N,N,N-triethyl ammonium L-Phenylalanine esters as potential antimicrobial agents

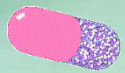
Presented by
Nausheen Joondan
Prakashanand Caumul*
Sabina Jhaumeer-Laulloo

Department of Chemistry, University of Mauritius
*Corresponding author: p.caumul@uom.ac.mu

The microorganisms represent a serious health issue as a result of intrinsic resistance of several species due to:

Excessive use of antibiotics

Poor adherence to antibiotics treatment



Therefore, there is a need to develop more antimicrobial compounds with a lower probability of resistance mechanism.

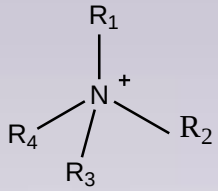
Quaternary ammonium compounds(Quats)

- Do not interfere with microorganism's metabolic pathway
- Less probability of resistance

Zasloff, M, Nature, 415, 2002, 89–395.

Quaternary ammonium compounds (Quats)

General structure



where R_1- R_4 = alkyl or aryl

These compounds cause a generalized damage of cytoplasmic membrane so that the positively charged "head" of the molecule interacts with negatively charged membrane components followed by penetration of nonpolar tenside constituent of its hydrophobic part. The crucial first step at the membrane destruction is in this case the decrease of its electrical potential by Coulombic interactions followed by incorporating in the bacterial membrane.

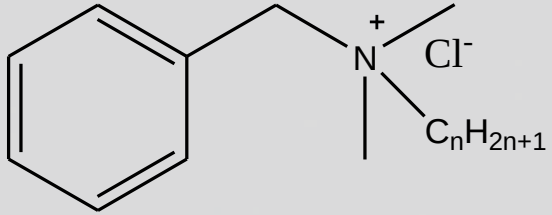
Antibacterial activity of QUATS

- **Possess better antibacterial property**
- **Affinity for anionic biological membrane due to Coulombic attraction**

P. Gilbert, L.E. Moore, J. Appl. Microbiol, 99, 2005, 703- 715.

Quats as antibacterial compounds

There are several different types of quats used as biocides, with benzalkonium being the most common.

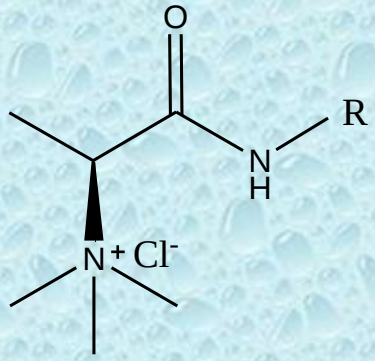


•Benzalkoniums have been shown to be effective as biocides, but recent data indicates that various bacterial strains were found to be resistant to benzalkonium.

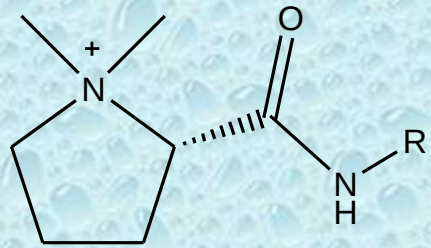
•Some quaternary ammonium compounds have toxic effects towards mammalian cells and can be used only for topical applications.

J. N. Mbithi, V. J. Springthorpe and S A Sattar, Appl. Environ. Microbiol, 56, 1990, 3601

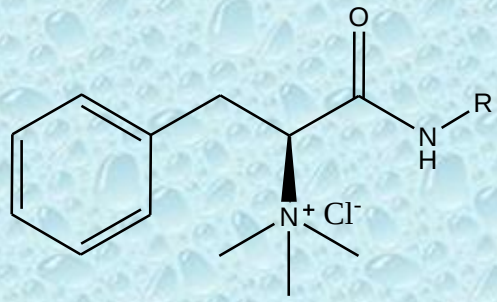
To overcome the problem of toxicity, quaternary ammonium amphiphiles derived from natural precursors such as amino acids have been known.



Alanine derived quat
R= $C_{16}H_{33}$



proline derived quat
R= $C_{16}H_{33}$



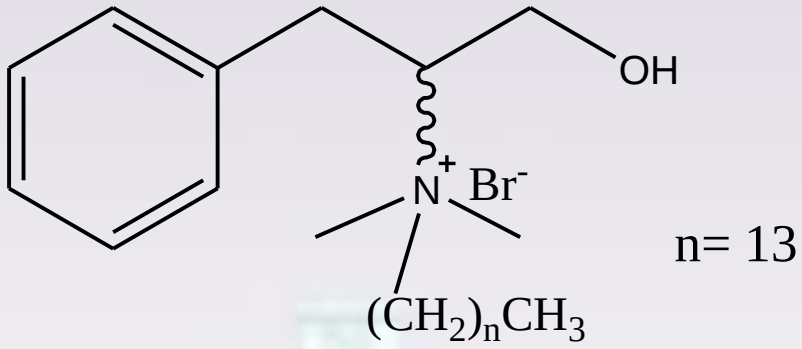
phenyalanine derived quat
R= $C_{16}H_{33}$

Examples of quats derived from amino acids

V. Jadhav, S. Maiti, Biomacromolecules, 9, 2008, 1852-1859.

Quats derived from phenylalanine as antibacterial agents

The following quat derived from phenylalanine was found to have high antibacterial activity with MIC 8.5 µM with respect to *S. aureus ATCC 6538*



Since quat derived from phenylalanine was found to have good antibacterial activity, it was envisaged to synthesise novel quats derived from this particular amino acid as potential antibacterial agents.

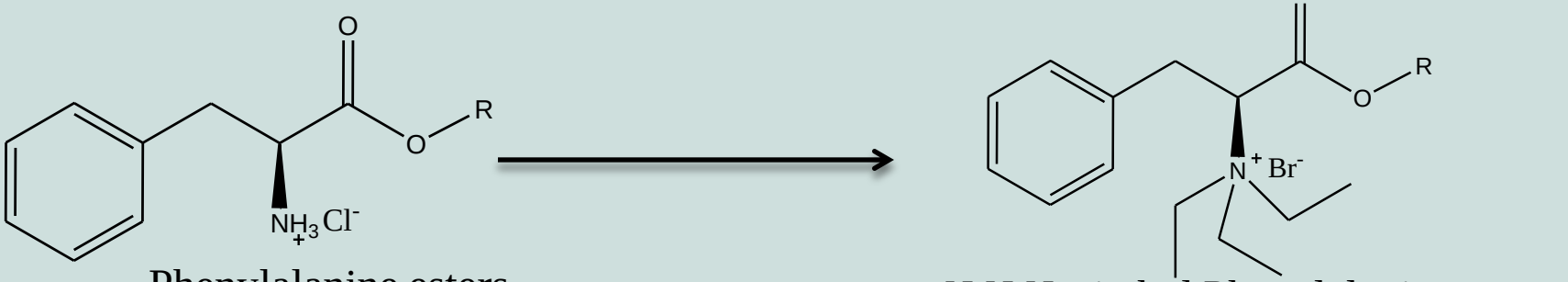
Lukac . M, Cent. Eur. J. Chem, 8, 2010 , 194–201.

Synthesis of *N*,*N*,*N*-triethyl phenylalanine esters

N,*N*,*N*-triethyl phenylalanine esters were synthesised novel quaternary ammonium compounds as potential antibacterial agents.

They were prepared by esterification of phenylalanine followed by tethering the amino group of the phenylalanine moiety with ethyl groups

Esters of increasing chain length were quaternised since antibacterial is also affected by chain length



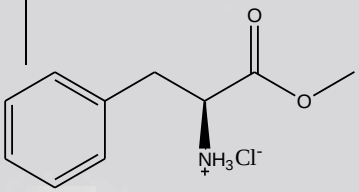
Phenylalanine esters

N,*N*,*N*-triethyl Phenylalanine esters

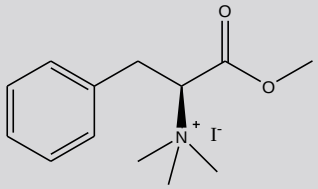
Compared to phenylalanine esters, these quats have:

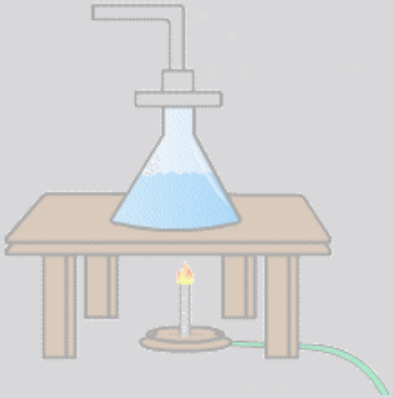
- Permanent positive charge on the head group
- Less pH dependent

Attempted synthesis of *N,N,N*-triethyl phenylalanine methyl ester

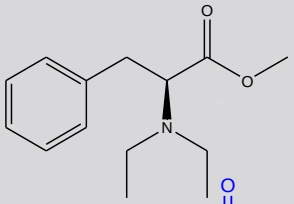


$NaHCO_3$/ MeI, methanol, room temperature

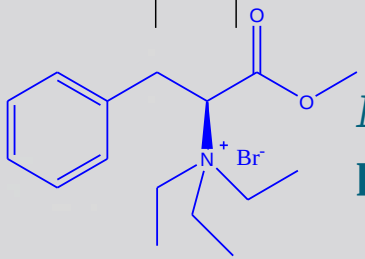




$NaHCO_3$/EtI, ethanol, room temperature

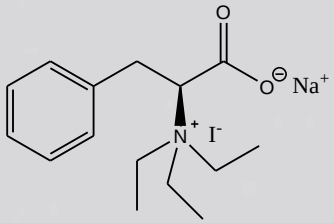


$NaHCO_3$/ EtBr, 90°C sealed tube



***N,N,N*-triethyl ammonium phenylalanine methyl ester**

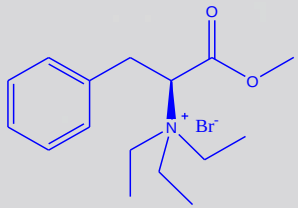
NaOH/EtI, 90°C sealed tube



TEA/ EtI, 90°C, sealed tube

No reaction

K_2CO_3/EtBr/ 90°C sealed tube.



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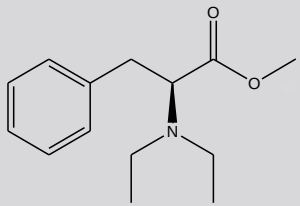
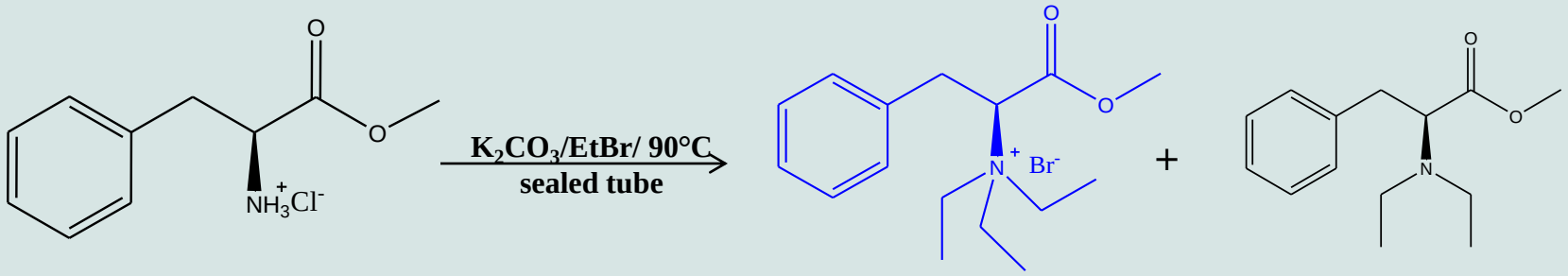


Synthesis of *N*,*N*,*N*-triethyl phenylalanine methyl ester

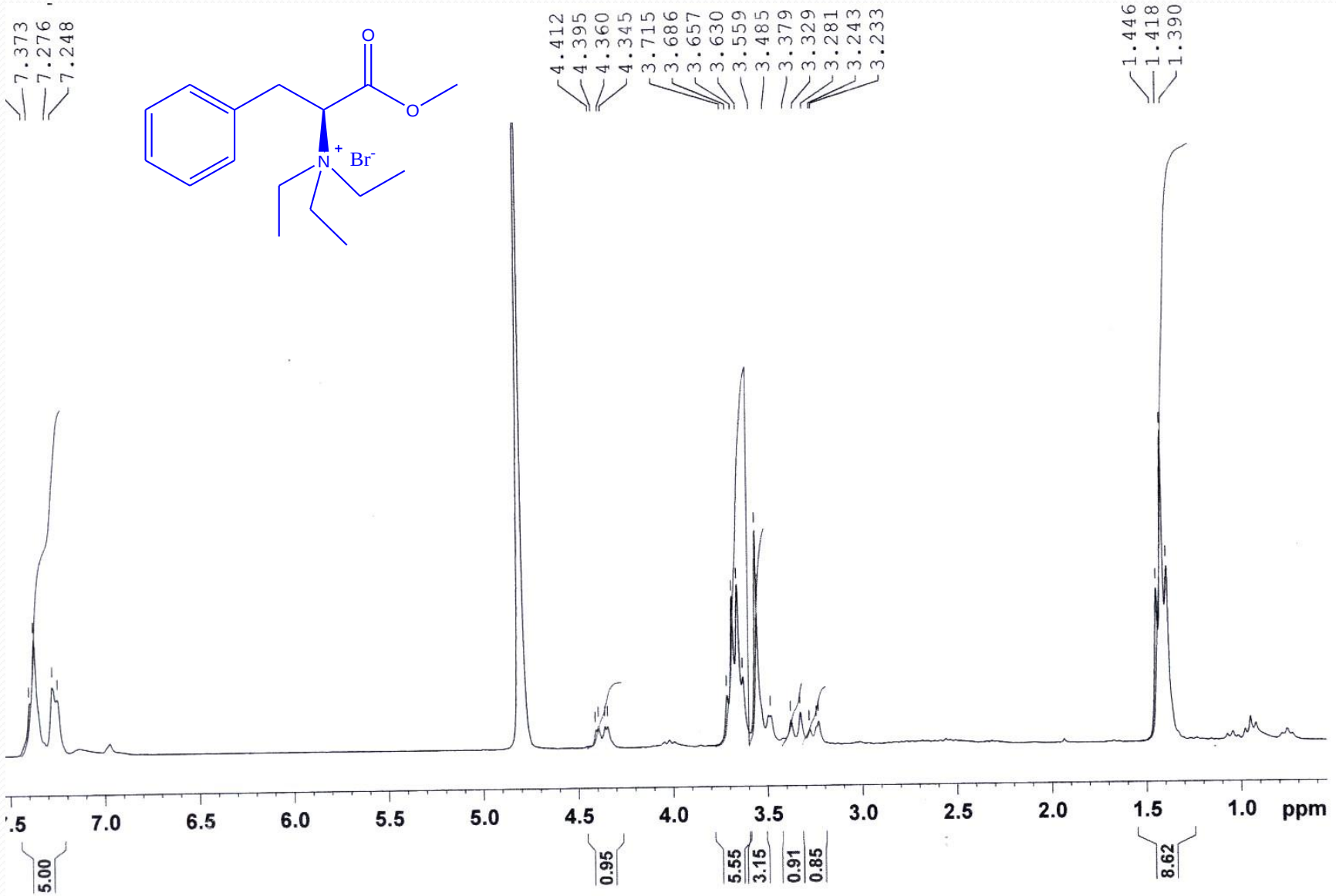


Compound 1a

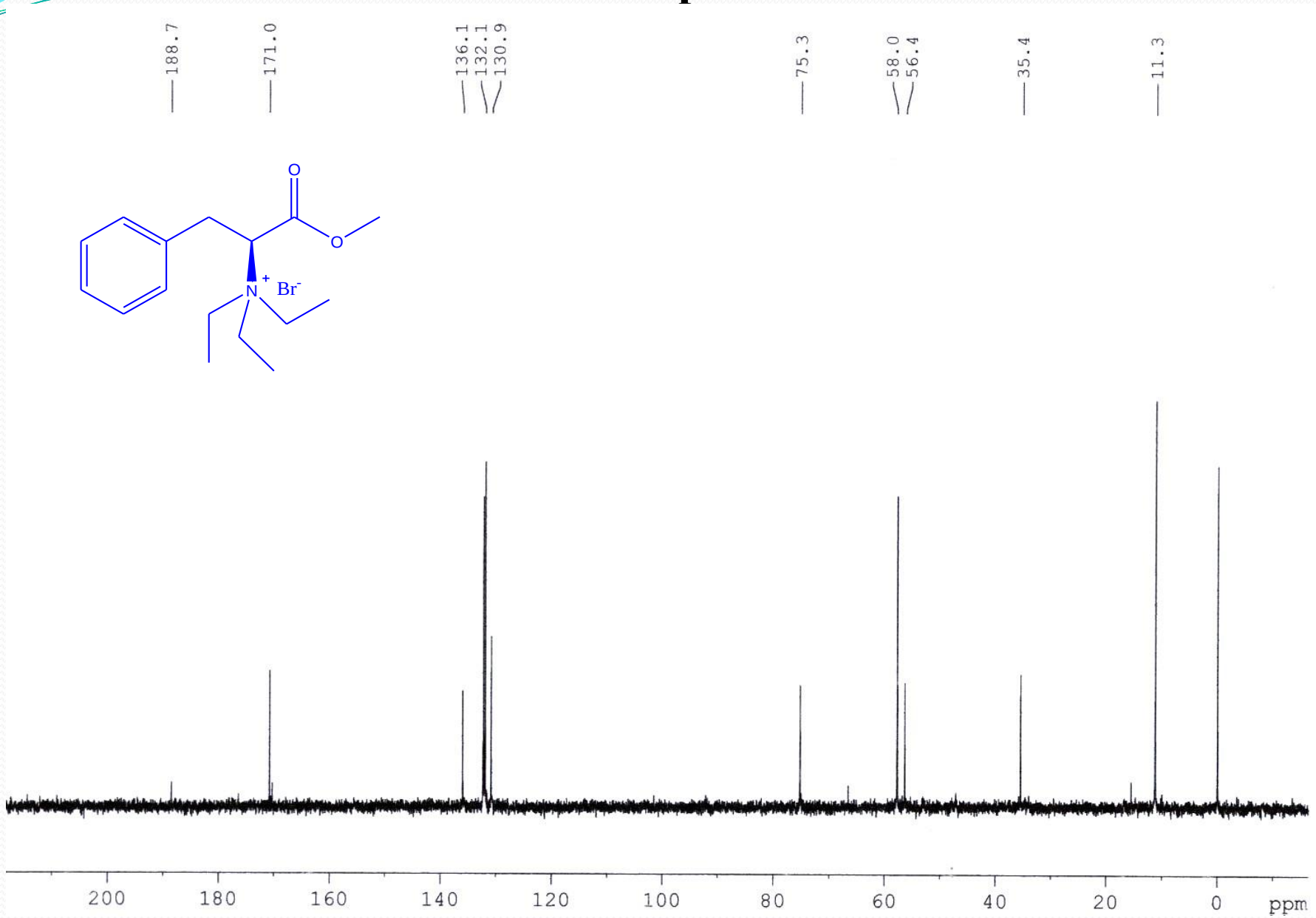
Compound 1b (Major product)

Quaternisation of phenylalanine methyl ester with ethyl groups gave rise to a mixture of the desired product (**1a**) with the diethyl derivative (**1b**) as the major product.

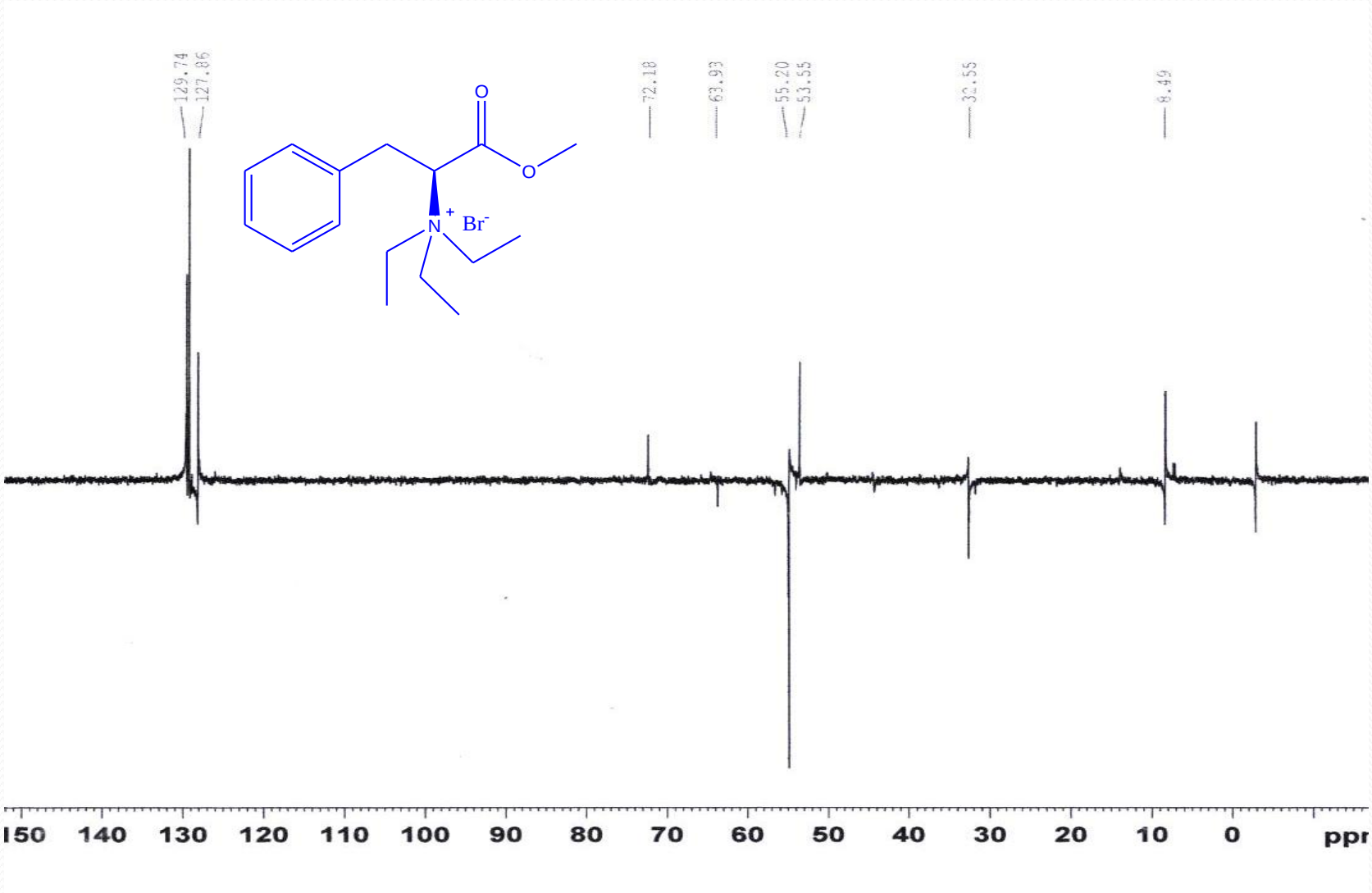
^{1}H NMR of Compound 1a



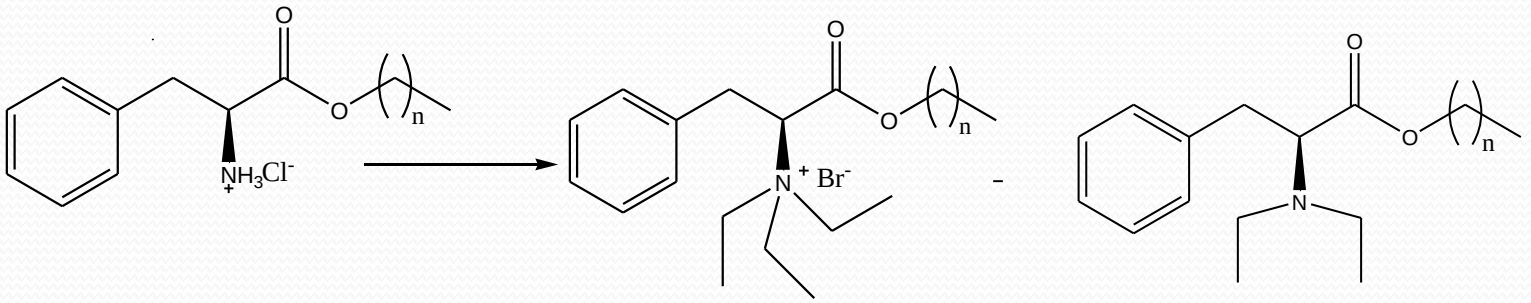
^{13}C NMR of Compound 1a



DEPT spectra of Compound 1a



Effect of alkyl ester chain length on the quaternisation of phenylalanine esters



n=0-3

***N,N,N*-triethyl phenylalanine esters**

Major product

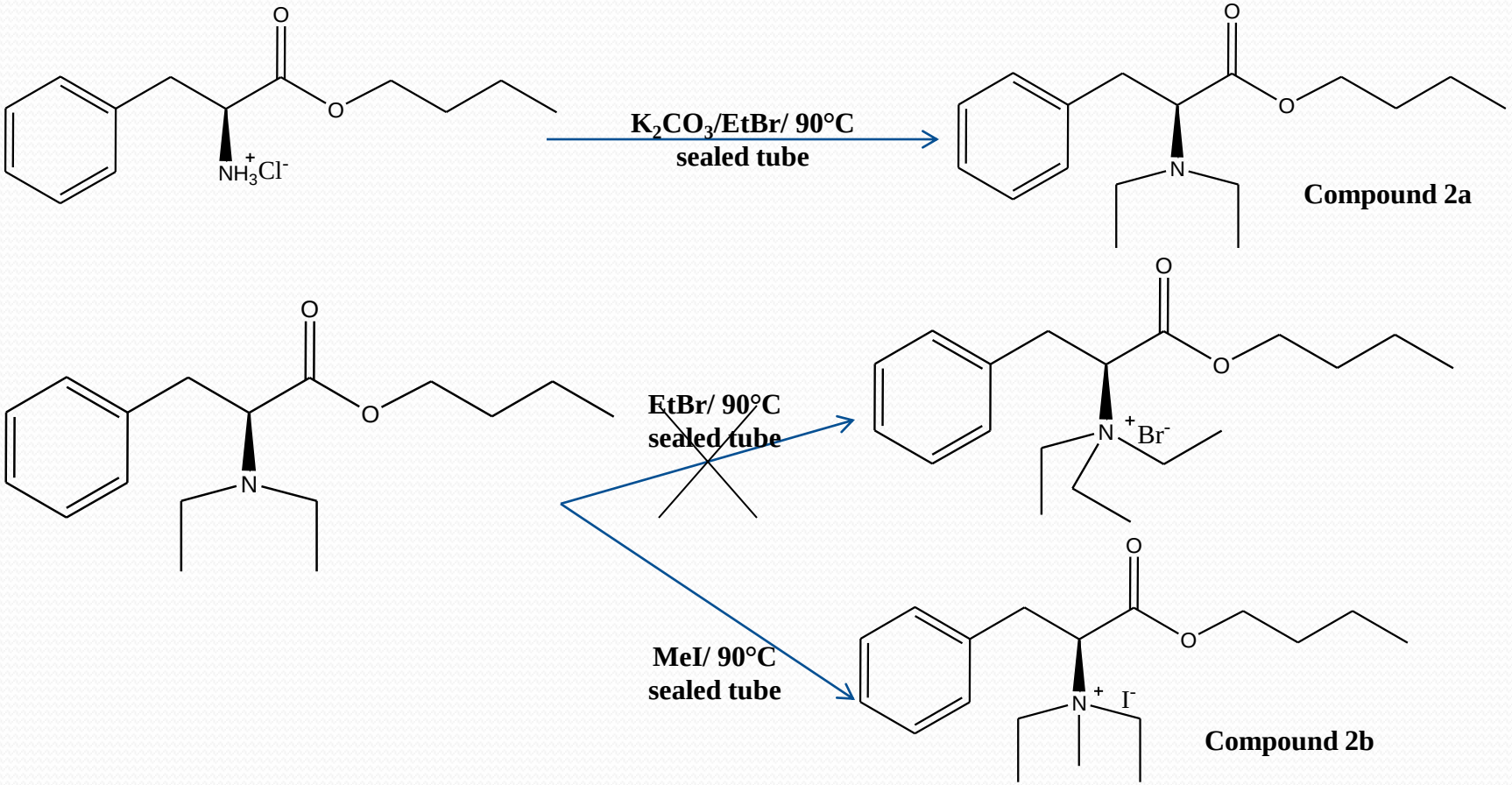
A **B**

n	Yield %	
	A	B
0	35	63
1	28	70
2	6	76
3	0	78

An increase of the alkyl ester chain length from methyl to propyl ester causes a decrease in the yield of the quaternary ammonium compound and an increase in the diethyl derivative.

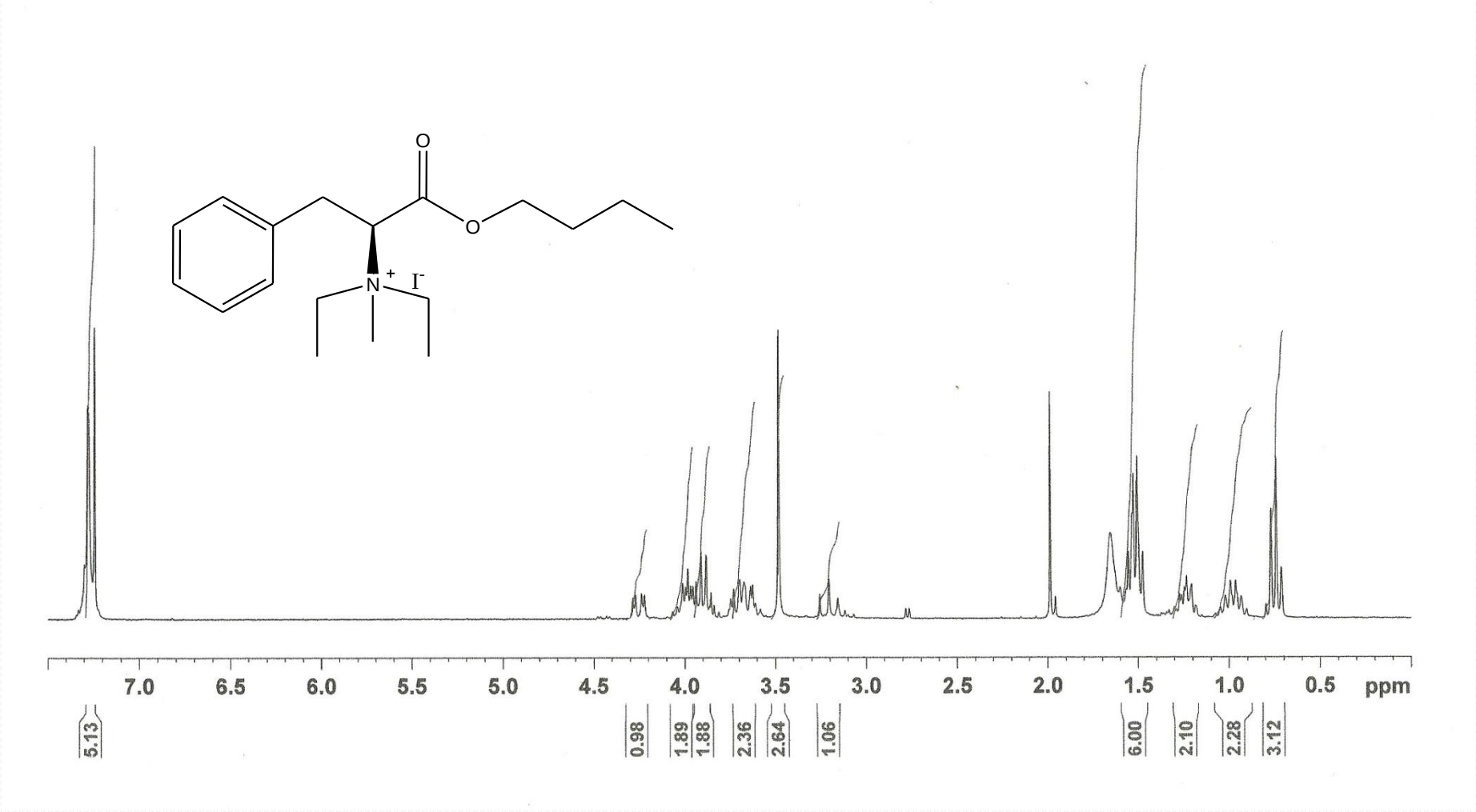
In the case of the butyl ester, the diethyl derivative was formed as the only product.

Attempted synthesis of quats from L-Phenylalanine butyl ester

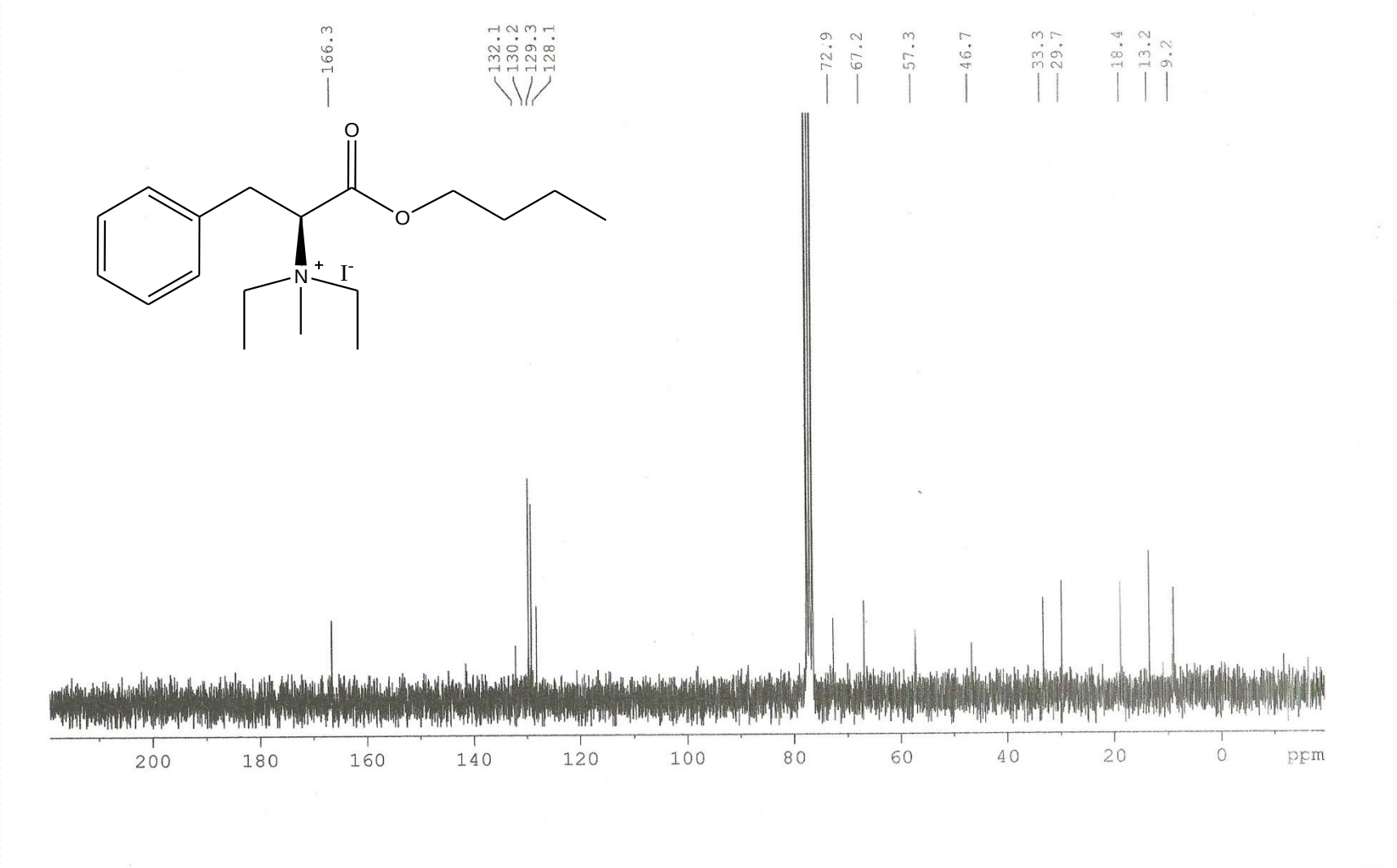


With alkyl ester chain length > C_3, quaternisation of phenylalanine esters with ethyl groups is not favoured due to steric hindrance. However, quaternisation of the diethyl derivative with a methyl group gave rise to the desired quaternary ammonium compound (2b).

^{1}H NMR of Compound 2b



^{13}C NMR of Compound 2b



Antibacterial screening of *N,N,N*-triethyl ammonium phenylalanine methyl ester

The Kirby-Bauer disc diffusion method was used to determine the activity of the compounds at 100 mg/mL . CTAB (cetyl trimethyl ammonium bromide) was used as positive control.

Microorganisms

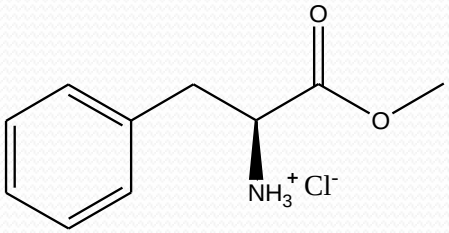
Gram positive bacteria:

- ***B. cereus* ATTC 11778**
- ***S. aureus* ATCC 29213**

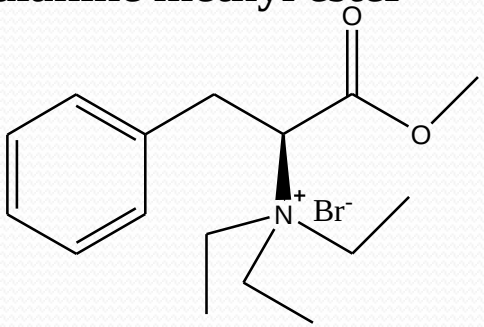
Gram negative bacteria

- ***S. typhimurium* ATCC 14028**
- ***P. aeroginosa* ATTC 27853**

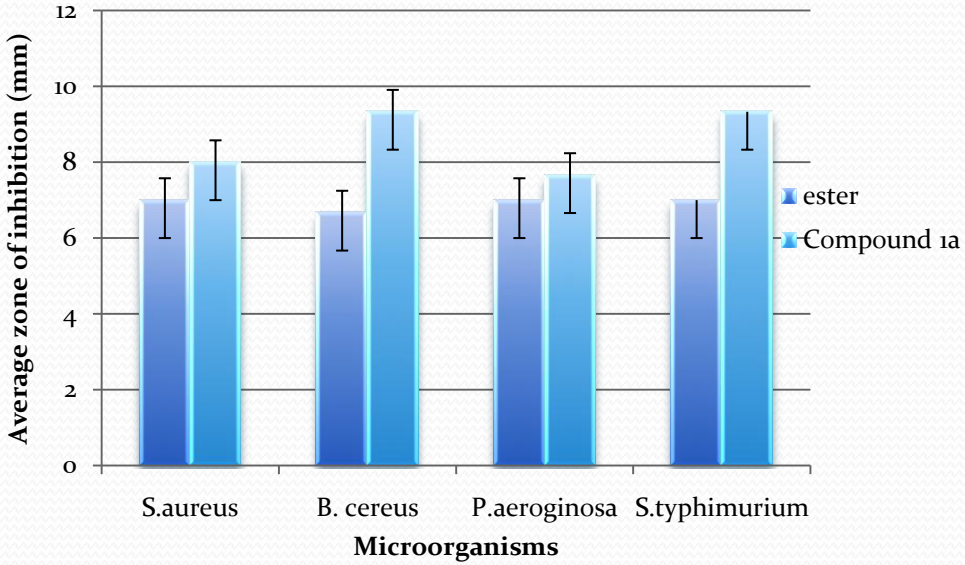
Activity of *N,N,N*-triethyl ammonium phenylalanine methyl ester (Compound 1a) with respect to phenylalanine methyl ester



Phenylalanine methyl ester



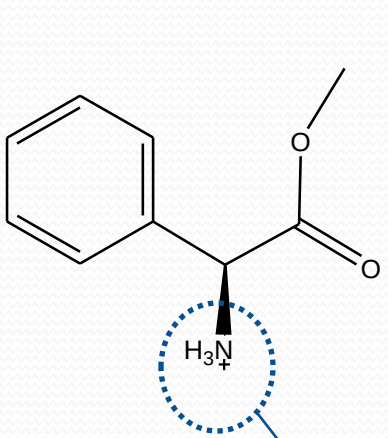
Compound 1a

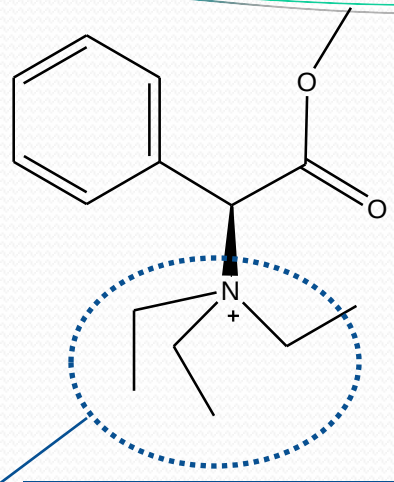


- **The activity of the *N,N,N*-triethyl ammonium phenylalanine methyl ester (Compound 1a) was compared to that of the unquaternised phenylalanine methyl ester by measuring their zone of inhibition at 100 mg/mL.**
- **In all cases, the zone of inhibition measured with respect to compound 1a was larger than that of the ester.**

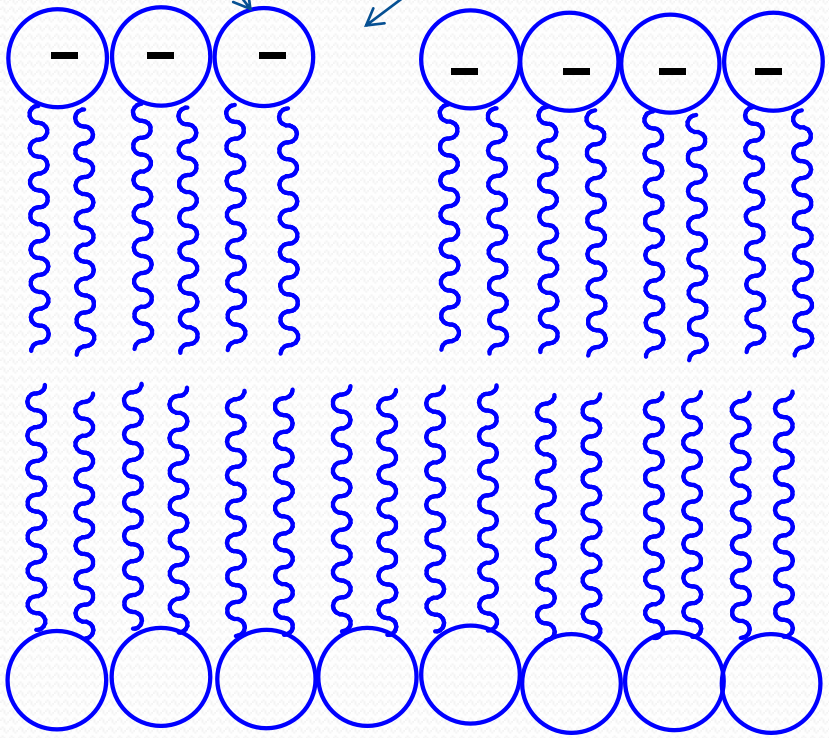
Possible mode of action of *N*,*N*,*N*-triethyl ammonium phenylalanine methyl ester

Phenylalanine methyl ester





Compound 1a



The higher activity of compound 1a might be attributed to a higher affinity of the compound for the membrane due to the extra ethyl groups which provide more hydrophobic interactions

Conclusion

- Compound 1a was found to be more active against the bacteria tested compared to phenylalanine methyl ester.

- This can be due to the fact that the quaternary ammonium moiety provides a more favorable environment for the electrostatic interaction between the negative oxygen group of the phospholipid than the primary amino group.

Acknowledgement

- **ECSOC 2013**
- **The University of Mauritius**
- **The Tertiary Education Commission**

Thank You!!