Synthesis of a new polyaminopolycarboxylic acid (BPHA) and its labeling with ^{99m}Tc

Guozheng Liu

(Isotope Department, China Institute of Atomic Energy, Beijing 102413*, P. R. China)*

Boli Liu

(Chemistry Department, Beijing Normal University, Beijing 100875*, P. R. China)*

Summary

N, N′—Bis(2—aminoethyl)propanediamine hexaacetic acid (BPHA) has been prepared as a tetrachloride salt. It has been characterized by ^{1}H NMR, Positive FAB MS, and elemental analysis. BPHA is easily labeled with ^{99m}Tc in the pH range of 2—5 using $SnCl_2 \cdot 2H_2O$ as reductant. The radioactive complex accumulates in both kidneys and liver and is fast excreted.

Key Words: polyaminocarboxylic acid, organic synthesis, radiolabeling, technetium, biodistribution.

Introduction

It has been noticed that the basal atoms of square pyramidal technetium compounds with multiple bonds TcX ($X = O^{2-}$, S^{2-}, N^{3-}, $R-N^{2-}$) pointing to the apices are very squeezed[1]. So 5—membered rings based on basal atoms are suitable. It has been discovered that ethyleneglycol and 1,2—propanediol can form complexes with the TcO^{3+} core but 1,3—propanediol can′t[2]. In a six—coordinated system, both linkages between the two coordinating atoms forming five and six—membered rings are acceptable. If there is no multiple bond in a six—coordinated system, the coordinating atoms are not so squeezed as the basal atoms of TcO^{3+} compounds. It has been deduced that too many adjacent two—atom—across bidentates may not be favorable to a six—coordinated system. In the literature, all technetium compounds of polyamino-polycarboxylates prepared in single crystals have a coordination number of six or even seven[3-5], but the polyamine chains of the

CCC 0362–4803/98/020097–08$17.50

Received 26 June 1997
Revised 10 September 1997

polyaminopolycarboxylate ligands studied so far are all polyethyleneamines. We hope to synthesize a polyamino-polycarboxylate ligand containing a propylene group. Our choice was N,N′—bis(2—aminoethyl)propanediamine hexaacetic acid(BPHA).

Triethylenetetraamine hexaacetic acid (TTHA) and diethylenetriamine pentaacetic acid (DTPA) were prepared from the corresponding polyamines, formaldehyde and sodium cyanide some fourty years ago[6] and later from polyamines and chloroacetic acid[7,8]. However, although N, N′ — bis (2 — aminoethyl) — 1, 3 — propanediamine was synthesized long ago, its acetic acid derivative BPHA has not been obtained.

EDTA and DTPA have been labelled with ^{99m}Tc and extensively studied for renal imaging[9,10]. ^{99m}Tc—DTPA is still widely used in nuclear medicine departments. Polyaminopolycarboxylic acids are also used as bifunctional chelating agents conjugated to monoclonal antibodies and other polypeptides such as Octreotide. The radionuclides for labelling can be ^{99m}Tc, ^{111}In, ^{67}Ga, ^{186}Re, ^{188}Re, etc. ^{111}In tagged DTPA—Phe^1—Octreotide is in clinical use for tumor imaging. In this paper, we wish to report the synthesis of BPHA and its labeling with ^{99m}Tc.

Experimental

Synthesis of BPHA · 4HCl

N,N′—Bis(2—aminoethyl)—1,3—propanediamine was prepared according to the literature method[11]. Sodium hydroxide (26.4g, 0.66mol) in 50 mL water was added slowly into a solution of chloroacetic acid (62.4g, 0.66mol) dissolved in 50 mL water. N,N′—Bis (2—aminoethyl)—1,3—propanediamine (16g, 0.10 mol) in 20 mL water was also added under stirring. The reaction proceeded at 70—80℃. Another sodium hydroxide(26.4g, 0.66 mol) in 50 mL water was droping down slowly to maintain the pH at 9—10. After the addtion of NaOH solution, the reaction mixture was kept at room temperature over night. The solution was neutralized to pH=2—3 with concentrated HCl and was concentrated to about 80 mL. During

the concentration solid sodium chloride crystallized and was removed by filtration. Concentrated HCl (50mL) was added and newly formed NaCl was removed again. The solution was decoloured by actived charcoal and concentrated to a thick fluid. Recrystallization with glacial acetic acid gave out 48. 33g brown crude product (59%). Anal. $C_{19}H_{32}O_{12}N_4 \cdot 4HCl$: C, 33. 85(34. 88); H 5. 70(5. 55); N, 7. 84 (8. 56). Recrystallization again with water—aceton (1 : 6. 5) gave white powder. Anal. $C_{19}H_{32}O_{12}N_4 \cdot 4HCl$: C, 34. 43 (34. 88); H, 5. 62 (5. 55); N, 8. 25(8. 56); Cl, 20. 85(21. 67). ^1H NMR (200 MHz, D_2O) δ: 2. 06(hump, 2H), 3. 10 —3. 36(m,12H), 3. 60(s,8H), 3. 94(s,4H). Positive FAB MS(NBA) m/z (relative abundance >20%): 509(100), 451(92), 393(60), 369(60), 350(38), 335 (27).

Labeling of BPHA with ^{99m}Tc

All common chemicals were reagent grade. The $^{99m}Mo/^{99m}Tc$ generator was from the China Institute of Atomic Energy. The paper used for paper chromatographic analysis was 100% cellulose (100g/m^2). The labeling efficiency was determined using two developing solvents separately. The R_f valuces of radioactive components are shown in Table 1. Radioactivity was determined in an NaI(Tl) well counter.

Table 1. The R_f valuces of radioactive components

Components	$^{99m}TcO_4^-$	$^{99m}TcO_2 \cdot xH_2O$	^{99m}Tc—BPHA
Acetone	0. 9—1. 0	0. 0—0. 3	0. 0—0. 3
Saline	0. 6—0. 8	0. 0—0. 3	0. 8—1. 0

A series of 2. 00 mL solutions with different pH values in vials were formulated to observe the effect of pH. In a 20 mL beaker were mixed 5. 00 mL BPHA · 4HCl (10. 00 mg/mL), 1. 00 mL $SnCl_2 \cdot 2H_2O$ in 2 N HCl (10. 00 mg/mL), 10. 00 mL

deionized water. The pH value was adjusted to 1—5 by dropping in 2 N NH_4OAc and then to 5—7 by substituting 2 N Na_2CO_3. Sample solution (2.00mL) was taken out and transfered to a vial at each selected pH. To every vial was added 0.2—1.0 mCi $^{99m}Mo/^{99m}Tc$ generator eluent. Paper chromatography was done on each solution five minutes later.

Formulated similarly were a series of solutions (2.00mL) at pH=4 containing 5.00 mg BPHA · 4HCl, 0.010—1.000 mg $SnCl_2 \cdot 2H_2O$, and 0.2—1.0 mCi $^{99m}TcO_4^-$. The influence of the amount of stannous ion could be obtained by determining the labeling efficiency with the above method.

The influence of the amount of BPHA · 4HCl was determined by formulating a series of 2.00 mL solutions containing 1.0—5.0 mg BPHA · 4HCl and 50 μg $SnCl_2 \cdot 2H_2O$ at pH=4.00. 0.2—1.0 mCi $^{99m}TcO_4^-$ was introduced in each vial. The labeling efficiency was determined five minutes later.

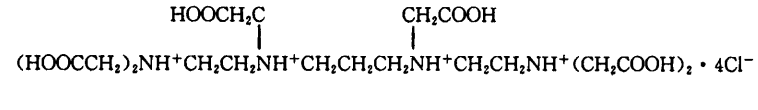
Preliminary biodistribution study of ^{99m}Tc—BPHA

A 2.00 mL solution at pH=4.00 containing 5.00 mg BPHA · 4HCl, 100 μg $SnCl_2$, and 0.3 mCi $^{99m}TcO_4^-$ was formulated. Kunming white mice (sixteen, 18—22g each) were divided into four groups. Each mouse was injected with 0.15 mL of the radioactive solution into the tail vein. The four groups were sacrificed separately by exsanguination at 2, 5, 15, and 30 minutes. The organs were weighed and the radioactivities determined.

Results and Discussion

The reaction between the polyamine and chloroacetic acid proceeds easily, but the separation process for the product is difficult. Although DTPA and TTHA have been obtained as free acids, we failed to obtain the free acid of BPHA because of the

high solvability of BPHA — NaCl complex. By protonation of the amine and carboxylate groups and co—ions effect of Cl^- in concentrated HCl, NaCl was successfully removed. BPHA was finally obtained as a tetrachloride salt. The structure of BPHA • 4HCl is shown as follows:



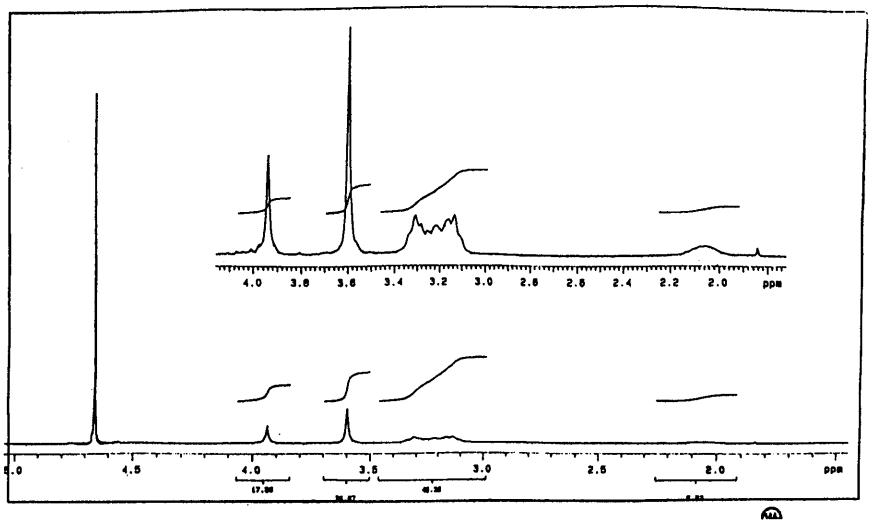


Fig 1. ^{1}H NMR spectrum of BPHA • 4HCl in D_2O

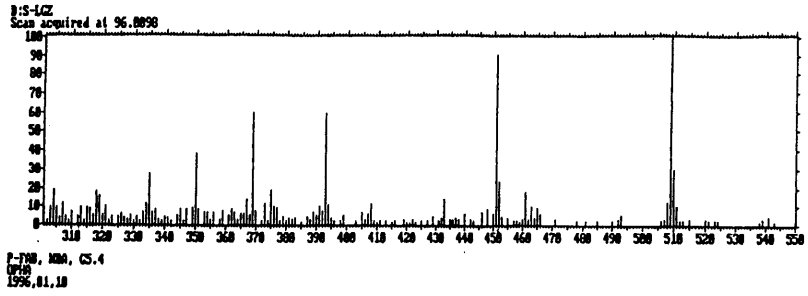


Fig 2. Positive FAB MS of BPHA • 4HCl

The ^{1}H NMR spectrum of BPHA • 4HCl is shown in Fig 1. In contrast to DTPA and TTHA[12,13], the chemical shift of the terminal acetic acid groups are

more upfield than that of the central acetic acid groups. This may parallel their difference in solution behavior. The positive FAB MS of BPHA • 4HCl is shown in Fig 2. The characteristic breaks of the chemical bonds are the breaks of N—CH_2COOH and N— H, which corresponds to the series of 509(100), 451(91), 393(60), 335 (29). Lin350 (38) is the result of the backbone break losing the fragment — $CH_2CH_2NH^+(CH_2COOH)_2$. Line 369(60) is in doubt.

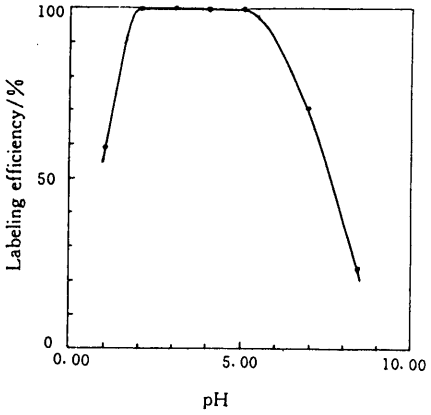


Fig 3. Influence of pH on the labeling efficiency of BPHA

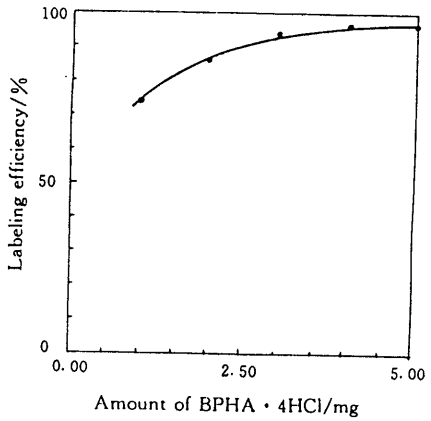


Fig 4. Influence of the amount of BPHA • 4HCl on the labeling efficiency

The radioactive complex is easily formed in the pH range 2—5 as shown in Fig 3. The decrease of labeling efficiency at pH<2 may result from the dissociation difficulty of protonated amine groups. The untagged ^{99m}Tc is mainly in the form of hydrolyzed reduced technetium, while at pH>7 the decrease of labeling efficiency results from the hydrolysis of the stannous ion. The solution becomes turbid when the pH≥7. The radioactivity not complexed by BPHA is mainly pertechnetate. The variation of the amount of $SnCl_2 \cdot 2H_2O$ under optimum pH condition (pH=4) indicates that a labeling efficiency of greater than 95% can be achieved in the examined range of 10 μg—1.000 mg $SnCl_2 \cdot 2H_2O$. However, the amount of BPHA • 4HCl significantly affects the labeling efficiency as shown in Fig 4. The labeling efficiency

drops off as the amount of BPHA • 4HCl is decreased. To ensure a labeling efficiency >95%, the conditions of pH=2−5 with the amount of BPHA • 4HCl>0.4mg are required in a 2.00 mL solution.

Table 2. Biodistribution of ^{99m}Tc−BPHA in mice (ID%/g)*

Organs	2 min	5 min	15 min	30 min
Blood	14.72±1.96	11.75±1.86	5.14±0.52	2.26±0.30
Brain	0.51±0.09	0.33±0.08	0.29±0.04	0.19±0.01
Heart	4.72±0.81	4.28±0.53	2.47±0.24	1.06±0.23
Liver	16.31±1.66	32.35±8.29	26.59±4.17	8.78±4.29
Kidneys	18.47±2.65	38.53±9.23	46.81±8.20	14.35±3.84
Spleen	6.48±1.58	13.58±5.53	9.57±2.20	3.62±1.42
Lungs	21.79±5.16	40.53±11.90	10.78±4.02	5.11±2.39

* Mean±s.d. of four animals.

Preliminary biodistribution results of ^{99m}Tc−BPHA in mice are shown in Table 2. Similar to ^{99m}Tc−DTPA, ^{99m}Tc−BPHA is taken up in kidneys and is excreted rapidly. But accumulation in the liver is unexpected. The reasons for this remain unclear and need to be elucidated by further research work.

Conclusion

A new polyaminopolycarboxylic acid, BPHA, was prepared as a tetrachloride salt. It was easily labelled with ^{99m}Tc in the pH range 2−5 with $SnCl_2 \cdot 2H_2O$ as the reductant. The radioactive complex is stable. A preliminary biodistribution study shows that it accumulates both in the kidneys and the liver.

References

1. Liu Guozheng, Liu Boli. —J Nucl Radiochem. 19(3):28 (in Chinese) (1997)
2. Hwang L L—Y, Ronca N, Solomon N A, et al. —Int. J. Appl. Radiat. Isot. 36(6): 475 (1985)
3. Anderegg G, Muller E, Zollinger K, et al. —Helv. Chim. Acta, 66:1593 (1983)
4. Burgi H B, Anderegg G, Blauenstein P. —Inorg. Chem., 20: 3829(1981)
5. Deutsch E — Coord. Chem. Rev. 44:191 (1982)
6. Frost A E. — Nature, 178 : 323 (1956)
7. Geigy J R. — Fr M843 (Oct 30, 1961)
8. Yashunskii V G, Samoilova O I. — Zh Vses. Khim. Obshchestva, im D. I. Mendelleeva, 8:360(1963)
9. Eckelman W C, Meinken G, Richards P. — J. Nucl. Med., 13(8): 577 (1972)
10. Agha N, Persson R B R. —Nuclearmedizen, 16:30 (1977)
11. Liu Guozheng, Shao Jianming. —Chemical Reagents, 18(1):50 (In Chinese) (1996)
12. Sandoval A A, Sandoval M W, Lin E, et al. —J. Magn. Resonance, 3:258 (1970)
13. Lin E, Sandoval A A, Cheng K L, et al. —J. Magn. Resonance, 4: 301 (1971)

