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# Reductive Amination with [$^{11}$C]Formaldehyde: A Versatile Approach to Radiomethylation of Amines

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## ABSTRACT

Carbon-11 radiolabeled amines constitute a very important class of radioligands that are widely used for positron emission tomography (PET) imaging. Radiolabeling of amines is often achieved through radiomethylation using [$^{11}$C]$CH_3I$ or [$^{11}$C]$CH_3OTf$ under basic conditions in a strictly anhydrous environment. Functional groups such as hydroxyl and carboxyl groups that are often present in the molecules are normally base sensitive and require protection and deprotection, which substantially prolongs and complicates the radiolabeling process. Here we report a versatile approach to a series of C-11 radiolabeled amines prepared through reductive amination using [$^{11}$C]formaldehyde. Using a variety of substrates bearing different functional groups, we demonstrate the general utility of this method. In contrast to conventional radiomethylation methods, the reductive amination using [$^{11}$C]formaldehyde can be carried out in an aqueous environment relatively quickly without the need of protection of base-sensitive functional groups.

**Keywords:** C-11 Formaldehyde; Radiomethylation; Reductive Amination; Positron Emission Tomography; Radiolabelling

## 1. Introduction

Molecular imaging has become an indispensible tool in biomedical research. To date, a variety of molecular imaging modalities have been established that complement each other in terms of sensitivity and resolution. Among them, positron emission tomography (PET) and magnetic resonance imaging (MRI) are widely used for clinical studies while optical imaging including multiphoton microscopy, bioluminescence imaging, and fluorescent molecular tomography (FMT) are widely used for preclinical studies. While each imaging modality is designed to detect different signals ranging from high-energy gamma rays to low-energy radiofrequency, use of these imaging modalities largely depends on the availability of endogenous or exogenous molecular probes carrying the required specific signals that can be readily detected at the targets of interest.

Thus, significant efforts have been made to develop molecular probes in line with advancements in mechanical design and optimization of imaging devices. While different sets of chemical and pharmacological properties are required for different modalities, development of molecular probes for PET represents a unique challenge in many aspects. This is largely due to the fact that PET probes must be labeled with positron-emitting radionuclides such as carbon-11 or fluorine-18. The inherent short half-lives of these positron emitters (20 min for C-11 and 110 min for F-18) make radiolabeling a very timeconstrained process. In addition, these radionuclides need to be produced by a cyclotron not only in trace quantities but also in very limited forms. For example, C-11 labelling is normally achieved through radiomethylation using [$^{11}$C]methyliodide [1] or [$^{11}$C]methyltriflate [2]. As a result, the chemical reactions with [$^{11}$C]$CH_3I$ or [$^{11}$C]$CH_3OTf$ must be carried out under very strict, often air- and moisture-free conditions. Often times, a trace amount of water present either in the solvents or reagents reduces radiochemical yields significantly or even abolishes the reactions. Other reactive functional groups such as hydroxyl or carboxyl groups present in the precursors also need to be protected before radiolabeling and subsequently deprotected after radiolabeling, which further prolongs radiosynthesis time and complicates the purification process.

Radiomethylation has been the primary reaction used to radiolabel alkylated amines, which constitute a very important class of PET radiotracers. Various amines have been identified as radiotracers for PET imaging of various molecular targets [3-18]. Given the fact that most amine radiotracers possess functional groups that are

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either base-sensitive or can be readily methylated, C-11 radiolabeling of those functionalized amines must undergo extensive protection and deprotection processes before they are subjected to radiomethylation with [$^{11}$C]$CH_3I$ or [$^{11}$C]$CH_3OTf$ under basic conditions. In order to skip the protection/deprotection steps, efforts have been made to radiolabel certain functionalized amines based on different reactivity toward C-11 methylating reagents. For example, for the radiolabeling of PIB, direct radiomethylation was achieved in an HPLC loop filled with unprotected precursor using [$^{11}$C]$CH_3OTf$ [19]. This is because [$^{11}$C]$CH_3OTf$ reacts with amino groups faster than hydroxyl groups. However, this method is applicable only to certain functionalized amines and is limited to small-production.

In addition to direct methylation, another important approach to synthesizing alkylated amines is reductive amination, which has long been used in organic synthesis. In this reaction, amines are first carbonylated with aldehydes, ketones, or carboxylates to form imines followed by subsequent reduction. To apply this reaction to radiolabeling, C-11-radiolabeled aldehyes, ketones and carboxylic acids need to be prepared. In 2000, Perrio-Huard and coworkers radiolabeled [$^{11}$C]amines through reductive amination using [$^{11}$C]magnesium halide carboxylates, which was converted directly from [$^{11}$C]$CO_2$. In 2003, Van der Meij and coworkers synthesized [$^{11}$C]acetone and used it for reductive amination for radiolabeling of 1-phenyl-piperazine [20]. In the meantime, Langstrom and coworkers established a general approach to [$^{11}$C]ketones through Suzuki coupling [21]. Subsequently, various [$^{11}$C]ketones were transformed to [$^{11}$C]amines through reductive amination. .

Recently, Hooker *et al*. has reported a simple and direct method for the preparation of C-11 formaldehyde ([$^{11}$C]HCHO, see general procedure for synthesis) [22]. This opens the way to radiolabel methylated amines through reductive amination. The advantage of reductive amination over conventional radiomethylation is that the reaction can be carried out in aqueous solution. So the reagents are not necessarily anhydrous. In addition, base-sensitive functional groups present in the molecule do not interfere with the reaction, which eliminates protection /deprotection steps. In this study, we report C-11 labelling of a series of amines through reductive amination using C-11 formaldehyde in order to demonstrate the general utility of this method.

## 2. Results and Discussion

The [$^{11}$C]$CH_3I$ was first converted to [$^{11}$C]formaldehyde in the presence of TMAO and directly used for subsequent reductive amination without further purification (see general procedure for $^{11}$C-reductive amination of target compounds) [23]. To test the versatility of the reaction for C-11 radiolabeling of amines, we used a series of amine precursors bearing functional groups. Functional groups such as hydroxyl or carboxyl are reactive toward [$^{11}$C]$CH_3I$ or [$^{11}$C]$CH_3OTf$ under conventional radiolabeling conditions and ought to be protected first. Through reductive amination with [$^{11}$C]formaldehyde, the aniline derivatives bearing hydroxyl group (1a, 2a, 3a) were directly radiomethylated in good radiochemical yield. In contrast to radiomethylation with [$^{11}$C]$CH_3I$ or [$^{11}$C]$CH_3OTf$, which must be carried out in strictly anhydrous solvents, the reductive animation can be performed in aqueous solvent like PBS. For compounds that are not water-soluble, they can be first dissolved in DMF and diluted with PBS. Thus, the whole process does not require use of any anhydrous solvents or reagents. The reductive amination was fast and could be completed within 5 min. After the reaction, the product can be directly purified by HPLC with >95% purity. The geometry of the hydroxyl group relative to the amino group did not exert significant impact on the radiochemical yield (see Purification for compounds 1b - 7b) [24]. Compared to radiomethylation of 2a and 3a, radiomethylation of 1a gave a relatively lower yield. This may be due to the fact that a cyclic hydrogen bond exists between the amino group and the hydroxyl group in the para-position, deactivating the amino group toward [$^{11}$C]formaldehyde.

The striking versatility of reductive methylation with [$^{11}$C]formaldehyde was also demonstrated by radiomethylation of amines bearing a free carboxyl group (4a). With no protection of the carboxyl group, the amino group can be readily radiomethylated. While conventional radiomethylation using [$^{11}$C]$CH_3I$ or [$^{11}$C]$CH_3OTf$ often requires refluxing at temperature over 120˚C, the reductive amination reaction can be run at relatively low temperatures. The radiochemical yield was significantly improved when the reaction temperature was elevated to 90˚C. Interestingly, adding aqueous PBS to the reaction media dramatically increases the radiochemical yield. At 90˚C, the radiochemical yield was increased ca. 5-fold when the reaction was run in DMF-PBS (1:3) versus in DMF alone.

Radiomethylation using [$^{11}$C]formaldehyde was found to be selective for primary amines. The reaction selectivity for primary amino group was demonstrated in the reductive methylation reaction of serotonin (5a), a widely studied neurotransmitter. Previous radiolabeling of serotonin with [$^{11}$C]$CH_3I$ or [$^{11}$C]$CH_3OTf$ required protection and deprotection of both the hydroxyl and the secondary amino group. However, under the reductive methylation condition, the primary amino group was selectively methylated. The radiomethylation reaction was also temperature sensitive. The radiochemical yield was significantly improved when the reaction was carried out at elevated temperature.

Using this radiomethylation method, we carried out radiosynthesis of PIB using non-protected radiolabeling precursor (6a). Current radiolabeling protocol that has been standardized for clinical trials requires protection of the 6-hydroxyl group with MOM and subsequent deprotection. Without protection, both hydroxyl and amino groups can be methylated, which requires separation by HPLC. Using [$^{11}$C]$CH_3OTf$, a loop method has been adapted by which the reaction can be run directly in the loop of HPLC before being injected into the separation column [19]. However, this loop method is only suitable for production of a small quantity under strictly anhydrous conditions. In contrast, only the amino group of 6a is radiomethylated by reductive amination, which can be run in aqueous media with comparable radiochemical yields. The hydroxyl group remained intact under the reaction condition [25].

Recently, we reported [$^{11}$C]MeDAS (7b) as a PET radioligand for myelin imaging [26]. Compared to previously reported radiolabeling using [$^{11}$C]$CH_3OTf$, radiosynthesis of [$^{11}$C]MeDAS was also achieved in aqueous solution through reductive amination in a much shorter time albeit a relatively lower radiochemical yield.

## 3. Experimental Section

**General procedure for synthesis of [$^{11}$]$CH_2O$:** The target gas (99.5% Nitrogen and 0.5% oxygen) was loaded on the cyclotron target and was bombarded by the cyclotron beam at 40 μA for 5 to 10 min. After that, the cyclotron-generated $^{11}CO_2$ was delivered into a three-neck reaction vessel equipped with water-cooling system and reduced to [$^{11}$C]$CH_3OH$ by lithium aluminum hydride (LAH, 0.1 M) solution in tetrahydrofuran (1.2 ml). Then hydriodic acid 57 wt% in water (0.9 ml) was added into the vessel to generate the [$^{11}$C]$CH_3I$ at 120˚C, which was concurrently distilled and trapped into a dry 3-ml conical reaction vial with screw cap which was previously filled with a mixture of trimethylamine *N*-oxide (TMAO, 5 mg) and DMF (200 μL) at –40˚C. Trapping of [$^{11}$C]$CH_3I$ was monitored by measuring the radioactivity in the isotope calibrator until the maximal value was attained. Then, the sealed vial was heated to 70˚C and maintained for 2 min. After that, the reaction vial was cooled to room temperature by dry ice in 2 min.

**General procedure for $^{11}$C-reductive amination of target compounds:** All of the mixture in the 3-ml reaction vial was collected and transferred into a dry 1-ml conical reaction vial with screw cap which was previously filled with target molecular compound (1 mg), sodium cyanoborohydride (5 mg) and sodium phosphate buffer (0.04 M, pH 7.0, 600 μL). Then, the sealed vial was heated to 70˚C or 90˚C and maintained for 5 min. After that, the vial was cooled to room temperature by dry ice in 2 min. In order to increase the radiochemical yield of C-11 reductive amination of target compounds, we assayed the effect of the solvent and the reaction temperature. We found if we used aqueous sodium phosphate buffer (0.04 M, pH 7.0, 600 μL) as reaction solvent, and the reaction temperature was 90˚C, the radiochemical yield was significantly increased (for example, see **Table 1**, entry 4- 11).

**Purification for compounds 1b - 3b:** The radiolabeled reaction mixture was directly loaded on to a semi-preparative HPLC (Phenomenex Luna C18 10 μm, 10 × 250 mm) column and was eluted with a mobile phase containing water and acetonitrile (5% for first 2 min, then a linear gradient from 5% to 95% in 15 min) at a flow rate of 3 ml/min and UV absorbance at 254 nm The retention times for compounds 1b, 2b and 3b are 12.82 min, 12.86 min and 12.22 min, respectively. Purification for compound 4b: The radiolabeled reaction mixture was directly loaded onto a semi-preparative HPLC (Phenomenex Luna C18 10 μm, 10 × 250 mm) column and was eluted with a mobile phase containing $NH_4Cl$/HCl buffer (pH 3.0) and acetonitrile (a linear gradient from 20 to 100% in 20 min) at a flow rate of 3 ml/min and UV wavelength at 254 nm. The retention time for compound 4b is 10.95 min. Purification for compound 5b: The radiolabeled reaction mixture was directly loaded onto a semi-preparative HPLC (Phenomenex Luna C18 10 μm, 10 × 250 mm) column and was eluted with a mobile phase containing water and acetonitrile (5% for first 2 min, then a linear gradient from 5 to 95% in 25 min) at a flow rate of 3 ml/min and UV absorbance at 254nm The retention time for compound 5b is 5.68 min.

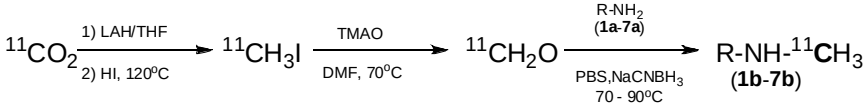
**Purification for compounds 6b and 7b:** To the radiolabeled reaction mixture was added 10 ml of water. The mixture was then passed through a Waters Light C-18 Sep-Pak cartridge previously conditioned with 10 ml of ethanol followed by 10 ml of water to remove non-organic impurities. The Sep-Pak cartridge was washed with another 10 ml of water and dried with a rapid air bolus. Then, the C-18 Sep-Pak cartridge was eluted with 1ml of ethanol, and the radiolabeled product was collected and loaded onto a semi-preparative HPLC (Phenomenex Luna C18 10 μm, 10 × 250 mm) column and was eluted with a mobile phase containing water and acetonitrile (4:6, v/v) at a flow rate of 3 ml/min and UV wavelength at 365 nm The retention times for 6b and 7b were 4.83 min and 6.53 min, respectively.

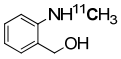
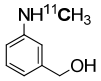
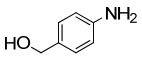
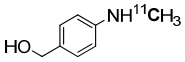
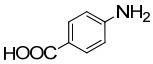
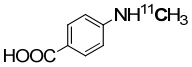
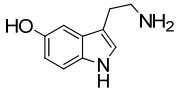
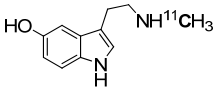
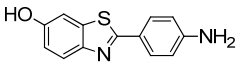
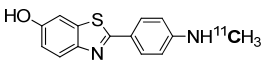
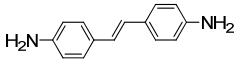
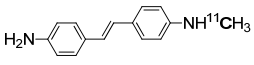
## 4. Conclusion

In conclusion, reductive amination using [$^{11}$C]formaldehyde proves to be a versatile approach to C-11 radiolabeling of amines. As demonstrated with different substrates bearing functional groups that are sensitive to

**Table 1. $^{11}$C-Reductive Amination of Target Compounds[a].**




| Entry | Precursor | Product | Solvents | Temp (˚C) | Reaction Time (min) | Purity (%)[d] | Radiochemical Yield (%)[e] |
|---|---|---|---|---|---|---|---|
| 1 |  **1a** |  **1b** | DMF-PBS (1:3) | 70 | 5 | >95 | 23.7 |
| 2 |  **2a** |  **2b** | DMF-PBS (1:3) | 70 | 5 | >95 | 31.6 |
| 3 |  **3a** |  **3b** | DMF-PBS (1:3) | 70 | 5 | >95 | 33.3 |
| 4 |  **4a** |  **4b** | DMF-PBS (1:3) | 70 | 5 | >95 | 1.8 |
| 5[b] | | | DMF | 90 | 5 | >95 | 3.0 |
| 6 | | | DMF-PBS (1:3) | 90 | 5 | >95 | 14.8 |
| 7 |  **5a** |  **5b** | DMF-PBS (1:3) | 70 | 5 | >95 | 2.1 |
| 8 | | | DMF-PBS (1:3) | 90 | 5 | >95 | 26.5 |
| 9[b] |  **6a** |  **6b** | DMF | 70 | 5 | >95 | 1.6 |
| 10[b] | | | DMF | 90 | 5 | >95 | 2.6 |
| 11[c] | | | DMF-PBS (1:1) | 90 | 5 | >95 | 7.9 |
| 12 |  **7a** |  **7b** | DMF-PBS (1:3) | 70 | 5 | >95 | 28.7 |

[a]Conditions: LAH/THF (0.1M, 1.2 ml), HI (57 wt% in $H_2O$, 0.9 ml), TMAO (5 mg), DMF (200 µL), Target compounds 1a - 7a (1 mg), $NaCNBH_3$ (5 mg), PBS (0.04 M, pH 7.0, 600 µL); [b]Conditions: add DMF (200 µL) for dissolving TMAO firstly, then add DMF (600 µL) for dissolving target compounds 4a and 6a in the next step; [c]Conditions: add DMF (200 µL) for dissolving TMAO first, then add DMF (200 µL) for dissolving target compound 6a and dilute the mixture with PBS (0.04 M, pH 7.0, 400 µL); [d]Radiochemical purity determined by HPLC; [e]Radiochemical yield is decay-corrected to $^{11}CH_2O$ radioactivity.

[$^{11}$C]$CH_3$I or [$^{11}$C]$CH_3$OTf, this method radiolabels only the amino group, which can be carried out in aqueous solvents in a short time.

## 5. Supplementary Material

Detailed experimental procedures for the cold synthesis of compounds 1b-3b and 7b, and $^1$H and $^{13}$C NMR spectra; HPLC spectra for hot synthesis of compounds 1b - 7b.

## 6. Acknowledgements

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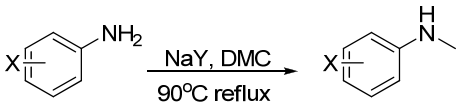
## Supplemental Information

### 1. General Information

All chemicals, unless otherwise stated, were purchased from Sigma-Aldrich and used without further purification. $^1$HNMR spectra were obtained at 400 MHz on an Inova 400 NMR system using 5 mm NMR tubes (Wilmad 528-PP) in $CDCl_3$ or DMSO-$d_6$ (Aldrich or Cambridge Isotopes) solutions at room temperature. Chemical shifts are reported as d values relative to internal TMS. HR-ESIMS were acquired under the electron spray ionization (ESI) condition. Radiochemical purity was determined by an Agilent 1100 high-pressure liquid chromatography (HPLC) system equipped with UV and Raytest gamma count detectors.

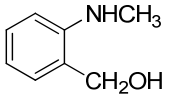
### 2. Procedures and Experimental Data

**Preparation of 2-(methylamino)-benzyl alcohol (1b) and 4-(methylamino)-benzyl alcohol (3b)**



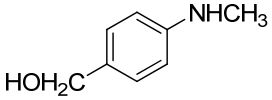

**1a** X = *o*-$CH_2OH$ **1b**
**3a** X = *p*-$CH_2OH$ **3b**

**2-(Methylamino)-benzyl alcohol** (**1b**)



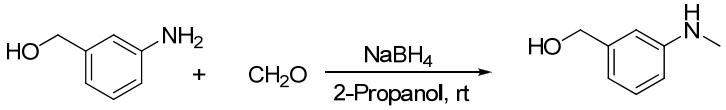

To a 25 ml two-neck flask fitted with a magnetic stir bar were added **1a** (1.5 mmol, 190 mg), zeolite NaY (1 mg, dried at 70˚C overnight) and 5 ml of anhydrous DMC. The resulting mixture was then stirred at room temperature for 5 min followed by refluxing at 90˚C for 24 hrs. Once the reaction was completed (monitored by TLC), the mixture was filtered and washed with $CH_2Cl_2$. The solvent was combined and removed by rotary evaporation. The crude product was purified by flash chromatography (Hexane/Ethyl acetate = 3:1, v/v) to yield **1b** (150 mg, 1.1 mmol, 73%). **1b**: $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 7.27-7.24 (m, 1H), 7.08 - 7.05 (m, 1H), 6.69-6.65 (m, 2H), 4.61 (S, 2H), 2.86 (S, 3H). HRMS (ESI) calcd. for $C_8H_{11}NO$ (m/z M + $H^+$):138.09134, Found 138.09135. HPLC (Phenomenex Luna C18 5 µm, 10 × 250 mm, water and acetonitrile (5% for first 2 min, then a linear gradient from 5 to 95% in 15 min, flow rate: 3 ml/min, UV 254nm), retention time is 12.82 min.

**4-(Methylamino)-benzyl alcohol (3b)**




To a 25 ml two-neck flask fitted with a magnetic stir bar were added **3a** (1.5 mmol, 190 mg), zeolite NaY (1 mg, dried at 70˚C overnight) and 5 ml of anhydrous DMC. The resulting mixture was then stirred at room temperature for 5 min followed by refluxing at 90˚C for 24 hrs. Once the reaction was completed (monitored by TLC), the mixture was filtered and washed with $CH_2Cl_2$. The solvent was combined and removed by rotary evaporation. The crude product was purified by flash chromatography (Hexane/Ethyl acetate = 3:1, v/v) to yield **3b** (72 mg, 0.53 mmol, 35%). **3b**: $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 7.24-7.17 (m, 2H), 6.61-6.57 (m, 2H), 4.54 (S, 2H), 2.83 (S, 3H). HRMS (ESI) calcd. for $C_8H_{11}NO$ (m/z M + $H^+$):138.09134, Found 138.09135. HPLC (Phenomenex Luna C18 5µm, 10 × 250 mm, water and acetonitrile (5% for first 2 min, then a linear gradient from 5 to 95% in 15 min, flow rate: 3 ml/min, UV 254 nm), retention time is 12.22 min.

**Preparation of 3-(methylamino)-benzyl alcohol (2b)**

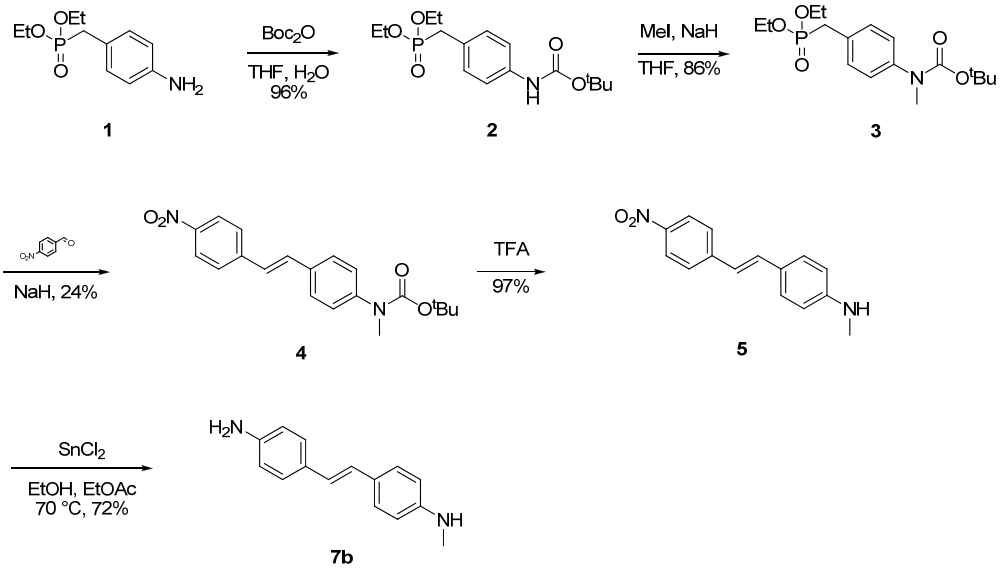



**2a** **2b**

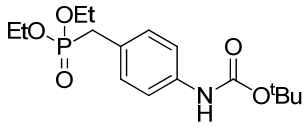
To a suspension of **2a** (1.0 mmol, 123 mg) and formaldehyde (1.1 mmol, 33.0 mg) was added 2-propanol (1.5 ml). After 3 hrs of continuous agitation at room temperature, $NaBH_4$ (1.5 mmol, 60 mg) was added and the reaction mixture was allowed to stir overnight. Once the reaction was complete (monitored by TLC), the mixture was filtered and washed with $CH_2Cl_2$. The solvent was removed by rotary evaporation. The crude product was purified by chromatography (Hexane/Ethyl acetate = 2:1, v/v) to yield product **2b** (40 mg, 0.29 mmol, 29%). $^1$HNMR(400 MHz, $CDCl_3$): $\delta$ 7.27 - 7.24 (m, 1H), 7.08 - 7.05 (m, 1H), 6.69 - 6.65 (m, 2H), 4.61 (S, 2H), 2.86 (S, 3H). HRMS (ESI) calcd. for $C_8H_{11}NO$ (m/z M + $H^+$):138.09134, Found 138.09134. HPLC (Phenomenex Luna C18 5 µm, 10 × 250 mm, water and acetonitrile (5% for first 2 min, then a linear gradient from 5 to 95% in 15 min, flow rate: 3 ml/min, UV 254 nm), retention time is 12.86 min.

**Preparation of (*E*)-*N*-methyl-4-(4-nitrostyryl)aniline (7b)**



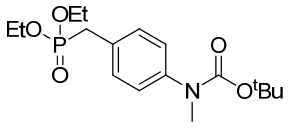

**Preparation of *tert*-butyl (4-((diethoxyphosphoryl) methyl)phenyl)carbamate (2)**.




**2**

To a 100 ml round bottom flask fitted with a magnetic stir bar were added diethyl 4-aminobenzylphosphonate (**1**, 2.500 g, 10.28 mmol) and di-*tert*-butyl dicarbonate (2.240 g, 10.26 mmol) in THF (15 ml) and water (6 ml). The reaction was stirred at room temperature overnight. THF was removed in vacuo and the remaining residue was suspended in ethyl acetate and water. The aqueous layer was extracted three times with ethyl acetate. The organic layers were combined and washed twice with water and once with brine. The organic layer was dried over $MgSO_4$, filtered, and concentrated to give **2** as a white powder (3.393 g, 96%) and was used without further purification. $^1$H-NMR ($CDCl_3$, 400 MHz): $\delta$ 7.31 (d, J = 8.4 Hz, 2H), 7.21 (m, 2H), 6.47 (br s, 1H), 4.00 (m, 4H), 3.09 (d, $^2J_{HP}$ = 21.2 Hz, 2H), 1.51 (s, 9H), 1.24 (td, $^3J_{HH}$ = 6.8, $^4J_{HP}$ = 0.4 Hz, 6H).

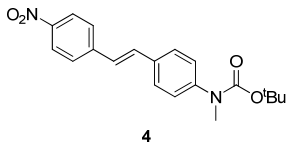
**Preparation of *tert*-butyl (4-((diethoxyphosphoryl) methyl)phenyl)(methyl)carbamate (3)**.




**3**

Compound **2** (503 mg, 1.46 mmol) was added to an oven dried 25 ml round bottom flask fitted with a magnetic stir. The flask was purged with argon then dry THF (3.0 ml) was added to dissolve **2**. The flask was cooled to 0˚C. NaH (93.0 mg, 2.33 mmol, 60% dispersion in mineral oil) was placed in a 2 ml vial and washed with hexanes (1.5 ml × 3). The NaH was suspended in dry THF (6.0 ml) and added to the solution of **2** under positive argon pressure. MeI (200 µL, 2.91 mmol, 2.28 g/ml) was added to the reaction mixture under positive argon pressure. The reaction was stirred at 0˚C under argon then slowly warmed to room temperature and stirred overnight. The reaction was quenched with water. THF was removed in vacuo. The remaining residue was suspended in ethyl acetate and water and the aqueous layer was extracted three times with ethyl acetate. The organic layers were combined and washed twice with water and once with brine. The organic layer was dried over $MgSO_4$, filtered, and concentrated to give **3** as a yellow oil (451 mg, 86%) and was used without further purification. $^1$H-NMR ($CDCl_3$, 400 MHz): $\delta$ 7.25 (m, 2H), 7.16 (d, J = 8.4 Hz, 2H), 4.01 (m, 4H), 3.23 (s, 3H), 3.12, $^2J_{HP}$ = 21.6 Hz, 2H), 1.43 (s, 9H), 1.24 (t, $^3J_{HH}$ = 7.0 Hz, 6H).

**Preparation of (*E*)-*tert*-butyl methyl(4-(4-nitrostyryl) phenyl)carbamate (4)**



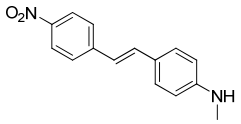

**4**

An oven dried 50 ml round bottom flask fitted with a magnetic stir bar was purged with argon and **3** (957 mg,

2.68 mmol) was added in dry DMF (3.0 ml). In a 2 ml vial, NaH (377 mg, 9.42 mmol, 60% dispersion in mineral oil) was washed with hexanes (1.5 ml × 3) then added to the solution of **3** in 9.0 ml DMF under positive argon pressure. The reaction was stirred under argon at room temperature for 20 minutes. 4-Nitrobenzaldehyde (383 mg, 2.53 mmol) was added to the reaction mixture in dry DMF (6.0 ml) under positive argon pressure. The reaction was stirred overnight under argon at room temperature. The reaction was quenched with water and the aqueous phase was extracted three times with ethyl acetate. The organic layers were combined and washed twice with water and once with brine. The organic layer was dried over $MgSO_4$, filtered, and concentrated. The crude red-orange solid was recrystallized in hot EtOH to give **4** as red crystals (529 mg, 24%). $^1$H-NMR ($CDCl_3$, 400 MHz) : $\delta$ 8.22 (ddd, J = 9.6, 4.4, 2.4 Hz, 2H), 7.63 (ddd, J = 9.2, 4.4, 2.4 Hz, 2H), 7.51 (ddd, J = 9.6, 4.8, 2.8 Hz, 2H), 7.29 (m, 2H), 7.24 (d, J = 16.4 Hz, 1H), 7.09 (d, J = 16.4 Hz, 1H), 3.29 (s, 3H), 1.48 (s, 9H).

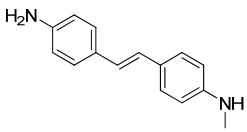
**Preparation of (*E*)-*N*-methyl-4-(4-nitrostyryl)aniline (5)**




**5**

Compound **4** (522 mg, 1.47 mmol) was added to a 10 ml round bottom flask fitted with a magnetic stir bar and dissolved in neat TFA (6.4 ml, 83 mmol, 1.48 g/ml). The reaction was stirred at room temperature for 2 h. The reaction was quenched with 4M NaOH (~40 ml) and the aqueous solution was extracted with ethyl acetate three times. The organic layers were combined and washed twice with water and once with brine. The organic layer was dried over $MgSO_4$, filtered, and concentrated to give **5** as a red solid (365 mg, 97%) and was used without further purification. $^1$H-NMR ($CDCl_3$, 400 MHz): $\delta$ 8.18 (m, 2H), 7.56 (m, 2H), 7.41 (m, 2H), 7.20 (d, J = 16.2 Hz, 1H), 6.92 (d, J = 16.2 Hz, 1H), 6.61 (m, 2H), 3.97 (br s, 1H), 2.89 (s, 3H).

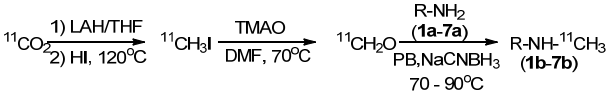
**Preparation of (*E*)-4-(4-aminostyryl)-N-methylaniline (7b)**




**7b**

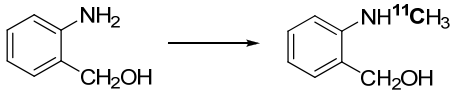
Compound **6** (355 mg, 1.40 mmol) was added to a 250 ml round bottom flask fitted with a magnetic stir bar in ethyl acetate (60 ml) and ethanol (30 ml). Tin (II) chloride (4.72 g, 24.9 mmol) was added to the solution of **6**. A water condenser was attached and the reaction was stirred at 70˚C overnight. The solvent was removed in vacuo. The residue was suspended in 4 M NaOH to precipitate the product. The mixture was filtered through a fine glass frit and washed several times with 4 M NaOH followed by water until the pH of the wash water was 7. Then, the solid on the frit was washed with ethyl acetate into a clean flask. That solution was dried over $MgSO_4$, filtered, and concentrated to give the crude product. The crude product was dissolved in $CH_2Cl_2$ and silica gel. The solvent was carefully removed leaving the crude product absorbed onto silica gel. This solid was added to the top of a silica column and purified by flask chromatography with $Et_2O/Et_3N$ (49:1). Concentration in vacuo gave **7b** as a light orange solid (224 mg, 72%). $^1$H-NMR ($CDCl_3$, 400 MHz): $\delta$ 7.36 (ddd, J = 9.2, 4.8, 2.8 Hz, 2H), 7.32 (ddd, J = 9.2, 4.8, 2.8 Hz, 2H), 6.90 (d, J = 16.2 Hz, 1H), 6.85 (d, J = 16.2 Hz, 1H), 6.67 (ddd, J = 9.2, 4.8, 2.8 Hz, 2H), 6.61 (ddd, J = 9.2, 4.8, 2.8 Hz, 2H), 3.72 (br s, N*H*$CH_3$ + N*H*$_2$, 3H), 2.86 (s, 3H). HR-ESIMS: *m*/*z* calcd for $C_{15}H_{16}N_2$ ($M + H^+$), 225.1386; found, 225.1385. HPLC (Phenomenex Luna C18 5μm, 10 × 250mm, water : acetonitrile = 4:6 (v/v), flow rate: 3ml/min, UV 365 nm), retention time is 5.63 min.

**General procedure for synthesis of $^{11}CH_2O$**




The cyclotron-made [$^{11}$C]carbon dioxide was transferred into a three-neck reaction vessel with water-cooling system and reduced to $^{11}CH_3OH$ by lithium aluminum hydride (LAH, 0.1M solution in THF). Once the reaction mixture is dry enough, hydriodic acid (0.9 ml, 57 wt% in water) was added into the vessel to generate labeled methyl iodide $^{11}CH_3I$ at 120˚C. $^{11}CH_3I$ was concurrently distilled and trapped in a dry 3-ml conical reaction vial with a screw cap which was previously filled with a mixture of trimethylamine *N*-oxide (TMAO, 5 mg) and DMF (200 μL) at –40˚C. Trapping of [$^{11}$C] methyl iodide was monitored by measuring the activity in an isotope calibrator until the maximum value was attained. Then the reaction mixture was sealed and heated at 70˚C for 2 minutes in a heating block. After cooling to room temperature in an ice bath, $^{11}CH_2O$ was ready for use.

**Synthesis of $^{11}$C-1b**

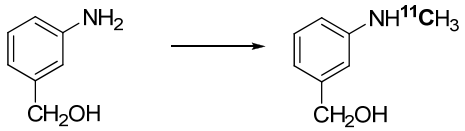



**$^{11}$C-1b**

$H^{11}CHO$ generated in the above reaction vial was transferred into a dry 1-ml conical reaction vial with screw cap which was previously filled with (2-aminophenyl)methanol (1 mg), sodium cyanoborohydride (5 mg) and sodium phosphate buffer (0.04 M, pH 7.0, 600 μL). The sealed vial was then heated to 70˚C and maintained for 5 min. After cooling to room temperature, the radiolabeled reaction mixture was directly loaded onto a semi-preparative HPLC (Phenomenex Luna C18, 10μm, 10 × 250 mm) column and was eluted with a mobile phase containing water and acetonitrile (5% for first 2 min, then a linear gradient from 5 to 95% in 15 min) at a flow rate of 3 ml/min and a UV absorbance at 254 nm. The retention time of $^{11}$C-**1b** is 12.82 min. Identification of $^{11}$C-**1b** and radiochemical purity were verified by co-injection with the non labeled cold standard of **1b** which has the same retention time on the UV and radioactive chromatograms. The radiochemical purity of $^{11}$C-**1b** was >95% after HPLC purification. The decay-corrected radiochemical yield of [$^{11}$C]**1b** obtained after HPLC purification was 23.7% based on the radioactivity of [$^{11}$C]methyl iodide trapped.

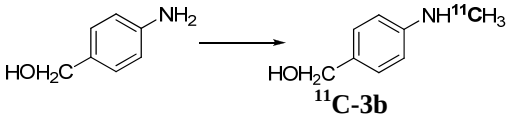
**Synthesis of $^{11}$C-2b**




**$^{11}$C-2b**

$H^{11}CHO$ generated in the above reaction vial was transferred into a dry 1-ml conical reaction vial with screw cap which was previously filled with (3-aminophenyl)methanol (1 mg), sodium cyanoborohydride (5 mg) and sodium phosphate buffer (0.04 M, pH 7.0, 600 μL). The sealed vial was then heated to 90˚C and maintained for 5 min. After cooling to room temperature, the radiolabeled reaction mixture was directly loaded on to a preparative HPLC (Phenomenex Luna C18, 10μm, 10 × 250 mm) column and was eluted with a mobile phase containing water and acetonitrile (5% for first 2 min, then a linear gradient from 5 to 95% in 15 min) at a flow rate of 3 ml/min and a UV wavelength at 254 nm. The retention time is 12.86 min. Identification of $^{11}$C-**2b** and radiochemical purity were verified by co-injection with the non labeled cold standard of **2b** which has the same retention time on the UV and radioactive chromatograms. The radiochemical purity of $^{11}$C-**2b** was >95% after HPLC purification. The decay-corrected radiochemical yield of [$^{11}$C]**2b** obtained after HPLC purification was 31.6% base on the radioactivity of [$^{11}$C]methyl iodide trapped.

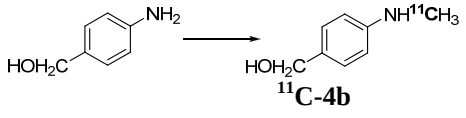
**Synthesis of $^{11}$C-3b**




**$^{11}$C-3b**

$H^{11}CHO$ generated in the above reaction vial was transferred into a dry 1-ml conical reaction vial with screw cap which was previously filled with (4-aminophenyl) methanol (1 mg), sodium cyanoborohydride (5 mg) and sodium phosphate buffer (0.04 M, pH 7.0, 600 μL). The sealed vial was then heated to 90˚C and maintained for 5 min. After cooling to room temperature, the radiolabeled reaction mixture was directly loaded on to a preparative HPLC (Phenomenex Luna C18, 10 μm, 10 × 250 mm) column and was eluted with a mobile phase containing water and acetonitrile (5% for first 2 min, then a linear gradient from 5 to 95% in 15 min) at a flow rate of 3 ml/min and a UV wavelength at 254 nm. The retention time is 12.22 min. Identification of $^{11}$C-**1b** and radiochemical purity were verified by co-injection with the non labeled cold standard of **3b** which has the same retention time on the UV and radioactive chromatograms. The radiochemical purity of $^{11}$C-**3b** was >95% after HPLC purification. The decay-corrected radiochemical yield of [$^{11}$C]**3b** obtained after HPLC purification was 33.3% base on the radioactivity of [$^{11}$C]methyl iodide trapped.

**Synthesis of $^{11}$C-4b**



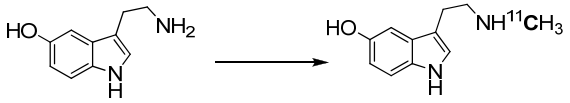

**$^{11}$C-4b**

$H^{11}CHO$ generated in the above reaction vial was transferred into a dry 1 ml conical reaction vial with screw cap which was previously filled with 4-aminobenzoic acid (1 mg), sodium cyanoborohydride (5 mg) and sodium phosphate buffer (0.04 M, pH 7.0, 600 μL). The sealed vial was then heated to 70˚C and maintained for 5 min. After cooling to room temperature, the radiolabeled reaction mixture was directly loaded on to a preparative HPLC (Phenomenex Luna C18, 10μm, 10 × 250 mm) column and was eluted with a mobile phase containing $NH_4Cl$/HCl buffer (pH 3.0) and acetonitrile (a linear gradient from 20 to 100% in 20 min).at a flow rate of 3 ml/min and a UV wavelength at 254 nm. The retention time for compound **4b** is 10.95 min. Identification of $^{11}$C-**4b** and radiochemical purity was verified by co-injection with the non labeled cold standard of **4b** which has the same retention time on the UV and radioactive chromatograms. The radiochemical purity of $^{11}$C-**4b** was >95% after HPLC purification. The decay-corrected radiochemical yield of [$^{11}$C]**4b** obtained after HPLC purification was 1.8% base on the activity of

[$^{11}$C]methyl iodide trapped. In order to increase the radiolabelling yield of [$^{11}$C]**4b**, we used either DMF 600μL or sodium phosphate buffer (0.04 M, pH 7.0, 600 μL) as reaction solvent, the reaction temperature was set to 90°C and maintained for 5 min. The radiochemical yield of 3.0% and 14.8% can be attained based on the radioactivity of [$^{11}$C]methyl iodide trapped.

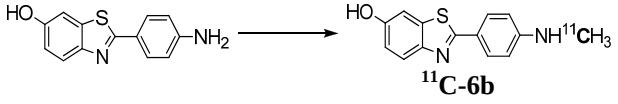
**Synthesis of $^{11}$C-5b**




**$^{11}$C-5b**

H$^{11}$CHO generated in the above reaction vial was transferred into a dry 1-ml conical reaction vial with screw cap which was previously filled with 3-(2-aminoethyl)-1H-indol-5-ol (1 mg), sodium cyanoborohydride (5 mg) and sodium phosphate buffer (0.04 M, pH 7.0, 600 μL). The sealed vial was then heated to 70˚C and maintained for 5 min. After cooling to room temperature, the radiolabeled reaction mixture was directly loaded on to a preparative HPLC (Phenomenex Luna C18, 10μm, 10 × 250 mm) column and was eluted with a mobile phase containing water and acetonitrile (5% for first 2 min, then a linear gradient from 5 to 95% in 25 min).at a flow rate of 3 ml/min and a UV wavelength at 254 nm. The retention time is 5.68 min. Identification of $^{11}$C-**5b** and radiochemical purity was verified by co-injection with the non labeled cold standard of **5b** which has the same retention time on the UV and radioactive chromatograms. The radiochemical purity of $^{11}$C-**5b** was >95% after HPLC purification. The decay-corrected radiochemical yield of [$^{11}$C]**5b** obtained after HPLC purification was 2.1% base on the activity of [$^{11}$C]methyl iodide trapped. In order to increase the radiolabelling yield of [$^{11}$C]**5b**, we set the reaction temperature to 90˚C and maintained for 5 min. The radiochemical yield increased to 26.5% based on the radioactivity of [$^{11}$C]methyl iodide trapped.

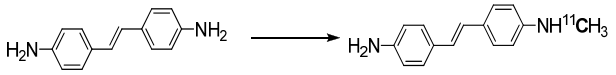
**Synthesis of $^{11}$C-6b**




**$^{11}$C-6b**

H$^{11}$CHO generated in the above reaction vial was transferred into a dry 1-ml conical reaction vial with screw cap which was previously filled with 2-(4-aminophenyl) benzo[d]thiazol-6-ol (1 mg), sodium cyanoborohydride (5 mg) and sodium phosphate buffer (0.04 M, pH 7.0, 600 μL). The sealed vial was then heated to 70˚C and maintained for 5 min. After cooling to room temperature, the reaction mixture was diluted with water and was passed through a Waters C-18 Sep-Pak cartridge previous conditioned with ethanol then water to remove the non-organic impurities. The Sep-Pak cartridge was washed with 10 ml of water and dried with a rapid air bolus, and the radiolabeled product was eluted with ethanol and was loaded onto a preparative HPLC (Phenomenex Luna C18, 10 μ C18 10 × 250 mm) column and was eluted with a mobile phase containing water and acetonitrile (4:6 v/v) at a flow rate of 3 ml/min and a UV wavelength at 365 nm. The retention time is 4.83 min. Identification of $^{11}$C-**6b** is verified by coinjection with the non labeled cold standard **6b**, which has the same retention time on the UV and radioactive chromatograms. The radiochemical purity of $^{11}$C-**6b** after HPLC preparation is greater than 95%. The radiochemical yield was approximately 10%.

**Synthesis of $^{11}$C-7b**



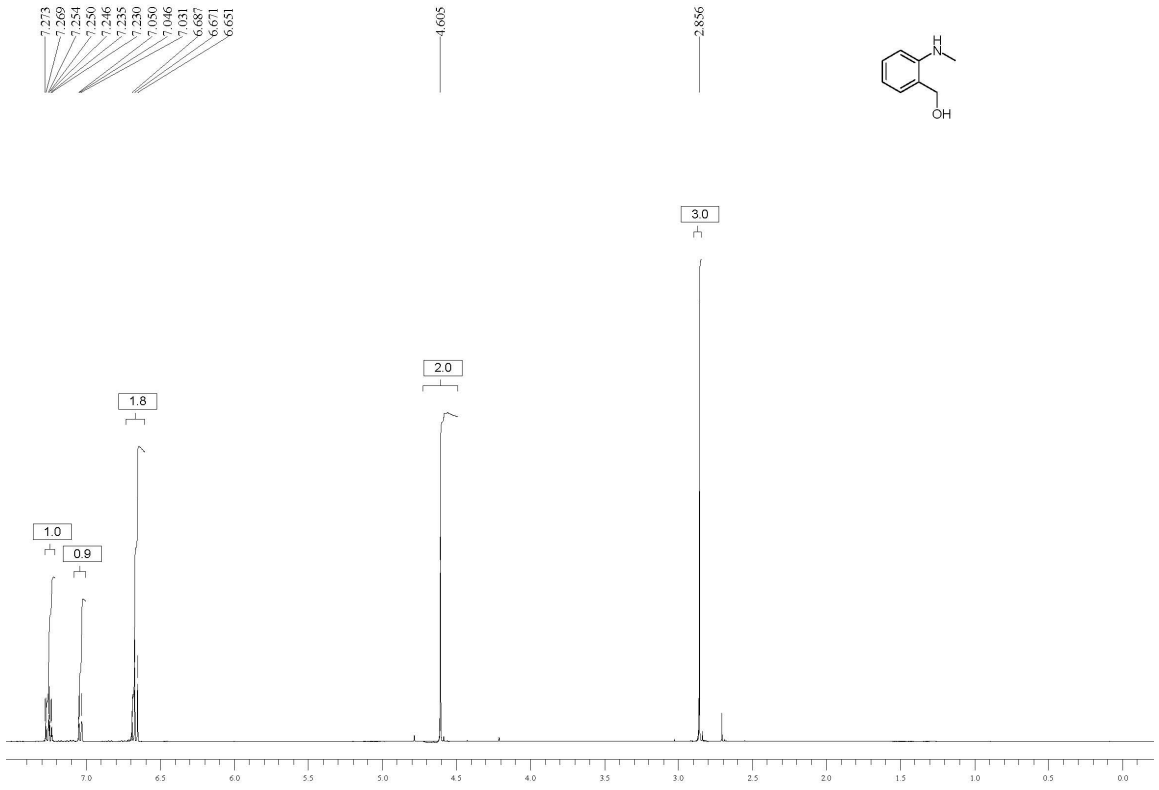

**$^{11}$C-7b**

H$^{11}$CHO generated in the above reaction vial was transferred into a dry 1-ml conical reaction vial with screw cap which was previously filled with (E)-4,4'-(ethene-1,2-diyl)dianiline (1 mg), sodium cyanoborohydride (5 mg) and sodium phosphate buffer (0.04 M, pH 7.0, 600 μL). The sealed vial was then heated to 70°C and maintained for 5 min. After cooling to room temperature, the reaction mixture was diluted with water and was passed through a Waters C-18 Sep-Pak cartridge previous conditioned with ethanol then water to remove the non-organic impurities. The Sep-Pak cartridge was washed with 10 ml of water and dried with a rapid air bolus, and the radiolabeled product was eluted with ethanol and was loaded onto a preparative HPLC (Phenomenex Luna C18, 10μ C18 10 × 250 mm) column and was eluted with a mobile phase containing water and acetonitrile (4:6 v/v) at a flow rate of 3 ml/min and a UV wavelength at 365 nm. The retention time is 6.53 min. Identification of $^{11}$C-**7b** and radiochemical purity was verified by co-injection with the non labeled cold standard of **7b** which has the same retention time on the UV and radioactive chromatograms. The radiochemical purity of $^{11}$C-**7b** was >95% after HPLC purification. The decay-corrected radiochemical yield of [$^{11}$C]**7b** obtained after HPLC purification was 28.7% based on the activity of [$^{11}$C]methyl iodide trapped.

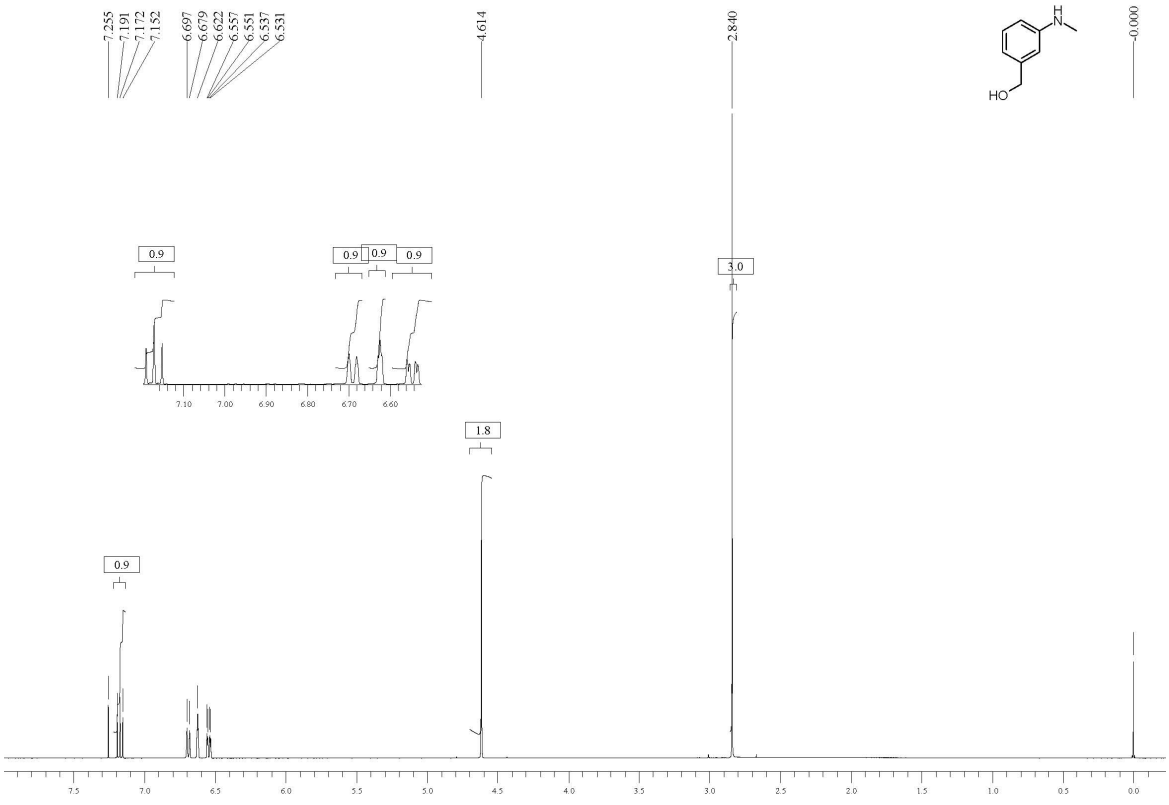
## 3. $^{1}$HNMR Spectra of Novel Compounds

**2-(Methylamino)-benzyl alcohol (1b)**

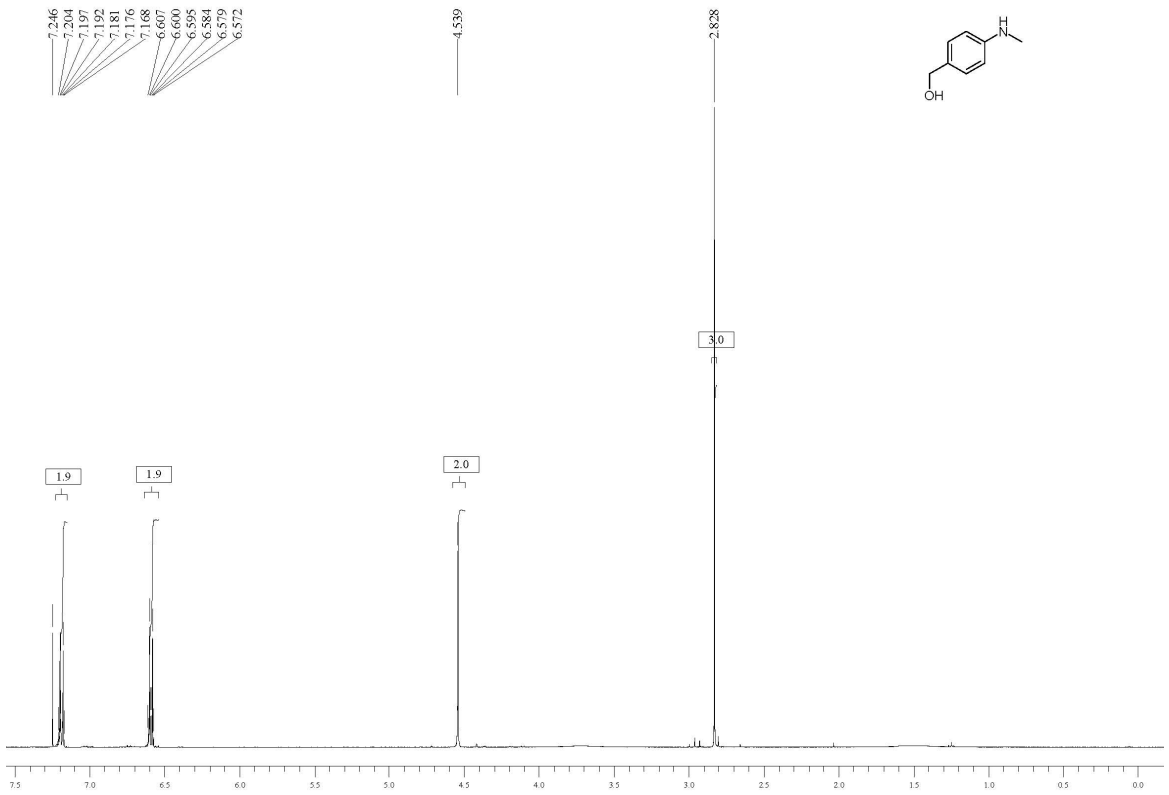



**3-(Methylamino)-benzyl alcohol (2b)**

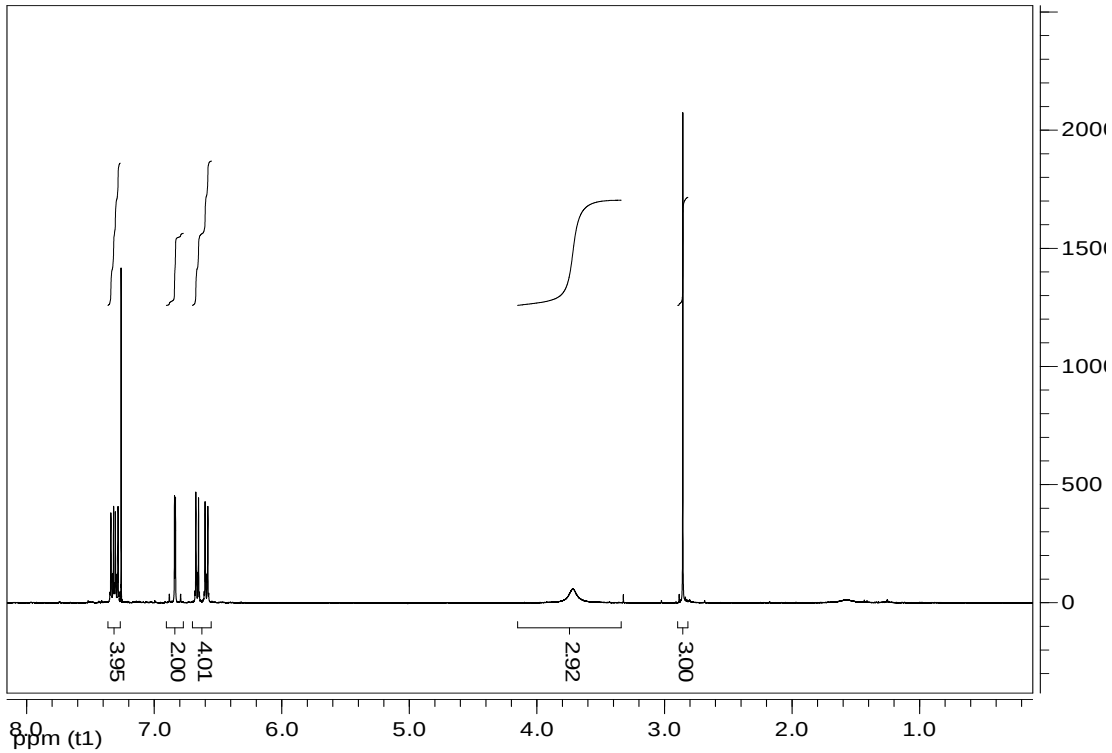


**4-(Methylamino)-benzyl alcohol (3b)**



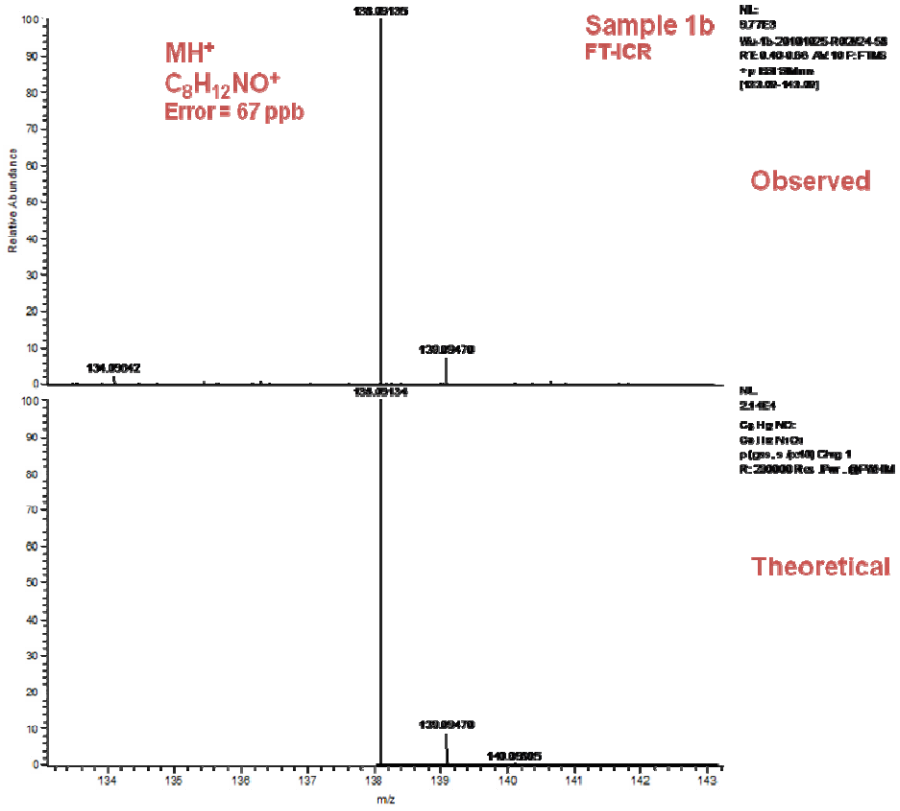

**(*E*)-4-(4-aminostyryl)-N-methylaniline (7b)**



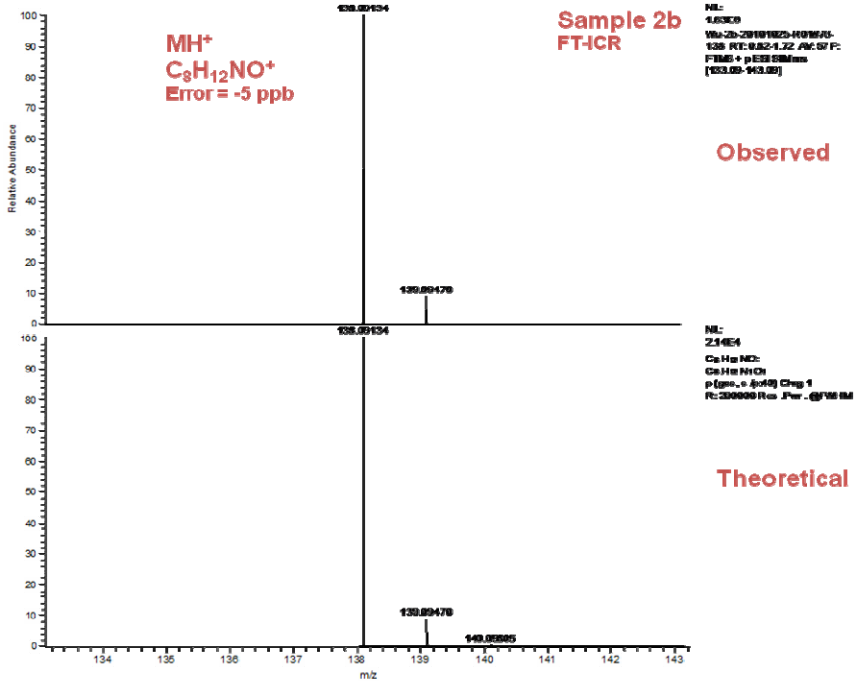

 

## 4. HRMS(ESI) Spectra of Novel Compounds

2-(Methylamino)-benzyl alcohol **(1b)**

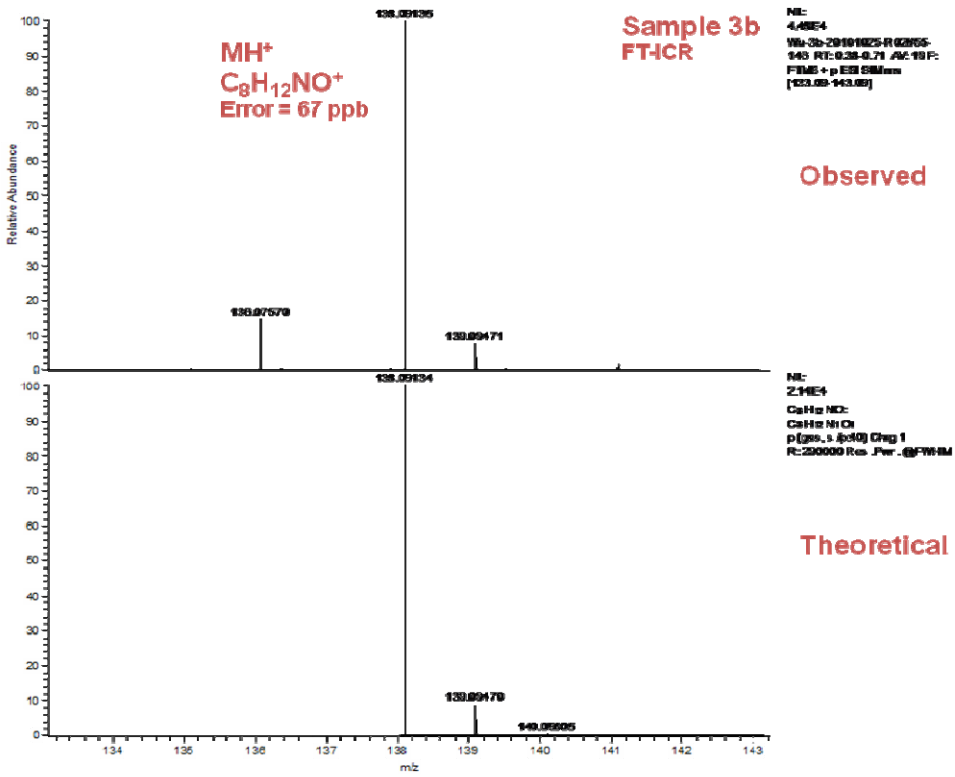



3-(Methylamino)-benzyl alcohol (**2b**)

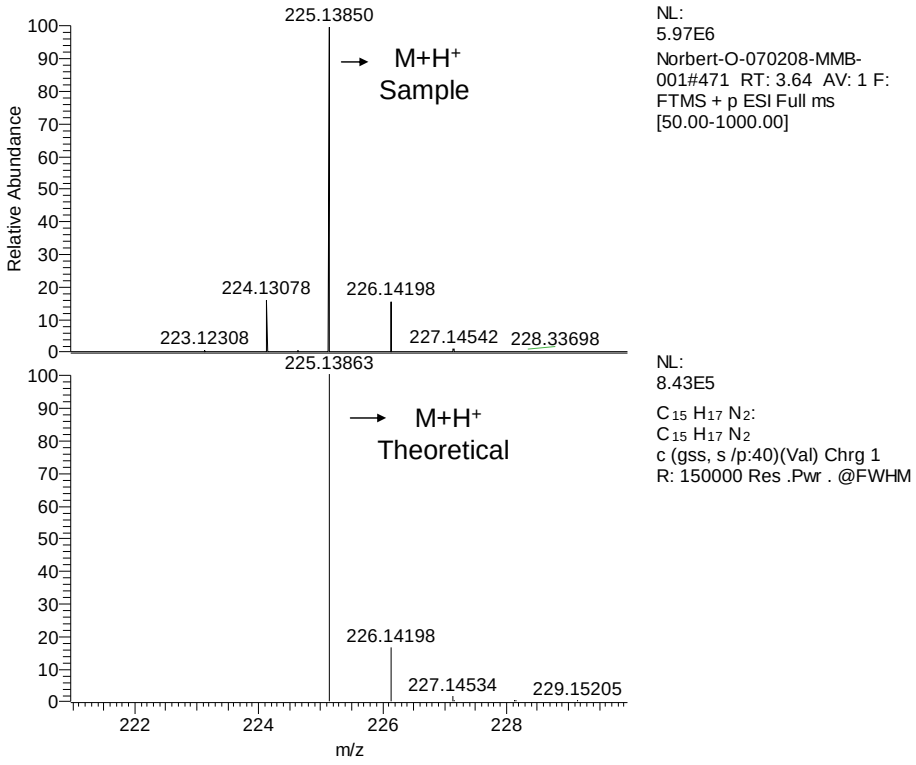


4-(Methylamino)-benzyl alcohol (**3b**)




(*E*)-4-(4-aminostyryl)-N-methylaniline **(7b)**

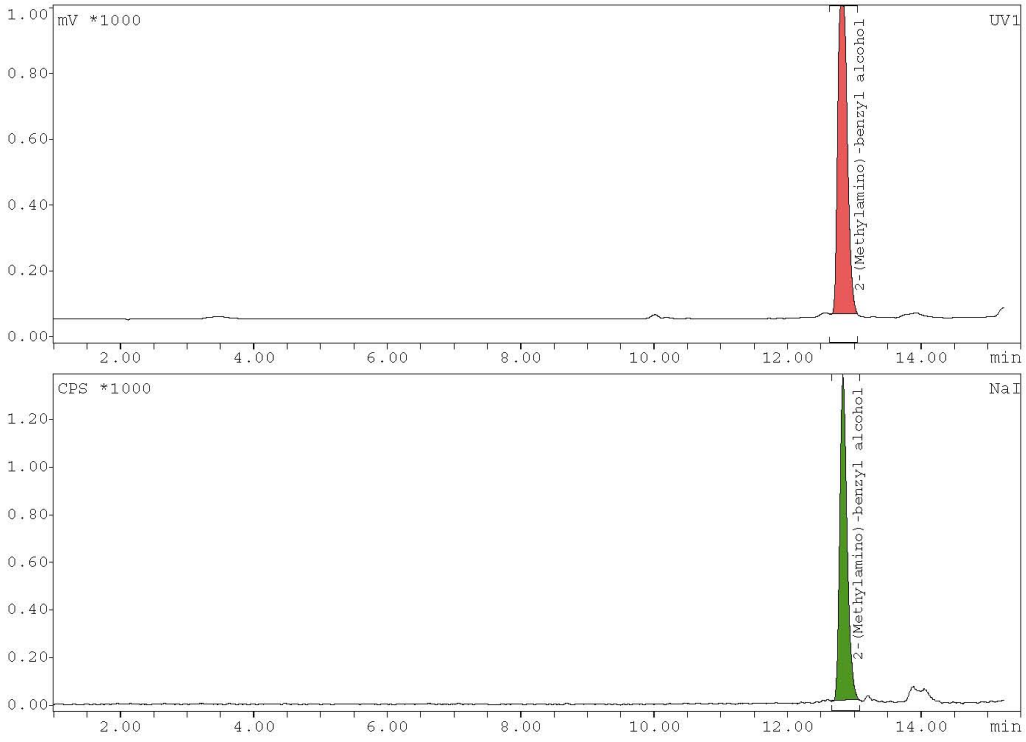


## 5. HPLC Spectra for both Cold and Hot Syntheses

### $^{11}$C-**1b**

Measurement 090109-05-2Mono-R PETNET Page 1/1
C:\GINA_LC\RUOSHI51109\090109-05-2Mono-R Print date: 9/1/2009




**Sample description**

| | | |
|---|---|---|
| Measurement: | 090109-05-2Mono-R, injection : | 9/1/2009 12:17 PM |
| Method: | RUOSHI51109 from: | 9/1/2009 9:53 AM |
| Station number: | 3 | |

**Preparation**

| Preparation | Sign/Lot. |
|---|---|
| | |

**Integration NaI**

| Substance | R/T | Type | Area | %Area |
|---|---|---|---|---|
| | min | | Counts | % |
| 2-(Methylamino)-benzyl alcohol | 12.83 | BB | 10326.19 | 100.00 |
| Total | | | 10326.19 | |
| Total area | | | 17619.88 | |

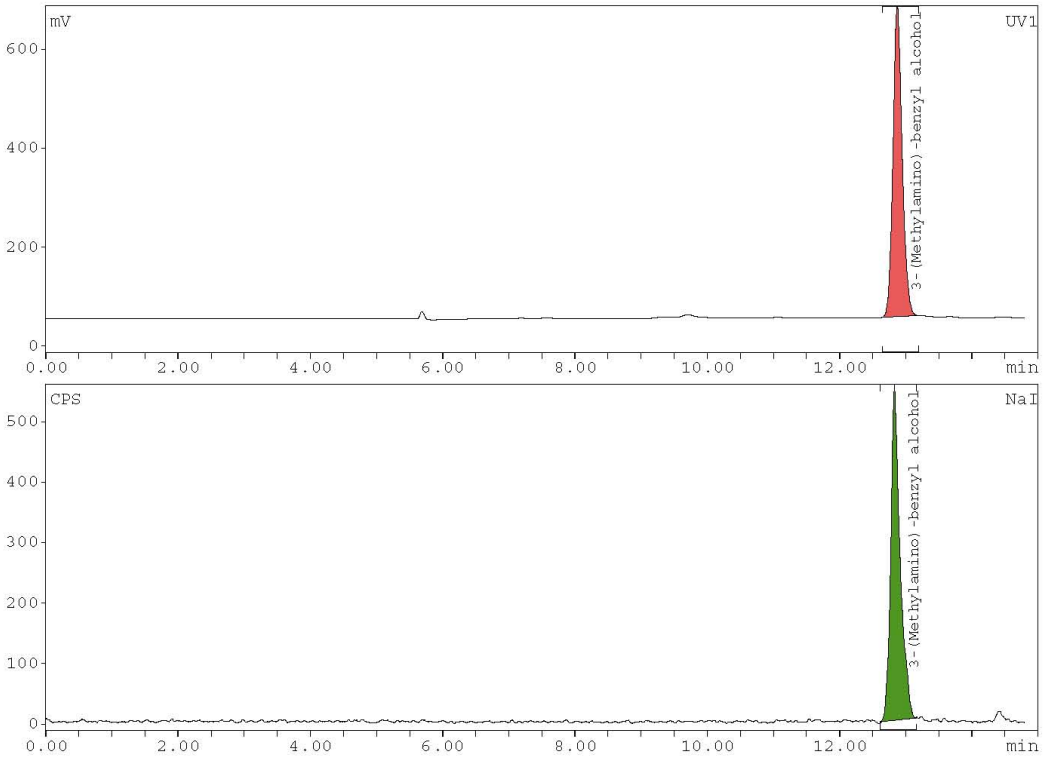
**Integration UV1**

| Substance | R/T | Type | Area | %Area |
|---|---|---|---|---|
| | min | | mV*s | % |
| 2-(Methlamino)-benzyl alcohol | 12.82 | BB | 9549.361 | 100.00 |
| Total | | | 9549.361 | |

$^{11}$C-**2b**

Measurement 092309-02-3Mono-R PETNET Page 1/1
C:\GINA_LC\RUOSHI51109\092309-02-3Mono-R Print date: 9/23/2009




**Sample description**

| | | |
|---|---|---|
| Measurement: | 092309-02-3Mono-R, injection : | 9/23/2009 9:55 AM |
| Method: | RUOSHI51109 from: | 9/23/2009 8:14 AM |
| Station number: | 3 | |

**Preparation**

| Preparation | Sign/Lot. |
|---|---|
| | |

**Integration NaI**

| Substance | R/T | Type | Area | %Area |
|---|---|---|---|---|
| | min | | Counts | % |
| 3-(Methylamino)-benzyl alcohol | 12.83 | BB | 5407.194 | 100.00 |
| Total | | | 5407.194 | |
| Total area | | | 9713.344 | |

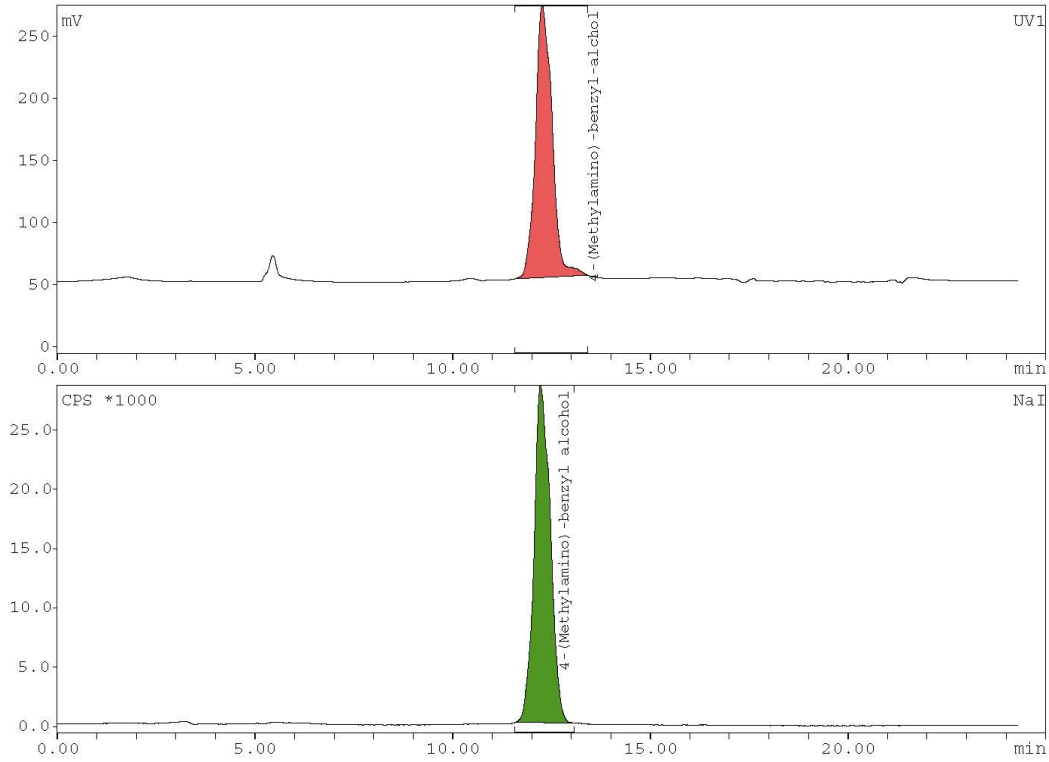
**Integration UV1**

| Substance | R/T | Type | Area | %Area |
|---|---|---|---|---|
| | min | | mV*s | % |
| 3-(Methylamino)-benzyl alcohol | 12.87 | BB | 6053.017 | 100.00 |
| Total | | | 6053.017 | |

$^{11}$C-**3b**

Measurement 091809-04-4Mono-R PETNET Page 1/1
C:\GINA_LC\RUOSHI51109\091809-04-4Mono-R Print date: 9/18/2009




**Sample description**

| | | |
|---|---|---|
| Measurement: | 091809-04-4Mono-R, injection : | 9/18/2009 11:32 AM |
| Method: | RUOSHI51109 from: | 9/18/2009 9:34 AM |
| Station number: | 3 | |

**Preparation**

| Preparation | Sign/Lot. |
|---|---|
| | |

**Integration NaI**

| Substance | R/T | Type | Area | %Area |
|---|---|---|---|---|
| | min | | Counts | % |
| 4-(Methylamino) | 12.22 | BB | 816318.0 | 100.00 |
| Total | | | 816318.0 | |
| Total area | | | 1081630.0 | |

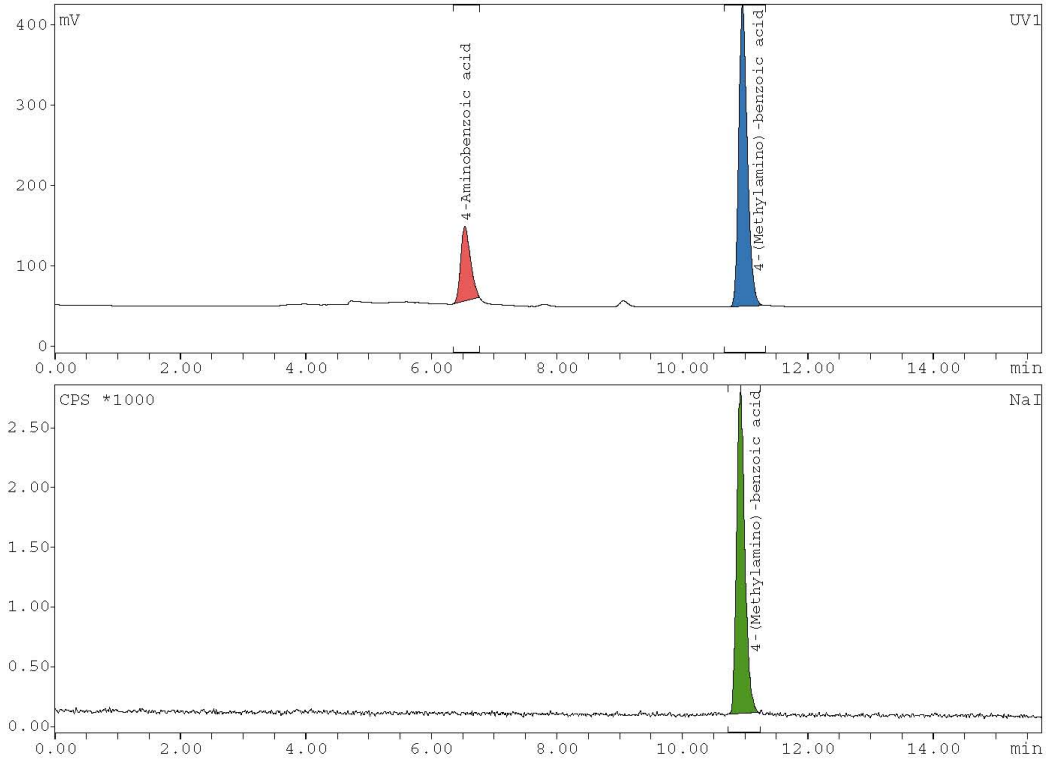
**Integration UV1**

| Substance | R/T | Type | Area | %Area |
|---|---|---|---|---|
| | min | | mV*s | % |
| 4-(Methylamino) | 12.27 | BB | 6428.992 | 100.00 |
| Total | | | 6428.992 | |

## $^{11}$C-**4b**

Measurement 100809-03-Bacid-me-R PETNET Page 1/1
C:\GINA_LC\RUOSHI51109\100809-03-BACID-ME-R Print date: 10/8/2009




**Sample description**

| | |
|---|---|
| Measurement: | 100809-03-Bacid-me-R, injection :10/8/2009 11:21 AM |
| Method: | RUOSHI51109 from: 10/8/2009 8:59 AM |
| Station number: | 3 |

**Integration NaI**

| Substance | R/T | Type | Area | %Area |
|---|---|---|---|---|
| | min | | Counts | % |
| 4-(Methylamino) | 10.93 | BB | 24611.93 | 100.00 |
| Total | | | 24611.93 | |
| Total area | | | 126071.75 | |

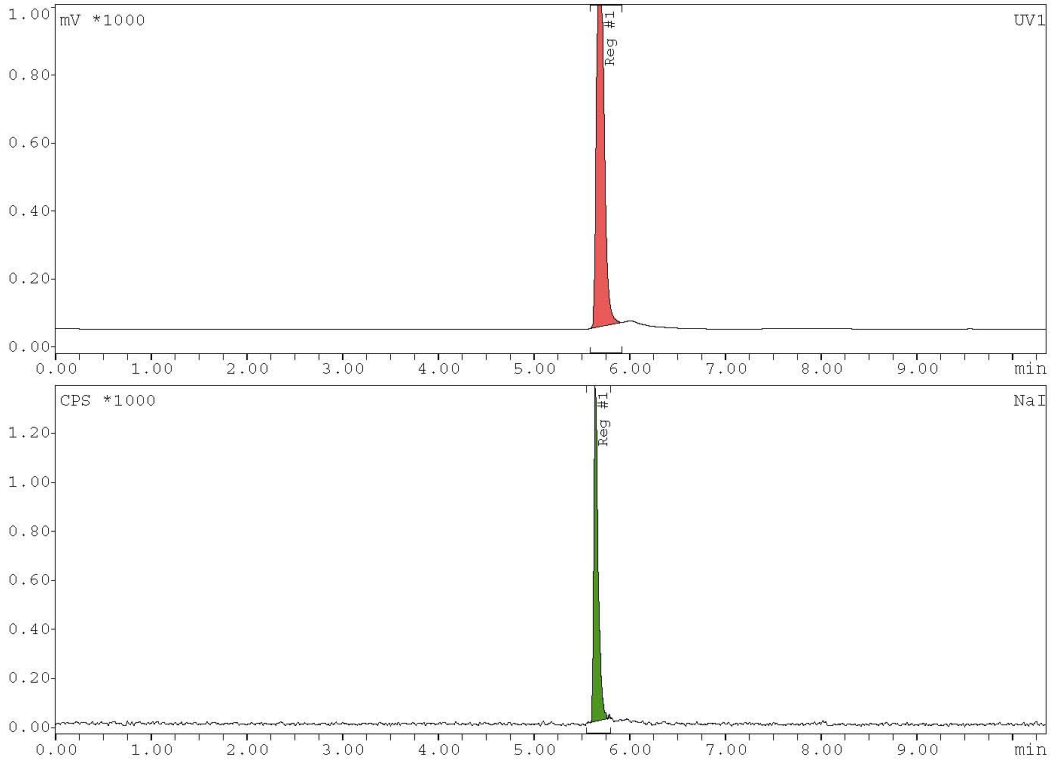
**Integration UV1**

| Substance | R/T | Type | Area | %Area |
|---|---|---|---|---|
| | min | | mV*s | % |
| 4-Aminobenzoic | 6.53 | BB | 977.288 | 21.45 |
| 4-(Methylamino) | 10.97 | BB | 3578.181 | 78.55 |
| Total | | | 4555.468 | |

$^{11}$C-**5b**

Measurement 100709-07-Serotonin-me-R PETNET Page 1/1
C:\GINA_LC\RUOSHI51109\100709-07-SEROTONIN-ME-R Print date: 10/7/2009




**Sample description**

| | | |
|---|---|---|
| Measurement: | 100709-07-Serotonin-me-R, injection : | 10/7/2009 11:44 AM |
| Method: | RUOSHI51109 from: | 10/7/2009 8:47 AM |
| Station number: | 3 | |

**Integration NaI**

| Substance | R/T | Type | Area | %Area |
|---|---|---|---|---|
| | min | | Counts | % |
| Reg #1 | 5.65 | BB | 4316.156 | 100.00 |
| Total | | | 4316.156 | |
| Total area | | | 13862.438 | |

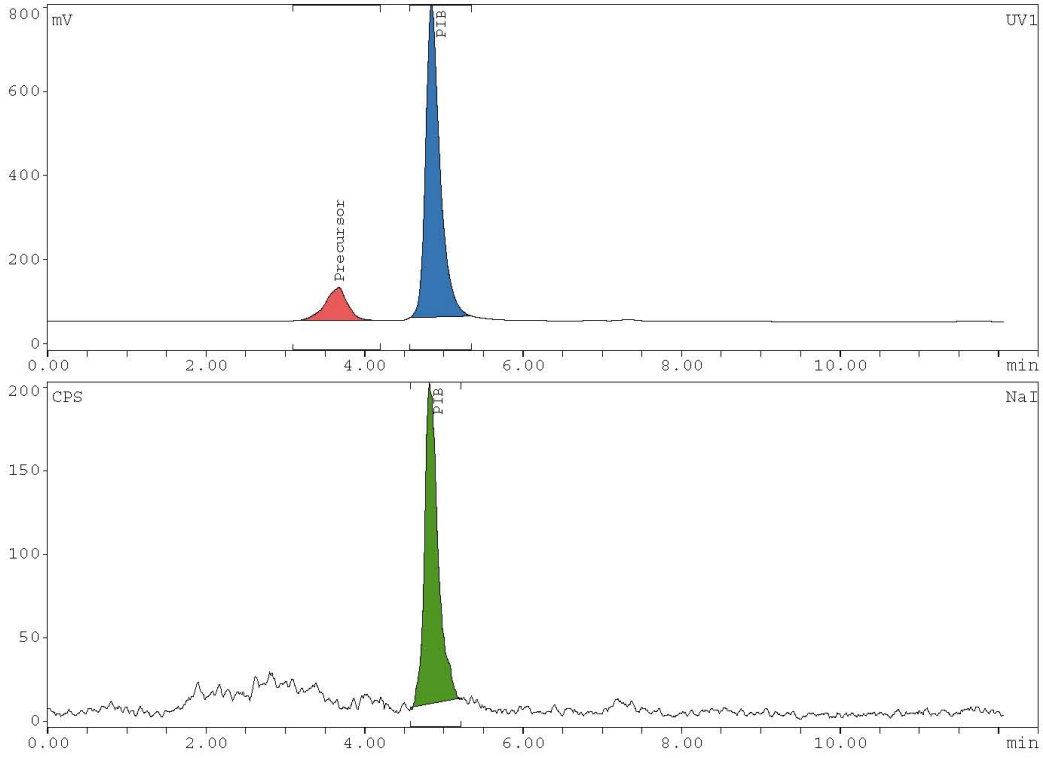
**Integration UV1**

| Substance | R/T | Type | Area | %Area |
|---|---|---|---|---|
| | min | | mV*s | % |
| Reg #1 | 5.68 | BB | 5860.755 | 100.00 |
| Total | | | 5860.755 | |

## $^{11}$C-**6b**

Measurement 091609-06-PIB-R PETNET Page 1/1
C:\GINA_LC\RUOSHI51109\091609-06-PIB-R Print date: 9/16/2009




**Sample description**

| | | |
|---|---|---|
| Measurement: | 091609-06-PIB-R, injection : | 9/16/2009 11:08 AM |
| Method: | RUOSHI51109 from: | 9/16/2009 9:26 AM |
| Station number: | 3 | |

**Preparation**

| Preparation | Sign/Lot. |
|---|---|
| | |

**Integration NaI**

| Substance | R/T | Type | Area | %Area |
|---|---|---|---|---|
| | min | | Counts | % |
| PIB | 4.83 | BB | 2260.728 | 100.00 |
| Total | | | 2260.728 | |
| Total area | | | 8295.188 | |

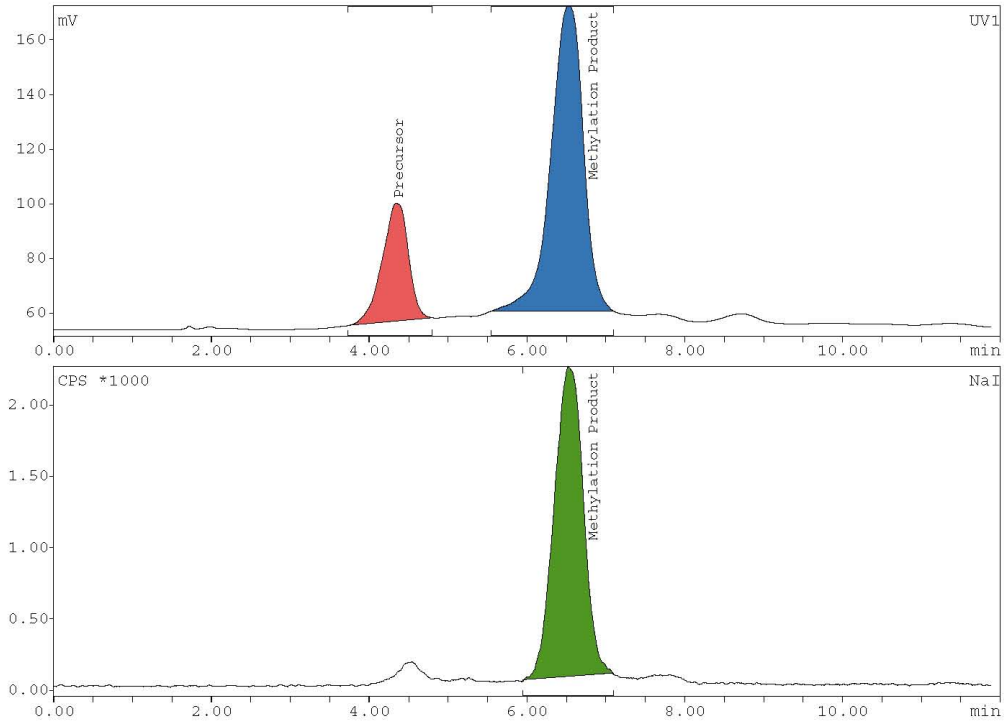
**Integration UV1**

| Substance | R/T | Type | Area | %Area |
|---|---|---|---|---|
| | min | | mV*s | % |
| Precursor | 3.67 | BB | 1540.636 | 14.12 |
| PIB | 4.85 | BB | 9372.823 | 85.88 |
| Total | | | 10913.459 | |

$^{11}$C-**7b**

Measurement 080709-07-DAS-R PETNET Page 1/1
C:\GINA_LC\RUOSHI51109\080709-07-DAS-R Print date: 8/7/2009




**Sample description**

| | | |
|---|---|---|
| Measurement: | 080709-07-DAS-R, injection : | 8/7/2009 10:43 AM |
| Method: | RUOSHI51109 from: | 8/7/2009 10:31 AM |
| Station number: | 3 | |

**Preparation**

| Preparation | Sign/Lot. |
|---|---|
| | |

**Integration NaI**

| Substance | R/T | Type | Area | %Area |
|---|---|---|---|---|
| | min | | Counts | % |
| Methylation Pro | 6.53 | BB | 55813.94 | 100.00 |
| Total | | | 55813.94 | |
| Total area | | | 95407.13 | |

**Integration UV1**

| Substance | R/T | Type | Area | %Area |
|---|---|---|---|---|
| | min | | mV*s | % |
| Precursor | 4.35 | BB | 993.630 | 23.71 |
| Methylation Pro | 6.53 | BB | 3196.689 | 76.29 |
| Total | | | 4190.319 | |