Improved Synthesis of No-Carrier-Added [*I]MIBG and Its Precursor

Friedrich Hammerschmidt, a,∗ Herbert Kvaternik, a,b,c Anna Schweifer, a† Kurt Mereiter, d Reingard M. Aigner b

a Institute of Organic Chemistry, University of Vienna, Währingerstraße 38, 1090 Vienna, Austria
Fax +43(1)42779521; E-mail: friedrich.hammerschmidt@univie.ac.at
b Department of Nuclear Medicine, Medical University of Graz, Auenbruggerplatz 9, 8036 Graz, Austria
Fax +43(316)38512151; E-mail: herbert.kvaternik@medunigraz.at
c Radiopharmaceuticals, Seibersdorf Labor GmbH, 2444 Seibersdorf, Austria
d Institute of Chemical Technologies and Analytics, Vienna University of Technology, Getreidemarkt 9/164SC, 1060 Vienna, Austria

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† Deceased December 29, 2010

Abstract: 3-(Trimethylstannyl)benzyl alcohol was coupled in a Mitsunobu reaction with bis(Boc)-guanidine to give bis(Boc)-protected 3-(trimethylstannyl)benzylguanidine used as precursor for [*I]MIBG. Radioiodination with [*I]iodine generated from [*I]NaI and chloramine-T, removal of Boc groups, and purification by HPLC gave no-carrier-added tracer [*I]MIBG (81% radiochemical yield, 99% chemical purity) used for imaging tumors of neuroendocrine origin. The structures of bis(Boc)-guanidine and bis(Boc)-protected 3-(trimethylstannyl)benzylguanidine were secured by single crystal X-ray structure analyses.

Key words: Mitsunobu reaction, electrophilic aromatic substitution, medicinal chemistry, [*I]iodine, halogenation

m-Iodobenzylguanidine (MIBG) labeled with radioiodine 131I or 123I is an analogue of the adrenergic neuron-blocking agent guanethidine and of norepinephrine being mainly biosynthesized in the adrenal medulla. 1 Since 1980 [*I]MIBG is well-established in diagnostic scintigraphy and widely applied in imaging tumors of neuroendocrine origin, particularly neuroblastoma and pheochromocytoma. 2–4 [*I]MIBG as a norepinephrine analogue is selectively taken up by actively neuroendocrine cells such as those of the adrenal medulla, sympathetic ganglia, and cardiac tissue. 5–7 Our synthesis of [*I]MIBG is also based on iodoestannylation of 3-(trimethylstannyl)benzylguanidine. 14,15 Initially, the preparation of this precursor was tried in two steps by a literature procedure. 16 First, 3-iodobenzylamine was treated with hexamethylditin and tetrakis(triphenylphosphine)palladium(0) to obtain the corresponding 3-(trimethylstannyl)benzylamine, which was described as a crystalline solid (mp 78 °C). 16 However, it was found to be an oil (bp 98 °C/1 mmHg), which was also supported by literature. 17 Furthermore, the yield of stannylation was unsatisfactorily low (25%). The second step was the conversion of the amine with cyanamide in the presence of a catalytic amount of HCl to the guanidine, which was claimed to be an oil. We repeated this transformation, but the result was inconclusive.

Therefore, it was decided to prepare the amine and the guanidine by a different approach (Scheme 1). Halogen–metal exchange in 3-iodobenzyl alcohol (1a) with t-BuLi (3.2 equiv) at −78 °C in anhydrous THF followed by 1.5 equivalents of Me3SnCl gave at best 23% of 3-(trimethylstannyl)benzyl alcohol (2). The yield could be increased to 69%, when t-BuLi was replaced by n-BuLi (3.2 equiv, 90 min, −78 °C, 1.5 equiv of Me3SnCl, THF). The yield was even better (93%), when 3-bromobenzyl alcohol (1b)
was used as the substrate (2.3 equiv of $n$-BuLi for halogen–lithium exchange at –78 °C in THF for 90 min, 2.1 equiv of Me$_3$SnCl). This method was used previously for the preparation of 3-(tributylstannyl)benzyl alcohol.$^{18}$

Scheme 1 Synthesis of precursor 6

The 3-(trimethylstannyl)benzyl alcohol (2) was converted into the azide$^{17}$3 by the Mitsunobu reaction$^{19}$ in 82% yield and then reduced$^{17}$ to the oily benzylamine 4 in 70% yield. The amine had been prepared earlier from (3-bromobenzyl)dimethylamine by Koldobsky et al. in three steps.$^{17}$

The guanidinylation of 4 could have been effected, for example, by reaction with cyanamide,$^{16}$ Mukaiyama’s reagent, and thioureas,$^{20}$ or $N,N'$-bis(tert-butoxycarbonyl)-$N'$-triflylguanidine.$^{21}$ None of this method was tried, as it had been found$^{22}$ in the meantime that the Mitsunobu reaction of alcohol 2 with $N,N'$-bis(tert-butoxycarbonyl)guanidine could give a protected precursor in high yield, which could be purified by flash column chromatography. Alternatively, the benzyl alcohol could be transformed into the bromide, which is then reacted with the sodium salt of $N,N'$-bis(tert-butoxycarbonyl)guanidine.$^{23}$ As the Mitsunobu reaction was our first choice, the protected guanidine was prepared first and the literature procedures were optimized (Scheme 2). Commercially available $S$-methylisothiourea hemisulfate was Boc-protected$^{24}$ in 91% yield in the biphasic system NaHCO$_3$/H$_2$O/Boc$_2$O/CH$_2$Cl$_2$. $N$-tert-Butyloxycarbonylmethylisothiourea (8), formed as a side product (8%), could be removed easily by flash chromatography. Exchange of the methylthio group for amine with ammonia in methanol furnished the bis(Boc)-protected guanidine 9a in 95% yield.$^{22}$ Interestingly, Feichtinger et al. reported that 9b was formed from guanidine and Boc$_3$O under strong alkaline conditions in 59% yield.$^{21}$ In the literature, structures$^{19,22}$ 9a and in one case$^{21}$ 9b are given for bis(Boc)-guanidine without providing evidence. To solve this ambiguity, a single crystal X-ray analysis was performed proving structure 9a for the solid state (Figure 1).

Finally, 9a and 3-(trimethylstannyl)benzyl alcohol (2) were reacted in a Mitsunobu reaction with Ph$_3$P/DIAD (diisopropyl azodicarboxylate) in anhydrous toluene to give the desired precursor 10, which was isolated by flash chromatography (87%) (Scheme 3).$^{22}$ Satisfyingly, precursor 10 crystallized (from hexanes) to give colorless crystals. The same precursor had been prepared earlier as an oil by the reaction of 3-iodobenzyl bromide with the sodium salt of bis(Boc)-guanidine, followed by exchange of the iodine for trimethyltin using Pd(Ph$_3$P)$_4$/Me$_6$Sn$_2$ (57%).$^{15}$ Iododestannylation and removal of protecting groups furnished the tracer.

When this work was finished, we found that the bis(Boc)-protected guanidine is commercially available. Thus, our synthesis comprises just two steps starting from 3-bromo-benzyl alcohol, which is also commercially available. As the precursor was described as an oil$^{14,15}$ (chemical purity 94%) in the literature and ours was crystalline, and although the $^1$H NMR spectra were virtually identical, we decided to perform a single crystal X-ray structure analysis (Figure 2). Satisfyingly, it proved the structure of com-

Figure 1 Molecular structure of 9a with hydrogen bonds as dashed lines

Scheme 2 Improved synthesis of bis(Boc)-guanidine 9a
Synthesis of No-Carrier-Added [\(^{131}\)I]MIBG

Radioiodination of the precursor \(N,N'\text{-bis(Boc)}\)-\(N\)-(3-trimethylstannyllbenzyl)guanidine (10) with no-carrier-added \([^{131}\text{I}]\text{NaI and N-chlorosuccinimide (NCS) as an in situ oxidizing agent has been recently reported. Rossouw et al. described the use of 2000 \(\mu\text{g} \) of NCS as an oxidant – a 10-fold mass excess relative to precursor 10 – which appeared to us as nonoptimal. We argued that the large excess of NCS could interfere with the final purification of the tracer by HPLC or by using a solid phase cartridge. Therefore, it was decided to replace NCS with chloramine-T, also a well known oxidizing agent.\(^{25,26}\) The reaction of 200 \(\mu\text{g} \) of 10 dissolved in MeOH–AcOH with no-carrier-added \([^{131}\text{I}]\text{NaI and only 60} \ \mu\text{g} \) of chloramine-T at room temperature resulted in the almost quantitative formation of the bis(Boc)-protected 3-[\(^{131}\text{I}\)]iodobenzylguanidine intermediate \([^{131}\text{I}]\text{I1]. Here, the labeling reaction was monitored by radio TLC. Since the reaction mixture contained acetic acid, a slight removal of the Boc groups from \([^{131}\text{I}]\text{I1} \) was observed already during labeling. After deactivation of the oxidant with sodium metabisulfite, the complete removal of the protecting groups was performed with \(\text{CF}_3\text{CO}_2\text{H} \) at 110 °C for 10 minutes to give the crude \([^{131}\text{I}]\text{MIBG ([^{131}\text{I}]\text{I2]}. After neutralization with NaOH, the labeled \([^{131}\text{I}]\text{MIBG was purified by semi-preparative HPLC. Up to 98% of the starting radioactivity could be isolated as \([^{131}\text{I}]\text{MIBG. A small amount of unreacted \([^{131}\text{I}]\text{NaI was detected in the reaction mixture, but no significant quantities of by-products were found. The solvent was gently removed at 35 °C under reduced pressure from the pooled fractions containing \([^{131}\text{I}]\text{MIBG. The dry residue was redissolved in phosphate-buffered saline–5% EtOH. To prevent decomposition by radiolysis 2,5-dihydroxybenzoic acid and benzyl alcohol were added as preservatives.}^{27,28}\) \([^{131}\text{I}]\text{MIBG was isolated with an overall radiochemical yield of 81 ± 3% (n = 4).}

The purity of \([^{131}\text{I}]\text{MIBG was assessed with radio TLC and radio HPLC by comparison with an authentic nonradioactive reference sample. The amount of cold MIBG (2 \(\mu\text{g} \) in a batch of \([^{131}\text{I}]\text{MIBG, was determined by HPLC, resulting in a specific activity of >11 GBq/\text{mol. The radiochemical purity of the isolated \([^{131}\text{I}]\text{MIBG was >99% and the final formulations remained stable for more than 20 hours.}

In conclusion, we have developed a concise synthesis of a trimethylstannylated precursor, which allows a no-carrier-added labeling with radiiodine to form \([^*\text{I}]\text{MIBG. As a novel approach, the radiolabeling reaction was carried out successfully using chloramine-T as oxidant. It was shown that the \([^*\text{I}]\text{MIBG could be obtained in high yield and purity under mild reaction conditions. The labeling process appears to be amenable for automation, which is applicable to all radioiodine isotopes and allows large scale routine production of \([^*\text{I}]\text{MIBG for radiopharmaceutical use.}

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mmol), di(tert-butyl) carbonate (0.85 g, 2.25 mmol, 2 equiv) and Ph,P (0.443 g, 1.689 mmol, 1.5 equiv) in anhyd toluene (10 mL) was cooled in an ice bath under argon. 3-(Trimethylstannyl)benzyl alcohol (2; 0.305 g, 1.126 mmol) dissolved in anhyd toluene (2 mL) and DIAD (0.321 g, 0.31 mL, 1.689 mmol, 1.5 equiv) were added and the stirring was continued for 5 h at r.t. The solvent was evaporated under reduced pressure and the residue was flash chromatography (CH2Cl2–hexanes, 3:1) to give precursor 9a (0.793 g, 95%) as colorless crystals; mp 144–145 °C (hexanes). The 1H NMR spectrum was identical to the one reported in the literature;24 no 13C NMR spectrum was given.

N,N'-Bis(tert-butoxycarbonyl)-3-(3-trimethylstannylbenzyl)guanidine (10)

1H NMR (CDCl3): δ = 8.06–7.74 (m, 4 H), 7.45–7.14 (m, 4 H), 4.67 (s, 2 H), 1.47 and 1.33 (2 s, each 9 H), 0.25 [s, (117/119)Sn,C = 35.9 Hz], 128.2 [3 C, (117/119)Sn,C = 45.9 Hz], 65.6, –9.6 [3 C, (117/119)Sn,C = 10.7 Hz], 128 ± 2.25 mmol, 2 equiv) and Ph,P (0.443 g, 1.689 mmol, 1.5 equiv) in anhyd toluene (10 mL) was cooled in an ice bath under argon. 3-(Trimethylstannyl)benzyl alcohol (2; 0.305 g, 1.126 mmol) dissolved in anhyd toluene (2 mL) and DIAD (0.321 g, 0.31 mL, 1.689 mmol, 1.5 equiv) were added and the stirring was continued for 5 h at r.t. The solvent was evaporated under reduced pressure and the residue was flash chromatography (CH2Cl2–hexanes, 3:1) to give precursor 10 (0.503 g, 87%) as colorless crystals; mp 89–90 °C (hexanes, r.t. to –18 °C). IR (Si, film): 3411, 2970, 1718, 1629, 1240, 1149 cm–1. The 1H NMR spectrum is identical with that of literature;25 no 13C NMR and IR spectra were given.

Crystal Structure

Data: C9H23N4O6, FW = 259.31, colorless prism of 0.57 ± 0.28 × 0.26 mm from hexanes, T = 100(2) K, triclinic, space group P-1, α = 11.6836(5) Å, β = 16.1253(6) Å, γ = 16.3857(6) Å, V = 884.42(2) Å3, β = 84.200(2)°, γ = 70.064(2)°, V = 2887.15(19) Å3. Z = 8 (Z’ = 4), D = 1.193 g·cm–3, μ = 0.091 mm–1, λ(MoKα) = 0.71073 Å. Of 68471 reflections measured, 16711 were unique. Refinement of F2 using the program SHELXTL31 concluded with R = 0.0664 and wR2 = 0.1229 for 689 parameters and all data. The structure contains four chemically identical but crystallographically different molecules assembled in bis-NH···N hydrogen bonded pairs.

N,N'-Bis(tert-butoxycarbonyl)-N-(3-trimethylstannylbenzyl)guanidine (10)

A solution of N,N'-bis(tert-butoxycarbonyl)guanidine (9a; 0.583 g, 2.252 mmol, 2 equiv) and Ph,P (0.443 g, 1.689 mmol, 1.5 equiv) in anhyd toluene (10 mL) was cooled in an ice bath under argon. 3-(Trimethylstannyl)benzyl alcohol (2; 0.305 g, 1.126 mmol) dissolved in anhyd toluene (2 mL) and DIAD (0.321 g, 0.31 mL, 1.689 mmol, 1.5 equiv) were added and the stirring was continued for 5 h at r.t. (TLC: hexanes–EtOAc, 5:1, Rf = 0.85). The solvent was evaporated under reduced pressure and the residue was flash chromatography (CH2Cl2–hexanes, 3:1) to give precursor 10 (0.503 g, 87%) as colorless crystals; mp 89–90 °C (hexanes, r.t. to –18 °C). IR (Si, film): 3380, 2970, 1716, 1629, 1240, 1149 cm–1. The 1H NMR spectrum is identical with that of literature;25 no 13C NMR and IR spectra were given.

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Anal. Caled for C29H39N3O4Sn: C, 49.24; H, 6.89; N, 8.20. Found: C, 49.57; H, 6.99; N, 8.27.

Crystal Structure
Crystall data: C29H39N3O4Sn, FW = 512.21, colorless oval of 0.45 × 0.34 × 0.28 mm from DMF, T = 100(2) K, monoclinic, space group P21/c, a = 11.8026(7) Å, b = 12.7568(6) Å, c = 16.6750(10) Å, β = 102.388(1)°, V = 2452(3) Å3, Z = 4, Dc = 1.388 g cm−3, μ = 1.071 mm−1, Λ(MoKα) = 0.71073 Å. Of 4417 reflections measured, 7150 were unique. Refinement of F2 using the program SHELEX31 concluded with R = 0.0252 and wR2 = 0.0626 for 271 parameters and all data.

3-[131I]Iodobenzylguanidine ([131I]MIBG, [131I]12)
The labeling was performed in a sealed conical vial. To a solution of 0.05 M phosphate-buffered saline (pH 4.5)–EtOH–benzyl alcohol (100 μL), and [131I]NaI (in 0.1 M NaOH, 6 μL, 45.9 MBq) the labeling was initiated by the addition of an aq solution of chloramine-T (20 μL, 2.8 mg/mL). The reaction mixture was stirred at r.t. for 10 min to form the intermediate [131I]11 (TLC: MeOH–2 M ammonia–1 M NH4NO3, 27:2:1; Rf = 0.77). The reaction was quenched with aq Na2S2O5 (50 μL, 4 mg/mL). Afterwards, CF3CO2H (100 μL) was added and the sealed vial was heated for 15 min at 110 °C to give [131I]12. The reaction mixture was adjusted to pH 4 with aq 2 M NaOH (600 μL) and the crude product was purified by hPLC [Prontosil C18-asf-EP2 column, 5 μm, 10 × 250 mm, Bischoff; liquid phase: MeOH–0.05 M phosphate buffer (pH 4.5) (1:1), 3 mL/min; tR = 11 min]. To the isolated fraction (44.6 μL, 4 mg/mL). Afterwards, CF3CO2H (100 μL) was added and the sealed vial was heated for 15 min at 110 °C to give [131I]11. The reaction mixture was adjusted to pH 4 with aq 2 M NaOH (600 μL) and the crude product was purified by hPLC [Prontosil C18-asf-EP2 column, 5 μm, 10 × 250 mm, Bischoff; liquid phase: MeOH–0.05 M phosphate buffer pH 4.5 (4:6); flow rate: 1 mL/min; detection: λ = 254 nm; tR = 8.2 min.}

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References
(30) CCDC 873052 (9a) and 859213 (10) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre via www.ccdc.ac.uk/data_request/cif.