ISSN 1070-3632, Russian Journal of General Chemistry, 2015, Vol. 85, No. 7, pp. 1763–1767. © Pleiades Publishing, Ltd., 2015.

Synthesis, Characterization, and Antibacterial Activities of Cr(III), Co(III), Ni(II), and Mn(III) Complexes of Heptadentate Schiff Base Ligand Derived from Tris(2-aminoethyl)amine[1]

S. J. Matin and R. R. Khojasteh

Department of Chemistry, North Tehran Branch, Islamic Azad University, Tehran, Iran
e-mail: s.jalalimatin@yahoo.com

Received April 5, 2015

Abstract—This is the first study of the antibacterial activity of heptadentate Schiff base ligand (trensal) and its Cr(III), Co(III), Ni(II), and Mn(III) complexes that have been synthesized from the heptadentate Schiff base ligand derived from salicylaldehyde and tris(2-aminoethyl)amine. Heptadentate Schiff base ligand and its complexes were characterized by elemental analysis, ^{1}H and ^{13}C NMR, IR, and UV–Vis spectroscopy. The products were tested *in vitro* against the bacterial species, *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa,* and *Bacillus cereus* by disc diffusion and micro-broth dilution methods. All compounds demonstrated potent antibacterial activity higher than the Schiff base ligand. Staphylococcus aureus was the most susceptible bacterial species to Co(III) complex while *Escherichia coli* and *Pseudomonas aeruginosa* required a relatively higher minimum inhibition concentrations of Cr(III) and Ni(II) complexes. Co(III) complex was the most potent inhibitor against *Pseudomonas aeruginosa*. Mn(III) complex was active against *Pseudomonas aeruginosa*.

Keywords: heptadentate Schiff base complexes, antibacterial effects, tris(2-aminoethyl)amine, salicylaldehyde

DOI: 10.1134/S1070363215070312

INTRODUCTION

Schiff bases condensation reactions are used in synthesis of a wide range of macrocyclic compounds. The Schiff bases can be coordinated to a metal center by N atom of imine or a substituent in the aromatic ring (e.g., O, S,) and form a stable complex [1, 2]. Among synthesized heptadentate Schiff base complexes are Cryptate [3], Cu(II) N_7 Schiff base [4] and two In(III) complexes [5]. Heptadentate (N_4O_3) Schiff base potential ligands derived from condensation reactions of tris(2-aminoethyl)amine (tren) with various ring substituted salicylaldehydes have been prepared and their coordination chemistry with lanthanide ions has been extensively studied [6, 7]. In the complexes each tripodal heptadentate Schiff base ligand (trensal) effectively encapsulated the lanthanide ion and enforces a seven-coordination geometry.

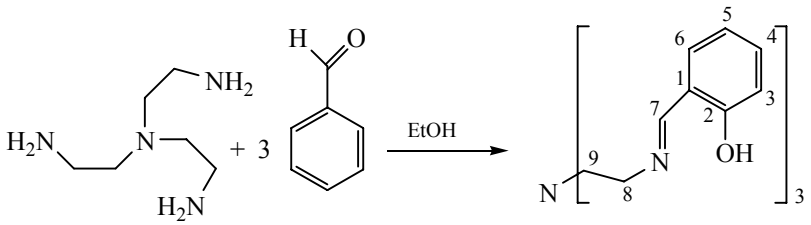
Complexes of fully condensed potentially heptadentate (N_4O_3) Schiff base ligands derived from condensation of tris(3-aminopropyl) amine and salicylaldehyde were synthesized [8]. Potential applications of macrocyclic Schiff base as supramolecular devices are contrast agents in magnetic resonance imaging (MRI), radiopharmaceuticals and as models for biological systems [9–12]. Over recent years the increasing microbial resistances to antibiotics initiated considerable interest in synthesis of new compounds with potential effects against pathogenic bacteria and fungi [13, 14]. Biological activities of Schiff bases are attributed to formation of stable chelating complexes with the transition metals present in the cells. The present study is devoted to the synthesis and characterization of some new complexes of trensal potentially heptadentate Schiff base formed by condensation of salicylaldehyde and tris(2-aminoethyl)amine. Structures of the products have been elucidated on the basis of analytical and spectral data. The complexes have been screened for biological activity against some kinds of bacteria (G^+ and G^-).

RESULTS AND DISCUSSION

Physical measurements. The macrocyclic ligand was synthesized by condensation of salicylaldehyde

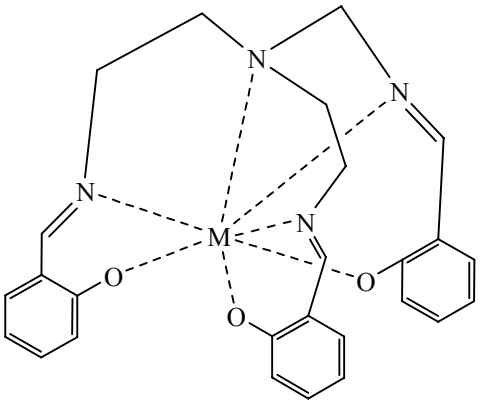
[1] The text was submitted by the authors in English.

Scheme 1. Synthesis of the ligand.



with tris(2-aminoethyl)amine in ethanol (Scheme 1). The reaction proceeded smoothly to give the corresponding Schiff base ligand in high yield. The ligand was soluble in common organic solvents. The Schiff base and Co(III) and Mn(III) complexes were prepared according to the slightly modified methods presented in publications [15–17]. All complexes (Scheme 2) were stable at room temperature and insoluble in water but soluble in DMSO. Purity of the Schiff base ligand and its complexes was monitored by TLC. IR spectrum of the free ligand demonstrated the characteristic C=N band at 1637 cm^{-1} that was shifted to lower frequencies in the spectra of the metal complexes (1612–1597 cm^{-1}), indicating the bonding of nitrogen of the azomethine group to the metal ion via electrons donation from nitrogen to the vacant d-orbital of the metal ion. The ligand spectrum band at 3450 cm^{-1} (stretching and deformation of the phenolic OH) was not recorded in the spectra of complexes indicating deprotonation of the hydroxyl group and coordination via oxygen. Participation of oxygen in formation of the C–O–M bond was confirmed by the shift of the ligand spectrum Ph–OH band (1247 cm^{-1}) in the spectra of complexes. Therefore, IR spectra provided strong evidence for complexation of the potentially heptadentate ligand.

Scheme 2. The proposed chemical structure of the complexes.



M = Cr(III), Co(III), Ni(II), or Mn(III).

Electronic spectra of the Schiff base ligand and its complexes were recorded in DMSO. The absorption bonds at 257, 313, and 420 nm were attributed to the transitions $\pi \rightarrow \pi^*$ (aromatic ring), $n \rightarrow \pi^*$ (C–O), and $n \rightarrow \pi^*$ (C=N) respectively. In the metals complexes the bands were shifted to some extent due to involvement of the imine nitrogen in coordination with the metals ions.

^{1}H NMR spectrum of the ligand demonstrated the signal at 13.65 ppm of phenolic O<u>H</u> that was not recorded in the spectra of complexes which indicated the coordination bond formation between metal ion and oxygen due to its deprotonation. Formation of the complexes was also confirmed by the characteristic peak of the imine hydrogen. The phenolic <u>C</u>–OH signal observed at 161.33 ppm in ^{13}C NMR spectrum of the ligand had the different shift in the spectra of complexes.

Biological activity. *Disc diffusion method.* The Schiff base and its complexes demonstrated antibacterial activity against some bacterial strains (Table 1). Co(III) complex demonstrated the highest antibacterial activity against the *Bacillus cereus* and Ni(II) complex was the least active against the *Pseudomonas aeruginosa*. A comparative study of the ligand and its complexes (inhibition zones values, mm) indicated that complexes exhibited higher anti-microbial activity than the free ligand. The data revealed that the effect of products on gram-positive bacteria was higher than gram-negative bacteria. Gram-negative organisms have an outer membrane which provides an efficient control over the uptake of toxic substances, hence, generally are more resistant to antibacterial agents [18].

Micro-broth dilution minimum inhibition concentration. The MICs (minimum inhibition concentrations) of the compounds were also determined because the comparison of sizes of the inhibition zones was not trustworthy. The antibacterial activity data (Table 2) indicated that all complexes

Table 1. Inhibition zones (mm) of complexes and the ligand against bacterial strains

Compound	Diameter of inhibition zone *D*, mm			
	Pseudomonas aeruginosa	*Escherichia coli*	*Bacillus cereus*	*Staphylococcus aureus*
Ligand (L)	7	8	12	9
Cr–L	10	12	14	13
Ni–L	8	10	20	10
Co–L	17	17	25	20
Mn–L	11	21	14	11
Chloramphenicol	8	15	–	14
Imipeneme	–	25	20	20
Cephradine	–	–	–	8
Ampicilin	–	11	–	14
DMSO	0	0	0	0

Table 2. Minimum inhibition concentration

Compound	Concentration, mg/mL			
	Pseudomonas aeruginosa	*Escherichia coli*	*Bacillus cereus*	*Staphylococcus aureus*
Ligand (L)	1.25	0.62	0.31	0.62
Cr–L	1.25	0.62	0.31	0.31
Ni–L	1.25	0.62	0.15	0.31
Co–L	0.15	0.15	0.15	0.07
Mn–L	0.62	0.15	0.31	0.31

exhibited a slightly different antimicrobial activity compared to that of the control drugs. The lowest MIC was detected for Co(III) complex against *Staphylococcus aureus* and the highest was measured for Cr(III) and Ni(II) complexes against *Pseudomonas aeruginosa*. Overall, the complexes exhibited higher antibacterial activity than the free ligand.

It can be explained on the basis of chelation theory [19], according to which the surrounding cell wall is composed of the lipid membrane that allows only lipid-soluble substances to enter the cell. Due to interaction of the ligand orbitals and the metal ions and the presence of donor groups in a complex polarity of the metal ions is decreased. Such factors increase the interaction between a complex and lipid cells promoting the penetration of complexes into a cell. LPSs are the main components of cells walls of gram-negative bacteria. The Schiff base can penetrate the bacterial cell membrane by coordination of the metal ion via oxygen or nitrogen donor atom to LPS which damages the outer cell membrane and inhibits growth of the bacteria. So, the synthesized Schiff base ligand has moderate inhibitory effects on the growth of tested microorganisms. To the best of our knowledge this is the first study of the antibacterial activity of heptadentate Schiff base ligand complexes.

EXPERIMENTAL

Materials and spectral measurements. All starting materials were purchased from Aldrich and Merck and used without further purification. IR spectra were recorded in KBr discs on a Perkin-Elmer 78 spectrophotometer. UV–Vis spectra were recorded in DMSO on a Shimadzu UV-1601 spectrophotometer. CHN analysis was carried out on a Heraeus instrument (Vario EL). Melting point was determined on a Buchi SMP-20 capillary melting point apparatus. ^{1}H and ^{13}C NMR spectra were measured in DMSO-d_6 solution by a Brucker AMX 250 MHz spectrometer with TMS as an internal standard. Metals were determined using a Shimadzu (A.A) 680 G atomic absorption spectrophotometer. Isolated bacteria were purchased from Persian Type Culture Collection(PTCC).

Synthesis of tris[2-(salicylaldeneimino)ethyl]-amine. Tris[2-(salicylaldeneimino)ethyl]amine (trensal)

was prepared according to the method presented in the publication [15]. To a solution of salicylaldehyde (0.06 mol, 7.3 g) in absolute ethanol (25 mL) was added tris(2-aminoethyl)amine (0.02 mol, 2.92 g) in absolute ethanol (25 mL). The solution was refluxed for 1 h. The bright yellow microcrystals were filtered off and dried in vacuum (Scheme 1). Yield 89.12%, mp 191–193ºC. IR spectrum, ν, cm^{-1}: 3450.32 (O–H), 2939.49 (C–H), 1637.24 (C=N), 1068.53 (N–C), 1247.06 (C–O), 1573.31 (C=C). UV–Vis spectrum, λ_{max}, nm: 254, 338, and 414. ^{1}H NMR spectrum, δ, ppm: 2.84 t (6H, H^9), 3.59 t (6H, H^8), 8.23 s (3H, H^7), 6.87 s (3H, H^6), 6.74 t (3H, H^5), 7.30 t (3H, H^4), 6.84 d (3H, H^3), 13.65 s (3H, OH). ^{13}C NMR spectrum, δ_C, ppm: 55.33 (C^9), 57.39 (C^8), 166.62 (C^7), 116.96, 118.75, 118.84, 131.97, 132.60, 161.33 (C^1 to C^6). Found, %: C 70.68; H 6.63; N 12.21.$C_{27}H_{30}N_4O_3$. Calculated, %: C 70.74; H 6.55; N 12.22.

Synthesis of the complexes *(general procedure).* To a solution of the ligand (3 mmol) in 20 mL of ethanol was added hydrated metal nitrate (3 mmol) and sodium acetate (3 mmol) in 20 mL of ethanol. The reaction mixture was stirred at room temperature overnight followed by refluxing for 5 h. The reaction solution was left in refrigerator overnight. The precipitate was filtered off and washed with cold absolute ethanol and dried in vacuum. Co(III) and Mn(III) complexes were synthesized according to the method presented in publications [16, 17] with slight modifications.

Sodium[tris(2-(salicylaldeneimino)ethyl)amine]–Ni(II) (I). Light yellow solid, yield 70%, mp 339–341ºC. IR spectrum, ν, cm^{-1}: 3060 $(C–H)_{arom}$, 2949 $(C–H)_{aliph}$, 1597 (C=N), 1524, 1460 (C=C), 1075 (C–O), 1291 (C–N), 537 (M–N), 418 (M–O). UV–Vis spectrum, λ_{max}, nm: 257, 317, and 375. ^{1}H NMR spectrum, δ, ppm: 2.64 t (6H, H^9), 3.00 t (6H, H^8), 8.18 t (3H, H^7), 7.47 br (3H, H^6), 7.09 br (3H, H^5), 7.61 br (3H, H^4), 6.91 br (3H, H^3). ^{13}C NMR spectrum, δ_C, ppm: 50.61 (C^9), 56.84 (C^8), 166.27 (C^7), 116.61, 118.33, 118.44, 131.63, 132.27, 161.01 (C^1 to C^6). Found, %: C 63.05; H 6.3; N 10.6. Ni $C_{27}H_{27}N_4O_3$. Calculated, %: C 63.07; H 5.25; N 10.9.

[Tris(2-(salicylaldeneimino)ethyl)amine]–Co(III) (II). Dark green solid, yield 70.29%, mp 297–299ºC. IR spectrum, ν, cm^{-1}: 3055 $(C–H)_{arom}$, 2946 $(C–H)_{aliph}$, 1634 (C=N), 1545, 1470 (C=C), 1051 (C–O), 1288 (C–N), 540 (M–N), 420 (M–O). UV–Vis spectrum, λ_{max}, nm: 265, 310, and 370. ^{1}H NMR spectrum, δ, ppm: 1.04 t (6H, H^9), 3.45 t (6H, H^8), 8.10 (3H, H^7), 6.50–7.48 m (aromatic ring). ^{13}C NMR spectrum, δ_C, ppm: 54.95 (C^9), 59.89 (C^8), 166.11 (C^7), 116.22, 118.61, 121.15, 132.67, 133.97,159.64 (C^1 to C^6). Found, %: C 62.02; H 5.4; N 10.83. Co $C_{27}H_{27}N_4O_3$. Calculated, %: C 63.04; H 5.25; N 10.89.

[Tris(2-(salicylaldeneimino)ethyl)amine]–Cr(III) (III). Yellow green solid, yield 72.61%, mp 341–343ºC. IR spectrum, ν, cm^{-1}: 3070 $(C–H)_{arom}$, 2950 $(C–H)_{aliph}$, 1596 (C=N), 1524, 1460 (C=C), 1075 (C–O), 1290 (C–N), 537 (M–N), 410 (M–O). UV–Vis spectrum, λ_{max}, nm: 257, 307, and 404. ^{1}H NMR spectrum, δ, ppm: 1.03 t (6H, H^9), 2.64 t (6H, H^8), 8.20 (3H, H^7), 7.55 br (3H, H^6), 7.01 br (3H, H^5), 7.68 br (3H, H^4), 6.94 br (3H, H^3). ^{13}C NMR spectrum, δ_C, ppm: 50.86 (C^9), 51.36 (C^8), 170.72 (C^7), 112.56, 118.76, 122.52, 133.53, 135.48, 169.70 (C^1 to C^6). Found, %: C 63.62; H 5.61; N 10.91. Cr $C_{27}H_{27}N_4O_3$. Calculated, %: C 63.90; H 5.32; N 11.04.

[Tris(2-(salicylaldeneimino)ethyl)amine]–Mn(III) (IV). Olive drab solid, yield 92.72%, mp 299–301ºC. IR spectrum, ν, cm^{-1}: 3052 $(C–H)_{arom}$, 2946 $(C–H)_{aliph}$, 1612 (C=N), 1546, 1469 (C=C), 1022 (C–O), 1295 (C–N), 461 (M–N), 405 (M–O). UV–Vis spectrum, λ_{max}, nm: 256, 317, and 410. ^{1}H and ^{13}C NMR data were not obtained because the complex was paramagnetic. Found, %: C 63.51; H 5.4; N 10.97. Mn $C_{27}H_{27}N_4O_3$. Calculated, %: C 63.53; H 5.29; N 10.98.

Bacterial species. In the study we used two gram-negative bacteria and two gram-positive bacteria. The standard strains of the following microorganisms were used as test organisms: *Staphylococcus aureus* (PTCC 1112), *Escherichia coli* (PTCC 1330), *Pseudomonas aeruginosa* (PTCC 1074), and *Bacillus cereus* (PTCC 1015).

Preparation of cultures for antimicrobial susceptibilities. Two types of media were used (Müller-nutrient broth and Müller–Hinton agar). Bacterial isolates were grown in each medium for 24 h at 37ºC. The inoculum density of each bacterial isolate was standardized with 0.5 McFarland turbidity standard. The suspension had a final inoculum of 5×10^8 cfu/mL (colony-forming unit).

Antimicrobial activity. The *in vitro* antibacterial screening of the Schiff base ligand and its complexes dissolved in DMSO has been carried out against four bacteria. We determined antimicrobial activity of compounds by disc diffusion and micro-broth dilution methods.

Disc diffusion method (Table 1). Müller–Hinton agar medium (38 g of Müller–Hinton agar and 3 g of agar in 1000 mL of distilled water) was prepared and sterilized. A small amount of each bacteria was placed on the side of the plate. A sterile loop was used to spread the bacteria in one direction from the initial site of inoculation. The plates were incubated for 24 h at 37ºC for bacteria growth. A bit of each bacteria was dissolved in the sterile distilled water tube similar to 0.5 McFarland turbidity standard (the suspension had a final inoculum of 1.5×10^8 cfu/mL). The plates, with respect to the number of samples, were inoculated with bacteria by two sterile cotton swabs. The substance (0.02 g) was dissolved in 1 mL of DMSO. The sterile blank discs (Whitman no. 1 filter paper, 5 mm diameter) were dipped in 0.1 mL of each sample and placed on plates at specified intervals by sterile forceps. After an incubation period of 24 h at 37ºC the diameter of each zone of inhibition was measured with a ruler (mm). Cephradine (30 μg/disc), Ampicillin (10 μg/disc), Imipeneme (10 μg/disc) and Chloramphenicol (30 μg/disc) were used as standards for the antibacterial activity measurements. The separate study carried out with pure DMSO demonstrated no activity of the solvent against any bacterial strains. The experiment was repeated three times using the same procedure conditions.

Micro-broth dilution minimum inhibition concentration. The MICs were determined with micro-broth dilution. 13 Sterile tubes containing 1 mL of Mueller nutrient broth medium were prepared and sterilized for each substance. 0.02 g of each substance was dissolved in 1 mL of DMSO and the first tube was filled in with 1 mL of the sample. This was followed by pipetting of 1 mL of the solution from the first tube and its addition to the second tube. Then, 1 mL of the solution from the second tube was added to the third tube. This process was repeated for all 12 tubes. As a result the concentration of each tube was half of the previous one. The extra solution (1 mL) from the last tube was discarded. Thus, the thirteenth tube acted as a control. Upon incubation for 24 h at 37ºC a bit of a bacteria was dissolved in the sterile distilled water tube similar to 0.5 McFarland turbidity standard. Specified amounts of bacteria suspension was added to all tubes except the tube number 12 (control sample) to make the concentration in all tubes to be 5×10^5 cfu/mL. The tubes were placed in an incubator at 37ºC for 24 h, upon which MIC values of the substances were determined (Table 2).

REFERENCES

1. Mohamed, G.G., Qmar, M.M., and Hindy, A.M.M., *Spectrochim. Acta, Part A*, 2005, vol. 62, nos. 4–5, p. 1140. DOI:10.1016/j.saa.2005.03.031.
2. Liu, Y., Zhao, B.-T., Zhang, H.-Y., Wada, T., and Inoue, Y.J., *Chem. Soc. Perkin Trans*., 2001, vol. 7, p. 1219. DOI: 10.1039/B008444L.
3. Mathieu, F. and Weiss, R., *J. Chem. Soc. Chem. Commun*., 1973, vol. 21, p. 816a. DOI: 10.1039/C3973000816A.
4. Drew, M.G.B., Nelson, J., and Nelson, S.M., *J. Chem. Soc. Dalton. Trans*., 1981, vol. 8, p. 1685. DOI: 10.1039/DT9810001685.
5. Riesen, A., Kaden, T.A., Ritter, W., and Macke, H.R., *J. Chem. Soc. Chem. Commun*., 1989, vol. 8, p. 460. DOI: 10.1039/C39890000460.
6. Bernhardt, P.V., Flanagan, B.M., and Riley, M.J., *Aust. J. Chem*., 2000, vol. 53, p. 229. DOI: 10.1071/CH99175.
7. Kanesato, M., Nagahara, K., lgarashi, K., Sato, K., Kikkawa, Y., and Goto, M., *Inorg. Chim. Acta.,* 2011, vol. 367, p. 225. DOI: 10.1016/j.ica.2010.12.001.
8. Keypour, H., Salehzadeh, S., and Parish, R.V., *Molecules*, 2002, vol. 7, p. 140. DOI: 10.3390/70200140.
9. Caravan, P., Ellison, J.J., McMurry, T.J., and Lauffer, R.B., *Chem. Rev*., 1999, vol. 99, no. 9, p. 2293. DOI: 10.1021/cr980440x.
10. Zhang, S., Wu, K., and Sherry, A.D. *Angew. Chem. Int. Ed*., 1999, vol. 38, no. 21, p. 3192. DOI: 10.1002/(SICI)1521-3773(19991102)38:21<3192::AID-ANIE3192>3.0.CO;2-#.
11. Shaker, A., Lobna, N., Mohamed, A., and Mohamed, I., *Russ. J. Gen. Chem*., 2013, vol. 83, no. 12, p. 2460. DOI: 10.1134/S1070363213120438 .
12. Sedaghat, S. and Ghammamy, S., *Russ. J. Gen. Chem.,* 2013, vol. 83, no. 4, p. 722. DOI:10.1134/s1070363213040191.
13. Kulakov, I.V., *Russ. J. Gen. Chem*., 2009, vol. 79, no. 7, p. 1585. DOI: 10.1134/S1070363209070275.
14. Pilyugin, V.S., Sapozhnikov, Yu.E., Davydov, A.M., Chikisheva, G.E., Vorob'eva, T.P., Klimakova, E.V., Kiseleva, G.V., Kuznetsova, S.L., Davletov, R.D., Sapozhnikova, N.A., and Yumadilov, R.Kh., *Russ. J. Gen. Chem*., 2006, vol. 76, no. 10, p. 1653. DOI: 10.1134/S1070363206100264.
15. Chakraborty, J., Samanta, B., Pilet, G., and Mitra, S., *Inorg. Chem. Commun*., 2007, vol. 10, no. 1, p. 40. DOI: 10.1016/j.inoche.2006.09.002.
16. Hai-yan, Z., Jing, L., Yong-heng, X., Zhi-feng, P., Feng-ying, B., Mao-fa, G., and Shu-yun, N., *Chem. Res. Chinese Univ.,* 2010, vol. 26, no. 5, p 687.
17. Elerman, Y. and Kabak, M., *Acta. Cryst., Sect. C*., 1994, vol. 50, no. 11, p. 1694. DOI: 10.1107/S0108270194005871.
18. Vaara, M., *Micro. Rev*. 1992, vol. 56, no.3, p. 395.
19. Raman, N., Sobha, S., and Mitu, L., *J. Saudi. Chem. Soc*., 2013, vol. 17, no. 2, p. 151. DOI: 10.1016/j.jscs.2011.03.003.