SYNTHESIS AND ANTIMICROORGANISM ACTIVITY OF MIRAMISTIN

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Abstract

Quaternary ammonium compounds are known for their rich physicochemical and biological characteristics, and are widely used in many areas of life such as surface active properties and anti-microbial properties. Miramistin is a typical compound with full physicochemical and biological properties of quaternary amino compounds, has antimicrobial properties and is widely used in the medical and pharmaceutical fields. This article presents the results of research on the synthesis and optimization of the miramistin synthesis process based on the use of different starting compounds. In addition, the antimicroorganism properties of miramistin were tested and showed potential application as an antimicroorganism.

Keywords: *Miramistin; quaternary ammonium compounds; antimicroorganism.*

1. Introduction

Quaternary ammonium compounds (QACs) are compounds obtained by replacing all four hydrogen atoms of the ammonium cation NH_4^+ with four functional groups R_1, R_2, R_3, and R_4, which are usually hydrocarbons such as alkyl or aryl (Fig. 1). According to this definition, quaternary ammonium compounds are very diverse, encompassing an extremely wide range of compounds and derivatives. Up to now, their boundaries, classification, and even subclasses have not been clearly distinguished [1].

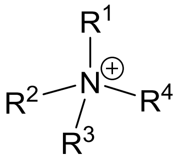

Fig. 1. Quaternary ammonium compounds structural formula.

Regarding physical properties, QACs are well soluble in polar solvents such as water and alcohol, but their solubility decreases significantly with increasing alkyl chain length. A notable physicochemical property of QACs is their ionic conductivity. Their solutions are very good electrolytes because they have both a hydrophilic positively charged N^+ center and a long hydrophobic alkyl chain [1]. Regarding chemical properties,

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if reactions on substituent group are not considered, QACs do not react with electrophiles, oxidants and strong acids; durable and stable to most nucleophine agents; durable at high temperatures and QACs will decompose with particularly strong bases. They also undergo rearrangement or dealkylation under harsh conditions in the presence of extremely potent nucleophines such as thiolates. The most typical examples in this case are the two phenomena of Sommelet-Hauser and Stevens arrangement [2].

The substituents in QACs will directly affect the physical, chemical and biochemical properties of QACs, including their antibacterial ability. According to experimental and calculated data of the study, QACs usually have high water solubility, low volatility, and low to moderate hydrophobicity ($\log K_{ow} < 4$). However, when changing the substituent groups with suitable hydrocarbon groups such as aryl or alkyl with more than 10 carbon atoms, QACs have highly hydrophobic properties ($\log K_{ow} > 9$) and begin to appear active surface movement [1], [3]. As predicted, if continue to increase the length of the hydrocarbon groups on substituent groups, the hydrophobic properties and surface activity will continue to increase. In addition, as mentioned above, QACs have a permanently positively charged center, although undissociated salts can exist in solution and possibly even on the surface. This facilitates the adsorption of QACs to negatively charged solids or microbial membranes (composed of negatively charged phospholipid groups). These properties of QACs make them resistant to microorganisms and have a variety of other applications such as surfactants, preservatives, antistatic agents, softeners, dispersants, etc. [4]. Particularly during the COVID-19 pandemic, the widespread use of QACs as antimicrobials has significantly increased the pandemic response [5].

Miramistin is a surfactant based antibacterial drug developed in Russia. It has antibacterial effects on gram (+) and gram (-) bacteria, aerobic and anaerobic bacteria, spore producing and non spore producing bacteria. Miramistin is one of the typical QACs, where R^1 and R^2 are $-CH_3$ groups, R^3 is benzyl group and R^4 is 3-(myristoilamino) propyl group (Fig. 2). With such a molecular structure, miramistin is both highly hydrophobic and has strong surface activity properties [6]. This positively contributes to the ability of microorganisms to adhere and adsorb on membranes. Therefore, miramistin is commonly used in the field of pharmaceutical chemistry, especially in the group of disinfectants, antiseptics and biological weapons prevention in the military field [7], [8].

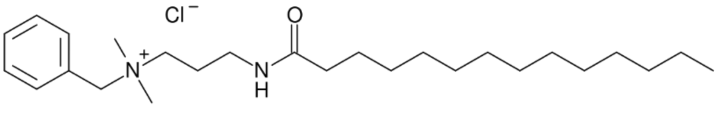


Fig. 2. Miramistin structural formula.

In this study, the authors synthesized miramistin from the starting material 3-dimethylaminopropylamine, reacting with myristic acid or myristoyl chloride to obtain the intermediate product N-(3-(dimethylamino)propyl)tetradecanamide (hereinafter collectively called amidoamine). After purification, the intermediate product was subjected to quaternary benzylation with benzyl chloride to obtain miramistin. Next, we tested the antimicroorganism activity of miramistin.

2. Experiment

2.1. Chemicals and equipment

Chemicals and reagents were purchased from commercial suppliers and used without special instructions. The nuclear magnetic resonance (NMR) spectra have been recorded with Brucker Avance 600 spectrometers at Vietnam Academy of Science and Technology. ^{1}H NMR and ^{13}C NMR spectra were obtained in DMSO-d_6 using tetramethylsilan (TMS) as internal standard, operating at 600 MHz and 151 MHz, respectively. Chemical shifts (δ) are expressed in ppm and coupling constants J are given in Hz. Abbreviations are as follows: s (singlet), d (doublet), t (triplet), m (multiplet). The Fourier-transform infrared spectroscopy (FT-IR) has been recorded with Spectrum Two spectrometer at Le Quy Don Technical University. Antimicrobial activity was tested based on the Broth microdilution method at Vietnam Academy of Science and Technology. Tested on 7 strains of microorganisms and fungi including: Staphylococcus aureus (SA), Bacillus subtilis (BS), Lactobacillus fermentum (LF), Salmonella enterica (SE), Escherichia coli (EC), Pseudomonas aeruginosa (PA), Candida albicans (CA).

2.2. Synthesis of miramistin

2.2.1. Synthesis of N-(3-(dimethylamino)propyl)tetradecanamide

Path a: The myristic acid (4.57 g, 20 mmol), molecular sieve 3A (2.5 g) and toluene (100 mL) was added to a 250 mL double-necked flask, the mixture stirred at 80°C under N_2. 3-dimethylaminopropylamine (3.1 mL, 24 mmol) was slowly added to the the reaction system over 0.5 hours, then the reaction was carried out at 140°C for 20 hours. After the reaction is completed, filter the mixture to remove molecular sieve 3A powder. Water (30 mL) was added and extracted with ethyl acetate (20 mL × 3). The combined organic layer, then the solution was cooled and maintained at 0-5°C until complete precipitation. Filtration is carried out to collect solids and washed with cold acetone, dry under low pressure to obtain the product **3a** (3.78 g, 60.5%).

Path b: The 3-dimethylaminopropylamine (2.51 mL, 20 mmol), triethyl amine (3 mL, 24 mmol) and 1,2-dichloroethane (20 mL) was added to a 100 mL flask, the mixture was cooled to 0°C and stirring under nitrogen atmosphere. Myristoyl chloride (5.44 mL, 20 mmol) was added into the reaction mixture drop wise maintaining 0°C and the stirring was continued for 6 hours at room temperature. After the reaction is completed, water (20 mL) was added and extracted with dichloromethane (20 mL × 3). The combined organic layer was washed with brine (20 mL) and dried (Na_2SO_4), then the solution was cooled and maintained at 0-5°C until complete precipitation. Filtration is carried out to collect solids and washed with cold acetone, dry under low pressure to obtain the product **3a** (4.1 g, 53.4%).

N-(3-(dimethylamino)propyl)tetradecanamide (*3a*). IR ν (cm^{-1}) 3303, 2917, 2849, 1639, 1548, 1261, 719; ^1H NMR (600 MHz, DMSO-d_6) δ 7.72 (s, 1H, $N\underline{H}$); 3.04 (dd, J = 12.8; 6.6 Hz, 2H, $C\underline{H}_2$); 2.17 (t, J = 7.2 Hz, 2H, $C\underline{H}_2$); 2.10 (s, 6H, $C\underline{H}_3$); 2.02 (t, J = 7.4 Hz, 2H, $C\underline{H}_2$); 1.52 – 1.45 (m, 4H, $C\underline{H}_2$); 1.24 (s, 20H, $C\underline{H}_2$); 0,86 (t, J = 6.9 Hz, 3H, $C\underline{H}_3$).

2.2.2. Synthesis of miramistin

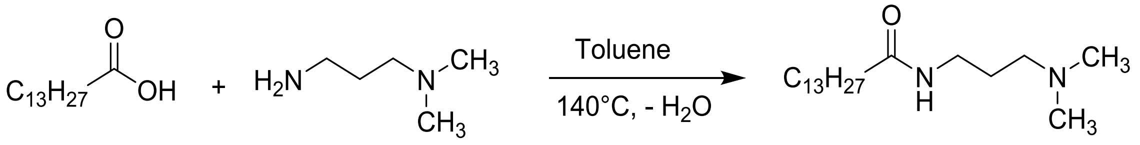
The N-(3-(dimethylamino)propyl)tetradecanamide **3a** (1.56 g, 5 mmol), acetone (25 mL) was added to a 100 mL double-necked flask, the mixture stirred at 60°C under N_2. Benzyl chloride (0.75 mL, 5.5 mmol) was added drop wise, reflux for 4 hours. After the reaction is completed, cooled the mixture and maintain at 0-5°C until complete precipitation. Filtration is carried out to collect solids and washed with cold acetone, and dried to give the target product **4a** (3.52 g, 87%).

Miramistin (4a). IR ν (cm^{-1}) 3374, 2922, 2852, 1646, 1547, 1456, 728, 702; ^1H NMR (600 MHz, DMSO-d_6) δ 8,24 (s, 1H, $N\underline{H}$); 7.59 (d, J = 7.0 Hz, 2H, $C\underline{H}$); 7.50 (dd, J = 13.3; 7.1 Hz, 3H, $C\underline{H}$); 4.61 (s, 2H, $C\underline{H}_2$); 3.31 (dd, J = 9.8; 6.6 Hz, 2H, $C\underline{H}_2$); 3.11 (d, J = 5.9 Hz, 2H, $C\underline{H}_2$); 2.99 (s, 6H, $C\underline{H}_3$); 2.07 (t, J = 7.5 Hz, 2H, $C\underline{H}_2$); 1.98 – 1.93 (m, 2H, $C\underline{H}_2$); 1.47 – 1.43 (m, 2H, $C\underline{H}_2$); 1.22 (s, 20H, $C\underline{H}_2$); 0.84 (t, J = 6.9 Hz, 3H, $C\underline{H}_3$); ^{13}C NMR (151 MHz, DMSO-d_6) δ 172.42; 132.96; 130.13; 128.76; 128.10; 65.98; 6.33; 49.19; 35.44; 35.36; 31.25; 29.01; 28.97; 28.92; 28.81; 28.72; 28.66; 25.18; 22.63; 22.04; 13.86.

3. Results and discussion

Myristic acid and myristoyl chloride were selected to react with 3-dimethylaminopropylamine to synthesize the intermediate compound N-(3-(dimethylamino)propyl)tetradecanamide by two different pathways.

In the first pathway, the method of synthesizing amidoamine **3a** from myristic acid was researched. The reaction was conducted between myristic acid and 3-dimethylaminopropylamine, using toluene as the reaction solvent, reflux at 140°C within 20 hours and the product N-(3-(dimethylamino)propyl)tetradecanamide was obtained with a yield of 60.5% (Fig. 3).



1a **2a** **3a, 60.5 %**

Fig. 3. Synthesis of amidoamine ***3a*** *from myristic acid.*

The chemical structure of amidoamine **3a** was determined by FT-IR and ^{1}H NMR spectrum. In the IR spectrum, the following characteristic peaks appear: valence vibration of -NH at 3303 cm^{-1}, valence vibration of carbonyl group (C=O) at 1639 cm^{-1}, deformation vibration of N-C=O at 1548 cm^{-1} and typical peaks for C-H bond valence vibrations at 2917 cm^{-1} and 2849 cm^{-1} (Fig. 4).

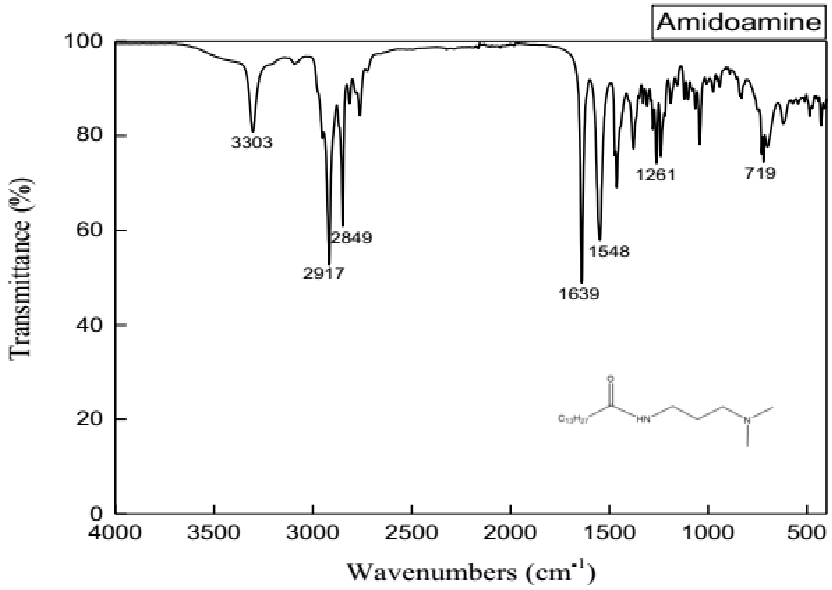


Fig. 4. FT-IR spectrum of amidoamine ***3a****.*

In the ^{1}H NMR spectrum of product **3a** (Fig. 5), all characteristic signals of the protons present in the molecule also appear, 40 protons resonances were observed. In which, some characteristic peaks appear as follows: 1 proton of -NH group gives a resonant singlet signal at position 7.72 ppm; 6 protons of $-N(CH_3)_2$ gives a resonant singlet signal at position 2.10 ppm; the triplet peak at 0.86 ppm is the 3 protons of the CH_3 group at the end of the long chain.

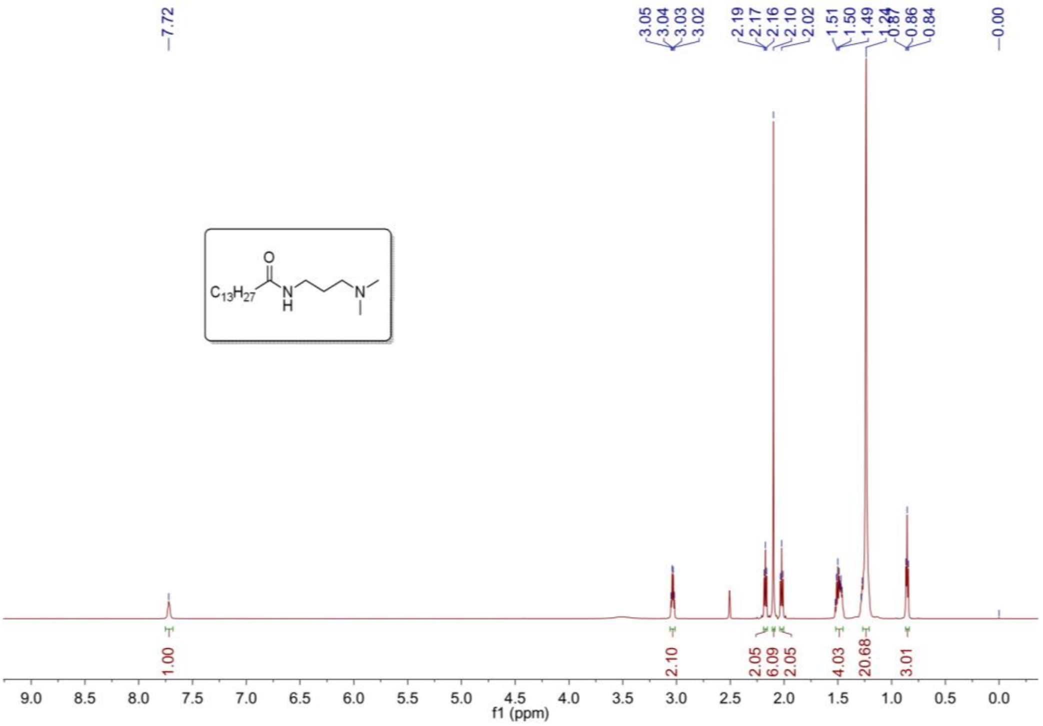
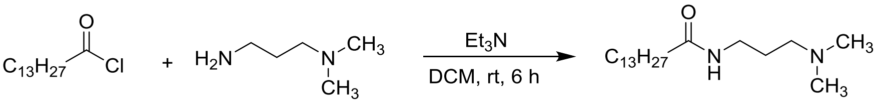


*Fig. 5. ^1H NMR spectrum of amidoamine **3a**.*

In the second pathway, amidoamine **3a** is prepared from myristoryl chloride using trimethylamine as catalyst, dichloromethane as solvent, the mixture is stirred for 6 hours at room temperature and the product N-(3-(dimethylamino)propyl) was observed with a reaction yield of 53.4% (Fig. 6).

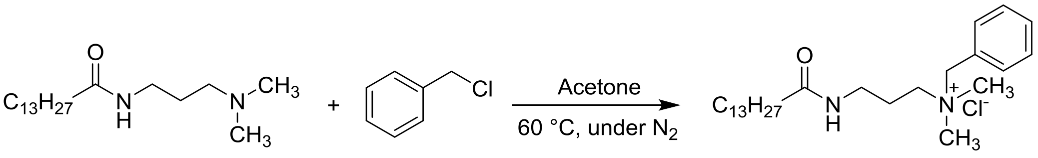


1b **2a** **3a, 53.4 %**

*Fig. 6. Synthesis of amidoamine **3a** from myristoyl chloride.*

The obtained results show that both methods are capable of preparing amidoamine compounds with average efficiency. However, in the first pathway, the reaction is carried out in a longer time, at a higher temperature and the yields are similar than the second method. Compared to the first pathway, the second pathway uses milder reaction conditions. Therefore, we chose the second pathway to synthesize amide intermediate compounds.

After synthesizing the intermediate product amidoamine **3a**, we proceeded to synthesize miramistin. N-(3-(dimethylamino)propyl)tetradecanamide **3a** reacts with benzyl chloride in acetone solvent to form miramistin product as a white solid, with a yield of 87% (Fig. 7). The chemical structure of miramistin was characterrized by FT-IR, ^{1}H NMR and ^{13}C NMR spectrum.



miramistin, 87 %

Fig. 7. Synthesis of miramistin.

FT-IR spectrum of miramistin product (Fig. 8) shows the valence vibration peak of the -NH group at 3374 cm^{-1}, the valence vibration peak of the carbonyl group (C=O) at 1646 cm^{-1}, the specific peak of the -CH$_2$ group at 2922 cm^{-1} and 2852 cm^{-1}, in addition, the characteristic peaks represent bending vibrations in the aromatic ring appears at 728 cm^{-1} and 702 cm^{-1}.

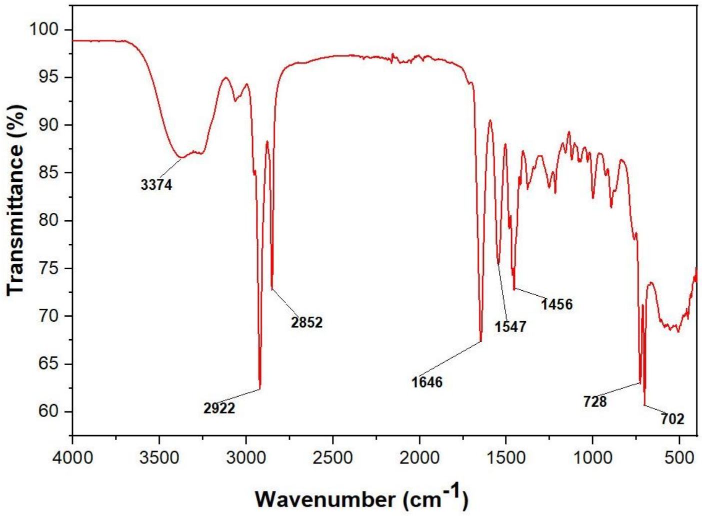


Fig. 8. FT-IR spectrum of miramistin.

The ^{1}H NMR spectrum of miramistin is shown in Fig. 9. Some characteristic peaks appear as follows: 1 proton of -NH group gives a resonant singlet signal at 8.84 ppm; at 7.47-7.59 ppm is the characteristic signal for 5 protons on the aromatic ring (ArH); the singlet at δ = 4.61 ppm represents 2 linear protons in the benzyl group; 6 protons of -N(CH$_3$)$_2$ gives a resonant singlet signal at position 2.99 ppm; the triplet peak at 0.84 ppm is the 3 protons of the -CH$_3$ group at the end of the long chain. In the ^{13}C NMR spectrum, 26 carbon resonances were observed (Fig. 10).

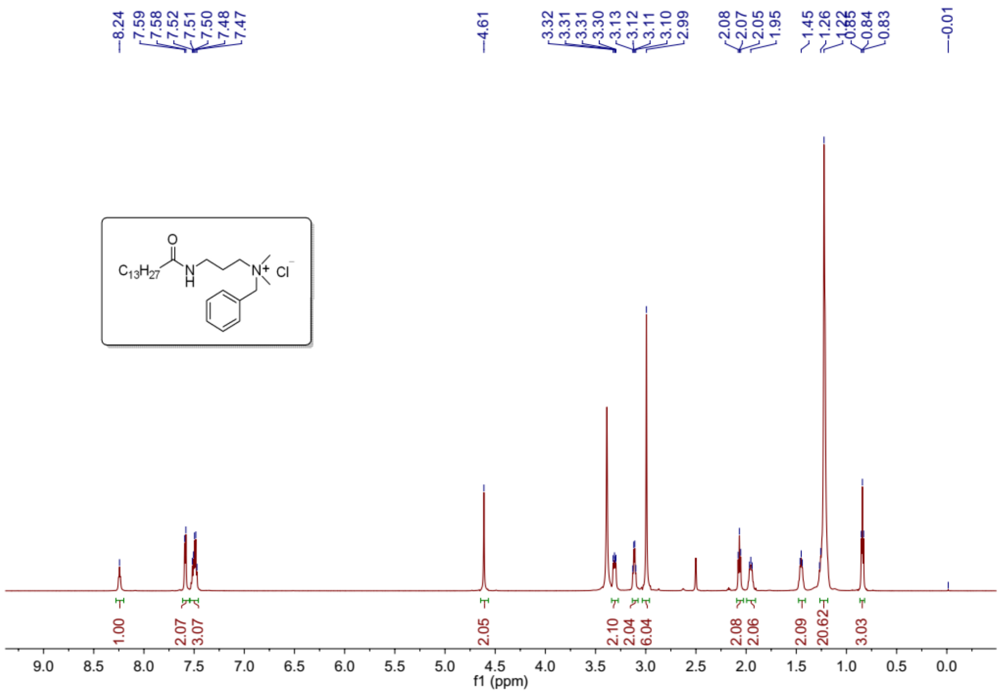


Fig. 9. ^{1}*H NMR spectrum of miramistin.*

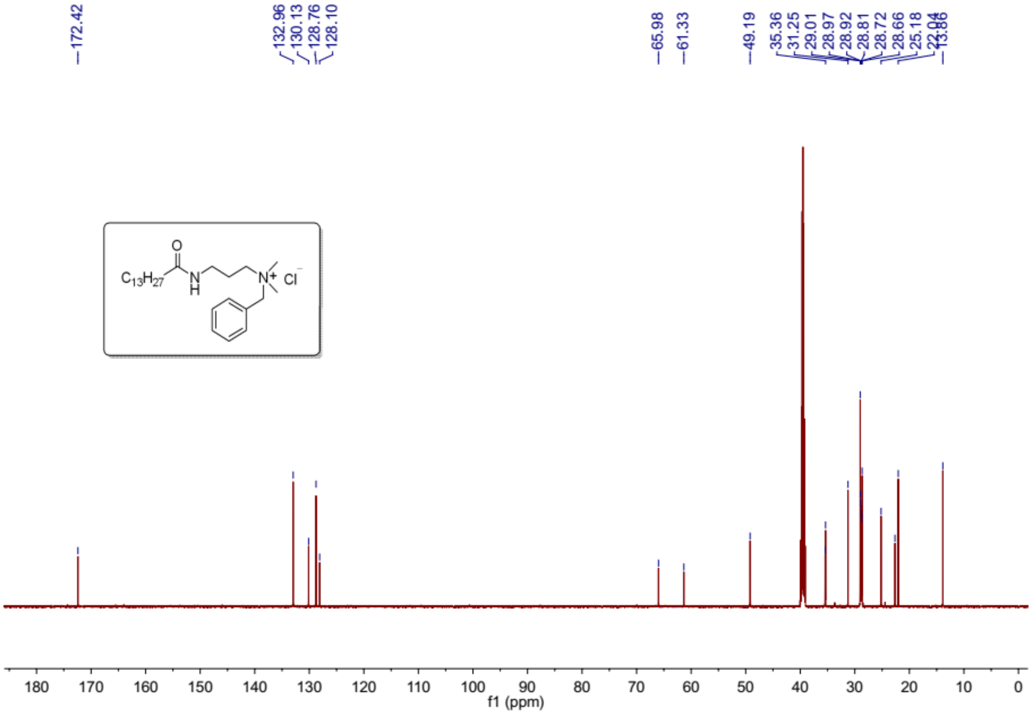


Fig. 10. ^{13}*C NMR spectrum of miramistin.*

After successfully synthesizing miramistin, we tested the antibacterial activity using the broth microdilution method. Tested on 7 strains of microorganisms and fungi including: Staphylococcus aureus (SA), Bacillus subtilis (BS), Lactobacillus fermentum (LF), Salmonella enterica (SE), Escherichia coli (EC), Pseudomonas aeruginosa (PA), Candida albicans (CA). The results of the antibacterial activity test are shown in Tab. 1. Miramistin test samples were diluted at concentrations of 128, 32, 8, 2, 0.5 µg/mL for testing. At concentrations of 128 and 32 µg/mL, the test sample showed good antibacterial effect against all 7 tested microorganisms, however, for CA fungus, the effect was only 67% at a concentration of 128 µg/mL and 58% at a concentration of 32 µg/mL. Diluting the concentration to 8 µg/mL, the test sample was only effective against gram (+) microorganisms, while the antibacterial effect against gram (-) microorganisms was significantly reduced, and SE bacteria were ineffective. The test sample at a concentration of 2 µg/mL was only effective against 2 strains SA and LF with 100% efficiency and had almost no antimicroorganism ability against the remaining microbial strains such as BS, SE, EC, PA and CA. In addition, the test sample was almost ineffective against all tested microbial strains when diluted to a concentration of 0.5 µg/mL.

To evaluate the effectiveness of miramistin, the authors compared the test sample with reference samples including Ampicillin, Cefotaxime, and Nystatin. Research results show that, for gram (+) microbial strains such as SA, BS and LF, the IC_{50} and MIC values of miramistin are approximately equal to those of Ampicillin, proving that miramistin has very has many potential applications as antimicroorganism.

Tab. 1. Test results for antimicroorganism activity

Name	**Concent-ration (µg/mL)**	**% Inhibition of microbial and fungi**						
		Gram (+)			**Gram (-)**			**Fungi**
		SA	**BS**	**LF**	**SE**	**EC**	**PA**	**CA**
Miramistin	128	100	100	100	100	100	100	67
	32	100	100	100	92	100	100	58
	8	100	100	100	0	20	47	41
	2	100	0	100	0	0	0	12
	0.5	9	0	0	0	0	0	0
	IC_{50}	1.18 ± 0.09	5.00 ± 0.46	1.25 ± 0.08	21.04 ± 1.57	17.00 ± 1.46	9.36 ± 0.89	6.50 ± 0.47
	MIC	2	8	2	32	32	32	> 128
Ampicillin	IC_{50}	0.02 ± 0.005	3.62 ± 0.15	1.03 ± 0.07				
	MIC	0.125	32	32				
Cefotaxime	IC_{50}				0.43 ± 0.05	0.007 ± 0.002	4.34 ± 0.15	
	MIC				32	0.5	8	
Nystatin	IC_{50}							1.32 ± 0.05
	MIC							8

4. Conclusion

The target product miramistin was successfully synthesized in two steps. Step one, synthesize amidoamine by reaction between 3-dimethylaminopropylamine and myristic acid or myristoyl chloride. The yields of the two methods are 60.5% and 53.4% respectively, however the method using myristoyl chloride has gentler reaction conditions. Step two, miramistin is synthesized by the reaction between amidoamine and benzyl chloride in acetone solvent, the yield is 87%. The chemical structure of the intermediate compound and miramistin was demonstrated by FT-IR, ^{1}H NMR and ^{13}C NMR spectra. Antimicroorganism test results show that miramistin has good antibacterial properties, at concentrations of 2-8 µg/mL, effective against gram (+) microorganisms such as SA, LF, BS and good effect against most strains of microorganisms when used at concentrations of 32 µg/mL.

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QUY TRÌNH TỔNG HỢP VÀ KHẢ NĂNG KHÁNG VI SINH VẬT KIỂM ĐỊNH CỦA MIRAMISTIN

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Tóm tắt: Các hợp chất amoni bậc bốn đã được biết đến với các đặc điểm hóa lý, sinh học phong phú, được ứng dụng rộng rãi trong nhiều lĩnh vực của đời sống như tính chất hoạt động bề mặt, tính kháng vi sinh vật. Tiêu biểu là miramistin, một hợp chất với đầy đủ các tính chất điển hình của một amoni bậc bốn, có khả năng kháng vi sinh vật và được ứng dụng nhiều trong lĩnh vực y tế, hóa dược. Bài báo trình bày kết quả nghiên cứu tổng hợp miramistin trên cơ sở sử dụng các hợp chất đầu khác nhau. Ngoài ra, tính kháng vi sinh vật của miramistin đã được thử nghiệm và cho thấy tiềm năng ứng dụng làm thuốc diệt khuẩn và kháng sinh.

Từ khóa: *Miramistin; hợp chất amoni bậc bốn; kháng vi sinh vật.*

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