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Research Article

# A tin precursor for the synthesis of no-carrier-added [*I]MIBG and [$^{211}$At]MABG

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**Abstract:** Radioiodinated MIBG has shown considerable promise as an imaging agent for cardiac and oncologic applications, and also as a targeted radiotherapeutic for treating patients with neuroendocrine tumors. This radiolabeled agent, synthesized at a no-carrier-added level, has demonstrated advantages over the carrier-added preparation in preliminary clinical studies. Earlier we developed a silicon precursor from which both radioiodinated MIBG and the α-particle-emitting $^{211}$At analog [$^{211}$At]MABG could be synthesized at a no-carrier-added level. In order to increase the practicality of this approach, we have developed a synthesis of a tin precursor in two steps from a readily available starting material. This tin precursor, *N*, *N*′-bis(*tert*-butyloxycarbonyl)-3-(trimethylstannyl)benzylguanidine (Bis-Boc MTMSBG) was evaluated for the synthesis of n.c.a. [*I]MIBG and [$^{211}$At]MABG via halodestannylation. The radiochemical yields were 83 ± 9% ($n = 7$), 30 ± 21% ($n = 2$), 77 ± 2% ($n = 2$), and 66 ± 7% ($n = 4$) for labeling with $^{131}$I, $^{124}$I, $^{125}$I, and $^{211}$At, respectively. Copyright © 2007 John Wiley & Sons, Ltd.

**Keywords:** MIBG; tin precursor; radioiodine; Astatine-211

## Introduction

Radioiodinated *meta*-iodobenzylguanidines (MIBG) are one of the most prominent types of radiopharmaceuticals with more than 2400 research articles involving MIBG having been published since their discovery in 1980. MIBG labeled with $^{123}$I and $^{131}$I has been routinely used for diagnostic imaging of the pathophysiology of heart and neuroendocrine tumors such as neuroblastoma.[1,2] The synthesis of [$^{124}$I]MIBG, useful for positron emission tomography (PET), has also been reported.[3] In addition, [$^{131}$I]MIBG has been used extensively in the treatment of neuroendocrine tumors,[4] and the feasibility of using $^{125}$I-labeled MIBG for therapy has been explored.[5]

Radiolabeled MIBG preparations currently used in the clinic are synthesized by the isotopic exchange method.[6,7] This preparation method has several intrinsic disadvantages. First, the uptake-1 mechanism responsible for MIBG transport is saturable[8,9] and second, the high amounts of unlabeled MIBG present in therapy formulations can cause pharmacological effects. To avert these potential problems, we have developed a method to synthesize radioiodinated MIBG at a no-carrier-added (n.c.a.) level from a silicon precursor.[10] It was possible to synthesize *meta*-[$^{211}$At]astatobenzylguanidine ([$^{211}$At]MABG), a MIBG analog tagged with the α-particle-emitting heavy halogen $^{211}$At, using the same precursor.[11] Other methods to synthesize n.c.a. [*I]MIBG have been described subsequently.[12–15]

Our original method involves the radiohalogenation of a silicon precursor, which was synthesized in multiple steps.[10] Although better radiochemical yields can be obtained from a tin precursor,[16] at that time, our attempts to synthesize a tin precursor of MIBG were not successful. However, within the last 10–15 years, methods for the synthesis of protected guanidine derivatives have been developed[17] and these methods have made the synthesis of guanidine-containing compounds very facile. Unlike the free guanidines, protected derivatives are nonpolar, and therefore, are amenable to normal-phase chromatographic purifications. For example, we have reported the synthesis of *N*, *N*′-bis(*tert*-butyloxycarbonyl)-3-iodobenzylguanidine

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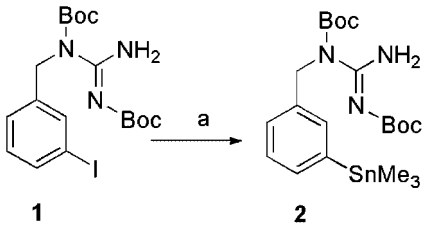


**Figure 1** Scheme for the synthesis of *N*, *N*′-bis(*tert*-butyloxycarbonyl)-3-(trimethylstannyl)benzylguanidine.

(Bis-Boc MIBG; **1**; Figure 1), which is a protected derivative of MIBG.[18] The current paper extends this approach to the synthesis of the corresponding tin precursor, *N*, *N*′-bis(*tert*-butyloxycarbonyl)-3-(trimethylstannyl)benzylguanidine (Bis-Boc MTMSBG; **2**; Figure 1). We describe its conversion to n.c.a. MIBG labeled with $^{131}$I, $^{125}$I, and $^{124}$I and to [$^{211}$At]MABG.

## Results and discussion

Several clinical trials have been undertaken to evaluate n.c.a. [*I]MIBG.[13,19–22] A recent report lamented the limited availability of n.c.a. [*I]MIBG as a possible cause for the lack of interest in exploiting the potential advantages of this tracer.[23] Molecular Insight Pharmaceuticals, Inc. (MIP; Boston, MA) in collaboration with MDS Nordion (Vancouver, Canada) is optimizing the synthesis of n.c.a. [*I]MIBG on a large scale (personal communications), and MIP has just initiated a phase I clinical study to determine its dosimetry, metabolism and pharmacokinetics. Nonetheless, n.c.a. [*I]MIBG is currently not available to most research groups wishing to utilize this tracer for studies in cell culture, animal models and clinical studies.

In the work reported herein, we have presented an alternative strategy for the synthesis of n.c.a. [*I]MIBG and [$^{211}$At]MABG. In addition to its ease of radiohalogenation compared with labeling via a silicon precursor, there was another motivation for exploring the synthesis of MIBG from a tin precursor. This tin precursor can be made from commercially available materials in two steps compared to the four steps used for the synthesis of silicon precursor we reported earlier.[10] The method used by MIP to synthesize n.c.a. [$^{131}$I]MIBG is that of Hunter and Zhu[12] using the resin-bound tin precursor. While labeled MIBG can be prepared with ease using this precursor, the precursor is not commercially available and its synthesis may be too difficult for many laboratories. On the other hand, the tin precursor described herein can be made easily. In addition, we speculate that the synthesis of a solid support-immobilized tin precursor such as those described by Hunter and Zhu[12] and Valliant *et al.*[15] might be easier using the protected MIBG described in this study. It should be pointed out that Samnick *et al.*[13] have reported the synthesis of n.c.a. [$^{123}$I]MIBG from a tin precursor; however, they refer to another publication for the synthesis of the tin precursor. This publication is a meeting abstract and actually describes the synthesis of some fatty acid tin derivatives.[24]

There are a number of ways by which the tin precursor **2** can be synthesized. The synthesis of both 3-tributylstannylbenzyl alcohol[25] and 3-tributylstannylbenzylamine[10] has been reported. The tin precursor **2** could have been prepared from these by the guanidinylation methods reported.[26,27] However, we opted to convert **1** to **2** by the palladium-catalyzed stannylation (Figure 1). A yield of 57% was obtained for this conversion and the NMR and mass spectral data were consistent with the structure of **2**. Lack of stability does not seem to be an issue because a batch that was synthesized more than 2 years ago and stored at 4°C is still intact.

Radiochemical yields for the radioiododestannylation of **2** (Figure 2) were dependent on the radionuclide (Table 1) presumably due to differences in factors including source, specific activity, oxidation state, impurities and age. With $^{131}$I, the yields were very good, the average yield being 83 $\pm$ 9% ($n = 7$), comparable to yields reported for the synthesis of n.c.a. [$^{131}$I]MIBG from the silicon precursor.[10] However, the reaction time is longer for the current method. We used a reaction time of 10 min in this study and have not explored the possibility of using shorter time periods. Although Boc groups are expected to be removed under the initial reaction conditions namely at 70°C in acetic acid, to ensure their complete removal the reaction mixture was additionally treated with TFA necessitating the use of an extra 10 min. Only two reactions each were conducted for $^{125}$I and $^{124}$I. The radiochemical yield for [$^{125}$I]MIBG synthesis (77 $\pm$ 2%; $n = 2$) was slightly lower than that obtained for [$^{131}$I]MIBG; however, the HPLC of its reaction mixture showed, like in the case of $^{131}$I, mainly one radioactive peak corresponding to MIBG (Figure 3). Radiochemical yields for



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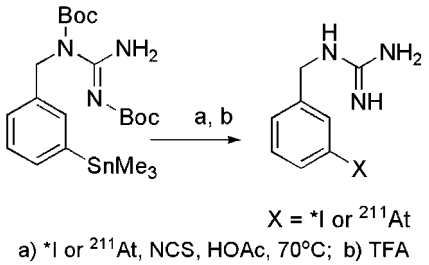


**Figure 2** Radiohalogenation of the tin precursor to [*I]MIBG and [$^{211}$At]MABG.

**Table 1** Radiochemical yields for the synthesis of [*]MIBG and [$^{211}$At]MABG from tin precursor

| Serial number | Isotope | Starting radioactivity (μCi) | %Radiochemical yield[a] |
|---|---|---|---|
| 1 | Iodine-131 | 880 | 86 |
| 2 | — | 763 | 65 |
| 3 | — | 845 | 91 |
| 4 | — | 1059 | 80 |
| 5 | — | 792 | 91 |
| 6 | — | 125 | 79 |
| 7 | — | 1331 | 86 |
| 8 | Iodine-125 | 310 | 75 |
| 9 | — | 279 | 78 |
| 10 | Iodine-124 | 2800 | 44 |
| 11 | — | 1110 | 15 |
| 12 | Astatine-211 | 1824 | 35 |
| 13 | — | 435 | 45 |
| 14 | — | 3204 | 66 |
| 15 | — | 6486 | 70 |
| 16 | — | 3087 | 56 |
| 17 | — | 3634 | 70 |

[a]Not corrected for decay.

$^{124}$I were 44 and 15% for the two reactions. Contrary to $^{125}$I runs, the HPLC of the crude reaction mixture was rather messy (Figure 3). Lower radiochemical yields (20–40%) for $^{124}$I-labeling, compared to $^{125}$I (60–70%), have been reported for the synthesis of *N*-hydroxysuccinimidyl 4-[*I]iodobenzoate.[28] Addition of carrier sodium iodide to improve the radiochemical synthesis of $^{124}$I-labeled Bolton–Hunter reagent has been recommended.[29] More reactions need to be done in order to optimize the synthesis of n.c.a. [$^{124}$I]MIBG for routine use in PET.

The radiochemical yields for labeling **2** with $^{211}$At were 57 ± 15% ($n = 7$; not decay corrected). However, it should be noted that in reaction 12 (Table 1), there was a radioactive peak with a retention time corresponding to **1** and 50% of the injected radioactivity was associated with this. Also for reaction 13, the HPLC of crude reaction mixture showed one major radioactive peak corresponding to MABG. Average yields for reactions 14–17 is 66 ± 7%. This is lower than that reported for the synthesis of [$^{211}$At]MABG from the silicon precursor.[11] This result, and to a certain degree that obtained with $^{131}$I, is counterintuitive but the nature of the solvent might have a role in the observed difference. The solvent used for radiohalodesilylation was trifluoroacetic acid and we have observed higher yields for radioiododesilylation with this solvent compared to acetic acid.[10] However, the lability of carbon–tin bonds to trifluoroacetic acid precludes the use of this solvent in halodestannylation reactions. Radiochemical purity of all labeled MIBG derivatives was more than 95% as demonstrated by HPLC (Figure 4) and radio-TLC (data not shown). No significant peaks were seen in the UV profiles of these HPLC runs.

## Experimental

### General

All chemicals were purchased from Sigma-Aldrich unless otherwise noted. Sodium [$^{125}$I]iodide and sodium [$^{131}$I]iodide with specific activities of 2200 and 1200 Ci/mmol, respectively, were obtained from Perkin Elmer Life and Analytical Sciences (Boston, MA). Sodium [$^{124}$I]iodide with a specific activity of ~2800 Ci/mmol was obtained from IBA Molecular (Sterling, VA). *N*, *N*′-bis(*tert*-butyloxycarbonyl)-3-iodobenzylguanidine (bis-Boc-MIBG; **1**) was synthesized as reported before.[18]

Aluminum-backed sheets (Silica gel 60 F254) were used for analytical TLC, and normal-phase column chromatography was performed using silica gel 60, both obtained from EM Science (Gibbstown, NJ). Column chromatographic fractions were collected using a Gilson model 203 microfraction collector (Middleton, WI) or an ISCO Foxy 200 fraction collector (Lincoln, NE), and the products were identified by TLC. In some cases, an ISCO UA-6 UV-visible detector was placed between the column outlet and the fraction collector to identify products. Preparative thick layer chromatography was performed using 20 × 20 cm,



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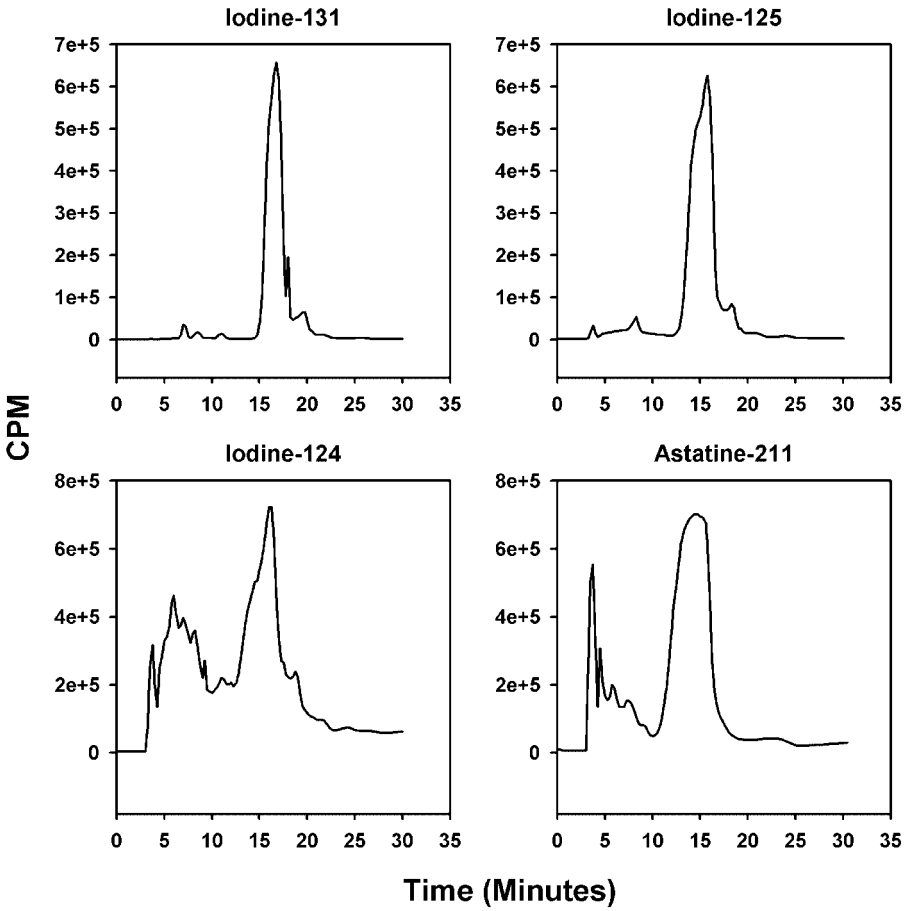


**Figure 3** Typical HPLC radiochromatograms of reaction mixtures obtained from the radiohalodestannylation of **2**.

1000 μm plates (Whatman, Clifton, NJ). Before applying the sample, the plates were run in ethyl acetate to remove any adsorbed impurities. Radio-TLC was initially analyzed using a System 200 Imaging Scanner (BioScan, Washington, DC); sheets were then cut into strips and counted using an automated gamma counter (LKB 1282, Wallac, Finland). High-pressure liquid chromatography was performed using a Beckman System Gold HPLC equipped with a Model 126 programmable solvent module, a Model 168 diode array detector, a Model 170 radioisotope detector, and a Model 406 analog interface module. Reversed-phase chromatography was performed using a Waters XTerra C18 column (4.6 × 250 mm, 5μ). Proton NMR spectrum was obtained on a Varian Mercury 300 spectrometer. Chemical shifts are reported in δ units; solvent peaks are referenced appropriately. Mass spectral data were obtained using an Agilent LCMS system (Agilent 1100 LC/MSD Trap SL; Agilent Technologies, Palo Alto, CA). High-resolution mass spectral data were obtained using JEOL SX-102 high-resolution mass spectrometer.

### *N*, *N*′-bis(*tert*-butyloxycarbonyl)-3-(trimethylstannyl)-benzylguanidine (bis-Boc-TMSBG; 2)

A mixture of **1** (101 mg; 0.21 mmol), hexamethylditin (550 mg; 1.7 mmol), and $(Ph_3P)_2PdCl_2$ (17 mg; 0.024 mmol) in 10 ml of dry dioxane was heated at 100°C for 1–2 h. The precipitated palladium was removed by filtration through a bed of Celite®, and the Celite® bed was washed with ethyl acetate. Solvents were removed from the combined filtrate by rotary evaporation. The residual orange oil was loaded onto a bed of silica gel, and the nonpolar byproducts were eluted with hexanes. The silica bed was further flushed with ethyl acetate to elute the product and other more polar byproducts. Ethyl acetate fractions were concentrated and the resultant crude mixture was subjected to preparative thin layer chromatography using 10% (v/v) ethyl acetate/hexanes as the eluent to yield 61 mg (57%) of **2** as a colorless oil: $^1$H NMR ($CDCl_3$): 0.27 (s, 9H [$^{119}$Sn-H d]), 1.35 (s, 9H), 1.49 (s, 9H), 5.18 (s, 2H), 7.18–7.30 (m, 2H), 7.38 (dd, 1H), 7.42 (d, 1H), 9.44 (br s, 2H). MS (LCMS) *m/z*: cluster peaks



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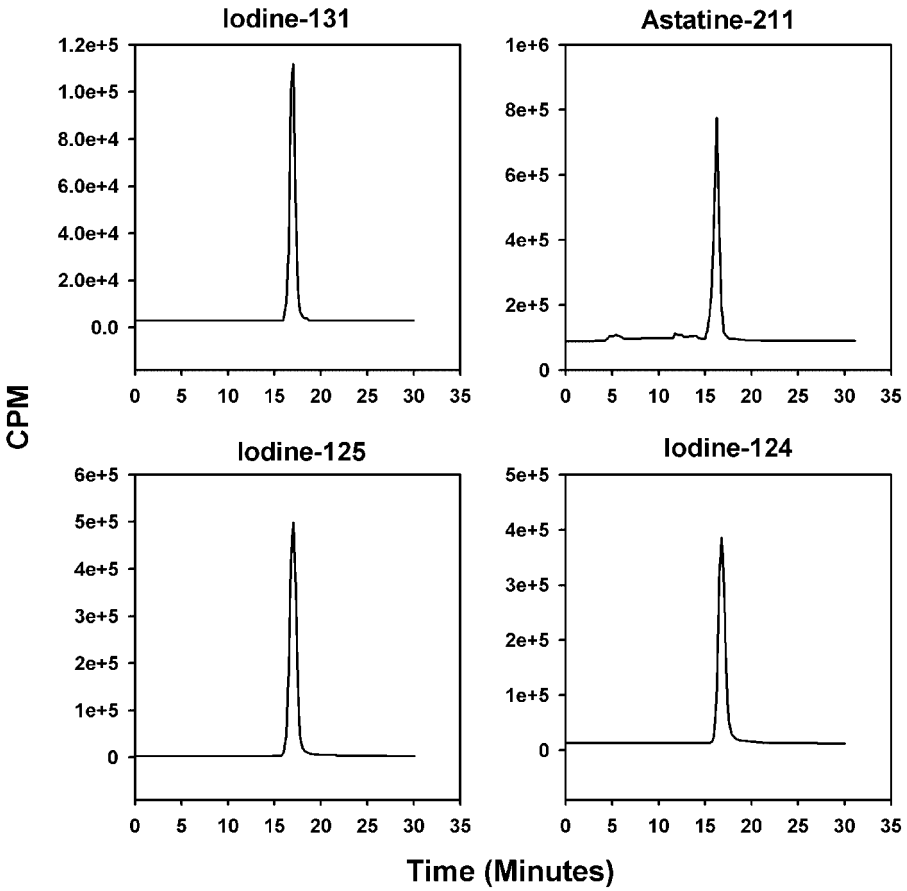


**Figure 4** Radiochromatograms of purified [*I]MIBG and [$^{211}$At]MABG. No detectable peaks were seen in the UV profile.

at 514.1 ($MH^+$), 458.1 ($MH–C_4H_8)^+$; 402.0 ($MH–2C_4H_8)^+$. HRMS ($FAB^+$) calcd. for $C_{21}H_{36}N_3O_4{}^{118}Sn$ ($MH^+$): 512.1716. Found: 512.1718 ± 0.0005 ($n = 4$).

## No-carrier-added [*I]MIBG

*N*-chlorosuccinimide (10 μl of 0.3 M in acetic acid) and **2** (50 μg in 10 μl acetic acid) were added to a 2-dram vial containing ~1 mCi of $^{131}$I in 1–2 μl of 0.1N NaOH. The vial was capped and after thoroughly mixing the contents by vortexing, it was heated at 70°C in an oil bath for 10 min. Trifluoroacetic acid (100 μl) was added to the reaction mixture, which was further heated at 70°C for 10 min. The solvents were evaporated with a gentle stream of argon. Further co-evaporation with 3 × 25 μl MeOH was done to ensure complete removal of residual acetic acid. The radioactivity was taken in μ of MeOH and injected onto a reversed-phase HPLC column that was eluted isocratically with 0.1% TFA in 80:20 water:acetonitrile at a flow rate of 1 ml/min. The fractions containing the radioactivity peak corresponding to MIBG ($t_R = 16.8$ min) was collected, and concentrated by solid-phase extraction as described before.[30] [$^{125}$I]MIBG and [$^{124}$I]MIBG were synthesized similarly. Iodine-124 came as a more dilute solution, and therefore, it was necessary to evaporate the radioactivity solution (25–35 μl) with an argon stream to a volume less than 2 μl before subjecting it to the reaction conditions.

## [$^{211}$At]MABG

The $^{211}$At activity was produced on the Duke University Medical Center CS-30 cyclotron via the $^{209}$Bi(α, 2n)$^{211}$At reaction by bombarding natural bismuth metal targets with 28 MeV α-particles using an MIT-1 internal target system (Cyclotron Inc., Napa, CA).[31] The radioactivity was condensed into a Teflon tube[32] immersed in a dry ice/ethanol bath and subsequently extracted from the tube into methanol. Because $^{211}$At is carrier free (no stable astatine isotopes), its specific activity is assumed to be the theoretical value (~435 Ci per μmol).



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*N*-chlorosuccinimide (3 μmol in 10 μl of acetic acid) and the tin precursor (0.2 mg in 10 μl of acetic acid) were added to a solution of $^{211}$At in methanol (3–7 mCi in about 50 μl) in a $\frac{1}{2}$-dram vial. The vial was vortexed and heated in an oil bath at 70°C for 10 min. The methanol was evaporated with a gentle stream of argon and TFA (100 μl) was added. The vial was further heated at 70°C for another 10 min and TFA was evaporated from the reaction mixture with an argon stream. The radioactive residue was reconstituted in 25 μl of methanol and injected onto HPLC. The remainder of the procedure was the same as described above for [*I]MIBG.

## Conclusions

We have described a synthesis for a tin precursor that was prepared in two steps from commercially available materials. This could be converted to radioiodinated MIBG and [$^{211}$At]MABG in reasonable yields and excellent radiochemical purity. This novel tin precursor provides an additional tool for the synthesis of radiohalogenated MIBG derivatives at a no-carrier-added level.

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