Preparation of an imaging agent for cerebral muscarinic acetylcholine receptor, (R,S)^{131}I-QNB

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Abstract The method to synthesize a high affinity muscarinic receptor antagonist (R,S)I-QNB[(R)-(-)-1-azabicyclo [2,2,2]oct-3-yl-(S)-(+)-α-hydroxy-α-(4-[127I]iodophenyl)-α-phenyl acetate] from 4-nitrobenzophenone with improvement compared to literatures was reported in this article. IR, MS and ^{1}HNMR characterized the final product. (R,S)^{131}I-QNB was prepared using Cu(I) assisted iodine exchange labeling, and showed by TLC that the radiolabeling yield(RLY) was over 80%, and radiochemical purity(RCP) was over 95%. Stability of the labeled compound was also determined. It was found that (R,S)^{131}I-QNB dried by nitrogen blowing can stay at 4-10℃ for a week without change of RCP.

Keywords (R,S)I-QNB, Preparation, ^{131}I labeling, Cerebral muscarinic acetylcholine receptors

CLC numbers R817, O615.2, Q51

1 Introduction

Muscarinic receptors are one type of colinergic receptors. They widely spread in peripheral(PNS) and central nervous system(CNS). In PNS, all organs innervated by the parasympathetic nervous system, such as heart, blood vessels, have muscarinic Acetylcolinergic receptors(mAchrs). CNS also comprises a complex network of mAchrs, which mediate some effects of colinergic drugs, and are involved in many CNS diseases such as Alzheimer's diseases(AD). AD are manifested through changes of mAchrs levels and functions in the brain.[1-3] By now imaging of mAchrs activity has become a leading diagnostic and research tool, and it is necessary to seek a perfect tracer which has high affinity and subtype selectivity to mAchrs and can also penetrate blood-brain barrier. At present, a number of tracers have been synthesized for SPECT or PET, among which halogenated derivatives of QNB were reported to tally with the above request most.[4] (R,S)I$^{123/125}$-QNB (the same thing as (R,R)I$^{123/125}$-QNB mentioned in literature before mid-1992)[5,6] has been widely used for *in vivo* imaging of mAchrs in SPECT for its high affinity and selectivity when binding to muscarinic cholinergic sites.[2,7,8] F^{18}MeQNB was reported to have high selectivity to M2 subtype, which may make it superior in early stage diagnosis of AD.[9,10] But the difficulty and high cost to synthesize F^{18}MeQNB prevent its further use. The purpose of our study was to prepare (R,S)I^{131}-QNB, with higher chemical and labeling yield, and to provide convenience for relevant research and clinical use henceforth.

2 Materials and methods

2.1 Instruments

Bio-Rad FT-IR spectrometer (made in USA), YANADIMOTO melting point instrument (uncorrected, made in Japan), Varian Model AM-400 Proton Nuclear Magnetic Resonance Spectrometer (USA), Varian MAT2.2 Mass Spectrograph(USA), Bio-Rad GS250 Molecular Imager(USA), Packard Cobra γ-counter (USA).

2.2 Reagents

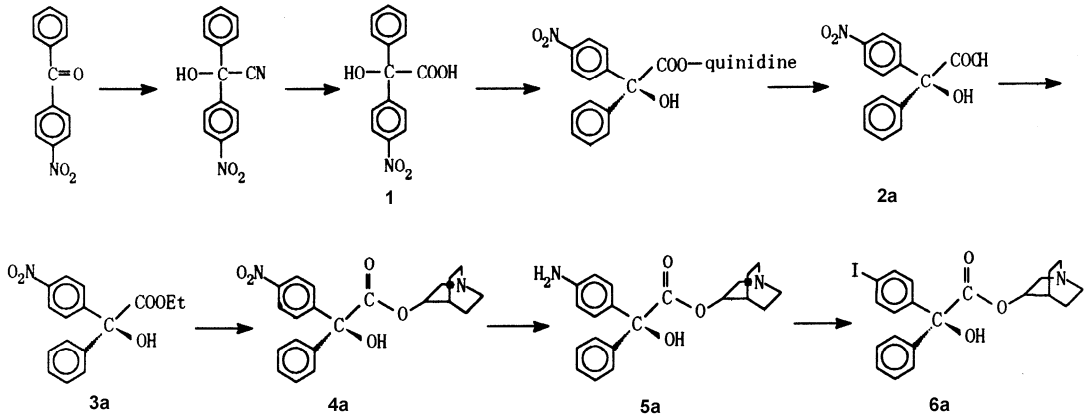
4-Nitrobenzophenone(99+%), R-(-)-3-quinuclidinol (99+%, GC), trimethylsilyl cyanide(98%) and trifluoromethanesulfonic anhydride(99+%) were purchased from ACROS ORGANICS; Quinidine(CP), sodium nitrite(AR), urea(AR), sodium iodide(AR), copper(II) sul-

Supported by the Foundation for Outstanding Young Members of Health Ministry in China(9925)
Received date: 2002-03-18

phate(CP), ascorbic acid(AR) and gentistic acid(CP) were purchased from Shanghai Chemical Co.; 5%Pd-C was obtained from Jiangsu Chemical Industry Institute; ^{131}I-NaI was from China Institute of Atomic Energy.

2.3 The preparation of (R, S) I-QNB

The reaction route is as follows:



2.3.1 Racemic α-Hydroxy-α-(4-nitrophenyl)-α-phenylacetic acid (**1**)

4-nitrobenzophenon (9 g, 39.6 mmol) and 500 mg of zinc iodide were dissolved in 120ml of dry dichloromethane. Then trimethylsilyl cyanide (5 g, 50 mmol) was added dropwise to this stirred solution. After the mixture was stirred at room temperature under a nitrogen blanket for 72 h, the solvent was removed under vacuum to yield a brown oil, which was then suspended in 80 mL of 3N HCl and stirred for 24 h at room temperature. The formed semisolid was filtered and added to a solution of 40 mL glacial acetic acid/10 N HCl (1∶1), and refluxed at 110℃ for 24 h, then evaporated under vacuum to remove the solvent. Saturated aqueous solution of sodium carbonate was then added with stirring until the mixture was pH9. After the basic solution was extracted with dichloromethane to remove the unreacted 4-nitrobenzophenone, the aqueous layer was acidified with 5 N HCl until pH2, and extracted with dichloromethane. The later extracts was combined and washed with 3 N HCl, dried over anhydrous sodium sulphate and concentrated under vacuum to obtain an oil, which slowly solidified to yield the racemic acid(**1**) as an off-white solid: 6.5 g (23.8 mmol), yield 60.1%, mp 112-114 ℃; IR(KBr): 3480(OH), 1720(C=O), 1520 1352(NO_2)cm^{-1}.

2.3.2 (S)-α-Hydroxy-α-(4-nitrophenyl)-α-phenylacetic acid (**2a**)

Quinidine(2.4 g, 7.3 mmol) and racemic acid (**1**)(2 g, 7.3 mmol) were dissolved in boiling ethanol. Then the solution was cooled to room temperature and stayed for a day. The salt crystallized was filtered and recrystallised by 4 times from the same solvent until it had a constant mp (113-114℃) and rotation $[\alpha]_D^{30}$ (c 0.02, methanol) +145°, yield 50%. The quinidine salt was then stirred with excess 6 M HCl for 2 h, and the mixture was extracted with dichloromethane. The extract was dried over anhydrous sodium sulphate, and the solvent was removed under vacuum to obtain (S)-acid(**2a**) as a yellow oil, yield 87.3%, IR(KBr): 3480(OH), 1716(C=O), 1520 1352(NO_2)cm^{-1}.

2.3.3 (S)-(+)-Ethyl-α-hydroxy-α-(4-nitrophenyl)-α-phenyl acetate (**3a**)

The (S)-acid(**2a**)(1.4 g, 5.1 mmol) was dissolved in anhydrous ethanol(10 mL), and 0.2 mL of trifluoromethanesulfonic anhydride was added. After the solution was heated and refluxed for 12 h and concentrated under vacuum to remove the solvent, the residue was partitioned between ethyl acetate and saturated aqueous solution of sodium bicarbonate. The organic layer was separated, washed with water and dried over anhydrous sodium sulphate, and the solvent was removed to afford **3a**(1.4 g, 4.6 mmol), yield 90%, IR(KBr): 3487(OH), 2984(C-H), 1731(C=O), 1245(C-O), 855 758 701(Ar-H) cm^{-1}.

2.3.4
(R)-(-)-1-Azabicyclo[2,2,2]oct-3-yl-(S)-(+)-α-hydroxy-α-(4-nitrophenyl)-α-phenyl acetate (**4a**)

R-3-Quinuclidinol (0.89 g, 7 mmol) was added in 70 mL anhydrous benzene, and the solution was refluxed for an hour with a water separator to remove trace water. Then 0.1 g sodium was added, and the mixture refluxed with stirring for another hour. After removal of the residual sodium, **3a** (1.4 g, 4.6 mmol) was added, and the mixture was reacted under reflux with stirring for 1 h, then evaporated under vacuum to remove the solvent. The residue was partitioned between ethyl acetate and water, and the organic layer separated, washed with water, dried over anhydrous sodium sulphate, then the solvent was removed. The residue now was crystallized from acetonitrile, and the formed crystals can be recrystallized from the same solvent for purifying to obtain **4a**(1.09 g, 2.85 mmol), yield 62%, mp 164-165℃; TLC[silica gel, MeOH/NH_4OH(98:2)]: Rf 0.6; IR(KBr): 2942 2876(C-H), 1729(C=O), 1253(C-O)cm^{-1}.

2.3.5 (R)-(-)-1-Azabicyclo[2,2,2]oct-3-yl-(S)-(+)-α- hydroxy-α-(4-aminophenyl)-α-phenyl acetate (**5a**)

The nitro compound **4a**(0.512 g, 1.34 mmol) was dissolved in 30 mL ethanol, and 5% Pd-C(100 mg) was added to the solution. Then hydrogen was piped into the reaction container with solution stirred at ambient temperature and pressure until uptake of hydrogen ceased. The reaction solution was then filtered, and removal of the solvent in the filtrate to afford **5a** as an off-white solid (0.425 g, 1.21 mmol), yield 90%, mp 172-175℃; TLC[silica gel, MeOH/ NH_4OH(98:2)]: Rf 0.52; IR(KBr): 3475 3370(NH_2), 2962 2878(C-H), 1726(C=O), 1232(C-O)cm^{-1}; EIMS: M+ 352(7), 226(6), 198(100).

2.3.6
(R)-(-)-1-Azabicyclo[2,2,2]oct-3-yl-(S)-(+)-α-hydroxy-α-(4-[^{127}I]iodophenyl) -α-phenyl acetate (**6a**)

The solution of **5a** (0.42 g, 1.2 mmol) in 12 mL 10%H_2SO_4 and 4 mL acetone was cooled to 0-5℃. With stirring an aqueous solution of sodium nitrite (0.17 g, 2.4 mmol, 4 mL) was added dropwise. Maintaining the same temperature the mixture was stirred for 15 min, then urea (0.14 g, 2.4 mmol) was added with stirring to remove the excess sodium nitrite until the test paper (starch/KI) can not be colored by the solution. Then after a solution of sodium iodide (0.36 g, 2.4 mmol) in water (4 mL) was added dropwise, and stirred for 20 min, the reaction mixture was warmed to room temperature and stirred over night. The solution was adjusted to pH11 with 5 N NaOH, and extracted with dichloromethane. The combined extracts was dried over anhydrous sodium sulphate and concentrated under vacuum. The residue was then purified by column chromatography[silica gel, $CHCl_3$/CH_3 OH/NH_4OH=90/10/0.5] to obtain a foamy product, which was crystallized from acetonitrile to afford the final product **6a** (186 mg, 0.4 mmol), yield 33%, mp 140-142℃; TLC[silica gel, $CHCl_3$/CH_3OH/NH_4OH (90/10/0.5)]: Rf 0.55; IR (KBr): 3458(OH), 2947 2873 (C-H), 1735(C=O), 1236(C-O)cm-1; EIMS: M+ 463(14), 337 (3), 309 (100), 126 (97); 1H-NMR(DMSO-d6+D_2O): δ1.14-1.25(2H,m,CH_2),1.41-1.54(2H,m,CH_2),1.82(1H,m, CH),2,32-2.58(5H,m,2CH_2+O-CHCHHN),3.01-3.05(1H, m,O-CHCHHN),4.77-4.79(1H,m,O-CH),7.12-7.14(2H,d, Ar-H), 7.27(5H,s,Ar-H), 7.65-7.67(2H,d,Ar-H).

2.4 The preparation of (R,S)^{131}I-QNB

(R,S)^{131}I-QNB was prepared with Cu(I) assisted iodine exchanging labeling.[13,15]

In a V vial were added 60 μg copper(Ⅱ) sulphate, 10 mg ascorbic acid, 3 mg gentistic acid, 20 μg **6a** (43 nmol, in 20 μL 50% aqueous solution of ethanol), 200 μL ethanol and 100 μL water. Then $Na^{131}I$ (3.7×10^7Bq) was added to the mixture, and the V vial was septum sealed, heated at 100℃ for 1 h. The mixture was then cooled, with radiolabeling yield determined. After diluted with water (100 μL), the solution was extracted with dichloromethane (0.5 mL×2). The combined extract was dried through a short column stuffed with sodium sulphate, and radiochemical purity was determined. Then it was dried under nitrogen blowing for future use.

2.5 Determination of RLY and RCP

2.5.1 TLCa

Xinhua No.1 filter paper sheets as solid phase and eluted with $CHCl_3$/ CH_3OH/NH_4OH=90/10/0.5. The Rf values of (R,S)^{131}I-QNB and ^{131}I are 0.8~1.0 and 0~0.1, respectively.

2.5.2 TLCb

GF-254 silica gel thin layer plate as solid phase and mobile phase was $CHCl_3/CH_3OH/NH_4OH$(90/10/0.5). When finished, the plate was exposed to GS250 Phosphor Imaging Screen-BI for half an hour, then imaged and analyzed in GS250 molecular imager. The Rf values of (R,S)^{131}I-QNB and ^{131}I are 0.55 and 0, respectively.

2.6 Stability of (R,S)^{131}I-QNB

The labeled compound prepared as above-mentioned procedure was allowed to stand at room temperature (4-10℃) under two different conditions. One was drying through nitrogen blowing, and the other was dissolving in 30% aqueous solution of ethanol. RCP was determined with TLC every day during the examination period.

3 Results and discussions

3.1 The synthesis and labeling of (R,S)^{131}I-QNB

We have improved the methods in literatures[11-13] to prepare (R,S)^{127}I-QNB, so as to enhance the yields in some key steps. We also used a simpler method in labeling and purifying in stead of the method in literatures,[12-14] with a higher radiochemical yield(53%, uncorrected for radionuclide decay).

3.2 Radiolabeling yield and radiochemical purity

The result of TLCa showed that the radiolabeling yield was over 80%, and radiochemical purity was over 95%. The result in the GF-254 silica gel plate imaged by GS250 is as follows.

The spot of (R,S)^{127}I-QNB in Fig.1 was obtained by staining on its fluorescent spot with isotope. The Rf value of the labeled compound is equal to that of the unlabeled, which proves the labeled compound is nothing but (R,S)^{131}I-QNB. Area integral of its chromatographic peak shows that the RCP is over 95%.

3.3 Stability

The RCP of the labeled compound dried by nitrogen was still over 95% after standing for a week. When it was dissolved in 30% aqueous solution of ethanol, the RCP descended to over 90%, and standed for 6 h without evident change. When the solution was standed after one day, the RCP descended to 87%. It descended to 77% after one week.

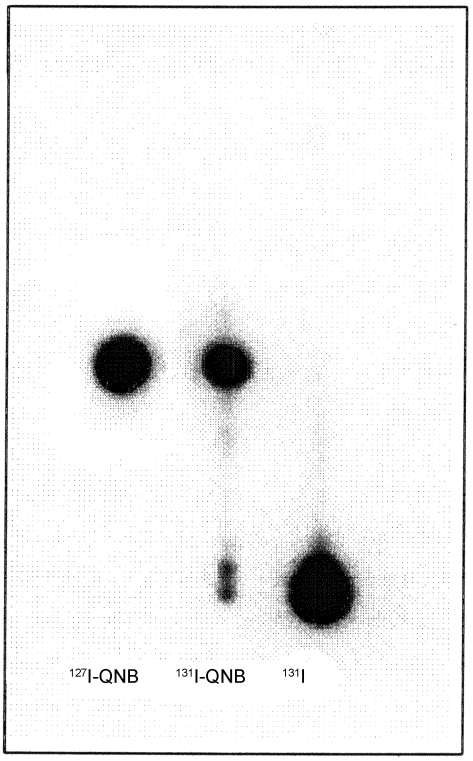


Fig.1 The result of (R,S)^{131}I-QNB in TLC[GF-254 silica gel board, $CHCl_3/CH_3OH/NH_4OH$(90/10/0.5)] imaged by GS250.

According to the results above, we concluded that the preparation of (R,S)^{131}I-QNB with Cu(I) assisted iodine exchanging labeling is a simple and feasible method, with high radiolabeling yield and radiolabeled compound's stability, which can facilitate research and clinical use for imaging of muscarinic receptors in CNS.

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