

Synthesis, Characterization and Antibacterial Activity of Copper (II) and Nickel (II) Complexes with Hexaazamacrocyclic Ligand

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ABSTRACT

Copper(II) and nickel(II) complexes of 14-membered hexaazamacrocyclic ligand with C-methyl substituent have been synthesized by one-pot reaction and characterized by elemental analyses, FT-IR, electronic spectra, and LC-MS. Complex types are $[\text{Cu}(\text{C}_{18}\text{H}_{42}\text{N}_6)(\text{ClO}_4)_2]$ (**1**), $[\text{Ni}(\text{C}_{18}\text{H}_{42}\text{N}_6)](\text{ClO}_4)_2$ (**2**), and $[\text{Cu}(\text{C}_{22}\text{H}_{50}\text{N}_6)(\text{ClO}_4)_2]$ (**3**). The results of spectroscopic techniques indicated that both complexes **1** and **3** containing copper(II) are octahedral geometry, whereas the complex **2** is a square-planar geometry in which the metal center coordinates to the four nitrogen atoms of macrocyclic ligand in a square planar fashion. All complexes were also screened for their antibacterial activity against on two bacteria strains. These results show that copper(II) and nickel(II) complexes were tested for antibacterial activity against gram positive, *Staphylococcus aureus* which was more active than the against gram negative, bacteria *Escherichia coli*. The results showed a good antibacterial activity of all complexes which suggests their potential applications as antibacterial agents.

Keywords: Macrocyclic ligand; Antibacterial activity; Copper (II); Nickel (II) complexes

Introduction

The hexaazacyclotetradecane macrocyclic complexes are readily synthesized in a one-pot reaction containing metal(II) salt for example, either copper(II) or nickel(II), bidentate ligand (ethylenediamine or 1,2-diaminopropane), and formaldehyde that has been utilized for cyclization to link two amine moieties and a primary amine (R-NH₂), such as propylamine [1], benzylamine [2], (R)-(+)-1-(1-naphthyl)ethylamine [3], 4-aminobutyric acid [4], and ethanolamine [5], which are function capable of pendant arm. The hexaazamacrocyclic ligands and their

complexes have been synthesized by the one-pot reaction which involves one step of reactions and gives a high yield. To obtain high stability through (i) inclusion of macrocyclic ligands such as 14-membered, 16-membered, 18-membered macrocyclic ligands and so on, (ii) the number and type of the donor atoms present in the ligand and their relative positions within the macrocyclic skeleton of donor atoms and (iii) size of the chelate rings such as five-membered and six-membered ligands are formed on complexation as well as the metal cation dimension and preference for a certain

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stereochemistry [10]. The hexaazacyclotetradecane macrocyclic complexes are a rapidly developing area in view of their various applications such as magnetic properties [6,7], catalytic performance [8,9], anti-microbial activity [10], and sensing [11].

The problems of drug-resistant bacteria are likely to raise, thus the need to invent a new type of antimicrobial that is effective and safe. In the last several years, the attention in this field was oriented to inorganic species among the organic ones. However, many complexes have shown a good antimicrobial activity and only a few are used as metallo antibiotics such as antiseptics or antimicrobial. Good anti-bacterial activity has been obtained for complexes bearing a biocation and a multidentate ligand having a proved anti-bacterial activity. Among biocations, copper(II) or nickel(II) are preferred for having: (i) the low human toxicity associated with both the presence of the albumin in the plasma and the metalotionein in the cytosol, (ii) the easiness to change its oxidation state, and (iii) the known biological activity including the ability to inhibit enzymes [12].

In this work, we report the synthesis of 14-membered hexaazamacrocyclic complexes with pendant arms obtained by one-pot reaction of 1,2-diaminopropane and both butylamine and hexylamine linking to obtain 14-membered hexaazamacrocyclic ligands by formaldehyde in presence of copper(II) and nickel(II) salt. The complexes were characterized by spectroscopic techniques using elemental analyses, FT-IR, electronic spectra, and LC-MS. In addition, the anti-bacterial activity results for these complexes are also reported.

Materials and Methods

Materials and Instruments

Copper acetate monohydrate, nickel acetate tetrahydrate, 1,2-diaminopropane, formaldehyde, butylamine, hexylamine, and perchloric acid (70%) were all purchased from commercial sources and used as received. IR

spectra (4000-400 cm^{-1}) were recorded with FT-IR spectrophotometer as a KBr disk. The visible spectra were recorded with a Shimadzu Lambda-1600 UV-Vis spectrophotometer in DMSO. Elemental analysis of CHN was performed using a CE instruments Flash EA 1112 series, Thermo Quest analyzer. Mass spectra were recorded by electro-spray ionization (ESI) technique operating in the positive ion mode by directly injecting the solution into a mass spectrometer (Waters Micromass).

Safety note: Perchlorate salts of metal complexes are potentially explosive. Only a small amount of material should be prepared, and it should be handled with care.

Synthesis of 1 and 2

To a 99.9% absolute ethanol (10 mL) of $\text{Cu}(\text{OAc})_2 \cdot \text{H}_2\text{O}$ (1.9965 g, 10 mmol) were added 1,2-diaminopropane (1.72 mL, 20 mmol) in 99.9% absolute ethanol (10 mL). After the mixture was a clear purple solution, 37% formaldehyde (3.2 mL) was then added, stirring until the mixture was a dark blue solution and then butylamine (2.0 mL, 20 mmol) was added with continuous stirring. The mixture of reactants was refluxed for 48 h and the purple-red solution was cooled to room temperature and filtered to remove any insoluble solids. After the amount of concentrated perchloric acid was added dropwise, the mixture was filtered and stored at room temperature to form purple-red solids.

Complex 2, the synthetic procedure is similar to that complex 1 except $\text{Ni}(\text{OAc})_2 \cdot 4\text{H}_2\text{O}$ (2.4886 g, 10 mmol) was used instead of $\text{Cu}(\text{OAc})_2 \cdot \text{H}_2\text{O}$ to obtain yellow solids.

Synthesis of 3

For complex 3, the synthetic procedure was similar to the one mentioned above, where $\text{Cu}(\text{OAc})_2 \cdot \text{H}_2\text{O}$ (1.9965 g, 10 mmol) was used, but hexylamine (2.6 mL, 20 mmol) was used instead of butylamine.

In vitro antibacterial activity

The *in vitro* antibacterial screening effects of the complexes were tested against two bacterial strains, namely *Staphylococcus*

aureus (*S. aureus* ATCC 29213) and *Escherichia coli* (*E. coli* ATCC 25922). All bacteria were inoculated into Nutrient Broth (NB) and incubated for 24 h. The disk diffusion method from the protocol, which was described by the National Committee for Clinical Laboratory Standard (NCCLS, 2004), was used for preliminary screening. The antibacterial diffusion tests were carried out as described by Berghe and Vlietink [13] using a cell suspension of about 1.5×10^6 CFU/mL obtained from the McFarland Turbidity Standard N° 0.5. Wells (6 mm diameter) were punched in the agar plates. Complexes dissolved in DMSO (25, 50 and 100 mg/mL) were added to these wells. A blank containing neat DMSO solvent (negative control) was also run parallel in the same plate. The plates were incubated at 37°C for 24 h and antibacterial was determined by measuring inhibition zone diameter. Tetracycline was used as the reference drug in positive control. All the experiments were repeated three times and the average values are presented.

Table 1. Elemental analysis for the complexes.

Complexes	Formula weight	Found (<i>Calc.</i>) %		
		C	H	N
[Cu(C ₁₈ H ₄₂ N ₆)(ClO ₄) ₂] (1)	604.97	35.73 (35.75)	6.99 (7.02)	13.89 (13.90)
[Ni(C ₁₈ H ₄₂ N ₆)](ClO ₄) ₂ (2)	600.16	36.02 (36.05)	7.05 (7.07)	14.01 (14.04)
[Cu(C ₂₂ H ₅₀ N ₆)(ClO ₄) ₂] (3)	661.12	39.97 (40.00)	7.62 (7.64)	12.71 (12.72)

FT-IR spectra of complexes

FT-IR spectra (Fig. 1) of the complexes exhibit characteristic bands of the expected functional groups and the relevant data are summarized in Table 2. FT-IR spectra of the complexes showed sharp band in the range of 3205-3240 cm⁻¹ are assigned to stretching vibration of secondary amine (-NH) group, indicating that the proposed condensation has occurred. The absence of a band between 1720-1740 cm⁻¹, corresponding to carbonyl group (C=O) of aldehydic moiety, further confirms the

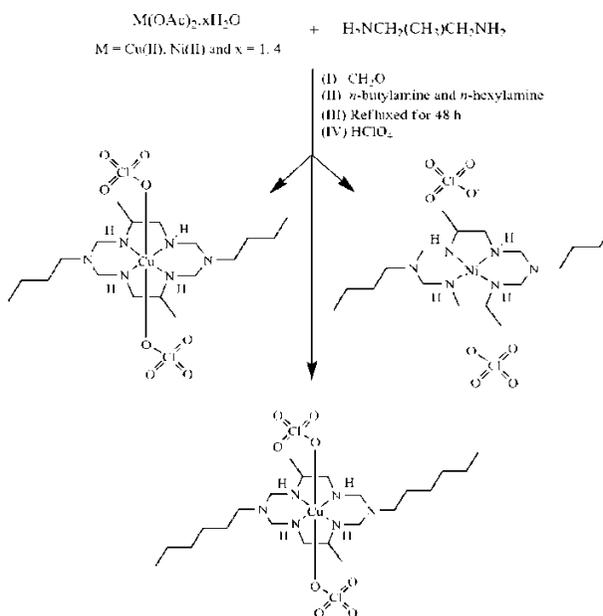
Results and Discussion

Synthesis for the complexes

The complexes with the hexaazamacrocyclic ligand were synthesized in one-pot condensation reaction of transition metal salts in which either copper(II) acetate monohydrate or nickel(II) acetate tetrahydrate reacted with ethylenediamine, formaldehyde, and butylamine or hexylamine in a 1:2:4:2 mole ratio (Scheme 1). The powder products were soluble in organic solvents such as DMSO, MeCN, DMF, and acetone. Unfortunately, all efforts failed to grow a single crystal suitable for X-ray crystallography.

These complexes were investigated and characterized by using FT-IR, LC-MS, and UV-Vis studies. The elemental analyses are summarized in Table 1 and are in accordance with the proposed formula for the complexes.

condensation. A medium intensity band appearing in the range 2970-2930 cm⁻¹ was assigned to $\nu(sp^3 \text{ C-H})$. In the region of 1100 cm⁻¹, there were two peaks, at 1125-1122 and 1089-1120 cm⁻¹, which could be assigned to vibration of perchlorate ions. The splitting of this peak clearly explained the presence of coordinated perchlorate. In all complexes, a band seen at 450-440 cm⁻¹ was probably due to the formation of M-N bonds [3,14].



Scheme 1. Synthetic route of the complexes

Table 2. FT-IR vibrational frequencies (cm^{-1}) for the complexes.

Complexes	$\nu(\text{N-H})$	$\nu(\text{C-H})$	$\delta(\text{C-H})$	$\nu(\text{Cl-O})$	$\delta(\text{Cl-O})$	$\nu(\text{M-N})$
$[\text{Cu}(\text{C}_{18}\text{H}_{42}\text{N}_6)(\text{ClO}_4)_2]$ (1)	3238	2963	1385	1121	626	441
$[\text{Ni}(\text{C}_{18}\text{H}_{42}\text{N}_6)](\text{ClO}_4)_2$ (2)	3208	2965	1383	1113	625	445
$[\text{Cu}(\text{C}_{22}\text{H}_{50}\text{N}_6)(\text{ClO}_4)_2]$ (3)	3238	2933	1384	1120	624	447

Notation: ν = stretching vibration and δ = bending vibration

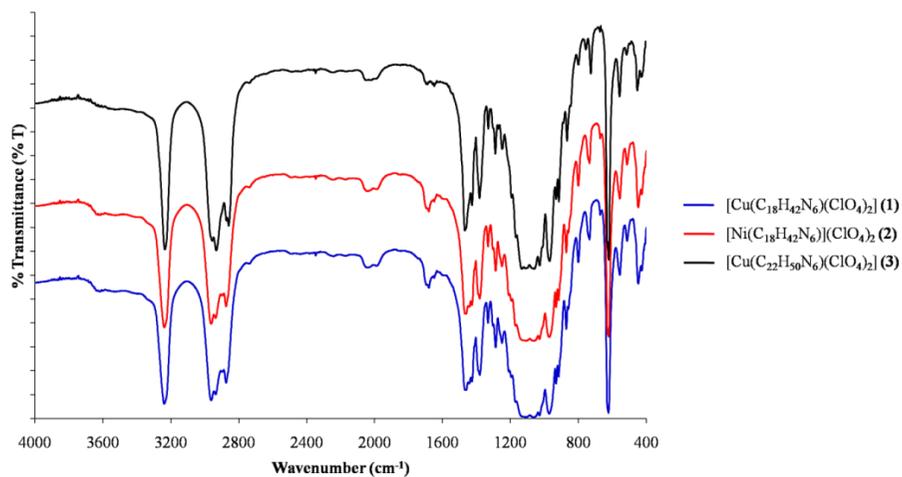


Fig. 1. FT-IR spectra of the complexes.

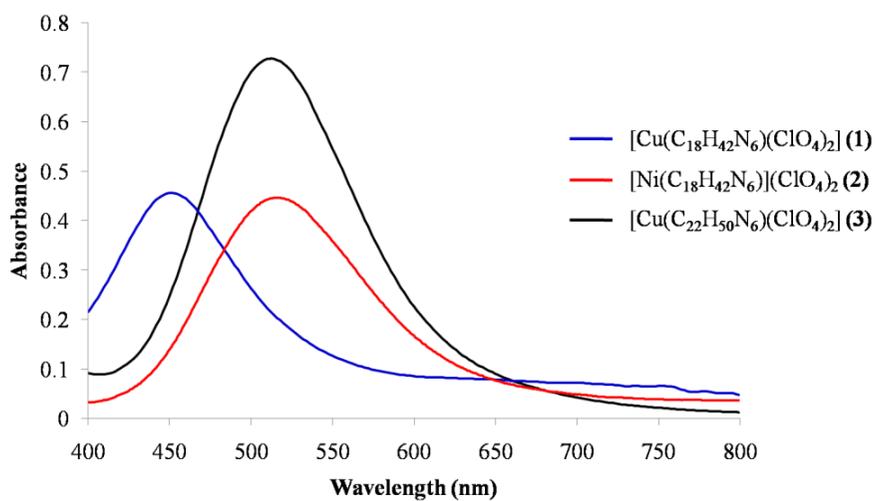


Fig. 2. The electronic spectra of complexes.

UV-Vis spectra of complexes

The electronic spectral data (Fig. 2.) of violet-red complexes **1** and **3**, and yellow complexes **2** in which these complexes are soluble in DMSO and show a visible absorption maximum at 515, 449, and 505 nm for complexes **1**, **2**, and **3**, respectively. It can be seen that those spectra at the $\lambda_{\max} = 515$, 449 and 505 nm which are assigned to d-d transition of Cu (II) and Ni (II) ions [15-17].

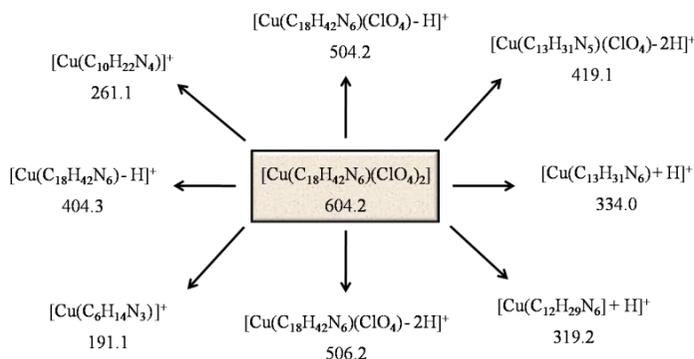
Mass spectra for the complexes

The electron spray ionization (ESI) mass data of **1**, **2**, and **3** complexes were

studied in positive mode. The ESI-MS data show the parent ion peaks due to molecular ions $[M]^+$ and $[M+2]^+$. The proposed molecular formulas for complexes are confirmed by comparing their molecular formula weights with m/z values [18-21]. The molecular ion peak $[M]^+$ was obtained for various complexes which are at 504.2 for complex 1, $[\text{Cu}(\text{C}_{18}\text{H}_{42}\text{N}_6)(\text{ClO}_4)]^+$, 500.2 for complex 2, $[\text{Ni}(\text{C}_{18}\text{H}_{42}\text{N}_6)]^+(\text{ClO}_4)$, and 561.2 for complex 3, $[\text{Cu}(\text{C}_{22}\text{H}_{50}\text{N}_6)(\text{ClO}_4)_2]^+$. The mass fragmentation pattern of complex 1 is shown in Scheme 2 and Fig. 3.

Table 3. Mass spectral data for the complexes.

Complexes	Precursor ion	Mass (m/z)		Fragment ions (m/z)
		Theoretical	Observed	
$[\text{Cu}(\text{C}_{18}\text{H}_{42}\text{N}_6)(\text{ClO}_4)_2](\mathbf{1})$	$[\text{M}-2\text{ClO}_4]^+$	506.2	504.2	419.1, 404.3, 319.2, 261.1, 247.1, 204.1, 191.1
$[\text{Ni}(\text{C}_{18}\text{H}_{42}\text{N}_6)](\text{ClO}_4)_2(\mathbf{2})$	$[\text{M}-2\text{ClO}_4]^+$	502.2	500.2	229.7, 172.7, 156.7, 140.7
$[\text{Cu}(\text{C}_{22}\text{H}_{50}\text{N}_6)(\text{ClO}_4)_2](\mathbf{3})$	$[\text{M}-2\text{ClO}_4]^+$	563.2	561.2	484.2, 461.2, 319.5, 217.5



Scheme 2. Fragmentation pattern of complex 1 observed in the ESI mass spectra

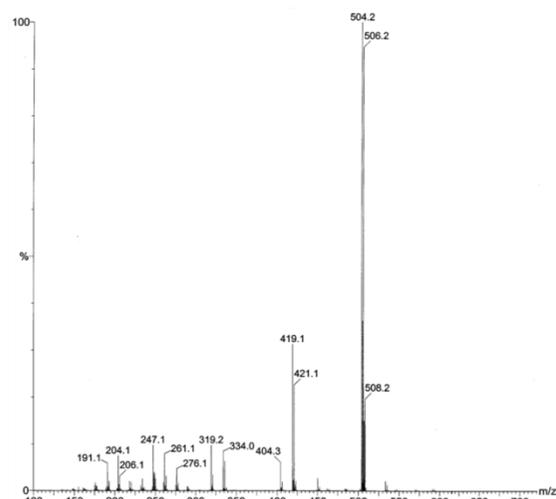


Fig. 3. Mass spectra of complex 1.

In vitro antibacterial activity

The copper (II) and nickel(II) complexes were screened *in vitro* for their antibacterial activity against two bacterial strains by the agar disc diffusion method. The sensibility of the bacterial strains towards all complexes determined by measuring the diameter of the inhibition zones is given in

Tables 4 and 5 and a histogram is shown in Fig. 4 and 5. It is observed that all synthesized complexes show good inhibitory activity against bacteria but less than tetracycline as a standard antibiotic. The copper(II) complexes of **1** and **3** showed more potent activity against both gram-positive and gram-negative bacteria than complex **2** with nickel(II) metal. All complexes showed better antibacterial activity against *E. coli* than *S. aureus* because of a gramnegative bacterium, a thin cell wall in these investigations, which is in accord with previous studies [22]. As a result, diffusion into cells of *E. coli* and *S. aureus* was greater than complexes and can inhibit the growth of bacteria. According to Tweedy's chelation theory [23] the polarity of the copper(II) and nickel(II) ions reduces owing to the partial sharing of its positive charge with the donor groups from the chelate ring, which however increases the lipophilic nature of the central metal atom, that favors its permeation more efficiently through the lipid layer of the micro-organism to destroy them more aggressively [24-26].

Table 4. Inhibition zone (mm \pm SD) of complexes in *E. coli* bacterial gram-negative.

Complexes	25 mg/mL	50 mg/mL	100 mg/mL	Tetracycline
[CuL ¹ (ClO ₄) ₂] (1)	18.30 \pm 0.58	20.67 \pm 0.58	26.33 \pm 0.58	30.00 \pm 0.00
[NiL ¹](ClO ₄) ₂ (2)	17.30 \pm 0.58	18.67 \pm 0.58	23.00 \pm 0.00	30.00 \pm 0.00
[CuL ² (ClO ₄) ₂] (3)	21.00 \pm 0.47	22.30 \pm 0.47	24.33 \pm 0.00	30.00 \pm 0.00

Table 5. Inhibition zone (mm \pm SD) of complexes in *S. aureus* bacterial gram-positive.

Complexes	25 mg/mL	50 mg/mL	100 mg/mL	Tetracycline
[CuL ¹ (ClO ₄) ₂] (1)	13.33 \pm 0.58	14.00 \pm 0.58	15.00 \pm 0.00	30.00 \pm 0.00
[NiL ¹](ClO ₄) ₂ (2)	10.67 \pm 0.00	13.00 \pm 0.00	14.00 \pm 0.00	30.00 \pm 0.00
[CuL ² (ClO ₄) ₂] (3)	15.00 \pm 0.47	17.33 \pm 0.47	20.33 \pm 0.47	30.00 \pm 0.00

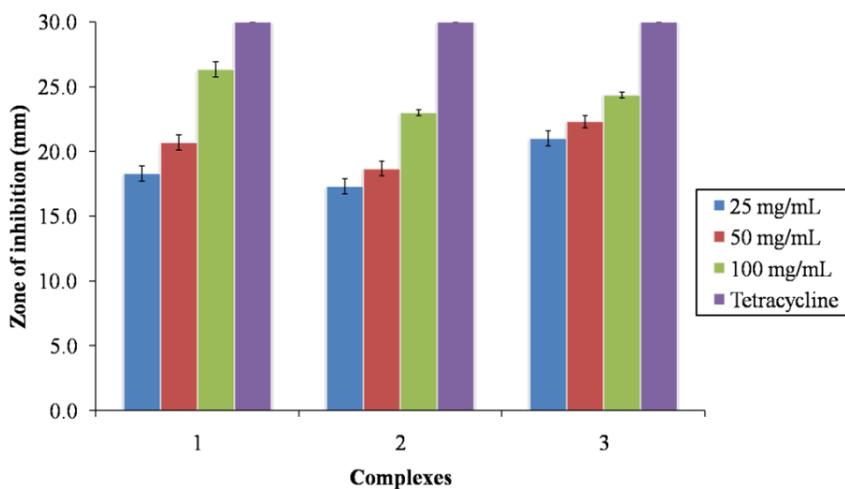


Fig. 4. The antibacterial activity of *E. coli* for the complexes.

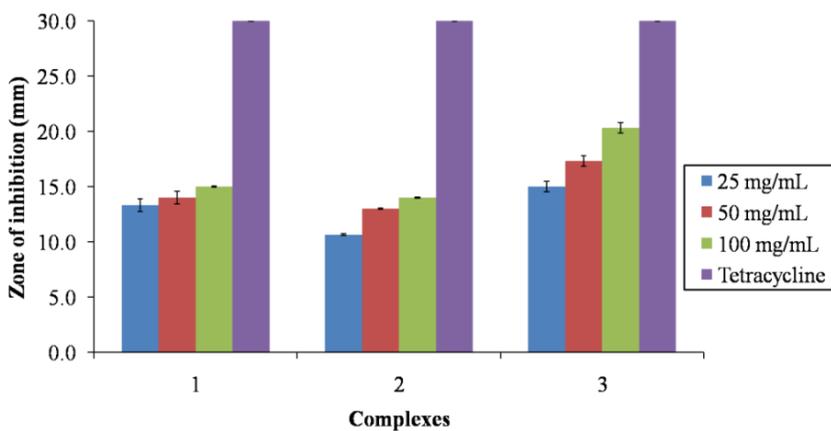


Fig. 5. The antibacterial activity of *S. aureus* for the complexes.

Conclusion

Copper(II) and nickel(II) complexes containing hexaazamacrocyclic ligand were prepared by one-pot reaction from the starting materials of 1,2-diaminopropane, formaldehyde, butylamine and/or hexylamine. All complexes were characterized by FT-IR, electronic spectra, LC-MS and elemental analyses. Both complexes **1** and **3** have an octahedral geometry, while the complex **2** has a square-planar geometry. The antibacterial activity test revealed that all complexes have better antibacterial activities against gram negative than gram positive bacteria. However, these complexes showed lower antibacterial activity than the standard drug.

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