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# Syntheses of stable-isotope labeled [M + 7] and [M + 6] 2-(methylamino)imidazole

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## Summary

Stable isotope-labeled 2-methylaminoimidazole (M + 7 and M + 6) was required as an intermediate in the synthesis of mass labeled drug candidates. These two isotopomers were synthesized with total yields of 24 and 36%, respectively. Labeled 2-aminoimidazole (M + 4) was prepared from labeled isothiourea (M + 3) and 2-aminoacetaldehyde dimethyl acetal (M + 1 and M + 2). The (M + 1) version of 2-aminoacetaldehyde dimethyl acetal was obtained in two steps starting with potassium [$^{15}$N]phthalimide, while the (M + 2) version was prepared from the reduction of diethoxyacetamide with $LiAlD_4$. Two different approaches for the preparation of 2-methylaminoimidazole from aminoimidazole were explored. Attempts to prepare protected 2-aminoimidazole to couple with $CH_3I$ (M + 4) to form the desired labeled 2-methyl-aminoimidazole failed. However, methylation was achieved by applying *N*-formamidation followed by deutero-reduction. These successful syntheses allowed us to selectively label with nitrogen, carbon or hydrogen isotopes at most of the positions of 2-methylaminoimidazole. 

**Key Words:** 2-[$^{13}$C,$^{2}H_2$]Methyl[$^{15}$N]amino[2-$^{13}$C–1,3-$^{15}N_2$]imidazole; 2-[$^{2}H_2$]-methyl[$^{15}$N]amino[2-$^{13}$C-1-$^{15}$N-3-$^{2}$H]imidazole; N-1H-[2-$^{13}$C,1,3-$^{15}N_2$] Imidazol-2-yl-[$^{13}$C,$^{15}$N]formamide; N-1H-[2-$^{13}$C,4-$^{2}$H,1,3-$^{15}N_2$]Imidazol-2-yl-formamide; [$^{13}$C,$^{15}N_2$]thiourea

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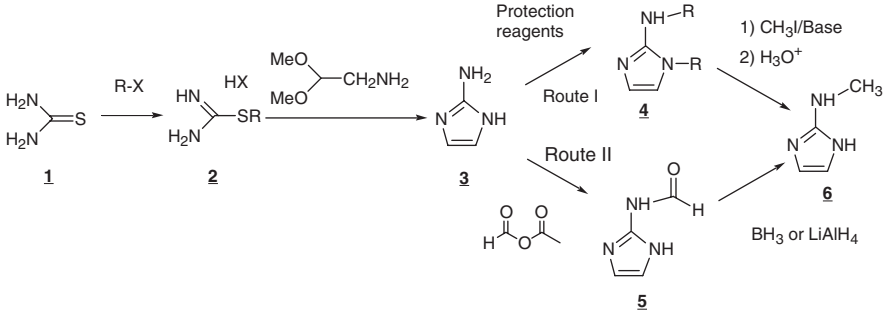
## Introduction

Stable isotope-labeled 2-methylaminoimidazole was required as an intermediate in the synthesis of mass labeled drug candidates. In this paper we wish to report efficient syntheses of 2-[$^{13}$C, $^{2}H_2$]methyl-[$^{15}$N]amino-[2-$^{13}$C –1,3-$^{15}N_2$]imidazole HCl salt **14** (M + 7) and 2-[$^{2}H_2$]methyl-[$^{15}$N]amino-[2-$^{13}$C-1-$^{15}$N-4-$^{2}$H]imidazole HCl salt **20** (M + 6).

The syntheses of stable isotope-labeled 2-methylaminoimidazole and 2-aminoimidazole were not reported in the literature before, although unlabeled 2-aminoimidazole was prepared from *S*-methylisothiourea and 2-aminoacetaldehyde dimethyl acetal.[1] Considering the supply of labeled starting materials, two routes to synthesize isotope-labeled 2-methylaminoimidazole (M + 7 and M + 6) were proposed and studied (Scheme 1).




**Scheme 1. Two possible synthetic approaches to 2-(methylamino)imidazole**

## Results and discussion

An initial thought was to introduce relatively cheap $^{13}CD_3I$ into 2-aminoimidazole (Route I, Scheme 1), so that the mass of the methylated molecule could be increased by 4 units. In order to achieve the goal, the amino group at the 2 position of imidazole must be activated, while the amino group in the imidazole ring must be protected. There are several general methods to introduce a methyl group into a protected amine.[2–5] However, most of them are not suitable for making 2-methylaminoimidazole from 2-aminoimidazole since the two amino groups give rise to non-selective methylation.

Protection of imidazole **3** was unsuccessful using *t*-butyldimethylsilyl chloride (excess), isobutyl or ethyl chloroformate, or 2-nitrobenzene-

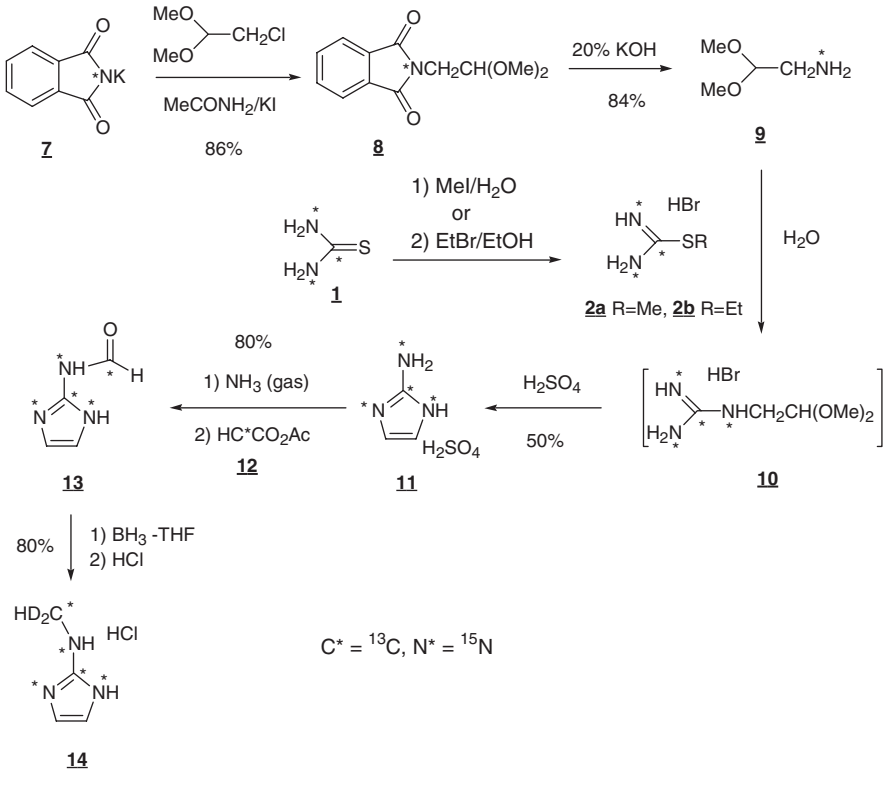
 

sulfonyl chloride. The treatment of imidazole **3** with excess *t*-butyldimethylsilyl chloride only provided mono substituted compound. The further methylation by MeI in the presence of *n*-BuLi gave an undesired 1-methyl-2-aminoimidazole. From the reaction of imidazole **3** with isobutyl chloroformate, a mono $N_1$-substituted compound as a major by-product was separated in 59% yield. With excess other reagents, such as ethyl chloroformate and 2-nitrobenzene-sulfonyl chloride, the reactions afforded a complicated mixture. Considering the difficulties in preparing a suitable protected imidazole for methylation by MeI, an alternate route (Route II, Scheme 1) involving *N*-formamidation and reduction was studied. Our investigation showed that the formamidation of 2-aminoimidazole with acetic formic anhydride provided only 2-formamidoimidazole **5** in good yield.[6] Reduction of 2-formamidoimidazole **5** with $BH_3THF$ afforded the desired 2-methylaminoimidazole **6** in high yield.

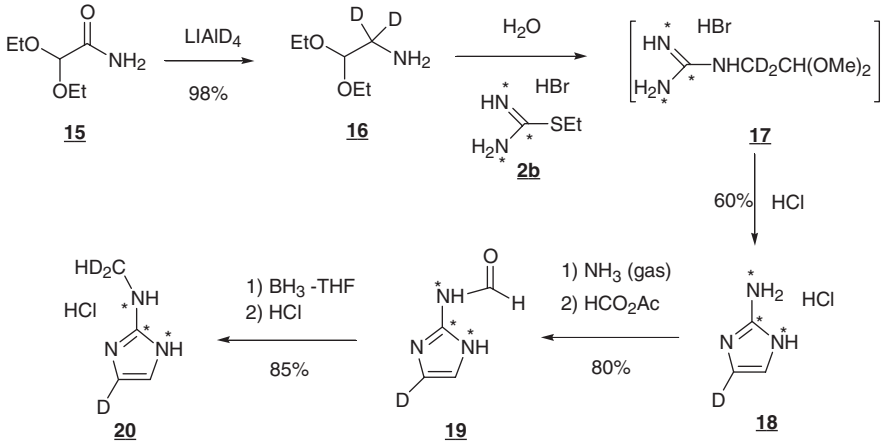
The entire synthesis of the title compound (M + 7) using Route II is shown in Scheme 2. By using acetamide as solvent instead of DMF in the *N*-alkylation of potassium [$^{15}$N]phthalimide with chloroacetaldehyde dimethyl acetal, we were able to obtain product **8** in 86% yield (vs 59% with DMF). Hydrolysis of compound **8** with 20% KOH solution afforded 2-[$^{15}$N]aminoacetaldehyde dimethyl acetal **9** with high purity and yield (84%). Initially, [$^{13}$C, $^{15}N_2$]*S*-methylisothiourea **2a** was synthesized by the treatment of [$^{13}$C, $^{15}N_2$]thiourea **1** with methyl iodide. Compound **2a** was then heated with 2-[$^{15}$N]aminoacetaldehyde dimethyl acetal in water followed by the treatment with concentrated $H_2SO_4$ to obtain 2-[$^{13}$C,$^{15}N_3$]aminoimidazole in 30% yield.[1] However, changing reagents from MeI to EtBr, and solvents from $H_2O$ to EtOH allowed us to achieve a higher yield of 2-[$^{13}$C, $^{15}N_3$]aminoimidazole **11** (50% vs. 30%). The reaction of 2-[$^{13}$C, $^{15}N_3$]aminoimidazole **11** with mixed anhydride **12** derived from 95% [$^{13}$C]formic acid in the presence of $MgSO_4$ provided 2-[$^{13}C_2$, $^{15}N_3$]formamidoimidazole **13** in 80% yield. Compound **13** was then reduced using $BD_3THF$ to provide the desired product **14** as the HCl salt in 80% yield.

The synthesis of the (M + 6) mass version of 2-methylaminoimidazole **20** is shown in Scheme 3. Commercially available diethoxyacetamide **15** was reduced with $LiAlD_4$ to form 2-amino-[2-$^2H_2$]acetaldehyde diethyl acetal **16** in 98% yield by a modified known procedure.[7] The latter was heated with [$^{13}$C, $^{15}N_2$]*S*-ethylisothiourea in water first and then further treated with concentrated HCl instead of concentrated $H_2SO_4$. The treatment with HCl provided the desired product in a higher yield




**Scheme 2. Synthesis of 2-(methylamino)imidazole (M + 7)**




**Scheme 3. Synthesis of 2-(methylamino)imidazole (M + 6)**

(60%) of [$^{13}$C, $^{2}$H, $^{15}N_2$]2-aminoimidazole hydrochloride **18** than the $H_2SO_4$ treatment. The formamidation and reduction reactions of compound **18** were carried out applying the same procedures as for the preparation of compound **14**. The final compound **20** was easily isolated as a HCl salt by the addition of 1 M HCl in ether to the free base of **20**.

## Experimental

All reactions were carried out under an atmosphere of nitrogen unless otherwise stated. $^{1}$H and $^{13}$C NMR spectra were recorded on a Varian Gemini 200 or 400 MHz. Chemical purity of all labeled compounds was determined by HPLC and GC-MS or LC-MS. Purifications were done by flash column chromatography on Biotage Flash 40 system. $LiAlD_4$ (98 at% D), and $BD_3$-THF (1 M, 99 at% D) were purchased from Cambridge Isotope Lab. [$^{15}$N] potassium phthalimide (98 at% $^{15}$N), [$^{13}$C, $^{15}N_2$]thiourea (99 at% $^{13}$C, 99 at% $^{15}$N) and [$^{13}$C]formic acid (95–96%, 99 at% $^{13}$C) were purchased from Isotec. All prepared stable-isotope labeled compounds contained less, 0.1% of unlabeled material based on LC-MS analysis, unless otherwise stated.

### *N-(1,1-Dimethoxylethyl-[$^{15}N$]phthalimide* **8**

A mixture of acetamide (26.0 g, 440 mmol), potassium iodide (7.50 g, 45 mmol) and [$^{15}$N] potassium phthalimide (10.15 g, 54.5 mmol) was heated to 110°C with stirring. To this suspension was added slowly chloroacetaldehyde dimethyl acetal (7.0 ml, 61.5 mmol) for 40 min. The reaction temperature was raised to 150°C for 1 h. The mixture was stirred at 150°C for another 1 h. After the reaction mixture was cooled to room temperature, water (50 ml) was added to the mixture. The off-white solid product **8** was collected by filtration, washed with water ($3 \times 5$ ml), and dried under vacuum (11.09 g, 86%). M.P. 98–100°C; $^{1}$H NMR ($CDCl_3$) δ 7.87-7.82 (m, 2H), 7.73-7.69 (m, 2H), 4.76 (t, $J = 5.82$ Hz, 1H), 3.81 (dd, $J = 1.11$ Hz, $J = 5.68$ Hz, 2H) 3.37 (s, 6H); $^{13}$C NMR ($CDCl_3$) δ 168.52 (d, $J = 12.7$ Hz), 134.49, 132.50 (d, $J = 7.9$ Hz), 123.84, 100.50, 53.68, 39.25 (d, $J = 10.4$ Hz); MS (ESI): 237 [(M + 1) + 1], 236, 205, 147, [M + 0] < 1%.

*[$^{15}N$]Aminoacetaldehyde dimethyl acetal* **9**

Compound **8** (7.0 g, 29.7 mmol) was suspended in KOH aqueous solution (20%, 100 ml), and heated at 100°C for 2 h. After cooling to room temperature, the reaction mixture was extracted with $CHCl_3$ ($5 \times 15$ ml). The $CHCl_3$ extracts were washed with brine (10 ml), and dried over $MgSO_4$. The solvent was evaporated under vacuum to give [$^{15}$N]aminoacetaldehyde dimethyl acetal **9** as a colorless liquid (2.65 g, 84%). $^1$H NMR ($CDCl_3$) δ 4.28 (t, $J = 5.13$ Hz, 1H), 3.38 (s, 6H), 3.77 (dd, $J = 5.13$ Hz, $J = 1.06$ Hz, 2H), 1.15 (2H); $^{13}$C NMR ($CDCl_3$) $\delta$ 106.27, 54.50, 44.14 (d, $J = 3.8$ Hz); MS (ESI): 107 [(M + 1) + 1].

*S-Ethyl-[$^{13}C$, $^{15}N_2$]isothiouronim* **2b**

A suspension of [$^{13}$C, $^{15}N_2$]thiourea (2.0 g, 25.3 mmol) in EtOH (20 ml) and bromoethane (16 ml) was heated at 55°C for 5 h to form a clear solution. Additional bromoethane (16 ml) was added, and the mixture was stirred overnight at 55°C. The solution was cooled to room temperature, and then poured into 500 ml of dry ether with stirring. The oily residue solidified after cooling in an ice bath. The precipitate was filtered off and washed with cold ether ($2 \times 10$ ml) to give S-ethyl-[$^{13}$C, $^{15}N_2$]isothiouronim **2b** as white crystals (4.54 g, 96%). M.P. 88-90°C; $^1$H NMR (DMSO-$d_6$) δ 9.26 (bd, $J = 15.2$ Hz, 2H), 8.81 (bd, $J = 8.43$ Hz, 2H), 3.43-3.13 (m, 2H), 1.27 (t, 3H, $J = 7.33$ Hz); $^{13}$C NMR (DMSO-$d_6$) δ 170.09 (t, $J = 17.0$ Hz), 25.04, 14.34 (d, $J = 1.8$ Hz); MS (ESI): 108 [(M + 3) + 1].

*[$^{13}C$, $^{15}N$]2-Aminoimidazole hydrosulfate* **11**

To a solution of S-ethyl-[$^{13}$C, $^{15}N_2$]isothiouronim **2b** (2.875 g, 15.4 mmol) in water (10 ml) was added [$^{15}$N] aminoacetaldehyde dimethyl acetal **9** (1.689 g, 15.5 mmol). The mixture was heated at 90°C for 1.5 h, and then distilled to remove EtSH. Water (10 ml) was added to the residue. The aqueous phase was extracted with $CHCl_3$ ($3 \times 10$ ml), and evaporated and dried under vacuum to give crude *N*-(2,2-dimethyloxyethyl)guanidine hydrobromide **10** as a glassy semi-solid (3.512 g). $^1$H NMR (DMSO-$d_6$) δ 4.481 (t, 1H), 3.33 (s, 6H), 3.274 (t, 2H); $^{13}$C NMR (DMSO-$d_6$) δ 157.33 (q, $J = 21.2$ Hz), 101.81, 54.26, 42.78 (d, $J = 10.5$ Hz); MS (ESI): 152 [(M + 3) + 1], 120, 102.

This crude product was dissolved in water (50 ml) and concentrated $H_2SO_4$ (1.0 ml), and heated at 110°C for 40 min. The mixture was evaporated with EtOH (3 × 5 ml) and dried under vacuum to give [$^{13}C$, $^{15}N_3$]2-aminoimidazole $H_2SO_4$ salt **11** as a semi-solid (1.42 g, 50%), which was used for the next reaction without further purification. $^1H$ NMR (DMSO-$d_6$/TFA) δ 6.85 (m, 2H); $^{13}C$ NMR δ 147.19 (q, $J = 20.80$ Hz), 113.60 (m); MS (ESI): 88 [(M + 4) + 1].

### *N-1H-[2-$^{13}C$, 1,3-$^{15}N_2$]Imidazol-2-yl-[$^{13}C$, $^{15}N$]formamide* **13**

To acetic anhydride (2.0 ml) was added [$^{13}C$]formic acid (95–96%, 0.8 ml) and $MgSO_4$ (0.2 g) at 0°C. The resulting suspension was warmed to 50°C, and stirred for 5 h. After cooling to room temperature, the anhydride **12** was used in the following reaction without purification.

2-[$^{13}C$, $^{15}N_3$]Aminoimidazole $H_2SO_4$ salt **11** (crude, 2.85 g, ca. 15 mmol) was suspended in a mixture of THF and MeOH (40 ml/5 ml). Dry $NH_3$ gas was passed through the suspension for 2 h. The precipitate was removed by filtration and the filtrate was concentrated under vacuum to give free 2-[$^{13}C$, $^{15}N_3$]aminoimidazole. To a solution of [$^{13}C$]acetic formic anhydride in dry THF (10 ml) was added a solution of 2-[$^{13}C$, $^{15}N_3$]aminoimidazole in THF (40 ml). The mixture was stirred at room temperature for 25 h, and then concentrated to dryness. The residue was dissolved in water (18 ml), and extracted with ether (10 ml) and $CHCl_3$ (10 ml). The aqueous solution was evaporated under vacuum to form about 10 ml of solution, which was neutralized (pH 7) with saturated aqueous $K_2CO_3$ solution. The resulting solid was collected by filtration. Drying under vacuum afforded N-1H-[2-$^{13}C$, 1,3-$^{15}N_2$]imidazol-2-yl-[$^{13}C$, $^{15}N$]formamide **13** (1.39 g, 80%). M.P. 203-205°C; $^1H$ NMR (DMSO-$d_6$/TFA) δ 8.515 (dq, 1H, $J_{N-C} = 207$ Hz, $J_{C-N-C} = 9.44$ Hz), 7.321 (m, 2H) [$^1H$ NMR for unlabeled compound (DMSO-$d_6$/TFA) δ 8.20 (br S, 1H), 6.70 (S, 2H)]; $^{13}C$ NMR (DMSO-$d_6$/TFA) δ 160.84 (d, $J = 9.1$ Hz), 137.22 (q, $J = 21.3$ Hz), 115.10 (m); MS (ESI): 117 [(M + 5) + 1], 115, 108.

### *2-[$^{13}C$, $^2H_2$]Methyl[$^{15}N$]amino[2-$^{13}C$ –1,3-$^{15}N_2$]imidazole HCl salt* **14**

To a cold suspension of [$^{13}C_2$, $^{15}N_3$]2-formamidoimidazole **13** (0.330 g, 2.84 mmol) was injected a solution of $BD_3$THF (1 M, 5.6 ml, 5.6 mmol) for 30 min at 0–2°C. The resulting suspension was warmed to room

temperature, and stirred overnight. After the reaction mixture was cooled to 0°C, MeOH (anhydrous, 8 ml) was added. The reaction solution was adjusted to pH = 2 by adding a solution of 2 M HCl in MeOH. The solution was then heated to reflux for 3 h. After cooling, the mixture was filtered through Celite and concentrated under vacuum. The oily residue was repeatedly concentrated under vacuum from methanol (3 × 5 ml) and then dissolved in ethyl acetate (10 ml). A dry solution of 1 M HCl in ether was added to the mixture to afford 2-[$^{13}$C, $^{2}H_2$]methyl[$^{15}$N]amino[2-$^{13}$C –1,3-$^{15}N_2$]imidazole HCl salt **14** as a white powder (0.318 g, 80%), which was collected by filtration, and dried under vacuum at 40°C. M.P. 141–143°C; $^{1}$H NMR (DMSO-$d_6$/TFA) δ 7.92 (d, 1H), 6.91 (b, 2 H), 3.53 (dt, 1H) [$^{1}$H NMR for unlabeled compound (DMSO-$d_6$/TFA) δ 7.90 (d, 1H), 6.90 (S, 2H), 2.8 (d, 3H)]; $^{13}$C NMR (DMSO-$d_6$/TFA) δ 148.03 (q), 113.50 (m), 29.20 (m); MS (ESI): 105 [(M + 7) + 1].

### *[2-$^{2}H_2$]Aminoacetaldehyde diethyl acetal* **16**

To a suspension of $LiAlD_4$ (10.0 g, 238 mmol) in dry THF (90 ml) was added dropwise a solution of 2,2-diethoxylacetamide (20.0 g, 135.8 mmol) in dry THF (50 ml) at 0°C. The resulting mixture was stirred at 25°C for 30 min, then heated at 75°C for 15 h. The mixture was cooled and quenched with $H_2O$ (10 ml), 15% NaOH(10 ml) and $H_2O$ (30 ml). After stirring for 30 min, the mixture was filtered to remove the white precipitate. The mother liquor was evaporated to give [2-$^{2}H_2$]aminoacetaldehyde diethyl acetal **16** as a colorless liquid (18.0 g, 98%). The product was used in the next reaction without further purification. $^{1}$H NMR ($CDCl_3$) δ 4.39 (s, 1H), 3.68 (m, 2H), 3.3.53 (m, 2H), 1.58 (bs, 2H), 1.20 (t, 6H); C NMR ($CDCl_3$) 104.03, 62.66, 44.12 (t), 15.59; MS (ESI): 136 [(M + 2) + 1], [M + 0] < 1%.

### *[$^{13}$C, $^{2}$H, $^{15}N_2$]2-Aminoimidazole hydrochloride* **18**

To a solution of *S*-ethyl-[$^{13}$C, $^{15}N_2$]isothiouronim **2b** (18.7 g, 99.5 mmol) in water (10 ml) was added [$^{2}H_2$] aminoacetaldehyde dimethyl acetal **16** (18.0 g, 133.3 mmol). The mixture was heated at 90°C for 1.5 h, and then distilled to remove EtSH. Water (30 ml) was added to the residue. The aqueous phase was extracted with $CHCl_3$ (3 × 10 ml), and evaporated and dried under vacuum to give *N*-(2,2-dimethyloxyethyl)[$^{13}$C, $^{2}H_2$,

$^{15}N_2$]guanidine hydrobromide **17** as a glassy foam (25.1 g, 98%). $^1$H NMR (DMSO-$d_6$) δ 4.52 (s, 1H), 3.60 (m, 2H), 3.46 (m, 2H), 1.09 (t, 6H); $^{13}$C NMR (DMSO-$d_6$) δ 157.8 (t, $J = 21.2$ Hz), 119.1 (t), 100.3, 62.8, 15.9; MS (ESI): 181 [(M + 5) + 1].

This crude product was dissolved in concentrated HCl (40 ml), and heated at 110°C for 20 min. The mixture was evaporated under vacuum with EtOH (3 × 5 ml) to give [$^{13}$C, $^2$H, $^{15}N_2$]2-aminoimidazole hydrochloride **18** (7.38 g, 60%) as a foam, which was used for the next reaction without further purification. $^1$H NMR ($D_2O$) δ 6.82 (bs, 1H); $^{13}$C NMR ($D_2O$) δ 147.5 (t); MS (ESI): 88 [(M + 4) + 1].

### *N-1H-[2-$^{13}$C, 4-$^2$H, 1,3-$^{15}N_2$]Imidazol-2-yl-formamide* **19**

The same procedure as for the preparation of N-1H-[2-$^{13}$C, 1,3-$^{15}N_2$]imidazol-2-yl-[$^{13}$C, $^{15}$N]formamide **13** was followed. From crude **18** (8 g, 64.8 mmol) product **19** was obtained as a brownish powder (5.96 g, 80%). $^1$H NMR (DMSO-$d_6$) δ 8.18 (bs, 1H), 6.71 (m, 1H); $^{13}$C NMR (DMSO-$d_6$) δ 140.5 (bm); MS (ESI): 116 [(M + 4) + 1].

### *2-[$^2H_2$]Methyl[[$^{13}$C, $^2$H, $^{15}N_2$]aminoimidazole HCl salt* **20**

The same procedure was followed as for the preparation of 2-[$^{13}$C, $^2H_2$, $^{15}$N]methyl[[$^{13}$C, $^{15}N_2$] aminoimidazole HCl salt **14**. From **19** (2.0 g, 17.4 mmol) product **20** was obtained as a white solid (2.06 g, 85%). M.P. 142–143°C; $^1$H NMR (DMSO-$d_6$) δ 12.29 (t, 1H), 7.80 (db, 1H), 6.88 (m, 1H), 2.81 (m, 1H); $^{13}$C NMR (DMSO-$d_6$) δ 148.4 (t); MS (ESI): 104 [(M + 6) 1].

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