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SYNTHESES AND RADIOCHEMICAL STUDIES OF ^{99m}Tc-COMPLEXES OF POLYDENTATE TRIS-AMINEOXIME LIGANDS

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SUMMARY

Two new polydentate amineoxime ligands, Tris[1',1'-dimethyl-2'-oximino propyl)-2-aminoethyl]amine (I) and Tris[(1'-methyl-2'-oximinopropyl)-2-aminoethyl]amine (II) possessing trisubstituted nitrogen atoms which would possibly provide an inherent capping as well as ensure stereochemical rigidity, were synthesised. The radiochemical studies of these ligands with ^{99m}Tc were carried out and complexation yields were optimised by varying the reaction conditions. The ^{99m}Tc complexes were characterised by TLC, paper electrophoresis and solvent extraction studies. Radiochemical purity of these complexes were improved considerably on extraction of the complexes in chloroform, evaporation and reconstitution in saline containing stannous ions. The uptake of the ^{99m}Tc complexes with the two ligands prepared under optimised conditions were tested in biodistribution studies using Swiss mice.

Key words : polydentate ligands, tris-amine-oximes, ^{99m}Tc complexes, biodistribution

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INTRODUCTION

The BATOs (1,2) (boronic acid adducts of technetium dioximes) are a series of neutral lipophilic technetium complexes formed by template synthesis from $^{99m}TcO_4^-$, a vicinal dioxime, a boronic acid and halide ion. Of the large number of such complexes prepared and investigated, two BATOs have found application as myocardial and cerebral perfusion imaging studies (3,4). Bearing in mind the structural features in the BATO complex CardioTec® (5), it was interesting to envisage novel ligand systems comprising of an array of amine oxime donors that could possibly mimic the BATO complexes. Unlike as in the case of the BATOs, wherein three dioxime ligands are bound to the Tc(III) centre in a trigonal configuration via six nitrogens, our efforts were directed towards the designing of novel ligands incorporating a set of three amines and three oxime donors. An additional feature which is incorporated in the BATOs is the mono-capping of the molecule by a boronic acid derivative and this feature is attributable to the size of technetium and the coordination geometry associated with the heptacoordination of the metal (6). In the present case, incorporating a tri-substituted nitrogen atom in the starting material could draw the structural analogy between the envisaged ligand and that of the BATOs.

The synthetic strategy involves the condensation of the synthon viz. tris-(2-amino)ethylamine with moieties bearing the oxime functionality. The tri-substituted nitrogen atom not only provides the inherent capping of the resultant ligand as is characteristic of BATO complexes but also possibly ensures stereochemical rigidity of the complex which would result on complexation with ^{99m}Tc. While in the case of the synthesis of ligand I, the moiety bearing the oxime group is 2-chloro-2-methyl-3-oximinobutane (7), that in the case of ligand II, it is 2,3-butanedione monoxime. The ligands, which would thereby result from the condensation reaction, would be structurally similar but differing in the number of alkyl groups which will possibly influence the lipophilicity of the resultant complexes with ^{99m}Tc.

The radiochemical studies of the ligands with ^{99m}Tc were carried out and complexation yields were optimised by varying the reaction conditions such as pH, ligand concentration and stannous tartrate concentration. The ^{99m}Tc complexes were characterised by standard quality control techniques (8) viz. TLC, PC, paper electrophoresis and solvent extraction studies.

The ^{99m}Tc complexes of the two ligands prepared under optimised conditions were used for biodistribution studies in Swiss mice and their modes of clearances were studied.

EXPERIMENTAL

Tris-(2-aminoethyl)amine, 2,3-butanedione monoxime, isoamyl nitrite, 2-methyl-2-butene and sodium borohydride were purchased from Aldrich Chemical Company, USA. Anhydrous methanol and ethanol were prepared according to standard procedures. Stannous tartrate and $SnCl_2.2H_2O$ were obtained from Sigma Chemical Company USA. $^{99m}TcO_4^-$ was eluted from a ^{99}Mo-^{99m}Tc column generator developed in our laboratory. Chloroform, used for solvent extraction studies was purified as per standard procedure and distilled. All radiochemical studies were performed using deoxygenated saline. Flexible silica gel plates IB-F(7.5 x 2.5 cm) were from J.T. Baker Chemical Company, N.J. Whatman 1 chromatography paper (30 x 2.5 cm) was used for paper electrophoresis.

^{1}H-NMR spectra were recorded using a Varian VXR 300S spectrometer, using $CDCl_3$ as the solvent and tetramethyl silane (TMS) as the internal reference. FT-IR spectra were recorded using KBr pellets on Nicolet 5 DXB spectrometer and mass spectra were recorded on Shimadzu QP 1000 spectrometer operating at 70 eV using direct insertion probe.

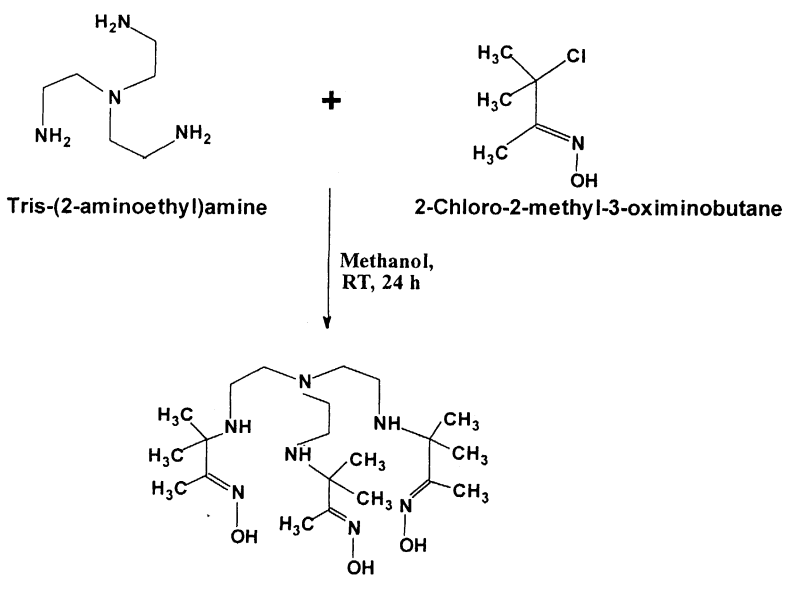
Syntheses

Ligand I : ***Tris[1',1'-dimethyl-2'-oximino propyl)-2-aminoethyl]amine***

The synthesis of ligand I was achieved via a condensation reaction of 2-chloro-2-methyl-3-oximino butane freshly prepared prior to use. To a

stirred ice-cold solution of isoamyl nitrite (40.5 mL, 0.3 mol) and 2-methyl-2-butene (33.8 mL, 0.3 mol) was added concentrated HCl (75 mL) dropwise. The solution turned bluish-green with precipitation. The reaction mixture was kept at 0°C overnight to complete the precipitation. The precipitate was filtered under cold condition, washed with cold aqueous methanol, dried and stored at 0°C.

To a portion of 2-chloro-2-methyl-3-oximinobutane (3.3g, 0.02 mol) suspended in anhydrous methanol (70 mL), was added tris-2-(aminoethyl)amine (1.4 mL, 0.006 mol) under stirring at 0°C. The reaction mixture was kept stirring at 0°C for 2 h. Initial addition of the tris-amine yielded a white precipitate which disappeared on further addition. The reaction mixture was kept stirring at room temperature overnight. Concentration of the reaction mixture under rotary evaporation resulted in a thick viscous product. Distilled water (50 mL) was added to the reaction mixture and pH was adjusted with 2M NaOH to ~9.5. Extraction with CH_2Cl_2 (5x15 mL), drying the pooled organic extracts over anhydrous Na_2SO_4 and solvent removal yielded 2.0 g (73 %) of the crude product. Purification by repeated column chromatography on silica gel using methanol-ammonia (98:2) as the eluant gave the pure product as a single



Ligand I

spot on TLC (4% NH_4OH in MeOH). The pure product was obtained as a while solid (0.63g) from the pooled fractions. IR (KBr, ν cm^{-1}) : 3276 (br, -OH, -NH), 1663 (oximine), 1382,1364 (gem methyl). ^{1}H-NMR ($CDCl_3$, δ ppm): 1.23(s, 18H, gem methyl), 1.83(s, 9H, $C\underline{H}_3$-C=NOH), 2.45-2.51 (m, 6H, >$NCH_2C\underline{H}_2NH$-), 2.56-2.62 (m, 6H, >$NC\underline{H}_2CH_2NH$-), 2.99 (t, J=6.2Hz, 3H, -NH-). MS (m/z, rel.int.): 344 [2.5, M^+-(3xNH_2OH)]. 313(2), 285(5), 255(9.2), 222(11.7), 215(79.8), 196(25.2), 182(15.1), 170(10), 156(74.7), 99(100).

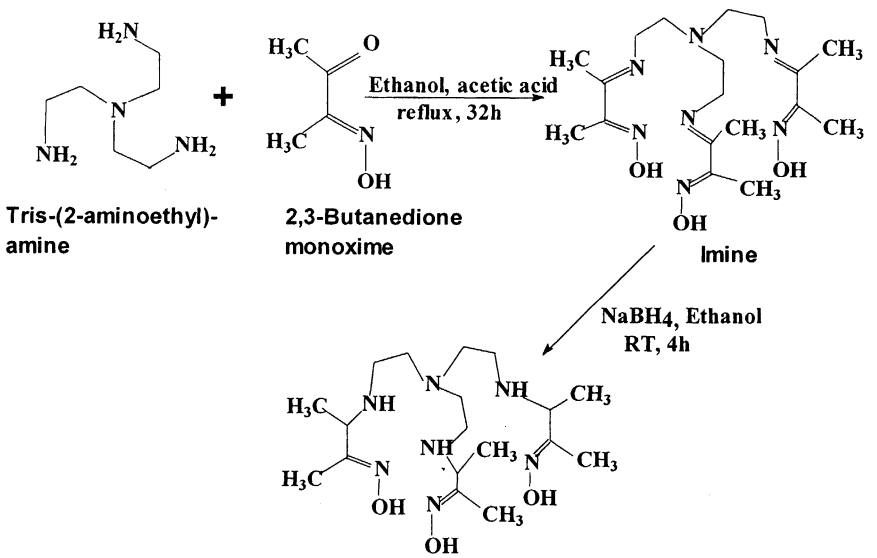
Ligand II: ***Tris[(1'-methyl-2'-oximinopropyl)-2-aminoethyl]amine***

A mixture of 2,3-butanedione monoxime (7.5g, 0.07mol) and tris-2-(aminoethyl)amine (3.6g, 0.024mol) was refluxed in dry ethanol (75 mL) with 0.1mL of acetic acid, for a period of 32h. The progress of the reaction was monitored using TLC (1% NH_4OH in MeOH). The reaction mixture was extracted initially with ether (3x15mL) and then (4x15mL) after saturation of the aqueous layer with brine. The pooled organic extracts were dried over anhydrous Na_2SO_4 and then concentrated under vacuum to yield the imine as a dark red viscous product (6.8g, 70%).

In the next step, to a stirred solution of the imine (6.8g, 0.02mol) in anhydrous ethanol (70 mL) was added $NaBH_4$ (1.35g, 0.03mol) in small instalments. The reaction mixture was kept stirring at room temperature for 4h while the progress was monitored on TLC using 8% NH_4OH in MeOH. The reaction mixture was worked up by addition of water (50 mL) followed by extraction in $CHCl_3$ (5x20 mL) after saturation with brine. The pooled organic extracts were dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to yield the crude product (2.8g, 40%) as a viscous oil. Column chromatographic purification of the crude product on silica gel using 1% NH_4OH in MeOH as the eluant yielded the pure product as a yellow liquid. IR (neat, ν cm^{-1}) : 3289 (br, -OH, -NH), 1650 (oximine).^{1}H-NMR ($CDCl_3$, δ ppm): 1.22(d, J=6.77Hz, 9H, -NHCHC$\underline{H}_3$), 1.83(s, 9H, $C\underline{H}_3$-C=NOH), 2.42-2.72 (m, 12H, >$NCH_2C\underline{H}_2NH$- and >$NC\underline{H}_2CH_2NH$-), 2.86 (t, J=6.2Hz, 3H, -NH-), 3.23-3.33(m, 3H, -NHC$\underline{H}$CH_3). MS (m/z, rel.int.): 401 [0.46, M^+]. 294(0.24), 200(0.5), 166(3.99), 115(4.5), 97(14.6), 56(100)..

Complexation studies with ^{99m}Tc

Complexation studies of the ligands with ^{99m}Tc were carried out using stannous tartrate as the reducing agent. Optimisation of the reaction conditions such as pH, ligand concentration and reaction time were made in order to obtain the maximum complexation yields.



Tris-(2-aminoethyl)-amine

2,3-Butanedione monoxime

Imine

Ligand II

The protocol involved mixing 0.1mL (0.1mM-5mM) of the ligand in methanol, 0.5 mL of acetate buffer (0.5 M, pH 4), 1.0 mL of $^{99m}TcO_4^-$ (10-20 MBq/mL obtained from a column generator) and nitrogen purged saline to a final volume of 4.8 mL. A saturated solution of stannous tartrate (0.2 mL) was added to the above solution and allowed to react at room temperature for 5-10 min. The resultant complexes were characterised by standard quality control techniques such as TLC, paper electrophoresis and solvent extraction. Reaction conditions were optimised by varying the ligand concentration, pH (buffers- acetate pH 3, phosphate pH 5 and 7 and bicarbonate pH 9 and 12) and reaction time.

Biodistribution

Male Swiss mice (20-25 g) were injected in tail vein with 0.1-0.3 mL of the reaction mixture containing ~370-550 kBq of activity. The mice were sacrificed at 10 min, 30 min, 2 h and 4 h post-injection. Tissues and organs were excised, rinsed, weighed and counted in a NaI(Tl) well counter. The percent injected dose (ID) in each tissue was calculated from the above data. The % ID in the blood was calculated by measurement of the activity in 0.5 to 1 g of blood withdrawn by cardiac puncture immediately after sacrifice and assuming the whole blood volume as 6.5 % of the body weight. Uptakes in respective organs are expressed as %ID/organ. All the animal experiments were carried out in compliance with the relevant national laws relating to the conduct of animal experimentation.

RESULTS AND DISCUSSION

The peaks observed in the ^{1}H-NMR spectra and the ion peaks observed in the EI mass spectra were consistent with the expected structure of the ligands.

The ligands I and II showed maximum complexation yields of 60-65% at a ligand concentration of $5x10^{-5}$ M/L at pH 4 with 0.2 mL of a saturated solution of stannous tartrate and 10-20 MBq/mL of $^{99m}TcO_4^-$. Complexation was not observed above pH 5 for both the ligands.

Radiochemical purity of the complexes were improved from 60-65% to 90-96 % (n = 10) by back extraction, wherein, the chloroform extract of the complex was evaporated with a slow stream of nitrogen gas and reconstituted with saline containing stannous tartrate. The complexes were characterised by thin layer chromatography in saline and acetonitrile, paper electrophoresis in 0.025M PO_4^{-3} buffer, pH 7.5 and solvent extraction in chloroform. In TLC/saline, the major activity remained at the point of spotting with minimum activity at the solvent front eliminating the possibility of TcO_4^- (R_f 0.9-1.0) impurity being present in the reaction mixture. TcO_2 impurity (R_f =0) was inferred to be minimal as observed in

TLC/acetonitrile wherein the major activity was observed at R_f = 0.9-1. Electrophoresis pattern showed that the complexes are neutral.

Table 1 : Radiochemical purity of the reconstituted complexes on storage as estimated by solvent extraction studies

	% Radiochemical Purity	
Time	**Ligand I**	**Ligand II**
5 min	93	96
30 min	81	80
2 h	80	72
4 h	79	70

The extractability of the complexes in chloroform indicated that the complexes are lipophilic. The distribution ratios as obtained by repeated partitioning of the complexes (n = 3) between chloroform and saline with ligand I was 89 ± 11 and that of ligand II was 61 ± 3 thereby reflecting the higher lipophilicity of ligand I in comparison to that of ligand II. The radiochemical purities of the complexes prepared after back extraction were estimated by solvent extraction in chloroform and the stabilities of the reconstituted complexes as a function of radiochemical purity, with time, have been described in Table 1.

The results of the biodistribution studies of the complexes with ligand I and II are tabulated in Tables 2 and 3, respectively.

The brain uptakes of the ^{99m}Tc complexes of I and II in Swiss mice were 0.43(0.09)% and 0.41(0.12)%, respectively at 1 min post injection. Since the blood activities of the ^{99m}Tc complexes at 1 min post injection were high, the uptakes in the heart which otherwise could have been considered to be significant (being 1.03(0.04)% and 1.8(0.0)% respectively) did not evoke much interest towards use of these complexes as myocardial agents. Minimum activity in the stomach for both the ^{99m}Tc-complexes indicated the absence of formation of $^{99m}TcO_4^-$ *invivo*. The clearances of the

Table 2 : Biodistribution pattern of the ^{99m}Tc complex with Ligand I

Organ	% injected dose/organ, mean (std. deviation), n=3			
	1 min	10 min	30 min	120 min
Blood	45.2(9.4)	17.7(5.1)	13.5(2.3)	10.3(3.4)
Liver	19.2(2.2)	13.4(2.1)	11.6(0.6)	10.7(2.5)
Intestine	7.1(0.7)	12.4(2.4)	17.7(3.4)	27.8(1.3)
Kidney	4.6(0.3)	6.1(0.7)	6.7(0.4)	4.9(0.9)
Stomach	0.8(0.23)	0.7(0.6)	0.9(0.4)	0.5(0.08)
Heart	1.8(0.0)	0.53(0.2)	0.45(0.08)	0.4(0.1)
Brain	0.41(0.1)	0.23(0.1)	0.1(0.04)	0.2(0.08)
Lungs	3.8(0.7)	1.2(0.4)	1.8(1.3)	0.9(0.1)
Femur	0.38(0.02)	0.23(0.07)	0.2(0.03)	0.14(0.3)
Muscle	22.7(7.0)	10.6(0.7)	10.2(3.0)	6.3(1.0)
Spleen	0.45(0.06)	0.2(0.05)	0.21(0.04)	0.07(0.01)

CONCLUSION

Two novel polydentate amine-oxime ligands were synthesised and characterized. The radiochemical studies of these ligands with ^{99m}Tc under optimized conditions yielded neutral, lipophilic complexes in high yields. The reconstituted complexes, used for biodistribution studies in Swiss mice showed *invivo* clearance via the hepatobiliary route. Efforts are underway to prepare and characterise the ^{99}Tc and inactive Re labeled complex in order to obtain a better insight of the formulation of the ^{99m}Tc complexes.

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Table 3: Biodistribution pattern of the ^{99m}Tc complex with Ligand II

Organ	% injected dose/organ, mean (std. deviation), n=3			
	1 min	10 min	30 min	120 min
Blood	33.3(1.5)	8.6(1.7)	7.2(1.1)	7.8(4.3)
Liver	21.1(2.1)	14.5(2.2)	16.9(0.3)	12.9(8.7)
Intestine	8.4(0.5)	9.0(0.3)	14.2(0.65)	23.8(1.2)
Kidney	7.5(1.6)	5.5(0.3)	5.9(0.3)	6.3(1.9)
Stomach	0.71(0.1)	0.35(0.12)	0.4(0.06)	0.5(0.2)
Heart	1.03(0.04)	0.4(0.25)	0.2(0.04)	0.34(0.1)
Brain	0.43(0.1)	0.1(0.01)	0.09(0.03)	0.2(0.15)
Lungs	4.4(0.5)	1.2(0.1)	1.7(0.2)	1.3(0.6)
Femur	0.5(0.07)	0.24(0.04)	0.3(0.1)	0.3(0.2)
Muscle	21.7(0.5)	14.4(4.8)	20.2(6.7)	12.1(3.0)
Spleen	0.4(0.02)	0.4(0.2)	0.24(0.03)	0.24(0.1)

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