

## Synthesis of *N*-Quaternary Ammonium [<sup>3</sup>H] and [<sup>99m</sup>Tc]Polyazamacrocycles, Potential Radiotracers for Cartilage Imaging

Colette NICOLAS, Michèle BOREL\*, Jean-Claude MAURIZIS, Nadine GALLAIS,  
Maryse RAPP, Monique OLLIER, Michel VERNY, Jean-Claude MADELMONT.

INSERM U 484, Rue Montalembert, B.P. 184, 63005 Clermont-Ferrand Cedex,  
France.

### SUMMARY

The [<sup>3</sup>H] and [<sup>99m</sup>Tc] synthesis of three new compounds, *N*-[3-(pyridinio)propyl] cyclam (NPPC), *N*-[3-(triethylammonium)propyl] cyclam (NTPC) and *N*-[3-(triethylammonium)propyl]-15ane-N5 (NTP 15-5), potential tracers for cartilage, is reported. NPPC and NTPC were labelled with tritium on the ethyl group of cyclam with a specific radioactivity of 89 MBq/mmol. The [<sup>99m</sup>Tc]-labelling of the three quaternary ammonium chelates was realized with a high specific activity in the range 20-25 MBq/μmol.

**KEY WORDS:** polyazamacrocycles, tritiated cyclam, <sup>99m</sup>Tc cyclam, cartilage radiotracer.

### INTRODUCTION

In previous studies, we have demonstrated that the acetylcholinesterase reactivators quaternary ammonium-oximes were rapidly and strongly concentrated in

\*Author to whom correspondence should be addressed

cartilaginous tissues such as intervertebral disks and articular cartilages after intravenous injection to rats (1,2). This property is due to a binding of the quaternary ammonium function of the drugs to the anions of the acidic functions of the cartilage proteoglycans (3). These results encouraged us to design molecules presenting a quaternary ammonium function capable of binding to the cartilaginous tissue and another function able to bind  $^{99m}\text{Tc}$  in order to obtain new radiodiagnostic agents for the articular cartilage imaging. For this purpose, potential radiodiagnostic agents must preserve their affinity for this tissue when the quaternary ammonium is bound to a macrocycle, and the stability of the chelate *in vivo* must be sufficient to carry radioactivity in targets compatible with scintigraphic examination.

In the present study, we describe the [ $^3\text{H}$ ] and [ $^{99m}\text{Tc}$ ] synthesis of three new molecules, *N*-[3-(pyridinio)propyl] cyclam (NPPC), *N*-[3-(triethylammonio)-propyl] cyclam (NTPC) and *N*-[3-(triethylammonio)propyl]-15ane-N5 (NTP 15-5) (Figure 1). [ $^3\text{H}$ ]-Labelling was used on animal models to verify the targeting of the quaternary ammonium group towards cartilage and [ $^{99m}\text{Tc}$ ] labelling was performed to determine the *in vivo* stability of such complexes as a function of the macrocycle.

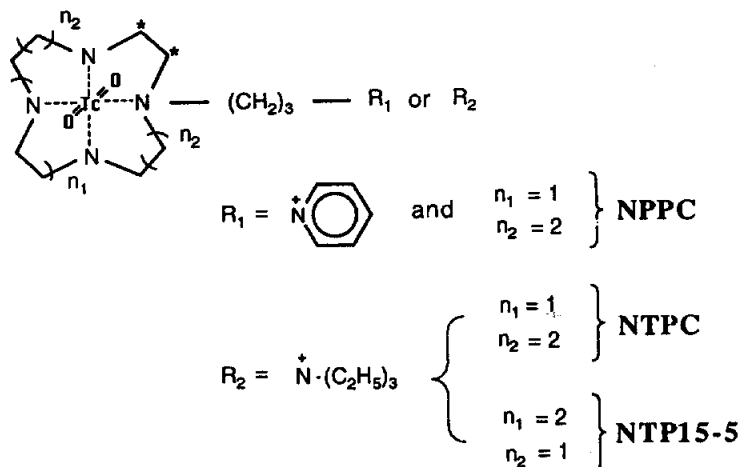


Figure 1. Structure of NPPC, NTPC and NTP 15-5

## RESULTS AND DISCUSSION

[<sup>3</sup>H]NPPC **9a** and [<sup>3</sup>H]NTPC **9b** were synthesized as outlined in Scheme 1. Quaternarization of aliphatic or aromatic structures was carried out to determine the influence of the nature of the quaternary ammonium on the affinity towards cartilage. [<sup>3</sup>H]cyclam **5** was prepared following the transition metal "template" procedure from Nickel Complex **2** (**4**) without isolating the intermediate compounds **2**, **3**, **4** in 20% radiochemical yield and a specific radioactivity of 89 MBq/mmol.

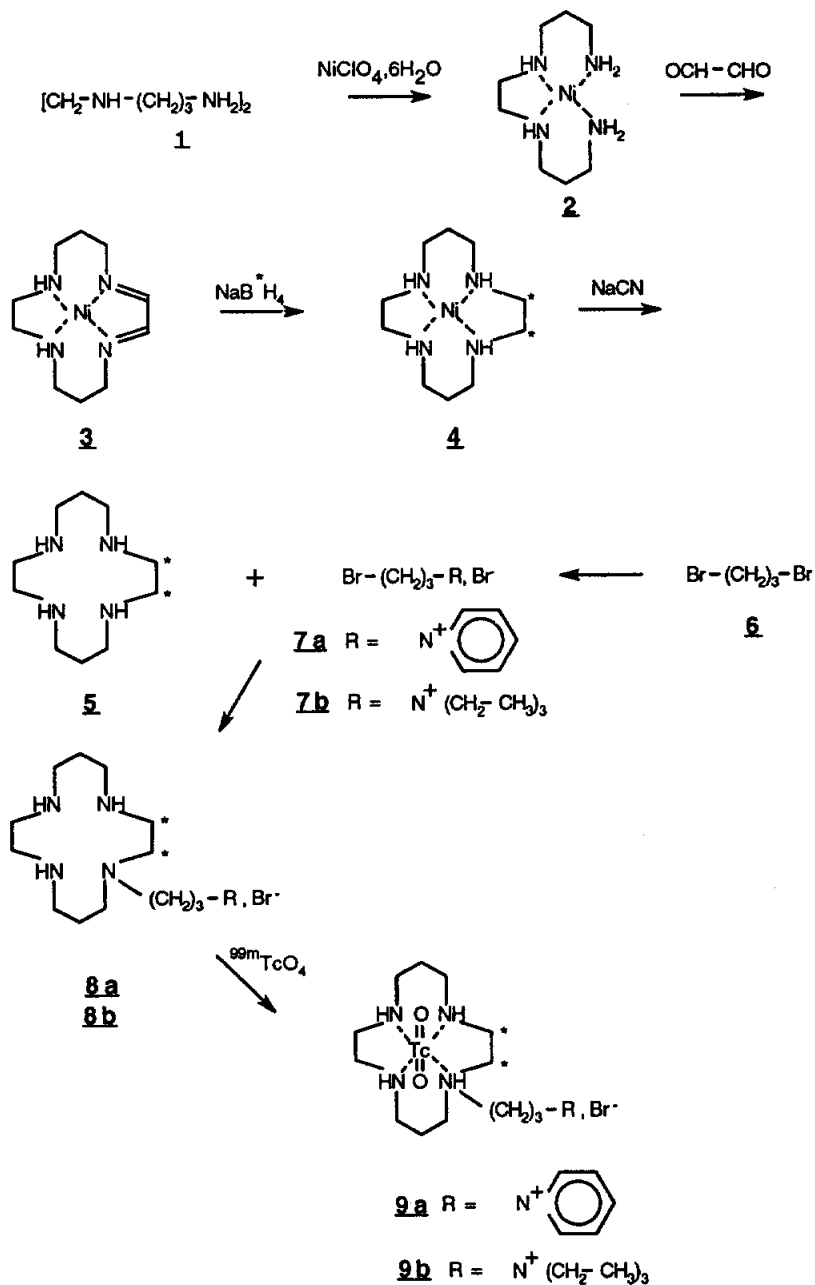
Although most of the *N*-monofunctionalized polyazamacrocycles were prepared with a ten fold excess of macrocycle (**5**), in our case we have used stoichiometric conditions between [<sup>3</sup>H]cyclam and the 3-bromopropyl ammonium compounds **7a** or **7b** to obtain *N*-[3-(pyridinio)propyl] or *N*-[3-(trimethylammonio)propyl] [<sup>3</sup>H]cyclam (**8a** or **8b**) in 80 % and 88% yields respectively. Then these compounds were labelled with <sup>99m</sup>Tc by the classical method (TcO<sub>4</sub><sup>-</sup>/SnCl<sub>2</sub>·2H<sub>2</sub>O) to give the [<sup>3</sup>H] and [<sup>99m</sup>Tc] doubly labelled complexes **9a** and **9b**. The yields and purities were determined using both thin layer chromatography and high pressure liquid chromatography. All [<sup>99m</sup>Tc] complexes were stable in solution at pH<8 for 24 h or longer.

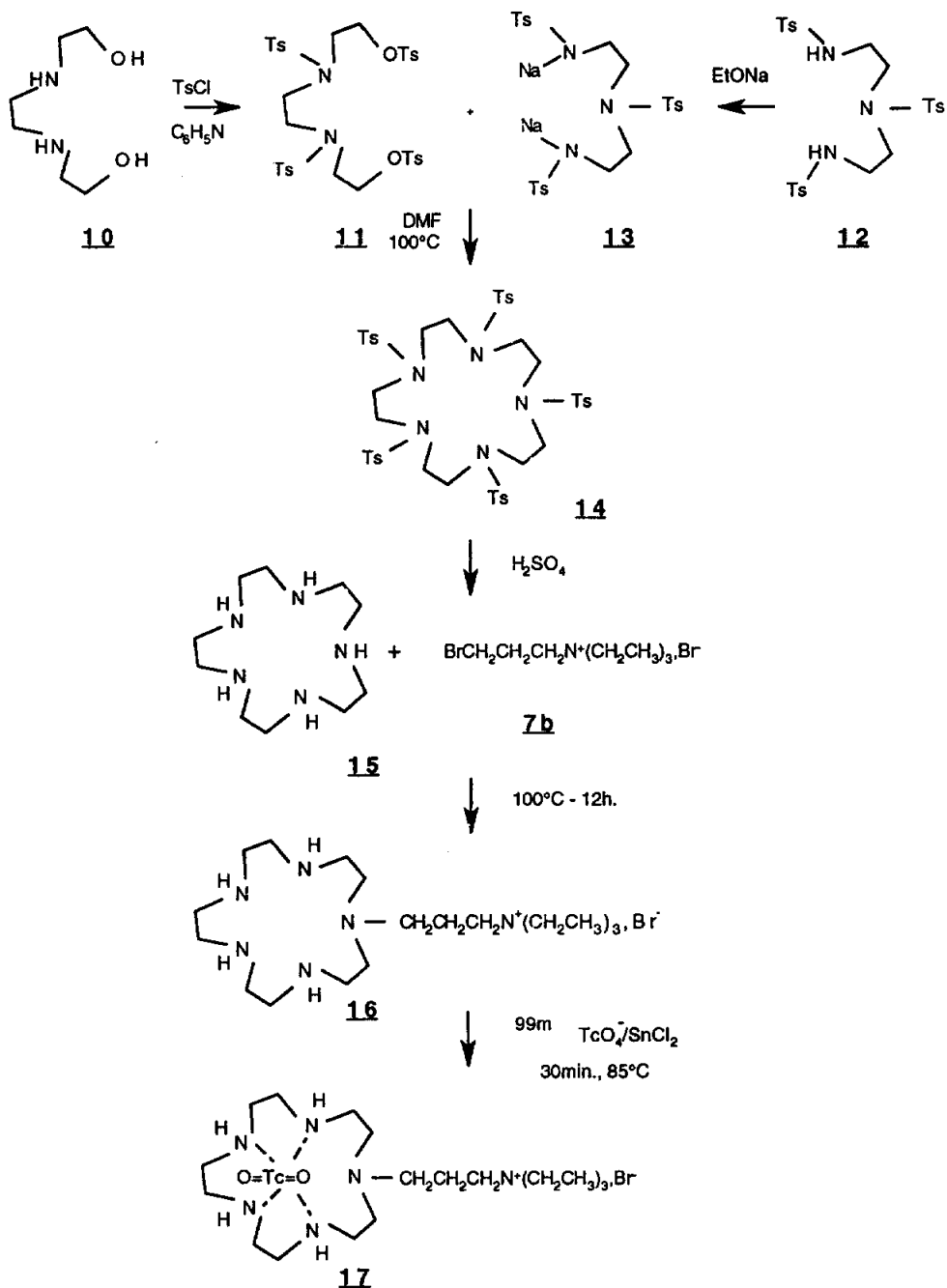
[<sup>99m</sup>Tc]NTP 15-5 **17** was synthesized according to Scheme 2 (6). This compound was prepared in order to determine the influence of the macrocycle structure on the stability of the technetium chelates *in vivo*.

The specific activities of the [<sup>99m</sup>Tc] complexes were in the range 20-25 Mbq/μmol.

## EXPERIMENTAL

**Chemistry. General Comments.** Analytical thin layer chromatography (TLC) was conducted on precoated silica gel plates (Merck 60F-254 and RP18F-254S) with both detection by UV at 254 nm and visualization by iodine. The radioactive spots were scanned and recorded on an automatic TLC- multichannel linear analyzer Berthold LB 2832. Infrared Spectra (IR) were recorded on a Perkin Elmer 398 Spectrometer. Proton-carbon correlations were obtained from 2D-NMR spectra

Scheme 1. Synthesis of [ $^{99\text{m}}\text{Tc}$ ] and [ $^3\text{H}$ ] NPPC and NTPC



Scheme 2. Synthesis of [ $^{99\text{m}}\text{Tc}$ ] NTP 15-5

which were performed on a Bruker AM 200 spectrometer. Chemical shifts ( $\delta$ ) were reported in parts per million relative to the internal tetramethylsilane (TMS) standard. Electrospray mass spectra (ESI-MS) were obtained from CNRS, Vernaison (France). Solutions of  $\text{Na}^{99\text{m}}\text{TcO}_4$  were eluted from  $^{99}\text{Mo}$ - $^{99\text{m}}\text{Tc}$  generator (Elumatic III International CIS, Centre Jean Perrin, Clermont-Ferrand, France).  $\text{NaB}^3\text{H}_4$  was purchased from CEA, Saclay (France), (T641 M, 4 GBq).

### **[ $^3\text{H}$ ] cyclam 1,4,8,11-Tetraaza [2,3- $^3\text{H}$ ]cyclotetradecane 5**

The nickel complex 2 was prepared by adding dropwise over 4 min, *N,N'*-bis(2-aminopropyl)ethylene diamine 1 (2.6 g ; 15 mmol) to a solution of nickel (II) perchlorate,  $6\text{H}_2\text{O}$  (5.5 g ; 15 mmol) in  $\text{H}_2\text{O}$  (50 mL) then stirring for 1 h. To the red-brown solution 2 cooled to ca  $5^\circ\text{C}$ , was added over 5 min 30 % glyoxal (3 mL ; 15 mmol) and the mixture allowed to stand 4 h at room temperature. The solution 3 was cooled again to  $5^\circ\text{C}$  and treated with  $\text{NaB}^3\text{H}_4$  (4 GBq ; 29 mg ; 0.7 mmol) and then with  $\text{NaBH}_4$  (1.1 g ; 28.5 mmol) in small portions over 1 h. The mixture was stirred at room temperature for 2h then heated for 20 min at  $90^\circ\text{C}$ . The hot solution 4 was rapidly filtered.  $\text{NaCN}$  (3 g ; 60 mmol) was added to the filtrate under a ventilated hood and the resulting solution was heated at reflux for 2 h at  $100^\circ\text{C}$  then cooled.  $\text{NaOH}$  pellets (1.5 g ; 37.5 mmol) were added and the mixture was evaporated until semi-solid and extracted 4 times with 50 mL portions of  $\text{CH}_2\text{Cl}_2$ . The organic layers were combined, dried over  $\text{Na}_2\text{SO}_4$  and evaporated under reduced pressure ; the crude [ $^3\text{H}$ ]cyclam 5 was dissolved in  $\text{CH}_2\text{Cl}_2$  (40 mL) and precipitated by  $\text{Et}_2\text{O}$ . Chemical yield: 1.65 g, 6.3 mmol (42 %). Radiochemical yield: 20 % ; Specific radioactivity: 89 MBq/mmol ;  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ )  $\delta$ : 1.70-1.85 (4 H, m, 2 x  $\text{CH}_2\text{-CH}_2\text{-CH}_2$ ), 2.65 (8H, s, 2 x  $\text{N-CH}_2\text{-CH}_2\text{-N}$ ), 2.70-2.75 (8 H, t, 2 x  $\text{N-CH}_2\text{-CH}_2\text{-N}$ ).

### **1-(3-Bromopropyl)pyridinium bromide 7a**

To a solution of 1,3-dibromopropane 6 (5 g ; 24.75 mmol) in  $\text{Ac}_2\text{O}$  (25 mL) pyridine (2 mL ; 24.75 mmol) was added dropwise. The solution was then stirred overnight at room temperature. 1-(3-Bromopropyl)pyridinium bromide 7a was filtered and washed with acetone and dried under vacuum. Yield : 85 % ;  $^1\text{H-NMR}$  ( $\text{D}_2\text{O}$ )  $\delta$ : 2.35

(2H, m, CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>), 3.26 (2H, t, Br-CH<sub>2</sub>), 4.57 (2H, t, CH<sub>2</sub>-N), 7.85, 8.33, 8.65, 8.68 (5 H, ttd, Pyr).

**(3-Bromopropyl)triethylammonium bromide 7b**

This compound was prepared as 7a but with heating at 60°C overnight. Yield : 78 %  
<sup>1</sup>H-NMR (D<sub>2</sub>O) δ : 1.17 (9H, t, 3 x CH<sub>3</sub>), 2.11 - 2.22 (2H, m, CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>), 3.13-3.27 (6 H, m, 3 x N<sup>+</sup>-CH<sub>2</sub>, CH<sub>3</sub>), 3.46 (2H, t, Br-CH<sub>2</sub>-).

**1-(3-Pyridiniopropyl)-1,4,8,11-tetraaza-[2,3-<sup>3</sup>H]-cyclotetradecane : [<sup>3</sup>H] NPPC 8a**

[<sup>3</sup>H] Cyclam 5 (100 mg ; 0.5 mmol) and 1-(3-bromopropyl)pyridinium bromide 7a (151.5 mg ; 0.5 mmol) were dissolved in water (5 mL) and heated up to 95°C under vigorous stirring for 16 h. After cooling, the aqueous solution was extracted with CH<sub>2</sub>Cl<sub>2</sub> to remove the residual [<sup>3</sup>H]-Cyclam, then evaporated to dryness. The residue was taken up with absolute EtOH, precipitated by addition of concentrated HCl (0.12 mL), filtered, washed with EtOH then Et<sub>2</sub>O to yield [<sup>3</sup>H] NPPC 8a (200 mg ; 80 %). Specific radioactivity : 89 MBq/mmol.

<sup>1</sup>H-NMR (D<sub>2</sub>O) δ : 2.01 (4 H, t, CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>), 2.40 (2H, m, CH<sub>2</sub>-(propyl), 3.08 (2H, m, N-CH<sub>2</sub>), 3.25 (8 H, t, 4 x N-CH<sub>2</sub>), 3.42 (8 H, s, 2 x N-CH<sub>2</sub>-CH<sub>2</sub>-N), 4.59 (2H, m, CH<sub>2</sub>-Pyr), 8.0, 8.45, 8.78-8.81 (5H, ttd, Pyr).

**1-(3-Triethylammoniopropyl)-1,4,8,11-tetraaza-[2,3-<sup>3</sup>H]-cyclotetradecane: [<sup>3</sup>H]NTPC 8b**

This product was synthesized as 8a with a yield of 88 %. Specific radioactivity : 89 MBq/mmol. <sup>1</sup>H-NMR (D<sub>2</sub>O) δ : 1.03 (9H, t, 3 x CH<sub>3</sub>), 1.85-1.92 (6H, m, 3 x CH<sub>2</sub>), 3.03-3.15 (18H, m, 9 x N-CH<sub>2</sub>), 3.28 (8H, s, 2 x N-CH<sub>2</sub>CH<sub>2</sub>-N).

**[<sup>99m</sup>Tc] Labelling of [<sup>3</sup>H] NPPC 9a and [<sup>3</sup>H] NTPC 9b**

General procedure : In a sterile and under vacuum glass vial (15 mL), closed with rubber stopper and aluminium capsule were introduced successively : a solution of ligand [<sup>3</sup>H NPPC or <sup>3</sup>H NTPC] (5 mg ; 10<sup>-3</sup> mmol) in physiological serum (1 mL), sodium pertechnetate [<sup>99m</sup>TcO<sub>4</sub> ; 925 MBq (25 mCi)] in physiological serum (1 mL).

The vial was heated to 85°C for 5 min (metal bath). Then a freshly prepared deoxygenated aqueous solution of SnCl<sub>2</sub>, 2H<sub>2</sub>O (1 mL ; 9 mmol) was added. The vial was heated at 85°C for 30 min. The radiochemical yield was close to 100 %. Labelling control TLC, silica gel (MeOH/H<sub>2</sub>O, 85/15) : [<sup>99m</sup>Tc] **9a** or **9b** : *R<sub>f</sub>* = 0; <sup>99m</sup>TcO<sub>4</sub><sup>-</sup> : *R<sub>f</sub>* = 0,8.

If necessary, a purification by elution on a Sephadex G25 column with physiological serum was done.

### **Tetratosyl-*N,N'*-bis (2-hydroxyethyl) ethylene diamine 11**

A solution of *p*-toluene sulfonylchloride (77 g ; 0.4 mol) in pyridine (100 mL) was added over 2 h under vigorous stirring to a solution of *N,N'*-bis(2-hydroxyethyl) ethylene diamine **10** (14.8 mg ; 0.1 mol) in pyridine (200 mL), cooled to 0°C under nitrogen. The solution was stirred for 4 h while the temperature was allowed to rise to 25°C.

The solution was poured into a mixture of ice (250 mL) and concentrated HCl (250 mL) under stirring. The tetratosyl derivative **11** was filtered, washed with H<sub>2</sub>O, then with MeOH and finally recrystallized from MeOH. TLC, silica gel (CH<sub>2</sub>Cl<sub>2</sub>/EtOH, 98/2) : *R<sub>f</sub>* = 0.50 ; IR (KBr)  $\nu$  : 3270 cm<sup>-1</sup> (no NH band) ; <sup>1</sup>H-NMR (CDCl<sub>3</sub>/TMS)  $\delta$  : 2.43 (12 H, s, 4 x CH<sub>3</sub>), 3.29 (4 H, s, 2 x CH<sub>2</sub>N), 3.35 (4 H, t, 2 x N-CH<sub>2</sub>), 4.13 (4H, t, 2 x CH<sub>2</sub>O), 7.31-7.78 (16 H, m, 4 x C<sub>6</sub>H<sub>4</sub>).

### ***N,N',N''*-tri-*p*-Tosyl diethylene triamine, disodium salt 13**

*N,N',N''*-tri-*p*-Tosyldiethylene triamine **12** (56.4 g ; 0.1 mol) was added to sodium ethoxide (0.2 mol) in absolute ethanol (150 mL). After heating for 1h at 80°C the solvent was removed under reduced pressure to give the disodium salt **13** which was dried under vacuum and KOH pellets.

### ***N*<sup>1</sup>,*N*<sup>4</sup>,*N*<sup>7</sup>,*N*<sup>10</sup>,*N*<sup>13</sup>-Pentatosyl-1,4,7,10,13-penta-azacyclopentadecane 14**

In a 1000 mL three necked round-bottomed flask fitted with two dropping funnels, a magnetic stirrer and a nitrogen inlet, DMF (100 mL) was warmed to 100°C. The tetratosyl derivative **11** (76.4 g ; 0.1 mol) in DMF (200 mL) and the disodium salt



**13** (61 g ; 0.1 mol) in DMF (200 mL) were added simultaneously and slowly (2 h) through the dropping funnels while maintaining the temperature at 100-110°C under stirring. The brown solution was stirred for 5h at 110°C and overnight at room temperature, then poured into ice and water under vigorous stirring. The precipitate was filtered, washed with H<sub>2</sub>O, MeOH and Et<sub>2</sub>O. After drying under vacuum at 80°C, N<sup>1</sup>,N<sup>4</sup>,N<sup>7</sup>,N<sup>11</sup>,N<sup>13</sup>-pentatosyl-[15ane-N5] **14** was dissolved in CH<sub>2</sub>Cl<sub>2</sub> then precipitated by MeOH addition. Yield : 70% ; TLC : silica gel (CH<sub>2</sub>Cl<sub>2</sub>/EtOH , 98/2) R<sub>f</sub> : 0.40 ; IR (KBr) : no NH band. <sup>1</sup>H-NMR (CDCl<sub>3</sub>/TMS) δ : 2.44 (15 H, s, 5 x CH<sub>3</sub>), 3.27 (20 H, s, 5 x CH<sub>2</sub>CH<sub>2</sub>), 7.30-7.70 (20 H, 2 x d, 5 x C<sub>6</sub>H<sub>4</sub>)

#### 1,4,7,10,13-penta-azacyclopentadecane or 15 ane-N5 **15**

Pentatosyl derivative **14** (35.5 g ; 36 mmol) was treated at 110°C during two days with concentrated H<sub>2</sub>SO<sub>4</sub> (150 mL) under a dry argon atmosphere. The cooled solution was poured slowly through a dropping funnel into Et<sub>2</sub>O (500 mL) which was cooled with an ice bath. The grey-white precipitate was filtered rapidly and at once washed with Et<sub>2</sub>O then dissolved in H<sub>2</sub>O (100 mL), treated with a large excess of NaOH pellets (20 g), evaporated then extracted by CHCl<sub>3</sub> to give **15**. Yield : 80 % ; TLC : silica gel (CH<sub>2</sub>Cl<sub>2</sub>/EtOH, 98/2) R<sub>f</sub> = 0 ; IR (KBr) : NH (3270 cm<sup>-1</sup>). <sup>1</sup>H-NMR (CDCl<sub>3</sub>/TMS) δ : 2.45 (5H, s, 5 x NH), 2.76 (20 H, s, 5 x CH<sub>2</sub>-CH<sub>2</sub>) ; <sup>13</sup>C-NMR (CDCl<sub>3</sub>) δ : 48.30 (s, N-CH<sub>2</sub>).

#### 1-[(3-Triethylammonio)propyl]-1,4,7,10,13-penta-azacyclopentadecane: NTP-15-**5 16**

In a 100 mL round-bottomed flask, a solution of **15** (2.15 g ; 0.01 mol) in H<sub>2</sub>O (50 mL) was added to 3-(bromopropyl)triethylammonium bromide **7b** (3.03 g ; 0.01 mol). The mixture was warmed to 90°-100°C for 12h under nitrogen. After evaporation, the residue was washed twice with CH<sub>2</sub>Cl<sub>2</sub> then dissolved in EtOH (100 mL). After treatment with 10N HCl (4 mL) and cooling, the white cottony precipitate was filtered, washed with EtOH and Et<sub>2</sub>O to give **16**. Yield : 80 % ; TLC : silica gel (0,1 N-HCl) R<sub>f</sub> = 0.45 ; RP18 (EtOH) R<sub>f</sub> = 0.12 ; <sup>1</sup>H-NMR (D<sub>2</sub>O) δ : 1.04 (9H, t, 3 x CH<sub>3</sub>), 1.72 (2 H, m , CH<sub>2</sub>), 2.53 (2 H, t, N-CH<sub>2</sub>), 2.81 (4 H, t, N-CH<sub>2</sub>), 2.95 (2H,

t, CH<sub>2</sub>-N), 3.10 (6H, m, 3 x N-CH<sub>2</sub> CH<sub>3</sub>), 3.16 (4 H, s, 2 x NH-CH<sub>2</sub>), 3.29 (12 H, s, 3 x N-CH<sub>2</sub>CH<sub>2</sub>N); <sup>13</sup>C-NMR (D<sub>2</sub>O-DMSO-d<sub>6</sub>) δ: 8.38 (3 x CH<sub>3</sub>), 17.46 (C-CH<sub>2</sub>-C), 44.78 (3 x N-CH<sub>2</sub>CH<sub>2</sub>-N), 47.03 (2 x N-CH<sub>2</sub>), 49.63 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>-N), 50.71 (2 x N-CH<sub>2</sub>), 54.25 (3 x N<sup>+</sup>-CH<sub>2</sub>CH<sub>3</sub>), 55.77 (N-CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>); ESI-MS : 357.3 (M<sup>+</sup>).

### [<sup>99m</sup>Tc]Labelling of NTP 15-5 17

The general procedure previously described was applied to the labelling of NTP 15-5 by <sup>99m</sup>Tc.

### Acknowledgement

The authors would like to thank J.Lefrançois for her help in typing this manuscript.

### References

1. Garrigue H., Maurizis J.C., Nicolas C., Madelmont J.C., Godeneche D., Hulot T, Morge X., Demerseman P., Sentenac-Roumanou H, Veyre A. *Xenobiotica* **20**: 699-709 (1990).
2. Garrigue H., Maurizis J.C., Madelmont J.C., Nicolas C., Meyniel J.M., Louvel A., Demerseman P, Sentenac-Roumanou H, Veyre A. *Xenobiotica* **21** : 583-595 (1991).
3. Maurizis J.C., Ollier M., Nicolas C., Madelmont J.C., Garrigue H., Veyre A. *Biochem. Pharmacol.* **44** : 1227-33 (1992).
4. Barefield E.K., Wagner F., Herlinger A.W., Dahl A.R. *Inorganic Syn* **16** : 220-225 (1975).
5. Ketring A.R., Troutner D.E., Hoffman T.J., Stanton D.K., Volkert W.A., Holmes R.A. *Int. J. Nucl. Med. Biol.* **11** : 113-119 (1984).
6. Richman J.E., Atkins T.J. *J. Am. Chem. Soc.* **96** : 2268-70 (1974).