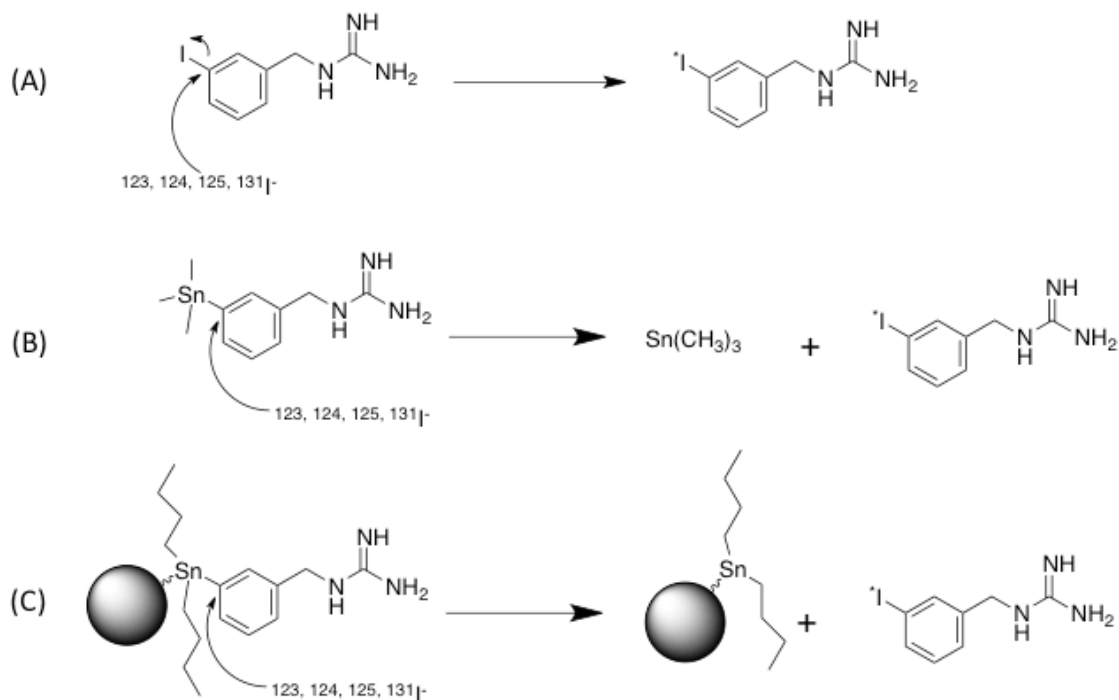


Figure 3. Current Methods of MIBG Production

There are three current methods of production for MIBG. The first method relies on a radioiodine-for-iodine substitution where unlabeled or “cold” MIBG is reacted with I-123, 124, 125, or 131.⁵ (Figure 3A) However, once hot MIBG is produced it cannot be isolated from cold MIBG due to chemical similarity. This results in a product that has very low in specific activity. (*I-123 MIBG SA = 1mCi/mmol*)⁶ Another method of production relies on trialkyl tin substitution with radioiodine.⁷ (Figure 3B) Using this method, hot MIBG can be isolated from the reactant resulting in a product that is higher

in specific activity (SA). One drawback of this method is trialkyl tin formation. Testing for tin must be performed as trialkyl tin has been shown to be toxic.⁸ To alleviate tin by-products, a resin based method was developed. Benzylguanadine is attached to the resin through a dibutyl tin group and substitution with radioiodine results in [^{*}I]MIBG being released into the liquid phase of the reaction mixture where the tin precursor and by-product remain attached to the resin and can easily be removed from solution.⁹ (Figure 3C) This method also produces MIBG in high specific activity but without a toxic tin by-product.

Iodonium Based Approach:

The aims of this project are to develop a general method of nucleophilic radioiodination for aromatic tracers and to then use this method for the synthesis of radiolabeled MIBG. Preliminary studies with stable I-127 have shown that asymmetrical diaryliodonium salts (Figure 4A) can be iodinated with high yields. In this approach, the wanted product is attached to the more electron withdrawing benzene ring. (Figure 4B) When the solution is heated, radioiodine preferentially bonds to the electron withdrawing benzene ring. (Figure 4C) Methoxide is usually used for the R₁ group. With this approach, it is possible to produce radiolabeled MIBG by using a protected diaryliodonium salt precursor.

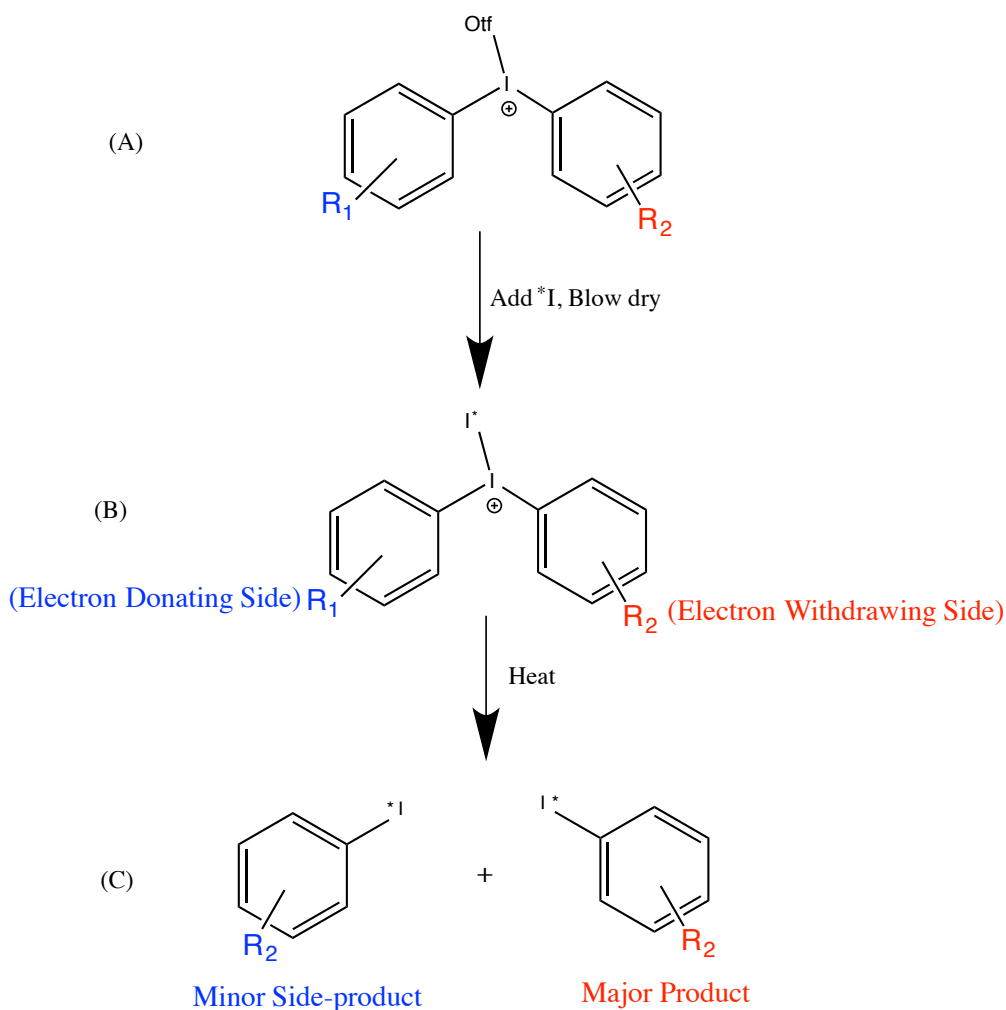


Figure 4. General Schematic for Precursor Based Approach

Materials and Methods

Materials:

All chemicals/solvents were purchased from Sigma-Aldrich and were used as received unless otherwise stated. I-125 was commercially purchased from PerkinElmer Inc as sodium iodide (Na^*I) in either a 0.1M or 10^{-5} M NaOH solution. (4-((2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)methyl)phenyl)(4-methoxyphenyl)iodonium and (E)-(3-((N,N'-bis(tert-butoxycarbonyl)-3,5-dimethyl-4-oxo-1,3,5-triazinane-1-

carboximidamido)methyl)phenyl)(4-methoxyphenyl)iodonium were provided by Ground Fluor Pharmaceuticals.

General Procedure for the Optimization of Chemistry:

Iodomaleimide Synthesis: 5mg of the maleimide precursor ((4-((2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)methyl)phenyl)(4-methoxyphenyl)Iodonium) was dissolved in 400 μ L of CH₃CN. Varying amounts of 1M AcOH was added to the reaction vial with 1 μ L of Na^{*}I. The dissolved precursor was added to the vial and the solution was evaporated with gas. The residual was reconstituted in a 50% CH₃CN:toluene labeling solution (125 μ L of CH₃CN + 125 μ L of toluene) and was heated at 100°C for 30 minutes.

Reaction pH: Reactions were performed using varying amounts of added 1M AcOH. Silica radioTLC in 30% EtOAc:hexane was then performed to determine labeling yields of product and side-product. One trial was conducted with no added acid and additional trials were done with 0.5, 1.5, and 3 μ mol of added acid.

MIBG Synthesis: The remaining procedure was performed using the protected MIBG precursor. ((E)-(3-((N,N'-bis(tert-butoxycarbonyl)-3,5-dimethyl-4-oxo-1,3,5-triazinane-1-carboximidamido)methyl)phenyl)(4-methoxyphenyl)Iodonium) 5 mg of MIBG precursor was dissolved in 400 μ L of CH₃CN. 2.5 μ L of 1M AcOH was added to the reaction vial with 1 μ L of Na^{*}I. The dissolved precursor was then added to the

reaction vial and the solution was blown dry with gas. After the solution was completely dried, it was then redissolved in a labeling solution. Different 250 μ L labeling solutions were tested. (CH_3CN , DMF, 10% CH_3CN :toluene, and 50% CH_3CN :toluene) The effect of the labeling solution was determined on both labeling yields and transfer percentages. The redissolved solution was then heated at 85°C for 30 minutes. Silica radioTLC in 100% EtOAc was then performed to determine percent yield of all products. The reaction was then blown dry with gas again before deprotection.

Deprotection: The reaction residue was redissolved in 100 μ L of 6M HCl and was then heated at 100°C for either 7 minutes or 15 minutes. The solution was then neutralized using 60 μ L of 10M NaOH. RadioTLC was then performed in 100% EtOAc to analyze the reaction.

HPLC Purification: The final solution was injected into a semi-prep C-18 HPLC column with a flow rate of 1mL/min. The ideal HPLC solvent system was determined. Isocratic mobile phase solutions (30-20% CH_3CN : KH_2PO_4) were evaluated to determine best separation. Radiolabeled MIBG was collected off the column 15-18 minutes after injection.

Optimizing Specific Activity: Leftover precursor was removed from the reaction before deprotection to improve specific activity. (Figure 7) An HLB sep pack was conditioned first using 5 mL of EtOH and then 5 mL of water. The reaction was diluted up to 10 mL with water and loaded onto the HLB sep pack. The HLB cartridge was

rinsed using 6 mL of 40% CH₃CN:H₂O and the protected MIBG was eluted using 2 mL of EtOH. The SA of the solution was then compared to the SA of the previous solutions made without use of an HLB sep pack before deprotection.

Results and Discussion

Reaction pH:

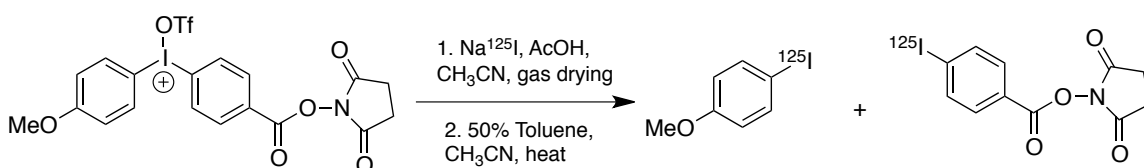


Figure 5. Iodomaleimide Reaction

The iodomaleimide synthesis was used to determine the ideal pH for the reaction. The maleimide precursor was dissolved in CH₃CN and was evaporated in the presence of AcOH. (Figure 5) The residual was then reconstituted in a 50% CH₃CN:toluene labeling solution. The redissolved solution was then heated at 100°C for 30 minutes. RadioTLC was then performed to determine incorporation of radioiodine in the product. Iodomaleimide did not require deprotection and no additional purification was performed.

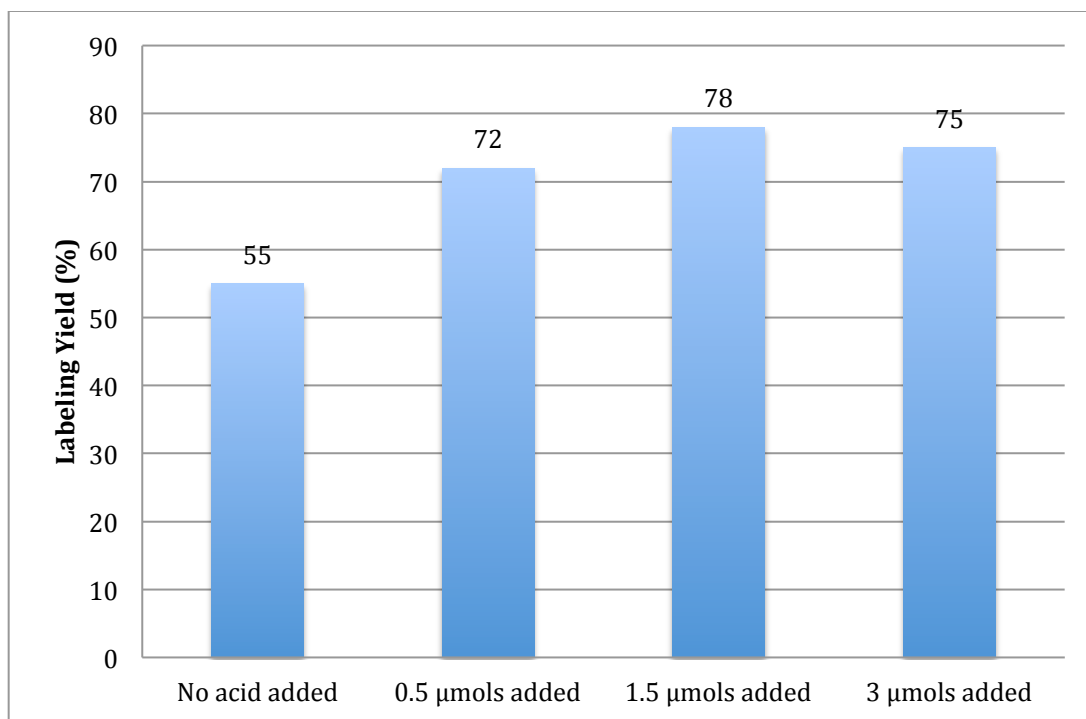


Figure 6. [^{125}I]Iodomaleimide Yields Compared to Acid Added

[^{125}I]Iodomaleimide labeling yields increased as more 1M AcOH was added to the reaction. The addition of 1M AcOH also decreased the formation of iodoanisole side-product. (Figure 5) It was found that with 3 μmol of added acid, labeling yields did not improve. The ideal amount of added acid was determined to be greater than 1.5 μmol but less than 3 μmol. 2.5 μL of added AcOH resulted in a 2.4 μmol acidic surplus when reacted with 1 μL of Na^{*}I in 0.1M NaOH. The optimal amount of acid was determined to be a 2.4 μmol surplus. All subsequent reactions were performed using a 2.4 μmol surplus of 1M AcOH.

MIBG Synthesis:

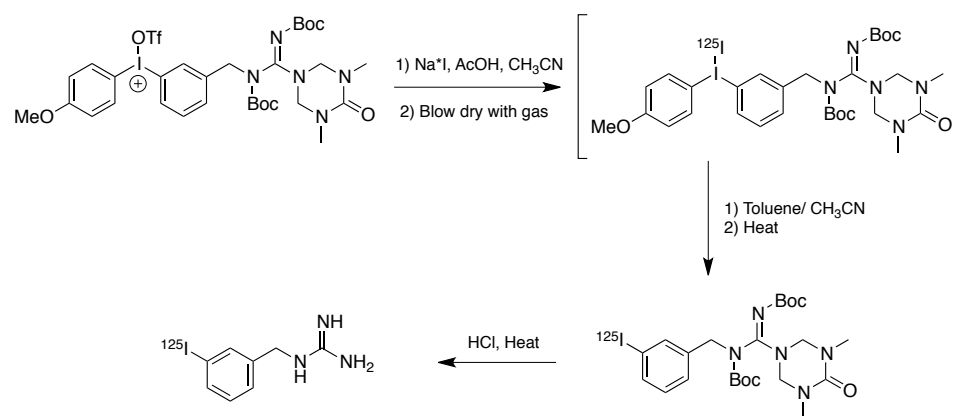


Figure 7. MIBG Reaction

The MIBG synthesis was used for the remaining procedure. The MIBG precursor was dissolved in CH₃CN and was evaporated in the presence of AcOH. The residual was then reconstituted in a labeling solution. (CH₃CN, DMF, 10% CH₃CN:toluene, or 50% CH₃CN:toluene) The redissolved solution was then heated at 85°C for 30 minutes. RadioTLC was then performed to determine incorporation of radioiodine in the product. The solution was evaporated once gain. The solution was redissolved in HCl and deprotection was performed at 100°C. The solution was neutralized with NaOH and was then injected onto an HPLC system.

MIBG									
Solvent	N (Ibl)	% Yield	% Transfer	% Anisole	% Total Product	% Total Anisole			
10% CH ₃ CN	4	88	1.9	70	-	5.9	0.9	61.3	4.1
50% CH ₃ CN	4	80	2.2	81.33	± 0.6	10.1	1.2	64.7	8.2
DMF	3	54	2.1	91	-	16.7	2.1	49.1	15.2
100% CH ₃ CN / 30'	3	56	6.5	80	2.6	15.7	2	44.8	12.6
100% CH ₃ CN / 120'	3	71	2.2	80	2.6	11.1	0.7	56.9	8.9

Table 1. The Effect of Labeling Solution on Total Yield

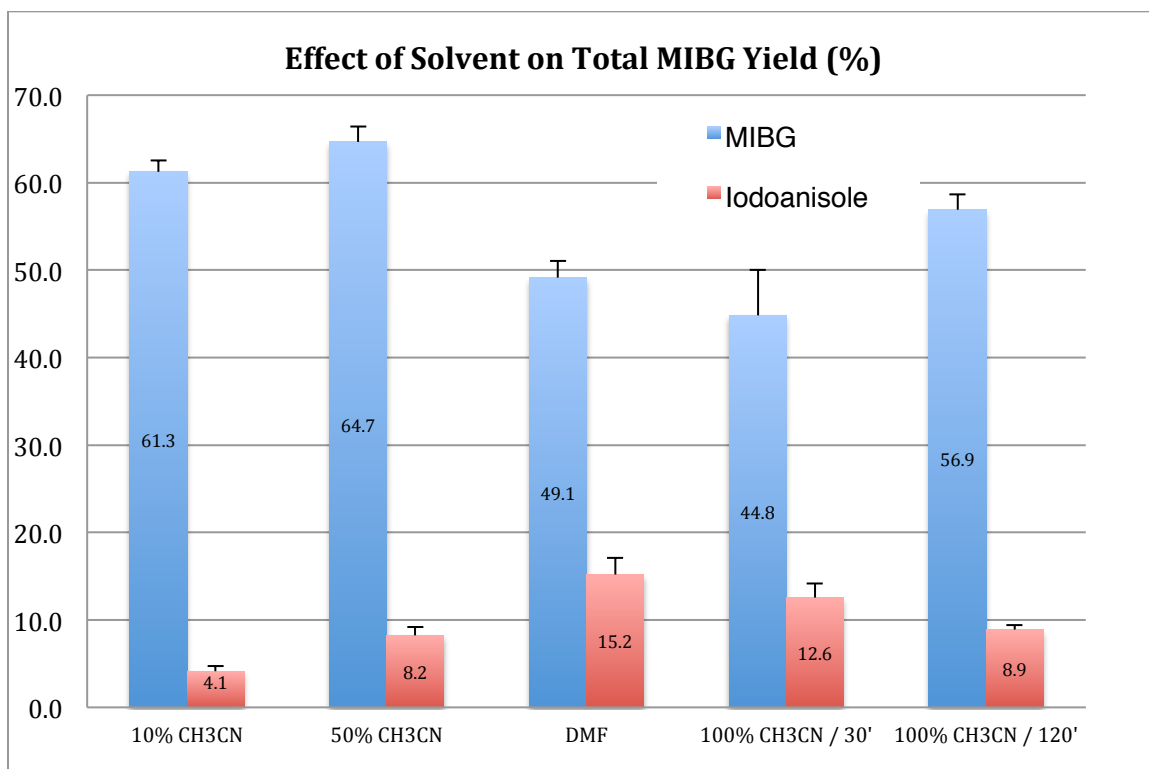


Figure 8. Graph of [¹²⁵I]MIBG Total Yields in Different Labeling Solutions

The labeling solutions tested were CH₃CN, 10% CH₃CN:toluene, 50% CH₃CN:toluene, and DMF. When a CH₃CN:toluene solution was used, CH₃CN was added first and the vial was shaken vigorously to ensure that all reactants were collected off the walls of the vial and are fully dissolved. 50% CH₃CN:toluene was found to be the best labeling solution because it gave the highest labeling yield and transfer percentage. Although 10% CH₃CN:toluene gave similar yields with less formation of iodoanisole, the large volume of added toluene caused activity to stick to the vial and made transferring the solution difficult. 100% CH₃CN and DMF gave good transfer percentages but labeling yields were found to be low. (Table 1) The addition of toluene as a solvent during heating shortens the reaction because nonpolar solvents changes the dielectric

nature of the solution shortening reaction times when charged reactants are being converted to neutral products. Therefore, 50% CH₃CN:toluene (125 µL CH₃CN + 125 µL toluene) was determined to be the best labeling solution and it was used in all subsequent reactions.

Deprotection Time:

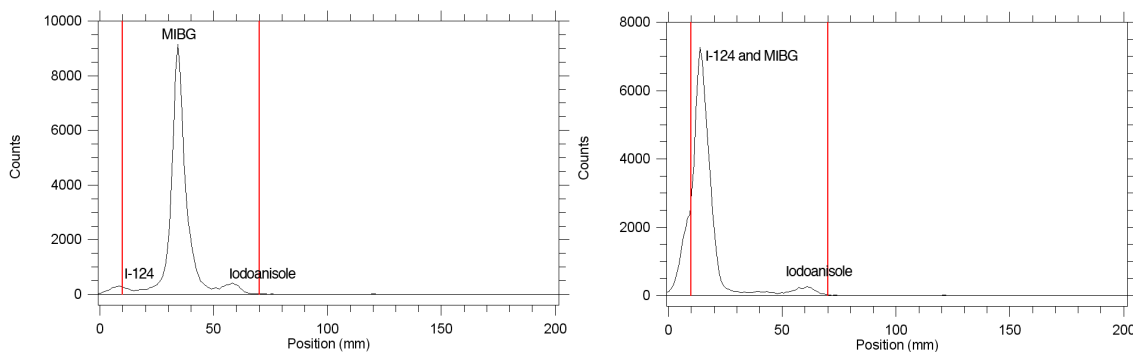


Figure 9. RadioTLC for [¹²⁴I]MIBG Before (Left) and After (Right) 7 Minute Deprotection Time. Performed on silica plate in 100% EtOAc

Both 7 and 15 minute deprotection times were tested. After deprotection radioTLC was performed to determine if protecting groups on the primary and secondary amines were completely removed. Removal of protecting groups resulted in increased polarity of the compound causing it to stick at baseline on a silica plate in 100% EtOAc. It was determined that after 7 minutes in 100°C, protecting groups on MIBG were completely removed. (Figure 10) Results from HPLC after deprotection showed formation of meta-chlorobenzylguanadine (MCBG). The nucleophilicity of chloride in HCl lead to the formation of this unwanted by-product. It was also found that a longer deprotection time allowed more formation of MCBG. Therefore, 7 minutes was chosen to be the best deprotection time because it was sufficient to fully deprotect the product and it reduced the formation of MCBG.

HPLC Solvent:

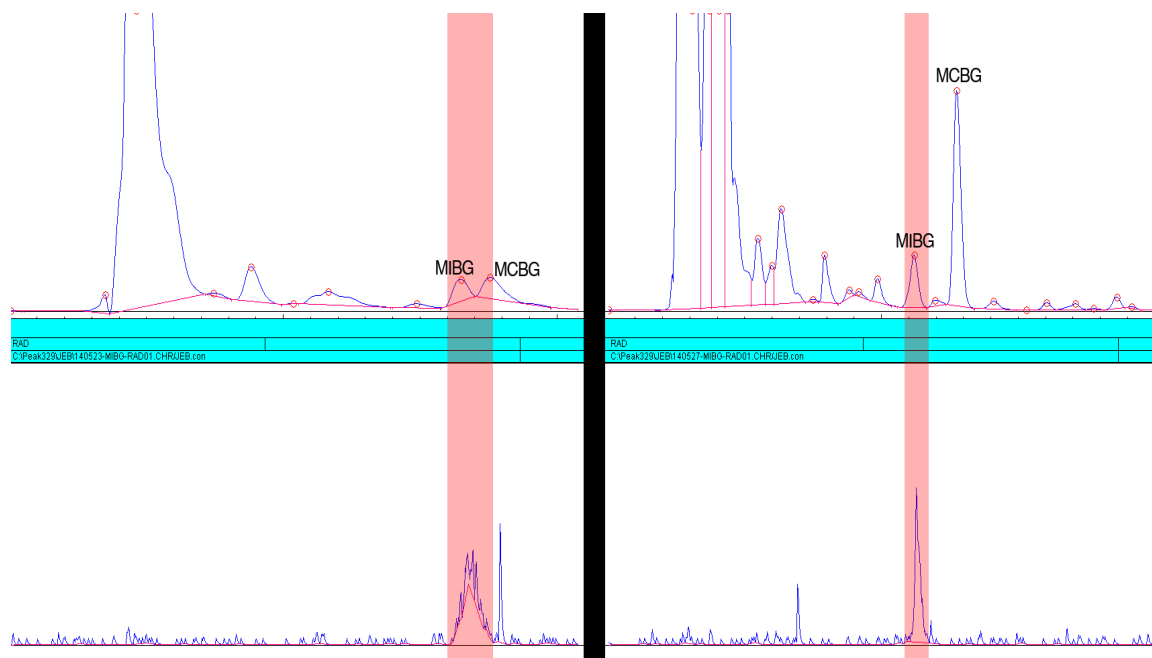


Figure 10. HPLC Traces for [¹²⁵I]MIBG in 25% CH₃CN:KH₂PO₄ (Left) and 20% CH₃CN:KH₂PO₄ (Right)

The solvent system used during HPLC purification must allow for clear separation of labeled MIBG from unwanted by-products such as iodoanisole and MCBG. Solvent systems of different polarities were used to purify labeled MIBG. 30%, 25%, and 20% CH₃CN:KH₂PO₄ mixtures were tested. The solution was passed through a semi-prep C-18 column at a rate of 1mL/min. It was found that the 25% solution was far too polar, resulting in an overlap between the MIBG and MCBG peaks. (Figure 11) This overlap was not seen in 20%. The 20% solution was chosen to be ideal because it allowed collection of labeled MIBG without bleed through of MCBG.

Overall Yield:

Three trials were performed using the 20% solvent system. Based on injections from standards, MIBG was known to elute off the column at minutes 15-18 after injection. *I-MIBG was collected from the column during this period of time and activity measurements were taken. The recovered activity was compared to the initial activity and an overall percent yield for the whole procedure was calculated. (Table 2) It was found that an average of 56% of the initial activity was present in the form of labeled MIBG. Results were consistent across the three trials with a SEM value of 1.1%.

Trial	Initial Activity (μCi)	Recovered Activity (μCi)	Percent Yield (%)
1	300	171	57%
2	1310	710	54%
3	830	480	58%
Average:			56% $\pm$ 1.1%

Table 2. Results of HPLC Purification. Three trials conducted using a 25% $\text{CH}_3\text{CN}:\text{KH}_2\text{PO}_4$ solvent system. Sample collected during minutes 15-18.

Optimization of SA:

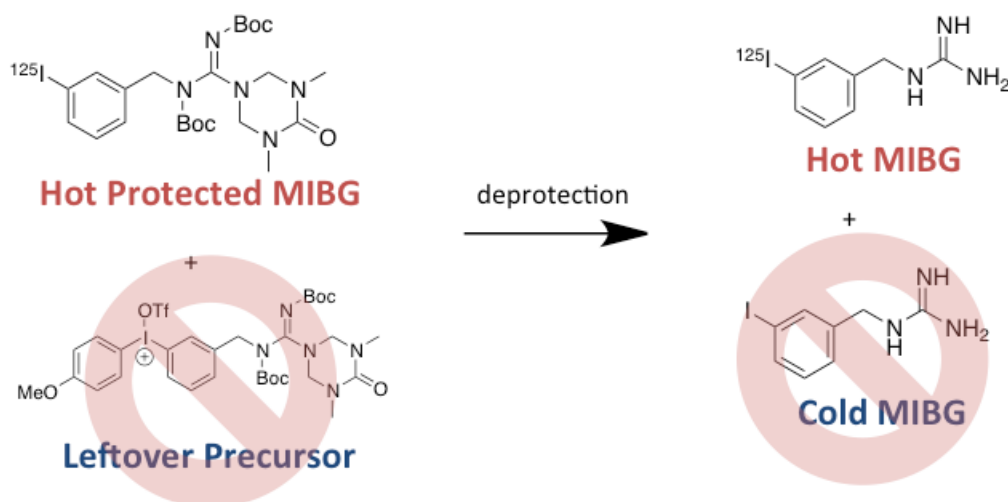


Figure 11. Removal of Leftover Precursor Before Deprotection To Improve Specific Activity

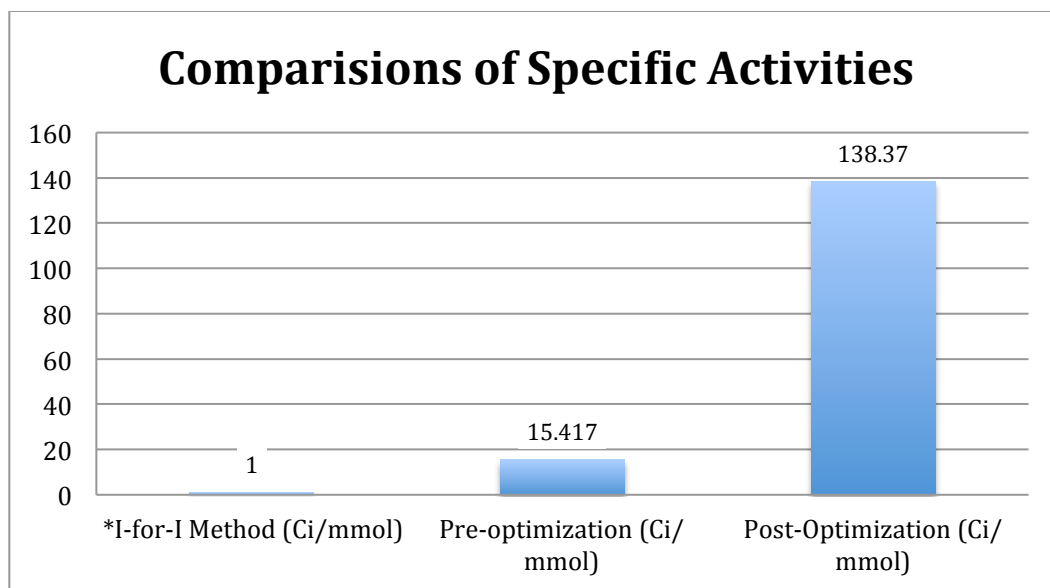


Figure 12. Comparison of Specific Activities Before and After Optimization Procedure

Removing leftover precursor before deprotection using an HLB sep pack was found to significantly increase specific activity. Removal of precursor increased SA from 15.42 Ci/mmol to 138.37 Ci/mmol. (Figure 12) Results from HPLC also show that removal of precursor led to less formation of MCBG during deprotection. Removal of the precursor before heating prevents nucleophilic attack from chloride and also prevents splitting of the precursor into additional cold MIBG. (Figure 11) In comparison to the radioiodine-for-iodine exchange method, the newly optimized procedure produces a product that is 137 fold greater in SA.

Conclusions

These results show that radioiodination of MIBG can be achieved through a diaryliodonium salt precursor without the addition of carrier or the use of a proprietary resin. After labeling, 85% of activity was present in the form of MIBG and only 9% was

in the form of iodoanisole. It was also found that 56% of the initial activity was present in the form of labeled MIBG at the end of purification. These yields are comparable to those in procedures that rely only on isotopic exchange between cold MIBG and iodide. Additionally, the addition of no carrier in this procedure results in a solution that has significantly higher specific activity.

The effects of reaction pH, deprotection time, labeling solution, and HPLC solvent were examined on this reaction. It was found that an acidic surplus of 1M AcOH produced the highest amount of labeled product and the least amount of labeled side-product. A deprotection time of 7 minutes was found to be sufficient to fully deprotect the product. Longer deprotection times were found to only increase formation of the by-product MCBG. Labeling solutions containing both CH₃CN and toluene were found to produce best yields in regards to both labeling percentage and transfer percentage. A 20% CH₃CN:KH₂PO₄ solution was chosen for HPLC because it allowed for separation of MIBG from MCBG while retaining a reasonable elution time.

Removal of leftover precursor before deprotection significantly increased the specific activity of the product. This optimization increased specific activity of the procedure 8-fold. When compared to the radioiodine-for-iodine method, the post-optimized procedure produced MIBG 137-fold higher in specific activity. Removal of precursor also prevented the formation of MCBG, making HPLC purification easier. The results of cell studies were inconclusive and the assessment of the studies is ongoing. These results show that iodonium chemistry is a novel and effective way to radioiodinate aromatic tracers and that this method may be applicable for similar compounds.

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