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SYNTHESIS OF A [^{99m}Tc] PHARMACOMODULATED CYCLAM - A POTENTIAL RADIOTRACER FOR FUNCTIONAL BRAIN IMAGING

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SUMMARY

Condensation of 2{4-[2-(diethylamino) ethoxy] phenyl}ethanol tosylate with a large excess of 1,4,8,11 tetraazacyclotetradecane (CYCLAM) in DMF gave N-2-{4-[2-(diethylamino)ethoxy]-phenyl}-ethyl-1,4,8,11-tetraazacyclotetradecane : **4** which was isolated and purified through its Cu-complex. [^{99m}Tc] **4** was prepared (80 % yield - 99 % radiochemical purity) using a commercial Sodium [^{99m}Tc] pertechnetate generator. Compound **4** is made up of a nitrogen crown ether analog moiety allowing [^{99m}Tc] labelling, mono N-functionalized with an aryl-alkyl ether group previously labelled with [^{125}I] and studied in detail.

Key-words : monofunctionalized Cyclam, ^{99m}Tc labelling, functional brain radiotracer, Nuclear Medicine

INTRODUCTION

In an earlier paper, we reported that some [^{125}I] iodinated compounds were promising agents for functional brain imaging. Four of them (figure 1) were shown to exhibit an interesting crossing of the blood brain barrier (1). Compounds **5** and **6** gave stable values of the brain-to-blood ratio (about 4,0) within the first hour after injection suggesting that these agents might be useful for brain scintigraphy. Compounds **7** and **8** displayed high levels of activity in the brain, at short times (≤ 15 min). The uptake in the brain, expressed as ID/g brain was 2,6-1,5, suggesting they may be suitable for studies of cerebral blood flow. Iodine-123 can be used to label pharmaceuticals. Although its γ energy is convenient, it is not an ideal nuclide, on account of its cost and availability.

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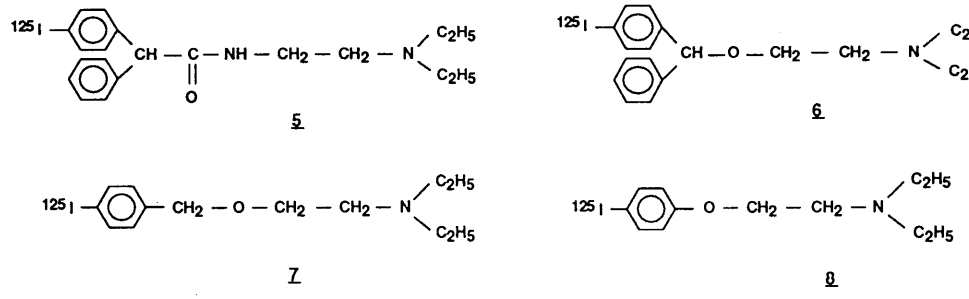
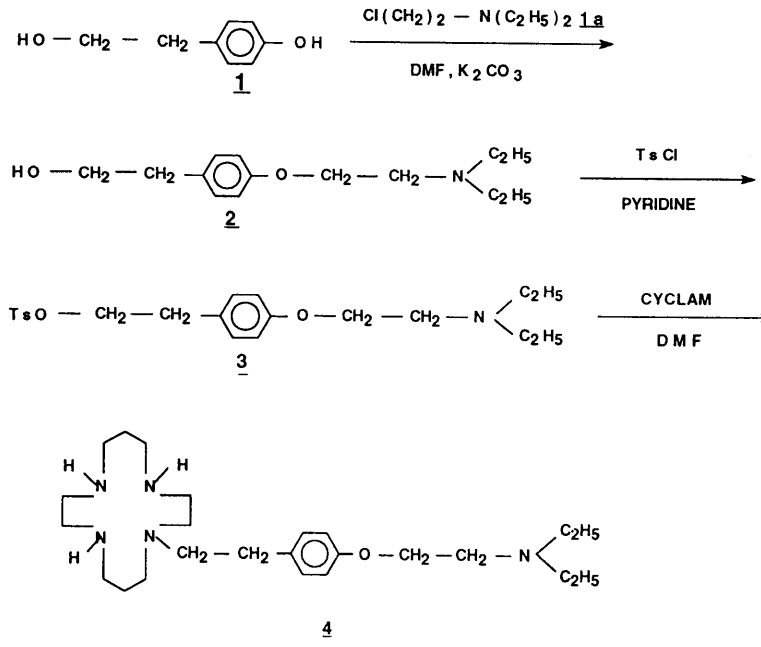


FIGURE 1: Agents for functional brain imaging

Presently about 80 % of the radiopharmaceuticals used in nuclear medicine are [^{99m}Tc] compounds. The reason for the preeminent position of ^{99m}Tc in clinical studies is its extremely favorable physical and nuclear characteristics. The 6h half-life is long enough to allow a radiochemist to carry out radiopharmaceutical synthesis and for nuclear medicine practitioners to collect useful images. At the same time it is short enough to permit the administration of millicurie amounts of ^{99m}Tc radioactivity without significant radiation dose to the patient. The monochromatic 140 keV photons are readily collimated to give images of superior spatial resolution. Furthermore, ^{99m}Tc is readily available at low cost in the form of ^{99m}Tc pertechnetate from commercial ^{99}Mo-^{99m}Tc generators. However, $^{99m}TcO^-_4$ is negatively charged and no effective chemistry exists that can be used to attach the negatively charged pertechnetate anion to a pharmaceutical molecule. Therefore, it is necessary to reduce Tc (VII) of $^{99m}TcO^-_4$ to a lower oxidation state to produce a stable ^{99m}Tc-pharmaceutical complex. Most of the ^{99m}Tc radiopharmaceuticals available in routine clinical use are ^{99m}Tc chelates.

A promising approach is to couple the pharmaceutical molecule with a chelating agent that can bind ^{99m}Tc. We have investigated ^{99m}Tc chelating agents. The resulting complexes must, of course, be sufficiently stable to withstand exposure to physiological conditions. We recently reported (2) that macrocyclic amines (cyclam: 1,4,8,11-Tetraazacyclotetradecane, pentaazacyclopentadecane), bisaminothiols (BAT), dithiocarbamates, react rapidly with ^{99m}Tc to form kinetically and thermodynamically stable complexes . Since cyclam is commercially available we chose to develop the synthesis of the N-functionalized macrocycle **4**, (scheme 1) capable of complexing ^{99m}Tc. Troutner and al. (3) described some N-functionalised azamacrocycles complexing [^{99m}Tc] and capable of crossing the blood-brain barrier. The only contribution of the azamacrocycle portion of this molecule is to complex Tc.



SCHEME 1 : Synthesis of a mono N - functionalized Cyclam

RESULTS AND DISCUSSION

The results reported here suggest that the monofunctionalization of azamacrocycles using an excess of macrocycle over the alkylating agent (6) is a suitable method for the preparation of the new ligand **4**.

2-(4-Hydroxyphenyl) ethanol **1** and 2-diethylaminoethyl chloride hydrochloride **1a** were condensed under alkaline conditions (K_2CO_3) in N,N-dimethylformamide (DMF) at 100°C, affording compound **2** in 60% yield. **2** reacts with toluene-4-sulfonyl chloride (TsCl) affording the tosyl derivative **3** in good yield. The reaction of an excess of cyclam with the alkylating agent **3** (5 : 1 ratio) favors the formation of the mono-substituted derivative **4** (60 % yield). The purification of compound **4** was easily performed via its Cu-complex. The latter was obtained as a copper chloride methanol solution. The dried solid Cu-complex was recrystallised from acetone.

Pure free ligand **4** was regenerated under alkaline conditions (KOH) after treatment with an aqueous solution of potassium cyanide (4). Technetium-99m-labelling was performed by using the principle of ligand exchange. This technique uses the vial B of commercial kit TCK-17 (International CIS Bio) as a source of stannous pyrophosphate, which was added under nitrogen to the functionalized form

of Cyclam 4 in aqueous solution (5). The[^{99m}Tc]-complex was purified by liquid chromatography on sephadex G25 column using a bicarbonate buffer pH = 9,2 as mobile phase. The radiochemical yield of isolated compound was in the range of 70-80 %.

To characterize the positive charge of the complex, chromatography on a DOWEX 50 WX2 cationic Column with H_2O as eluant was performed. The feasability of a neutral and lipophilic ^{99m}Tc-complex such as ^{99m}Tc-bisaminothiol (BAT) with Tc = O or Tc ≡ N core is under investigation .

EXPERIMENTAL

General comments. ^{99m}Tc as sodium pertechnetate was eluted from a ^{99}Mo-^{99m}Tc generator (Elumatic III International CIS) kindly supplied by the Centre Jean Perrin, Clermont-Ferrand. All chemicals were purchased from commercial suppliers and used as received. Nuclear magnetic resonance (NMR) spectra were performed on a Bruker AM 200 (4.5 T) spectrometer. Chemical shifts (δ) are reported in parts per million relative to the internal tetramethylsilane standard. Infrared (IR) spectra were recorded on a Perkin Elmer 398 spectrometer. Melting points (mp) were determined on a digital melting point apparatus (Electrothermal) . Analytical thin layer chromatographies (TLC) were performed on precoated silica gel plates (Merck 60 F 254, 0,2 mm thick Merck RP 18 F S, 254 0,25 min thick or aluminium oxide neutral (Merck 60 GF 254) with both detection by ultra violet light at 254 mm and visualization by iodine. The radioactive spots were scanned and recorded on an automatic TLC-multichannel linear analyzer (LB 2832 Berthold). The crude material 4 was purified by preparative chromatography on a silica gel column eluted with CH_2Cl_2 and CH_2Cl_2/EtOH (98 : 2).

2-{4-[2-(diethylamino)ethoxy]-phenyl}-ethanol [2]

A solution of 1 (13,8 g, 0,1 mol) in DMF (100 ml) was treated with K_2CO_3 (14,1g, 0,11 mol) stirred at 100°C for 30 min. Then it was cooled to 60°C and 2-diethylaminoethyl chloride (17,2 g, 0,1 mol) was added. This mixture was left with continuous stirring for 16 h, then evaporated under vacuum to dryness. The residue was treated with HCl in ether to give the corresponding salt.

NaOH 1N (100 ml) was then added to the crude HCl salt, the solution was stirred for 10 min and extracted with 3 x 100 ml of dichloromethane. The organic layer was washed several times with water and dried over anhydrous $MgSO_4$ to give 2 as a yellow oil. (Yield : 60 %).

TLC : silica gel (dichloromethane/éthanol 70/30) Rf = 0,25

IR ν : 3500-3100 cm^{-1} (OH), 2800-3000 cm^{-1} (CH, CH_2, CH_3), 1600-1460 cm^{-1} (CH arom) 1240 cm^{-1} (C-O).

^{1}H-NMR (DMSO-d_6) δ : 0,98 (t, 6H, CH_3) 2,50-2,60 (m, 6H, $CH_2C_6H_5$, N CH_2CH_3), 2,80 (t, 2H, CH_2 N), 3,50-3,57 (m, 2H, CH_2OH) 3,98 (t, 2H, OCH_2), 4,60 (1s, 1H, OH, exchangeable with D_2O), 6,79-7,12 (dd, 4H, C_6H_4)

2-{4-[2-(diethylamino)ethoxy]-phenyl}-ethanol Toluene-4-sulfonate 3

2,4 g (12,6 Mmol) of toluene-4-sulfonyl chloride (TsCl) in a minimum amount of pyridine, freshly distilled on potassium hydroxide, were added to 2,50 g (10,4 mmol) of alcohol **2** and stirred at - 20°C overnight. The solution was evaporated under reduced pressure. The residue was dissolved in CH_2Cl_2, washed three times with 10 mL of water and dried over anhydrous $MgSO_4$. After evaporating the solvent in vacuo, the crude material was purified using silica gel column chromatography with CH_2Cl_2-EtOH (98 : 2) to give the pure product **3**. (Yield : 65 %).

TLC : silica gel (dichloromethane/ethanol, 70/30) Rf = 0,57 ; (dichloromethane/ethanol, 95/5) Rf = 0,35

I R ν : 2800-3000 cm^{-1}(CH, CH_2, CH_3), 1600-1460 cm^{-1}(CH arom) 1350 cm^{-1}(SO_2), 1240 cm^{-1} (C=O)

^{1}H-NMR (DMSO-d_6) δ : 1,07 (t, 6H, CH_3) 2,43 (t, 3H, $CH_3C_6H_4$), 2,65 (q, 4H, NCH_2 CH_3), 2,87-2,94 (m, 4H, CH_2 N,$CH_2C_6H_4$), 4,01 (t, 2H, CH_2OS), 4,15 (t, 2H, OCH_2), 6,76-7,02, 7,26-7,71 (2 dd, 8H, C_6H_4)

N-2-{4-[2-(diethylamino) ethoxy]-phenyl}-ethyl-1,4,8,11-tetraazacyclotetradecane 4

A suspension of Cyclam (1 g, 5 mmol) in dry DMF (50 mL) was heated at 110°C under nitrogen until dissolution. A solution of **3** (391 mg, 1 mmol)in dry DMF (10 ml) was added dropwise. After stirring and heating at 110°C for 1 h the mixture was then left overnight (12 h) at 0°C. The excess of cyclam, precipitated from the crude mixture, was collected and the solvent evaporated. The resulting oil was dissolved in CH_2Cl_2 (100 mL), washed with 0,1N $NaHCO_3$ (20 ml) and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure to give a yellow oil.

Purification : Crude monofunctionalized Cyclam **4** was purified via its complex with cupric chloride.

(a) Synthesis of the Cu2+ complex of N-2-{4-[2-(diethylamino) ethoxy]-phenyl}-ethyl-1,4,8,11-tetraazacyclotetradecane

An equimolar solution of 4 and $CuCl_2$, in methanol gave a green solution. After being stirred for 1 h at 65°C, the mixture was evaporated to dryness on a rotary evaporator. The blue solid was washed with acetone and recrystallised from acetone-methanol (66/100).

(b) Synthesis of free ligand 4

Potassium cyanide (337 mg, 5 mmol) was added to the aqueous solution of the pure crystallised Cu- complex (278 mg, 0,5 mmol) and the solution heated at 70°C for 30 min. The mixture rapidly turned colorless. The solution was cooled and solid KOH was added until pH = 12 and the mixture extracted with 3 x 20 ml portions of CH_2Cl_2. the combined organic extracts were dried over Na_2SO_4 and evaporated. (Yield : 62 %).

TLC : Aluminium oxide neutral (Ethanol Rf = 0,30) ; Aluminium oxide neutral (Ethanol $(C_2H_5)_3$ N 95/5 Rf = 0,75)

I R ν : 2800-3000 cm^{-1} (CH, CH_2, CH_3), 1600-1460 cm^{-1}(CH arom), 1240 cm^{-1} (C - O)

^{1}H-NMR ($CDCl_3$) δ : 1,10 (t, 6H, CH_3), 1,61-1,89 (m, 3H, NH exchangeable with D_2O), 2,51-2,75 (m, 28 H, $CH_2C_6H_5$, N CH_2CH_3), HNCH_2, CH_2 N cyclam), 2,85 (t, 2H, CH_2 N), 4,01 (t, 2H, OCH_2), 7,01-7,30 (dd, 4H, C_6H_4) .

Measured mass (FAB+, glycerol matrix) of molecular ion : 420,0 calculated for $C_{24}H_{45}N_5O$: 419,6

[^{99m}Tc] labelling :

Sodium [^{99m}Tc] pertechnetate (7 mCi, 259 Mbq, 1 mL) was added to a reactor vial fitted with a septum containing the vial B of commercial kit TCK-17 (International CIS Bio) (0,5 ml) and the product 4 (12 mg, 28μmol). The mixture was stirred and heated (80°C) for 30 min.

The $[TcO_2]^+$ - complex was separated from the reaction mixture by chromatography on a Sephadex G25 column (eluent : bicarbonate buffer pH = 9,2). Under these conditions the [^{99m}Tc]-complex was eluted first, the impurity : pyrophosphate-complex (20-30 %) remained on the column.

The radiochemical purity of the complex was determined by TLC on silica gel with methylethylcetone as mobile phase or on reverse phase RP18 in MeOH-CH_3CN-THF-$AcONH_4$ (3:3:2:2, v/v). The average radiochemical purity was 99 %

The positive charge of the complex, was characterized by chromatography on a Dowex 50WX2 cationic column with H_2O as eluent. All the radioactivity was retained on the column.

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