Synthesis of serotonin transporter imaging agent [^{125}I]ADAM

LU Chun-Xiong, WU Chun-Ying, JIANG Quan-Fu, CHEN Zheng-Ping,
ZHANG Tong-Xing, LI Xiao-Ming, WANG Song-Pei
(Jiangsu Institute of Nuclear Medicine, Wuxi 214063)

Abstract The synthesis of serotonin transporter imaging agent [^{125}I] -2-((2-((dimethylamino)methyl)phenyl) thio)-5-iodophenylamine([^{125}I] ADAM) was reported. The chemical structure of the labeling precursor 5- (tributylstannyl) -2-((2-((dimethylamino)methyl)phenyl)thio)phenylamine and all its intermediates were verified by IR,^{1}HNMR and MS. The radioiodinated compound was prepared using iododestannylation reaction by hydrogen peroxide. Final radiochemical purity was above 95% determined by TLC.

Key words Serotonin transporter, [^{125}I] ADAM, Synthesis
CLC number R817

1 Introduction

Abnormalities in the serotonin transporter (SERT) have been implicated in several neurologic and psychiatric disorders, such as depression, suicide, schizophrenia, drug addition and eating disorders.[1,2] In addition, SERT is the primary target for the widely prescribed antidepressant agent.[3] In order to study the above-mentioned neurologic and psychiatric disorders and the mode of action of antidepressant agents in humans, it is of great need to have high affinity and specificity SERT radioligands for both SPECT and PET studies.

ADAM(2-((2-((dimethylamino)methyl)phenyl)thio)-5-iodophenylamine)(8) displays an extremely potent binding affinity toward SERT (K_i= 0.013nmol/L). ADAM also shows more than 1,000-fold selectivity for SERT over norepinephrine transporter (NET) and dopamine transporter (DAT) (K_i=699 and 840 nmol/L, for NET and DAT, respectively). The radiolabeled compound [^{125}I] ADAM presents an excellent brain uptake in rats(1.41% dose at 2 min post intravenous (IV) injection), and consistently displays the highest uptake (between 60-240 min post IV injection) in hypothalamus, a region with the highest density of SERT. The specific uptake of [^{125}I]ADAM(**8**) in hypothalamus exhibiting the highest target-to- nontraget ratio((hypothalamus–cerebellum)/ cerebellum was 3.97 at 120 min post IV injection).[4]

In this paper, we report the synthesis and radiolabeling of ADAM.

2 Materials and methods

2.1 General

All reagents were purchased from commercial suppliers and used without further purification unless otherwise stated. When reactions were worked up by extraction with dichoromethane (CH_2Cl_2), ethyl acetate (EtOAc) or ethyl ether (Et_2O), organic solutions were dried with anhydrous Na_2SO_4 and concentrated with a rotary evaporator under reduced pressure.

Melting points were determined on Yanadimoto apparatus and uncorrected. Column chromatography was performed using silica gel, 100~200mesh. ^{1}HNMR spectra were recorded on AM spectrometer at 400 MHz, with $CDCl_3$ as solvent and tetramethylsilane (TMS) as the internal standard (0 ppm). Mass spectra were run on Varian MAT 2.2 spectrometer.

Supported by a grant (RC2002068) from "135" Medicinal Momentous Project of Jiangsu Province and by a grant from Department of Personnel, Jiangsu Province.
Received date: 2004-11-09

2.2 Synthesis of [^{125}I]ADAM[5]

The synthesis procedure is shown in Fig.1.

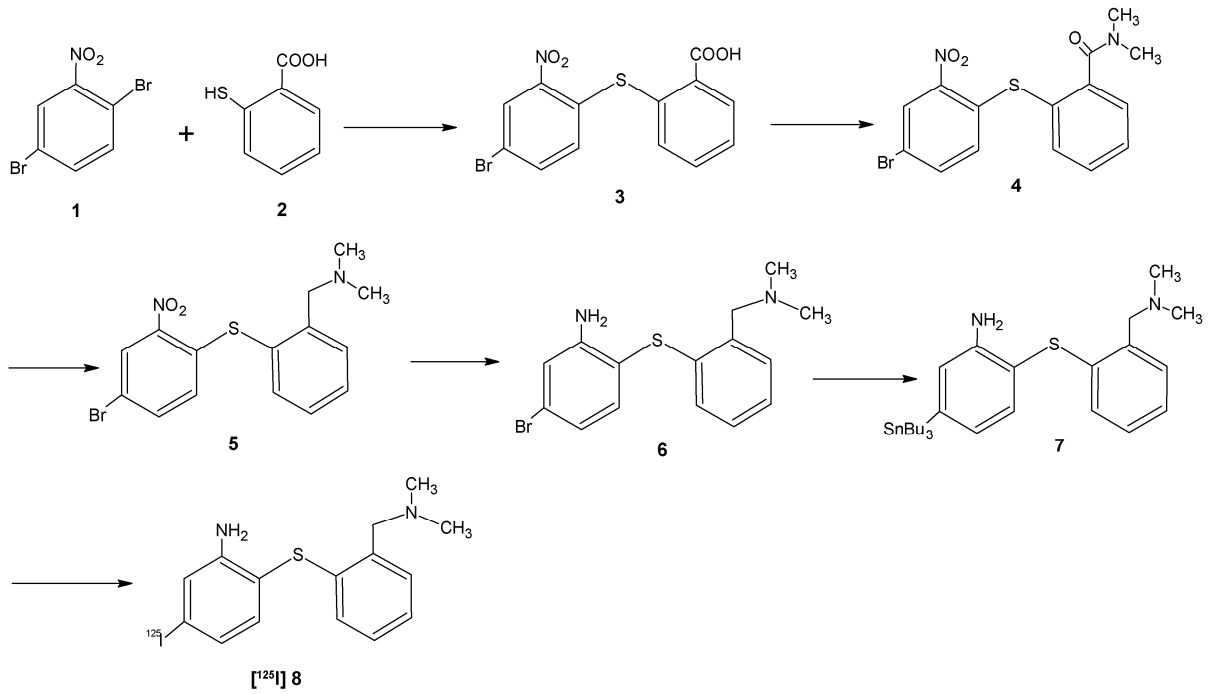

Fig.1 Synthesis and radiolabeling of ADAM.

2.2.1 2-(4-bromo-2-nitrophenylsulfanyl) benzoic acid[**3**]

A mixture of 2,5-dibromonitrobenzene ([**1**], 11.8 g, 42 mmol), thiosalicylic acid ([**2**], 6.2 g, 40 mmol), Cu powder (0.73 g) and K_2CO_3 (12.7 g) in DMF (100mL) was heated at 65℃ overnight, cooled to room temperature and poured to ice water. The mixture was filtered under reduced pressure. The filtrate was made acidic with the addition of 6 mol/L HCl and extracted with CH_2Cl_2 (100mL×3). The combined organic layers were washed once with water, dried and concentrated. The crude product was crystallized form EtOH to provide **3** (14g, 98%) as yellow solid, mp 162~164℃ (yield 74%, mp 160-161℃[5]). IR（cm^{-1}）: 3083, 1680, 1532, 1344; MS: 354, 352 (M-1); ^{1}HNMR: δ 8.26 (d,1H), 8.06~8.12 (dd,1H), 7.42~7.58 (m,4H), 6.90~6.94 (d,1H).

2.2.2 2-(4-bromo-2-nitrophenylsulfanyl)-N,N-dimethylbenzamide[**4**]

A solution of compound **3** (2.0g, 5.65 mmol) in thionyl chloride (15.0 mL) was refluxed for 4 h, cooled to room temperature and excess thionyl chloride was removed under reduced pressure. The residue was then redissolved in THF (15.0 mL). And to this solution was added N,N-dimethylamine (5.0 mL, 40% solution in water) at 0℃ and then was stirred 1h. The reaction mixture was diluted with H_2O and extracted with CH_2Cl_2 (30mL×4). The combined organic layers were washed once with H_2O, dried and concentrated. Column chromatography of the crude products on silica gel and elution with EtOAc/hexane (3:2) afforded compound **4** (2.0g, 96.6%) as yellow solid, mp 126~128℃ (light yellow solid, mp 115-116℃,[4] yield 77.1%, thick oil,[5] yellow solid, mp 112.5-114℃[6]). IR (cm^{-1}): 3095, 1631, 1511, 1332; MS: 383, 381 (M+1), 405, 403 (M+Na); ^{1}HNMR: δ8.28 (d,1H), 7.37~7.58 (m,5H), 6.76~6.80 (d,1H), 3.00 (s,3H), 2.80 (s,3H).

2.2.3 2-(4-bromo-2-nitrophenylsulfanyl)-N,N-dimethyl-benzylamine[**5**]

To a solution of **4** (3.0 g, 8.5 mmol) in THF (15.0 mL), cooled at 0℃, was introduced the BH_3·THF complex (20.0 mL, 1 mol/L solution in THF, 20.0 mmol) via a syringe. The reaction mixture was refluxed at 70℃ for 2h and then stirred overnight at room temperature. The reaction mixture was cooled to 0℃ and concentrated HCl added. The solvent was removed *in vacuo*. The aqueous phase was diluted with H_2O (50 mL) and heated to reflux for 20 min. After cooling down to room temperature, the mixture was adjusted to pH 8 with 10% $NaHCO_3$ and extracted with CH_2Cl_2 (15mL×4). The combined organic layers were dried and concentrated under reduced pressure. Column chromatography on silica gel and elution with EtOAc/hexane (3:2) afforded compound **5** (2.5g, 86.5%) as little yellow solid, mp 97~98℃ (colorless oil,[4] mp 92-93℃,[5] mp 96-98℃[6]). IR(cm^{-1}): 2816, 1586, 1522, 1334; MS: 243

[M-N(CH_3)$_2$-Br], 276, 278 [M-N(CH_3)$_2$-NO_2], 322, 324 [M-N(CH_3)$_2$], 369, 367 (M+1), 391, 389 (M+Na); ^{1}HNMR: δ8.36 (d,1H), 7.62~7.66 (1d,1H), 7.46~7.56 (m,2H), 7.30~7.40 (m,2H), 6.56~6.60 (d,1H), 3.58 (s,2H), 2.10 (s,6H).

2.2.4 5-bromo-2-[2-(dimethylaminomethylphenysulfanyl)]-phenylamine[**6**]

To a solution of compound **5** (1.02 g, 2.78 mmol) in MeOH (30 mL) was added concentrated HCl (8 mL). The suspension was cooled to 0℃, $SnCl_2$ (1.9 g, 10 mmol) was added and the reaction mixture stirred overnight at room temperature under nitrogen. The mixture was then diluted with H_2O (80 mL), and extracted with EtOAc (30mL×2). The organic layers were discarded. The aqueous layer was adjusted to pH 10 with 1mol/L NaOH and extracted with EtOAc (50mL×4). The combined organic layers were washed once with H_2O, dried and concentrated under reduced pressure to give compound **6** (0.8g,86%) as a colorless oil (yield 78%,[4] yield 92.7%[5]). IR(cm^{-1}): 2856, 1605, 1471, 1221; MS: 213[M-N(CH_3)$_2$-Br], 292, 294 [M-N(CH_3)$_2$], 339, 337 (M+1), 361 (M+Na); ^{1}HNMR: δ6.80~7.35 (m,7H), 4.65 (s,2H), 3.58 (s,2H), 2.25 (s,6H).

2.2.5 2-((2-((dimethylamino)methyl)phenyl)thio)-5-(tri-n-butyltin)-phenylamine[**7**]

To a solution of compound **6** (0.3g, 0.89mmoL) in triethylamine (6.0mL), bis (tributyltin) (3mL) and tetrakis (triphenylphosphine) palladium (0) (60mg, 0.048mmol) were added. The mixture was heated at 100℃ in a sealed bottle for 48h. The solvent was removed under reduced pressure and the residue was purified successively by column chromatography on silica gel to give compound **7** (0.3g, 61.6%) as a colorless oil (yield 59%[4]). IR (cm^{-1}): 2954, 2922, 1602, 1463; MS: 548 (M+1), 571 (M+Na); ^{1}HNMR: δ6.78~7.80 (m,7H), 4.45 (s,2H), 3.60 (s,2H), 2.38 (s,6H), 0.9~1.70 (m,31H).

2.2.6 Radiolabeling of [^{125}I]ADAM ([^{125}I] **8**)

The tin compound **7** (50μg in 50μL of ethanol), [^{125}I] sodium iodide (~37MBq), and 1 mol/L HCl (100μL) were placed in a sealed vial. To this mixture, 100μL of H_2O_2 (3% solution in water) was added via a syringe at room temperature. The iodination reaction was terminated after 10 min by an addition of saturated $NaHSO_3$ and the resulting solution was neutralized by adding a saturated $NaHCO_3$ solution. The yield of reaction was 90% determined by TLC. The R_f of [^{125}I]$^-$ was 0~0.1 and the R_f of [^{125}I] ADAM was 0.9~1.0 (ethyl ether/ethanol=19/1). The mixture was extracted with ethyl acetate. The radiochemical purity was above 95% determined by TLC.

3 Results and discussion

Synthesis of radiolabeling precursor **7** is outlined in Fig.1. Oya[4] and Shiue[6] reported that 2-(4-bromo-2-nitrophenylsulfanyl)-N,N-dimethylbenz amide (**4)** was prepared by the reaction of 2,5-dibromonitrobenzene (**1**) and 2-thio-N,N-dimethylbenzamide. But 2-thio-N,N-dimethylbenzamide is unstable and very difficult to be prepared. We used 2,5-dibromonitrobenzene (**1**) (commercially available) and thiosalicylic acid (**2**, commercially available) to couple to afford the benzoic acid **3**, which was taken to amide **4** by first converting **3** to acid chloride with thionyl chloride, then reacting the acid chloride with N,N-dimethylamine. Reduction of the amide functionality in **4** with borane-THF complex led to the benzylamine **5**. Reduction of the nitro group in compound **5** with tin (Ⅱ) chloride under acidic conditions provided the phenylamine **6**. Compound **6** is the bromo derivative of target compound, ADAM. The bromo to iodo transformation was achieved via a tributyltin intermediate, **7**, which was prepared by a palladium(0)-catalyzed coupling reaction with bis (tributyltin), with which the tributyltin group replaced the bromo group. The total yield of the multi-steps was 42% and it was higher than the literature (30%).[4] Finally compound **7** was treated with radioactive [^{125}I] sodium iodide in oxidative condition (H_2O_2) to produce the labeled compound [^{125}I]ADAM (**8**) in excellent yields.

References

1 Mann J J, Huang Y Y, Underwood M D *et al*. Arch Gen Psychiatry, 2000, **57**: 729-738

2 Meltzer C C, Smith G, Dekosky S T *et al*. Neuropsychopharmacology, 1998, **18**: 407-430

3 Frazer A. J Clin Psychiatry, 1997, **58** (Suppl **6**): 9-25

4 Shunichi Oya, Seok-Rye Choi, Catherine Hou *et al*. Nuclear Medicine and Biology, 2000, **27**: 249-254

5 Yiyun Huang, Dah-Ren Hwang, Zhihong Zhu *et al*. Nuclear Medicine and Biology, 2002, **29**: 741-751

6 Shiue G G, Ping Fang, Chyng-Yann Shiue. Applied Radiation and Isotopes, 2003, **58**: 183-191