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An Efficient Synthesis of *trans*-*N*-{2-[4-(2-Methoxyphenyl)piperazinyl]ethyl}-*N*-(2-pyridyl)-*N*-(4-tosyloxymethylcyclohexane)carboxamide, A Precursor of [^{18}F]Mefway for Imaging 5-HT$_{1A}$ Receptor

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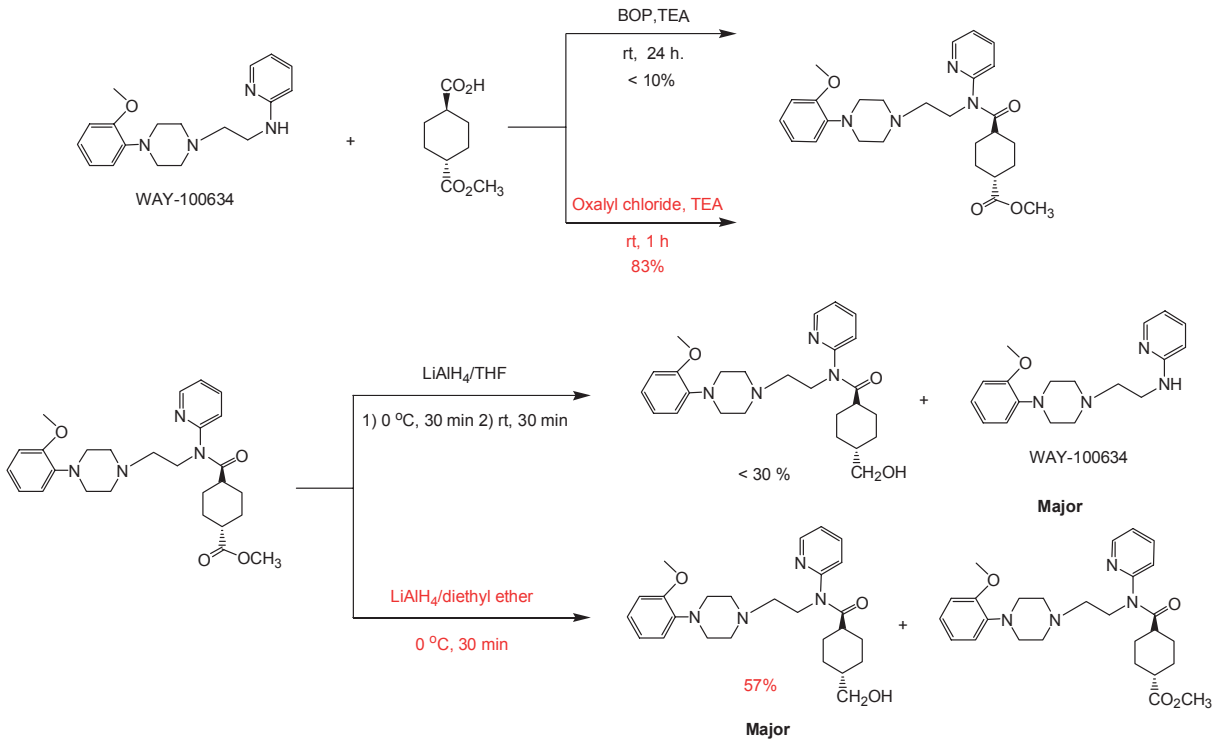
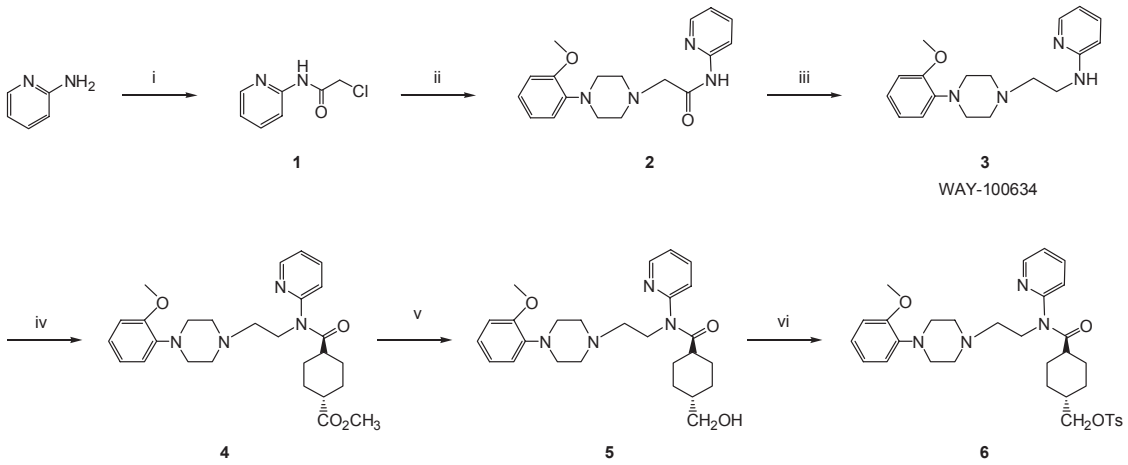
The serotonin (5-HT) system, one of the most important neurotransmitter systems, has been classified into seven subtypes (5-HT$_{1-7}$). Among these subfamilies, 5-HT$_{1A}$ receptors in the central nervous system are strongly implicated in psychiatric disorders such as depression, anxiety and schizophrenia.[1,2] Thus molecular imaging agents for the 5-HT$_{1A}$ have been intensively studied since the past decade.[3,4] Positron emission tomography (PET) as a non-invasive imaging technique with a high-sensitivity (10^{-9} – 10^{-12} M) and quantitative property, provides the means to visualize receptor densities in living system.[5,6] PET can play an important role both in assessing the neuropsychiatric disorders and in therapies with already developed pharmaceuticals.[7,8,9]

It is known that only high affinity agonists bind to receptors while antagonists' binding is relatively insensitive to their affinity.[10,11] Thereby, a number of antagonist tracers have been developed as PET imaging agents on the basis of the WAY-100635 which is a selective antagonist for 5-HT$_{1A}$ receptors at both somatodendritic and postsynaptic receptor sites.[12-16] Structural analogues were designed to have stability to metabolism



Scheme 1. Key steps in synthesis of the precursor



Scheme 2. Synthesis of Mefway precursor. Reagents and conditions: i) choroacetyl chloride, TEA, CH_2Cl_2, rt, 79%, ii) 1-(2-methoxyphenyl)-piperazine, K_2CO_3, NaI, DMF, 80 °C, 84%, iii) $LiAlH_4$, THF, rt, 76%, iv) oxalylchloride, *trans*-4-carbomethoxycyclohexane-1-carboxylic acid, CH_2Cl_2, rt, 83%, v) $LiAlH_4$, Et_2O, 0 °C, 57%, vi) Ts_2O, TEA, CH_2Cl_2, rt, 75%.

with high affinity, and selectivity for the 5-HT_{1A}. The most important drawback of these compounds is the significant defluorination that causes low-quality images due to contamination of ^{18}F-fluoride ion in the skull. Recently, Neil Saigal *et al*. reported [^{18}F]Mefway ((*N*-2-{2-[4-(2-methoxyphenyl) piperazinyl]ethyl}-*N*-(2-pyridyl)-*N*-(4-^{18}F-fluoro-methylcyclo-hexane) carboxamide) as a potent PET agent for 5-HT_{1A} receptors. This compound had not only high target-to-non target ratios in receptor rich regions but also little defluorination.[17]

However, synthesis of its precursor, *trans*-*N*-{2-[4-(2-methoxyphenyl)piperazinyl]ethyl}-*N*-(2-pyridyl)-*N*-(4-tosyloxymethylcyclohexane)carboxamide, prior to the fluorination was quite inefficient with the low overall yield for the development of radiopharmaceutical due to the significant breakdown of amide bond, i.e., up to 70% of starting substrate, during the reduction of carbomethoxy group toward WAY-100635 derivative. Thereby, an efficient synthesis of *trans*-[^{18}F]Mefway precursor is needed which we would like to describe with adaptation of acid chloride-assisted coupling reaction and an improved reduction method.

WAY-100634 (**3**) was prepared as previously described.[8] Reaction of 2-aminopyridine with chloroacetyl chloride at room temperature provided the 2-(chloroacetyl)amidopyridne (**1**) as a intermediate. The treatment of the intermediate with 1-(2-methoxyphenyl)piperzines in DMF at 80 °C in the presence of K_2CO_3 and NaI gave the corresponding *N*-2-[2-{4-(2-methoxyphenyl)-1-piperazinyl}ethyl]amidopyridine (**2**), which is subsequently reduced to desired product **3**, using $LiAlH_4$ in THF at room temperature.

The WAY-100634 was coupled to *trans*-4-carbomethoxycyclohexanecarbonyl chloride in dichloromethane in the presence of triethylamine at room temperature, yielding *trans*-*N*-2-{2-[4-(2-methoxy-phenyl)piperazinyl]ethyl}-*N*-(2-pyridyl)-*N*-(4-carboxymethylcyclohexane) carboxamide (**4**). In this reaction, we used oxalyl chloride as a coupling agent instead of benzotriazole-1-yloxytris-(dimethylamino)-phosphonium hexafluoro-phosphate (BOP) to enhance reactivity of carboxylic acid. This method improved the reaction yield up to 83% higher than previously reported method using BOP.[17] Specific reduction of carbomethoxy group in **4** was conducted in $LiAlH_4$ in Et_2O at 0 °C to provide *trans*-*N*-2-{2-[4-(2-methoxyphenyl) piperazinyl]ethyl}-*N*-(2-pyridyl)-*N*-(4-hydroxymethylcyclohexane)carbox-amide (**5**) in a 57% yield. This new procedure improves the reaction yield significantly compared to the previous protocol.[17]

The treatment of same substrate **5** with $LiAlH_4$ in THF facilitated significant breakdown of the amide bond to yield compound **3** in more than 60% yield due to the possible presence of adventitious water in THF or unusual reactivity of $LiAlH_4$ in THF solution.[17] For the preparation of the tosylated Mefway precursor **6**, the hydroxyl compound **5** was reacted with *p*-toluenesulfonyl anhydride at room temperature in dichloromethane in the presence of triethylamine to afford the precursor of [^{18}F]Mefway in 75% yield.

In conclusion, we developed an efficient synthetic pathway for the preparation of the precursor of [^{18}F]Mefway, which consisted of the improved the acid chloride coupling reaction to activate carboxylic acid and proper reduction condition to suppress breakdown of amide bond.

Experimental Section

***N*-2-(2-Chloroethyl)amidopyridine (1).** Chloroacetyl chloride (2.39 mL, 32.97 mmol) was slowly added to the mixture of 2-aminopyridine (1.88 g, 19.98 mmol) and TEA (4.30 mL, 30.69 mmol) in dry dichlorometane (100 mL) at 0 °C. The reaction mixture was stirred at room temperature under an Ar atmosphere for 3 h. The organic layer was extracted with CH_2Cl_2, washed with water, and dried over anhydrous $MgSO_4$. The residue was purified by flash column chromatography (3:1 hexane/ethyl acetate) to give product (2.68 g, 79%) as a white solid. (Caution! VERY IRRITATIVE SOLID) ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.20 (s, 2H), 7.09-7.14 (m, 1H), 7.77 (m, 1H), 8.22-8.28 (m, 2H), 9.45 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz)

δ 42.8, 114.3, 120.5, 139.1, 147.1, 150.4, 164.7; HRMS (FAB^+, *m*-nitrobenzylalcohol): Calcd for $C_7H_8ON_2Cl$: 171.0325, Found: 171.0324.

***N*-2-[2-{4-(2-Methoxyphenyl)-1-piperazinyl}ethyl]amido-pyridine (2).** The mixture of 1-(2-methoxy)piperazine (0.20 mL, 1.17 mmol) and K_2CO_3 (0.40 g, 2.93 mmol) in DMF (8 mL) was stirred at 80 °C for 1 h. After cooling down to room temperature, a solution of compound (**1**) (0.20 g, 1.17 mmol) in DMF (2 mL) and sodium iodide (0.025 g, 0.17 mmol) were added to the mixture. The reaction mixture was stirred at 80 °C for 3 h, cooled to the room temperature. The organic layer was extracted with ethyl acetate, washed with water, and dried over anhydrous $MgSO_4$. The residue was purified by flash column chromatography (50:1 CH_2Cl_2/MeOH to 20:1 CH_2Cl_2/MeOH) to give product (0.32 g, 84%) as pale yellow oil. ^1H NMR ($CDCl_3$, 300 MHz) 2.82-2.85 (m, 4H), 3.18 (s, 4H), 3.24 (s, 2H), 3.87 (s, 3H), 6.86-7.06 (m, 5H), 7.69-7.75 (m, 1H), 8.25-8.33 (m, 2H), 9.64 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 50.6, 53.8, 55.4, 62.3, 111.2, 113.9, 118.4, 119.9, 121.0, 123.2, 138.3, 140.9, 148.0, 151.0, 152.3, 169.2; HRMS (FAB^+, *m*-nitrobenzylalcohol): Calcd for $C_{18}H_{23}O_2N_4$: 327.1821, Found: 327.1818.

***N*-2-[2-{4-(2-Methoxyphenyl)-1-piperazinyl}ethyl]-*N*-(2-pyridinyl)amine (3).** 1 M $LiAlH_4$/THF (6.70 mL, 6.70 mmol) was slowly added to a solution of compound (**2**) (0.73 g, 2.24 mmol) in dry THF (10 mL) at 0 °C. The mixture was stirred at room temperature under an Ar atmosphere for 3 h. After quenching with saturated aqueous NH_4Cl at 0 °C for 30 min, the mixture was filtrated with ethyl acetate. The organic layer was extracted with ethyl acetate, washed with water, and dried over anhydrous $MgSO_4$. The residue was purified by flash column chromatography (50:1 CH_2Cl_2/MeOH to 20:1 CH_2Cl_2/MeOH) to give product (0.53 g, 76%) as pale yellow oil. ^1H NMR ($CDCl_3$, 300 MHz) δ 2.68-2.72 (m, 6H), 3.10 (s, 4H), 3.36-3.41 (m, 2H), 3.87 (s, 3H), 5.14 (s, 1H), 6.40-6.43 (d, 1H, J = 8.4 Hz), 6.54-6.59 (m, 1H), 6.85-7.04 (m, 4H), 7.39-7.45 (m, 1H), 8.08-8.11 (m, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 38.5, 50.7, 53.1, 55.3, 56.8, 107.0, 111.1, 112.7, 118.2, 121.0, 122.9, 137.3, 141.3, 148.2, 152.3, 158.8; HRMS (FAB^+, *m*-nitrobenzylalcohol): Calcd for $C_{18}H_{25}ON_4$: 313.2028, Found: 313.2030.

***trans*-*N*-2-{2-[4-(2-Methoxyphenyl)piperazinyl]ethyl}-*N*-(2-pyridyl)-*N*-(4-carboxymethylcyclohexane)carboxamide (4).** Oxalylchloride (0.19 mL, 2.13 mmol) was added to a solution of *trans*-4-carbomethoxycyclohexane-1-carboxylic acid (0.20 g, 1.05 mmol) in dry CH_2Cl_2 (10 mL), and the mixture was refluxed for 2 h. After removing solvent and unreacted oxalylchloride in vacuo, the product was re-dissolved in dry CH_2Cl_2. Compound (**3**) (0.22 g, 1.05 mmol) and TEA (0.16 mL, 1.14 mmol) were added to the previous product at 0 °C. The reaction mixture was stirred at room temperature under an Ar atmosphere for 2 h. After the mixture was washed with 10% aqueous $NaHCO_3$ (100 mL), the organic layer was extracted with CH_2Cl_2, and dried over anhydrous $MgSO_4$. The residue was purified by flash column chromatography (ethyl acetate, 0.1% v/v TEA) to give product (0.28 g, 83%) as pale yellow oil. ^1H NMR ($CDCl_3$, 300 MHz) δ 1.17-1.25 (m, 2H), 1.62-1.66 (m, 2H), 1.84-1.96 (m, 4H), 2.25-2.29 (m, 2H), 2.58-2.63 (m, 6H), 2.98 (s, 4H), 3.62 (s, 3H), 3.84 (s, 3H), 3.95-4.00 (m, 2H), 6.83-7.00 (m, 4H), 7.24-7.31 (m, 2H), 7.74-7.80 (m, 1H), 8.51-8.53 (m, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 27.8, 28.3, 41.3, 42.1, 44.8, 50.1, 51.4, 53.0, 55.2, 55.6, 111.0, 117.9, 120.7, 121.9, 122.4, 122.8, 138.2, 140.9, 149.2, 152.0, 175.5, 176.0; HRMS (FAB^+, *m*-nitrobenzylalcohol): Calcd for $C_{27}H_{37}O_4N_4$: 481.2815, Found: 481.2814.

***trans*-*N*-2-{2-[4-(2-Methoxyphenyl)piperazinyl]ethyl}-*N*-(2-pyridyl)-*N*-(4-hydroxymethylcyclohexane)carboxamide (5).** 1 M $LiAlH_4$/diethyl ether (0.16 mL, 0.16 mmol) was slowly added to the solution of compound (**4**) (0.076 g, 0.16 mmol) in diethyl ether (5 mL) at 0 °C. The reaction mixture was stirred for 30 min at 0 °C under an Ar atmosphere, and quenched with saturated aqueous NH_4Cl. The organic layer was extracted with ethyl ether, and then evaporated solvent under reduced pressure. The residue was purified by gravity column chromatography using neutral silica gel (30:1 CH_2Cl_2/MeOH) to give product (0.041 g , 57 %) as colorless oil. ^1H NMR ($CDCl_3$, 300 MHz) δ 1.58-1.85 (m, 10H), 2.59-2.63 (m, 6H), 2.98 (s, 4H), 3.37-3.39 (d, 2H, J = 6.0 Hz), 3.84 (s, 3H), 3.96-4.01 (t, 2H, J = 6.9 Hz), 6.83-6.98 (m, 4H), 7.22-7.28 (m, 2H), 7.74-7.76 (m, 1H), 8.51-8.53 (m, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 28.4, 28.9, 39.6, 42.3, 45.2, 50.6, 53.3, 55.3, 56.1, 68.3, 111.1, 118.1, 120.9, 122.3, 122.8, 138.1, 141.3, 149.3, 152.2, 155.8, 176.0; HRMS (FAB^+, *m*-nitrobenzylalcohol): Calcd for $C_{26}H_{37}O_3N_4$: 453.2866, Found: 453.2870.

***trans*-*N*-2-{2-[4-(2-Methoxyphenyl)piperazinyl]ethyl}-*N*-(2-pyridyl)-*N*-(4-tosyloxymethylcyclohexane)carboxamide (6).** *p*-Toluenesulfonyl anhydride (0.018 g, 0.056 mmol) and TEA (0.0072 mL, 0.050 mmol) were added to the solution of compound (**5**) (0.021 g, 0.046 mmol) in CH_2Cl_2 (5 mL). The reaction mixture was stirred at room temperature under an Ar atmosphere for 48 h. After the mixture was washed with 10% aqueous $NaHCO_3$ (100 mL), the organic layer was extracted with CH_2Cl_2, and dried over anhydrous $MgSO_4$. The residue was purified by gravity column chromatography using neutral silica gel (20:1 CH_2Cl_2/MeOH) to give product (0.022 g, 75%) as colorless oil. ^1H NMR ($CDCl_3$, 300 MHz) δ 1.25-1.78 (m, 10H), 2.44 (s, 3H), 2.57-2.61 (m, 6H), 2.97 (s, 4H), 3.74-3.76 (d, 2H), 3.84 (s, 3H), 3.96-3.98 (d, 2H), 6.83-6.96 (m, 4H), 7.25-7.33 (m, 4H), 7.72-7.77 (m, 3H), 8.51-8.53 (m, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 21.6, 28.0, 28.5, 36.4, 41.8, 45.3, 50.6, 53.4, 55.3, 56.1, 74.9, 111.1, 118.1, 120.9, 122.2, 122.4, 122.8, 127.8, 129.8, 132.9, 138.2, 141.3, 144.7, 149.3, 152.2, 155.8, 175.6; HRMS (FAB^+, *m*-nitrobenzylalcohol): Calcd for $C_{33}H_{43}O_5N_4S$: 607.2954, Found: 607.2954.

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