

# Synthesis, Spectroscopic Properties and Antibacterial Activity of Copper Tetraalkyltetrapyridinoporphyrazinium Derivatives

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The paper examined the synthesis of cationic copper tetraalkyltetrapyridinoporphyrazinium derivatives bearing N-alkyl chains of different length: methyl, dodecyl, hexadecyl. The compounds have been characterized through spectral and elemental analysis. The antibacterial activity of cationic copper tetramethyltetrapyridinoporphyrazinium salt (iodide, chloride, bromide) was evaluated by using a Gram-negative bacteria, *Escherichia coli*, that can be detected in urogenital tract and sometimes in water.

**Keywords:** copper tetrapyridinoporphyrazine, alkylation, Gram-negative bacteria

Cationic phthalocyanines were proposed recently for photodynamic inactivation of bacteria, also phthalocyanine-like compounds with combination of hydrophilic and hydrophobic substituents may be used in phototherapy of cancer [1].

## Experimental part

First was synthesized copper 3,4-tetrapyridinoporphyrazine by cyclotetramerization of 3,4-pyridinedicarbonitrile with copper acetate in the presence of triethylamine, in ethanol, at reflux, 12h (fig.1).

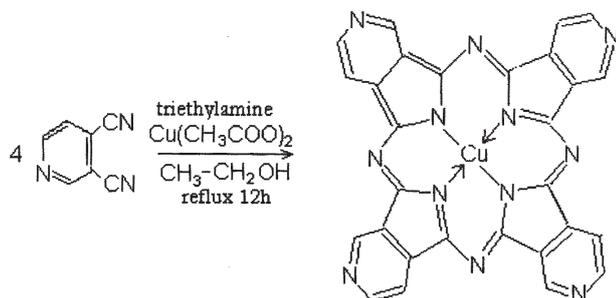
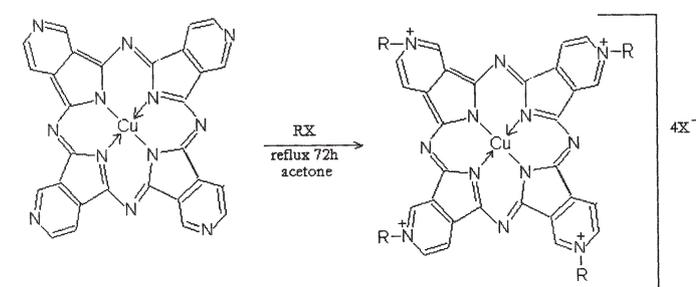


Fig.1. Synthesis of 3,4-tetrapyridinoporphyrazine

After reflux, copper 3,4-tetrapyridinoporphyrazine was treated for alkylation with alkyl halide, in acetone. The method comprises the following sequence of reaction (fig.2):



R = -CH<sub>3</sub>; -(CH<sub>2</sub>)<sub>11</sub>CH<sub>3</sub>; -(CH<sub>2</sub>)<sub>15</sub>CH<sub>3</sub>

X = I; Cl; Br

Fig.2. Alkylation of copper 3,4-tetrapyridinoporphyrazine

For the synthesis of the 3,4-pyridinedicarbonitrile were used 15g of 3,4-pyridinedicarboxylic acid (0.09mol), that was stirred and refluxed 48h in 200mL ethanol, with 7.5mL concentrate sulphonic acid (fig.3).

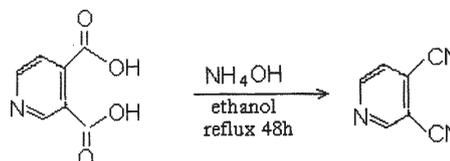


Fig.3. Synthesis of 3,4-pyridinedicarbonitrile

After 48h, the solvent was neutralized with NaOH solution 3M. The solid was removed with diethyl ether, filtered and the solvent was evaporated.

The solid was distilled to obtain the ester of 3,4-dicarboxypyridine [1].

After that, the ester was treated with NH<sub>4</sub>OH and stirred 48h.

A white precipitate was obtained. This was filtered to obtain 8.24g of 3,4-pyridinedicarbonitrile.

## Synthesis of copper 3,4-tetrapyridinoporphyrazine

A solution of 3,4-pyridinedicarbonitrile (0.0040mol) and copper acetate (0.014 mol) in 80mL ethanol was stirred at reflux for 12h. After that 520mL triethylamine was added and solvent was evaporated. The solid solved in dichloromethane and was washed four times with water. Purification was made by layer chromatography on silicagel column using benzene-acetone(1:1) as eluent to remove the copper acetate in excess. To precipitate it was added a mixture of dichloromethane:methanol (1:1), was filtered and after that was washed with small quantities of methanol.

The product was dried on vacuum. 5.37g of copper porphyrazine (yield=62%), a green compound, was obtained [3], that is soluble in DMF, DMSO, ethyl acetate, but is insoluble in water.

## Synthesis of copper 3,4-tetrapyridinoporphyrazinium tetraiodide

A mixture of copper 3,4-pyridinoporphyrazine (0.47mol) and 5mL of methyl iodide in 5 mL acetone was stirred at reflux 72h. The solvent was removed at the vacuum, the solid was suspended in cyclohexane to precipitate and was filtered. After that the solid was re-precipitated in ethanol-water(1:1) and the solid was washed with hexane and then,

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Compound	Alkyl halide	Copper porphyrzine	Yield (%)
1	Methyl iodide (5mL)	3g	95
2	Dodecyl iodide (8.83g)	3g	94
3	Hexadecyl iodide (10.53g)	3g	91
4	Methyl chloride (1.51mL)	3g	95
5	Dodecyl chloride (6.1g)	3g	94
6	Hexadecyl chloride (7.78g)	3g	91
7	Methyl bromide (2.85mL)	3g	95
8	Dodecyl bromide (8.86g)	3g	94
9	Hexadecyl bromide (9.12g)	3g	91

**Table 1**  
REACTION CONDITIONS FOR SYNTHESIS OF COMPOUNDS 1-9

with dichloromethane in excess. 6.17g of copper 3,4-tetrapyrindino-porphyrzinium tetra-iodide (yield 92%) were obtained.

For the other compounds the reaction conditions are shown in the table 1.

### Results and discussions

The compounds were characterized by elemental analysis, UV-VIS, IR spectroscopy [4] and *Escherichia coli* test. The analyses confirm the proposed structures.

The results are shown in the following tables:

Compound	Formula / molecular weight	C%		H%		N%		I/Cl/Br %		Cu%	
		calc.	det.	calc.	det.	calc.	det.	calc.	det.	calc.	det.
1	C <sub>36</sub> H <sub>24</sub> N <sub>12</sub> I <sub>4</sub> Cu/ M=1198	33.33	33.63	1.85	1.74	12.94	12.92	46.90	46.97	4.96	4.90
2	C <sub>88</sub> H <sub>112</sub> N <sub>12</sub> I <sub>4</sub> Cu/ M=2008	52.58	51.98	5.57	6.53	8.36	8.39	30.27	29.96	3.18	3.23
3	C <sub>96</sub> H <sub>144</sub> N <sub>12</sub> I <sub>4</sub> Cu/ M=2136	53.93	54.31	6.74	6.69	7.86	7.56	28.46	28.73	2.99	2.87
4	C <sub>36</sub> H <sub>24</sub> N <sub>12</sub> Cl <sub>4</sub> Cu/ M=830	52.04	52.34	2.89	3.01	20.24	20.12	17.10	17.46	7.71	7.61
5	C <sub>88</sub> H <sub>112</sub> N <sub>12</sub> Cl <sub>4</sub> Cu/ M=1542	68.48	68.23	7.26	7.03	10.89	11.05	9.20	8.99	4.15	4.32
6	C <sub>96</sub> H <sub>144</sub> N <sub>12</sub> Cl <sub>4</sub> Cu/ M=1670	68.98	68.52	8.62	8.33	10.05	9.87	8.50	8.75	3.83	3.92
7	C <sub>36</sub> H <sub>24</sub> N <sub>12</sub> Br <sub>4</sub> Cu/ M=1008	42.85	43.00	2.38	2.45	16.65	15.98	31.74	30.89	6.34	6.23
8	C <sub>88</sub> H <sub>112</sub> N <sub>12</sub> Br <sub>4</sub> Cu/ M=1720	61.39	61.51	6.51	6.49	9.76	10.02	18.60	18.74	3.72	3.93
9	C <sub>96</sub> H <sub>144</sub> N <sub>12</sub> Br <sub>4</sub> Cu/ M=1848	62.33	61.99	7.79	7.97	9.09	8.89	17.31	17.13	3.46	3.63

**Table 2**  
ELEMENTAL ANALYSIS OF COMPOUNDS 1-9

### *Escherichia coli* test

*Escherichia coli* recovered from water was used [5]. Bacterial cultures grown under the same conditions. *E. coli* strain was grown aerobically at 37°C, using a liquid medium (EC Blue (17.4g/L)). When we see the turbidity in the aliquots, we transfer the bacteria on the GEAM medium, on the Petri plates. The plates were incubated for 48h, at 37°C. Viable bacteria were monitored and their number calculated by counting the number of colony forming units.

**Table 3**  
UV-VIS SPECTRA

Compound	UV		VIS	
	$\lambda_{\max}$ [nm]	$\epsilon_{\max}$ [L·mol <sup>-1</sup> ·cm <sup>-1</sup> ]	$\lambda_{\max}$ [nm]	$\epsilon_{\max}$ [L·mol <sup>-1</sup> ·cm <sup>-1</sup> ]
1	352	3.716x10 <sup>4</sup>	677	2.430 x10 <sup>4</sup>
2	387	5.321x10 <sup>4</sup>	683	1.996 x10 <sup>4</sup>
3	360	3.922 x10 <sup>4</sup>	687	2.547 x10 <sup>4</sup>
4	357	4.113 x10 <sup>4</sup>	677	3.824 x10 <sup>4</sup>
5	387	3.345 x10 <sup>4</sup>	683	2.458 x10 <sup>4</sup>
6	363	4.364 x10 <sup>4</sup>	687	2.478 x10 <sup>4</sup>
7	385	3.893 x10 <sup>4</sup>	677	3.259 x10 <sup>4</sup>
8	383	4.434 x10 <sup>4</sup>	683	2.491 x10 <sup>4</sup>
9	361	4.361 x10 <sup>4</sup>	687	3.127 x10 <sup>4</sup>

**Table 4**  
IR SPECTRA (KBr)

Compound	$\nu_{C-H}$	$\nu_{C=C}$	$\delta_{C-H}$
1	3050 (pi),2920 <sub>as.</sub> ,2845 <sub>sim</sub>	1636, 1604	1301,1251,1111,746,618
2	3050(pi),2970 <sub>as.</sub> ,2870 <sub>sim</sub>	1608,1600	1250,1110,950,810,660
3	3050(pi),2968 <sub>as.</sub> ,2870 <sub>sim</sub>	1600,1460	1230,1118,920,815,770,620
4	3050(pi),2930 <sub>as.</sub> ,2840 <sub>sim</sub>	1635,1600	1290,1229,1115,735,615
5	3050(pi),2980 <sub>as.</sub> ,2825 <sub>sim.</sub>	1602,1600	1257,1120,920,790,683
6	3050(pi),2960 <sub>as.</sub> ,2893 <sub>sim</sub>	1600,1505	1259,1136,912,823,783,640
7	3050(pi),2923 <sub>as.</sub> ,2798 <sub>sim.</sub>	1640,1609	1332,1260,1200,750,620
8	3050(pi),2974 <sub>as.</sub> ,2875 <sub>sim</sub>	1609,1599	1256,1125,948,824,663
9	3050(pi),2978 <sub>as.</sub> ,2798 <sub>sim</sub>	1603,1470	1260,1200,973,840,766,623

This is the witness plate(A).  
The second sample (B) was obtained in the same way, but the compound was added before the incubation, in dark, on the GEAM medium.

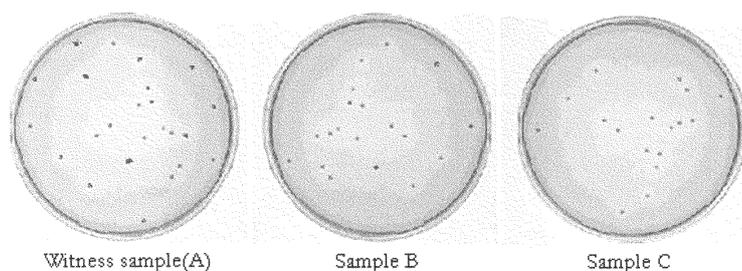


Fig. 4. Escherichia coli-samples with the compound 1

The third sample (C) was irradiated with visible light in presence of compounds, using a 150W lamp.

We observe a diminuation of the number of colony forming in the the samples where we use the compounds 1 and 4.

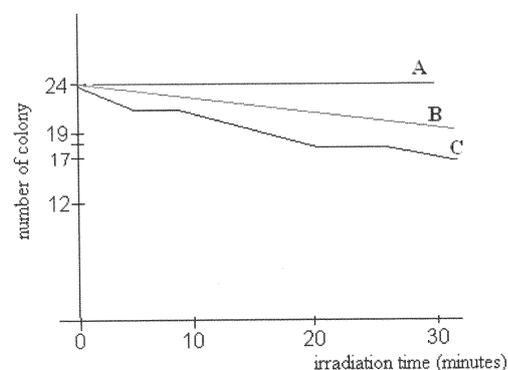


Fig. 5. The graphic of number of colony decrease after irradiation in presence of compound 1 - sample A; B; C

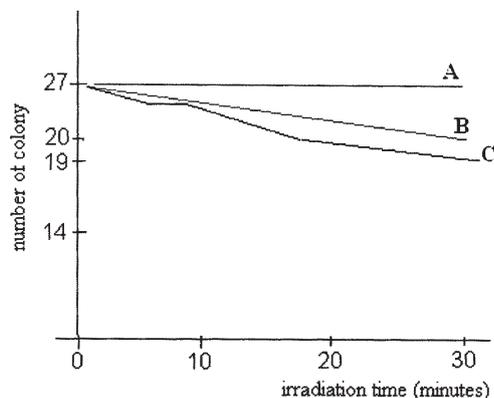


Fig. 6. The graphic of number of colony decrease after irradiation in presence of compound 4 - sample A; B; C

The results are shown in the following figures:

### Conclusions

Cationic tetraalkyltetrapyrroline derivatives bearing N-alkyl chains of different length were synthesized from copper tetrapyrroline. The photodynamic activity was evaluated by using a Gram – negative bacteria (*Escherichia coli*) that is inactivated by two dyes (1 and 4), with or without exposure to visible light. These compounds produced a decrease of *E.coli* cell survival, then they showed a bactericidal activity.

### References

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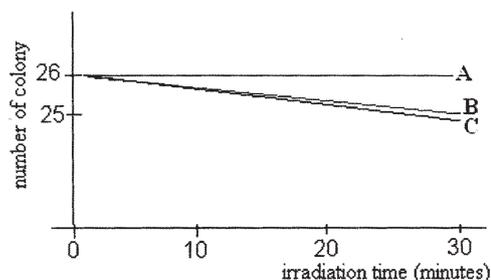


Fig. 7. The graphic of number of colony decrease after irradiation in presence of compound 7 - sample A; B; C

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