Synthesis, characterization and ^{11}C- radiolabeling of amino phenyl benzothiazoles: Structural effects on the alkylation of amino group.

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Abstract: Several amino phenyl benzothiazoles were prepared with a view to using them as amyloid binding agents for imaging β-amyloid in Alzheimer's disease. These precursors were radiolabeled with ^{11}C-positron-emitting radioisotope using an automated synthesizer and selected radiolabeled compounds were further purified by HPLC. Our results demonstrate that changes in structure have a major influence on the radioactive yield and the ease with which the radiolabel can be introduced. Amino phenyl benzothiazoles with an attached isopropyl group resisted dialkylation perhaps due to steric hindrance caused by this group. Straight chain attachment of methyl, ethyl, butyl and crotyl groups in the structure decreased the radiochemical yield. Notably, the o-amino phenyl benzothiazole derivatives were difficult to alkylate despite stringent experimental conditions. This reactivity difference is attributed to the hydrogen bonding characteristics of the o-amino group with the nitrogen atom of the thiazole ring.

Introduction: Dementia is a generic term used to describe a collection of conditions which lead to the progressive loss of cognitive function. Presently 30 million people worldwide are affected and this number is expected[1] to rise to over 80 million by 2050. The most common form of dementia is Alzheimer's disease (AD) accounting for 50-70% of all cases[2]. Progressive neuronal loss occurs in AD and the formation of β-amyloid plaques and neurofibrillary tangles within the brain tissue have been hypothesised to play a role in pathogenesis[3-5]. Efforts to develop imaging agents to aid the diagnosis of AD and monitor its progression have focussed on small molecules that bind to the Aβ-amyloid peptide.

In the past decade, benzothiazole derivatives have been found to exhibit remarkable selectivity towards amyloid and several aminophenyl benzothiazole derivatives have been investigated as potential imaging probes. Todd et al[10] provide a comprehensive review of the development of these compounds for amyloid imaging. The [^{11}C]-6-Me-BTA was prepared by methylation of 4-(6-methyl-2-benzothiazolyl) aniline using [^{11}C] methyl iodide[11]. Additional modification by elimination of the 6-methyl group in the structure gave [^{11}C]BTA which showed improved uptake and washout characteristics in normal mice and specific amyloid binding in transgenic mice *in vivo* and in human AD brain homogenates[12,13]. The 6-hydroxy substituted amino methyl benzothiazole derivative is one of the most successful radiolabeled imaging probes to date and has been named as Pittsburgh Compound B (PIB).[14-24] Here we describe the synthesis, characterization and ^{11}C- radiolabeling of aminophenyl benzothiazole derivatives as an extension to previously described compounds. We introduced alkyl groups on the amino arm in the structure of the PIB compound and examined the reactivity towards alkylation. The rationale for examining the o-aminophenyl benzothiazole compounds is to investigate the presence of intramolecular hydrogen bonds which may confer a more planar molecular structure which is thought to be important for binding to amyloid plaques. These intramolecular hydrogen bonds are lacking in p-aminophenyl benzothiazoles.

Experimental: All chemicals were purchased from Aldrich/Sigma Chemical Company and used as received. Anhydrous solvents were also obtained from Aldrich/Sigma in Sure/Seal™ bottles and transferred to reaction vessel via cannula under nitrogen atmosphere. Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker 400, 500 or 700 , 900MHz instruments and chemical shifts are reported in parts per million relative to tetramethylsilane, which was the internal standard. ^{13}C NMR spectra were recorded in $CDCl_3$ on the same instruments by using proton decoupling technique. The chemical shifts reported for ^{13}C NMR spectra are referenced to chloroform at 77.0ppm.

p-Aminophenyl benzothiazole: A flask was charged with 30 ml of polyphosphoric acid and heated to 220^{o}C over an oil bath. p-Amino benzoic acid (5.48g, 0.036mole) was added in aliquots and the contents were stirred to dissolve the amino benzoic acid to form a homogenous solution. A brown solution resulted after the addition of the acid. After this period, 5.0g (0.04mole) of o-aminothiophenol was transferred into the mixture when a vigorous reaction took place forming a dark brown slurry. The reaction mixture was heated to 220^{o}C and stirred for additional 8 hours maintaining the temperature. After this period, the mixture was allowed to cool to 100^{o}C and the viscous dark liquid was carefully poured into a beaker containing 500ml of ice/water mixture. A precipitate was obtained which was filtered and washed with 4 x250 ml of water. The resulting solid was once again placed in an Erlenmeyer flask and a solution of sodium carbonate (50g/250ml water) was added and the mixture was stirred for 1h. The insoluble precipitate was filtered off and washed with additional water (2x200ml) and dried under vacuum to yield a light greenish yellow powder. The product was further purified by crystallization using methanol/water (80:20) to furnish the required p-amino phenyl benzothiazole as light yellow needles (5.4g, 0.22mol, 66% yield). Selected IR bands :3429, 3178, 1636, 1604, 1475, 1433, 1312, 1250, 1228, 1182, 1159, 963, 827, 760 cm^{-1}; ^{1}H NMR (400MHz) (d_6-DMSO), δ ppm: 8.02-7.98 (m, 2H), 7.77-7.73 (d, 2H, J=8.0Hz), 7.45-7.44 (t, 1H), 7.36-7.31 (t, 1H), 6.69-6.65(d, 2H, J=8.0Hz), 5.89 (s, 2H); ES+ mass: m/z calculated for $C_{13}H_{10}N_2S$: 226; found 227 (M+1}

p-N-methyl aminophenyl benzothiazole: A similar procedure was adopted for the preparation of this compound except p-N-methyl amino benzoic acid was used and the greenish grey product was further recrystallized using methanol/water mixture (75/25) to obtain dark shiny crystals of the desired product (3.5g, 0.014mol, 35% yield).

IR: 3307, 1654, 1609, 1540,1513, 1466, 1440, 1416, 1342, 1316, 1286, 1255, 1227, 1181, 1161, 1111, 1074, 1007, 962, 864, 824, 758 cm^{-1}; ^{1}H NMR (200MHz) (d_6-DMSO)δ ppm: 8.0-7.89 (m, 2H), 7.84-7.80(d, 2H, J=8.0Hz), 7.48-7.45 (t, 1H), 7.35-7.28 (t, 1H), 6.66-6.62 (d, 2H, J=8.0Hz), 6.47 (bs, 1H), 3.28

s, 3H); ^{13}C NMR (d_6-DMSO)δ ppm: 168.8, 154.6, 153.2, 134.4, 129.4, 126.9, 124.9, 122.6, 122.4, 120.7, 112.2, 29.9; EI mass calculated for $C_{14}H_{12}N_2S$: 240, found : 241 (M+1).

Mono alkylation of Aminophenyl bezothiazoles: The mono alkylation reaction was achieved using a standard procedure described below. The appropriate alkyl iodide/bromide was used for the mono alkylation reactions. As an example, to prepare p-N-isopropylamino phenyl benzothiazole, 0.452g (2 mmol) of p-Amino phenyl benzothiazole was placed in a round bottom flask, 40 ml of acetonitrile was added and the contents stirred to form a homogenous solution. To this mixture was added, with stirring, 0.3g (2.2 mmol) of anhydrous potassium carbonate. Using a dry syringe, 0.250g (2 mmol) of isopropyl bromide was introduced into the flask and the contents were allowed to reflux for 9 hours. After completion of the reaction, the contents were cooled to room temperature and the mixture was filtered. The solvent was removed under vacuum and the contents were taken up in ethyl acetate (30ml) and washed with water and dried over anhydrous sodium sulphate and filtered. Rotary evaporation of the solvent gave a residue which was column chromatographed over an alumina basic column using hexane/ethylacetate (90:10) to obtain the desired compound. (0.136g, 50% yield). ^{1}H NMR ($CDCl_3$)δ ppm; 7.99-7.96 (d,1H,J=9.0Hz), 7.92-7.89 (d, 2H, J=9.0Hz), 7.85-7.82 (1H, d, J=9.0Hz); 7.46-7.41 (t, 1H), 7.33-7.26 (t, 1H), 6.63-6.60 (d, 2H, J=9.0Hz); 3.92 (s, 1H), 3.75-3.67 (m,1H), 1.25-1.24 (d, 3H, J=6.0Hz); EI mass calculated for $C_{16}H_{16}N_2S$: 268, found : 269 (M+1).

p-N-ethyl- Aminophenyl benzothiazole: ^{1}H NMR ($CDCl_3$)δ ppm: 7.97-7.95 (d, 1H, J=), 7.90-7.88 (d, 2H, J=), 7.42-7.39 (t, 1H), 7.29-7.26 (t, 1H), 6.63-6.61 (d, 2H, J=), 3.95 (bs, 1H), 3.24-3.20 (m, 2H), 1.29-1.26 (t, 3H); ^{13}C NMR ($CDCl_3$):δ ppm: 168.7, 154.4, 150.7, 134.5, 129.1, 125.9, 124.2, 122.5, 122.3, 121.3, 112.3, 38.1, 14.7.

o-N-ethyl-Aminophenyl benzothiazole:^{1}H NMR ($CDCl_3$) δ ppm:8.85 (bs, 1H), 7.96-7.94 (d, 1H, J=), 7.87-7.85 (d, 1H, J=), 7.74-7.72 (d, 1H, J=), 7.46-7.43 (t, 1H), 7.36-7.30 (m,2H), 6.79-6.78 (d, 1H, J=), 6.69-6.67 (t, 1H), 3.40-3.34 (m,2H), 1.44-1.40 (t, 3H); ^{13}C NMR ($CDCl_3$):δ ppm: 169.6, 153.6, 147.5, 132.0, 130.7, 125.9, 124.7, 122.2, 121.2, 114.9, 111.3, 31.6, 14.6.

p- N-butyl-Aminophenyl benzothiazole : ^{1}H NMR ($CDCl_3$) δ ppm: 7.97-7.95 (d, 1H, J=), 7.89-7.88 (d, 2H, J=), 7.82-7.80 (d, 1H, J=), 7.42-7.39 (t, 1H), 7.30-7.28 (t, 1H), 6.63-6.61 (d, 2H, J=), 4.01 (bs, 1H), 3.18-3.15 (t, 2H), 1.65-1.59 (m, 2H), 1.45-1.41 (m,2H), 0.97-0.94 (t, 3H); ^{13}C NMR ($CDCl_3$):δ ppm: 168.8, 154.4, 150.8, 134.5, 129.1, 125.9, 124.2, 122.4, 122.3, 121.3, 112.2, 43.2, 31.5, 20.2, 13.9.

o-N-butyl-Aminophenyl benzothiazole: ^{1}H NMR ($CDCl_3$) δ ppm: 8.93 (bs, 1H), 7.92-7.90 (d, 1H, J=), 7.85-7.83 (d, 1H, J=), 7.72-7.70 (d, 1H, J=), 7.44-7.42 (t, 1H), 7.33-7.27 (m,2H), 6.78-6.76 (d, 1H, J=), 6.66-6.64 (t, 1H), 3.32-3.29 (m,2H), 1.80-1.75 (m, 2H), 1.59-1.54 (m,2H), 1.02-0.99 (t, 3H); ^{13}C NMR ($CDCl_3$):δ ppm: 147.7, 132.1, 130.7, 125.9, 124.7, 122.2, 121.1, 114.8, 111.3, 42.7, 31.3, 20.5, 13.9.

o-N-isopropyl-Aminophenyl benzothiazole: The use of potassium carbonate in acetonitrile did not yield the required compound. However a modification in the synthetic procedure was adopted for this precursor. In a flask was placed 0.113g (0.5 mmol) of o-aminophenyl benzothiazole and 10 ml of dimethyl sulfoxide and the mixture was stirred to form a homogenous solution. To this mixture was added 20mg (0.5 mmol) of powdered sodium hydroxide resulting into a deep orange brown solution. To this was added 0.062g (0.5mmol) of isopropyl bromide and the resulting mixture was stirred at room temperature for 6 hours. The contents were added to 50ml of ice/water mixture to obtain a precipitate. The precipitated product was extracted with ethyl acetate (50 ml) and the organic layer was separated and dried over anhydrous sodium sulphate. Filtration and rotary evaporation yielded a residue which was further purified by preparative thin layer chromatography to yield a deep yellow product (10% yield). ^{1}H NMR ($CDCl_3$):8.94 (1h< NH), 7.93 (d, J=7.92Hz, 1H), 7.85 (d, J=8.27 (1H), 7.72 (d, J=8.27Hz, 1H), 7.43 (t, J=7.24Hz, 1H), 7.33 (t, J=7.58Hz, 1H), 7.29 (t, J=7.58Hz, 1H), 6.79 (d, J=8.62Hz, 1H), 6.64 (t, J=7.24Hz, 1H), 3.82 (m, 1H), 1.36 (d, J=6.2Hz, 6H); ^{13}CNMR ($CDCl_3$) 169.6, 153.6, 146.7, 133.1, 132.0, 130.9, 125.9, 124.7, 122.2, 121.1, 114.6, 114.4, 111.8, 43.6, 22.9.

p-N-dimethyl-Aminophenyl benzothiazole: This compound was prepared using a similar procedure as before except that methyl iodide and p-methy- Aminophenyl benzothiazole was used as reactants in the presence of sodium hydroxide in dimethyl sulfoxide. The product was further purified by

preparative thin layer chromatography using hexane/ethyl acetate (80:20) (<15% yield) :^{1}H NMR ($CDCl_3$)δ ppm: 7.96-7.93 (m, 3H), 7.82-7.80 (d, 1H, J=), 7.42-7.39 (t, 1H), 7.29-7.27 (t, 1H), 6.73-6.71 (d, 2H) J=), 3.03 (s, 6H); ^{13}C NMR ($CDCl_3$)δ ppm: 168.8, 154.3, 152.1, 134.5, 128.8,125.9, 124.1, 122.2, 121.3, 111.7, 29.34

o-N-methyl- aminophenyl benzothiazole : Column chromatography using 100:5 hexane/ethyl acetate gave a deep yellow product. (yield <15%). ^{1}H NMR ($CDCl_3$): 8.79 (1H, NH), 7.96 (d,J=7.9Hz, 1H), 7.86 (d, J=7.8Hz, 1H), 7.73 (d, J=7.7, 1H), 7.44 (t, =7.7Hz, 1H), 7.34 (m, 2H), 6.77 (d, J=8.25Hz, 1H), 6.69 (t, J=7.36 1H), 3.04 (d, J=4.96Hz, 3H); ^{13}C NMR ($CDCl_3$) 169.1, 153.2, 148.0, 132.7, 131.7, 130.2, 125.5, 124.3, 121.8, 120.7, 114.6, 114.3, 110.4, 29.3.

p-N-crotyl- Aminophenyl benzothiazole: IR : 3399, 3002, 1611, 1529, 1474, 1455, 1336, 1313, 1255, 1226, 1179, 1123, 1101, 963, 827, 752,^{1}H NMR ($CDCl_3$)δ ppm: 8.0-7.81 (m, 4H), 7.47-7.43 (t, 1H), 7.34-7.26 (t, 1H), 6.67-6.63 (d, 2H, J=8.0Hz), 5.84-5.51 (m,2H), 3.77-3.74 (d,2H,J=6.0Hz), 1.76-1.71 (d, 3H); EI mass calculated for $C_{17}H_{16}N_2S$: 280, found : 281 (M+1).

o-N-crotyl -Aminophenyl benzothiazole was prepared in analogous method to that for the preparation of p-N-crotyl- Aminophenyl benzothiazole.

o-N-crotyl- Aminophenylbenzothiazole: ^{1}H NMR ($CDCl_3$)δ ppm: 9.02 (bs, 1H), 7.96-7.95 (d, 1H, J=), 7.85-7.84 (d, 1H, J=), 7.74-7.73 (d, 1H, J=), 7.45-7.43 (t, 1H), 7.34-7.32 (t, 1H), 7.30-7.29 (t, 1H), 6.78-6.77 (d, 1H, J=), 6.69-6.67 (t, 1H), 5.83-5.80 (m, 1H), 5.69-5.67 (d, 1H, J=), 3.92 (d, 2H), 1.76-1.75 (d, 3H, J=), ^{13}C NMR ($CDCl_3$)δ ppm: 169.6, 153.6, 147.4, 133.2, 132.0, 130.7, 127.7, 127.2, 125.9, 124.7, 122.3, 121.1, 115.3, 114.7, 111.5, 44.8, 17.88.

Cyclotron: Carbon-11 was produced as $^{11}CO_2$ via the $^{14}N(p,\alpha)^{11}C$ nuclear reaction by irradiating ultra-pure nitrogen gas containing 0.5 % oxygen with 16 MeV cyclotron produced protons. The cyclotron used is a *Cyclone®18 Twin* (IBA Molecular) dual ion source, 18 MeV fixed energy, negative ion accelerator. The gas target employed has an aluminium body with a volume of approximately 50

cm^3 and is filled to a pressure of 20 bar at room temperature with target gas. The target windows consist of a 12.5 µm thick titanium foil on the vacuum side and a 500 µm thick aluminium foil on the target side which effectively reduces the beam energy delivered to the target gas from 18 MeV to 16 MeV. The target yield at saturation is around 4.4 GBq/µA.

Radiochemistry: Conversion of the cyclotron produced $^{11}CO_2$ to [^{11}C]methyl iodide and subsequent radiolabelling was performed using a *Synthra MeI Plus* (Synthra GmbH) automated synthesis module. After cyclotron target irradiation, the $^{11}CO_2$ was transferred to the module and cryo-trapped using liquid nitrogen with any remaining target gas and unwanted by-products such as ^{11}CO directed to waste. The $^{11}CO_2$ was then reacted with hydrogen gas at 425°C over a nickel catalyst to form [^{11}C] methane. The [^{11}C] methane was purified by trapping on Carbosphere® at -120°C with any unreacted $^{11}CO_2$ directed to waste. [^{11}C]Methane was then released from the trap by heating and then reacted with iodide at 750°C to form the synthon [^{11}C] methyl iodide. Excess iodine was trapped on sodium hydroxide cartridges while the synthon was purified by trapping on Porapak® traps. The synthon was then released from the traps by heating under helium flow and directed to a reaction vessel for the radiolabelling reaction. Typically, $^{11}CO_2$ was converted to [^{11}C] methyl iodide with a yield of 30%. Typically the activity of trapped $^{11}CO_2$ at the end of the bombardment was 18-20GBq, the activity obtained after conversion of [^{11}C] methyl iodide was 8-10GBq (at the trap)

The radiolabelling was conducted as follows: For the [^{11}C] methylation of the aminophenyl benzothiazole precursors, 1-2 mg of the compound was weighed and was transferred into the reaction vessel containing a stirrer bar. Using a dry syringe, 0.5ml of dimethyl sulfoxide was introduced followed by 10-15 mg of anhydrous potassium carbonate and the reaction vessel was attached to the automated synthesizer (Figure 1) and the contents were stirred at room temperature. [^{11}C] methyl iodide produced from the Synthra automated system was bubbled to the reaction mixture for 5 minutes and the temperature of the reaction mixture was heated to 120^0C and held for 5 minutes before transferring to the product vial.

For the [^{11}C] methylation of the mono-alkylated aminophenyl benzothiazole precursors, 1-2 mg of the sample was introduced into the reaction vessel, followed by 0.5 ml of anhydrous dimethyl sulfoxide and then 10 mg of powdered potassium hydroxide was added to the mixture. The reaction was stirred at room temperature for 5 minutes and the radiolabelling was conducted as described previously. The total time of synthesis from the start to end was 12 minutes.

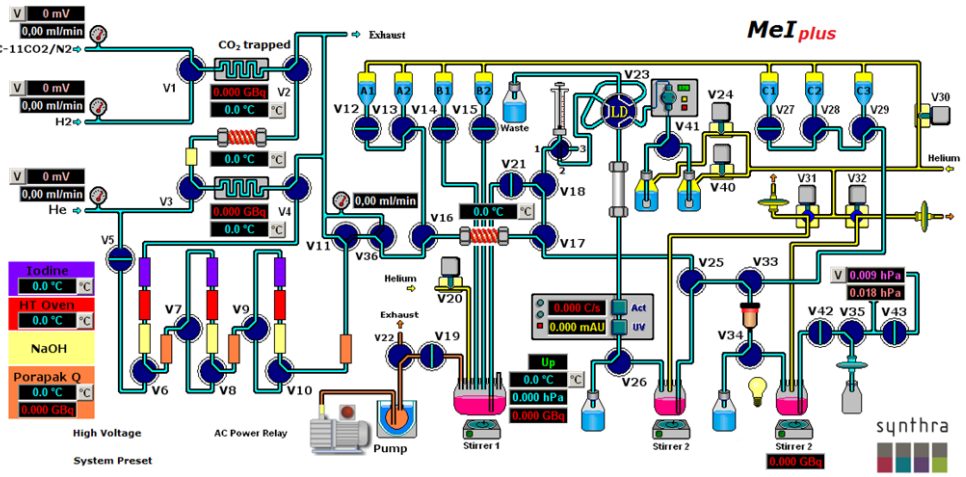


Figure 1: MeI Plus synthesiser and user interface showing schematic of synthesiser.

HPLC methods: The radiolabeled compounds were further purified using the following HPLC methods. HPLC analysis was performed using a Shimadzu model-LC 20AD instrument having a diode array detector, a quaternary pump, thermostatic column compartment, and a radiodetector (Bioscan *Flow Count* with a pin-diode detector) in conjunction with *LCsolutions* software computer operated system. The analytical column used for detection was a *Luna* (Phenomenex) 5 micron – reversed phase C-18 column of dimension 150/4.6 mm. The column was maintained at room temperature throughout the analysis. Aqueous mobile phase (A): 0.8%TEA (8ml) and H_2O (992 ml), pH adj ca 7.5 with 85% H_3PO_4 (ca 2.1 ml). Organic mobile phase (B): MeCN. Flow rate was maintained at 1ml/min throughout the analysis.

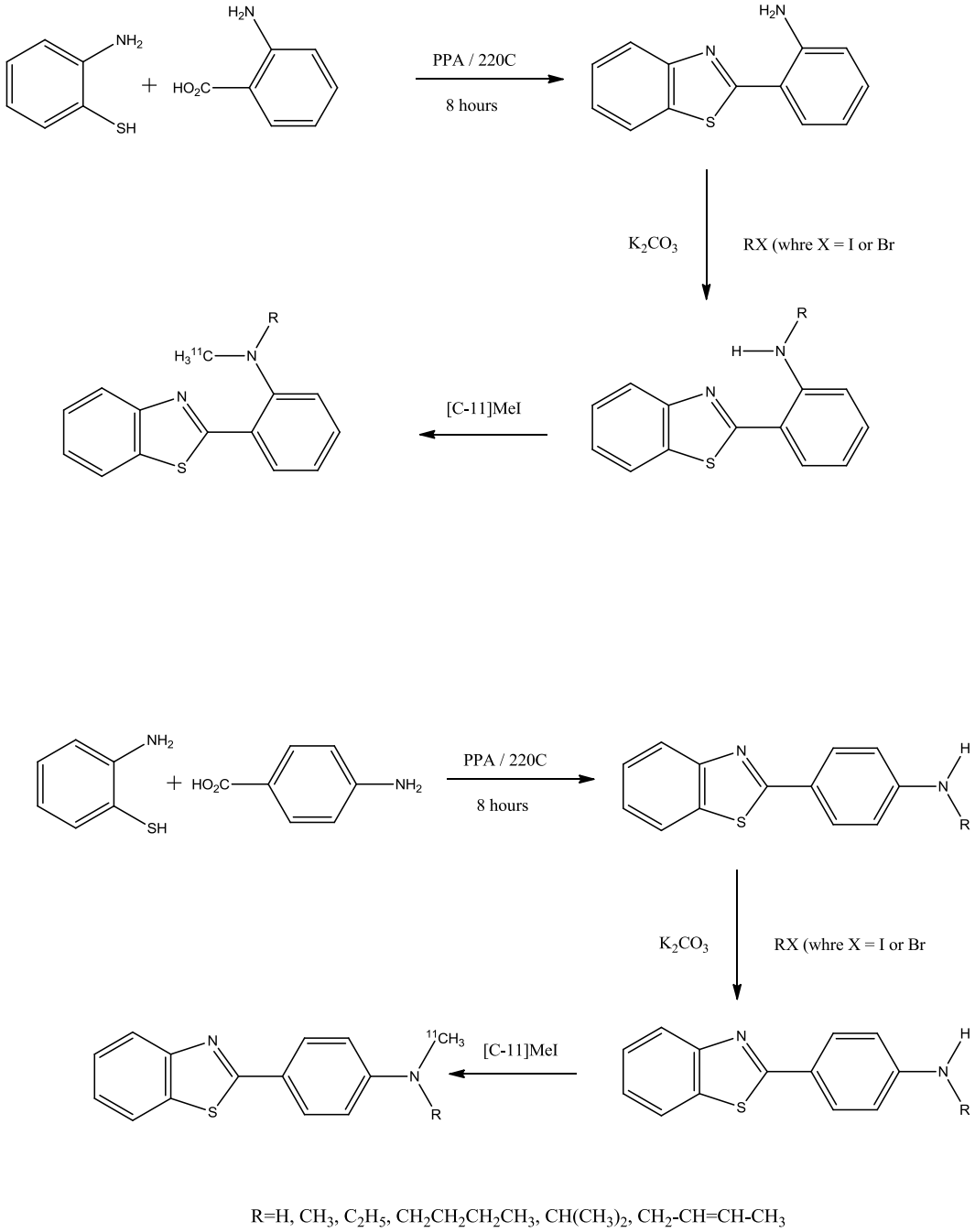
Method 1: 0-1min 50% MeCN(solvent B): 50% (solvent A); 1-25 min 50-95%(B); 25-30min 95% (B), 30-31 min 95-50% (B), 31-33 min 50%(B)

Method 2: 0-1min 50% MeCN(solvent B): 50% (solvent A); 1-10 min 50-95%(B); 10-17 min 95%(B), 17-31 min 95-50%(B),31-33 min 50%(B).

Results and discussion:

Synthesis and Radiolabeling of benzothiazole precursors:

The synthesis of aminophenyl benzothiazole was achieved following Scheme 1 :



R=H, CH_3, C_2H_5, $CH_2CH_2CH_2CH_3$, $CH(CH_3)_2$, CH_2-CH=CH-CH_3

Scheme 1: Synthesis of [^{11}C]-radiolabeled aminophenyl benzothiazoles

The o-and p-Aminophenyl benzothiazole precursors were prepared by the condensation of o-aminothiophenol with corresponding aminobenzoic acid in polyphosphoric acid at 220˚ C. The mono alkylated precursors were prepared by reacting the amino functionality with the corresponding alkyl halide. Radiolabeling was achieved by treating the appropriate benzothiazole precursors with [^{11}C]-methyl iodide. The identity of the radiolabeled compounds was confirmed by comparing the HPLC retention times with those of the cold reference standards. The primary focus of this paper was to examine the reactivity of the amino functionality of primary and secondary aminophenyl benzothiazole ligands with respect to methylation using [^{11}C] methyl iodide. Our investigations have demonstrated that the radiolabeling of unsubstituted aminophenyl benzothiazole was accomplished easily using sodium/potassium carbonate and methyl iodide, however the secondary amino compounds required much harsher conditions including higher temperatures, stronger base as well as more polar solvent such as dimethyl sulfoxide. Our radiolabeling experiments also demonstrated that the [^{11}C] methylation did not occur if bulkier alkyl groups (e.g. isopropyl) were present on the amino functionality. This highlights the possibility that steric hindrance impedes entry of the second alkyl group in the structure of the molecule. In contrast, when straight chain alkyl groups were present on the amino moiety (hydrogen, Methyl, Ethyl), the [^{11}C]–methylation was successful with reasonable radiochemical yields. Having gained the insight into the radiolabeling of the precursors, we plan to further elaborate this strategy for ortho aminophenyl substituted benzothiazoles to assess whether amyloid binding of these derivatives is diminished or enhanced. The existing literature is dominated by p and m-substituted aminophenyl benzothiazole derivatives.

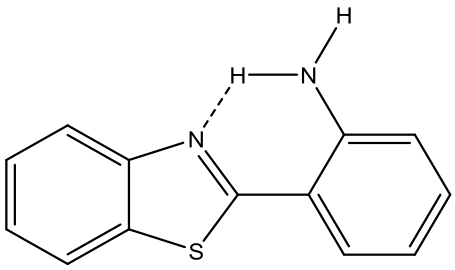
Effect of bulkier groups during dialkylation:

To further explore the contribution of steric hindrance to difficulties in alkylating o-aminophenyl and p-aminophenyl benzothiazole with bulkier groups, we attempted to synthesize the t-butyl group attached aminophenyl benzothiazole. However, the reaction did not yield the required t-butyl substituted product. This may be rationalized due to the formation of elimination products instead

of substitution. It is also known that at higher temperatures, the elimination product predominates as previously proposed [25,26]. In this regard we note that alkylation of the o-aminophenyl benzothiazoles was also difficult to achieve and yields were very low compared to that for p-aminophenyl benzothiazoles. We also failed to obtain isorpropyl-o-aminophenyl benzothiazole using standard reaction conditions (K_2CO_3/MeCN). However, we were able to prepare the isopropyl precursor with a stronger base (NaOH), albeit in very low yields. In contrast, the straight chain n-butyl derivatives of both aminophenyl benzothiazoles could be synthesised consistent with the notion that bulkier groups hinder alkylation. Further support comes from the relative ease with which both o and p- crotyl aminophenyl benzothiazoles could be prepared, presumably due to the straight chain nature of the halide.

Structural effects of aminophenyl benzothiazoles:

We examined the difference between the structural characteristics of o-aminophenyl benzothiazole and p-aminophenyl benzothiazole. Imparting further stability to the molecule, the o-aminophenyl benzothiazole forms intramolecular hydrogen bonding with the nitrogen atom of the thiazole ring as shown below:

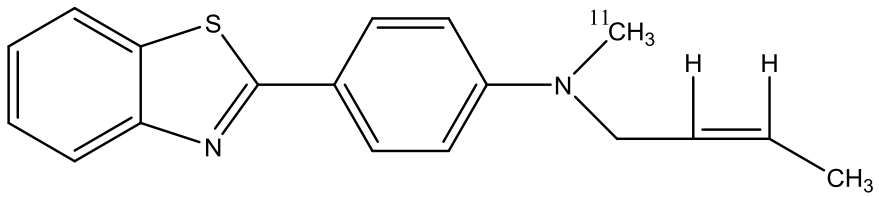


This has been previously proposed by Dey et al[27] to account for the solvatochromism of some aminophenyl benzothiazole derivatives. According to this proposal, the lone pair of electrons associated with the amino group is being forced to be parallel with the π-cloud, thereby increasing the resonance character of this group with the benzothiazole moiety. Greater difficulty in alkylation of the o-aminophenyl benzothiazole derivatives compared to p-aminophenyl benzothiazole derivatives may be due to the formation of the hydrogen bond in the former; additional energy

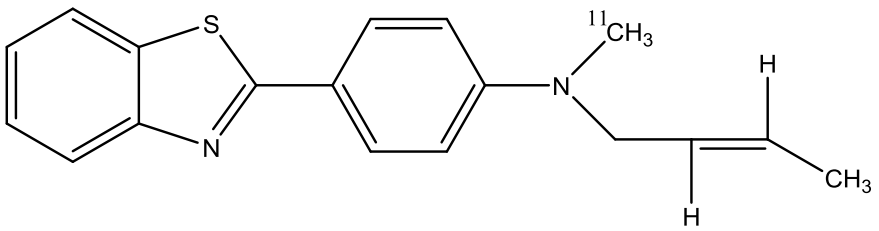
would thus be required to break the bond. A detailed account of the hydrogen bonding characteristics and density functional energy calculation is reported elsewhere [28].

The radiolabeling of the p-aminophenyl benzothiazole with [^{11}C]CH_3I went smoothly and the required mono alkylated product, the p-N-[^{11}C]methyl amino phenyl benzothiazole, was obtained even under mild reaction conditions (e.g. using K_2CO_3 as the base and dimethyl sulfoxide as the solvent). The radiolabeled compound had similar characteristics to the cold material synthesized using a similar strategy. The starting material p-aminophenyl benzothiazole had a HPLC retention time of approximately 6.0 minutes (method 1). The radiolabeled product had a retention time of 9.3 minutes and the cold alkylated product showed a consistent retention time of 9.25 minutes confirming the radioalkylation of the starting material.

We next examined the radiolabeling of a crotyl substituted-p- aminophenyl benzothiazole derivative. A facile radioalkyation of the crotyl derivative was achieved using [^{11}C]methyl iodide. (figure 2). The smaller hot peak in the chromatogram is due to the cis isomer methylated product (see scheme 2 below) present in the starting material as shown below (since the Aldrich starting material for this compound, (crotyl bromide) is only 87% trans and 13% cis.)



N-^{11}C-methyl, N'- (*cis*)-crotyl aminophenyl benzothiazole (minor peak)



N-^{11}C-methyl, N'- (*trans*)crotyl aminophenyl benzothiazole (major peak)

Scheme 2. Representation of cis (minor) and trans (major) isomers of crotyl aminophenyl benzothiazoles labelled with [$^{11}CH_3$].

The rationale for introducing the crotyl group in the structure of the p-aminophenyl benzothiazole was to increase the liphophilicity of the product. We next conducted experiments to radiolabel the mono methyl substituted p-aminopheny benzothiazole using a similar strategy. While radiolabeling took place, the yield of radiolabeled material was much lower than that for the parent p-aminophenylbenzothiazole. (figure 3, method 2). We rationalize that the bulkier group attached to the amine moiety perhaps causes steric hindrance towards the incoming methyl group and therefore lower radiolabeling capacity consistent with our earlier discussion. The cold isopropyl substituted aminophenyl benzothiazole showed a retention time of approximately 14 min (method 2) while the radiodialkylated product showed a retention time of 18 minutes respectively consistent with the dialkylated compounds retention time.

We next focused our attention to radiolabel the p-N-methyl aminophenyl derivative of benzothiazole in order to conduct a second alkylation using [^{11}C] methyl iodide. The radiolabeled compound which had an HPLC retention time of 9.8 min (method 2). We also see the peak at 7.5 min corresponding to the unlabelled starting material. The radiolabeled compound had similar retention time (9.8 min) of the cold sample demonstrating that the alkylation did occur and that we were able to get reasonable yield of the radiotracer.

We also examined the radiolabeling of o-aminophenyl benzothiazole precursors with [^{11}C] methyl iodide to establish the trend among the derivatives with the structure. Accordingly we chose as a start the o-aminophenylbenzothiazole compound. Both o-aminophenyl and p-aminophenyl benzothiazoles underwent mono alkylation with radiolabeled methyl iodide and the radioactive yield was also comparable. Figure 4 shows the mono alkylated [^{11}C] methyl group attached o-aminophenyl benzothiazole. The radiolabeling of this precursor was further confirmed using authentic cold precursor. Interestingly, the alkylated compound showed a retention time slightly lower than the

starting material perhaps due to its polarity and also the change in the position of the amino group in the structure of the molecule (see the o-amino and p-amino phenyl benzothiazoles retention times). This is further substantiated by a simple thin layer chromatographic profile of p-aminophenyl benzothiazole and o-aminophenyl benzothiazole. The former had a R_f value of 0.24 while the later had a R_f value of 0.62 in 20:80 ethylacetate/hexane medium. We also compared the yield of individual tracers of these substituted aminophenyl benzothiazoles and table 1 provides the data. For the individual derivatives reported in this paper, experiments were repeated to determine the yields. The reported yields are decay corrected yields based on MeI. We have also included the literature data for the C-11 PIB compound for the purposes of comparison with our results [29-32]. The yield of C11-PIB is higher compared to the yield of p-aminophenyl benzothiazole for mono alkylation and this is attributed either due to the use of weaker base (K_2CO_3) in our case compared to stronger base (KOH) used for the mono alkylation of C11-PIB [32] or due to the additional activation by the presence of ether group in the structure of PIB. From the table values the following trend in the radiochemical yield is observed: The p-amino methyl phenyl benzothiazole was obtained with 3±2% yield. Among the derivatives, the isopropyl substituted benzothiazole gave the lowest yield both in the o and the p- position in the structure. It is also evident from the table values that the dialkylation of the para substituted compound showed a yield of approximately 10±7% while the ortho substituted compound gave a value of 26±11%. In a similar fashion, the p-crotyl group substituted compound gave a yield of 17±11 % while the ortho compound gave a value of only 0.1%. The trend of higher yields for dialkylation versus monoalkylation for both p- and o-amino phenyl benzothiazoles may be explained by the use of sodium carbonate for monoalkylation whereas dialkylation was conducted using a stronger base such as sodium hydroxide.

In summary, we synthesized various substituted aminophenyl benzothiazoles and labelled them with [^{11}C] methyl iodide using an automated synthesizer. The mono alkylation of the aminophenyl benzothiazoles were accomplished using a weaker base like potassium or sodium carbonate while the dialkylation of these precursors required a stronger base like potassium hydroxide/sodium

hydroxide. In the case of para and ortho amino phenyl substituted benzothiazoles, the dialkylation was achieved and the yield of the radiolabel was comparable. Furthermore, the bulkier substituents on the aminophenyl benzothiazoles further hampered the dialkyation especially when we introduced isopropyl group in the structure of these aminophenyl benzothiazoles. Additionally we found that we were unable to synthesize the t-butyl group attached aminophenyl benzothiazoles using t-butyl bromide perhaps due to elimination reactions occurring at higher temperatures. Attempted preparation of these t-butyl substituted aminophenyl benzothiazole using our experimental conditions failed to yield the product. The difficulty in alkylation of the bulkier group attached aminophenyl benzothiazoles is rationalized due to steric hindrance caused by the bulkier group in the structure. Additionally we surmise that o-aminophenyl benzothiazole forms a hydrogen bond with the thiazole nitrogen while the p-aminopheny benzothiazole lacks such a hydrogen bond in the structure. It is also possible that the hydrogen bond causes further stability for the o-aminophenyl benzothiazoles resulting in the difficulty towards dialkylation compared to the p-aminophenyl benzothiazoles. Further work is in progress to evaluate the amyloid binding characteristics of the cold precursors and to examine the ability of the new ^{11}C- radiolabeled compounds to cross the blood-brain barrier and to bind to amyloid in the living brain.

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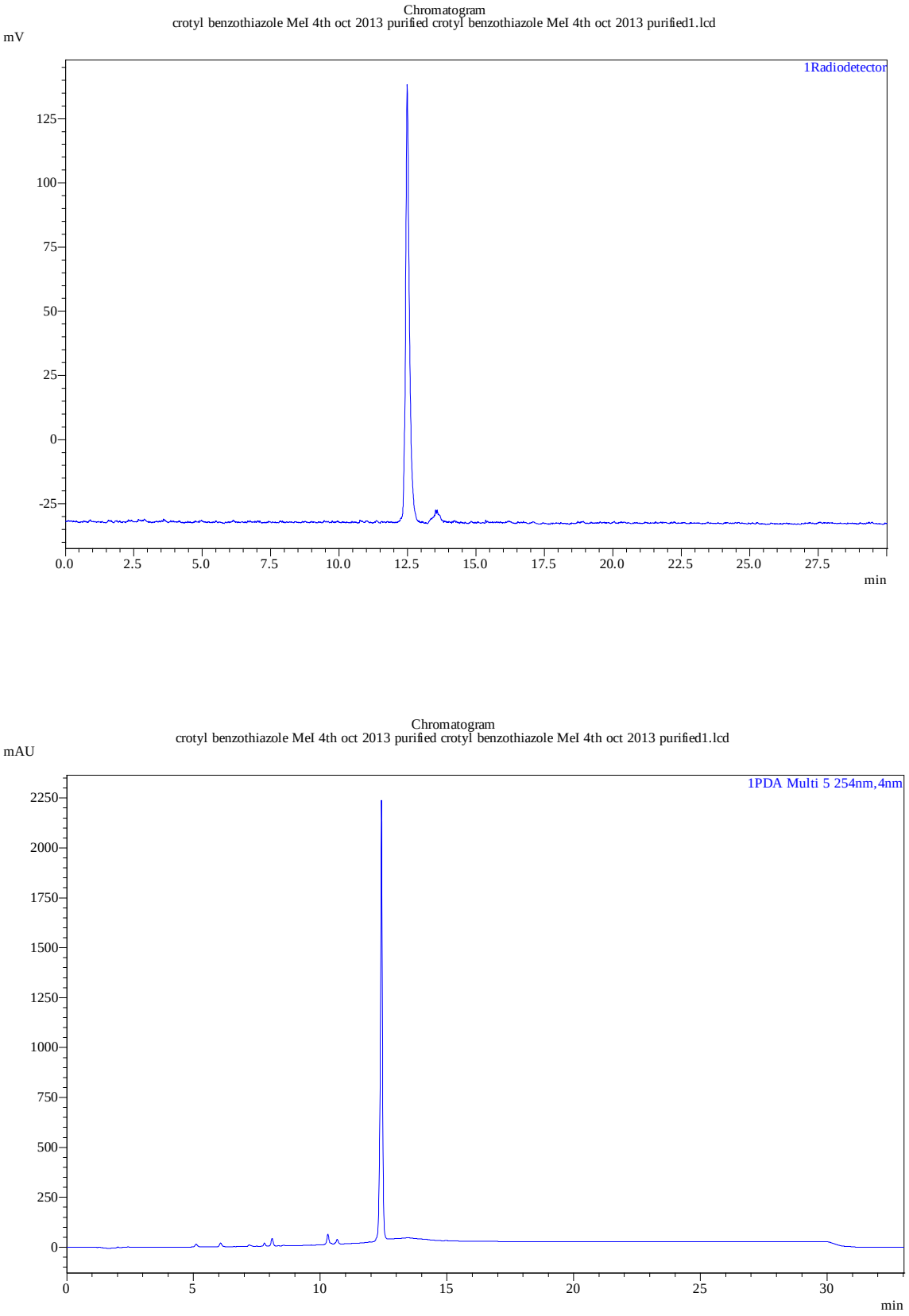
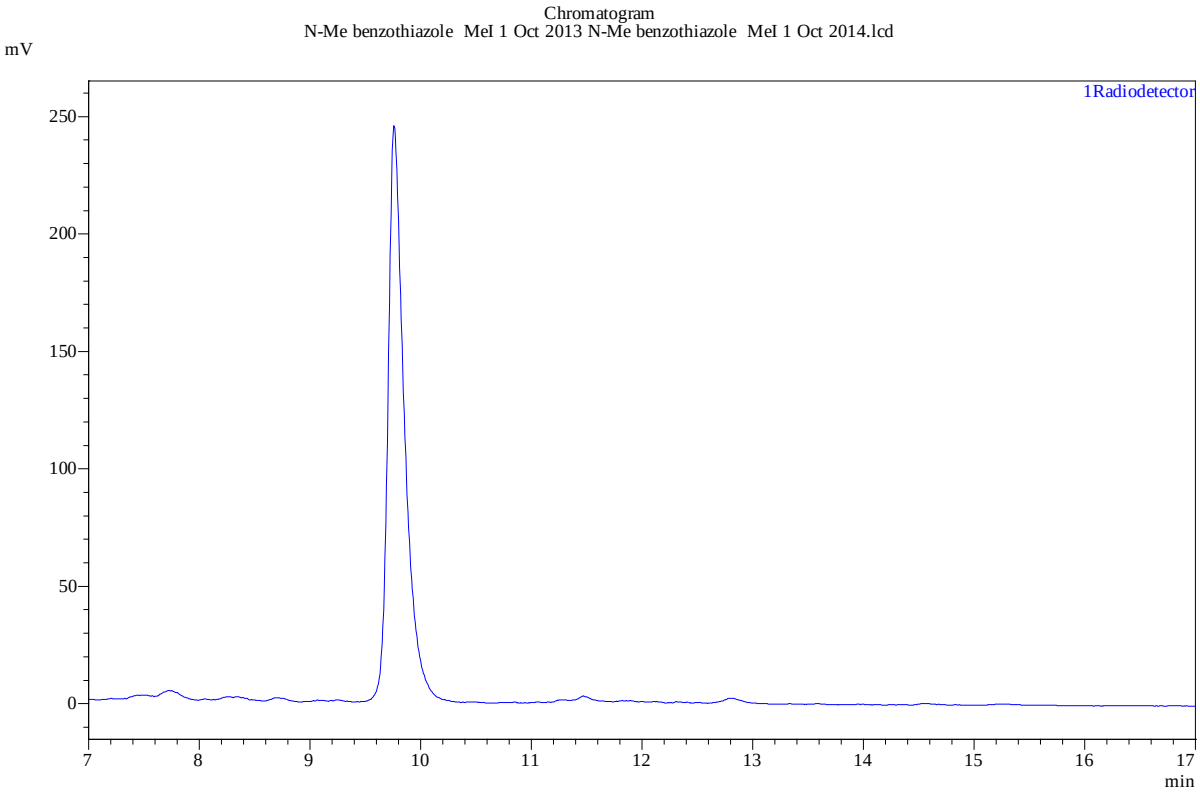


Figure 2. Chromatogram of hot and cold trace of purified ^{11}C-labeled crotyl-p-aminophenyl benzothiazole



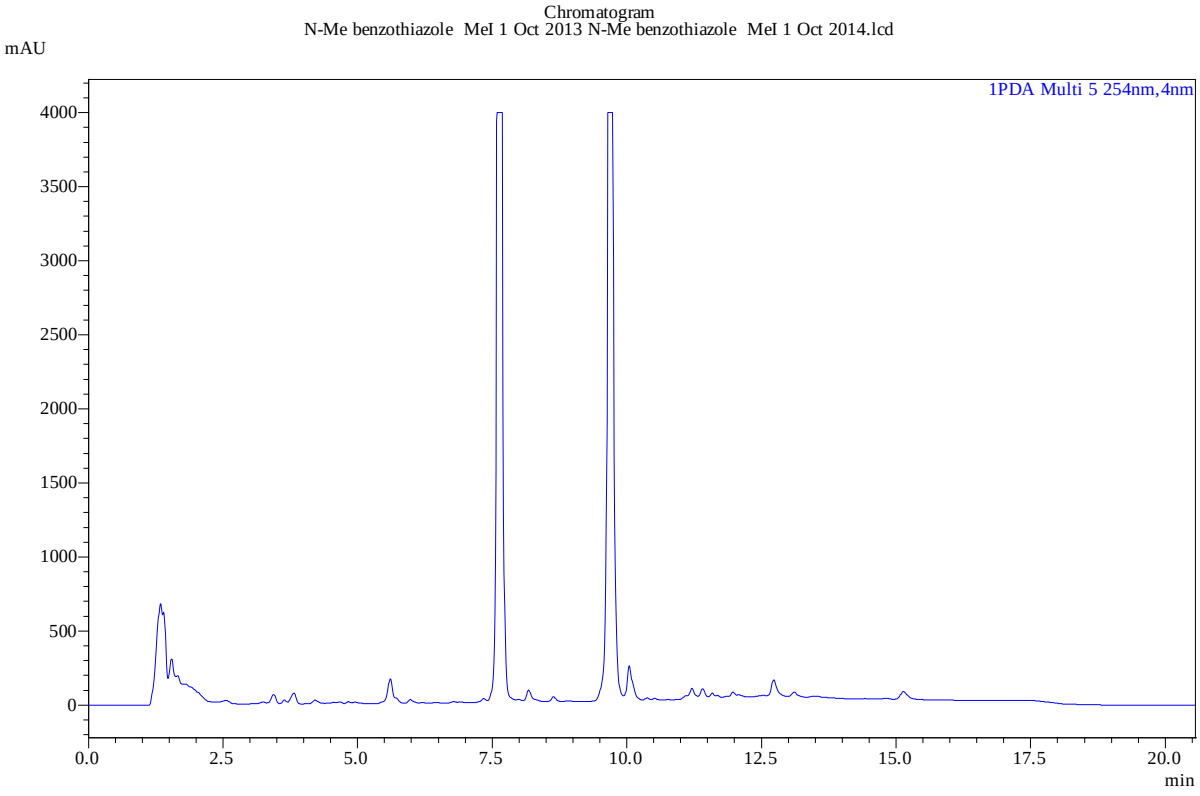
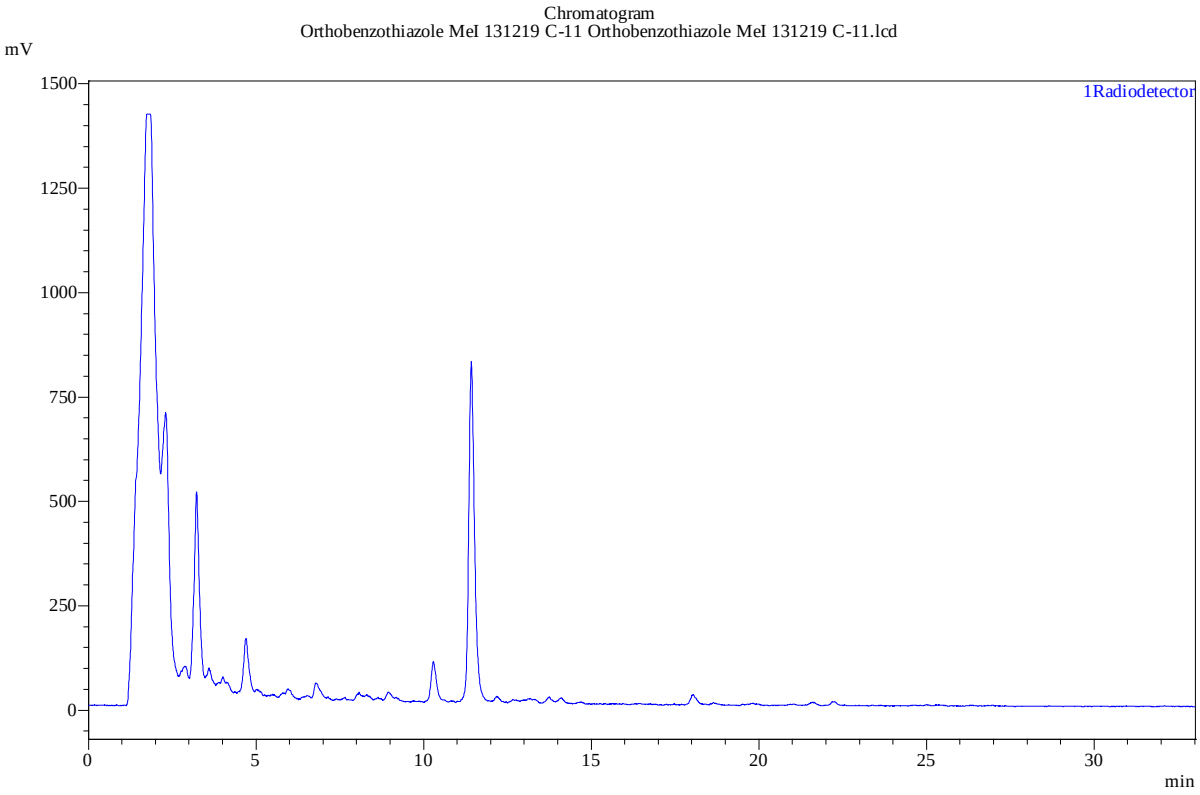


Figure 3. Chromatogram of hot and cold trace of ^{11}C-labeled methyl –p-aminophenyl benzothiazole



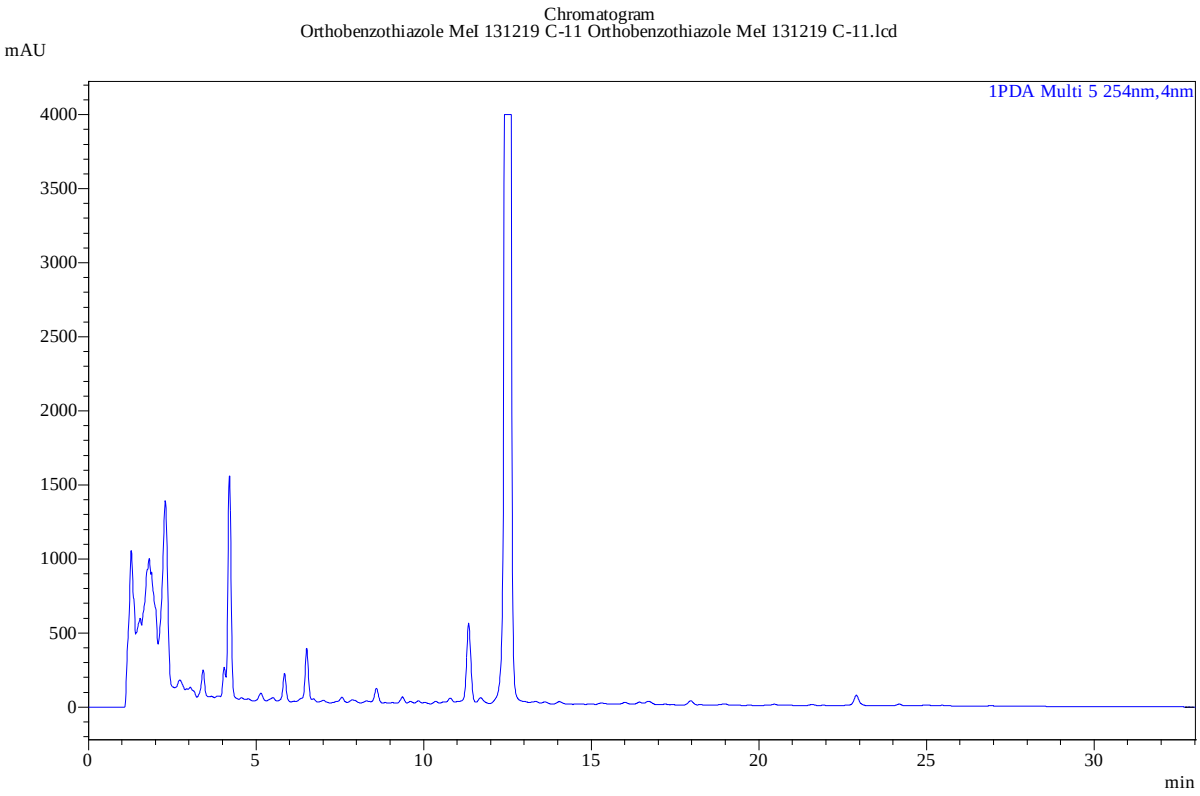
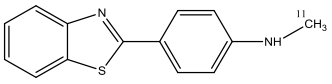
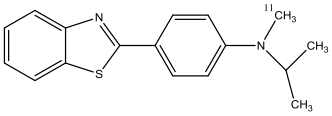
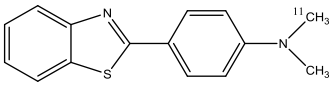
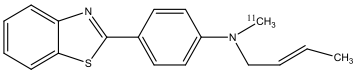
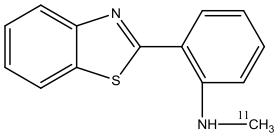
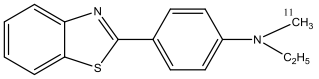
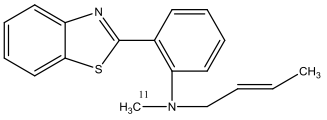
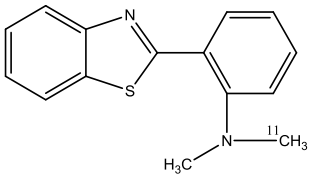
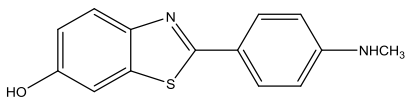


Figure 4. Chromatogram of hot and cold trace of ^{11}C-labeled o-aminophenyl benzothiazole

The percentage yield of radiolabeled compounds based on ^{11}CMeI

Compound	Yield (decay corrected)
	3±2.0
	0.6±0.2
	10±7.0
	17±12
	10±7.0
	31±5.0
	0.2±0.1
	26±11
	25±10 [29-32]