Est. 1984

ORIENTAL JOURNAL OF CHEMISTRY
An International Open Free Access, Peer Reviewed Research Journal

www.orientjchem.org

ISSN: 0970-020 X
CODEN: OJCHEG
2015, Vol. 31, No. (3):
Pg. 1751-1758

Synthesis, Characterization, and Antibacterial Activity of Cu(II), Ni(II), and Zn(II)Complexes of 14-Membered MacrocyclicTetraazaLigand

SITI FAIRUS M.YUSOFF[1]*, AMEERAAQEELASALLEHHUDDIN[1], LATIFAH M. YUSOFF[1], BOHARI M.YAMIN[1], NAZLINAIBRAHIM[2] and ONG WEI LENG[2]

[1]School of Chemical Sciences and Food Technology, [2]School of Biosciences and Biotechnology, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 Bangi, Selangor, Malaysia.
*Corresponding author E-mail: sitifairus@ukm.edu.my

http://dx.doi.org/10.13005/ojc/310356

(Received: March 06, 2015; Accepted: April 22, 2015)

ABSTRACT

The macrocyclic 5,5,7,12,12,14-hexamethyl-1,4,8,11-tetraazacyclotetradeca-7,14-dienium bromide salt (L*Br*) was synthesisedfor use in complexationreactions with various metal salts of copper, nickel, and zinc. Twelve complexes were synthesised; five of them were successfully obtained in a crystal form that was suitable for X-ray crystallographic analysis. All the complexes were analysedby microelemental analysis, Fourier Transform infrared spectroscopy, ultraviolet-visible spectroscopy,and magnetic susceptibility. The metals formed coordination bonds to the four aza nitrogen atoms,during the process of which the azomethine nitrogen atoms were deprotonated. The cationic charge in each complexwasbalanced with a bromide anion from the macrocyclic ligand. Deprotonation of the azomethine nitrogen atoms led to the formation of an acid/base complex with the metal salts.Preliminary studies for biological activity, based on the minimum inhibitory concentration (MIC) method, indicated that the macrocyclic complexes showed greater antibacterial activity than the bromide macrocyclicligand, which showedlower MIC valueswith *S. aureus*(6.3 mg/mL) and*E. aerogenes* and *B. Subtilis* (3.1 mg/mL).

Key words: Tetraaza, Macrocyclic, Copper, Nickel, Zinc Complexes, Antibacterial.

INTRODUCTION

Macrocyclic complexes and their cyclic ligands playa major role in expanding the field of bioinorganic chemistry.[1]Not surprisingly, the number of studies on complexes of macrocyclictetraaza ligands involving the synthesis ofnew ring systems,andthe investigation of its function and properties andthe discoveryof potential applications in the fields of industry, medicine and others,has grown.[2]However, there are only a fewcomplexes that involvea neutral macrocyclic ligand [$Me_6N_4H_2$]with either copper or zinc as the central metal. Examples which have been reported

are;[Cu($Me_6N_4H_2$)]ClO_4,[3] [CuI($Me_6N_4H_2$)]I H_2O,[4] [CuBr ($Me_6N_4H_2$)] Br.2H_2O,[5] [ZnI($Me_6N_4H_2$)] I_3,[6][Zn ($Me_6N_4H_2$)]ClO_4,[7]and $C_{18}H_{35}Br_3N_4O_2Zn_2$,[8]We have reported the preparation of our first copper complex,*catena*-poly[[5,5,7,12,12,14-hexamethyl-1,4,8,11-tetraazacyclotetradeca-1,7-iene) copper (II)-μ-chlorido [dichlorocuprate(II)-μ-chlorido].[9]

Previously, most of the macrocyclic complexes were obtained by a template reaction. For example, the complex [($Me_6N_4H_4$)·Ni] was derived from therecrystallisation reaction of tris(ethylenediamine) in acetone.[10]In the present work, we focus primarily on the synthesis of the macrocyclic complex via two separate reactions. Firstly, the synthesis of the protonated macrocyclictetraaza was first performed,followed by the complexation reaction. Since the macrocyclic ligand is protonated (Scheme 1), we may vary its counteranion.[11]However, the complexation reaction with the protonated form of the tetraazamacrocycleis still not widely reported. Therefore, we have conducted further studies on the complexation reaction using various transition metals.

The complexes of macrocyclictetraaza ligands with various transition metalsplay an importantroleby acting as an active part of metalloenzymes[12]since the structure of the macrocyclic complexes are similar to the ligand sites of metalloenzymes. Also, both macrocyclic compounds and their complexes have important applications in coordination chemistry and organic catalysis.[13,14]The complexes can also be used as biomimetic biological model compounds because of their similarities to natural proteins such as hemerythrin and enzymes.[15]Furthermore, these types of macrocyclic compound arealso used as catalysts for biological reactions such as epoxidation or hydrolysis of DNA anddioxygen binding,whilst also acting as active components ofantiviral drugs.[16] They are also usedas medical imaging agents.[17]Additionally, transition metal macrocyclic complexes have been well tested in tumour treatment methods,radio immunotherapy, and cancer diagnosis.[18]

In this paper, we report onthe complexation of various copper, nickel, and zinc metal salts with the protonated 14-membered ring tetraaza ligand, 5,5,7,12,14,14-hexamethyl-1,4,8,11-tetraazacyclotetradeca-7,14-dienium bromide. The antibacterial properties of both the complexes and the free ligand arealsoreported.

EXPERIMENTAL

All chemicals and solvents were reagent grade and used as received. C, H and N analyses were determined using Fison model EA 110. The complexes were dried in air. IR spectra (as KBR pellets) were recorded on a Perkin Elmer 400 FT-IR/FT-NIR. Electronic spectra in the 200-800 nm region were recorded on a 1800 Shimadzu spectrophotometer. Magnetic susceptibilities were measure at 25 °C by the Gouy method. The calculation were carried out using $\mu_{eff} = 2.84 \sqrt{\chi M_{coor}}.T$. The TLC of all the ligand and complexes confirmed their purity.

Synthesis of 14-membered tetraazamacrocyclic ligand

The macrocyclic ligand $C_{16}H_{38}N_4O_2Br_2$ (4)was synthesised by mixingammonium salt(0.9794g, 0.01 mol) (1)and ethylenediamine (0.601g, 0.01 mol) (2)in acetone(3) at 80°Cunder constant stirring for 2 h. The solution was then filtered and left overnight at room temperature for crystal growth. Yield: 65%; m.p. 113.4–125.3°C. Anal. Calcd for $C_{16}H_{38}N_4O_2Br_2$(FW478.32): C,40.2%; H, 7.9%; N, 11.1%. Found: C, 38.9%; H, 7.5%; N, 11.6%. NMR: ^{1}H, δ_H1.49(6H, s, CH_3), δ_H 2.06 (3H, s, CH_3), δ_H2.80 (2H, s, CH_2),δ_H3.42 (2H, t, CH_2),δ_H3.69 (2H, t, CH_2), and δ_H4.91 (2H, t, NH_2^+).

Synthesis of ($Me_6N_4H_2$)Br_2.2H_2O complexes with copper, nickel, and zinc

The complex was synthesised by stirring together a 1:1 mixture of $CuCl_2$(*L*(Cu^1) and ligand in methanol. The solution was thenfiltered and left at room temperature for evaporation.The same steps were repeated by changing the ligand to $CuBr_2$(*L*(Cu^2)), $Cu(NO_3)_2$(*L*(Cu^3)), $CuSO_4$(*L*(Cu^4)), $Ni(OAc)_2$(*L*(Ni^1)), $NiCl_2$(*L*(Ni^2)), $NiSO_4$(*L*(Ni^3)), $Ni(NO_3)_2$(*L*(Ni^4)), $ZnCl_2$(*L*(Zn^1)), $Zn(OAc)_2$(*L*(Zn^2)), $ZnSO_4$(*L*(Zn^3)), and $Zn(NO_3)_2$(*L*(Zn^4)). The analytical data are given in Table 1.

Antibacterial test

The metal salts, free ligands and the complexes formed were tested for antibacterial

activity.Three specieseach of Gram-positive and Gram-negative bacteria were chosen for the test. The complex properties werescreenedby using the minimum inhibitory concentration (MIC).

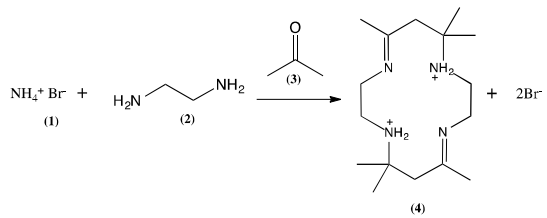
RESULTS AND DISCUSSION

Characterization of the Ligand

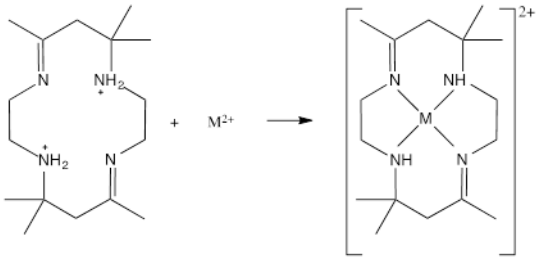
The reaction to synthesise the ligand (**4**) (Scheme 1)afforded a white crystalline solid in 65% yield. The melting point was 113.4-125.3 °C. Analysis of the microelemental data was in agreement with the expected formula of $C_{16}H_{38}N_4O_2Br_2$ (experimental: C = 38.9%, H = 7.5%, and N = 11.6%; theoretical: C = 40.2%, H = 7.9%, and N = 11.1%). The FTIR spectrum showed stretching bands at 1667 and 1228 cm^{-1},indicating the presence of C=N and C-N bonds, respectively. The sharp band at 3468 cm^{-1} and the broad band at 3012 cm^{-1} are due to the primary amino and water O-H stretches, respectively. The ^1H NMR spectrum confirmed the presence of methyl protons and protonated amine at 1.49, 2.06, and 4.89 ppm, respectively. The signals at 2.80, 3.42, and 3.69 ppm are due to the methylene protons. ^{13}C NMR further confirmed the presence of C=N and C-N bonds with the chemical shifts of 175.87 and 58.35 ppm, respectively. TheUV-VIS spectrum showeda single absorption peakat 238nm, which may be assignedasthe $\pi \rightarrow \pi^*$ electronic transition ofthe C=N chromophore.

Characterization of Metal Complexes

A suspension of ligand (*L*) in methanol at reflux reacts with metal(II) salts to generate the corresponding complexes (Scheme 2) in moderate yield (49-88%). Analytical and physical data (Table



Scheme 1: Synthesis of protonated tetraaza ligand



Scheme 2: Synthesis of tetraazamacrocyclic copper complex, M = Cu(II), Ni(II) and Zn(II)

Table 1 Elemental analysis and melting point data

Complexes	Colour	Yield (%)	Experimental (Theoretical)			Melting point
			C	H	N	(ºC)
L(Cu^1)	Purple	65	31.0(31.0)	5.0(6.8)	11.4(9.0)	208.0–208.3
L(Cu^2)	Purple	88	28.2 (28.7)	5.1 (5.7)	7.8 (8.4)	225.4–225.8
L(Cu^3)	Purple	69	26.5 (26.7)	4.7 (5.0)	7.6 (7.8)	217.6–218.0
L(Cu^4)	Purple	70	28.2 (28.9)	7.0 (7.2)	9.5 (8.5)	199.6 – 200.4
L(Ni^1)	Yellow	73	36.0 (36.1)	6.3 (6.4)	14.9 (10.5)	328.6 – 332.1
L(Ni^2)	Orange	65	13.0 (26.9)	4.7 (4.5)	24.2 (7.8)	319.7 – 325.0
L(Ni^3)	Green	55	9.7 (25.9)	5.2 (4.2)	14.4 (7.8)	328.0 – 328.4
L(Ni^4)	Green	49	6.1 (25.9)	5.0 (4.4)	7.2 (7.6)	327.9 – 331.0
L(Zn^1)	Yellow	70	30.3 (31.3)	6.1 (6.2)	15.2 (9.1)	198.0 – 200.0
L(Zn^2)	White	60	35.9 (36.7)	6.5 (6.5)	7.3 (10.7)	260.0 – 265.0
L(Zn^3)	White	64	29.9 (30.8)	5.5 (5.9)	7.2 (10.7)	240.0 – 243.0
L(Zn^4)	White	68	30.3 (30.7)	9.1 (9.3)	4.2 (11.1)	182.0 – 199.8

1) and spectral data (Table 2 and 3) showed that some of the complexes are compatible with the proposed structures. The complexes are coloured, stable in air partially soluble in common solvents.

The elemental analysis data for all complexes except*L*(Ni^2), *L*(Ni^3), and *L*(Ni^4)shows a high level of agreement with its theoretical value. However, the percentages of N atom for the complexes are quite different than the theoretical values. This abnormality was due to faulty detector for N atom at the time of measurement. Vibrations for N-H (amine), C-H (methyl), C-N (imine), and C=N (azomethine) in IR spectra are the most important vibrations that each complexmust contain. The presence of these indicates that the formed complexes may contain the original ring structure of the tetraazamacrocycle. Table 2 shows the FTIR frequenciesof the complexes. The absence of C=N (azomethine) vibrations in *L*(Ni^2), *L*(Ni^3), and *L*(Ni^4), indicates that their complexes may have different structures and that the tetraazamacrocycle may have undergone a ring opening.Table 3 shows the UV-VIS and magnetic moments (μ_{eff}) data for all the complexes formed. *L*(Zn^1), *L*(Zn^2), *L*(Zn^3), *L*(Zn^4) and *L*(Ni^2)do not show μ_{eff}effects or d-d transitions because all the zinc complexes are diamagnetic; the d-orbital (d^{10}) of zincis already full and no other

Table 2: FTIRstretching frequencies for the complexes

Complexes	Stretching frequencies (cm^{-1})			
	N-H(amine)	C-H(methyl)	C-N(imine)	C=N (azomethine)
L(Cu^1)	3158.4	2973.9	1169.7	1662.9
L(Cu^2)	3148.8	2969.8	1164.4	1670.8
L(Cu^3)	3158.7	2973.6	1169.3	1662.6
L(Cu^4)	3424.4	2968.7	1171.4	1632.7
L(Ni^1)	3424.7	2868.5	1165.7	1653.9
L(Ni^2)	3175.2	2919.1	1033.5	-
L(Ni^3)	3168.9	2921.9	1030.7	-
L(Ni^4)	3170.5	2919.1	1034.7	-
L(Zn^1)	3200.0	2964.0	1033.0	1656.0
L(Zn^2)	3211.2	2961.9	1107.9	1660.3
L(Zn^3)	3202.9	2968.3	1664.1	1159.6
L(Zn^4)	3118.1	2967.6	1084.1	1604.2

Table 3: UV-VIS and μ_{eff}data for the synthesised complexes

Complexes	μ_{eff}	Transition $\pi \rightarrow \pi^*$	Transitiond-d
L(Cu^1)	2.45	246.0	519.0
L(Cu^2)	2.31	237.5	520.5
L(Cu^3)	-	242.5	516.5
L(Cu^4)	2.01	234.0	519.0
L(Ni^1)	0.87	241.5	436.5
L(Ni^2)	-	276.0	-
L(Ni^3)	-	271.5	421.0
L(Ni^4)	1.39	277.0	-
L(Zn^1)	-	247.0	-
L(Zn^2)	-	247.0	-
L(Zn^3)	-	220.0	-
L(Zn^4)	-	250.0	-

possible transitions may occur. The absence of any *d-d* transition in *L*(Ni[4]), indicates that the product formed does not contain any nickel.

X-ray crystallography

Although all the productswere formed as solid crystals, not all weresuitable for X-ray crystallographic analysis. The crystals of the complexes formed wereanalysedusing Bruker APEX-II CCD fitted with Mo Ká radiation. Only copper tetraazamacrocylic complex (*L*(Cu[1])) andnickel (II) tetraazamacrocylic complex (*L*(Ni[1]))were suitable for X-ray analysis. The X-ray structure of each product complemented the results

Table 4: Crystal data and refinement parameters for *L*(Cu[1]) and *L*(Ni[1]) complexes

	***L*(Cu[1])**	***L*(Ni[1])**
Empirical formula	$C_{16}H_{36}Br_2CuN_4O_2$	$C_{16}H_{34}Br_2N_4NiO_2$
Formula weight	539.85	533.00
Crystal system	Monoclinic	Monoclinic
Space group	P 21/c	P 21/c
*A (*Å)	8.0453(8)	8.0402(5)
*B (*Å)	15.6095(19)	15.6070(10)
*c (*Å)	8.9229(10)	8.9200(6)
Á (°)	90	90
Â (°)	99.844(4)	99.800(2)
ã (°)	90	90
V(Å)	1104.1(2)	1102.98(12)
Z	2	2
F(000)	550	544
Density (mg/m³)	1.624	1.605
Absorption coefficient, μ (mm^{-1})	4.625	4.518
Crystal size (mm)	0.300 x 0.280 x 0.250	0.500 x 0.160 x 0.150
¸range for data collection (°)	2.882 to 25.496°	3.415 to 25.999
Goodness-of-fit on F2	1.106	1.089
Independent reflections/R_{int}	2055/0.1379	2155/0.0767
Data / restraints / parameters	2055 / 3 / 130	2155 / 0 / 126
R_1 [I> 2´(I)]	0.0485	0.0409
wR_2 [I> 2´(I)]	0.1017	0.0913

Table 5: Selected bond length and angle for *L*(Cu[1])and *L*(Ni[1])

Bond Lengths (Å)	***L*(Cu[1])**	**Bond Lengths (Å)**	***L*(Ni[1])**
Cu1-N1	1.904(5)	Ni1-N1	1.936(3)
M1-N2	1.929(5)	Ni1-N2	1.919(3)
N1-C5	1.280(8)	N1-C6	1.499(5)
N1-C1	1.477(8)	N1-C2	1.482(5)
N2-C2	1.465(9)	N2-C4	1.276(5)
N2-C3	1.496(8)	N2-C3	1.480(5)
Bond Angle (°)		Bond Angle (°)	
N1-Cu1-N2	86.02(2)	N2-Ni-N1	86.02(13)
C1-N1-Cu1	111.5(4)	C3-N2-Ni1	111.7(2)
C2-N2-Cu1	108.1(4)	C2-N1-Ni1	107.0(2)

obtained by elemental analysis, FTIR and UV spectroscopy.[21]The structural data for the complex of *L*(Cu[1])(Figure 1) and *L*(Ni[1])(Figure 2) showed good agreement withits elemental analysis data. Moreover, its FTIR proved that all theimportant vibrations were present. The UV-VIS analysis also showed the presence of the azomethine chromophore and *d-d* transition, strongly indicating that the product maintained its original tetraaza ligand structure.However, the structure of several related macrocyclic complexes have been reported for many times in the past years using different salt of metal (II) ions. The experimental details for the molecular structure and structure refinement data for the complexes of *L*(Cu[1])and *L*(Ni[1])are shown in Table 4. Selected bond distance and bond angles are listed in Table 5.

Antibacterial studies of the metal salts, synthesised tetraaza ligand and complexes

The free ligand and its complexes were tested as antibacterial agentswith the help of MIC tests. The compounds were dissolved in distilled water with an initial concentration of 25mg/mL.ThreeGram-positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis*,and *Enterococcus faecalis*) and threeGram-negative bacteria (*Escherichia coli*, *Enterobacteraerogenes*, and *Pseudomonas aeruginosa*) were selected for the test. The bacterial single colony was maintained in nutrient agar and wassubcultured in Mueller-Hinton broth for the tests. The solution was diluted eight times, and positive and negative controlswere duplicated three times for comparison using a 96 well plate. The results aretabulated in Table 4.

Table 6: Results of MIC tests using the tetraaza ligand, complexes and metal salts

Bacteria	Complex/Metal Salts						
	T_{Br}	*L*(Cu[1])	*L*(Ni[1])	*L*(Zn[1])	$CuCl_2$	$Ni(OAc)_2$	$ZnCl_2$
Staphylococcus aureus	-	6.3	25.0	3.1	1.6	-	0.9
Bacillus subtilis	25.0	3.1	25.0	25.0	0.8	1.6	3.1
Enterococcus faecalis	-	6.3	25.0	12.5	-	1.6	1.6
Enterobacteraerogenes	-	3.1	25.0	25.0	0.8	6.3	3.1
Pseudomonas aeruginosa	25.0	6.3	25.0	25.0	-	3.1	6.3
Escherichia coli	12.5	6.3	25.0	12.5	0.8	1.6	1.6

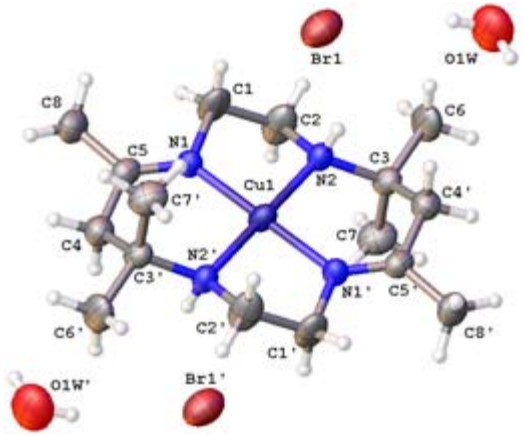


Fig. 1: Molecular structure of *L*(Cu[1]) drawn at 50% probability ellipsoid

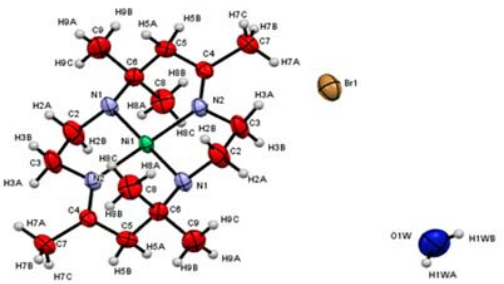


Fig. 2: Molecular structure of *L*(Ni[1]) drawn at 50% probability ellipsoid

Each macrocyclic complex exhibited higher antibacterial activity than its free ligand. The complexes and its free ligand (*L*) have similar structures, but the complex shows one difference in that it containsa metal ion that is bonded directly to four nitrogen atoms in the ring. The antibacterial activity of the complexes can be explained with the help of chelating reactions. Chelates reduce the polarity of the metal ion because of the overlapping of the ligand orbital and the sharing of the positive charge between the metal and the electron-donating group. Therefore, the complexes aremuch more stable than the free ligands due to the delocalisation of ð electrons. This helps the complexesin penetrating the lipid membrane and blocking the metal binding site on the enzyme within the microorganism.[22]In addition, the copper complex of tetraazamacrocycle*L*(Cu^1) also blocks the cell respiration process, preventing the synthesis of protein and therefore inhibiting the growth of the organism.[23]The MIC value for the metal salts used for the complexation reactions of*L*(Cu^1), *L*(Ni^1), and *L*(Zn^1) showed better results than their complexes. This is because the size of the metal salt molecule is much smaller than that of the complex,making it easier for the salt to penetrate the membrane cells.

CONCLUSION

Complexation reactions of tetraazamacrocyclic ligand with copper, nickel, and zinc resulted in the formation of new complexes. The complexationreaction of *L*(Cu^1)and *L*(Ni^1) involved the deprotonation of the amino nitrogen atoms, but with the same bromide counteranion from the ligand. The complexation reaction of *L*(Ni^3) and *L*(Ni^4) showed that opening of the tetraazaring occurred. *L*(Cu^1), *L*(Ni^1)and *L*(Zn^1) showed antimicrobial activity to the selected bacteria.

ACKNOWLEDGEMENTS

A.A.S.H. would like to thank the Universiti Kebangsaan Malaysia (UKM) for the MSc Scholarship (Zamalah, UKM). S.F.M.Y would like to thank MOSTI and theUniversitiKebangsaan Malaysia for the research grants (DLP-2013-009 and DIP-2014-16).

REFERENCES

1. El-Tabl. A.S. *Bull. Korean Chem. Soc.***2004**, *25*, 1757-1761
2. Sujatha, S., Balasubramanian, S., Fun, H.K., and Chinnakali, K.*Polyhedron***2008**, *27*, 1925-1929
3. Lu, T.H., Lee, T.J., Liang, B.F., and Chung, C.S. *J. Inorg. Nucl. Chem.***1981**,*43*, 2333-2336
4. Podberezskaya, N.V., Pervuknina, N.V., and Myachina, L.I. *J. Struct. Chem.***1986**, *27,*268-272
5. Shi, F.F., and He, X.L. *ActaCrystallogr. Sect. E: Struct. Rep. Online,* **2011**, *67*, 607
6. Heinlein, T., and Tebbe, K.F. *Z. Kristallogr.***1985,***170*, 170-171
7. Yang, Y.M. *ActaCrystallogr. Sect. E: Struct. Rep. Online,* **2005**, E61, m1618-m1619
8. Li, H.F., Shi, F.F., and Rong, R. *ActaCrystallogr. Sect. E: Struct. Rep. Online,* **2010**, E66, 1338
9. Yamin, B.M., Ismail, W., and Daran, J.C. *ActaCrystallogr. Sect. E: Struct. Rep. Online,* **2012**, E68, 886-887
10. Curtis, N.F., and House, D.A. *Chemistry and Industry***1961**,*6*, 1708-1709
11. Mohamad Wafiuddin Haji Ismail. Sintesisgaram 5,5,7,12,12,14-heksametil-1,4,8,11-tetraazasiklotetradeka-7,14-dienium, Pengkompleksandengan Logam &A ktiviti Biologi. Master Thesis, Graduate Studies Centre, UKM. (2012)
12. Chaudhary, A., Dave, S., Swaroop, R., and Singh, R.V. *J. Indian Chem. Soc.* **2002**, *79,* 371-373
13. Ciampolini, M., Nardi, N., Vantacoli, B., andMicheloni, M. *Coord. Chem. Rev.* **1992**, *120*, 223-236
14. Li, Z., Ding, H., and Wu, S. *Mater. Res. Bull.***2013**, *48*: 1920-1926
15. Najlaa al-Radadi, S., Sawsan Al-Ashqar, M., and Mohsen Mostafa, M. *J. Incl. Phenom. Macro. Chem.***2011**, *69,* 157-165
16. Frausto da Silva, J.J.R., and Williams, R.J.P. The Biological Chemistry of the Elements, Clarendon Press, Oxford. (1991)

17. Lauffer, R.B. *Chem. Rev.***1987**, *87*, 901-927
18. Norman, T.J., Parker, D., Smith, F.C., and King, D.J. *Supramol. Chem.***1995**, *4,* 305-308
19. Rohovec, J., Vojtisek, P., and Lukes, I. *Collection of Czechoslovak Chemical Communications***1999**, V64, 73-88
20. Kennedy, A.R., Lutta. S.T., Morisson, C.A., Okoth, M.O., and Orang'o, D.M. *ActaCrystallogr. Sect. E: Struct. Rep. Online,* **2011**, E67,682-683
21. Hassan, N.H., Ali, N.M., Yamin, B.M., AbdKarim, N.H. and AbdGhani, N.A. *The Malaysian J. Anal. Sci.***2014**, *18*, 562-571
22. Prayong, P., Barusrux, S.,and Weerapreeyakul, N. *Fitoterapia***2008**. *79*, 598-601
23. Sharma, R., Prabhat, Singh,.R.,Pawar, S. andChauhan, A. *J. Am. Sci.***2010**, *6*, 559-564